Study the role of rhizospheric bacterial community during the phytoremediation of distillery waste

THESIS

SUBMITTED TO
BABASAHEB BHIMRAO AMBEDKAR (A CENTRAL) UNIVERSITY, LUCKNOW

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW
प्रज्ञा शील करूणा
ESTABLISHED 1996

FOR THE DEGREE OF

Doctor of Philosophy

IN

Environmental Microbiology

Submitted By

Kshitij Singh

(Enrolment No. 1082/17)

Under the supervision of

Prof. Ram Chandra

DEPARTMENT OF ENVIRONMENTAL MICROBIOLOGY
SCHOOL FOR ENVIRONMENTAL SCIENCES
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A Central University)
VIDYA VIHAR, RAEBARELI ROAD, LUCKNOW-226025
UTTAR PRADESH, INDIA

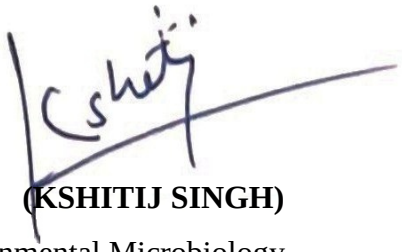
2022

Dedicated To My Parents

DECLARATION

I, **Kshitij Singh** hereby declare that the work which is being presented in the thesis entitled **"Study the role of rhizospheric bacterial community during the phytoremediation of distillery waste"** in partial fulfillment of the requirements for the award of the Degree of Doctor of Philosophy and submitted in the Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, Uttar Pradesh is an authentic record of my own work carried out during the period from February 2018 to September 2022 under the supervision of **PROF. RAM CHANDRA**, Head, Department of Environmental Microbiology, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow.

The matter presented in this thesis has not been submitted by me for the award of any other degree in any other University/Deemed University without proper citation. This is also declared that the thesis is essentially



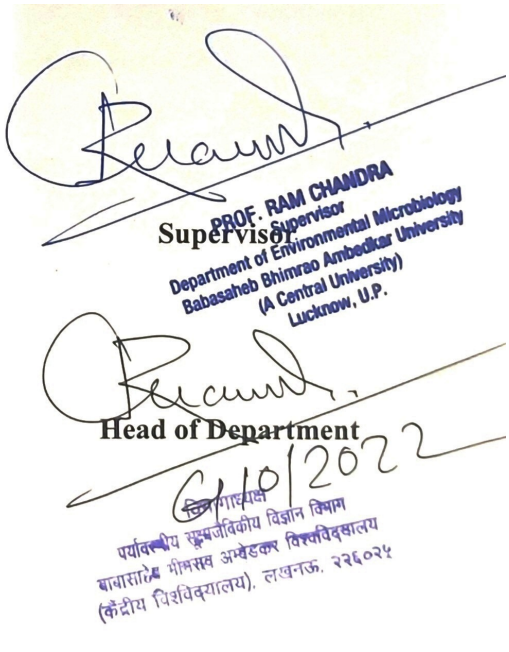
(KSHITIJ SINGH)
Department of Environmental Microbiology
School for Environmental Sciences
Babasaheb Bhimrao Ambedkar University
(A Central University),
Vidya Vihar, Raebareli Road,
Lucknow- 226025, Uttar Pradesh, India

CERTIFICATE

This is to certify that the dissertation report entitled **"Study the role of rhizospheric bacterial community during the phytoremediation of distillery waste"** submitted by **Mr. Kshitij Singh** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The thesis submitted to Babasaheb Bhimrao Ambedkar University, Lucknow satisfies all the requirements as stipulated in the Doctor of Philosophy (Ph.D.) regulation amended in 2017 and it is fit for submission and evaluation for the award of the degree of **Doctor of Philosophy** of the University.

Date: 06/10/2022



ACKNOWLEDGEMENT

*First and foremost I would like to thanks to my guide **prof. Ram chandra**, Head, Department of Environmental Microbiology, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow for his guidance, constructive criticism, endless patience, affection and cheerful encouragement throughout this study. His altruistic, benevolent and magnanimous nature has left an indelible mark in my heart and mind. Sir, you would always remain a source of inspiration in my whole life.*

*I owe gratitude to **Prof. Sanjay Singh,** Vice Chancellor, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow for providing excellent facility and research environment at the university which enable me to complete this research work and helping me to come up with the best possible outcome.*

*I extend my deep gratitude to **Department of Biotechnology, New Delhi** for financial support during this research work without which this study could not have been undertaken.*

*I would special thanks to my best friend, **Mr. Sunil Kumar**. He always helped me from start to end of my Ph.D.*

*I am extremely thankful to My Lab Members **Dr. Sangeeta Yadav**, **Ms. Sonam Tripathi, Mr. Ajay Kumar Singh, Mr. Adarsh Singh, Ms. Prerna Chaudhary** and **Mr. Zobair** for their valuable help and cooperation.*

I express my special thanks to all Faculties, Department of Environmental Microbiology, and School for Environmental Sciences, Babasaheb Bhimrao Ambedkar University, and Lucknow for his cooperation.

*I express my thanks to my lab staff **Mr. Sarju Singh and Vipin Kumar,** who were always helped whenever I required during experiments. They also supported to me for completion of thesis time to time as required.*

*Further, I would like to acknowledge **Dr. Ram Nageena Singh** for providing me the NGS analysis of rhizospheric bacterial community.*

*Finally, and most of all, I would like to give my special thanks to my **parent**, my uncles Mr. Harish Chandra, Mr. Bal Chandra, my brothers Gaurav, Saurabh, Shani, Raj and my sisters Garima and Priya, which always inspired me. It was their constant support, love, care, blessings advice and prayers that have been a driving force in all my efforts and brought me to this stage. I cannot begin to properly express my gratitude for all that you have done for me.*

Last but not the least I would like to thank my Lord Shiva, without whom nothing would be possible but with whom - everything is!

(Kshitij Singh)

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND SYMBOLS

α	**:**	Alpha
Al	**:**	Aluminium
$AlCl_3$		Aluminium chloride
amu	**:**	Atomic mass unit
ANOVA		Analysis of variance
AAS	**:**	Atomic absorption spectrophotometer
ATP	**:**	Adenosine triphosphate
APS	**:**	Ammonium per sulfate
ASS	**:**	Ammonium salt sugars
~	**:**	Approx
β	**:**	Beta
BD	**:**	Bacterial decolourised
$BaSO_4$	**:**	Barium sulfate
BLAST	**:**	Basic local alignment search tool
BOD	**:**	Biological oxygen demand
BSA	**:**	Bovine serum albumin
BSTFA	**:**	N,O-bis(trimethylsilyl)trifluoroacetamide
C	**:**	Carbon
$CaCl_2$	**:**	Calcium chloride
CaO	**:**	Calcium oxide
Cd	**:**	Cadmium
CEC	**:**	Cation exchange capacity
CFU	**:**	Colony forming unit
cm	**:**	Centimeter
COD	**:**	Chemical oxygen demand
Cr	**:**	Chromium
Cu	**:**	Copper
$CuSO_4$	**:**	Copper sulfate
CW	**:**	Constructed wetland
°C	**:**	Degree centigrade
DO	**:**	Dissolved oxygen
dNTP	**:**	Deoxynucleotide triphosphate
dATP	**:**	Deoxyadenosine triphosphate
dGTP	**:**	Deoxyguanosine triphosphate
dTTP	**:**	Deoxythymidine triphosphate
dCTP	**:**	Deoxycytidine triphosphate
DNA	**:**	Deoxyribo nucleic acid
DAD	**:**	Diode array detector
DSW	**:**	Distillery spent wash
eV	**:**	Electron volt
EC	**:**	Electrical conductivity
EDCs	**:**	Endocrine disrupting chemicals
EDTA	**:**	Ethylenediamine tetra acetic acid
EtBr	**:**	Ethidium bromide
F	**:**	Forward
Fe	**:**	Iron
$FeCl_3$	**:**	Ferric chloride
$FeSO_4$	**:**	Ferrous sulfate
Fig.	**:**	Figure
FT-IR	**:**	Fourier transform-infrared spectroscopy
λ	**:**	Wavelength

g	**:**	Grams
g L^{-1}	**:**	Gram per liter
GC-MS	**:**	Gas chromatography-mass spectrometery
GPM	**:**	Glucose peptone melanoidins
h	**:**	Hours
H_2S	**:**	Hydrogen sulfide
H_2O_2	**:**	Hydrogen peroxide
HCl	**:**	Hydrochloric acid
$HgCl_2$	**:**	Mercuric chloride
HNO_3	**:**	Nitric acid
HPLC	**:**	High performance liquid chromatography
HRT	**:**	Hydraulic retention time
IU	**:**	International unit
kV	**:**	Kilovolt
Kb	**:**	Kilo base
Kbp	**:**	Kilo base pair
kDa	**:**	Kilo Dalton
K_2HPO_4	**:**	Dipotassium hydrogen orthophosphate
L	**:**	Liter
LiP	**:**	Lignin peroxidase
Ltd.	**:**	Limited
M	**:**	Mol
mm	**:**	Millimeter
mM	**:**	Milimolar
m/z	**:**	Mass-to-charge ratio
µg	**:**	Microgram
µl	**:**	Microlitre
µm	**:**	Micrometer
mg	**:**	Milligrams
mL	**:**	Milliliter
mmol L^{-1}	**:**	Milimolar per liter
m^2/g		Metre square per gram
m^3d^{-1}	**:**	Cubic metre per days
mg L^{-1}	**:**	Milligram per liter
mg kg^{-1}	**:**	Milligram per kilogram
MEGA	**:**	Molecular evolutionary genetics analysis
$MgSO_4$	**:**	Magnesium sulfate
$MgCl_2$	**:**	Magnesium chloride
Mn	**:**	Manganese
MnP	**:**	Manganese dependent peroxidase/Manganese peroxidase
$MnCl_2$	**:**	Manganese chloride
MRPs	**:**	Maillard reaction products
MIP	**:**	Manganese independent peroxidase
MR	**:**	Methyl red
Min	**:**	Minutes
MW	**:**	Molecular weight
N	**:**	Normality
NaOH	**:**	Sodium hydroxide
Na_2SO_4	**:**	Sodium sulfate
ng·µL^{-1}	**:**	Nanogram per microliter
ng	**:**	Nanogram
nm	**:**	Nanometer
Ni	**:**	Nickel

NaCl	**:**	Sodium chloride
NaOH	**:**	Sodium hydroxide
NIST	**:**	National Institute of Standard and Technology
NCBI	**:**	National Center for Biotechnology Information
O	**:**	Oxygen
OTU	**:**	Operational taxonomic unit
OD	**:**	Optical density
(O-F) test	**:**	Oxidative-fermentative
OsO4	**:**	Osmium tetroxide
%	**:**	Percent
p	**:**	Para
pmol	**:**	Picomole
pUC	**:**	Plasmid vector UC
Pvt.	**:**	Private
pH	**:**	Potential of hydrogen
Pb	**:**	Lead
PAGE	**:**	Polyacrylamide gel electrophoresis
PCR	**:**	Polymerase chain reaction
PMDE	**:**	Post methanated distillery effluent
PMDS	**:**	Post methanated distillery sludge
QIIME		Quantitative Insights Into Microbial Ecology
R_f	**:**	Retardation factor
R	**:**	Reverse
RFLP	**:**	Restriction fragment length polymorphism
rRNA	**:**	Ribosomal ribonucleic acid
rDNA	**:**	Ribosomal deoxyribonucleic acid
RDP	**:**	Ribosomal database project
rpm	**:**	Revoultion per minute
RT	**:**	Retention time
SEM	**:**	Scanning electron microscope
EDS	**:**	Energy dispersive X-ray spectrometer
***Sau*3A**	**:**	*Staphylococcus aurous* restriction enzyme 3A
Sp.	**:**	Species
Sec	**:**	Seconds
SDS	**:**	Sodium dodecyl sulfate
SD	**:**	Standard deviation
SGA-MRPS	**:**	Sucrose-glutamic acid Maillard reaction Product
SCB	**:**	Sodium cacodylate buffer
SPSS	**:**	Statistical package for Social Sciences
T4	**:**	Bacteriophage T4
***Taq*1**	**:**	*Thermus aquaticus* type I
TMCS	**:**	Trimethylchlorosilane
TLC	**:**	Thin layer chromatography
TOC	**:**	Total organic carbon
TDS	**:**	Total dissolved solids
TS	**:**	Total solids
TSS	**:**	Total suspended solids
TN	**:**	Total nitrogen
TEM	**:**	Transmission electron microscope
TMS	**:**	Trimethylsilyl
TAE	**:**	Tri-acetate-EDTA
TEMED	**:**	N, N,N’N’-tetramethylethylenediamine

U	**:**	Unit
U mL^{-1} min^{-1}	**:**	Unit per milliliter per minute
UV	**:**	Ultraviolet
V	**:**	Volt
VS	**:**	Volatile solid
V3	**:**	Variable region third
V4	**:**	Variable region four
v/v	**:**	Volume/ volume
w/v	**:**	Weight/volume
X-Gal	**:**	5-Bromo-4-chloro-3-indoyl-β-D-galactopyranosides
Zn	**:**	Zinc
$ZnSO_4$	**:**	Zinc sulfate
3′	**:**	Three prime
5′	**:**	Five prime
<	**:**	Less than
>	**:**	Greater than

Chapter 1
Introduction

Chapter-1
Introduction

1.1 Introduction

Sugarcane molasses-based distilleries are sources of complex environmental pollutants due to the presence of various heavy metal-containing organic pollutants (Chandra et al., 2018). In India, there are more than 319 sugarcane molasses-based distilleries in operation that generate an approx. 1500 tons of sludge per day during the anaerobic digestion of spent wash (Chandra et al., 2018). The general process of alcohol production and effluent generation has been illustrated in Fig.1.1. The main source of effluent generation is the distillation stage of fermented molasses. During the distillation process, an average of 12-15 L spentwash is generated for each litre of ethanol produced. This reflects the magnitude of environmental pollution caused by effluent generated from the distillery sector all over India (Tripathi et al., 2021).

The sludges of distillery contained high iron (Fe), zinc (Zn), copper (Cu), chromium (Cr), cadmium (Cd), manganese (Mn), nickel (Ni), and lead (Pb) content, all present in concentrations above the prescribed limit in the environment recommended by US Environmental Protection Agency (USEPA), Environmental, U. S. (2006) (Firestone et al., 2010). In addition, the recent studies have highlighted that distilleries sludge contains several persistent organic compounds e.g. dodecanoic acid, octadecanoic acid, n-pentadecanoic acid, hexadecanoic acid, β-sitosterol, stigmasterol, β-sitosterol trimethyl ether, heptacosane, dotricontane, lanosta-8, 24- dien-3-one, 1-methylene-3-methyl butanol, and 1-phenyl-1-propanol as androgenic and mutagenic compounds. These compounds are listed by the USEPA, 2012, as endocrine-disrupting chemicals (EDCs). Due to the complexation of metals with organic pollutants, these organometallic compounds restrict the bioavailability of metals to plants. Consequently, their bioremediation and biodegradation process is seized. Therefore, these pollutants persist in environment for long time without much change. Hence, the generated sludge remains alkaline with a high pollution load they do not allow the microbial growth and any vegetation. Moreover, the existing vegetation is also damaged (Tripathi et al., 2021).

Distillery waste contains a high concentration of recalcitrant organic pollutants generated during the processing of sugarcane juice in sugar industries and alcohol production. In addition, distillery waste water also contains natural color contributing compounds such as polyphenols, caramels, melanoidins and alkaline degradation products of hexoses (ADPH) etc. (Tripathi et al., 2010). The major colourant compound present in distillery effluent is due to melanoidins (Singh et., 2021). Melanoidins are nitrogenous, heterocyclic brown pigments polymer generated during the chemical reaction between amino and carbonyl compounds at elevated temperatures (Singh et al., 2021). These compounds are synthesized by the reaction occurring between the amino groups of amino acids, peptides, or proteins and the carbonyl groups of reducing sugars. These polyphenolic compounds have antioxidant, anti-microbial, anticarcinogenic, free radical scavenging and metal chelating properties (Singh et al., 2021).

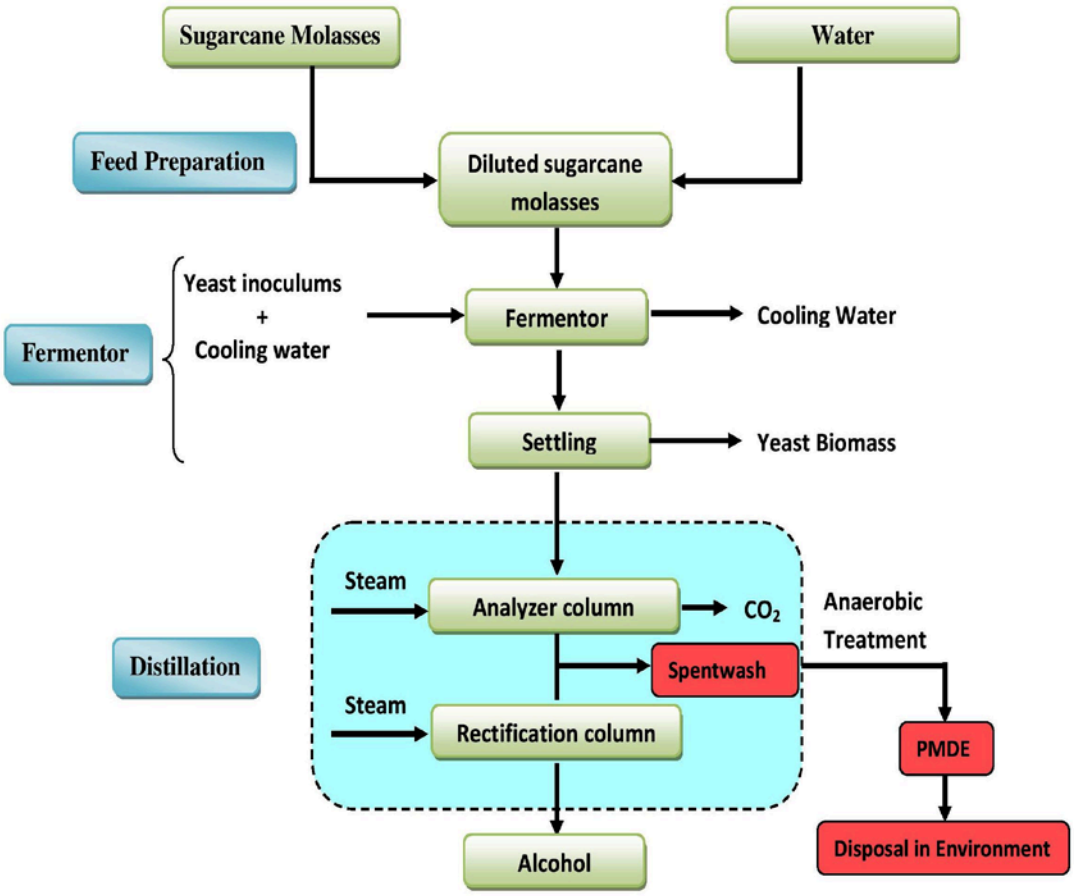


Fig.1.1. Flow diagram representation of ethanol production and PMDE disposal in environment.

Phenolic compounds are also reported to react with proteins during beer storage and form high molecular weight compounds and hazes (Aron and Shellhammer, 2010). The presence of polyphenols in distillery waste is largely depends on the source of molasses and sugar content in feed flow (Arimi et al., 2014). Polyphenols are categorized into three broad classes: phenolic acids, flavonoids, and tannins. The phenolic compounds detected in molasses based distillery waste includes benzoic acid and its derivatives (e.g., gallic acid), cinnamic acid and its derivatives (e.g., coumaric acid, caffeic acid, chlorogenic acid and ferulic acid) ring compounds (Chowdhary et al., 2018).

Fig.1. Figure showing the environmental impact of distillery waste at disposed site. (a) Discharge of PMDE (b) ecosystem affected after PMDE disposal at open area (c) View of distillery sludge discharged from methane reactor (d) Potential plants growing on disposed sludge showing phytoremediation

The discharge of distillery waste in water bodies without adequate treatment causes severe water pollution. Due to its high BOD, COD values, high sulphate, phosphate, and nitrogen content, it causes eutrophication of contaminated water resources (Bhateria and Jain 2016). The toxicity of distillery wastewater (DWW) to

aquatic organism has been reported by several researchers. Mahimaraja and Bolan (2004) have estimated the LC50 value of 0.5% by using a bio-toxicity test on fresh water fish *Cyprinus carpio* var. communis. Besides,, it was reported by some other researchers that respiratory process in *Cyprinus carpio* under DWW stress get affected resulting in a shift towards the anaerobic conditions at organ level during the sublethal intoxication (Ramakritinan et al., 2005).

Distillery waste also causes soil pollution and acidification in the case of inappropriate land discharge. Further, it has been also pointed out that it inhibits seed germination, reduces soil alkalinity, cause soil manganese deficiency and reduces the growth and yield of crop plants (Yadav and Chandra, 2019). In addition, Bharagava and Chandra (2010) have also reported that post methanated distillery effluent (PMDE) have deleterious effects on seed germination and seedling growth parameters in *Phaseolus mungo* (L). The inhibition in seed germination at higher PMDE content might be attributed due to high salt concentration and TDS, which increases high osmotic pressure (OP) and anaerobic conditions, respectively. These conditions affect various biochemical and physiological activities such as movement of solute, respiration and enzymatic process of seed germination. It has been also reported that high PMDE concentration also acts as an inhibitor for plant growth hormone(s) (auxin and gibberline), which play an important role in plant growth and development (Bharagava and Chandra, 2008). Moreover, Bharagava et al. (2008) have reported that at higher PMDE concentration, the entrance of potentially toxic trace elements into the protoplasm may result in the reduction of intermediate metabolites, which are responsible for the reduction in plant growth parameters.

Besides soil and water pollution, the residents of distillery waste contaminated area also face severe health problems such as irritation of eyes, skin allergies, headache, fever, vomiting sensation, and stomach pain etc. due to gaseous pollutants generated from wastes. Moreover, due to aquatic and soil pollution they also adversely affect to human health. All these problems might be due to the presence of high concentration of dissolved impurities like carbonates, bicarbonates, sulphates, calcium chloride, magnesium, iron, sodium, and potassium along with the colloidal impurities like coloring compounds, organic waste, finely divided silica and clay (Patil, and Prasad, 2020).

Some researchers have reported that excessive glycation process also destroys the essential amino acids, inactivation of enzymes, cross-linking of glycated extra-cellular matrix, inhibition of regulatory molecule binding sites, altered macromolecular recognition, abnormalities in nucleic acid function, endocytosis and increased immunogenicity etc. (Echavarría and Ibarz, 2012). Further, the genotoxic compounds can act at various levels in cells (causing gene, chromosome, or genome mutations), necessitating the use of a range of genotoxicity assays designed to detect these different types of mutations (Taylor et al., 2004). Glösl et al. (2004) have demonstrated that heated sugarcasein model melanoidins consisting variable sugars exhibit different mutagenic activity. For example, ketose sugars (fructose and tagatose) showed a remarkably high mutagenic activity as compared to their aldose isomers (glucose and galactose) and generated reactive oxygen species results in the breaking of DNA strands and mutagenesis.

In addition, some other maillard reaction products (MRPs) were also reported to induce chromosomal aberrations in Chinese hamster ovary cells and gene conversion in yeast cells. The mutagenicity and DNA strand breaking activity of glucose-glycine model melanoidins was also demonstrated by Chandra and Bharagava (2008), who reported that LMW fractions act as lipid sink and induced DNA damage, where the effect increases with increase in concentration.

Though, various bioremediation techniques have been reported for mitigation of environmental pollution by distillery waste in past decades (Rana et al., 2017). But phytoremediation has emerged as a promising green technology for the decontamination of organometallic polluted sites of various Industrial wastes including sugarcane molasses based distilleries (Tripathi et al., 2021; Montpetit and Lachapelle, 2017). Further, recent studies have also highlighted the phytoextraction potential of some native plants growing as weeds and grasses such as *Argemone Mexicana* (mexican poppy), *Chenopodium album* (goosefoots), *Rumex dentatus* (toothed dock), *Tinospora cordifolia* (giloy), *Calotropis procera* (madhaar), and *Basella alba* (pui)for metal-contaminated sources and other organometallic industrial sludge disposed sites (Rana et al., 2022). However, among the potential metal accumulators, several are known as food and medicinal plants (Shammi et al., 2016; Singh and Prasad, 2014) which may be well recorded for social awareness to maintain

the food and medicinal quality of crop plants or other medicinal plants because during phytoextraction of metals from sludge will damage the food quality or medicinal properties of their metabolites (Suman et al., 2018).

While many common native plants have been reported for more potential to grow on disposed sludge of distillery waste, however, the accumulation of heavy metals by these plants in the environment from the multi-metal contaminated site is not well studied (Kumar and Chandra, 2019). To understand the impact of complex industrial wastes in plants and explore their potential for phytoremediation, there is a need to identify the mutagenic and carcinogenic constituents of the distillery sludge and the metal-accumulation patterns in these native plants. Heavy metal pollution in soil, water, and food material is a major threat to human health. Heavy metals tend to accumulate in the soil, water, and plants in the environment, which could contaminate the food chain subsequently (Ali and Khan, 2019). For example, Indian mustard grown in distillery effluent irrigated soil showed significant accumulation of heavy metals (Cd, Cu, Fe, Mn, Ni, and Zn) in their edible parts (i.e. seeds and leaves) and could pose a health risk to humans (Bharagava et al., 2008). It is noteworthy that at <10% (v/v), the effluent showed an inducible effect on the growth of the mustard plant; while at >10% (v/v), the effluent showed an inhibitory effect on the various physiological parameters of the plants.

A cheaper, practical and ecologically relevant alternative is the bacterial assisted phytoremediation, association of plants with microorganisms that contribute to the degradation and removal of pollutants both from soil and wetland ecosystem (Bert et al., 2009). Bacterial assisted phytoremediation, referred to as a type of phytoremediation, involves the interaction of plant roots and the bacteria associated with these root systems to remediate soils containing elevated concentrations of organic compounds (Morel et al., 2006). These techniques could provide cost-effective methods of remediating soils and groundwater contaminated with metals, radionuclides, and various types of organics, with fewer secondary wastes and less environmental impact than would be generated using traditional remediation methods. Bacterial communities like *Bacillus, Rhodococcus, Paenibacillus, Acidovorax, Alcaligenes, Mycobacterium,* and *Pseudomonas,* etc. are also able to enhance the phytoremediation process. For example, they can increase metal availability via the production of polysaccharides and polymers (Wang et al., 2022).

Phytoremediation for in situ remediation for industrial wastewater being seen as an efficient, sustainable and cost-effective remediation technique compared to conventional physical–chemical techniques (Ali et al., 2013). During phytoremediation, biodegradation of pollutants is promoted by the synergy between plants and the microorganisms present in the rhizosphere, the region of soil that is directly influenced by root secretions. Successful phytoremediation is dependent on the survival and growth of plants on contaminated sites, as well as the ability of the rhizosphere to support an active soil microbial population (Hou et al., 2015). Phytoremediation is defined as the use of plants to destroy, sequester and remove toxic pollutants from the environment. However, this method also has many drawbacks. Therefore, phytoremediation associated with rhizospheric microorganisms has emerged as an acceptable agronomic remediation technology. The relationships that exist between plants and microbes in the rhizosphere play a key role in enhancing the efficacy of phytoremediation through a process known as 'bio-assisted phytoremediation' (Sharma et al., 2021).

Efficacy of phytoremediation strongly depends on a large and deep penetrating root system and a high transpiration rate (Yadav et al. 2018). When the pollutant is assimilated and translocated within plant tissues, metabolic transformation processes occur that are mediated by a great variety of enzymes for several types of substrates (Kvesitadze et al., 2015). The plant metabolic transformation process depends directly on the solubility and bioavailability of the pollutant, to make phytoremediation a feasible process. Physicochemical and structural properties determine the uptake of organic chemicals by plant roots from the soil (Delgado and Gómez, 2016).

The plant strategies for growth and phytoremediation at the polluted site involved various biochemical mechanisms for the amelioration of environmental pollutants. The mechanism of plant strategies is regulated through the production of glutathione, phytochelatins, metallothioneins, and various organic acids (Kumar et al., 2016). Therefore the plant may adopt phytoextraction, phytostabilization, phytovolatilization, or rhizofiltration strategies based on the nature of pollutants and plant species (Tripathi et al., 2021). The phytoextraction process is mediated by the combined action of bacteria and plants due to secretion of some organic acids from plant and bacteria facilitating plant growth and transpiration mechanism (Meena et al., 2021). Consequently, various heavy metals are accumulated in the plant from complex organometallic wastes. In addition to physico-chemical factors such as soil

quality temperature, moisture, and light characteristics that influence plant root growth and development, these rhizobacteria also affect the efficiecy of phytoremediation and known as Plant growth Promoting Rhizobacteria (PGPR) (Gouda et al., 2018). PGPR include bacteria that inhabit the rhizosphere, improve plant health and may also enhance plant growth. It is well established that only 1 to 2% of bacteria promote plant growth in the rhizosphere. PGPR promote plant growth and enhance phytoremediation through a variety of activities, such as synthesis is of phytochromes and plant hormones [e.g., indole acetic acid (IAA)], bio-control, promote nodulation in legumes, boost seedlings development, increase mineral phosphate solubilization, produce siderophores and acetyl-CoA carboxylase (ACC) (Etesami and Maheshwari, 2018). Through systemic acquired resistance and induced systemic resistance, PGPR also help plants to become more resistant to various plant pathogens and environmental stresses (Kamle et al., 2020). Several studies showed that PGPR species including: *Azotobacter, Pseudomonas, Rhodococcus, Methylobacterium, Kluyvera, Ochrobactrum, Variovox, Burkholderia, Bacillus, Brevibacillus*, *Flavobacterium, Xanthomonas, Azomonas* and *Brevundimonas*, have been successfully used to increase plant growth in phytoremediation (Tripathi et al., 2022; Khan et al., 2009; Yadav et al., 2019).

The general process of microbial colonization in rhizosphere includes bacteria, fungi, acticomycetes, protozoa and algae (Fig. 1.2). However, bacteria are the most abundant microbial present in the rhizosphere. The genera such as *Azospirillum*, *Azotobacter, Acetobacter diazotrophicus, Azoarcus* include bacterial species that have the ability to fix nitrogen biologically (Turan et al., 2016). Besides biological nitrogen fixation, phosphate solubilization is also an important phenomenon in the rhizosphere that enhances the nutrient availability to the host plant. Direct and indirect mechanisms are followed by PGPR for plant growth (Oleńska et al., 2020). The direct mechanism includes secretion of phyto-hormones i.e. auxin, decrease of plant ethylene levels and facilitates the uptake of nutrients from the environment through nitrogen fixation. Direct plant growth promotion by PGPR involves either providing plants with microbe-oriented compounds or helping in the absorption of several nutrients from the environment that are essential for plant growth. While, indirect mechanism PGPR prevents the deleterious effects of one or more phytopathogenic organisms (Jeyanthi, and Kanimozhi, 2018). This includes production of antagonistic

substances for inhibition of phathogenic microbes it is a type of biological control. Among these mechanisms PGPR adopted one or more for plant growth. PGPRs control the detrimental effects of pathogenic agents on plants to reduce the impact of diseases by producing growth inhibitors i.e. antibiotics, bacteriocins, siderophores, induction of systemic resistance, competition for nutrients and niches and lytic enzymes or by increasing natural resistance of host plant (Wang et al., 2018). Antagonistic activity of PGPR is regulated by several mechanisms including competition, parasitism, and siderophores or antibiotics production (Tariq et al., 2017). The mechanism for biocontrol by PGPR is induced systemic resistance (ISR) that manipulates the physical and biochemical properties of the host plant for controlling plant diseases. The direct mechanisms are biofertilization, stimulation of root growth, rhizoremediation, and plant stress control.

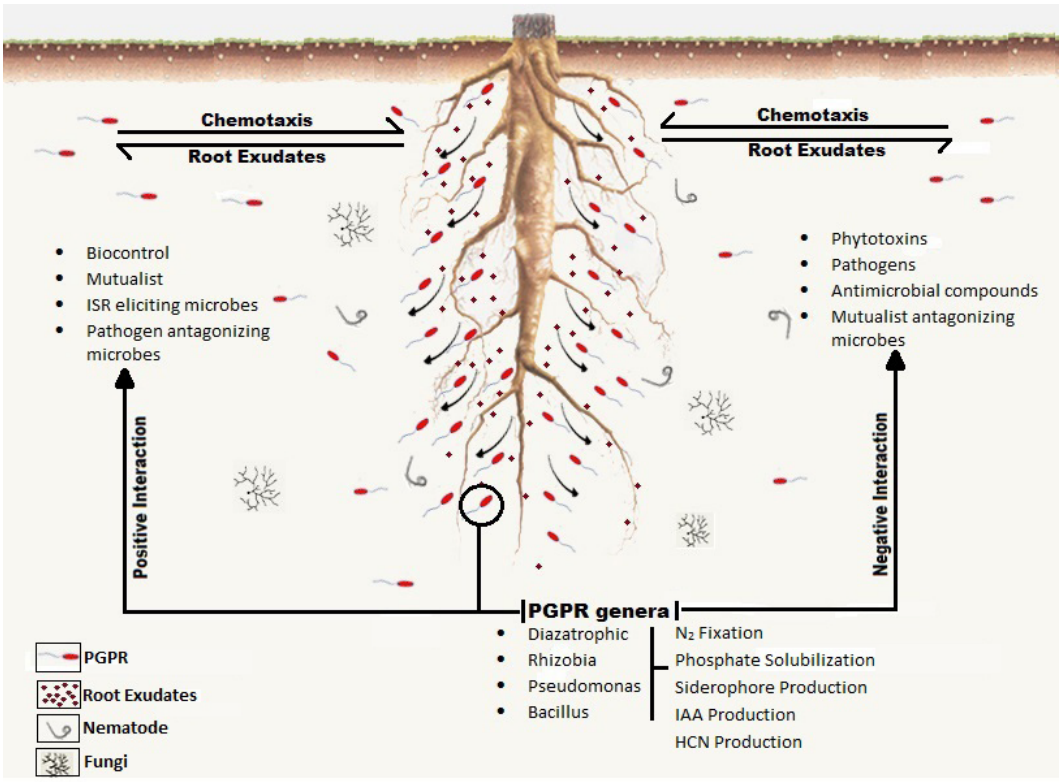


Fig.1.2. Ecology and Biodiversity of PGPR living in the Rhizosphere

PGPR and their interactions with plants are exploited commercially and hold great promise for sustainable agriculture. Applications of these associations have been investigated in maize, wheat, oat, barley, peas, canola, soy, potatoes, tomatoes, lentils, radicchio and cucumber (Gray and Smith, 2005). PGPR are agriculturally important

bacteria having specific symbiotic relationships with plants. PGPR serve as one of the active ingredients in biofertilizer formulation. Based on the interactions with plants, PGPR can be separated into symbiotic bacteria, whereby they live inside plants and exchange metabolites with them directly and free-living rhizobacteria, which live outside plant cells. Symbiotic bacteria mostly reside in the intercellular spaces of the host plant, but there are certain bacteria that are able to form mutualistic interactions with their hosts and penetrate plant cells. In addition to that, a few are capable of integrating their physiology with the plant, causing the formation of specialized structures. Rhizobia, the famous mutualistic symbiotic bacteria, could establish symbiotic associations with leguminous crop plants, fixing atmospheric nitrogen for the plant in specific root structures known as nodules. PGPR can also be termed as plant health promoting rhizobacteria (PHPR) or nodule promoting rhizobacteria (NPR) and are attached with the rhizosphere that is an important ecological environment of soil for plant-microbe interactions. Various species of rhizobacteria belonging to the genera *Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Bacillus, Bradyrhizobium, Burkholderia, Enterobacter, Flavobacterium, Klebsiella, Mesorhizobium, Pseudomonas,Rhodococcus, Streptomyces, Serratia* etc. have been reported to promote plant growth and antagonize plant pathogens.

By adopting various strategies, including the production of antioxidant enzymes, subcellular localization of metals, and organic acid exudation, these plant growth promoting bacteria, such as Pseudomonas and Bacillus, alleviate abiotic stresses in plants (e.g., heavy metal toxicity), enhance plant growth (Wang et al., 2020) and promote plant life (Sharma et al., 2020a). According to the Environmental Protection Agency (EPA), heavy metals such as Cu, Cd, Pb, As, Cr, Ni, and Hg are the most common environmental pollutants. Several studies showed that plant growth-promoting rhizobacteria (PGPR)-assisted phytoremediation were very effective in removing of heavy metal from contaminated soils (Dhiman et al., 2016; Sharma et al., 2021a, b). The application of PGPR is regarded to be a cost-effective and environmentally friendly treatment to enhance the clean-up process of contaminated soil by plants that are subjected to biotic and abiotic stress (Pandey et al., 2017; Sharma et al., 2020a, b). PGPR are helpful for plant growth enhancement and bioremediation of contaminated soil through sequestering or degrading heavy metals and other toxicants. Bioremediation is, therefore, an option that offers the

possibility to destroy or render harmless, various contaminants using natural biological activity. PGPR assist phytoremediation directly or indirectly through several mechanisms, such as increased nutrient uptake, suppressing pathogens by producing antibiotics and siderophores or bacterial and fungal antagonistic substances (hydrogen cyanide), phytohormone production and nitrogen. Many plants are shown to accumulate metals and biotransformation of recalcitrant compounds.

Fast community fingerprints of 16_S rDNA denatured gradient electrophoresis (DGGE) based on a polymerase chain reaction (PCR) showed bacterial phyla such as *Proteobacteria* and *Firmicutes* are commonly found in the rhizospheric zone of plants to remove heavy metals from different industrial contaminants (Marques et al., 2015). The development of mutually beneficial interaction between the host plant and rhizosphere micro-organisms leads to the enhancement of plant growth and resilience (Hansen et al., 2017). Hence, the use of appropriate rhizosphere microorganisms can simultaneously increase metal phyto-availability, decrease metal toxicity and stimulate the host plant biomass to accumulate a high level of metals (Shelake et al., 2018). The enhancement of interactions between plants and rhizosphere bacteria could be a critical component of phytoremediation technology. In heavy metals contaminated environment, the luxuriant growth of several indigenous plants suggested the phytoextraction capacity of these native plants against the heavy metals (Kumari et al., 2016). The use of native plants is strongly recommended, but little work has been carried out regarding using invasive species for phytoremediation. The invasive species often out compete native plants due to their adaptability and robustness (Traveset, et al., 2014).

The microbial population present in the rhizosphere or near the root zone can degrade the pollutant or make it more bioavailable, challenging the plant tolerance when exposed to environmental pollutant (Martin et al., 2014). Plant growth promoting rhizobacteria (PGPR) have also been used as inocula to further increase plant growth, reduce environmental stress and promote degradation by rhizosphere-associated microorganism (Hou et al., 2015). Mainly the studies have been focused only on the remediation of heavy metals through rhizospheric bacteria from the contaminated sites. But, the study on the removal and minimizing of organic pollutants from the polluted sites is not much studied so far.

Some native potential plants (weeds and grasses) grow on the discharge site of distilleries sludge; they have been reported for heavy metal phytoextraction by Chandra et. al. 2018. But, there rhizospheric bacterial community is not yet revealed by any researcher. Due to the specific variant of pollutants there may be indigenous rhizospheric bacterial community which is playing role in mineralizing and assisting phytoremediation of distillery waste. Thus, distillery waste may be responsible for the variation in the population dynamics of different tolerant group's rhizospheric bacterial community. Rhizospheric bacterial community may increase bioremediation of soil contaminated with distillery waste.

Several researchers have focused on bioremediation of environmental pollutants through rhizospheric bacterial community of various industries. The maximum work on distillery has been updated to the phytoremediation of heavy metal and organic compounds from distillery waste by Chandra et. al. 2018, Chandra and Kumar; 2017. But, there rhizospheric bacterial community assisting phytoremediation has not been revealed so far. Therefore, the combined effect of plant associated bacterial species or that of rhizosphere bacterial species and biotransformation of effluent by native indigenous bacteria for the decolorization and degradation of distillery waste will be a beneficial study for the removal of various environmental pollutants.

Keeping in view to the above hypothesis, the thesis has been compiled into eleven chapters; chapter first has introduced the basic information on the topic of thesis. The information related with number of sugarcane molasses based distilleries installed in India and their wastewater generated in the environment. Further, this chapter also provides comprehensive information on the distillery wastewater colorants, as well as its toxicological effects on environment, human and animal health. Additionally, the role of rhizospheric bacteria in mineralizing various organic and metallic compounds present in distillery sludge and effluent.

The background and rational of the study has been explained well in chapter 2. The chapter has highlighted to understand the problem of distillery waste i.e. organic pollutants, heavy metals, Post Methanated distillery effluent (PMDE), and their environmental impact. Some bacterial species and consortium also have been reported for their capability to degrade the various pollutants present in the distillery waste. The dark brown colourant in the distillery wastewater is basically caramelized sugar

which is known as melanoidins. The decolourizsation abilities of various bacteria have been reported in this chapter. The roles of some wetland plant for phytoremediation potential in different conditions are also mentioned. Though, the various attempts have been reported for decolourization and degradation of distillery wastewater at laboratory stages by using different bacterial consortium but all these studies have been reported at very low concentration of effluent. Therefore, this restrict for pilot-scale up-gradation of technology. Therefore, in the present study, the study has been focused for decolourisation and degradation of distillery wastewater at high concentration for feasibility of technology. Moreover, the hyperaccumulator herbaceous plants are also screened out for heavy metal phytoextraction potential from complex organo-metallic distillery sludge for eco-restoration of polluted site.

Further, chapter three has focused on five objectives as per topic of thesis. Chapter four has focused on characterization of organic pollutants and heavy metals from Post-Methanated Distillery Effluent (PMDE) and their environmental effect. The study has focused on the characterization of organic pollutants by using GC-MS technique and heavy metals through Inductively Coupled Plasma (ICP) from Post-Methanated Distillery Effluent (PMDE) and their environmental effect. Various unknown complex recalcitrant organometallic complexes with EDC properties which are a major source of environmental pollutants and health hazards are identified. The absorption maxima of PMDE were noted between 200–350 nm in the UV–Vis spectral analysis also indicated polymerized-form of Maillard product. Hexadecanoic acid, butyl ester, octadecanoic acid, monopalmitin 2 TMS, effusanin E, 1-(benzyl) -2-fluoro-2-phenyl-3-(p-toluene's sulfonyl) propane, and 24-hydroxy-3,4-secolanost-4,(28),8- died-3-nitrile were prominent organic compounds in the category of mutagenic and androgenic compounds characteristics, detected in PMDE. In addition, the presence of various activity in germinating Zea mays seeds and gel imaging at different concentrations of PMDE heavy metals also indicated the contribution of toxic property of PMDE, as resulting in phototoxic effect on seed germination with Zea mays and *Heteropneustes fossilis* with the degradation of lamellae in gill. Thus, this affirmed the source of various unknown mutagenic, carcinogenic, and EDCs compounds with organometallic complexes.

The fifth chapter summarizes the characterization of rhizospheric bacterial communities from hyperaccumulator plant growing on organometallic sludge during

phytoextraction for ecorestoration. The findings of study revealed that *P. hysterophorus* is as hyperaccumulator for various heavy metals from complex organometallic wastes during its growth on the disposed distillery sludge. The analysis of organic compounds showed degradation of pollutants present in sludge after growth of *P. hysterophorus*. Further, the histological observations of root by transmission electron microscopy (TEM) confirmed the deposition of metal granules in their tissue after accumulation by plant from distillery sludge. Besides, identified rhizospheric bacteria i.e. *Alcaligenes faecalis* (ON024323), *Cytobacillus firmus* (ON024324), *Bacillus* subtilis (ON024325) and, *Niallia circulans* (ON024326), also showed potential for plant growth promoting rhizobacteria activities which has supported the bacterial assisted phytoextraction potential of heavy metals from complex organometallic sludge of distillery from polluted site. This plant may be used as biotechnological tools for eco-restoration of polluted site by industrial waste as a green technology.

The sixth chapter showed the comparative assessment of phosphate, zinc and potassium solubilization by rhizospheric bacterial communities in *Phragmites communis* and *Typha spp. Phragmites communis* and *Typha spp.* is a naturally robust and vigorous primary species in many wetland environments worldwide. This plant has characteristic capacity to grow in different environmental conditions and can uptake, translocate, and accumulate a wide range of pollutants in both belowground and aboveground tissue. The ability of the plant to develop and grow in the polluted ecosystems allowed for the use of *Phragmites communis* in many types of sewage treatment plants also. To increase the efficiency of phytoremediation of a polluted natural or artificial aquatic ecosystem and to estimate the required purification time and accelerate the rate of its reclamation, the interaction processes between common *Phragmites communis* and soil microbes, metal accumulation, and ionic homeostasis in the hydrophyte purification systems should be further tested. The research carried out by interdisciplinary teams (plant physiologist, biochemist, geochemist, microbiologist, and agriculture and genetic engineer) in a short time can advance the efficiency of removing both metals and organic impurities.

Furthermore, the seventh chapter correlates bacterial community and organometallic pollutants during in-situ phytoremediation by *Parthenium*

hysterophorus and *Cannabis sativa* growing on organometallic pollutants-rich hazardous distillery sludge. The sequence analysis of 16S rRNA V3–V4 hypervariable region with Illumina MiSeq platform showed 230166 and 277515 OTUs derived from rhizospheric distillery sludge samples out of 305005 sequences read, respectively. The major genus detected in rhizospheric sludge sample were uncultured- *chloroflexi* (0.8%), *Candidatus-solibacter* (1.4%), *Bacillus* (1.4%), *Flavobacterium* (1.4%), others (26.2%), *Bdellovibrio (*0.6%) and unknown (42.0%). Our results suggested that rhizospheric bacterial communities associated with *Parthenium hysterophorus* and *Cannabis sativa* were substantially different in richness, diversity, and relative abundance of taxa compared to rhizospheric sludge. Further, the comparative organic pollutant analysis from non-rhizospheric and rhizospheric sludge samples through GC–MS analysis revealed the disappearance of several compounds and generation of some compounds as new metabolic products by the activity of rhizospheric bacterial communities. The results of this study will be helpful in understanding the role of rhizospheric bacterial communities responsible for degradation and detoxification of complex organometallic waste and, thus, can help in designing appropriate phytoremediation studies for eco-restoration of polluted sites.

The eighth chapter has focused on characterization of rhizospheric bacterial communities of *Typha aungustifolia* during rhizofilteration of distillery effluent in constructed wetland treatment system by integrating bacterial degraded PMDE with constructed wetland treatment at pilot scale to evaluate the feasibility of technology based on the promising result. Post-methanated distillery effluent (PMDE) was found dark brown with very high BOD, COD, TDS and metallic content beyond its permissible limit. Hence, due to high TDS bacterial growth was not possible. Therefore, the TDS reduction was optimised for bacterial growth at variable range of pH and concentration. The optimum reduction of TDS was obtained with application of ferric chloride (0.34%) within 6-8hrs of incubation. The detail value of TDS and other pollution parameters are shown in table (Table 8.1 and 8.3). 71.70% decolorization was observed. The efficiency of decolorization and TDS reduction of PMDE was evaluated with rapid mixing along with the variable pH. The optimum TDS reduction resulted 80% supernatant and 20% precipitation of colloidal material. Further, the PMDE supernatant is assessed for bacterial growth in presence of

variable nitrogen (0.5%, 1.0%, 1.5% & 2.0%) and carbon (0.5%, 1.0%, 1.5% & 2.0%) source to evaluate the bacterial growth for reduction of color from supernatant of PMDE. 79.45% decolorization was further observed. Subsequently, the biomass was separated and supernatant was passed through the constructed wetland treatment system with plantation of *Typha angustata*. The reduction of TDS was observed 87.46%, BOD 96.38% and COD 99.37%. While the color was reduced up to 90.49%, and the others physicochemical parameters also reduced up to 95%. Therefore, the current chapter showed Biological decolourisation of post-methanated distillery effluent (PMDE) in biphasic bacterial and wetland plant treatment system for environmental safety and identification of potential bacteria.

The chapter nine has summarized the whole thesis work and findings with research output in a systematic manner. The chapter ten has compiled the all cited references of thesis which are relevant to the topic and they are cited in each chapter. Each chapter has cited the recent references based on methodology and results. The available references are cited on synthesis of melanoidin, physico-chemical properties of distillery wastewater, impact of post methanated distillery wastewater and plant growth promoting rhizobacteria reported at polluted sites. The complete thesis has 200 cited references.

The last chapter has listed all the scientific output based on the thesis work. There is two original research paper published in peer reviewed journal of international repute with high impact factor. Two review paper are also published based on topic of thesis have been published in the international journal and three book chapter as invited chapter published CRC press. A certificate of participation and best oral presentation award has been provided in international conferences. One international patent also has been granted based on the work of thesis in credit of candidate.

Chapter 2
Review of Literature

Chapter-2

Review of Literature

Sugar and distilleries are known as agrobased industries in India. These industries are important source of economy (Lee et al., 2020). But, they are major source of environmental pollution and health hazards also. To maintain the ecological balance various pollution control rule were promulgated by Govt. of India but the industries are still facing challenge to meet out the prescribed standard of industrial waste disposal (Gronwall and Jonsson, A. C. (2017). Currently, the distilleries have been recommended to adopt the incineration of the spentwash with zero pollution discharge (Bhardwaj et al., 2019). There are several adversely environment affected area in different state of India due to distillery operations which needs ecological ecorestoration by innovative technologies (Ratna et al., 2021). Though, several technologies have been implemented for the industrial growth with sustainable development but still needs thorough research for pollution prevention and eco-restoration of polluted sites. Therefore, the current chapter focuses on the rational study of the distillery waste and its removal from the environment.

2.1. Distillery waste and their environmental pollution.

Distilleries are one of the most polluting industries generating large volume of wastewater having a serious environmental concern. Distillery effluent is characterized by dark brown color, acidic pH, high temperature, low dissolved oxygen (DO), high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) (Bezuneh, 2016). Distillery wastewater disposed onto the environment prior to treatment is hazardous and leads to soil and water pollution (Fuess and Garcia, 2014). The dark brown color of distillery effluent causes reduction of sunlight penetration, decreased photosynthetic activity and dissolved oxygen concentration in rivers, lakes and lagoons, hence becomes detrimental to aquatic life (Tiwari and Prakash, 2021). It also causes reduction in soil alkalinity and inhibition of seed germination. Different physicochemical and biological methods have been investigated for the treatment of distillery effluents (Prajapati and Chaudhari, 2015). In recent years, increasing attentions has been directed towards biological wastewater treatment methods. Bioremediation of wastewater using microorganisms is efficient and cost effective

method. Microorganisms as bacteria, fungi, and algae have been shown to exhibit bioremediation activities mainly due to their production of complex and non-specific enzymatic systems capable of degrading various forms of pollutants from wastewater (Morin-Crini et al., 2022). Distilleries can be categorized among the most polluting industries generating large volume of wastewater known as spent wash (Kulkarni et al., 2016). Distilleries generate wastewater at various stages in the manufacturing process as distillation, condenser cooling, fermenter cooling, fermentation and washing stages (Amenorfenyo et al., 2020). Larger amount of the effluent is produced at distillation and condenser cooling stages. The characteristics of the wastewater generated depend on the feed stock used. Distilleries are agro-based industries, which utilize agricultural products as sugar cane juice, sugar cane molasses, sugar beet molasses, corn, wheat, cassava, rice, barley as raw materials (Singh et al., 2019).

Distillery effluent is characterized by its acidic pH (4-5), dark brown color, high temperature (53-100°C), low dissolved oxygen (DO), high biochemical oxygen demand (BOD) (40,000-50,000 mg/L) and chemical oxygen demand (COD) (80,000-100,000 mg/L) (Kumar and Sharma, 2019). Apart from the high BOD and COD load, distillery effluent also contains significant amount of phenols (7,202 mg/L), chlorides (7,997 mg/L), sulphates (1,100 mg/L), nitrates, phosphates (1,625 mg/L) and heavy metals (Kumar and Sharma, 2019). The dark brown color of the effluent is mainly due to the formation of polymer melanoidin by a non-enzymatic browning reaction called Maillard reaction (Singh et al., 2019). Melanoidins are highly recalcitrant and have antioxidant properties which make them toxic to many microorganisms (Singh et al., 2021). Distillery wastewater disposed to the environment prior to treatment is hazardous and can be a major source of soil and water pollution (Tripathi et al., 2021). It induces toxic substances into water bodies as rivers, lakes and lagoons which adversely affect aquatic plants and animals (Chowdhary et al., 2020). The highly colored nature of the effluent also leads to the reduction of sunlight penetration in rivers, lakes or lagoons which, in turn, reduces oxygenation of the water by photosynthesis and hence becomes detrimental to aquatic life (Bashir et al., 2020). Disposal of distillery wastewater into soil is equally harmful, as it reduces soil alkalinity and manganese availability. It also imparts high concentration of heavy metals viz., copper, nickel, silver, cadmium, iron and mercury which are capable of

inhibiting seed germination and seeding growth (Bezuneh, 2016). According to various reports, application of distillery effluent for irrigation without proper monitoring might result in reduction of soil fertility by suppressing the activity of soil microorganisms as nitrogen fixing bacteria rhizobium and azotobacter (Saeed et al., 2021). Distillery effluent must be treated before it is disposed into the environment which helps to minimize the adverse effect posed by the effluent (Fito et al., 2019). There have been several treatment technologies explored so far for the reduction of the pollutants from distillery wastewater. Treatment methods can vary based on the chemical composition of the effluent as well as economic viability of the technology. Generally, wastewater treatment methods can be categorized as physical, chemical and biological methods. Physicochemical treatment methods such as adsorption, sedimentation, screening, coagulation, pH adjustment, reverse osmosis, ultrafiltration, flotation, oxidation, electrolysis, membrane filtration and evaporation have been used for treatment of distillery effluents (Gunatilake, 2015).

Physicochemical methods of wastewater treatment have so many drawbacks such as consumption of chemicals, high cost, large amount of sludge left after treatment, and possible formation of harmful by-products. As a result of this, in recent years, biological wastewater treatment using microorganisms has attracted the attention of researchers all over the world (Viswanaathan et al., 2022). Microbial degradation and decolorization of distillery effluents have been found as cost effective and environmental friendly alternative to physicochemical methods (Chowdhary et al., 2017). Various types of microorganisms as bacteria, fungi, and algae have been reported for their potential in degradation and decolorization of various industrial effluents including that of distilleries. Hence, this review discusses and summarizes the role of microorganisms in degradation and decolorization of distillery wastewater (Singh and Raj, 2020). Moreover, the mechanisms of microbial degradation of melanoidin by fungal, bacterial and algal systems are also discussed (Ravikumar et al., 2021).

2.2. Biological treatment of distillery waste

Microorganisms play a key role in bioremediation process and have been proven as an efficient, low cost and environmental friendly alternative to physicochemical methods

(Pande et al., 2020). Several microbial species including fungi, bacteria and algae have been studied for their capacity to degrade and decolorize toxic chemical pollutants present in various industrial wastewater including distilleries (Saeed et al., 2021). Free or immobilized cells have been studied widely for bioremediation of distillery wastewater (Shukla et al., 2020). Immobilizing microorganism on inert support material including alginate, polyacrylamide, agar, polystyrene, and polyurethane is more advantageous compared to that of free-cell (Susilowati et al., 2019). Some of the advantages include compact physical structure of carrier pellets, high biomass retention, reusability of culture and easier separation process. The potential of microorganisms in distillery wastewater treatment is highly dependent on the type of chemical composition of wastewater, nutrient, pH, temperature, oxygen and inoculum size (Papadopoulos et al., 2020)

2.2.1. Bacterial treatment

A wide variety of bacterial cultures as *Pseudomonas aeruginosa, Pseudomonas putida, Lactobacillus plantarum, Bacillus circulans, Bacillus megaterium, Bacillus firmus, Bacillus thuringiensis, Bacillus cereus, Lactobacillus hilgardii, Lactobacillus coryniformis, Xanthomonas fragariae* have been reported for their activity in degradation and decolorization of pollutants from distillery effluents (Pant and Adholeya, 2007).

Aerobic bacterial strains are very effective in bioremediating distillery effluents under aerobic conditions (Mohana et al., 2009). However, those bacterial strains are not economical due to high energy consumption for aeration thus; it was very difficult to apply those bacterial strains on an industrial scale (Białkowska, 2016). Considering this problem it is important to isolate bacterial strains that can degrade distillery wastewater under anaerobic condition (Maintinguer et al., 2007). Anaerobic bacterial strains are advantageous than that of aerobic strains due to low energy consumption hence, minimizes cost of wastewater treatment (Pant and Adholeya, 2007). Ohmomo et al. (1988) reported the first bacterial strain *Lactobacillus* hilgardii W-NS capable of decolorizing molasses melanoidins under anaerobic condition. This bacterial strain decolorized about 28% of molasses melanoidin under optimum condition. Another facultative anaerobic bacterial culture L-2 belonging to Lactobacillus showed similar decolorization of 31% for 12.5% (v/v)

diluted digested spent wash in 7 days of incubation. Along with decolorization this bacterial culture also removed 56.2% COD. Nakajima et al. (1999) observed decolorization yield of 35.5% using bacterial strain MD-32 within 20 days of cultivation under both thermophilic and anaerobic conditions. The COD and color removal efficiency of anaerobic bacterial strains is lower than that of aerobic bacteria. Hence, it is important to isolate bacterial strains capable of degrading and decolorizing toxic chemical pollutants under anaerobic conditions.

2.2.2. Algal treatment

Microalgae are unicellular microorganisms that are known for their capacity in bioadsorption and biodegradation of toxic chemical pollutants as phenols, heavy metals, pesticides, polycyclic aromatic hydrocarbons (PAHs), xenobiotics and melanoidins from wastewater (Touliabah et al., 2022). Utilizing microalgae for bioremediation purpose is advantageous compared to that of bacterial and fungal systems in many ways (Das et al., 2022). The first advantage is that, microalgae has a great potential in utilizing contaminants as ammonium, nitrate and phosphate as a nutrient hence minimizes the amount of externally added nutrient in case of fungi and bacteria (Gupta et al., 2019). Secondly fungi and bacteria require optimum condition for growth and bioremediation activity whereas microalgae can grow rapidly and adapt harsh conditions (Li et al., 2019). Thirdly microalgae produces valuable products as ethanol, methane, livestock feed, or it can also be used as organic fertilizer due to its high N:P ratio (Zhu et al., 2016). Hence, utilizing microalgae for bioremediation purpose is advantageous compared to fungal and bacterial system. Various species of microalgae as C*hlorella vulgaris, Oscillatoria boryana, Chlorella pyrenoidosa, Chlorella sorokiniana, Coenochloris pyrenoidosa, Nostoc muscorum, Neochloris oleoabundans, Phormidium valderianum, Chlorella zofingiensis,* and *Chlorella ellipsoidea* have been used in bioremediation of wastewater (Bezuneh, 2016).

The green microalgae belonged to the genera of *Chlorella* have been studied most widely due to its capacity of bioremediating toxic chemicals pollutants (Sutherland and Ralph, 2019). Valderrama et al. (2002) carried out research to develop a procedure for treatment of recalcitrant wastewater from ethanol and citric

acid production using first the microalga *Chlorella vulgaris* followed by the macrophyte *Lemna minuscula*. In the first stage of treatment, *Chlorella vulgaris* resulted in a reduction of ammonium ion 71.6%, phosphorus 28% and chemical oxygen demand 61% from 10% diluted wastewater within 4 days of treatment. Travieso et al. (2002) evaluated the performance of a laboratory-scale microalgae pond for secondary treatment of distillery wastewater previously digested in an anaerobic fixed bed reactor using *Chlorella vulgaris* SR/2. *Chlorella vulgaris* SR/2 removed volatile suspended solids (VSS) 78.8%, total solids (TS) 60.6%, total suspended solids (TSS) 53.4%, chemical oxygen demand (COD) 83.2% and biochemical oxygen demand (BOD) 88.0% from the effluent. More recently, Solovchenko et al. (2014) investigated phycoremediation of alcohol distillery wastewater with a novel *Chlorella sorokiniana* strain isolated from White Sea. This algal strain showed maximum reduction in chemical oxygen demand (COD) 92.5%, nitrate 95%, phosphate 77% and sulfate 35% within four days. Another marine cyanobacterium *Oscillatoria boryana* decolorized pure melanoidin pigment (0.1%) and crude pigment in the distillery effluent (5%) by about 75% and 60%, respectively, within 30 days of treatment (Kalavathi, 2021).

2.2.3. Mixed culture treatment

Several researchers studied the efficiency of mixed culture microorganisms for degradation and decolorization of distillery wastewater (Sankaran et al., 2015). The mixed microbial cultures exhibited increase in mineralization of effluents over that showed by individual cultures (Balapure et al., 2019). This might be due to the enhanced effect of coordinated metabolic interactions present in mixed community. Bharagava et al. (2009) observed enhanced growth, enzyme production and melanoidin degradation by mixed bacterial culture compared to axenic bacterial culture. In that report a mixed consortium comprised of *Bacillus licheniformis*, *Bacillus* sp. and *Alcaligenes* sp. showed melanoidin decolorization of about 73.79 and 69.83% for synthetic and natural melanoidins whereas axenic cultures decolorized 65.88, 62.56 and 66.10% synthetic and 52.69, 48.92 and 59.64% natural melanoidins, respectively. In another report, a mixed bacterial culture comprised of *Bacillus thuringiensis*, *Bacillus brevis* and *Bacillus* sp. exhibited two-to four fold increase in melanoidin decolorisation over that showed by any individual Bacillus isolate. Pant

and Adholeya developed a novel fungal consortium comprised of *Penicillium pinophilum, Alternaria gaisen, Aspergillus flavus, Fusarium verticillioides, Aspergillus niger* and *Pleurotus florida* for decolorization of distillery effluent using agricultural residues as a growth substrate. The fungal consortium was run on a bioreactor with undiluted distillery effluent for 40 days. In the first 14 days, 61.5% color and 65.4% COD removal was achieved (Ghosh et al., 2019).

2.3. Melanoidin as complex colour compound in the distillery wastewater

Melanoidins are nitrogenous, heterocyclic brown pigments polymer generated during the chemical reaction between amino and carbonyl compounds at elevated temperatures (Wang et al., 2011). These compounds are synthesized by the reaction occurring between the amino groups of amino acids, peptides, or proteins and the carbonyl groups of reducing sugars (Phisut and Jiraporn, 2013). A previous study also has revealed in a chemical reaction that carbohydrates and amino acids are the basic skeleton of the melanoidin compound (Cämmerer et al., 2002). The formation of melanoidin involves three significant steps:

Initial/First stage: Initially, compounds during reaction do not produce any color. Therefore, the spectrophotometer detection does not show any absorption spectrum in the range of about 280 nm. The reaction starts with the following two steps (Singh et al., 2021):

Step 1: Sugar–amine condensation
Step 2: Amadori rearrangement

At the initial stage, condensation of sugar-amine and the rearrangement of amadori product by the nucleophilic attack of the amine group to the electrophilic carbonyl group of sugar take place. It is basically a sugar dehydration reaction assisted by amine (Parker, 2015; Berger, 2007). The carbonyl groups reaction components are reducing sugars like glucose, aldose; they react with amino groups from amino acids, peptides or proteins, to give an N-substituted glycosylamine (Provost, 2019). This compound is also known as the Amadori compound and the reaction taking place at this level is reversible (Becker and Yu, 2013).

The Amadori product further releases the proton and forms a cationic base. It irreversibly loses the proton to form the final Amadori compound, i.e., N-substituted 1-amino-2-deoxy-2-ketose in enol and keto form according to the acidic and basic condition of the maillard reaction (Fig. 2a) (Amaya-Farfan and Rodriguez-Amaya, 2021). Compared with the sugar-amine condensation product, the final rearrangement Amadori compound is more stable because it degrades at high temperatures and decomposes rapidly in mild bases (Diez-Simon et al., 2019).

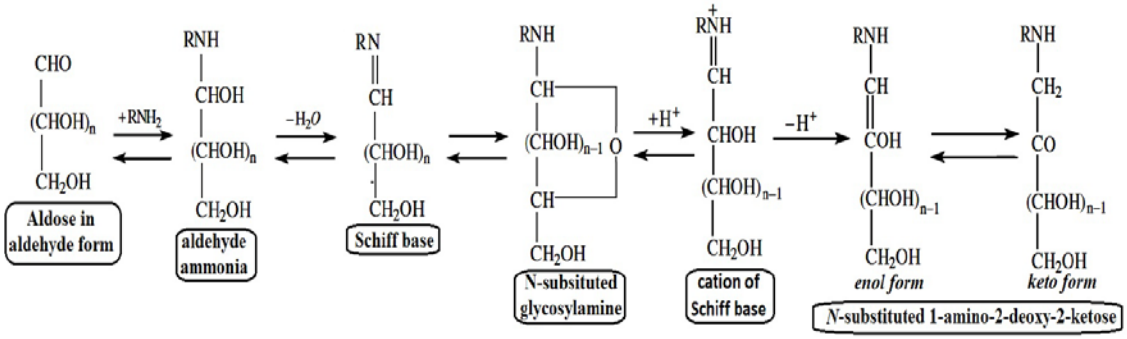


Fig.2a. Overall pathway of Sugar–amine condensation to form N-substituted glycosylamine and Amadori rearrangement, leading to the formation of Amadori compound, the N-substituted 1-amino-2-deoxy-2-ketose.

II) *Intermediate stage/Second stage:* At this stage, products remain colorless or pale yellow and show high absorption in the ultraviolet range. There are following three major stages (Yadav and Chandra, 2019):

Step 3: Dehydration of sugar

Step 4: Fragmentation of sugar

Step 5: Degradation of an amino acid (Strecker degradation)

Sugar dehydration occurs in two ways: Under the acidic condition, furfural formation takes place (when pentosanes are involved) or hydroxylmethylfurfural (HMF) (when 6 carbon compounds are involved) (Xu et al., 2020). While in alkaline conditions, there is a degradation of amadori product converted into N-substituted 1-amino-2-deoxy-2-ketose, which further convert into reductone (Ntakirutimana et al., 2022). This is a reversible reaction. Furthermore, this can convert through the fission process into furanone or *C*-methyl reductones and α-dicarbonyls, as shown in Fig. 2b (Gum and Gum, 2017). However, various compounds can increase the rate of furfural compounds formation (Chen et al., 2017). For example- Glycine can rapidly convert

xylose (5-carbon compound) into furfural and glucose (6-carbon compound) to hydroxylmethylfurfural (HMF) (Baptista et al., 2021). Hence, it is clear that amadori product dehydrate more easily than the N- substituted glycosylamine (original compound) or aldose (ALjahdali and Carbonero, 2019).

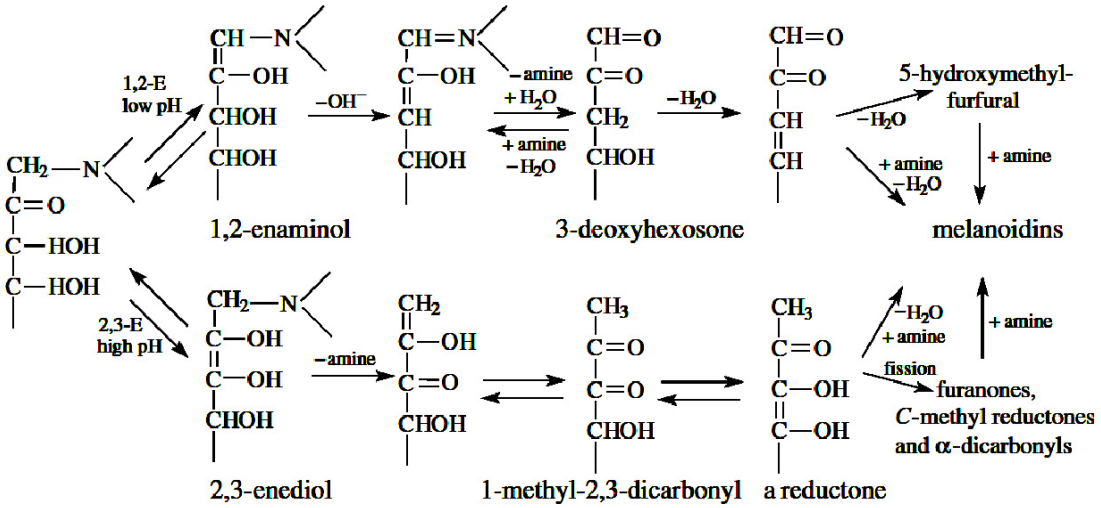


Fig.2b. The two major pathways from Amadori compounds to melanoidins formation (based on Hodge).

Furfural formation is often a relatively common method to detect food spoilage during storage for quality maintenance of food materials (Wibowo et al., 2015). It has been observed that reductone formation takes place from sugar by the loss of two water molecules, while in the formation of furfural compounds, there is a loss of three water molecules (Aalaei et al., 2019). Thus, reductones are compounds with - C (OH): C (OH) - group, as in ascorbic acid, as shown in Fig. 2b (Kurata and Otsuka, 2013). The sugar fragmentation reaction pathway occurs by retroaldolization (dealdolisation) process to some extent, oxidative fission also occurs. In this reaction, the fission product varies considerably in its color appearance (Sneddon, 2016). The fragmented sugar product with α-hydroxymethylcarbonyl compounds undergoes for browning reaction in water and amine compounds that polymerize fast with color formation (Nikolov, 2013). Strecker degradation is also known as the degradation of amino group compounds by carbonyl compounds through the oxidative degradation process with the production of CO_2 (Hidalgo et al., 2013). In this reaction amino acids first react with Schiff bases and undergo acid-catalyzed decarboxylation. After that, the Schiff base compound undergoes hydrolysis to synthesize amino and aldehyde

group compounds (shown in the below reaction) (Cui et al., 2021). The aldehyde compounds after synthesis contribute to the formation of flavoring compounds in food material. This further may convert into Melanoidins (Olaniran et al., 2017). Hence, it's clear that strecker degradation Product during thermal processing of food materials is mainly responsible for flavor and aroma generation. The compound synthesizes at this stage are heterocyclic in nature (Yu et al., 2021).

III) *Final stage:* This stage is mainly for polymerization and color production. The aldehyde produced previously reacts with alcoholic group compounds and gives further aldol (Adrian, 2019). The reaction is further divided into two parts as follows:

Step 6: Aldol condensation

Step 7: Aldehyde–amine condensation and formation of heterocyclic nitrogen compounds

The aldehyde produced previously in the strecker degradation during the intermediate stage can react with each other by aldol condensation; its reaction can be effectively catalyzed by amines (and mainly by their salts), egg albumin, and peptones (Appell et al., 2018). In addition, the other carbonyl compounds formed during lipid oxidation may also participate in the reaction (Hidalgo and Zamora, 2016). The aldehyde compounds containing the α, β-unsaturated group can react at low temperature with amine and form polymeric, heterocyclic colored compounds with high molecular weight, and an unknown structure called melanoidin, which may take place through several different routes as shown in Fig. 2.1 (Liu et al., 2020).

Most studies have been done on melanoidin food products like bakery products, cooked meat, roasted coffee, honey, sweet wine, etc. as an antioxidant at low concentration (Shaheen et al., 2021). Their formation occurs during the thermal processing of food material in cooking, baking, roasting, and storage, due to reducing sugar and free amino groups (Lund and Ray, 2017). However, melanoidin at high concentration in the environment has been reported as pollutant by several researchers when it is discharged from industrial activities and makes complexation with other environmental pollutants along with heavy metals due to its anionic properties (Chandra and Kumar, 2017). Various researchers have reported melanoidin pollution when it enters into the environment as a waste product (Kumar and Sharma, 2019).

The major source of melanoidin in the environment is the sugarcane molasses-based distilleries waste water, where melanoidin makes complexation along with various mutagenic and carcinogenic compounds (Chowdhary et al., 2018).

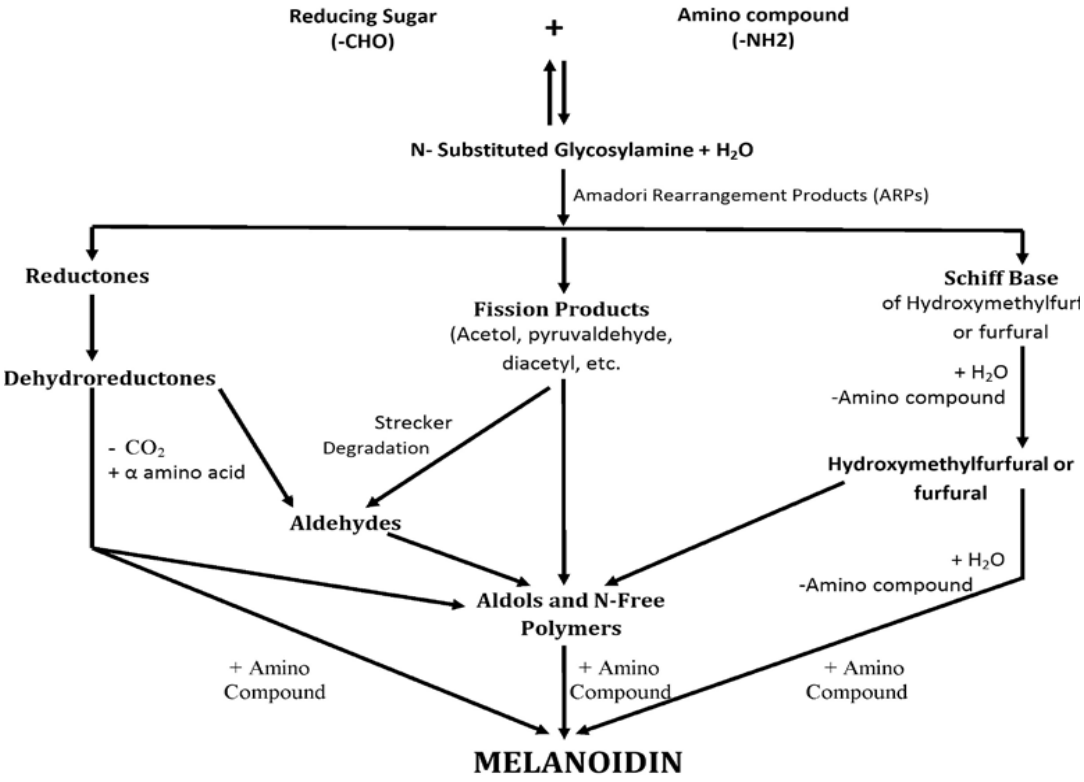


Fig. 2.1. Overall simplified chemical pathway of maillard reaction.

The effect of melanoidin has been reported for severe aquatic and soil pollution in the environment (Kumar et al., 2022). Due to severe threats to the environment, various attempts have been made globally by the researchers using the chemical and biological method, for degradation and decolorization of molasses-based melanoidin of distillery wastewater for environmental protection and its safe disposal (Kumar et al., 2020). Therefore, in the chemical analysis of distillery waste, there is a mixture of compounds with a variable absorption peak has been noted in the absorption spectrum between a range of 200-400 nm (Tripathi et al., 2022). The melanoidin compound directly causes adverse effects on aquatic and soil ecosystems due to high pollution parameters and the complexation tendency of melanoidin with other environmental pollutants and phenolic compounds (Chowdhary et al., 2017). The presence of various Endocrine Disrupting Chemicals & mutagenic compounds has been reported in the distillery waste (Chandra et al., 2018). Therefore, the discharge of distillery wastewater directly influences the aquatic ecosystem's light

penetration process, which alters the life cycle of flora and fauna and causes carcinogenic and lethal effects (Gupta et al., 2022). The inhibitory effect of distillery wastewater containing melanoidin has also been reported on seed germination & plant growth in several crops (Chowdhary et al., 2018; Chandra et al., 2018).

2.3.1. Decolorization of Melanoidin by Microorganisms

Several microorganisms such as bacteria and fungi show a good ability to decolorize the effluent of the melanoidin-based distillery industries. Thus, a better understanding of the microbial activities responsible for the degradation of melanoidins would contribute to enhancing the efficiency of the overall treatment system (Santal and Singh, 2013). In the degradation process, it would also be necessary to know the end product of melanoidins (Rane et al., 2018). Genetic improvement of isolates can be explored in the future for improving their decolorization efficiency (Wang et al., 2022). Thus, it can be suggested that microbial decolorization holds promise and can be exploited to develop a cost-effective, eco-friendly biotechnology package for the treatment of distillery effluent (Kapoor, 2018). More technically advanced research efforts are required for searching, exploiting new bacterial species, and improvement of practical application to propagate the use of bacteria for bioremediation of industrial effluents. Enzymatic studies would be employed to understand the mechanism of the degradation of melanoidins in future prospects. Such organisms could be used in bioreactors for treatment of wastewaters or scaling up for enzyme productions (Santal and Singh, 2013). Broader validation of these new technologies and integration of different methods in the current treatment schemes will most likely in the near future, render these both efficient and economically viable. Finally, microbial bioremediation involves a combination of microbiologists, biotechnologists, chemists, and engineers and is ideal to plug the ever-widening gap between the different disciplines (Tiwari and Gaur, 2019).

2.3.2. Phytoremediation mechanism of distillery waste remediation

Phytoremediation is a green technology that uses plant systems for remediation and rehabilitation of the contaminated sites (Khan, 2020). Plants have inbuilt enzymatic machinery capable of accumulating and degrading complex structural pollutants and can be used for cleaning of the contaminated sites (Ljaj et al., 2016). It is an

ecologically sound and sustainable reclamation strategy for bringing polluted sites into productive use but is still in an experimental stage; therefore it needs a lot of attention and scientific scrutiny (Kumar, 2021). The improper disposal of distillery effluent has become a global problem and there is very scanty information about its treatment using phytotechnologies (Bharagava, 2018). Singh et al. (2005) assessed the ability of aquatic macrophyte Potamogenton pectinatus L. to accumulate heavy metals, viz., Fe, Cu, Zn, and Mn, from distillery effluent and the related toxicity therein. The authors showed that *P. pectinatus* L. appears to be a suitable plant for phytoremediation of heavy metal distillery effluent as it accumulated significant amounts of Cu, Fe, Zn, and Mn from distillery effluent in its tissues during a period of 2 weeks. However, a decline in protein content of plants under heavy metals stress was observed during the treatment (Sorrentino et al.,2018). Sharma et al. (2011) screened 16 plant species to identify their tolerance and capacity at field phytoremediation of spent wash in the high rate evapotranspiration system. They observed tolerance of the biomethanated diluted spent wash for *Acacia farnesiana, Dendrocalamus strictus, Lawsonia inermis, Atriplex nummularia, Suaeda nudiflora, Arundo donax,* and *Phragmites karka*. A laboratory-scale constructed wetland (CW) employing Typha latipholia was used to treat diluted distillery effluent (Kumar, 2021)). A root zone of 1.5 3 0.3 3 0.3 m, filled with 75% sand and gravel and 25% soil was used and the diluted effluent was applied after 4 weeks of planting (Khazaleh and Gopalan, 2018). The system resulted in 76% COD reduction in 7 days which increased marginally to 78% COD reduction in 10 days. The BOD reduction was 22% and 47% on 7 and 10 days, respectively. In yet another instance, a distillery in northern India is presently employing CWs for polishing the effluent prior to land discharge for irrigation in the surrounding paddy fields (Pratap et al., 2021). The effluent is initially subjected to primary treatment which includes settling and anaerobic digestion in a structured media attached growth (SMAG)-type anaerobic reactor (Ambatkar and Mukundan, 2021). The primary treated effluent, with a COD of 28,000□35,000 mg L21 , is subjected to two-stage aeration to bring down the COD to 400 mg L21(Kumar, 2021) . Thereafter, it is directed to a CW before the final discharge. In another study, Billore et al. (2001) have demonstrated a four-celled horizontal subsurface flow (HSF) CWs for the treatment of distillery wastewater (DWW) after anaerobic treatment. The postanaerobic treated effluent had BOD of

2500 mg L21 and COD 14,000 mg L21 . A pretreatment chamber filled with gravel was used to capture the suspended solids (Ayaz et al., 2015). All the cells were filled with gravel up to varying heights and the third and fourth cells were planted with *T. latipholia* and *Phragmites karka*, respectively (Angassa et al., 2019). The overall retention time was 14.4 days and the treatment resulted in 64%, 85%, 42%, and 79% reduction in COD, BOD, total solids, and phosphorus, respectively (Chowdhary et al., 2018). Similarly, Olguın et al. (2008) evaluated the performance of subsurface flow-constructed wetlands (SSF-CWs) mesocosms planted with *Pontederia sagittata* and operating at two hydraulic retention times (HRTs), compared to unplanted SSF-CWs, for the treatment of diluted stillage subjected to no pretreatment apart from an adjustment to pH 6.0. The planted SSF-CWs were able to remove COD in the range of 80.24% to 80.62%, BOD5 in the range of 82.20% to 87.31%, total Kjeldel nitrogen (TKN) in the range of 73.42% to 76.07%, nitrates from 56% to 58.74%, and sulfates from 68.58% to 69.45%, depending on the HRT. However, phosphate and potassium were not removed. Mulidzi (2010) showed the impact of shorter retention time on the performance of CWs in terms of BOD, COD, and removal of other elements. The results showed an overall 60% COD removal throughout the year. This study also indicated the significant removal of other elements, namely, potassium, nitrogen, electrical conductivity, calcium, sodium, magnesium, and boron from DWW wastewater by CWs. A group of researchers from Mexico reported the use of phytofiltration technology based on *Azolla* sp., a free-floating and fast-growing aquatic plant, for treatment of anaerobically digested sugarcane ethanol stillage (Sa´nchez-Galva´n and Bolan˜os-Santiago, 2018). They demonstrated that developed phytofiltration is efficient for reducing the organic matter content, nutrients, and color intensity significantly in anaerobically digested stillage (ADS) under the tested conditions. The authors recommended that the conversion of nutrients from ADS into valuable *Azolla* biomass may provide an effective way to produce a very attractive feedstock for the production of a wide spectrum of biofuels. A single-phase system has provided good organic removal efficiency; however, a biphasic system is capable of optimizing the fermentation steps of each stage in separate fermenters (Nzeteu, 2018). Kumar and Sharma (2019) successfully treated anaerobically digested distillery effluent in a two-stage treatment process involving the transformation of recalcitrant coloring components of the effluent by aerobic bacterium *B. thuringiensis*

followed by a subsequent decline of a remaining load of pollutants by a macrophyte Spirodela polyrrhiza Schleiden. The biphasic system is the most appropriate treatment method for high-strength wastewater (Fito et al., 2018). A combination of bacterial pretreatment followed by CWs plant treatment system was investigated to determine its effect on the removal of heavy metals and detoxification of postmethanated distillery effluent (PMDE). This biphasic treatment of the effluent with *T. angustata* and *B. thuringiensis* (MTCC 4714) removed large quantities of various heavy metals at a range of effluent concentrations (i.e., 10%, 30%, 50%, and 100%) (Kumar and Chandra, 2020). A similar biphasic (two-step) treatment of the PMDE was carried out in a CW with *B. thuringiensis* followed by *T. angustata* L. by Chandra et al. (2008) which resulted in 98% to 99% COD, BOD, and color reduction after 7 days. The authors recommended that the bacterial pretreatment of PMDE integrated with CW will improve the treatment process of PMDE and promote safe disposal of hazardous distillery waste. Chandra et al. (2012) reported 96.0% and 94.5% reduction in COD and BOD values in a two-step sequential treatment of PMDE by bacteria and wetland plant Phragmites communis. He also characterized rhizosphere bacterial communities of P. communis and metabolic products generated during the sequential treatment of PMDE in CWs plant treatment system. A two-step sequential treatment for sugarcane molasses-based anaerobically treated distillery effluent was reported by Pant and Adholeya (2009a). In the first step, distillery effluent was treated in a hydroponic-based system using two plant species, namely, *Vetiveria zizanioides* and *P. karka*, to reduce (up to 84%) the high nitrogen content of the effluent. This first-step hydroponically treated distillery effluent was subjected for treatment by two fungus species in a bioreactor. Decolorization of effluent up to 86.33% was obtained with *Pleurotus florida* Eger EM1303 followed by *Aspergillus flavus*. This study recommended this distillery effluent treatment without the need for high dilutions and the addition of supplementary carbon sources. Restoration of habitats and in situ cleanup of contaminants can be achieved with significantly reduced remedial costs by this phytotechnology (Abdullah et al., 2020). Olguın et al. (2007) assessed the phytoremediation potential of Salvinia minima Baker compared to *S. polyrrhiza* in high-strength synthetic organic wastewater (HSWW) and also evaluated the growth characteristics of *S. minima* in various culture media, including anaerobic effluents from pig wastewater (PWAE). The authors concluded concluded that S. minima are a

better option than *S. polyrrhiza* for treating high-strength organic wastewater. Bharagava et al. (2008) studied the HM accumulation efficiency and its physiological effects in Brassica nigra L. plants grown in soil irrigated with different concentrations (25%, 50%, 75%, 100%, v/v) of PMDE after 30, 60, and 90 days treatment. This study concluded that *B. nigra* L. accumulate elevated concentration of Zn, Ni, Mn, Fe, Cu, and Cd due to increased amounts of cysteine and ascorbic acid (work as antioxidants) in leaves, shoot, and root of *B. nigra* L. at all the concentration and exposure periods of PMDE except at the 90-day period, where a decrease was observed at 100% PMDE concentration as compared to the respective control. Hatano and Yamatsu (2018) evaluated the facilitatory influence of melanoidin-like product (MLP) on phytoextraction efficiency in a medium including Cd21 or Pb21, the concentrations of which were adjusted near the regulation values of the Act in Japan. In this study, three Brassica species were tested due to their fast growth, high biomass productivity, and high heavy metal absorption, and the cultivation period was 2 months under sunlight. It was observed that both biomass and lead uptake were significantly increased by the addition of MLP, and almost all of the lead was accumulated in the root tissue. Therefore MLP was able both to detoxify lead ions and to improve their bioavailability in *Brassica* species. The author recommended that the phytoextraction of cadmium using these species was impractical under the Act. A research group from Japan have demonstrated that MLP from sugarcane molasses possesses the potential for an accelerator of phytoextraction efficiency of Japanese radish in the copper-contaminated media (Hatano et al., 2016). The result showed that MLP binds to all the metal ions examined and the binding capacity of MLP toward Cu21 seems to be the highest among them. The metal detoxification by MLP followed the order of Pb21 . Zn21 . Ni21 . Cu21 . Fe21 . Cd21 . Co21. Yadav and Chandra (2011) evaluated the heavy metals accumulation potential of *Typha angustifolia* and *Cyperus esculentus* growing in distillery and tannery effluent polluted wetland sites. The metal accumulation pattern in both macrophytes indicated that both macrophytes were root accumulators for Fe, Cr, Pb, Cu, and Cd (Galal et al., 2018). Simultaneously, chlorophyll, protein, cysteine, and ascorbic acid were also more induced in *T. angustifolia* and *C. esculentus*. In addition, Anatomical observation through transmission electron microscopy (TEM) in the root of *T. angustifolia* did not show any remarkable changes even after a higher accumulation of various metals in

the roots (Chandra and Kumar, 2017). However, the formation of multinucleolus in a shoot of 14.5 Success stories of phytoremediation of melanoidins 365 *T. angustifolia* was found to be evidence of extra protein synthesis for tolerance under stress conditions. Hence, *C. esculentus* was observed to have less tolerance for metals than T. angustifolia. Chandra et al. (2018) collected nine native plant species (grasses and weeds), viz., *B. alba, Calotropis procera, Tinospora cordifolia, Rumex dentatus, C. album, Pennisetum purpureum, Cynodon dactylon, Sacchrum munja*, and *Argemone mexicana*, which were abundantly grown on disposed distillery sludge containing a mixture of complex organic pollutants benzoic acid, 3,4,5- tris (TMS oxy), TMS ester, stigmasterol TMS ether, hexanedioic acid, dioctyl ester; benzene, 1-ethyl-2-methyl, 5α-cholestane, 4-methylene, campesterol TMS, β-sitosterol, and lanosterol, and also retains high quantities of Pb (31.22 mg kg21), Ni (15.60 mg kg21), Fe (5264.49 mg kg21), Mn (238.47 mg kg21), Cu (847.46 mg kg21), and Zn (43.47 mg kg21), which enhance the toxicity of sludge to the environment. The major identified organic compounds are listed under the EDCs also as per USEPA (2012). All the grown plants have been found to show hyperaccumulation properties of HMs from organometallic polluted sites mixed with androgenic and mutagenic compounds (Kumar et al., 2021). The observation of root tissues of all plant species through TEM analysis showed the formation of multivacuoles, multinucleolus, multinucleus, and mitochondria and opaque deposition of HMs granules in the cellular organelle of the plant cells which indicated the HMs tolerance mechanisms of the plant at an elevated concentration of HMs and other complex copollutants. The authors recommended that these native plants can be used for in situ phytoextraction of HMs from industrial waste polluted sites.

3.2. Challenges and future prospects

Technical and financial constraints are two significant obstacles that have hindered waste management improvements in developing countries (Inam et al., 2015). In most developing countries, there is a serious lack of technical expertise as well as engineering infrastructure preventing the transition of open dumps to landfills (Moh, 2017). Also given the low priority allocated to waste management, very limited funds are provided to the solid waste management sector by the governments (Kaza et al., 2018). The funds are often not sufficient to achieve the level of protection required for public health and the environment. Therefore mitigation of these challenges requires

large-scale financial support from the government for the upgrade of distillery effluent treatment technologies, especially in small-scale industries (Deng et al., 2017). Moreover, distillery industries are facing the following challenges during the treatment and safe disposal of effluent into the environment: (Chowdhary et al., 2020) 1. Melanoidins and polyphenols exhibit antioxidant and antimicrobial properties which make difficult the treatment of distillery effluent at industrial level. 2. There are several colorants instead of one type of pigment alone that may cause the undesirable color, which enhances the difficulties of decolorization and degradation of organic pollutant present in distillery effluent (Zhang et al., 2017). Understanding the structure and chemical characteristics of melanoidins are essential prior to development of a effective degradation technique (Faixo et al., 2021). 4. Lack of advanced processing techniques and waste treatment technologies in developing countries.

3.3. Conclusion:

The techniques available for the remediation of environmental industrial pollution involving complexes of organic, Inorganic compounds, heavy metals, sediments and groundwater are generally high-cost solutions. A cheaper, practical and ecologically relevant alternative is the bacterial assisted phytoremediation, association of plants with microorganisms that contribute to the degradation and removal of pollutants both from soil and wetland ecosystem. Efficacy of phytoremediation strongly depends on a large and deep penetrating root system and a high transpiration rate (Komives & Gullner, 2006). When the pollutant is assimilated and translocated within plant tissues, metabolic transformation processes occur that are mediated by a great variety of enzymes for several types of substrates (Sanderman, 1994; Coleman et al., 1997; Mezzari et al., 2004, 2005). The plant metabolic transformation process depends directly on the solubility and bioavailability of the pollutant, to make phytoremediation a feasible process. Physicochemical and structural properties determine the uptake of organic chemicals by plant roots from the soil. The microbial population present in the rhizosphere or near the root zone can degrade the pollutant or make it more bioavailable, challenging the plant tolerance when exposed to environmental pollutant (Pichtel & Liskanen, 2001; Merkl et al., 2005). Plant growth promoting rhizobacteria (PGPR) have also been used as inocula to further increase

plant growth, reduce environmental stress and promote degradation by rhizosphere-associated microorganism. Mainly the studies have been focused only on the remediation of heavy metals through rhizospheric bacteria from the contaminated sites. But, the study on the removal and minimizing of organic pollutants from the polluted sites is not much studied so far.

The sugarcane molasses-based distilleries discharge its waste as a Post-Methanated Distillery Effluent (PMDE) which is dark brown to black complex of various organic pollutants and heavy metals. PMDE retains high BOD (18,000–22,000 mg/l), COD (32,400–35,000 mg/l), colour (1, 50,000–1, 80,000 Co-Pt), sulphate (3,100–5,760 mg/l), phenol (4.0–4.2 mg/l), total suspended solids (TSS; 11,920–25,308 mg/l), total dissolved solids (TDS; 10,480–77,776 mg/l) and having heavy metals (Yadav. et. al. 2013). In distillery and fermentation industries, during the course of alcohol production, around 12–15 l of effluent are generated for per liter of alcohol produced and this large volume of effluent is the major source of soil and water pollution in the environment (Bharagava and Chandra; 2010). In India, there are more than 397 sugarcane molasses based distilleries releasing approximately 3.5×10^{13} kL spent wash annually (AIDA, 2016). There is an average sludge generation of 1500 tons per day during anaerobic digestion of spent wash (Kansal et al., 1998). Distillery sludge contains not only mixture of complex organic pollutants but also retains high quantity of heavy metals like Fe, Zn, Cu, Mn, Ni, and Pb (Chandra et. al. 2018). This reflects the magnitude of the environmental pollution caused by the waste generated from distillery sector. Disposal of distillery waste in aquatic environment reduces sunlight penetration, decreases both photosynthetic activity and dissolved oxygen content consequently damaging aquatic fauna and flora both. On land distillery waste causes reduction in soil alkalinity, inhibition of seed germination, damage to vegetation (Bharagava et al., 2008). Nevertheless, some native potential plants (weeds and grasses) grow on the discharge site of distilleries sludge; they have been reported for heavy metal phytoextraction by Chandra et. al. 2018. But, there rhizospheric bacterial community is not yet revealed by any researcher. Due to the specific variant of pollutants there may be indigenous rhizospheric bacterial community which is playing role in mineralizing and assisting phytoremediation of distillery waste. Thus, distillery waste may be responsible for the variation in the population dynamics of

different tolerant group's rhizospheric bacterial community. Rhizospheric bacterial community may increase bioremediation of soil contaminated with distillery waste.

Several researchers have focused on bioremediation of environmental pollutants through rhizospheric bacterial community of various industries. The maximum work on distillery has been updated to the phytoremediation of heavy metal and organic compounds from distillery waste by Chandra et. al. 2018, Chandra and Kumar; 2017. But, there rhizospheric bacterial community assisting phytoremediation has not been revealed so far. Therefore, the combined effect of plant associated bacterial species or that of rhizosphere bacterial species and biotransformation of effluent by native indigenous bacteria for the decolorization and degradation of distillery waste will be a beneficial study for the removal of various environmental pollutants.

Chapter 3
Objectives

Chapter-3

Objectives

The objectives of the study were as follows:

- Characterization of complex organic pollutants and heavy metals from Post-Methanated Distillery Effluent (PMDE) from different distilleries.
- Characterization of rhizospheric bacterial communities during phytoextraction of heavy metals from organometallic waste of distillery disposal site by hyperaccumulator plants (*Parthenium hysterophorus* and *Cannabis sativa).*
- Comparative assessment of phosphate, zinc and potassium solubilization by rhizospheric bacterial communities in *Phragmites communis* and *Typha spp.*
- Correlation between bacterial community and degradation of complex organic compounds in- situ bioremediation.
- Characterization bacterial communities during rhizofiltration of recalcitrant pollutants of distillery effluent in constructed wetland treatment system.

Chapter 4

Characterization of organic compound organic pollutants and heavy metals from Post-Methanated Distillery Effluent (PMDE) and their environmental effect.

Chapter-4

Characterization of organic compound organic pollutants and heavy metals from Post-Methanated Distillery Effluent (PMDE) and their environmental effect

4.1. Introduction

Organic compounds are any chemical compounds that contain carbon-hydrogen or carbon-carbon bonds at their most fundamental level. By way of clarification, an organic pollutant is an organic chemical that is released into an ecosystem and which causes pollution (however temporary or permanent) insofar as the chemical is harmful to or is destructive to the flora and/or the fauns of the ecosystem. Typically, and by virtue of the name, a pollutant is a chemical that is not indigenous to the ecosystem. However, if the discharged chemical is indigenous to the ecosystem (i.e., the organic chemical is a naturally occurring compound), it can be (should be) classed as a pollutant when it is released into the system in amounts that are in excess of the natural concentration of the organic chemical in the ecosystem, and by this increased concentration the chemical can cause harm to (or is destructive to) the flora and/or the fauna of the ecosystem.

Some organic chemicals are removed from the ecosystem by natural events, such as attack by indigenous bacteria (biodegradation) or by increasing the concentration of natural-occurring bacteria to remove the chemical from the ecosystem (bioremediation) (Speight and Arjoon, 2012). However, there are organic chemicals that are known as persistent organic pollutants (POPs) which are compounds that are resistant to environmental degradation through the various chemical and biological processes (Jacob, 2013). POPs, as the name implies, are not easily degraded in the environment due to their stability and low decomposition rates and, thus, have a long life in various ecosystems and often require other forms of removal such as physical or chemical methods of cleanup as well as the addition of nonindigenous microbes for cleanup (Speight and Lee, 2000; Speight and Arjoon, 2012). POPs also have the ability for long-range transport, and environmental contamination by POPs is extensive, even in areas where these chemicals have never

been used, and will remain in these environments for a considerable time (even years) and after restrictions implemented due to their resistance to degradation. POPs, like any organic chemical pollutant, can enter an ecosystem through the gas phase, the liquid phase, or solid phase and which can resist degradation and are mobile over considerable distances (especially in the gas phase or through transportation in river systems) before being redeposited in a location that is remote to the location of their introduction into the ecosystem. Furthermore, POPs can be present as vapors in the atmosphere or bound to (adsorbed on) the surface of soil or mineral particles and also have variable solubility in water. Furthermore, the capacity of the environment to absorb the effluents and other impacts of process technologies is not unlimited, as some would have us believe. The environment should be considered to be an extremely limited resource, and discharge of chemicals into it should be subject to severe constraints. Indeed, the declining quality of raw materials dictates that more material must be processed to provide the needed fuels. And the growing magnitude of the products and effluents from industrial processes has moved above the line where the environment has the capability to absorb such process effluents without disruption.

Different classes of organic pollutants in soil and water are broadly categorized in polychlorinated biphenyls (PCBs), polychlorinated hydrocarbons (PAHs), petroleum hydrocarbons, agrochemical and explosives (RDX, TNT).

However, there are several organic compound release from industry which are very much complex in nature, their property and impact are not known. Distilleries are among one of the most polluting industry in India due to discharged of various complex unknown compounds therefore, characterization of the pollutant is necessary from distillery. Discharged sugarcane molasses-based distillery effluent, also known as post-methanated distillery effluent (PMDE), is a dark-brown and highly complex chemically recalcitrant organometallic compound as a source of environmental pollutants (Yadav and Chandra, 2019). The PMDE has pH of 8.5, total suspended solids (21,000–40,700 mg L− 1), total dissolved solids (29,810 mg L−1), biological oxygen demand (8000–12,000 mg L−1), chemical oxygen demand (35,000–52,000 mg L− 1), nitrogenous compounds (15,284–28,696 mg L−1), and total sulfate (3875–4096 mg L− 1). In addition, there are phosphate (1625 mg L− 1), potassium at 537 mg

L−1, and chloride (7842–7997 mg L−1) as salts and phenolic compounds (6893–7202 mg L−1) (Bhargava and Chandra, 2010a, b). The dark color of PMDE is reported due to the thermal processing of various water-soluble compounds in sugarcane juice, such as cane pigments, phenolics, and different aminocarbonyl compounds, which are likely to form a complex polymer known as melanoidin at elevated temperature (Yadav and Chandra 2019). Melanoidin is a known nonenzymatic product, separated with sugarcane molasses as a by-product during the clarification of sugarcane juice (Chowdhary et al. 2018). The effect of melanoidin has been reported for severe aquatic and soil pollution in the environment. Due to severe threats to the environment, various attempts have been made globally by the researchers using the chemical and biological method, for degradation and decolorization of molasses-based melanoidin of distillery wastewater for environmental protection and its safe disposal (Singh et al., 2021). In various studies, the BOD/COD ratio has been described as degradability status (Abdalla and Hammam 2014; Choi et al. 2017). During the anaerobic treatment process, the spent wash becomes more viscous and dark because of microbial interaction with sulfur compounds (Nguyen and Juang 2013). The BOD/COD ratio (0.35) of an anaerobically degraded spent wash of distillery has been reported 0.35 based on the previous study. This indicated the nondegradability of waste in the presence of various complex compounds. In view of high concentration of pollutants in the effluent, bacterial community does not show any growth and bioconversion for degradation (Chandra et al. 2008a, b). Besides the complex organic compounds, there are substantial amounts of heavy metals reported in the PMDE, which increase its toxicity because of their strong binding tendency with the organic polymer component and their solubility under acidic conditions (Chandra et al. 2008a, b). These organometallic wastes along with the salts, phenolics, and chlorides, becomes more hazardous to the environment (Shon et al. 2006). In addition to the high organic content, PMDE also contains a complex of nitrogen (N), phosphorus (P), and potassium (K) as salts, which causes eutrophication of water bodies due to the presence of a significant amount of N and P as a source of nutrients to the microbial communities (Chowdhary et al. 2018). India and various other countries use this highly toxic wastewater for agricultural irrigation due to a lack of irrigation water. But, irrigation with distillery wastewater in agricultural fields contributes to soil pollution; as a result, the heavy metal uptake by crop plants

increases, influencing food quality and safety (Chhonkar et al. 2000). The high content of metals in food material poses various problems and induces disease due to accumulation in their tissue beyond the permissible limit. High amounts of lead (Pb) and arsenic (As) can cause chronic effects, including cancers of the kidney, bladder, skin, and lung, and may cause the health problems of plumbism, anemia, nephropathy, and gastrointestinal colic, in addition to affecting the central nervous system (Chhonkar et al. 2000). Moreover, in aquatic systems, periphyton, benthic invertebrates, and fish diversity have been reported due to the elevated concentration of iron in PMDE (Jaishankar et al. 2014). The accumulation of iron can cause substantial harm to aquatic organisms by clogging and hindering the breathing structures of fish (USEPA 2012). Free iron can also result in lipid peroxidation that severely damages mitochondria, microsomes, and other cellular organelles (Albretsen 2006). In children, the toxicity caused by excess iron accumulation is associated with symptoms of gastrointestinal breathing structure, vomiting, and diarrhea (Chang et al. 2011). Similarly, when copper accumulates in the body via ingested food, it causes acute gastrointestinal symptoms and development of liver cirrhosis and necrosis, with episodes of hemolysis and damage to renal tubules, and can even lead to coma (Hoffmann and McKiernan 2017). The hyperaccumulation of nickel is associated with skin allergy, lung fibrosis, and cancer of the respiratory tract (Rai et al. 2019). Plant growth and seed germination are also affected by different concentrations of PMDE. The high concentration of PMDE, has been reported for stunted stem growth and a reduced root system with inhibition of fruiting and flowering in *Phaseolus mungo* L. (Chandra et al. 2008a, b). Besides, recent studies have reported the presence of various recalcitrant organic pollutants which are listed under the screening list of endocrine-disrupting chemicals (EDCs) as emerging pollutants by the (USEPA 2012). The prominent compounds recently characterized by GC–MS in PMDE are hexanoic acid, butanoic acid, benzene propanoic acid, trimethylsilyl ester, monopalmitin 2-trimethylsilyl, effusion E, squalene, dithioerythritol 4-trimethylsilyl, octadecane, 1-methoxy ethoxy methyl-3-(pyridine-2-yl)-7-azaindole, and benzene acetic acid, alpha 4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester, Chemical Abstracts Service (CAS) in the previous study (Chandra et al. 2018). These compounds are reported as androgenic, mutagenic activity with EDCs properties discharged from various industrial wastewaters, sewage, and biomedical wastewater (Kostich et al. 2014;

Chandra et al. 2017). However, detailed characteristics regarding such hazardous compounds for environmental risk assessment have not been yet much known. Besides, distilleries discharge an average of 12–15 L of the spent wash per liter of alcohol produced by Indian distilleries (Chandra et al. 2018). In India, more than 397 distilleries generate approx. 28 × 1015 L of effluent annually, reflecting the magnitude of the environmental problem (AIDA 2016). Therefore, this study will reveal not only the presence of toxic organic pollutants but it will also establish the fate of PMDE in the aquatic ecosystem. The detailed toxic effects of PMDE on freshwater fish or other aquatic organisms have not been much reported. Hence, it is essential to reveal the nature of the compounds present in PMDE and their toxicological effects due to the complexation of various pollutants on aquatic and terrestrial ecosystems prior to its safe disposal.

4.2 Material and Methods

4.2.1. Sample collection and site description

The PMDE samples were collected from M/s Unnao Distilleries and Breweries, Unnao, Uttar Pradesh, India (26° 32′ N, 80° 30′ E). The industry produces ≈ 225 × 103/ L of ethanol per year, which generates ≈ 3.0 × 109 L PMDE annually 1 3 after anaerobic treatment (methanogenesis) (Chandra et al. 2018; Chandra and Kumar 2018). The PMDE sample was collected in presterilized 10.0-L plastic jerricans (Tarsons Products Pvt. Ltd.) from the distillery effluent discharge site. The samples were taken in triplicate and kept at 4 °C to avoid any thermal degradation. The same day it was brought to the laboratory and processed within 24 h for physicochemical analysis.

Fig.4.1. Sample collection. (a) View of M/s Unnao Distilleries and Breweries, Unnao (b) Post Methanated Distillery Effluent (PMDE) (c) Collecting PMDE (d) Collected PMDE

4.2.2. Reagents and chemicals

In this study, used chemicals and reagents were of analytical grade with a purity of > 99%. The chemicals were purchased from Merck (Merck KGaA, Darmstadt, Germany) and SRL (Sisco Research Laboratories, India), while other reagents and derivating chemicals for GC–MS analysis including were obtained from Sigma-Aldrich (St. Louis, MO, USA) and included.

4.2.3. Physico-chemical analysis of PMDE

The physicochemical properties of color, pH, BOD, COD, TDS, TSS, chloride, total N, ammonium N, phenolics, and sulfate were analyzed as per standard methods for

the examination of water and wastewater by the American Public Health Association (APHA 2011). The heavy metals analysis was done by atomic absorption spectrophotometry (ZEEnit 700, Analytic Jena, Germany) method after nitric acid–perchloric acid digestion as previously described method (Echavarria et al. 2013).

4.2.4. Extraction and identification of organic pollutants

Scanning electron microscopy and energy‐dispersive X‐ray Spectroscopy Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) were performed to assess the surface morphology of dissolved pollutants in PMDE. The samples were kept in an aluminum foil for drying in the oven for 4 to 5 h at 60 °C followed by keeping in desiccators for 2 h prior to ready for analysis. Samples were taken, and a very thin film of palladium and gold was deposited on the surface of the sample to conduct electricity. These samples were mounted on the electron micropore stubs. The SEM/ EDX analysis was carried with the help of a controlled field emission SEM (JOEL JSM-6330f, JOEL Ltd., Dearborn Rd, USA) equipped with a WVEX-EDS detection system to determine the morphology of the sample (Asberry et al. 2014).

4.2.5. Ultraviolet–visible and Fourier transform infrared spectroscopic analyses

To evaluate the absorption pattern of the various pollutants present in the PMDE, ultraviolet–visible (UV–Vis) absorption spectrum analysis was performed by a double-beam spectrophotometer (Evolution-201, Thermo Scientific, USA) at room temperature in the wavelength range of 200–700 nm. The Fourier transform infrared spectroscopy (FTIR) analysis of purified extract was performed by a spectrophotometer (Nexus-890, Thermo Electron Co., Yokohama, Japan) as described previously (Chandra et al. 2018). The FTIR
spectrum for the analysis of PMDE samples was done in the range of 400–4000 cm−1 (Causin et al. 2008).

4.2.6. Liquid–liquid extraction

The complex organic pollutants from the PMDE were extracted under alkaline conditions by ethyl acetate (Chandra et al. 2018). In the extraction, a fixed volume (10 mL) of the PMDE was mixed with an equal volume (10 mL) of ethyl acetate in a separating funnel (100 mL). The mixtures were continuously shaken for 2–4 h to mix

the solvent, with liquid– liquid extraction at irregular time intervals. After proper shaking, the solutions were kept to constant on separating funnel stand to separate the organic layer of solvent. Thereafter, the organic component was collected in a clean beaker; this process was repeated thrice to make complete extraction of organic pollutants. Finally, all the extracted organic solvents were pooled in a clean beaker. Subsequently, it was dehydrated by passing through anhydrous sodium sulfate. The extracted organic solvent-containing organic pollutants were dried by using rotavapor (Rotavapor RE 120; Buchi, Flawil, Sweden) at low pressure. Thereafter, the dried residue was dissolved in 1 mL of ethyl acetate and filtered using a 0.2 μm syringe filter (Millipore Ltd., Bedford, MA, USA); finally, it was used for FTIR and gas chromatography–mass spectrometry (GC–MS) analysis.

4.2.7. Derivatization of the extracted sample

An aliquot of the extracted sample of PMDE (300 μL) was transferred to GC vials and evaporated to dryness by nitrogen gas. Further, derivatization was performed with the addition of 50 μl pyridine and 80 μl trimethylsilyl BSTFA and TMCS to 300 μl samples. Lastly, the mixture was heated to 70 °C for 30 min with periodic shaking of the residue, after which the sample was loaded for GC–MS analysis as previously described (Chandra and Kumar 2017).

4.2.8. Gas chromatography–mass spectrometry analysis

The extracted organic contents from PMDE and sludge leachate were derivatized with trimethylsilyl (TMS), as previously described GC–MS analysis method (Chandra et al. 2017). In this process, 50 μl of pyridine and 80 μl trimethylsilyl BSTFA and TMCS were added to 300 μl samples. Further, the samples were heated to 70 °C for 30 min, with periodic shaking of the residues. Automatically, an aliquot (2.0 mL) of the silylated sample was injected into a GC–MS (Thermo Scientific Trace GC Ultra Gas Chromatograph, USA) as previously described (Chandra and Kumar 2017). The organic compounds were identified by comparing their mass spectra and retention times with those of compounds available in the National Institute of Standards and Technology (NIST) library (https ://www.nist.gov/).

4.2.9. Phytotoxicity assay with post-methanated distillery effluent

4.2.9.1 Seed germination experiment

The phytotoxicity assay of the PMDE was done with seed germination test of *Zea mays* L. Seed germination was evaluated at different concentrations of effluent using a Petri dish method as previously described (David and Rajan 2015). Ten sterilized seeds were put in different petri dish at equal spacing. The dishes contained the PMDE concentrations of 10%, 20%, 40%, 60%, 80%, and 100% (v/v), while tap water was used as a control in the same condition. The dishes were incubated at 28 °C till germination was observed (Bhargava and Chandra 2010). To make data statistically significant, three replicates were prepared for each concentration. The following formulae were used to determine the percentage toxicity and stress resistance index (Quaratino et al. 2007):

All experiments were performed at 4 °C to maintain the seed enzyme activity. The supernatant was used as a crude enzyme extract in the α-amylase activity test (Bhargava and Chandra 2010a). The reaction mixture (3 mL) contained 1.0 mL of 0.1 M acetate buffer, pH 4.8, 0.5 mL of enzyme extract, and 1.0 mL of 0.1% soluble starch solution diluted to 1.0 mL using acetate buffer. During the enzyme test, the enzyme extract was diluted to obtain an absorbance change of > 1.0 during enzyme assay. The reaction mixture was incubated at room temperature for 10 min, and the reaction was stopped by adding 1.0 mL of 0.1% iodine and 3 mL of 0.05 N HCl. Absorption was measured at 620 nm, and the decrease in absorbance was measured in terms of amylase activity (Bhargava and Chandra 2010a).

4.2.9.2. Enzyme concentration and purification

The supernatant with crude α-amylase enzyme was further concentrated by the addition of cold acetone (− 20 °C) in double volume. After centrifuging at 15,000 × g for 20 min, the precipitated proteins were collected. The acetone was removed, and the precipitated proteins were dissolved in 0.1 M sodium acetate buffer (pH 4.8). For further purification, the soluble proteins were passed through a Sephadex G-100 column (Sigma–Aldrich, USA) (80 cm × 2.0 cm) equilibrated with the same buffer. Two milliliters of protein fraction was eluted at the flow rate of 0.5 mL min− 1, and it was stored at − 20 °C until further analysis (Bhargava and Chandra 2010a).

4.2.9.3. Determination of enzyme molecular weight using SDS– PAGE

Seeds of each concentration treated with PMDE were homogenized in 0.1 M sodium acetate buffer to prepare enzyme extract (pH 4.8). Large particles in the solution were filtered out through two layers of cheesecloth, and the supernatant was centrifuged at 15,000 × g for 20 min. The enzyme molecular weight was estimated by SDS–PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) using 10% polyacrylamide gel. The standard markers α-amylase enzyme (Sigma–Aldrich, USA) and a protein ladder (Bangalore Genei, India) were used to determine the molecular weight of the enzyme. After performing gel electrophoresis, the gel was visualized and stored in a gel documentation system (Syngene, UK).

$$\text{Percentage of Phytotoxicity (\%)} = \frac{\text{Radicle length of control} - \text{Radicle length of the test}}{\text{Radicle length of control}} \times 100$$

$$\text{Index of Tolerance (\%)} = \frac{\text{Mean Length of the longest root in the treatment}}{\text{Mean length of the longest root in the control sample}} \times 100$$

4.2.9.4. Enzyme extract preparation and screening of α amylase activity

Seeds of each concentration treated with PMDE were homogenized in 0.1 M sodium acetate buffer to prepare enzyme extract (pH 4.8). Large particles in the solution were filtered out through two layers of cheesecloth, and the supernatant was centrifuged at 15,000 × g for 20 min. The enzyme molecular weight was estimated by SDS–PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) using 10% polyacrylamide gel. The standard markers α-amylase enzyme (Sigma–Aldrich, USA) and a protein ladder (Bangalore Genei, India) were used to determine the molecular weight of the enzyme. After performing gel electrophoresis, the gel was visualized and stored in a gel documentation system (Syngene, UK).

4.2.9.5. Toxicity of post‑methanated distillery effluent to a fish

An experiment for the toxicity was performed in accordance with Local/ National Guidelines of the ethical committee for experimentation in animals.

4.2.9.6. Fish collection and maintenance

Live and healthy adult Heteropneustes fossilis (35–45 g) were collected from the local fish market during the prespawning phase (July) as mentioned earlier (Mishra and

Chaube 2017). Fish were acclimatized for one week in the laboratory under normal photoperiod and temperature (12:12 h, light:dark and 25 •} 2 °C). During the acclimatization period, fish were fed ad libitum with goat liver daily.

4.2.9.7. Experimental setup

The PMDE toxicity test was performed to determine the effect in terms of the LC50 (Erhirhie et al. 2018) in a 24-h exposure. Fish were divided into four groups in duplicates (n = 2) with ten fish in each. Three groups were treated with a different concentration of the PMDE (1.0%, 5.0%, or 10%), and the fourth group was maintained as a control in plain freshwater. After 24 h, mortality data were recorded to calculate the LC50. Fish were killed in the laboratory, and the gills and liver were removed. Gills were stored in Bouin's fixative for anatomical study, and livers were stored at − 20 °C until catalase analysis. Catalase activity was used to check the stress level caused by exposure to the effluent.

4.2.9.8. Histological study

After 24 h in Bouin's fixative, trimmed gill pieces were dehydrated through a series of graded ethanol, followed by embedding in filtered paraffin (54 to 62 °C). Paraffin-embedded gills were sectioned with rotator microtome (Weswox, India) into 5-µm-thick sections. After passing through a series of graded alcohol and water, the sections were double- stained in hematoxylin and eosin, cleared in xylene, and
mounted in DPX to photomicrograph.

4.2.9.9. Catalase analysis

Liver tissues were weighed and processed for catalase (CAT) antioxidant enzyme activity (Keramati et al. 2010). In brief, 0.1 mL of supernatant was added to 2.9 mL of freshly prepared 30 Mm H2O2 in phosphate buffer (pH 7.0) and the optical density measured at 240 nm for 1.0 min with a UV–Vis spectrophotometer (EVOLUTION 201, Thermo Scientific).

4.2.9.9.1. Statistical analyses

The LC50 values with 95% confidence limits after 24 h were estimated by probit analysis with statistical software (IBM SPSS v. 16, 2018, USA). Data on the catalase analysis are presented as the mean} SEM. The significance of values obtained from

different groups was tested by using a oneway ANOVA ($P < 0.001$), and the intergroup comparisons were made by Newman–Keuls test ($P < 0.05$). Two separate tests with mean value were compared to verify the variation and the differences in the results, and Tukey's test was also used by following one-way ANOVA using GraphPad software (GraphPad Software, San Diego, CA, USA) (Kim 2014; Kumar and Chandra 2018). Each group had ten fish in duplicates for 24 h. Estimation was performed through probit analysis using SPSS software.

4.3. Results and discussion

4.3.1. Physicochemical characterization of post-methanated distillery effluent

The PMDE was dark brown with high color intensity (152,000 platinum–cobalt (Pt–Co) units) and had an odor similar to that of molasses with alkaline pH (8.53). The values of BOD and COD, and other pollution parameters were noted very high as shown in Table 1. Besides, the PMDE has been found an elevated level of different heavy metals, including Mn, Cr, Zn, Cu, Fe, Pb, Cd, Ni, Na, and K (Table 1). The BOD/COD ratio was found 0.38; this indicated the nondegradability of discharged effluent as reported in earlier studies regarding various wastewaters (Choi et al., 2017). The high BOD, COD of effluent might have contributed due to the presence of a high amount of dissolved organic matter along with calcium, magnesium, and nitrogen compounds as shown in Table 1. The amount of TDS and other organic compounds was corroborated with the previous analysis reported for anaerobically treated distillery spent wash (Chandra et al. 2008a, b; Sankaran et al. 2014). The levels of these parameters greatly exceeded the industrial discharge permissible limits of the regulatory agencies (USEPA 2002). The dark complex color might be due to the complex reactions of aminocarbonyl compounds, which showed strong binding ability with heavy metals and other cationic compounds (Yadav and Chandra 2019). The detailed mechanism for the formation of complicated coloring compounds in anaerobically treated distillery effluent has been also reported by previous studies (Sankaran et al. 2014). The alkaline pH of PMDE favored a stronger binding tendency of various soluble salts and heavy metals with the Maillard product, resulting in the complex structure of organometallic compounds (Chandra et al. 2018). In addition, the high levels of BOD, COD, TSS, TDS, volatile solids, total N, phenolics, and

sulfate and phosphate might be due to large amounts of several other unknown organics and inorganic compounds generated during the distillation process (Aniyikaiye et al. 2019). The high concentration of heavy metals in the PMDE might be supplemented due to corrosion of metallic pipes, fermentor vessels, and to some extent from raw material in sugarcane molasses (Bortoletto et al. 2018).

Table:- 4.1- Physico-chemical characteristics of PMDE wastewater.

All values are mean (n=3) ±SD in mg l^{-1} except color intensity (Co-Pt unit), pH, and temperature (^{0}C), (BOD): - at $p < 0.001$, [b] Less significant at $p < 0.05$

S.No.	Parameter	Values	Discharge Permissible limit (USEPA, 2002)	CPCB (2017)
1.	Color appearance	Dark Brown	--	--
2.	Color Intensity(Co-Pt)	152,000 ± 3.74	--	--
3.	Odor	Like molasses	--	--
4.	Ph	8.53 ± 0.01	8.00± 0.01[a]	7.54± 0.01
5.	BOD (mg/L^{-1})	17400.47 ± 19.18	40.00	47.00± 0.00
6.	COD (mg/L^{-1})	37193.68 ± 7.65	121.00[b]	79.00± 0.01
7.	TS (mg/L^{-1})	32000 ± 6.00	-	152± 0.01
8.	TDS (mg/L^{-1})	40,514.68 ± 6.55	50-70	70± 0.00
9.	VS (mg/L^{-1})	1058.35 ± 1.08		20± 0.01
10.	Chloride (mg/L^{-1})	2755 ± 4.30	750.00[b]	11.82± 0.01
11.	Total Nitrogen (mg/L^{-1})	1200.32 ± 4.16		9.90± 0.00
12.	Phenol (mg/L^{-1})	6895 ± 7.61	0.50a	-
13.	Sulphate (mg/L^{-1})	13,456.33 ± 3.55	750.00	
14.	Phosphate (mg/L^{-1})	309.96 ± 1.60	-	3.40± 0.01
15.	Calcium hardness as $Ca\ Co_3$	459±7.62	100-200[b]	10-20
	Trace Elements			
a)	Mn (mg/L^{-1})	8.25 ± 0.09	0.20[b]	0.15
b)	Cr (mg/L^{-1})	2.90 ± 0.027	0.05[b]	0.01
c)	Zn (mg/L^{-1})	16.65 ± 0.00	2.00	1.28
d)	Cu (mg/L^{-1})	2.50 ± 0.01	0.50[a]	0.19
e)	Fe (mg/L^{-1})	375.94 ± 0.36	2.00[a]	1.45
f)	Pb (mg/L^{-1})	2.48 ± 0.00	0.05	0.02
g)	Cd (mg/L^{-1})	BDL	BDL	BDL
h)	Ni (mg/L^{-1})	4.15 ± 0.00	0.10	0.04
i)	Na (mg/L^{-1})	108.95±0.07	0.04[a]	0.01
j)	K (mg/L^{-1})	137.34±	0.09[b]	0.02

4.3.2. Organic compounds in post-methanated distillery effluent

4.3.2.1. Scanning electron microscopy and energy dispersive X-ray spectroscopy

The SEM analysis revealed the morphology of the surface structure and the nature of recalcitrant organometallic pollutants present in PMDE as dissolved solids. The SEM

micrograph showed several sharp, rod-shaped crystalline structures of organometallic pollutants at 5000X (Fig. 1a). This indicated the presence of melanoidin as polymer, and the crystalline rod structure of organic polymer has been reported in other studies (Gomes et. al. 2019). These findings strongly supported our current observations, which were also corroborated with amorphous organometallic pollutants of the environment under scanning electron microscopy (Batista et al. 2018) as shown in Fig. 1a, b. The elemental analysis by SEM–EDX with their percent composition along with organic pollutants indicated the presence of metallic complex with other elements, i.e., O, Na, S, Cl, and K, in the sample analysis. The concentrations of these elements were noted above permissible levels (USEPA 2002), i.e., sodium by 40.21% and oxygen by 58.05%, followed by sulfur (0.68%), Cl (0.64%), and K (0.14%). The EDX elemental analysis was performed to confirm the elemental composition in the effluent to reveal the complexity. The high concentrations of various salts in the effluent indicated that it could be a major source of pollution in aquatic and terrestrial ecosystems.

4.3.4. Ultraviolet–visible and Fourier transform infrared spectroscopic analyses

Scanning of UV–Vis absorption spectra by spectrophotometry is the most commonly used method to detect the Maillard reaction product during the aminocarbonyl compound polymerization process (Martins and Boekel 2003). The scanning of UV–Vis absorption spectra absorption maxima within the range of 200–400 nm is shown in Fig. 1c. This result indicated a polymerized form of Maillard product in the PMDE as a dark-brown material that showed absorption in the UV range as basic property of melanoidin (Billaud et al. 2004). The absorption peaks were found at 290 nm, 350 nm, and 400 nm, but the absorption maximum of the spectral analysis was at λmax = 295 nm. This maximum is the characteristic property of melanoidin after completion of the polymerization reaction during its synthesis (Billaud et al. 2004). The various minor peaks in the absorption range indicated a mixture of various other pollutants. This property of the absorption spectra has also collaborated with the results of earlier studies (Echavarria et al. 2013; Chandra et al.

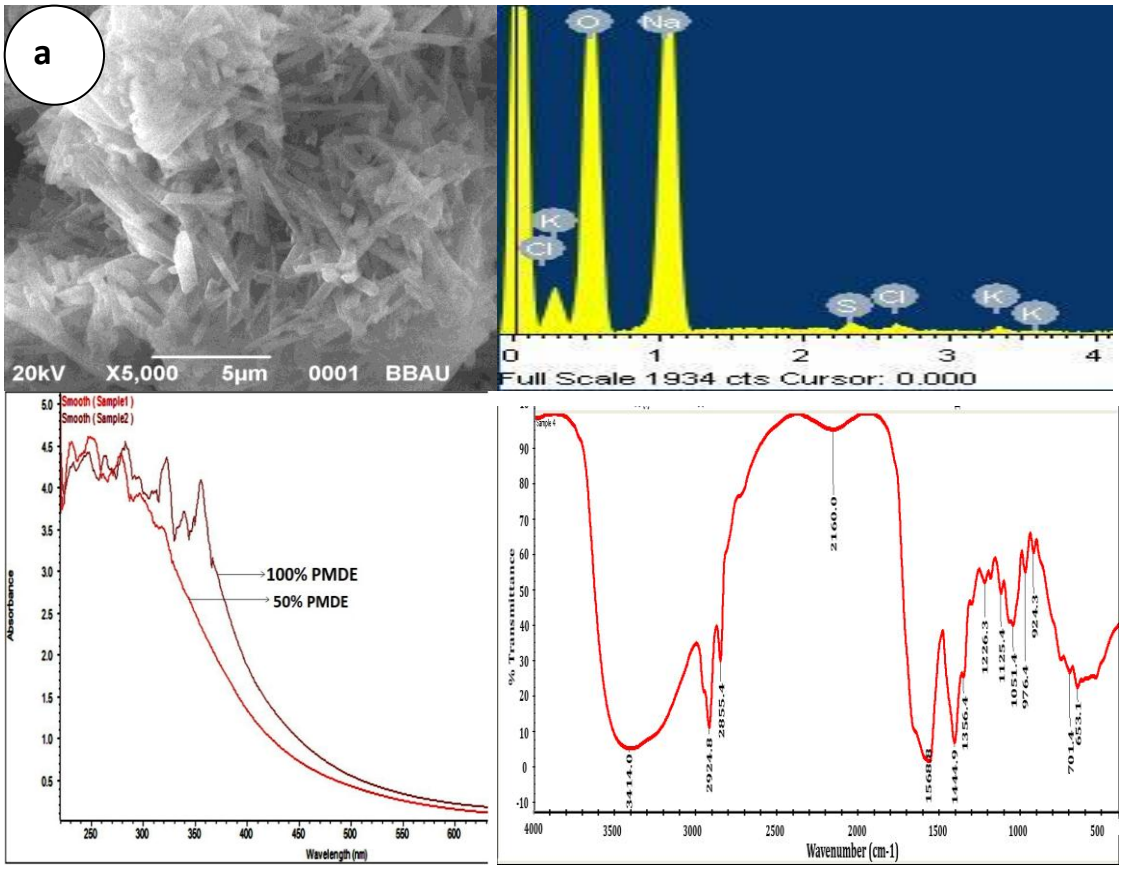


Fig.4.2.A-Surface topology of PMDE by SEM images B- Elemental analysis of the PMDE sample, C- UV-Vis spectral analysis D- Fourier transforms infrared (FTIR) spectra of PMDE sample.

The absorption spectra pattern of the PMDE sample at 50% concentration showed a similar absorption pattern, but some absorption peaks disappeared between 350 and 400 nm. This result explained the diminishing of organic compounds along with dilution. However, the absorption maxima at 295 nm remained constant, which indicated the major product of PMDE shown in Fig. 1c. Because the Maillard reaction product in the PMDE is derived from sugarcane molasses after the fermentation process, it contains heterogeneous compounds generated from sugarcane juice during the heating process. Hence, the mixed reaction products of the Maillard reaction are evidence in the variable ranges of absorption, as shown in Fig. 1c. However, the dominant peak at 295 nm indicated the abundance of early Maillard reaction products, in addition to those of the advanced stage of the polymerized product. This observation supported the complexity of the melanoidin structure reported previously

in their chemical structure (Gu et al. 2010). During the early stage in the production of melanoidin, various organic compounds, such as hydroxymethyl furfurals, reductones, and furanone, are also reported in the chain of polymerization process shown the absorption spectrum in the range of UV region (Echavarria et al. 2013). These findings had given strong support with a previous spectrophotometric analysis reported previously (Billaud et al. 2004). Similarly, in the FTIR analysis of organic pollutants in the PMDE, the stretching frequency of peaks indicated different functional groups at variable intensities of absorbance. The infrared spectra (500–4000 cm− 1) also indicated several functional groups. The peak values at 3610.8, 2923.8, and 2854.0 cm− 1 represented the O–H vibrational stretching in alcohols and carboxylic acids as reported previously (de Oliveira Silva et al. 2012; Kadam et al. 2013). The peak value of 3434.7 cm− 1 was attributed to an N–H amine group, and the peak value of 2031.0 cm− 1 represented C≡C stretching in the alkyne group. At stretching frequencies at 1737.5 and 1638.4 cm− 1, the C=O stretching of amides was indicated.

The spectrum region with strong intensity at 1576.9 and 1462.6 cm− 1 indicated compounds with a benzene ring. The band peak with medium intensity at 1378.8 cm− 1 represented –C–H stretching in an alkane group. The spectrum region between 1035.1 and 1294.1 cm− 1 was attributed to an acid and ester group, with a peak of strong intensity. The band at 968.5 and 892.7 cm− 1 was assigned to the =C–H small stretching vibration of the alkene group, as shown in Fig. 1d. The band at 724.0 cm− 1 was an indication of the C–Cl stretching vibration due to compounds with alkyl halide groups. These products are reported as intermediate by-products of the Maillard reaction in a separate study (Phisut and Jiraporn 2013). The results implied that the main organic pollutants in the PMDE were hydrocarbons, ketones, organic acids, fatty acids, aromatic compounds, phenolic and alcoholic compounds, along with some nitrogenous compounds as a complex structure. These compounds are reported to be generated as an intermediate stage in the Maillard reaction (Chandra and Kumar 2017). Hydrocarbons are the basic fragmented side products that are generated during each stage of the Maillard reaction, whereas the ketone group compounds are formed during the intermediate stage of amino acid degradation (Strecker degradation) of Melanoidin synthesis. Therefore, these compounds might be generated in the process of sugarcane juice heating in the sugar manufacturing

process. Phenolic compounds of high color are generated at the final stage of the Maillard reaction during aldol condensation of degraded amino acid compounds. Alcoholic and nitrogenous compounds are formed at the final stage of the Maillard reaction as various high and low molecular weight melanoidin polymers. Moreover, organic acids and fatty acids are generated during the fermentation of sugars and the digestion or breakdown of complex melanoidin compounds.

4.3.5. Gas chromatography–mass spectrometry/mass spectrometry analysis

The GC–MS/MS chromatogram of organic compounds extracted with ethyl acetate from the PMDE sample is shown in Fig. 2. The identified various organic compounds are listed in detail in Table 2 based on the mass-to-charge ratio (m/z) at various retention times (RTs). Most of the identified compounds were as residual organic compounds due to complexation with one another in the presence of metallic ions with raw material, including hexadecanoic acid, octadecanoic acid, propanoic acid, dodecanoic acid, and nonacosane, which are different forms of fatty acids that originated from the raw material of sugarcane molasses and juice (Chandra et al. 2017, 2018). In the recent report, some of these compounds were also listed under endocrine-disrupting chemicals (EDCs) as per the USEPA screening program list (USEPA 2012). However, the EDCs properties of various pollutants discharged from this industry are still unknown.

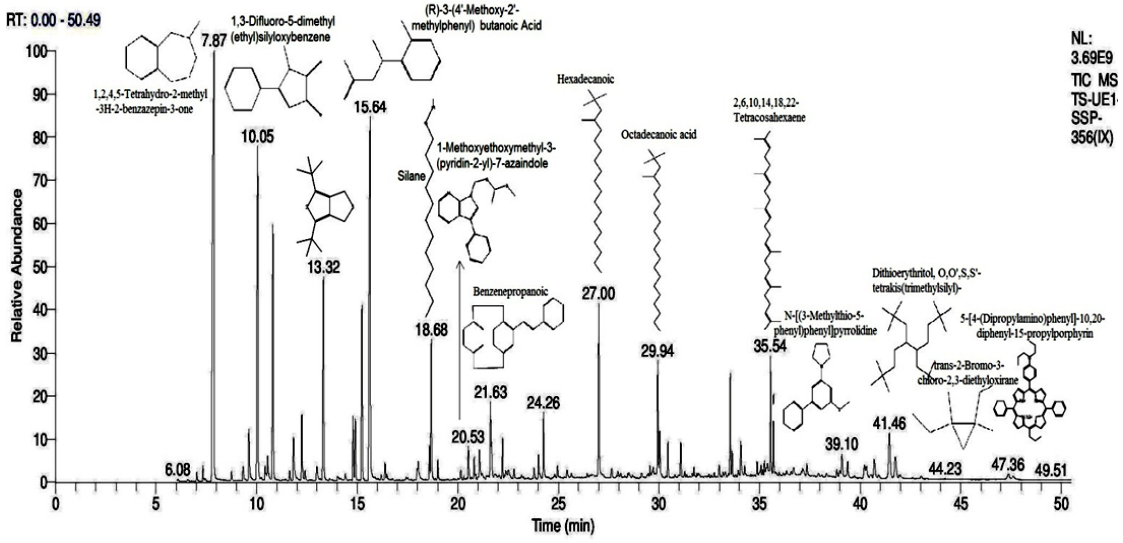


Fig.4.3: GC–MS chromatogram of TMS derivatized organic compounds extracted from PMDE

Table: 4.2 - Organic compounds identified by GC–MS analysis extracted with ethyl acetate (pH 8.0) from PMDE.

S.No.	RT	Source	COMPOUND	Toxicity
1.	6.08	Metabolic product in wastewater	ZINC(II)-à-ACETOXY-á FORMYLOCTAETHYLPORPHRIN	Bloody vomiting, liver failure, dilate the blood vessels.
2.	7.87	Volatile compounds in PMDE	3,7-Dioxa-2,8-disilanonane, 2,2,8,8-tetramethyl	Endocrine disruption, central nervous system damage,
3.	8.47	Residue in PMDE	Hexanoic acid, TMS ester	Endocrine disruption.
4.	10.05	Fatty acid residue	HEPTANOIC ACID TMS	Acute renal failure.
5.	11.54	Volatile substance	Cyclohexanecarboxylic acid,	Carcinogenic, allergy reaction.
6.	13.32	Plant Fatty acid residue	3,7-Dioxa-2,8-disilanonane,2,2,8,8-tetramethyl-5-[(TMS)oxy]- (CAS)	Hypertension, Stress, Osteoporosis, urinary stones,
7.	13.71	Principal sources of sulphur	Benzeneacetic acid, TMS ester	Gastrointestinal, Hematological, Respiratory (Nose to Lungs).
8.	15.64	Residue in PMDE	Nonanoic acid, TMS ester	strong eye irritant, vomiting
9.	16.35	Extracted in molasses	Benzenepropanoic acid, TMS ester	DNA damage, carcinogenic, allergy reaction, irritant dermatitis,
10.	18.68	Fatty acid residue	Decanoic acid, TMS ester	Inflammation, Fibrosis, Necrosis
11.	19.49	Residue in PMDE	4-Hydroxyphenylethanol, di-TMS	Parageusia, pink disease. Diarrhea Fever, Vomiting
12.	20.53	Chemical intermediate in PMDE.	Pentanedioic acid, 2-[(TMS)oxy]-,bis(TMS) ester	Carcinogenic, allergy reaction.
13.	21.63	Residue in PMDE	Dodecanoic acid, TMS ester (CAS)	inflammation and irritation
14.	23.81	Fatty acid residue	Octadecane	Cough. Sore throat. Skin & Eye Redness.
15.	24.26	Principal sources of sulphur	1,4-Benzenedicarboxylic acid,	Neurologic manifestations headache, nausea, vomiting,
16.	27.00	Volatile substance	1-(Benzyloxy)-2-fluoro-2-phenyl-3-(p-toluenesulsulfonyloxy)propane	Vision loss, Movement disorders , Prognosis
17.	27.84	Fatty acid residue	Hexadecanoic acid, trimethylsilyl ester	Degenerative bone disease, lung cancer,
18.	29.94	Fatty acid residue	Hexadecanoic acid, butyl ester	Difficulty Breathing Stomach Upset.
19.	30.17	Plant sterol	Nonacosane (CAS)	epigastria pain
20.	30.35	Fatty acid residue	Oleic acid, TMS ester	Carcinogens, Cardiovascular
21.	30.70	Fatty acid residue	Octadecanoic acid, TMS ester	Hypertension, Stress, Premenstrual syndrome, Osteoporosis,
22.	34.28	Bacterial degradation product	MONOPALMITIN 2TMS	Fatty Liver Disease (NAFLD), effect of Pancreas.
23.	35.54	Used as a chemical	Effusanin E	Toxicity to humans, including carcinogenicity
24.	36.35	Principal sources hydrogen sulphide gas	Enterolactone(2,3-bis(3-hydroxybenzyl)butyrolactone-di(TMS)	worsen asthma attacks, and aggravate existing heart disease
25.	39.10	Volatile substance	24-hydroxy-3,4-secolanost-4,(28),8-dien-3-nitrile	Endocrine disruption, central nervous system damage,
26.	41.46	Volatile substance	7-(Trimethylsilyloxy)-3-[4-(TMS)phenyl]-4H-1-benzopyran-4-one	kidney and proximal tubule cells,
27.	42.65	Plant sterol as residue in PMDE	Stigmasta-5,22-diene, 3-methoxy-, (3á,22E)-	Chest or Abdominal Pain Shock, Difficulty Breathing
28.	45.59	Volatile substance	2-(1-Phenylsulfanyloctyl)cyclohex-2-enone	Developmental toxicity, neurotoxicity
29.	48.67	Chemical intermediate in PMDE.	Benzeneacetic acid, à,4-bis[(TMS)oxy]-,trimethylsilyl ester (CAS)	Diarrhea Fever, Vomiting
30.	49.99	Acrylic acid used as a chemical intermediate.	(5,10,15,20-tetraphenyl[2-(2)H1]prophyrinato)zinc(II)	Inflammation and irritation

The source of other compounds detected in the effluent was either a bacterial metabolic product or a chemical additive, which can act as mutagens, carcinogens, or EDCs, along with metals and other metalloids in discharged effluent.

4.4. Phytotoxicity assay

4.4.1. Seed germination test

The environmental toxicity of the PMDE in soil was tested at various concentrations (control, 10%, 20%, 40%, 60%, 80%, and 100%) using the seed germination test of *Zea mays*. Seed germination and plumule growth were inhibited even at very low concentrations (10%) of PMDE. The higher concentrations not only inhibited seed germination, plumule, and radicle growth but also destroyed the seed viability with blackening of its embryo point. The seeds of *Zea mays* at 80% and 100% concentrations of the PMDE resulted to shrunk and turned black, as shown in Fig. 3a, b. The toxicity data at the different concentrations showed significant variation, as indicated by one-way ANOVA (Table 3). The amylase activity also showed reduction along with the increasing concentration of PMDE which is responsible for the conversion of starch into maltose mediated by alpha-glucosidase into glucose to supply energy in the germinating seed. The optimum α-amylase activity was noted 0.6 U in seeds treated with 10% (v/v) PMDE. Further, increase in PMDE concentration decreased the α-amylase production (> 10%). The inhibition in activity of α-amylase might has been noted due to the penetration of organic pollutants along with cumulative toxic effect of metallic compounds on the seed due to endosmosis effect of effluent concentration. These observations were corroborated with previous study reported in *Phaseolus mungo* L (Chandra and Bhargava 2004). Furthermore, the inhibition of root and shoot length also indicated an adverse effect on plant growth hormones (i.e., indole acetic acid (IAA), gibberellic acid, and cytokinins), which are responsible for root and shoot elongation. This observation concluded the inhibition of plant growth hormones at a high concentration of effluent along with adverse effect to seed germination as reported in separate study (Chandra et al. 2008a, b). The concentrations of heavy metals due to exceeding permissible levels in PMDE might be also responsible for the toxicity of the tested seed because the adverse effects of heavy metals on seed germination are well established (Mahimairaja et al. 2004).

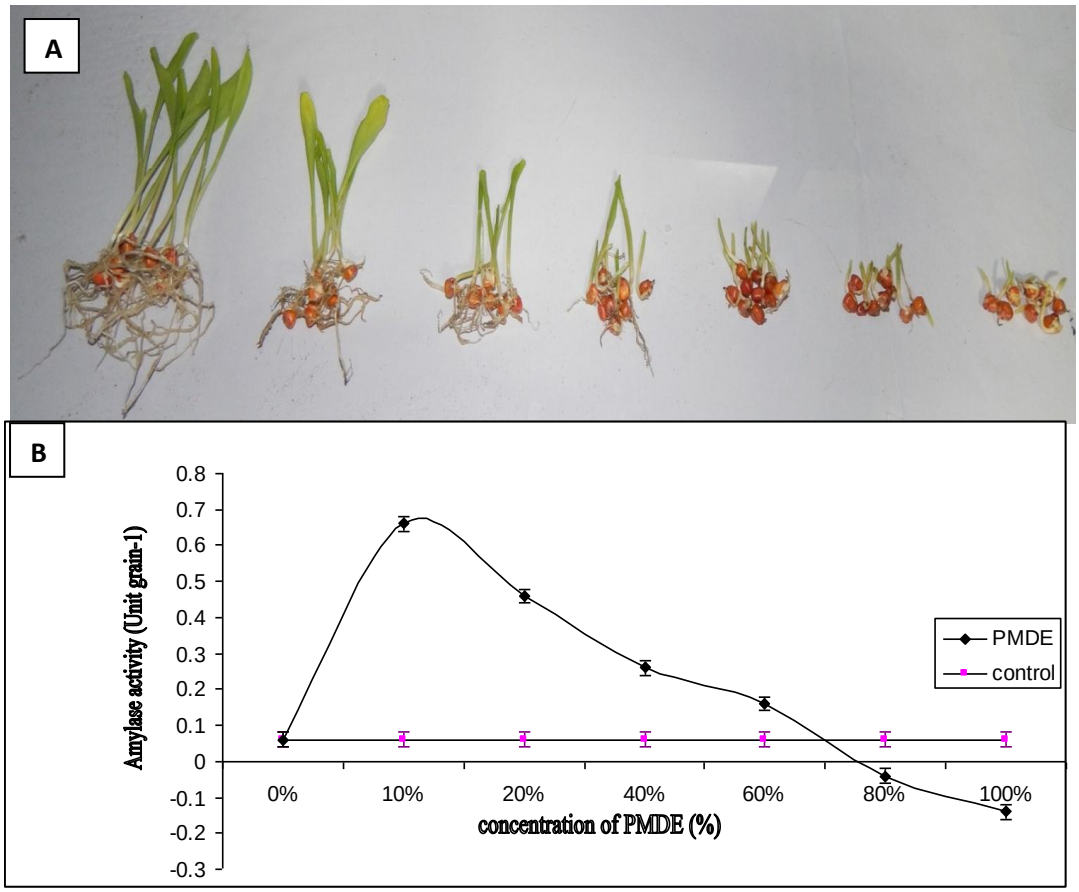


Fig. 4.4. A. Effect of toxicity of PMDE on seedling growth of *Zea-mays L*. against PMDE sample at various concentrations. **(1-** control**., 2-**10%**., 3-**20%., **4-**40%., **5-**60%., **6-**80%., **7-**100%) **B.** Mean acute alpha-amylase concentrations (U/mL) activity shown by Zea-mays seeds. Inset graph includes published acute PMDE concentrations (ug/dL) for the same sample. Error bars represent standard error of the mean and gel imaging at different concentration of PMDE (%).

Table: 4.3 - Effect of different concentrations of PMDE on seed germination and seedling growth of *Zea -mays.*

S.No.	No. of seed plated	Concentration (%)	Germination (%)	Germination index	Reduction in germination (%)	Radical length (cm)	Phytotoxicity (%)	Stress tolerance index.
1	10	Control (0)	100 ± 0.00	00 ± 0.00	00 ± 0.00	95.12 ± 0.82	00 ± 0.00	00 ± 0.00
2	10	10	80 ± 0.00	84.1 ± 0.04*	20 ± 0.00*	79.21 ± 0.07*	05.62 ± 0.23 ^{ns}	19.4 ± 0.04*
3	10	20	60± 0.00	71.0 ± 0.02*	40± 0.00*	63.35 ± 0.05 ^{ns}	16.61 ± 0.52*	24.5 ± 0.23 ^{ns}
4	10	40	40± 0.00	66.0 ± 0.00	60± 0.00 ^{ns}	35.51 ± 0.09 ^{ns}	67.90 ± 8.96*	47.6 ± 0.08 ^{ns}
5	10	60	10± 0.00	17.0 ± 0.01*	70 ± 0.00 ^{ns}	27.76 ± 0.08	82.68 ± 3.64	62.31 ± 0.10 ^{ns}
6	10	80	05± 0.00	09.0 ± 0.00	85± 0.00	12.21 ± 0.00	89.80 ± 3.99	71.1 ± 0.72
7	10	Effluent (100)	NG	-	-	-	-	-

All values are mean of three replicate ± SD., The statistical significance between the values of control to their respective concentrations was evaluated by ANOVA. PMDE: post-methanated distillery effluent.
* Significance level: $p < 0.05$. , ^{ns} Significance level: $p > 0.05$, *NG*=No Germination, *Control*= Tap water

4.4.2. Fish toxicity

The PMDE was noted very toxic to *H. fossilis*, though this is a known sturdy freshwater fish (Fig. 4.5a, b). The percentage of mortality significantly increased with an increase in the concentration of PMDE during 24-h incubation. Based on the mortality percentage, the estimated LC50 for 24 h was 3.663% PMDE. The toxicity of distillery effluent has been also reported in a previous study (Chowdhary et al. 2017). In the photomicrograph of gills, the level of effluent toxicity was well expressed after 24 h, compared with the control (Fig. 4.5a).

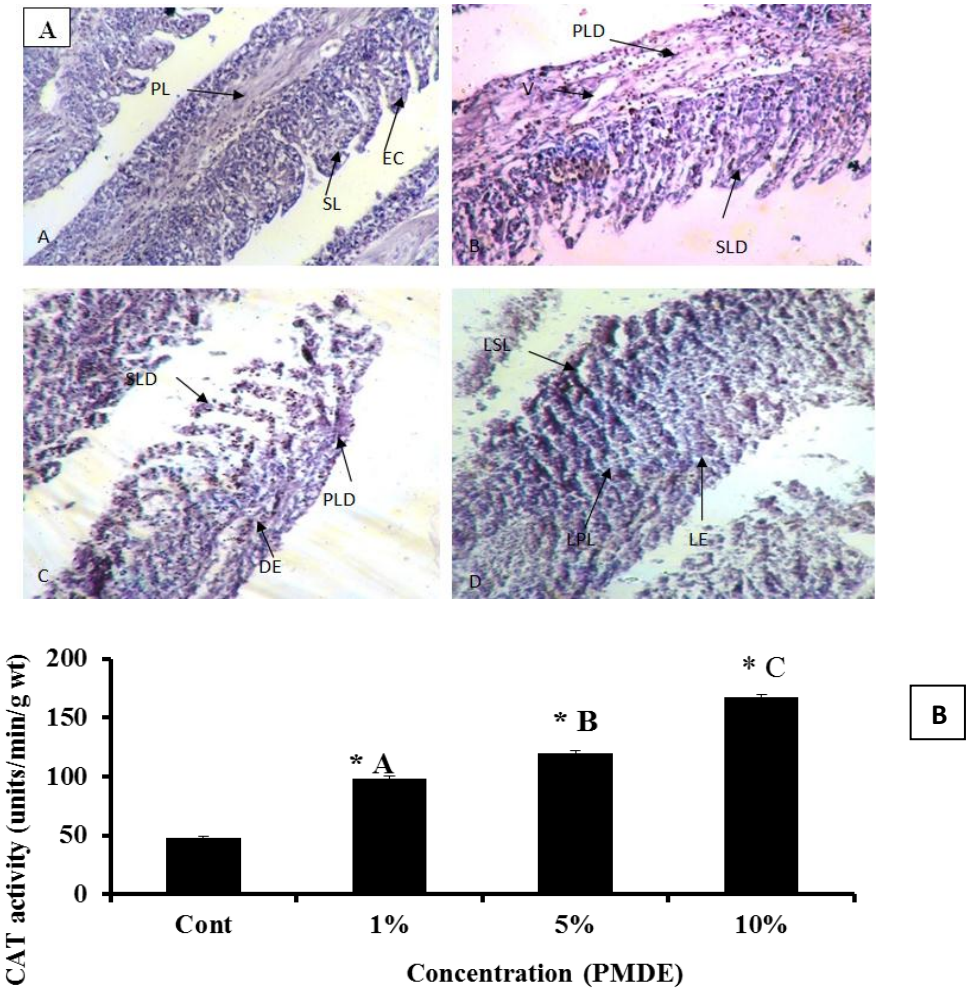


Fig.4.5. A. Concentration effect of post-methanated distillery effluent (PMDE; 1%, 5 %, 10 %) on gill histopathology of freshwater catfish, *Heteropneustes fossilis*, after 24 hr; (A) Control; (B) PMDE (1 %); (C) PMDE (5 %); (D) PMDE (10 %). Where PL: Primary lamellae; SL: Secondary lamellae; EC: Epithelial cell; PLD: Primary lamellae degeneration; SLD: Secondary lamellae degeneration; V: Vacuolation; DE: Damaged epithelium; LPL: Loss of primary lamellae; LSL: Loss of secondary lamellae; LE: Loss of epithelium. [Image captured with 20X; haematoxylin and eosine stained]. **B.** Catalase activity (units/min/g wt) in liver of *Heteropneustes fossilis* in different concentration of post-methanated distillery effluent (PMDE; 1%, 5 %, 10 %) with control. Each group had ten fish in duplicates for 24 hr. Values expressed as mean ± SEM. Data were analyzed by one way ANOVA ($P < 0.001$; *) and Newman- Kuels' test ($P < 0.05$; A, B, C). Groups superscripted with different letters are significantly different in intergroup comparison.

The abnormalities included degeneration of primary and secondary lamellae and epithelial cells and vacuolation. At a concentration of 10% PMDE, a total loss of the integrated structure of gill tissue was observed. In addition, toxicity was observed by a significant increase in CAT activity in the liver at increased concentrations of PMDE after 24 h (Fig. 4b). This result also indicated the activation of oxidative defense mechanisms against the generation of free radicals caused by the exposure of effluent. The present findings were corroborated with previous toxicity data of distillery effluent in terms of lethal concentration, nutritional biochemistry, and hematological parameters (Kumar and Gopal 2001; Ramakritinan et al. 2005; Shukla and Shukla 2013). The toxicity of catfish *Heteropneustes fossilis* during the reproductive phase (July) was noted LC50 value at 3.663% with 95% confidence limits of 9.231% and 0.143% after a 24-h exposure to PMDE. This supported the toxicity data observed previously for toxicity of fish with other pollutants (Bloch 1794).

4.5. Conclusion

This study revealed various unknown complex recalcitrant organometallic complexes with EDC properties which are a major source of environmental pollutants and health hazards. The absorption maxima of PMDE between 200–350 nm in the UV–Vis spectral analysis also indicated polymerized-form Maillard product. Hexadecanoic acid, butyl ester, octadecanoic acid, monopalmitin 2 TMS, effusanin E, 1-(benzyl) -2-fluoro-2-phenyl-3-(p-toluene's sulfonyl) propane, and 24-hydroxy-3,4-secolanost-4,(28),8- died-3-nitrile were prominent organic compounds in the category of mutagenic and androgenic compounds, detected in PMDE. In addition, the presence of various activity in germinating Zea mays seeds and gel imaging at different concentrations of PMDE heavy metals also indicated the contribution of toxic property of PMDE, as resulting in phototoxic effect on seed germination with *Zea mays* and *Heteropneustes fossilis* with the degradation of lamellae in gill. Thus, this affirmed the source of various unknown mutagenic, carcinogenic, and EDCs compounds with organometallic complexes.

Chapter 5
Characterization of rhizospheric bacterial communities from hyperaccumulator plant growing on organometallic sludge during phytoextraction for ecorestoration.

Chapter-5

Characterization of rhizospheric bacterial communities from hyperaccumulator plant growing on organometallic sludge during phytoextraction for ecorestoration

5.1 Introduction

Rhizospheric bacteria can establish deleterious, neutral or beneficial interactions with plants. Contaminant degrading rhizobacteria can be included among the plant-growth promoting rhizobacteria (PGPR) because the presence of contaminants, in general negatively affects the growth of the plant, and the elimination of the inhibiting chemicals will benefit the plant. Although contaminant degrading strains have been traditionally isolated from various environments, in the last two decades, an increasing number of studies have reported the isolation and identification of rhizospheric bacteria with contaminant-degrading abilities For a long period PGPR were mainly used for assisting plants to uptake nutrients from the environment or preventing plant diseases. Recently, the application of PGPR has been extended to remediate contaminated soils in association with plants. Of all the present contaminants, the profound impacts of organic and heavy metal pollutants have attracted worldwide attention. In order to eliminate or control the pollutants in soils, physical, chemical, and biological methods have been employed. It has obvious advantages over physicochemical remediation methods due to several merits: cost-effective, convenient, complete degradation of organic pollutants, and no collateral destruction of the site material or its indigenous flora and fauna.

Some organic contaminants can persist in the environment for a long time and bring great threat to human health. They mainly include: total petroleum hydrocarbons (TPHs) and polycyclic aromatic hydrocarbons (PAHs) coming from the exploration and consumption of fossil fuel, polychlorinated biphenyls (PCBs) widely used in the industrial process and are most degradation-resistant, and other chlorinated aromatics used as PCB replacement such as polychlorinated terphenyls (PCTs), halogenated compounds like perchloroethylene (PCE) and trichloroethylene (TCE) and pesticides like atrazine and bentazon. (Saleh et al., 2004). Heavy metals are the primary inorganic contaminants,

which include cadmium, chromium, copper, lead, mercury, nickel and zinc etc. This article reviews the applications of PGPR for bioremediation of these two main kinds of contaminants respectively. In contrast with inorganic compounds, microorganisms can degrade and even mineralize organic compounds in association with plants (Saleh et al., 2004). Hence discovery of effective pathways for degradation and mineralization of organic compounds may play an important role in the future. Phytoremediation strategies include: phytostabilization, where plants, either physically or by the action of the root exudates, help sequester the contaminant in the soil making it less bioavailable; phytovolatilization, where the plants take up the contaminant from the soil and transform it into a volatile compound that is released into the atmosphere for dispersal; phytoextraction, which involves the accumulation of toxic compounds in the harvestable part of a plant; rhizoremediation, involving the elimination of the contaminant by the microbes in the rhizosphere; and phytoremediation, a term which refers to the transformation of the contaminant by the plant metabolism (Ashraf et al., 2019).

In the rhizosphere, root-based interactions between plants and organisms are highly influenced by edaphic factors. However, the below-ground biological interactions that are driven by root exudates are more complex than those occurring above the soil surface. Plants and bacteria can interact with one another in a variety of different ways to absorb, degrade, or remove toxic contaminants from polluted soil and water. Bacteria that reside in different compartments of plants can synthesize several compounds that assist the plants to overcome stress, providing essential nutrients required for plant growth and development, improving plant defense systems against pathogens, and stimulating contaminants degradation. The interaction between bacteria and plant roots may be beneficial, harmful, or neutral for the plant, and sometimes the effect of a particular bacterium may vary as the soil and environmental conditions change.

The phytoremediation process may be influenced by various biotic and abiotic factors, and more work should be carried out to document the role of naturally adapted indigenous rhizosphere. Although plants and their associated bacteria degrade a wide range of hazardous organic pollutants, many compounds are degraded slowly or are not degraded at all. On the other hand, to efficient phytoremediation of metal contaminated site, it is first necessary to address the problem of metal bioavailability to plant. Finally, to employ bacteria assisted phytoremediation of either organic or metal contaminants on a

large scale in the environment, it will be necessary to convince regulatory bodies in various jurisdictions that the deliberate release of selected, or engineered, bacteria to the environment should be viewed not only as benign, but in fact as beneficial. This will require scientists to ensure, based on a thorough understanding of the processes and interactions between plants, pollutants, soil, and bacteria, that bacteria-assisted phytoremediation is a highly reproducible and dependable process.

Present chapter has presented the data of bacterial mediated phytoremediation by *Cannabis sativa* and *Parthenium hysterophorus*

5.2. Bacterial assisted phytoremediation of heavy metals and organic pollutants by *Cannabis sativa* as hyperaccumulator plants growing on distillery waste disposal site for ecorestoration of polluted site

Phytoremediation is a low-cost, high-potential technology for environmental sustainability (Tonelli et al., 2022). Till date several plant species have been characterize and identified for the phytoremediation of soils contaminated with heavy metals, herbicides, pesticides, explosives, radionuclides and fuel oil (Kennen and Kirkwood, 2015). During the phytoremediation process plants remove, transform, or stabilises toxins, including organic pollutants, at the growing site of sediments (Nedjimi, 2021). It has been demonstrated that the combination of plants and their associated microorganisms plays an important role in phytoremediation of contaminated soils (Nurzhanova et al., 2021). During the plant growth, plant secretes a diverse variety of secondary metabolites into the rhizosphere, which are easily access by rhizobacteria as a nutritional source. These metabolites include class of phytosterols, sugars, tricarboxylic acids, fatty acids, oxalic acid, polysaccharides, amino acids, phenols, and proteins (Hayat et al. 2017). Several microorganisms, on the other hand, defend the plant from pathogenic microbes and can release phytohormones in the rhizosphere, such as auxins, cytokinins, and gibberellins, which stimulate the synthesis of phytohormones and help the plant grow faster (Glick, 2020). But, selection of plant species for phytoremediation of pollutants at any site is determined based on plant verities, nature of pollutants along with environmental conditions and physiological status of soil (Tripathi et al., 2020). In the mechanism of phytoremediation, plant growth promoting rhizobacteria (PGPR) regulate the bioavailability of various nutrients to plants in soil through ligninolytic enzyme activity, such as lignin peroxidase (LiP), manganese peroxidase (MnP), and laccases (Lac), which dissolve complex organometallic compounds

for availability to plants (Bharagava et al., 2020; Tripath et al., 2021). This process is initiated by these microbes through colonization to root surface and involve in mechanism for plant prevention from toxicity through secretion and production of several regulatory compounds such as phytohormones, siderophore, metal binding proteins etc. (Manoj et al., 2020) Thus to account for this complexity a set of interaction is required i.e. adsorption to root, interaction with cell membrane, absorption to cell and translocation to the target tissue based on histological and physiological properties of plant (Gupta et al., 2016). Recent research has shown that bacterial assisted phytoremediation of heavy metals by various metal tolerant bacterial inoculants in laboratory conditions (Song et al., 2021). Metal tolerant bacterial enhancement in phytoextraction process of lead is also reported by two ornamental plants (Manzoor et al., 2019). Similarly, it has been reported that the arsenic tolerant strain of *Paracoccus versutus* and *Aeromonas caviae* inoculated in rhizospheric soil of *Adiantum capillus-veneris* have enhance the arsenic uptake (Marwa et al., 2020). Arsenic reducing bacteria also has been reported for the enhanced arsenic accumulation and tolerance by *Salix atrocinerea* (Navazas et al., 2021). Mitigation of nickel toxicity has been also reported by endophytic bacterial strains in Sesame (Naveed et al., 2020). However, little study has been conducted on bacterial assisted phytoremediation of organometallic contaminants in-situ from industrial waste, which could be a viable green solution for ecorestoration of polluted sites for long-term development. Moreover, the PGPR activity of bacteria in in-situ condition from the organometallic complex is reported by few researchers (Tripathi et al., 2021a; Tripathi et al., 2021b; Sharma et al., 2021; Sharma et al., 2020;). Therefore, the current research focuses on bacterial-assisted phytoremediation of heavy metal by *Cannabis sativa* growing on complex organometallic distillery waste disposal site. The *Cannabis sativa* was found growing luxuriantly on the disposed sugarcane molasses based distillery sludge. Therefore, to understand the mechanism of phytoextraction by *Cannabis sativa* growing on distillery sludge in situ condition, the organic compounds and heavy metals present in sludge were analyzed and monitored at three stages of plant growth. Simultaneously, the potential rhizobacterial species with PGPR activities were isolated, screened and identified along with phytoextraction process. In addition histological observation in tissue of plant for metal accumulation was also investigated (Chandra et al., 2018; Chandra et al., 2017).

5.2.1. Materials and Methods

5.2.1.1 *Study site and Sample collection*

The samples were collected from M/s Unnao Distilleries & Breweries, Unnao, Uttar Pradesh, India, which is located at 26° 32′ 0″ north latitude and 80° 30′ 0″ east longitude. The average annual temperature is 24-26°C, with seasonal variations of 14 to 40°C. This site has been previously reported by various scientist and researchers for production of high pollution mass with inorganic and organic pollutants (Tripathi et al., 2021a; Tripathi et al., 2021b; Chandra et al., 2018; Chandra et al., 2017). The sludge samples were collected at three periodic intervals in 30 days. As a control, a fresh sludge sample without plant growth was collected from the anaerobic digester plant's sludge disposal site, which was located within the premises of industry's. Second sampling was done after 30 days growth of *Cannabis sativa* on the disposed distillery sludge and the third sampling was done collected after 60 days growth of *Cannabis sativa* from the same site for the comparative analysis. Furthermore, the entire *Cannabis sativa* plant uprooted with rhizospheric soil and associated sludge samples, were carried in pre-sterilized polythene bags (HiMedia Laboratories Pvt.Ltd., Karnataka, India) for further analysis of accumulated heavy metal in various parts of growing plants. All the collected samples were transported to the laboratory in an ice-cold (4 °C) condition. (Tripathi et al., 2021a; Tripathi et al., 2021b.

5.2.1.2. Physico-chemical analysis of distillery sludge

Samples of distillery sludge were analysed in accordance with the method previously described by Kalra and Maynard (1991) for the determination of the pH, electrical conductivity, chloride (Cl-), sodium (Na+), and nitrate. While, the phenol content of sludge was determined using standard methods recommended by the American Public Health Association (APHA), (2012). As previously described, the pH and EC values of sludge (sludge: water = 1:2.5 w/v) samples were determined using an Orion meter (Model-960, Thermo Scientific, FL, USA) and an Orion conductivity meter, respectively (Chandra et al., 2008). After acid digestion, the total content of Fe, Zn, Cu, Mn, Ni, and Pb in a dry weight sample of sludge was determined using Inductively Coupled Plasma Mass Spectrometry (ICPMS-MS) (Agilent, 8900 ICP-MS Triple Quad, US) using method 3030H of the standard methods for water and wastewater examination (APHA, 2012).

Fig. 5.1. Sample collection: (a) Disposed sludge with no plant growth (b) growth of *Cannabis sativa* on disposed sludge near methane reactor (c) growth of *Cannabis sativa* at disposed site of contaminated sludge (d) uprooting of *Cannabis sativa* plant

5.2.1.3. Isolation, screening, and growth conditions of culturable rhizosphere bacteria

All the seven bacterial sp. were isolated from the rhizosphere of *Cannabis sativa* after sixty days growth on disposed distillery sludge. The isolation method was done as per general microbiological serial dilution method on nutrient agar (NA) petri dish (Pandey et al., 2019). The petri dish was incubated for 24 hours at 30± 2◦C until bacterial colonies appeared. Every colony was re-streaked for purification of strain (Abbasi et al., 2011).

5.2.1.4. Bioassays for Plant Growth Promoting Traits

5.2.1.4.1. Assay for indoleacetic acid (IAA) production

Bacterial cultures were grown at 28± 2◦C for 72 hours on their respective media. Fully grown cultures were centrifuged for 30 minutes at 3000 rpm. The supernatant was collected in separate test tube and two drops of orthophosphoric acid and 4ml of Salkowski reagent (35% of perchloric acid-50ml and 0.5M FeCl3-1ml solution) were

added. The appearance of a pink colour suggests the presence of IAA production (Linu et al., 2019).

5.2.1.4.2. Assay for NH_3 production

Peptone water was used to test the ammonia production of bacterial isolates. In each test tube, 10ml peptone water was used to inoculate freshly grown cultures, which were then incubated for 48–72 hours at 28± 2◦C. After that, 0.5 mL of Nessler's reagent was added to each test tube. The appearance of a brown to yellow colour was a positive sign of ammonia generation (Nivya, 2015).

5.2.1.4.3. Assay for N_2 production

For screening nitrogen fixing activity of bacterial isolates a glucose nitrogen free mineral medium (GNFMM) was used to screen the nitrogen fixing activity of bacterial isolates. Bromothymol blue indicator (BTB) was added to the medium in order to make it green in colour. The isolated rhizospheric bacterial strains were inoculated in GNFMM + BTB medium and kept in incubator at 28 ± 2◦C for 48–72 hours.

5.2.1.4.4. Assay for siderophore production

The PGPR strains were spotted on prepared chrome azurol S (CAS) agar plates and kept for incubation at 28 ± 2°C for 5 days. The change in colour of the medium from blue to yellow at the spotted area indicated the presence of siderophore formation. (Abo-Zaid et al., 2020).

5.2.1.4.5. Assay for Phosphate solubilization

To assess phosphate solubilization activity, isolated pure rhizospheric bacterial strains were spot-inoculated on Pikovskaya's agar plates with a metal loop and kept in incubator for 3 days at 30 °C. Clearing of zone formation surrounding the spotted colony was noted (Patel and Desai, 2015)

5.2.1.4.6. Assay for organic acid production

Pure rhizospheric bacterial cultures were cultivated for 24 hours at 37 °C in MRVP broth (pH 6.9). 2–3 drops of methyl red indicator were applied to an aliquot, and red colour was instantly detected for positive tests and yellow for negative tests. (Sultana, 2017).

5.2.1.4.7. Screening for ligninolytic activity of rhizospheric bacterial strains

By using a plate assay method, the pure bacterial strains were screened for diverse ligninolytic enzyme activity, such as manganese peroxidase (MnP), lignin peroxidase (Lip), and laccase (Lac) activity. For the screening, MSM with various substrates was utilised. Phenol red (0.1%) was used as a substrate for manganese peroxidase, while Azure B was used as a substrate for lignin peroxidase (0.002%). In contrast, laccase activity was identified in Nutrient Agar (NA) medium that contained CuSO4 (1mM), beef extract (3.5), peptone (5.0), NaCl (5.0), and guaiacol as substrate (in gm/l) (Verma and Ekta, 2015).

5.2.1.5. Rhizospheric bacterial strain identification and phylogenetic analysis

At first stage, isolation of genomic DNA was performed by inoculating isolated bacterial strain in nutrient broth (NB) at 30°C in temperature controlled shaker at 220 rpm for 24 hours. After incubation the bacterial strain was centrifuged at 4000 rpm for 10 minutes at 24°C. The supernatant was discarded, and the cell pellet was used to extract genomic DNA using the Bacterial Genomic DNA Isolation Kit (HiMedia) according to the manufacturer's instructions. The 16s ribosomal RNA genes were targeted using the primers P027F and 1378R, which were used to amplify a sequence of approximately 1500 bp from genomic DNA. One microliter of template DNA, 0.2 microliters of primers UNI-16-GT-F (AGAGTTTGATCCTGGCTCAG) and UNI-16-GT-R (GGTTACCTTGTTACG ACTT), 200 mL of each dNTP, 10X buffer, and 2 mM MgSO4, and one unit of High-Fidelity KOD Taq DNA Polymerase were used in a 25 mL PCR reaction. The following parameters were used during the cycle: Initial denaturation at 94°C for 4 minutes; 30 cycles of 30s denaturation at 94°C, 1 minute annealing at 63°C, and 1 minute extension at 68°C; and a final overall extension of 7 minutes at 68°C. The PCR product was purified using the PCR Purification Kit (Norgen Biotek, Canada), and Biokart India Pvt. Ltd. perform the sequencing. To compare sequences to homologous bacterial 16S ribosomal RNA sequences, NCBI (www.ncbi.nlm.nih.gov/BLAST) was used. CLUSTAL W was used to align the sequences, and the MEGA 11 software using the maximum likelihood (ML) technique was used to create a phylogenetic tree (Chandra and Kumar, 2017).

5.2.1.6. Plant samples digestion for heavy metals estimation

To evaluate the metal content of indigenous plants, they were uprooted and thoroughly cleansed with deionized water to remove sludge particles from the roots, followed by

rinsing with a 10 mmol L^{-1} calcium chloride solution. The plant's root, shoot, and leaves were separated and diced into small pieces, and the resulting biomass was oven-dried for 5 days at 70°C until it reached a consistent weight. In a muffle furnace, the dried plant components were ashed for 6 hours at 460°C.

In addition, to acquire the final product, the weighted ash from these samples was digested in 2% nitric acid (HNO_3) and filtered through a 0.45µm glass fibre filter (AOAC, 2002). Further, one gramme (1.0 g) of dried and sieved sediments was digested in 10 mL of HNO_3. When brown fumes started emitting, 5 ml of HNO_3 was added and the digestion was continued until brown fumes disappeared. The sample was then kept for chilling afterwards 5ml of double distilled water was added to complete the digestion (USEPA 3050-B) (Test methods for evaluating solid waste, 1996). Further, the sample was given for Atomic absorption spectroscopy (AAS) for determining the concentration of Fe, Cr, Zn Cu, Cd, Mn, Ni, and Pb (Kimbrough and Wakakuwa, 1989).

5.2.1.6.1. Metal accumulation efficiency

To analyse *Cannabis sativa's* metal accumulation potential, we calculated the bioaccumulation coefficient factor (BCF) and the translocation factor (TF). BCF is expressed by the ratio of metal accumulated in root to the metal accumulated in sludge while TF is expressed with the ratio accumulated in the shoot to root of plant. To determine a plant is hyperaccumulator or not, both the parameters i.e. BCF and TF have to be measure. A plant is said to be hyperaccumulator when measured value of BCF or TF of plant is greater than one (Malik et al., 2010).

5.2.1.6.2. TEM observations of root tissues histologically.

Young root segments (less than 2.0mm in length) from freshly uprooted *Cannabis sativa* were soaked in H_2S saturated water as a pretreatment and left to stand at room temperature for 30 minutes to precipitate trace components (Pourzarandian et al., 2004). After that, the root sample was dehydrated in 0.1M SCB (sodium cacodylate buffer, pH 7.2) and fixed in 2.5 percent glutaraldehyde (v/v) generated in sodium cacodylate (Ladd Research Industries, Williston, USA) buffer for 3 hours at 4 °C (pH 7.3). The root tissue was then rinsed three times with 0.1M SCB, with a ten-minute interval between each washing, and then post-fixed overnight in 1 percent OsO4 (osmium tetroxide). After rinsing with SCB

and dehydrating in a graded acetone series (50, 60, 70, 80, 90, 95, and 100 percent), the preserved tissue was embedded in an araldite-DDSA combination (Ladd Research Industries, Williston, USA). Using an ultramicrotome, slices were stained with uranyl acetate and lead citrate after backing at 60 °C (Cryo Leice EM UC7, Leica Microsystem, India). The TEM (FEI TecnaiTM G2 Spirit Twin, Hillsboro, USA) analysis of the segment was carried out with an accelerating voltage of 80 KV.

5.2.1.7. Analysis of organic contaminants from distillery sludge

5.2.1.7.1. Reagents and Chemical

The chemicals used in this study were obtained from Sigma-Aldrich i.e. N, O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA), sodium sulphate (Na_2SO_4, 99% pure), and TMCS (trimethylchlorosilane, 99% purity) (Saint Louis, MO, USA). In this study, the organic compounds extracted by using ethyl acetate (C4H8O2) as solvent from distillery sludge was purchased from Merck (Merck KGaA, Darmstadt, Germany). The reagents and chemical used in this study were of the highest grade for GC-MS analysis.

5.2.1.7.2. Detection of organic pollutants from distillery sludge by Solid-liquid extraction method

As previously mentioned by Chandra and Kumar (2017), Ethyl acetate was used to recover organic pollutants from distillery sludge, and it was used to detect a wide spectrum of organic compounds at a slightly alkaline pH. (i.e. 8.10). All three sludge samples (5.0 g) from various stages were weighed and placed in a 250 mL Erlenmeyer flask. 5 mL ethyl acetate was added to each flask individually and vigorously mixed to create a homogenised solution for the solubility of organic components. The samples were also subjected to vortex agitation (for 3 minutes), sonication (3 minutes on, 30 seconds off, three times), and centrifugation for 10 minutes at 5000rpm. To ensure complete extraction from sample the extraction procedure was repeated thrice. Following the process, the organic layer was separated from the precipitated sample and dehydrated by passing it through Na2SO4 before being dried under a stream of nitrogen gas. The dried residue was then diluted in 1.0 mL ethyl acetate and filtered using 0.22 m syringe filters.

5.2.1.7.3. *GC-MS analysis*

GC–MS analysis was executed with TSQ Quantum XLS triple quadrupole mass spectrometer with TriPlus auto sampler (Thermo Scientific, USA). According to Chandra

and Kumar (2017), the extracted samples were derivatised with trimethylsilyl (TMS) to enhance the mobility of all organic compounds before being injected to GC–MS analysis. A 2.0-mL aliquot of the derivatised material was injected into the GC–MS port of the equipment (Thermo Scientific, USA). The sample was separated by passing from DB-5 MS capillary column along with the helium gas at a flow rate of 1.1 mL^{-1}. The GC oven temperature was set to start at 65°C for 2 minutes, and then it was increase at a rate of 6°C min^{-1} to 230°C for 10 minutes, and then increased to 10°C min^{-1} to 290°C for 20 minute. The mass spectrum was obtained using a positive electron ionisation (+ EI) mode of the mass spectrometer with a 70 eV electron energy and a 7-minute solvent delay. The detected organic compounds were identified using the MS library NIST v. 1.0.0.12 that was provided with in the instrument software.

5.2.1.8. Results and discussion

5.2.1.8.1. Changes in distillery sludge's physicochemical properties

The physico-chemical parameters of fresh and 60 days disposed distillery sludge are given in Table 1. The measured pH value, Electrical conductivity (EC), sodium, phosphate, chloride, nitrate, phenol sulfate showed higher in disposed fresh distillery sludge. Additionally, value of trace elements i.e. Fe, Zn, Pb, Cd, Mn, Ni, and Cu are found higher than permissible limit and toxic to animal and human health because of their long term persistence in the environment (Onakpa et al., 2018; Rehman et al., 2018).

Sludge has an alkaline pH of 8.1, which results from reactivity of numerous salts and oxides i.e. carbonates, bicarbonates, sulphides, and hydroxides. They are mostly applied in the process of fermentation to modify the pH level (Chandra et al., 2018). The Value of EC has also been detected high due to various anions and cations present in sludge such as Na^{+}, Cl^{-}, NO^{-3}, PO_4^{3-} and SO_4^{2-}

Heavy metals have been found in large quantities in sludge as a result of the corrosive effects of sugarcane molasses and juice production. Further steps i.e. fermentation and distillation of sugarcane juices at last generates spentwash as waste. These processes could be the primary source of heavy metal in disposed distilleries sludge.

However, after 60 days of in-situ phytoremediation by *Cannabis sativa*, the sludge had reduced values of numerous physio-chemical parameters, as shown in Table 1. The original pH of distillery sludge was 8.1 (alkaline in nature), however it changed to 6.31 (slightly acidic). This could be due to rhizobacterial interaction with various heavy metal and complex organic components. Moreover, metabolites released from roots of *Cannabis sativa* such as amino acids, organic acids and numerous metal chelators, also reduces the pH of sludge.

These activity enhanced phytoextraction mechanism due to bioavailability of organic nutrients, ions and various metals from slightly acidic sludge. The EC of phytoremediated sludge after in-situ treatment was also found to be higher; this might be due to breakdown of organic pollutants by rhizobacterial activity and chelation of heavy metals which consequently increases the role of cations and anions in sludge. Except for EC, all physico-chemical parameters of in-situ phytoremediated sludge by *Cannabis sativa* after 60 days of growth were significantly greater ($p < 0.001$) than those of newly dumped distillery sludge.

Table 5.1. Physico-chemical properties of disposed distillery sludge before and after 60 days of In-situ of phytoremediation by *Cannabis sativa*

S.No.	Parameter	Fresh distillery sludge	Distillery sludge after In-situ phytoremediation (60days)	% Change
1.	pH	8.1 ± 0.00	6.31 ± 0.13	22.09
2.	EC (μS cm^{-1})	4.07 ± 0.11	5.71 ± 0.06	-40.29
3.	Sodium (mg kg^{-1})	43.21 ± 1.00	27.16 ± 0.23	37.14
4.	Chloride (mg kg^{-1})	1283.24 ± 3.12	716.12 ± 26.19	44.19
5.	Nitrate (mg kg^{-1})	86.19 ± 1.16	39.19 ± 1.02	54.53
6.	Phosphate (mg kg^{-1})	2213.61 ± 0.10	1439.53 ± 4.15	34.96
7.	Sulfate (mg kg^{-1})	149.03 ± 0.13	111.68 ± 0.16	25.06
8.	Phenol (mg kg^{-1})	499.13 ± 1.01	419.26 ± 0.05	16.00
	Trace Elements (mg kg^{-1})			

a.	Fe	2253.01 ± 0.16	257.65 ± 0.08	88.56
b.	Zn	41.17 ± 1.01	25.11 ± 0.30	39.00
c.	Cu	835.46 ± 0.01	201.15 ± 0.07	75.92
d.	Mn	243.17 ± 0.03	71.01 ± 0.16	70.79
e.	Ni	14.10 ± 0.04	3.01 ± 0.11	78.65
f.	Pb	32.12 ± 0.04	5.15 ± 0.16	83.96
g.	Cd	0.510 ± 0.71	1.31 ± 0.21	-156.86
h.	Cr	02.15 ± 1.07	0.91 ± 0.14	57.67

5.2.1.8.2. Isolation of PGP traits and its primary screening

The morphological and physiological properties of all identified rhizobacterial strains were examined. Isolated bacteria were further examined for PGPR parameters along with the ligninolytic enzymatic activity as shown in Table 5.1.

5.2.1.8.3. Indoleacetic acid (IAA) production

Among the seven isolates i.e. KS19, KS20, KS21, KS22, KS23, KS24, and KS25, all the isolates showed IAA production. Pink coloration was detected in their cell-free supernatant with inclusion of tryptophan in culture broth; maximum IAA production was recorded in strain KS20, KS22, KS23 and KS24 (17 µg ml−1) broth culture (Table 5.1.).

5.2.1.8.4. NH_3 production

All the isolates showed NH_3 production. The transition of brown to yellow colour was a positive indicator of ammonia generation. Maximum production of NH_3 was recorded in strain KS20 and KS24 (17 µg ml−1) broth culture (Table 5.1.).

5.2.1.8.5. N_2 production

Rhizospheric bacterial isolated strains were screened by changing the color of bromothymol blue (BTB) containing nitrogen free media, and these strains showed a color change in BTB containing media, indicating excretion of ammonia. Only strain KS20, KS22, KS23 and KS24 showed positive test for N_2 production (Table 5.1.).

5.2.1.8.6. Siderophore production

Among the seven strains only strain KS20, KS22, KS23 and KS24 showed the appearance of orange halos around their spots on the CAS agar medium indicating a positive test for siderophore production (Table 5.1.).

5.2.1.8.7. Phosphate solubilization

Among the seven strains only KS20, KS22, KS23, KS24 and KS25 showed significantly clearing of zone around the colonies (Table 5.1.). At 3 days of incubation, the Phosphate Solubilization Index (PSI) of phosphate solubilizing bacterial strains was measured by the following equation:

$$PSI = \frac{\text{Colony Diameter + Total diameter of halo zone}}{\text{Colony diameter}}.$$

The sequence of PSI was as follows:

KS23, (3.2 ± 0.1) > KS24 (2.7 ± 0.1) > KS25 (2.6 ± 0.1) > KS20 (2.0 ± 0.2)> KS22 (1.8 ± 0.3)

5.2.1.8.8. Assay for organic acid production

All bacterial isolates showed positive test for organic acid production. On adding 2 to 3 drops of methyl red indicator to all seven bacterial strains, only KS20, KS22, KS23 and KS24 showed red colour formation for positive test and KS19, KS21 and KS25 showed negative test for organic acid production (Table 5.1.).

5.1.8.8.9 .ligninolytic enzyme activity of rhizospheric bacterial strains

Four of the seven bacterial strains could produce ligninolytic enzymes. Using the plate assay method, strains were tested for Manganese peroxidase, Lignin peroxidase, and Laccase activity. For screening, MSM with various substrates was used. The presence of MnP activity was shown by the conversion of dark pink to yellow colour, the absence of blue colour indicated the presence of positive LiP, and the presence of brown colour halos indicated the positive test laccase activity (Table 5.1.).

Table 5.2.: Detection of plant growth promoting parameter of rhizospheric bacteria isolated from *Cannabis sativa* rhizosphere

ISOLATES	**IAA**	**NH_3**	**N_2 Fixation**	**Siderophore production**	**Phosphate Solubilization**	**Organic acid Production**	**Ligninolytic Enzyme**		
							Laccase	**MnP**	**LiP**
KS19	+	++	-	-	-	-	+	+	+
KS20	+++	+++	+	+	+	+	++	++	+
KS21	+	++	-	-	-	-	-	-	+
KS22	+	++	+	+	+	+	+	+	+
KS23	++	++	+	++	+++	++	+	++	+
KS24	+++	+++	+	+	++	++	+	+++	+
KS25	+	+	-	-	++	+	-	++	-

5.1.8.9. 16S rRNA gene analysis of rhizosphere bacteria and phylogenetic analysis

Purified isolated rhizosphere bacterial strains were identified on the basis of 16S rRNA sequencing. Among the seven rhizospheric bacterial strains i.e. KS19, KS20, KS21, KS22, KS23, KS24, and KS25, only four bacterial strains i.e. KS20, KS22, KS23 and KS24 could show most of the positive PGPR test along with ligninolytic enzymatic activity. 16S rRNA sequencing data, the isolated strains KS20, KS22, KS23 and, KS24 showed nearest relationships with *Bacillus cereus, Achromobacter denitrificans*, *Bacillus subtilis* and *Bacillus thuringiensis*. The nucleotide sequences of these potentially diverse rhizospheric bacteria were deposited in the National Center for Biotechnology Information (NCBI) database under accession numbers MW886231, MW886333, MW887524, and MW887525 respectively. Using the MEGA 11 software, a phylogenetic tree was generated that illustrated the relationships between bacterial species and the most closely related genera deduced from sequences of the 16S rRNA gene. (Fig.5) (Kumar and Chandra, 2018).

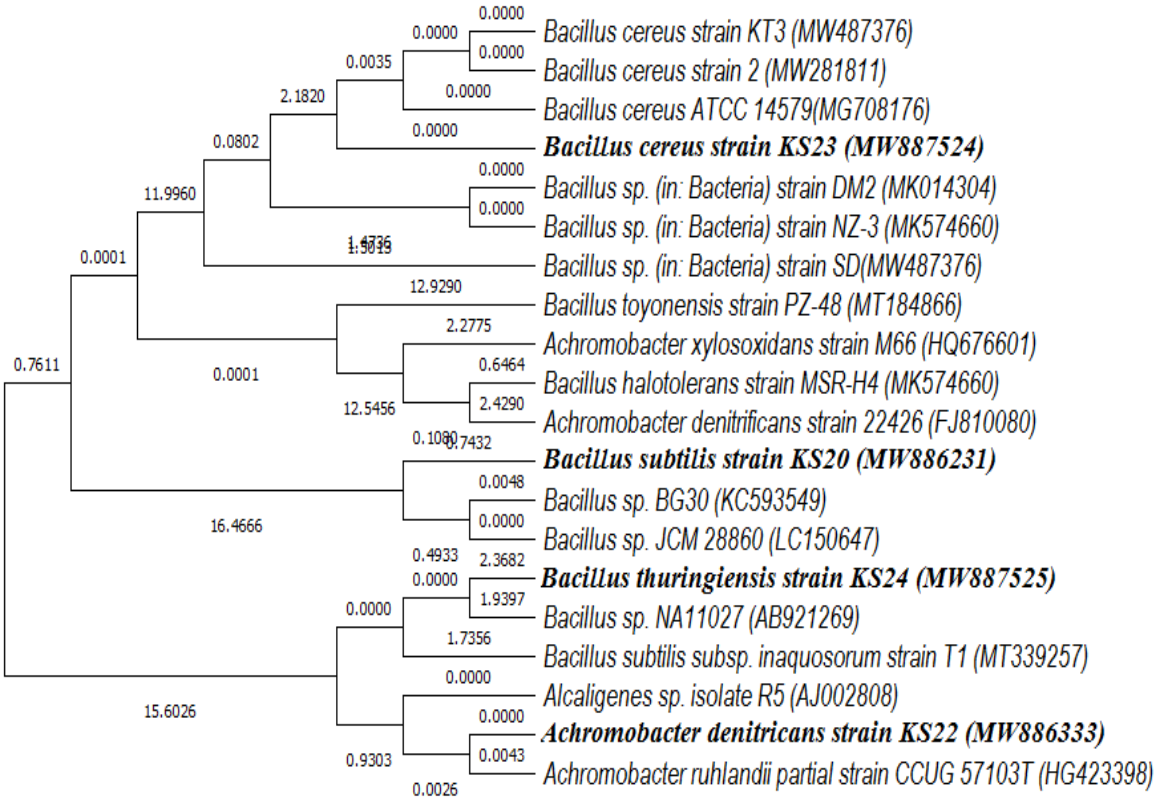


Fig. 5.3. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura K. and Nei; 1993). The tree with the highest log likelihood (-26859.98) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. This analysis involved 20 nucleotide sequences. There were a total of 1557 positions in the final dataset. Evolutionary analyses were conducted in MEGA XI

5.1.9.0. Accumulation of Heavy metals in Cannabis sativa

The distribution and accumulation of metals in plant components must be investigated in order to establish the role of plants in the rehabilitation of contaminated ecosystems (Baker et al., 2020).*Cannabis sativa* have the ability to accumulate various metals in a diverse pattern into their tissue (Fig.5.1). The highest accumulation of metal has been found in root and then in shoot followed by root tissue (Table 5.1.). Overall, the accumulation pattern of metal was noted as:

Fe (801.81 ± 0.123)> Cu (275.086 ± 0.069)> Zn (162.15 ± 0.085)> Mn (63.92 ± 0.093)> Pb (28.619± 0.192)> Ni (5.02 ± 0.078)> Cd (2.53± 0.085)> Cr (1.87 ± 0.079) mg kg^{-1}(Table 3).The heavy metals enter from the curricular cracks in root tissue and via diffusion of cell sap it accumulates in various parts of the plant. In accumulation pattern of

heavy metal, pH of soil and chemical nature of other organic co-pollutants plays a vital role in bioavailability of metals. Root exudates have been demonstrated to alter the pH of sludge, allowing heavy metals to move more freely in the soil. In addition, bacterial-plant interactions in the rhizosphere promote metal bioavailability in roots. (Chandrakala et al., 2019). Moreover, other factors, such as metal redox states, plant genotype, and metal heterogeneity, plant root system, and environmental conditions, all influence the metal accumulation pattern in plants. (Shahid et al., 2017).

Our findings showed that distillery sludge produced by methanogenesis of spent wash was initially alkaline in nature, including a variety of organometallic compounds. This complex organic waste has a high affinity for numerous trace elements (Migo et al., 1997). However, as a result of certain plant-microbe interactions, the organometallic bond breaks down, allowing metal to become available to the plant (Zhang et al., 2020). The accumulation of measured metals in *Cannabis sativa* root tissues was found to be higher in this study. This demonstrated that metal mobility in plant tissues is concentration dependent. The findings imply that a plant's metal tolerating strategy has evolved and is present in *Cannabis sativa* when it grows in metal-contaminated environments. Similar findings involving heavy metal accumulation have been observed in previous studies also. (Pavlenko-Badnaoui et al., 2019; Yabanli et al., 2014). *Cannabis sativa*, the tested plant, accumulated heavy metal concentrations that were higher than normal or phytotoxic levels, indicating that the plant has a hyperaccumulation propensity and the potential for use in phytoremediation of industrially contaminated areas.

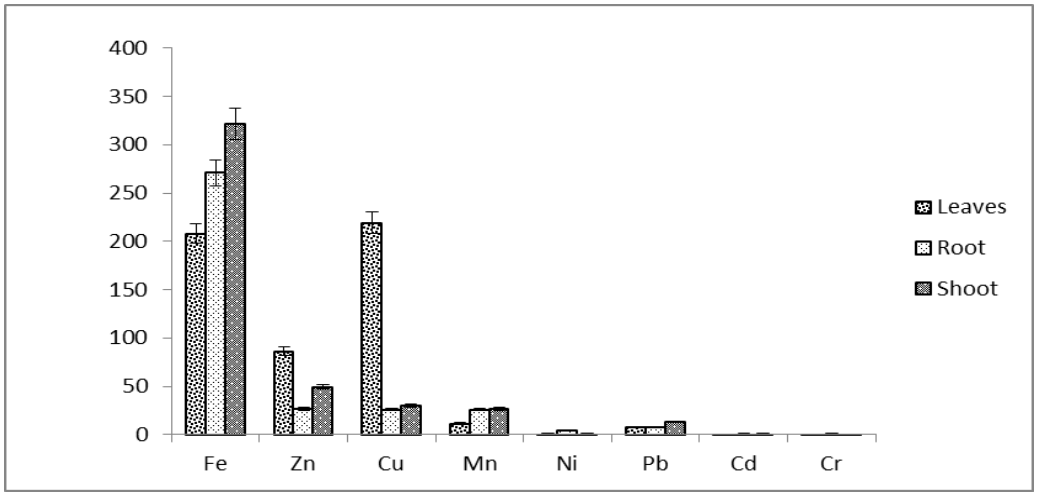


Fig.5.4. Accumulation of Fe, Zn, Cu, Mn, Ni, Pb, Cd and Cr in different parts of *Cannabis sativa* collected after 60 days from distillery sludge dumping site.

Table 5.3.: Heavy metal accumulation (mg kg^{-1} DW) in root, shoot and leaves of various plant species.

Plant	Plant parts	Fe	Zn	Cu	Mn	Ni	Pb	Cd	Cr
Cannabis sativa	Leaves	208.015 ± 0.075	86.016 ± 0.041	26.001 ± 0.041	11.69 ± 0.057	0.930 ± 0.003	8.250 ± 0.064	0.640 ± 0.041	0.490 ± 0.074
	Shoot	322.002 ± 0.024	49.315 ± 0.042	30.017 ± 0.004	26.54 ± 0.002	1.266 ± 0.071	13.054 ± 0.074	0.980 ± 0.010	0.510 ± 0.004
	Root	271.064 ± 0.024	26.819 ± 0.002	219.068 ± 0.024	25.69 ± 0.034	4.090 ± 0.004	7.315± 0.054	0.910 ± 0.034	0.870 ± 0.001
	Total	801.81 ± 0.123	162.15 ± 0.085	275.086 ± 0.069	63.92 ± 0.093	5.02 ± 0.078	28.619± 0.192	2.53± 0.085	1.87 ± 0.079
Accumulation pattern		S > R > L	L > S > R	R > S > L	S > R > L	R > S > L	S > L > R	S > R > L	R > S > L
	BCF	1.05	1.06	1.08	0.36	1.35	1.42	0.69	0.95
	TF	1.18	1.83	0.13	1.03	0.30	1.78	1.07	0.58

All the values are mean of three replicates (n=3)±standard deviation (SD), BDL: Below detection limit, R: Root, S: Shoot, L: Leaves

5.1.9.1. Translocation factor and Bioaccumulation coefficient factor

Cannabis sativa's strength to tolerate and accumulate heavy metals, which could be used as a technique for phytoextraction of metal-mixed endocrine disrupting chemicals (EDCs) polluted sites. According to previous studies, the BCF and TF values were utilized to evaluate the phytoextraction and phytostabilization capability of plant species. (Tauqeer et al., 2019; Ameh at al., 2019). The BCF refers to the most important plant feature in phytoremediation which is how plants take in metals, move them into their tissues, and store them in the aerial plant biomass (Ramana et al., 2021; Suthar et al., 2014). The result of BCF for each heavy metal in plant sample is shown in Table 5.1. In the study, it was discovered that the *Cannabis sativa* had high BCF values (> 1) for lead (Pb), nickel (Ni), copper (Zn), and iron (Fe), and low BCF values (<1) for cadmium (Cd) and manganese (Mn) as shown in Table 4. *Cannabis sativa* has a high BCF>1, indicating that it has the capacity to phytoextract Pb, Ni, Cu, Zn, and Fe from polluted sites. While, low BCF values (<1) for Cd and Mn, on the other hand, indicated that plants had difficulty in mobilising these metals in the root zone (Table 5.1.).

The order of BCF values for various metals were as Pb (1.42) > Ni (1.35) > Cu (1.08) > Zn (1.06) > Fe (1.05) > Cr (0.95) Cd > (0.69) > Mn (0.36) (Table 4). In various plants, the process of metal uptake and accumulation from soil to root is influenced by the concentration

accessible metals in soils, solubility sequences, evapo-transpiration rate, hydropotential, and photosynthetic activity of the plant (Hassan et al., 2019). TF, the ratio of shoot to root metals, indicates internal metal transportation from root to shoot. For different plant species, the TF of Fe, Zn, Cu, Mn, Ni, Pb, Cd and Cr were variable as shown in Table 4. The order of TF values for various metals were as Zn (1.83) > Pb (1.78) > Fe (1.18)> Cd (1.07) > Mn (1.03) Cr > (0.58) > Ni (0.30) Cu > (0.13). The maximum TF was detected for Zn (1.83) and the minimum was for Cu 0.13 (Table 4). The high TF values of certain metals may be due to high bioavailability of heavy metals, hydropotential, evapo-transpiration rate, and photosynthetic activity of plants, and increased biomass production to fast growth. According to Kumar et al (2021) these plants have important properties that can be utilized in phytoextraction of certain metals.

The high metal accumulation could be attributed to a well-developed detoxification mechanism based on the sequestration of heavy metal ions in vacuoles by binding them to appropriate ligands such as organic acids, proteins, and peptides in the presence of enzymes that are capable of functioning under high levels of metallicilous environment (Ghori et al., 2019).

Plant also showed phytostabilization of contaminated sites i.e. BCF with > 1 and TF < 1 for metal Cu and Ni (Table 5.1). Phytostabilisation is a plant-based method in which plant species are used to limit the mobility and bioavailability of heavy metals in the environment by absorbing them into the soil. Heavy metals can be immobilised by plants by adsorption on the surface of the roots, precipitation, and accumulating inside the roots or within the rhizosphere soil. By reducing metal mobility and leaching into ground water, as well as metal bioavailability and entry into the food chain, this technique helps to protect the environment (Ashraf et al., 2019).

5.1.9.2. Microscopic cellular observation in root of potential native plants after phytoextraction

The impact of heavy metals on cellular organisation is crucial to comprehending the morphological and physiological changes induced by heavy metals due to structural and functional complementarity (Khan et al., 2015). The TEM micrography of Cannabis sativa root revealed production of multi-vacuoles, multi-nucleolus, thickening of middle lamella,

round shaped multi-mitochondria, and modified nuclear structure with metal deposition in cell wall and cytoplasm as shown in Fig.5.2 (d-f). As a result of the high concentration of heavy metals in their cellular compartment and the greater amount of detoxification occurring in their cellular tissues, this indicated protein synthesis for their tolerance mechanism (Viehweger et al., 2014).

The additional development of nucleolus and vacuoles in the presence of heavy metals increases the production of ribosomes and mRNA, which in turn increases the production of novel proteins involved in heavy metal tolerance in plants (Kumar and Chandra, 2019). The increased number of mitochondria formation indicated the generation of more energy in the form of ATP inside the cell, which is required to combat heavy metal toxicity (Jin et al.,2008 and Alkhatib et al., 2013). Furthermore, the apparent deposition of metals in the form of electron dense granules in vacuoles, cell cytoplasm, and cell wall revealed the plant's predisposition for heavy metal hyperaccumulation. Depositions of heavy metals in the cell wall periphery of *Cannabis sativa* were also detected as shown in Fig 5.2(f). Heavy metal depositions along the cell wall play an important function in heavy metal tolerance by preventing free metal ions from circulating in the cytosol as shown in Fig 5.2 (f). The conclusion of these findings is supported by our previous findings (Chandra et al., 2018), which reported the formation of multi-vacuoles and depositions of metal granules in cell wall and cytoplasm in the root of *Cynodon dactylon* (Bermuda grass), *Saccharum munja* (munja), *Argemone Mexicana* (mexican poppy), *Pennisetum purpureum* (elephant grass), *Chenopodium* album (goosefoots) etc. growing under heavy metal stress as an adaptive strategy when there is a high concentration of metals in the environment and the presence of organic contaminants

Besides, the SEM image of root hairs of *Cannabis sativa* also revealed the presence of bacterial cell attached to the root surface which supported the role of rhizospheric bacteria for PGPR activities (Fig. 5.2 a-c). The isolated potential rhizospheric bacteria in current study were identified as *Bacillus cereus, Achromobacter denitrificans, Bacillus subtilis* and *Bacillus thuringiensis*. The spore forming features of these strains provides additional capability to survive in organometallic polluted environment. However, the other microbiome has been reported for survival in complex organometallic environment sludge (Kumar et al., 2021) which might be working as functional signature in the specific environment for growth of *Bacillus* species detected in our findings.

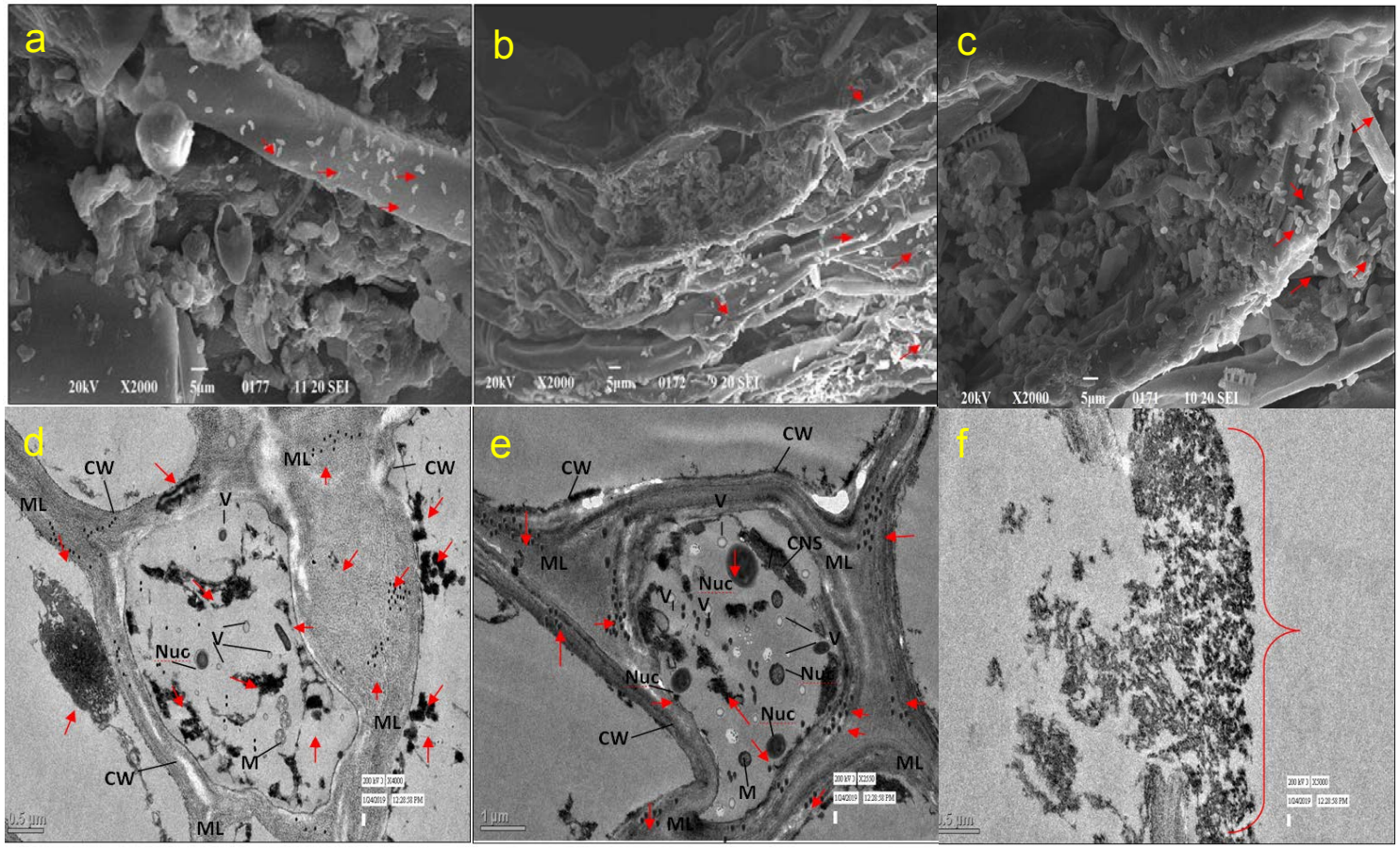


Fig.5.5.. (a-c) Scanning Electron Microscopy (SEM) images of rhizosphere of *Cannabis sativa.* Red arrow showing root adherence of rhizospheric bacteria on root surface of *Cannabis sativa*. (d-f) Transmission Electron Microscopy (TEM) images. Red arrow and marking showing the metal accumulation to root tissue of *Cannabis sativa.*V: Vacuole; CW: Cell wall; CM: Cell membrane; Nuc: Nucleus; ML: Middle lamella; N: Nucleus; Arrow (→) indicated metals deposition.

5.1.9.3. Characterization of organic pollutants

The GC-MS analysis of an ethylene acetate extracted sample from disposed distillery sludge revealed numerous significant peaks at 7.94, 10.57, 13.00, 18.57, 21.59, 26.16, 27.88, 29.70, 33.12, 37.56, 38.71, 41.03, 41.77, 44.33 and 50.26 as shown in Fig. 5.2 a. This indicated the presence of various complicated organic pollutants which are soluble in ethylene acetate. These compounds were identified by comparing the National Institute of Standards and Technology (NIST) library. These were identified as Diacetone alcohol, Pentasiloxane, Erythritol, 2,6-Bis(tert-butyl)phenol, 3-Chloropropionic acid, 2-Pyrrolidinone, Cyclic octaatomic sulphur, Cyclohexane, 1,3,5-Benzetriol, Lupan-3-ol, acetate, 2,4-Dihydroxybenzoic acid, 1-Octacosanol, 24-Ethyl-ë(22)-coprostenol, á-Sitosterol, Tris(2,4-di-tert-butylphenyl) phosphate (Table 5.2). Further, the organic compounds were also identified by GC-MS from same site of distillery sludge after 30 days growth of *Cannabis sativa*. The identified compounds were as Thiazolo[4,5-f]quinoline, 2,7,9-trimethyl-, 2-Methyl-1-methyl (tetramethylene)silyloxypropane, Tartronic acid, Heptanoic acid, 4-Heptanol, Phthalic acid, Silane, 1-Triethylsilyloxyheptadecane, Cannabinol, á-Sitosterol, Lanostan-18-oic acid, Quercetin, 11-hexavinylcyclo hexasiloxane, and 1,3,5,7,9,11,13-Heptaethylcycloheptasiloxane (Table 5), with altered RT and reduced peak (Fig. 5.2 b). This indicated the biotransformation and removal of several organic compounds due to phytoremediation of parent compound. The disappearance some compound revealed the degradation by rhizospheric microbial activities which facilitated for plant growth and accumulation of these pollutants as nutrient. While, most of the detected peaks at various RT were disappeared in the distillery sludge sample chromatogram of same site after 60 days growth of *Cannabis sativa* (Fig. 5.2 c). This result showed direct correlation of plant growth by using these organic compounds as nutrient (Sophia, and Shetty; 2020).

Subsequently, the analysis of PGPR activities by isolated bacterial strain from rhizospheric zone of Cannabis sativa revealed the production of IAA, ammonia, siderophores, organic acid, nitrogen fixing activity, phosphate solubilization with ligninolytic activity (Dixit et al.,2021). This confirmed that the rhizospheric bacterial population present in sludge played vital role for mineralization of complex organic compound for bioavailability to plant as nutrient. The role of rhizospheric bacterial population for the PGPR activity has been previously reported in the agricultural crop

(Vejan et al., 2016). Knowledge for role of rhizospheric bacteria present in complex organometallic for ecorestoration is very limited.

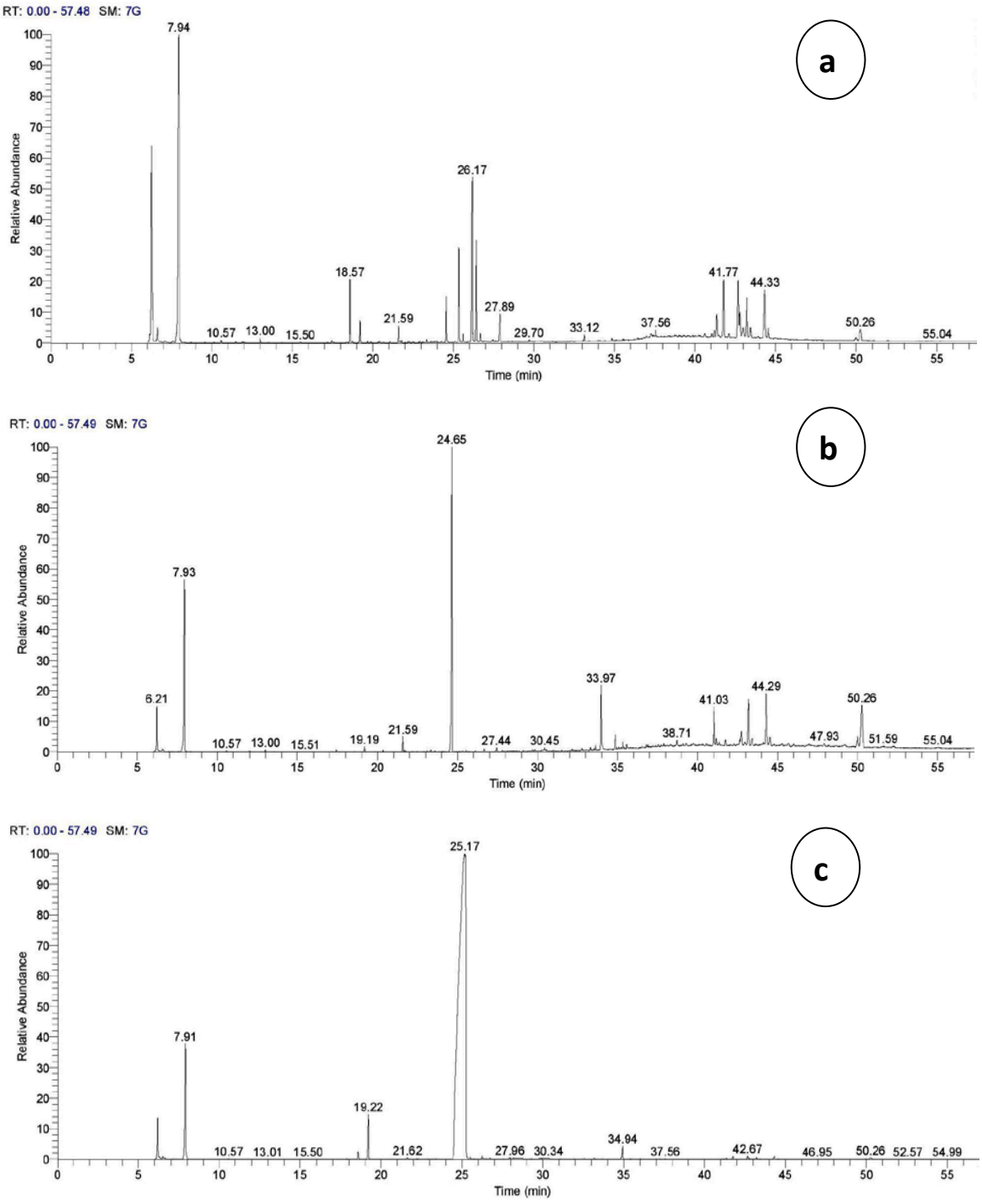


Fig. 5.6. Chromatogram of organic compounds extracted from distillery sludge (a) Initial stage, sludge without plant growth (b) Intermediate stage, sludge with 30 days plant growth (c) Final stage, sludge with 60 days plant growth

Table 5.4. : Detection of comparative list of various organic compounds by GC-MS extracted from distillery waste at various stages (a) Initial stage, sludge without plant growth (b) Intermediate stage, sludge with 30 days plant growth (c) Final stage, sludge with 60 days plant growth

S.No.	Retention Time (RT)	Initial (Fresh)	Intermediate	Final (Degraded)
1.	6.21	-	Thiazolo[4,5-f]quinoline, 2,7,9-trimethyl-	-
2.	7.90	-	-	Hexanoic acid
3.	7.93	-	2-Methyl-1-methyl (tetramethylene)silyloxypropane	-
4.	7.94	Diacetone alcohol	-	-
5.	10.57	Pentasiloxane	-	Pentasiloxane
6.	13.00	Erythritol	Tartronic acid	-
7.	13.01	-	-	Erythritol
8.	18.57	2,6-Bis(tert-butyl)phenol	-	-
9.	19.19	-	Heptanoic acid	-
10.	19.22	-	-	Undecanoic acid
11.	21.59	3-Chloropropionic acid,	4-Heptanol	-
12.	21.61	-	-	Dodecyl acrylate
13.	24.64	-	Phthalic acid,	-
14.	25.17	-	-	2-Fluoro-6-(4-thiomorpholinyl)benzonitrile
15.	26.16	2-Pyrrolidinone	-	-
16.	27.44	-	Silane	-
17.	27.88	Cyclic octaatomic sulfur	-	-
18.	27.96	-	-	Spiro(androstan-17,5'-furan-2'-one)
19.	29.70	Cyclohexane		-
20.	30.34	-	-	Bifenox
21.	30.45	-	1-Triethylsilyloxyheptadecane	-
22.	33.12	1,3,5-Benzetriol	-	-
23.	33.97	-	Cannabinol	-
24.	34.93	-	-	Ethanone
25.	37.56	Lupan-3-ol, acetate	-	4-Normethyl-9
26.	38.71	2,4-Dihydroxybenzoic acid	-	-
27.	41.03	1-Octacosanol	-	-
28.	41.77	24-Ethyl-ë(22)-coprostenol	-	-
29.	42.67	-	-	4'-(Salicylideneamino)acetanilide
30.	44.29	-	á-Sitosterol	-
31.	44.33	á-Sitosterol	-	-
32.	46.96	-	-	5H-Indeno[1,2-b]pyrazin-5-one
33.	47.93	-	Lanostan-18-oic acid	-
34.	50.26	Tris(2,4-di-tert-butylphenyl) phosphate	-	-
35.	50.26	-	Quercetin	-
36.	51.59	-	11-hexavinylcyclo hexasiloxane	-
37.	55.03	-	1,3,5,7,9,11,13-Heptaethylcycloheptasiloxane	-
38.	50.26	-	-	Quercetin

5.1.9.4. Conclusion

The physiochemical analysis of distillery sludge by ICP and GC-MS revealed for containing mixture of organometallic compounds at 8.5pH with high BOD COD value. Several compound at 8.5Ph with high BOD and COD value. Several compounds have been reported under list of androgenic chemical, this indicated mutagenic properties of pollutants. However, the growth of *Cannabis sativa* on sludge showed its potentiality for phytoremediation of these organic pollutants. Besides, the accumulation of metal granules tissue also revealed high phytoextraction properties of *Cannabis sativa* from complex organometallic waste. The BCF and TF data has been found > 1 of Fe, Zn, Cu, Mn, Ni, Pb, Cd, and Cr. This supported for hyperaccumulation activity of plant. The SEM analysis of root hairs had given a strong evidence for the growth of rhizospheric bacteria. The isolated bacterial population were identified as *Bacillus cereus Achromobacter denitrificans*, *Bacillus subtilis* and *Bacillus thuringiensis* with high PGPR attributes. The study concluded bacterial assisted phytoextraction of heavy metals from complex organometallic distillery sludge by *Cannabis sativa* for phytoremediation which is a green technology for ecorestoration of polluted site.

5.3. Evaluation of phytoremediation potential by rhizospheric bacteria of *Parthenium hysterophorus* growing on disposed distillery sludge for ecorestoration of polluted site

Various industry discharges complex organometallic waste containing hazardous compound (Wen et al., 2022). Presence of heavy metal with hazardous organic compounds aggravate their toxicity to ecosystem, therefore its degradability become a challenge because the complex organo-metallic waste imposes toxicity to the soil microbial community also which play a key role for biodegradability and bioconversion of the toxic pollutant (Kaur, 2021).

Distillery units in India are in a considerable number, where molasses and impure alcohol are still being used as raw materials for production of liquor (Mishra et al., 2019). The wastewater or spillage products from such distilleries contain huge quantity of dissolved organic matter, heavy metals, dyes etc., along with other pollutants (Tripathi et al., 2022). Currently, India is the third-largest producer of alcohol with the production of ~3.25 billion liters per annum from about 397 distilleries located in the tropical and subtropical regions of the country (Kumar and Chandra, 2020). Alcohol distilleries produce a huge amount of dark-brown sludge solid as a by-product of biomethanation of raw effluent (Kumar and Sharma, 2019). Besides, distillery sludge also comprises toxic organic acids, androgenic–mutagenic, and EDCs compounds (Tripathi et al., 2022). It is characterized by acidic pH and high content of phosphate, chloride, sodium, phenols, sulfate, organic matter, and heavy metals which enhances towards its toxicity into the environment (Chandra et al., 2018).

The disposal of toxic distillery sludge in the natural ecosystem is exposing the health risks to humans, plants, and animals through food chain contamination (Kumar et al., 2021). As per the Central Pollution Control Board, Ministry of Environment & Forests (MoEF), Government of India, alcohol distilleries are listed at the top of “Red Category” industries having a high polluting potential (Dhote et al., 2021).

Rhizosphere, as an important interface of soil and plant, plays a significant role in phytoremediation of contaminated soil by heavy metals, in which, microbial populations are known to affect heavy metal mobility and availability to the plant through release of chelating agents, acidification, phosphate solubilization and redox changes, and therefore, have potential to enhance phytoremediation processes (Seshadri et al., 2015). Obviously,

the rhizosphere contains a large microbial population with high metabolic activity compared to bulk soil (Singh et al., 2022). Especially, some plant growth-promoting bacteria associated with plant roots also may exert some beneficial effects on plant growth and nutrition through a number of mechanisms such as N_2 fixation, production of phytohormones and siderophores, and transformation of nutrient elements when they are either applied to seeds or incorporated into the soil (Etesami, H. and Maheshwari, 2018). The use of rhizobacteria in combination with plants is expected to provide high efficiency for phytoremediation (Etesami, H. and Maheshwari, 2018). Therefore, the potential and the exact mechanism of rhizobacteria to enhance phytoremediation of soil heavy metals pollution have recently received some attention (Khan and Bano, 2018).

The functioning of associative plant-bacterial symbioses in heavy-metal-polluted soil can be affected from the side of both the micropartner (plant-associated bacteria) and the host plant (Ma et al., 2016). Soil microbes play significant roles in recycling of plant nutrients, maintenance of soil structure, detoxification of noxious chemicals, and control of plant pests and plant growth. Thus, bacteria can augment the remediation capacity of plants or reduce the phytotoxicity of the contaminated soil (Yan et al., 2020). In addition, plants and bacteria can form specific associations in which the plant provides the bacteria with a specific carbon source that induces the bacteria to reduce the phytotoxicity of the contaminated soil (Sharaff et al., 2020). Alternatively, plants and bacteria can form nonspecific associations in which normal plant processes stimulate the microbial community, which in the course of normal metabolic activity degrades contaminants in soil (Mandal et al., 2016). Plants roots can provide root exudate, as well as increase ion solubility. These biochemical mechanisms increase the remediation activity of bacteria associated with plant roots (Sharma, 2021). To sum up, the adaptation capabilities of both partners of the associative symbiosis as well as the bioremediation potential of the microsymbiont are of importance in minimizing the detrimental effect of heavy-metal pollution (Lebrazi and Fikri-Benbrahim, 2018). As mentioned above, rhziobacteria secretion may play a major role among mechanisms of phytoremediation assisted by rhziobacteria (Singh et el., 2022).

The luxuriant growth of several indigenous plants on the contaminated environment indicated the phytoextraction potential of various heavy metals and remediation of organic co-pollutants (Tripathi et al., 2021).

The Santa-Maria, *Parthenium hysterophorus*, has been reported as a promising, and fast-growing, grows well in different climates and is attracted worldwide attention due to its ability to grow spontaneously in the polluted site together with its ability to accumulate heavy metals and has been recognized to be an invaluable resource for phytoremediation purposes.

Few studies have been reported for specific metal accumulation by *P. hysterophorus* in agricultural land and contaminated sites. Samreen and Khan (2017) reported *P. hysterophorus* as an hyperaccumulator weed for chromium and nickel in their pot experiment. Fazal and Asghari (2009) findings illustrated the utilization of *P. hysterophorus* for the remediation of lead-contaminated soil. Moreover, Madhulika et al.(2020) reported the plant as a boon for agricultural land under heavy metal contamination. But, the in-situ phytoremediation potential of polluted sites by *P. hysterophorus* is not yet reported. Thus, the present study aims to investigate the in-situ phytoremediation potential of *P. hysterophorus* growing on disposed distillery sludge contaminated with heavy metals and co-organic pollutants in comparison to freshly disposed distillery sludge. Simultaneously, the potential rhizobacteria were screened for its Plant growth promoting (PGP) attributes and identified along with phytoaccumulation process of *P. hysterophorus*. In addition histological observation in tissue of plant for metal accumulation was also examined (Chandra et al., 2018; Chandra et al., 2017)

5.3.1. Materials and Methods

5.3.1.1. *Selection of sampling site and sample collection*

The sample for experimental data was collected from M/s Unnao Distilleries & Breweries Limited, located in Unnao, Uttar Pradesh, India. The site is located in the outer region industrial area of Unnao (25.45◦N, 81.84◦E) (Fig. 1). The site has been selected due to luxuriant growth of

P. hysterophorus on disposed toxic organometallic distillery sludge (Chandra et al., 2018; Tripathi et al., 2021).

For the evaluation of phytoremediation potential of *P. hysterophorus* the sludge sample was collected at two periodic intervals. The fresh discharge from Post methanated distillery sludge was collected initially as a control. After 50 days of second visit it was found that *P. hysterophorus* was growing luxuriantly on the initially disposed sludge. All

the samples of both intervals were collected in presterilized polyethylene bags and container. Samples were stored at normal temperature and transported to the laboratory for further analysis.

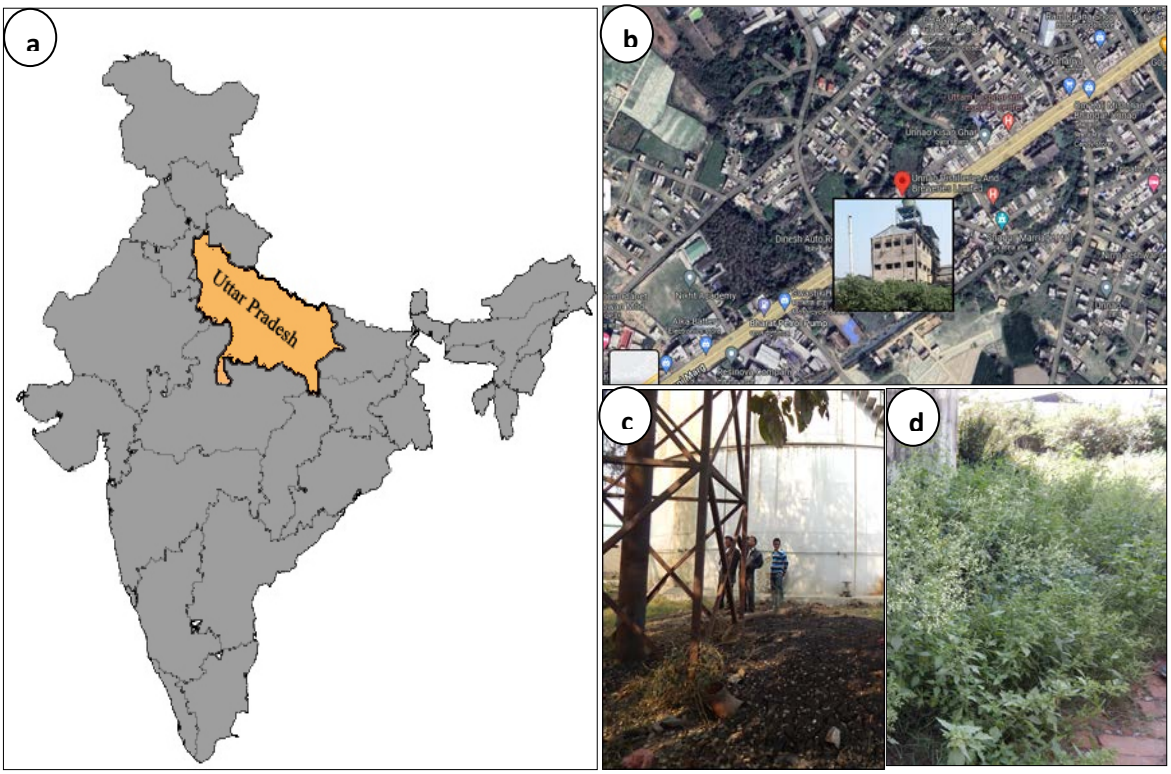


Fig. 5.3.1. Location map of sampling site Unnao Distilleries and Breweries Limited, Unnao, Uttar Pradesh, India (a-d). Sample was collected from the disposed site of distillery sludge. (a & b) showing the country and state of sample collection. (c) showing fresh disposed distillery sludge from anaerobic methane reactor. (d) is showing the growth of *Parthenium hysterophorus* on the disposed distillery sludge.

5.3.1.2. Physico-chemical analysis of distillery sludge

The sludge samples of both the stages were analyzed for physiochemical parameters i.e. pH value, measure of sludge salinity/electrical conductivity, phosphate, nitrate, sulfate, sodium and, chloride, by following the method described by Koottatep et al., (2021). While the phenolic content of sludge was carried out by using standard method prescribed in American Public Health Association (APHA), (2012). Moreover, the pH and EC value of sludge were measured by using Orion Star™ A211 Benchtop pH Meter (Cat No. STARA2210, ThermoFisher Scientific, USA) followed by method Wang et al., (2022).

Additionally, the metal content i.e. Fe, Zn, Cu, Mn, Ni, Pb, Cd and Cr in sludge were measured by acid digestion method recommended by Environmental Protection Agency (EPA 3050B). After digestion the digested sample was measured for metal analysis by using Inductively Coupled Plasma Perkin Elmer Optima 2100 DV (USA) and Cd concentration with GFAAS Perkin Elmer 900Z (USA). The calibrations of instruments were done on daily usage basis with standardized solutions.

Rest all the parameters of material and methods were as followed in section 5.2.1

5.3.4. Results and discussion

5.3.4.1. Changes in distillery sludge's physicochemical properties

The physico-chemical parameters of fresh and 50 days old distillery sludge were analyzed which has been shown in Table 5.3. The fresh sludge showed high toxic parameter and metallic content. This prevalent metal were Mn, Ni, , Pb, Cd Fe, Zn, Cu are found higher than permissible limit and toxic to animal and human health due to their persistence for long term in environment (Rehman et al.,2018; Onakpa et al., 2018). The measured pH value, Electrical conductivity (EC), sodium, phosphate, chloride, nitrate, phenol sulfate showed higher in disposed fresh distillery sludge.

Sludge has an alkaline pH of 8.3, which has resulted from addition of lime and oxidation of organic acids to CO_2 and its reaction with basic compounds like sulphides, and hydroxides which further generates carbonates and biocarbonates this increases the pH of the sludge generated during methanogenesis. They are mostly applied in the process of fermentation to modify the pH level (Chandra et al., 2018). The value of EC has also been detected high due to various anions and cations present in sludge such as Na^+, Cl^-, NO^{-3}, PO_4^{3-} and SO_4^{2}-.The corrosive impact of sugarcane-molasses and juice production, a high quantity of heavy metal has been detected in sludge. Further steps also include fermentation and distillation of sugarcane distilleries which at last is discharged as spentwash. These processes could be the primary source of heavy metal in disposed distilleries sludge.

However, after 50 days of in-situ phytoremediation by *P. hysterophorus*, the sludge had lower values of numerous physio-chemical parameters, as shown in Table 1.

The original pH of distillery sludge was found to be 8.3. (alkaline in nature) which changed into slightly acidic pH i.e. pH 6.71, this might be due to rhizobacterial interaction with various heavy metal and complex organic compounds. Moreover, metabolites released from roots of *P. hysterophorus* such as amino acids, organic acids and numerous metal chelators, also reduces the pH of sludge.

These activity enhanced phytoextraction mechanism due to bioavailability of organic nutrients, ions and various metals from slightly acidic sludge. The EC of phytoremediated sludge after in-situ treatment was also found to be higher; this might be due to breakdown of organic pollutants by rhizobacterial activity and chelation of heavy metals which consequently increases the role of cations and anions in sludge. Except for EC, all physico-chemical parameters of in-situ phytoremediated sludge by *P. hysterophorus* after 50 days of growth were significantly greater ($p < 0.001$) than those of newly dumped distillery sludge.

Table 5.5. Physico-chemical properties of disposed distillery sludge before and after 50 days of In-situ of phytoremediation by *Parthenium hysterophorus*

S.No.	Parameter	Fresh distillery sludge	Distillery sludge after In-situ phytoremediation (50days)	% Change
1.	pH	8.3 ± 0.07	6.71 ± 0.05	9.51
2.	EC (μS cm^{-1})	5.07 ± 0.12	6.01 ± 0.09	-18.54
3.	Sodium (mg kg^{-1})	45.37 ± 0.06	32.19 ± 0.12	29.05
4.	Chloride (mg kg^{-1})	1105.15 ± 0.02	601.05 ± 06.09	44.19
5.	Nitrate (mg kg^{-1})	91.08 ± 0.18	42.08 ± 0.12	53.79
6.	Phosphate (mg kg^{-1})	2305.01 ± 0.09	1501.13 ± 0.05	34.87
7.	Sulfate (mg kg^{-1})	153.12 ± 0.01	109.18 ± 0.06	28.69
8.	Phenol (mg kg^{-1})	501.03 ± 0.01	422.16 ± 0.15	15.74
	Trace Elements (mg kg^{-1})			
a.	Fe	2103.01 ± 0.01	415.02 ± 0.12	80.26
b.	Zn	52.02 ± 0.17	27.08 ± 0.90	47.94
c.	Cu	349.01 ± 0.41	198.08 ± 0.47	43.24
d.	Mn	162.05 ± 0.08	97.05 ± 0.09	40.11
e.	Ni	14.05 ± 0.06	4.23 ± 0.04	69.89
f.	Pb	37.01 ± 0.08	8.11 ± 0.68	78.08
g.	Cd	1.91 ± 0.05	1.02 ± 0.12	46.59
h.	Cr	1.15 ± 1.07	0.82 ± 0.24	28.69

5.3.4.2. Isolation of PGP traits and its primary screening

The morphological and physiological properties of all identified rhizobacterial strains were examined. Isolated bacteria were further examined for PGPR parameters along with the ligninolytic enzymatic activity as shown in Table 5.4.

5.3.4.2.1. Indoleacetic acid (IAA) production

Among the seven isolates i.e. KSPH1, KSPH2, KSPH3, KSPH4, KSPH5, KSPH6, KSPH7 and KSPH8, all the isolates showed IAA production. Pink coloration was detected in their cell-free supernatant with and without the inclusion of tryptophan in culture broth; maximum IAA production was recorded in strain KSPH2, KSPH4 and KSPH7 (17 μg ml−1) broth culture (Table 5.3.2).

5.3.4.2.2. NH_3 production

All the isolates showed NH_3 production. The transformation of brown to yellow colour was a positive indicator of ammonia production. Maximum production of NH_3 was recorded in strain KSPH2 and KSPH7 (17 μg ml−1) broth culture (Table 5.3.2).

5.3.4.2.3. N_2 production

Rhizospheric bacterial isolated strains were screened by changing the color of bromothymol blue (BTB) containing nitrogen free media, and these strains showed a color change in BTB containing media, indicating excretion of ammonia. Only strain KSPH2, KSPH4, KSPH6 and KSPH7 showed positive test for N_2 production (Table 5.3.2).

5.3.4.2.4. Siderophore production

Among the seven strains only strain and KSPH2, KSPH4, KSPH6 and KSPH7 showed the appearance of orange halos around their spots on the CAS agar medium indicating a positive test for siderophore production. (Table 5.3.2).

5.3.4.2.5. Phosphate solubilization

Among the seven strains only KSPH1, KSPH2, KSPH4, KSPH6 and KSPH7 showed significantly clearing of zone around the colonies (Table 5.3.2). At 3 days of incubation,

the Phosphate Solubilization Index (PSI) and Phosphate solubilizing efficiency (PSI) of bacterial strains were measured by the following equation:

$$PSI = \frac{\text{Colony Diameter + Clearing zone}}{\text{Colony diameter}}$$

The sequence of PSI was as follows:

KSPH6 (2.2 ± 0.3) > KSPH7 (2.0 ± 0.3) > KSPH2 (1.8 ± 0.2) > KSPH1 (1.6 ± 0.4)> KSPH4 (1.2± 0.1)

$$\text{Phosphate solubilizing efficiency} = \frac{\text{Solubilization diameter X 100}}{\text{Colony diameter}}$$

KSPH6 (120) > KSPH7 (100) > KSPH2 (80) > KSPH1 (60) > KSPH4 (40)

5.3.4.2.6. Assay for organic acid production

All bacterial isolates showed positive test for organic acid production. On adding 2 to 3 drops of methyl red indicator to all seven bacterial strains, only KSPH2, KSPH4, KSPH6 and KSPH7 showed red colour formation for positive test and KSPH1, KSPH3 and KSPH5 showed negative test for organic acid production (Table 5.3.2).

5.3.4.2.7. Ligninolytic enzyme activity of rhizospheric bacterial strains

Four of the seven bacterial strains could produce ligninolytic enzymes. Using the plate assay method, strains were tested for manganese peroxidase, lignin peroxidase, and laccase activity. For screening, MSM with various substrates was used. The presence of MnP activity was shown by the conversion of dark pink to yellow colour, the absence of blue colour indicated the presence of positive LiP, and the presence of brown colour halos indicated the positive test laccase activity (Table 5.3.2).

Table 5.6.: Detection of plant growth promoting parameter of rhizospheric bacteria isolated from *Parthenium hysterophorus* rhizosphere

ISOLATES	**IAA**	**NH_3**	**N_2 Fixation**	**Siderophore production**	**Phosphate Solubilization**	**Organic acid production**	**Ligninolytic Enzyme**		
							Laccase	**MnP**	**LiP**
KSPH1	+	+	-	-	+	+	-	+	-
KSPH2	++	+++	+	+	++	++	+	++	+
KSPH3	+	+	-	-	-	-	-	-	+
KSPH4	++	++	+	++	++	++	+	++	+
KSPH5	+	+	-	-	-	-	+	+	+
KSPH6	+	++	+	++	+	++	+	++	+
KSPH7	++	+++	+	+	+	+	++	+++	+

5.3.4.2.8. 16S rRNA gene analysis of rhizosphere bacteria and phylogenetic analysis

Purified isolated rhizosphere bacterial strains were identified on the basis of 16S rRNA sequencing. Among the seven rhizospheric bacterial strains i.e. KSPH1, KSPH2, KSPH3, KSPH5, KSPH6 and KSPH7, only four bacterial strains i.e. KSPH2, KSPH4, KSPH6 and KSPH7 could show most of the positive PGPR test along with ligninolytic enzymatic activity. 16S rRNA sequencing data, the isolated strains KSPH2, KSPH4, KSPH6 and KSPH7 showed nearest relationships with *Alcaligenes faecalis*, *Cytobacillus firmus*, *Bacillus* subtilis and, *Niallia circulans.* The nucleotide sequences of these potentially diverse rhizospheric bacteria were deposited in the National Center for Biotechnology Information (NCBI) database under accession numbers ON024323, ON024324, ON024325, and ON024326 respectively. Using the MEGA 11 software, a phylogenetic tree was generated that illustrated the relationships between bacterial species and the most closely related genera deduced from sequences of the 16S rRNA gene. (Fig.5.3.2) (Kumar and Chandra, 2018).

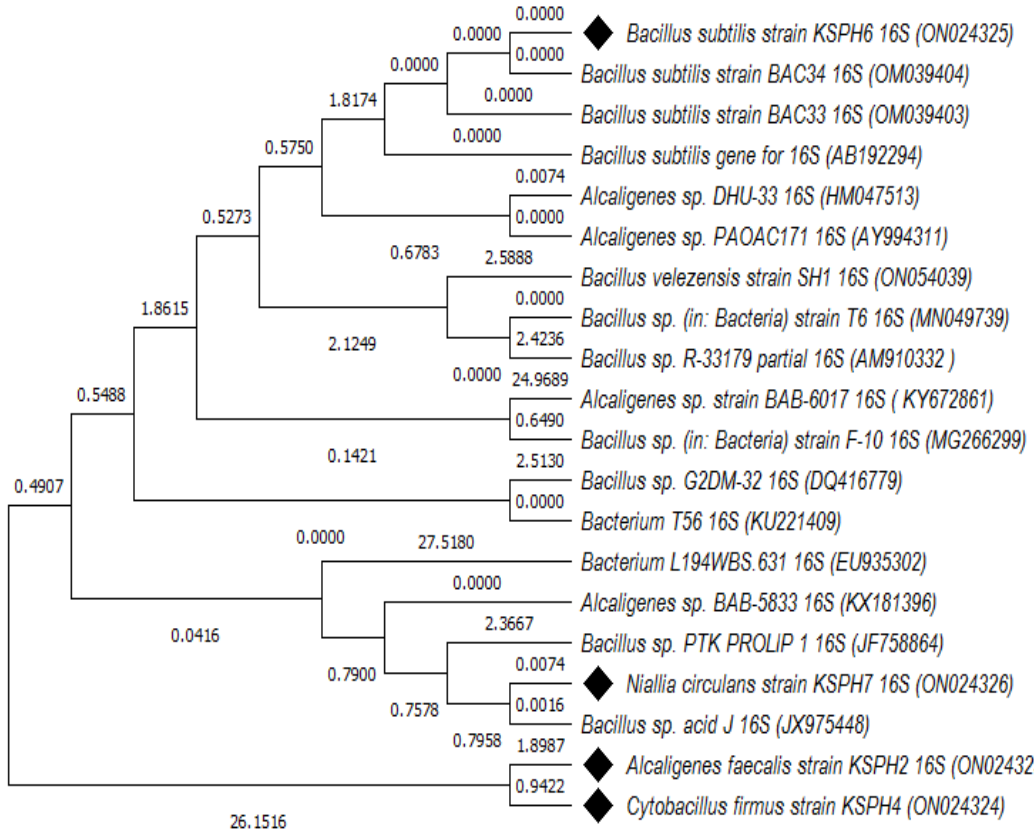


Fig. 5.3.2.. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (-30498.27) is shown. Initial tree(s) for the heuristic search were obtained

automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. This analysis involved 20 nucleotide sequences. There were a total of 1555 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021)

5.3.4.2.9 Accumulation of Heavy metals in P. hysterophorus

The distribution and accumulation of metals in plant components must be investigated in order to establish the role of plants in the rehabilitation of contaminated ecosystems (Ojuederie et al., 2017). *P. hysterophorus* have the ability to accumulate various metals in a diverse pattern into their tissue (Fig.5.3.3). The highest accumulation of metal has been found in root and then in stem followed by leaves (Table 3). Overall, the accumulation pattern of metal was noted as:

Fe (612.08 ± 0.112)> Cu (327.235 ± 0.136)> Zn (149.188 ± 0.101)> Mn (78.59 ± 0.107)> Pb (26.153± 0.159)> Ni (9.703 ± 0.16)> Cd (2.195± 0.183)> Cr (1.921 ± 0.153) mg kg^{-1}(Table 3).The heavy metals enter from the curricular cracks in root tissue and via diffusion of cell sap it accumulates in various parts of the plant. In accumulation pattern of heavy metal, pH of soil and chemical nature of other organic co-pollutants plays a vital role in bioavailability of metals. Root exudates have been demonstrated to alter the pH of sludge, allowing heavy metals to move more freely in the soil. In addition, bacterial-plant interactions in the rhizosphere promote metal bioavailability in roots (Chandrakala et al., 2019). Moreover, other factors, such as metal redox states, plant genotype, and metal heterogeneity, plant root system, and environmental conditions, all influence the metal accumulation pattern in plants (Zandi et al., 2022).

Our findings showed that distillery sludge produced by methanogenesis of spent wash was initially alkaline in nature, including a variety of organometallic compounds. This complex organic waste has a high affinity for numerous trace elements (Migo et al., 1997). However, as a result of certain plant-microbe interactions, the organometallic bond breaks down, allowing metal to become available to the plant (Zhang et al., 2020). The accumulation of measured metals in *P. hysterophorus* root tissues was found to be higher in this study. This demonstrated that metal mobility in plant tissues is concentration

dependent. The findings imply that a plant's metal tolerating strategy has evolved and is present in *P. hysterophorus* when it grows in metal-contaminated environments. Similar findings involving heavy metal accumulation have been observed in previous studies also. (Pavlenko-Badnaoui et al., 2019). *P. hysterophorus*, the tested plant, accumulated heavy metal concentrations that were higher than normal or phytotoxic levels, indicating that the plant has a hyperaccumulation propensity and the potential for use in phytoremediation of industrially contaminated areas.

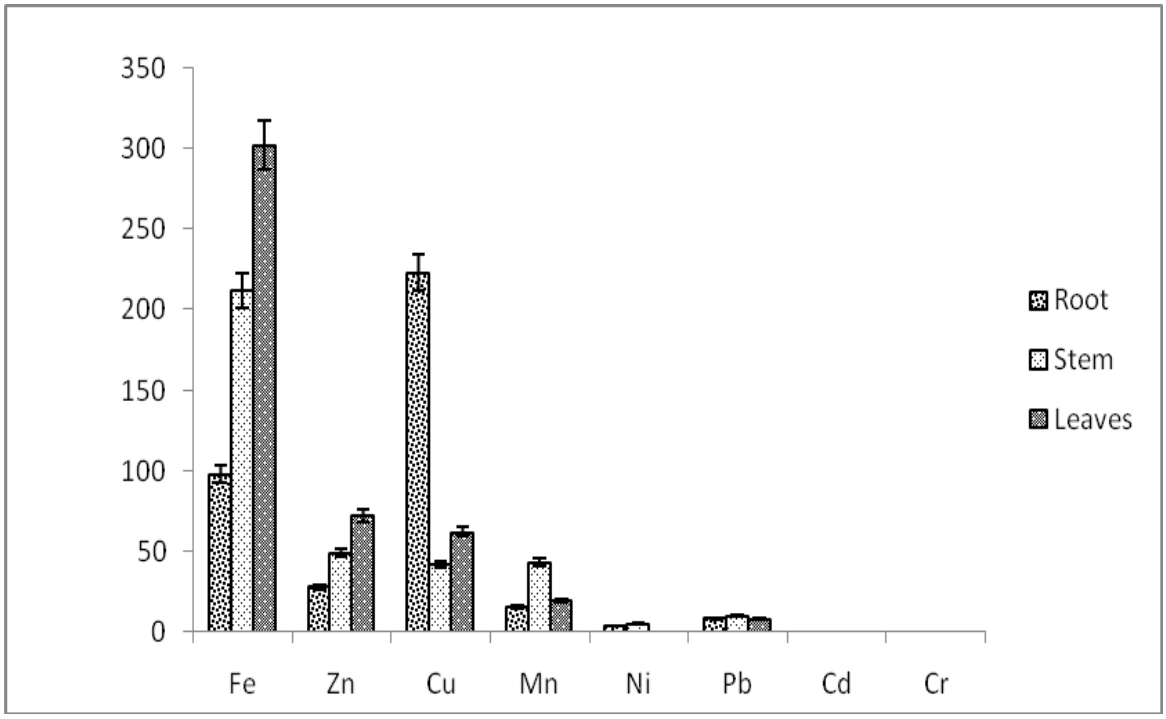


Fig.5.3.3. Accumulation of Fe, Zn, Cu, Mn, Ni, Pb, Cd and Cr in different parts of *Parthenium hysterophorus* collected after 50 days from distillery sludge dumping site.

Table 5.7: Heavy metal accumulation (mg kg^{-1} DW) in root, shoot and leaves of various plant species.

Plant	**Plant parts**	**Fe**	**Zn**	**Cu**	**Mn**	**Ni**	**Pb**	**Cd**	**Cr**
Parthenium hyterophorus	Leaves	302.014 ± 0.014	72.015 ± 0.014	62.075 ± 0.015	19.76 ± 0.014	0.985 ± 0.032	7.845 ± 0.047	0.320 ± 0.074	0.357 ± 0.041
	Stem	212.012 ± 0.074	49.075 ± 0.075	42.095 ± 0.054	43.35 ± 0.046	4.964 ± 0.074	9.954 ± 0.097	0.954 ± 0.094	0.654 ± 0.015
	Root	98.054 ± 0.024	28.098 ± 0.012	223.065 ± 0.067	15.48 ± 0.047	3.754± 0.054	8.354± 0.015	0.921 ± 0.015	0.910 ± 0.097
	Total	**612.08 ± 0.112**	**149.188 ± 0.101**	**327.235 ± 0.136**	**78.59 ± 0.107**	**9.703 ± 0.16**	**26.153± 0.159**	**2.195± 0.183**	**1.921 ± 0.153**
Accumulation pattern		R > S > L	L > S > R	R > L > S	R > L > S	R > S > L	S > R > L	S > R > L	R > S > L
	BCF	1.47	1.03	1.12	0.44	1.17	1.02	0.90	1.10
	TF	2.16	1.74	0.18	2.80	1.32	1.19	1.03	0.71

5.3.4.3.0. Translocation factor and Bioaccumulation coefficient factor

P. hysterophorus strength to tolerate and accumulate heavy metals, which could be used as a technique for phytoextraction of metal-mixed endocrine disrupting chemicals (EDCs) polluted sites. According to previous studies, the BCF and TF values were utilized to evaluate the phytoextraction and phytostabilization capability of plant species (Ghazaryan et al., 2019). The BCF refers to the most important plant feature in phytoremediation, which is how plants take in metals, move them into their tissues, and store them in the aerial plant biomass (Chandra et al., 2017). The result of BCF for each heavy metal in plant sample is shown in Table 5.3.3.

In the study, it was discovered that the *P. hysterophorus* had high BCF values (> 1) for iron (Fe), zinc (Zn), copper (Cu), nickel (Ni), Lead (Pb) and chromium (Cr), and low BCF values (<1) for manganese (Mn) and cadmium (Cd) and as shown in Table 3. *P. hysterophorus* has a high BCF>1, indicating that it has the capacity to phytoextract Fe, Zn, Cu, Ni, Pb and Cr from polluted sites, while low BCF values (<1) for Mn and Cd, on the other hand, indicated that plants had difficulty in mobilising these metals in the root zone (Table 5.3.3).

The order of BCF values for various metals were as Fe (1.47) > Ni (1.17) > Cu (1.12) > Cr (1.10) > Zn (1.03) > Pb (1.02) > Cd (0.90) > Mn (0.44) (Table 5.3.4). In various plants, the process of metal uptake and accumulation from soil to root is influenced by the concentration of accessible metals in soils, solubility sequences, evapo-transpiration rate, hydropotential, and photosynthetic activity of the plant (Chandra et al., 2018).

TF, the ratio of shoot to root metals, indicates internal metal transportation from root to shoot. For different plant species, the TF of Fe, Zn, Cu, Mn, Ni, Pb, Cd and Cr were variable as shown in Table 3. The order of TF values for various metals were as Mn (2.80) > Fe (2.16) > Zn (1.74) > Ni (1.32) > Pb (1.19) > Cd (1.03) > Cr (0.71) > Cu (0.18). The maximum TF was detected for Mn (2.80) and the minimum was for Cu 0.18 (Table 5.3.3). The high TF values of certain metals may be due to high bioavailability of heavy metals, hydropotential, evapo-transpiration rate, and photosynthetic activity of plants, and increased biomass production to fast growth. According to Kumar et al (2021) these plants have important properties that can be utilized in phytoextraction of certain metals (Saleem et al., 2020).

The high metal accumulation could be attributed to a well-developed detoxification mechanism based on the sequestration of heavy metal ions in vacuoles by binding them to appropriate ligands such as organic acids, proteins, and peptides in the presence of enzymes that are capable of functioning under high levels of metallicilous environment (Ghori et al., 2019). Metal transport from roots to shoots comprises long-distance translocation in the xylem and storage in the vacuole of leaf cells, both of which are influenced by element speciation, soil pH, and a variety of other parameters (Song et al., 2017).

Plant also showed phytostabilization of contaminated sites i.e. BCF with > 1 and TF < 1 for metal Cu and Cr (Table 5.3.3). Phytostabilisation is a plant-based method in which plant species are used to limit the mobility and bioavailability of heavy metals in the environment by absorbing them into the soil. Heavy metals can be immobilised by plants by adsorption on the surface of the roots, precipitation, and accumulating inside the roots or within the rhizosphere soil. By reducing metal mobility and leaching into ground water, as well as metal bioavailability and entry into the food chain, this technique helps to protect the environment (Ashraf et al., 2019).

5.3.4.3.1. Microscopic cellular observation in root of potential native plants after phytoextraction

The impact of heavy metals on cellular organisation is crucial to comprehending the morphological and physiological changes induced by heavy metals due to structural and functional complementarity (Khan et al., 2015). The TEM micrography of *P. hysterophorus* root revealed production of multi-vacuoles, multi-nucleolus, thickening of middle lamella, round shaped multi-mitochondria, and modified nuclear structure with metal deposition in cell wall and cytoplasm as shown in Fig.5.3.4. (a-c). As a result of the high concentration of heavy metals in their cellular compartment and the greater amount of detoxification occurring in their cellular tissues, this indicated protein synthesis for their tolerance mechanism (Viehweger et al., 2014).

The additional development of nucleolus and vacuoles in the presence of heavy metals increases the production of ribosomes and mRNA, which in turn increases the production of novel proteins involved in heavy metal tolerance in plants (Kumar and Chandra, 2019). The increased number of mitochondria formation indicated the generation of more energy in the form of ATP inside the cell, which is required to combat heavy

metal toxicity (Sun et al., 2022). Furthermore, the apparent deposition of metals in the form of electron dense granules in vacuoles, cell cytoplasm, and cell wall revealed the plant's predisposition for heavy metal hyperaccumulation. Depositions of heavy metals in the cell wall periphery of *P. hysterophorus* were also detected as shown in Fig 5.3.4 (c). Heavy metal depositions along the cell wall play an important function in heavy metal tolerance by preventing free metal ions from circulating in the cytosol as shown in Fig 5.3.4 (c). This discovery revealed novel information on plants' heavy metal detoxification mechanism, which is based on heavy metal ion sequestration in vacuoles and deposition on the cell wall.

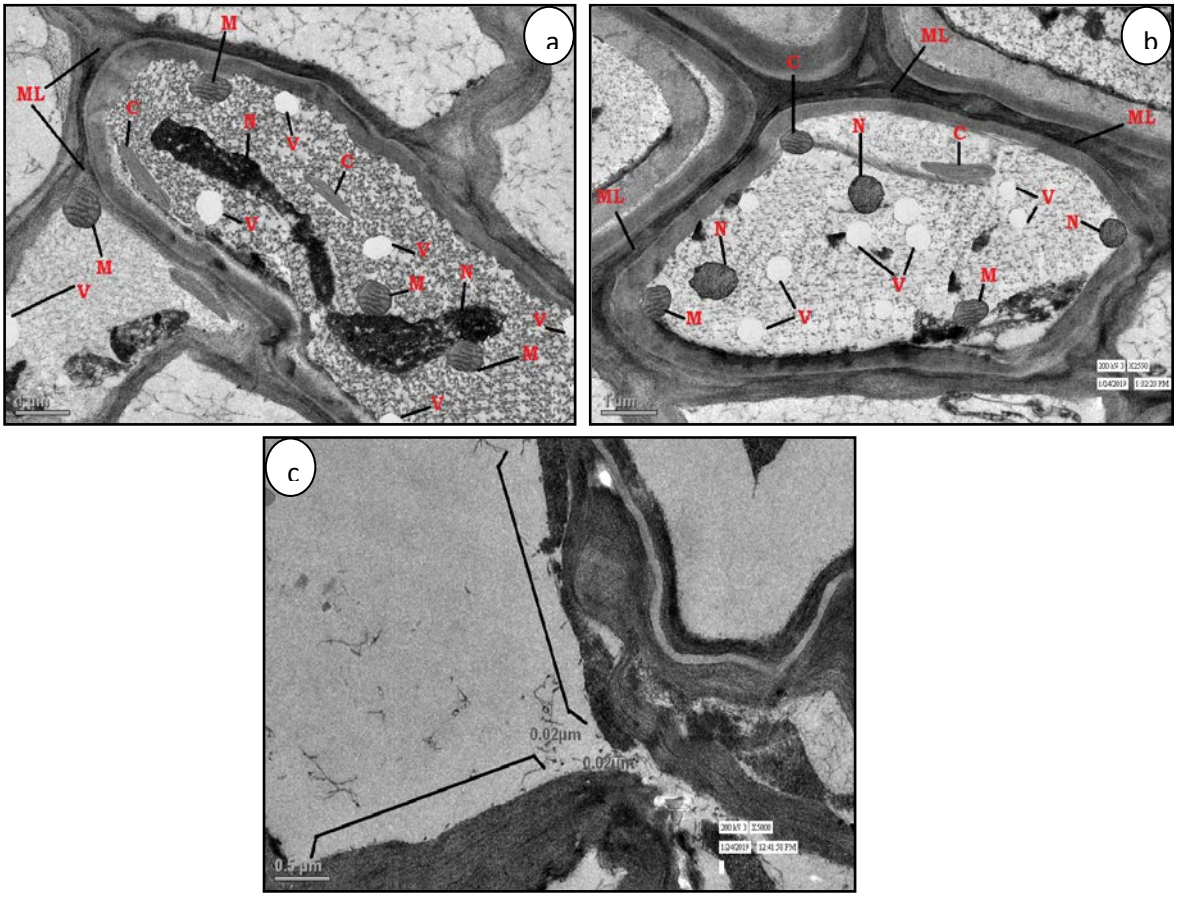


Fig. 5.3.4.. (a-c) Transmission Electron Microscopy (TEM) images. (a & b) Black arrow and marking showin the metal accumulation to root tissue of *Parthenium hysterophorus.*V: Vacuole; N: Nucleus; C: Chloroplast; the metal accumulation to root tissue of *Parthenium hysterophorus.*V: Vacuole; N: Nucleus; C: Chloroplast; M: Mitochondria, ML: Middle lamella; N: Nucleus; (c) Showing metal deposition at the periphery of cell wall. Arrow (→) indicated metals deposition.

The conclusion of these findings is supported by our previous findings (Chandra et al., 2018), which reported the formation of multi-vacuoles and depositions of metal granules in cell wall and cytoplasm in the root of *Cynodon dactylon* (Bermuda grass), *Saccharum munja* (munja), *Argemone Mexicana* (mexican poppy), *Pennisetum purpureum* (elephant grass), *Chenopodium* album (goosefoots) etc. growing under heavy metal stress as an adaptive strategy when there is a high concentration of metals in the environment and the presence of organic contaminants.

5.3.4.3.2. Characterization of organic pollutants

The GC-MS analysis from ethyl acetate and dichloromethane (1:1) revealed numerous peaks from fresh sludge and rhizospheric sludge collected after 50 days growth of plant at 6.19, 7.89, 7.92, 10.57, 13.00, 18.59, 19.19, 21.59, 23.34, 26.11, 26.64, 29.70, 30.12, 33.17, 33.96, 37.26, 38.71, 41.02, 41.78, 44.29, 44.36, 46.98, 47.54, 50.24, 50.25 and 51.57. This indicated the presence of various complicated organic pollutants which are soluble in ethyl acetate and dichloromethane. These compounds were identified by comparing the National Institute of Standards and Technology (NIST) library. The organic compounds from fresh distillery sludge were identified as 2-Oxovaleric acid, 1,3-Dioxolane, Heptanoic acid, 2-Propenoic acid, Oxalic acid, 1,2-Benzenedicarboxylic acid, Cyclopropane, Cannabinol, 2,4-Dihydroxybenzoic acid, 1-Octacosanol, á-Sitosterol, Pregn-4-ene-3-one, Quercetin- and, Tri-ruthenium dodecacarbonyl (Table 5). Further, the organic compounds were also identified by GC-MS from same site of distillery sludge after 50 days growth of *P. hysterophorus*. The identified compounds were as p-Toluenesulfonamide, Trimethyl(n-pentyl)silane, Alizarin, 2-Aminobenzoxazole, 2-Propenoic acid, Phthalic acid, Bisphenol A, Inabenfide, Silane, Hecogenin, á-Sitosterol, Pregn-4-ene-3,20-dione and Quercetin (Table 5), with altered RT and reduced peak (Fig. 5b). This indicated the biotransformation and removal of several organic compounds due to phytoremediation of parent compound. The disappearance some compound revealed the degradation by rhizospheric microbial activities which facilitated for plant growth and accumulation of these pollutants as nutrient. This result showed direct correlation of plant growth by using these organic compounds as nutrient (Sophia, and Shetty; 2020).

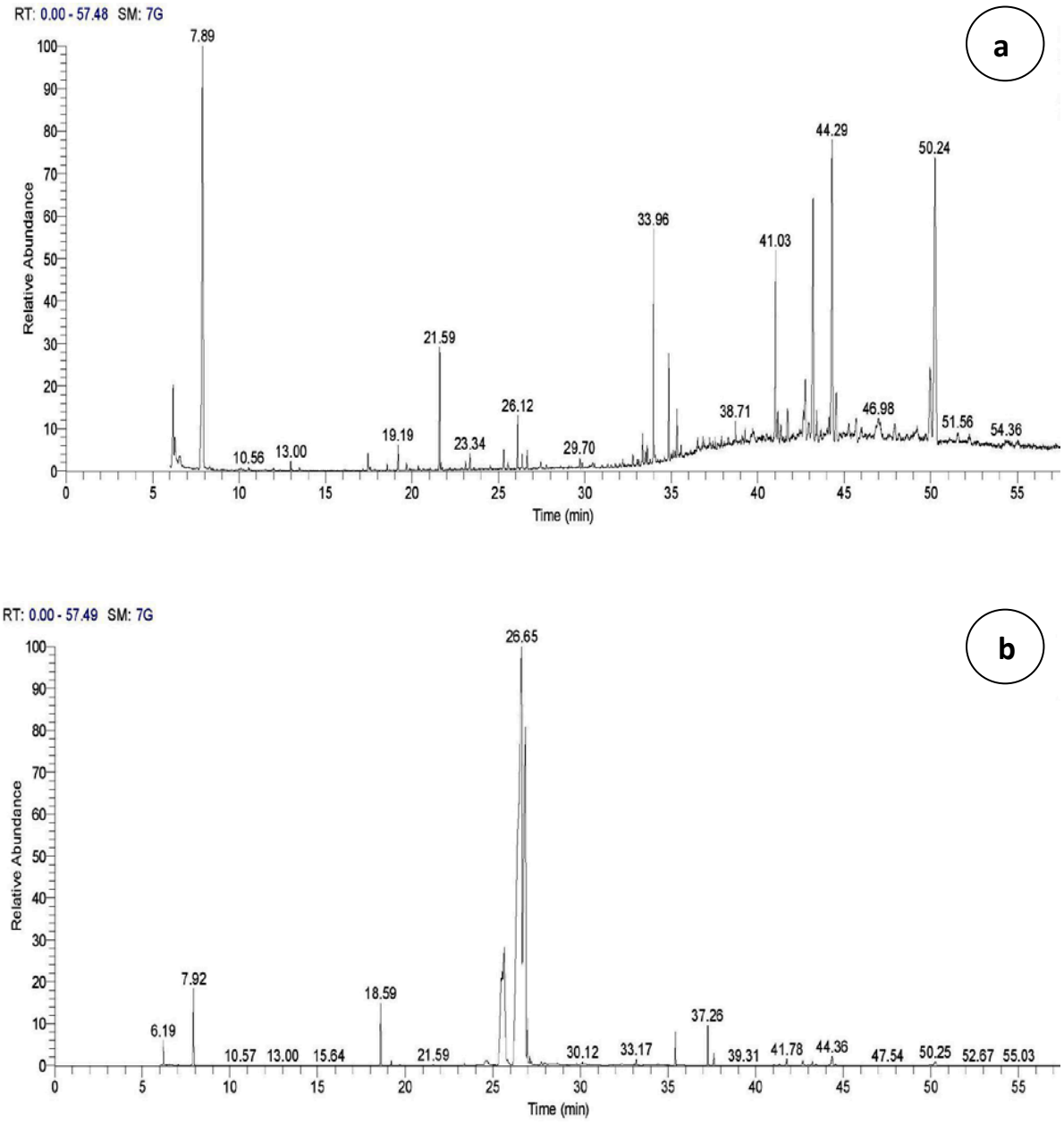


Fig. 5.3.5. Chromatogram of organic compounds extracted from distillery sludge (a) Initial stage, sludge without plant growth (b) Final stage, sludge with 50 days growth of *Parthenium hysterophorus.*

Subsequently, the analysis of PGPR activities by isolated bacterial strain from rhizospheric zone of *P. hysterophorus* revealed the production of IAA, ammonia, siderophores, organic acid, nitrogen fixing activity, phosphate solubilization with ligninolytic activity. This confirmed that the rhizospheric bacterial population present in

sludge played vital role for mineralization of complex organic compound for bioavailability to plant as nutrient. The role of rhizospheric bacterial population for the PGPR activity has been previously reported in the agricultural crops (Kalam et al., 2016). Knowledge for role of rhizospheric bacteria present in complex organometallic for ecorestoration is very limited.

Table 5.8: Detection of comparative list of various organic compounds by GC-MS extracted from distillery waste at various stages (a) Initial stage, sludge without plant growth Final stage, sludge with 50 days plant growth

S.No.	Retention Time (RT)	Initial (Fresh)	Final (Degraded) after 50 days growth of plant
1.	6.19	-	p-Toluenesulfonamide
2.	7.89	2-Oxovaleric acid	-
3.	7.92	-	Trimethyl(n-pentyl)silane
4.	10.57	-	Alizarin
5.	13.00	1,3-Dioxolane	
6.	18.59	-	2-Aminobenzoxazole
7.	19.19	Heptanoic acid	-
8.	21.59	2-Propenoic acid	2-Propenoic acid
9.	23.34	Oxalic acid	-
10.	26.11	1,2-Benzenedicarboxylic acid	-
11.	26.64	-	Phthalic acid
12.	29.70	Cyclopropane	-
13.	30.12	-	Bisphenol A
14.	33.17	-	Inabenfide
15.	33.96	Cannabinol	-
16.	37.26	-	Silane
17.	38.71	2,4-Dihydroxybenzoic acid	-
18.	41.02	1-Octacosanol	-
19.	41.78	-	Hecogenin
20.	44.29	á-Sitosterol	-
21.	44.36	-	á-Sitosterol
22.	46.98	Pregn-4-ene-3-one	-
23.	47.54	-	Pregn-4-ene-3,20-dione
24.	50.24	Quercetin-	-
25.	50.25	-	Quercetin
26.	51.57	Tri-ruthenium dodecacarbonyl	-

Conclusion

The findings of study revealed that *P. hysterophorus* is as hyperaccumulator for various heavy metals from complex organometallic wastes during its growth on the disposed distillery sludge. The analysis of organic compounds showed degradation of pollutants present in sludge after growth of *P. hysterophorus*. Further, the histological observations of root by transmission electron microscopy confirmed the deposition of metal granules in their tissue after accumulated by plant from distillery sludge. Besides, identified rhizospheric bacteria i.e. *Alcaligenes faecalis* (ON024323), *Cytobacillus firmus* (ON024324), *Bacillus* subtilis (ON024325) and, *Niallia circulans* (ON024326), also showed potential for plant growth promoting rhizobacteria activities which concluded the bacterial assisted phytoextraction potential of heavy metals from complex organometallic sludge of distillery from polluted site. This plant may be used as biotechnological tools for eco-restoration of polluted site by industrial waste as a green technology.

Chapter 6

Comparative assessment of phosphate, zinc and potassium solubilization by rhizospheric bacterial communities in *Phragmites communis* and *Typha spp*

Chapter-6

Comparative assessment of phosphate, zinc and potassium solubilization by rhizospheric bacterial communities in *Phragmites communis* and *Typha spp.*

6.1 Introduction

Soil contaminated with heavy metals (HMs) and organic pollutants is a complex problem due to their persistence in the soil as well as their adverse impacts on ecosystems (Ali et al., 2019). Most pollutants are released into the environment due to the anthropogenic activities, generating a large amount of waste in the form of heavy metals (HMs) and organic pollutants (Thakare et al., 2021).

Phytoremediation is considered as a cost-effective and promising technique that employs plants to remove both organic and inorganic pollutants from the soil (Balkrishna et al., 2022). The effectiveness of phytoremediation can be enhanced by the judicious selection of plant species to absorb and transport pollutants from the soil (Jeelani et al., 2020). There have been some useful results showing that phytoremediation could be an excellent alternative to chemical and mechanical methods in the remediation of contaminated sites (Hussain et al., 2022). The efficiency of phytoremediation doubly contaminated with organic pollutants and HMs is a complex issue because different types of pollutants may interact with each other and/or with plants and their rhizospheric biota (Ratna et al., 2021). In addition, high metal concentrations may suppress microbial activity in soils, thereby affecting the biodegradation of organic pollutants (Fei et al., 2020). The growth and pollutant removal by wetland plants may be influenced by an interaction of Cd and PAHs (Jeelani et al., 2018).

Aquatic macrophytes are indispensable in the natural water purification process owing to assimilation of heavy metals by plant organs, increase in biological diversity in the rhizosphere area and promotion of a variety of chemical and biological reactions (Prica *et al.*, 2019). They show significant diversity in their capacity to take up heavy metals and transfer them to aboveground organs (Javed et al., 2019). Their ability to take up and accumulate heavy metals in plant organs depends on plant species and age, but also on

environmental factors such as temperature, salinity and pH (Yaashikaa et al., 2022). The wetland macrophyte *Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae) is a helophytic perennial plant species typical of different wetland ecosystems. It is a robust and highly productive grass, with shoots up to 4 m tall and an extensive system of rhizomes and stolons involved in its vegetative propagation. *Phragmites australis* (common reed) is highly tolerant in relation to most abiotic factors, such as temperature and salinity. It also demonstrates high tolerance of heavy metal pollution and inhabits very clean to highly polluted sediments and waters (Saxena et al., 2019). Due to its fibrous roots and their large contact areas, as well as to its production of large amounts of aboveground biomass, common reed was found to be very efficient in accumulation of heavy metals.

Emergent aquatic macrophytes represent a diverse group of plants with an enormous potential for the removal/degradation of a variety of contaminants (Ammeri et al., 2022). Wetland plants can potentially enhance the removal and/or stabilization of metals (Saleh et al., 2020) and may also promote organic pollutant biodegradation (a) directly by rhizospheric root exudates , and (b) indirectly through the buildup of organic carbon. Emergent wetland species such as Phragmites australis, a truly cosmopolitan grass that flourishes in wetlands, has a high biomass production and provides essential ecosystem services (Eller et al., 2017). There is strong evidence that P. australis has been used widely for the phytoremediation of sites co-contaminated with metal and organic pollutants (Chirakkara et al., 2016).

Phragmites communis and *Typha spp*.are two macrophytes commonly present in natural and artificial wetlands. Roots of these plants engage in interactions with a broad range of microorganisms, collectively referred to as the microbiota. The microbiota contributes to the natural process of phytodepuration, whereby pollutants are removed from contaminated water bodies through plants. The outermost layer of the root corpus, the rhizoplane, is a hot-spot for these interactions where microorganisms establish specialized aggregates designated biofilm. Earlier studies suggest that biofilm-forming members of the microbiota play a crucial role in the process of phytodepuration. However, the composition and recruitment cue of the *Phragmites*, and *Typha* microbiota remain poorly understood. We therefore decided to investigate the composition and functional capacities of the bacterial microbiota thriving at the *P. australis* and *T. latifolia* root–soil interface.

Advanced industrialisation has led to the vast release of anthropogenic contaminants such as hydrocarbons, pesticides and heavy metals into the environment (Rasheed et al., 2020). Today, the contamination of aquatic and terrestrial environments with heavy metals represents a global problem that threatens aquatic ecosystems, agriculture and human health (Bashir et al., 2020). Diverse technological techniques have been developed to reduce concentrations of heavy metals in the environment (e.g., chemical precipitation, membrane filtration), but most of them, although effective, have proved to be expensive and not eco-friendly (Shrestha et al., 2021). The increasing need for remediation of contaminated sites has led to the development of cost-effective and eco-friendly biotechnologies like phytoremediation, which relies on the potential of naturally occurring plant species to extract, sequester and detoxify metal pollutants (Mahajan and Kaushal, 2018).

Color of sugarcane molasses-based distillery effluent is mainly due to complex biopolymer known as melanoidins. It is generated through the maillard reaction between the amino and carbonyl groups in organic substances (Tripathi et al., 2022). There are almost 300 distilleries in India generating highly colored spent wash in order of 13–15 times ethanol produced (AIDA, 1994). It has very high in biological oxygen demand (BOD, 40,000–42,000 ppm), chemical oxygen demand (COD, 82,000–90,000 ppm), total solids (TS, 5900–6500 ppm), phenolics (5–6 ppm), sulfate (6000–6500 ppm), and heavy metals (45–50 ppm) which constitute a major source of aquatic and soil pollution in India (Chaturvedi et al., 2006). It is highly colored components leads to the reduction of sunlight penetration in rivers, lakes and lagoons which in turn decreases both photosynthetic activity and dissolved oxygen, causing harm to aquatic life (Singh et al., 2019). Disposal of spent wash on land is equally harmful, causing a reduction in soil alkalinity, inhibition of seed germination, and damage to vegetation (Saleem et al., 2022). The conventional biological process such as activated sludge treatment is inefficient decolorizing the melanoidin containing effluent even after extended aeration (Fito et al., 2019).

Studies showed rhizosphere bacterial communities propagate at high speed, have large quantities, and have a strong ability to metabolize (Fang et al., 2021), which play a very important part in phosphorus removal, nitrogen removal, nitrogen fixation and conversion, organic matter decomposition and inorganic conversion, heavy metal removal, et. Rhizosphere bacterial communities are the main force involved in the degradation of

pollutants and play an important role in maintaining the ecological balance of wetland systems and achieving ecological purification.

The root zone of *Phragmites communis* and *Typha spp*. is rich in dissolved oxygen as well as in organic carbon, providing optimal conditions required for microbial colonization. Mycorrhizal ecotypes of *Phragmites communis* and *Typha spp*. have also been reported (Uddin et al., 2017), although mycorrhization (mostly by the members of the Glomeraceae family, e.g., *Glomus fasciculatum*, The rhizosphere of *P. communis* contains a variety of aerobic microbes (Yan et al., 2018), e.g., *Microbacterium hydrocarbonoxidans, Achromobacter xylosoxidans* and a number of species belonging to the genera Bacillus and Pseudomonas. In general, most bacterial species of the rhizospheric microbial community act as plant growth promoters (PGP), especially in wetland plant species. Kumar et al. (2020) reported the presence of Nitrosomonaslike ammonia oxidizing bacteria (AOB), indicating that nitrification occurs under waterlogged conditions. Srivastava et al. (2014) reported specific root zone-associated microbial community including *Acidobacteria, Actinobacteria, Nitrospirae* and *Spirochaetes* in *P. australis*. Bacteria are known to immobilize metal by precipitation and adsorption. Dissolution ability of immobilized zinc to zinc carbonate, zinc phosphate and zinc oxide in adequate quantity is not common amongst the cultivable bacteria. *Pseudomonas Acinetobacter, Gluconacetobacter, Bacillus Thiobacillus thioxidans, Thiobacillus ferroxidans* and facultative thermophilic iron oxidizers have been identified for their ability to solubilize zinc.

The current study showed the growth of *Phragmites communis* and *Typha spp*. in distillery effluent as a potential and strong evidence for bacterial assisted phytoremediation and decolorization of effluent (Kataki et al., 2021). The isolated bacteria were checked for the various PGPR activities and solubilization of phosphate, potassium and zinc to assist phytoremediation

6.2. Material and methods

6.2.1. Isolation, screening, and growth conditions of culturable rhizosphere bacteria

All the ten bacterial sp. were isolated from the rhizosphere of *Phragmites communis* and *Typha spp*. The isolation method was done as per general microbiological serial dilution method on nutrient agar (NA) petri dish (Sharma et al., 2022). The petri dish was

incubated for 24 hours at 30± 2°C until bacterial colonies appeared. Every colony was re-streaked for purification of strain (Fig. 6.1.) (Weselowski et al., 2016).

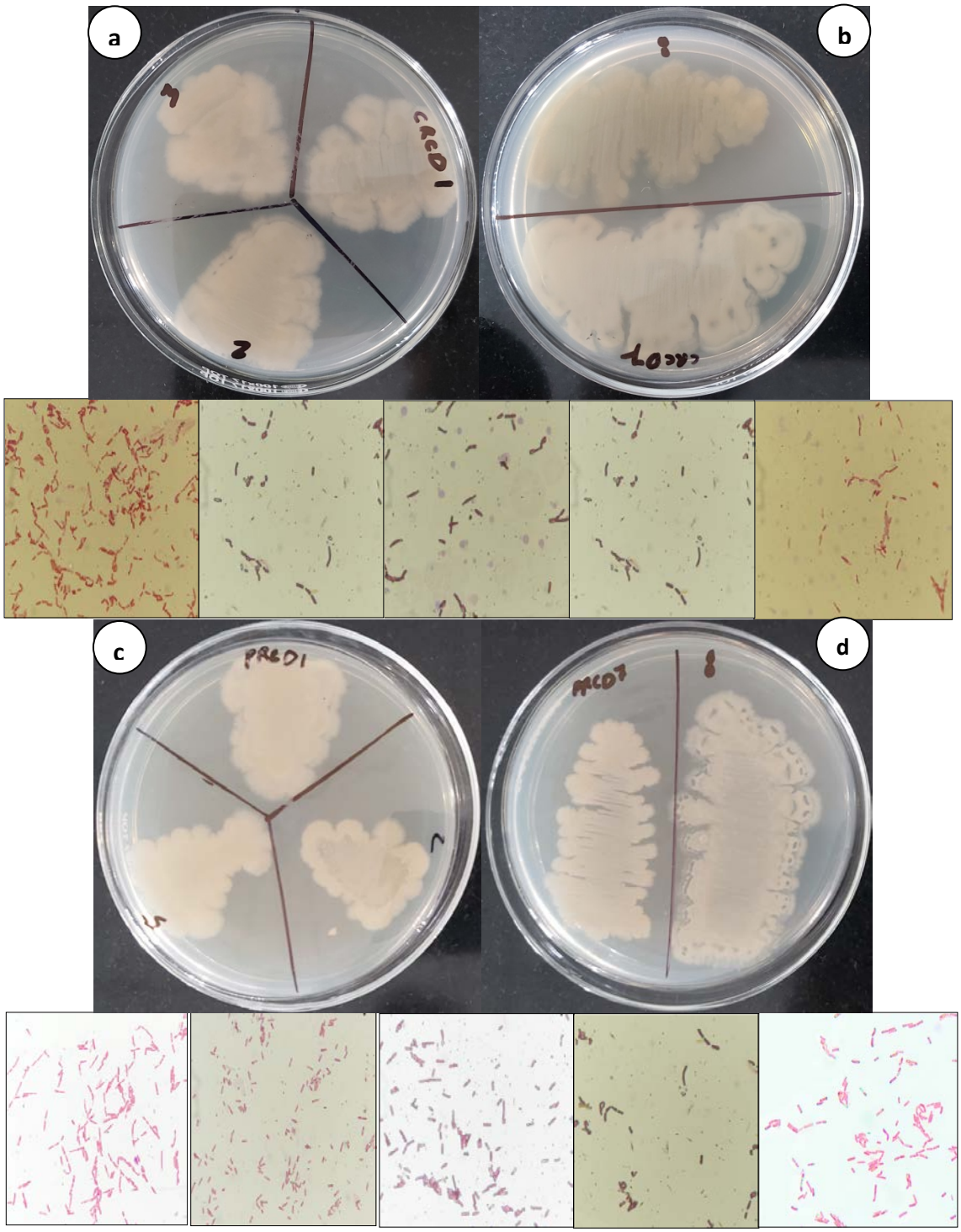


Fig. 6.1. Isolated bacteria from rhizosphere of *Phragmites communis* and *Typha spp*. and its gram staining (a & b) Isolated bacteria from rhizosphere of *Phragmites communis* (c & d) Isolated bacteria from rhizosphere of *Typha spp*. and its gram staining

6.2.2. Bioassays for Plant Growth Promoting Traits

6.2.2.1. Assay for indoleacetic acid (IAA) production

Bacterial cultures were grown at 28± 2◦C for 72 hours on their respective media. Fully grown cultures were centrifuged for 30 minutes at 3000 rpm. The supernatant was collected in separate test tube and two drops of orthophosphoric acid and 4ml of Salkowski reagent (35% of perchloric acid-50ml and 0.5M FeCl3-1ml solution) were added. The appearance of a pink colour suggests the presence of IAA production (Rawal and Saraf, 2020).

6.2.2.2. Assay for NH_3 production

Peptone water was used to test the ammonia production of bacterial isolates. In each test tube, 10ml peptone water was used to inoculate freshly grown cultures, which were then incubated for 48–72 hours at 28± 2◦C. After that, 0.5 mL of Nessler's reagent was added to each test tube. The appearance of a brown to yellow colour was a positive sign of ammonia generation (Ezati et al., 2019).

6.2.2.3. Assay for N_2 production

For screening nitrogen fixing activity of bacterial isolates a glucose nitrogen free mineral medium (GNFMM) was used to screen the nitrogen fixing activity of bacterial isolates. Bromothymol blue indicator (BTB) was added to the medium in order to make it green in colour. The isolated rhizospheric bacterial strains were inoculated in GNFMM + BTB medium and kept in incubator at 28 ± 2◦C for 48–72 hours.

6.2.2.4. Assay for siderophore production

The PGPR strains were spotted on prepared chrome azurol S (CAS) agar plates and kept for incubation at 28 ± 2°C for 5 days. The change in colour of the medium from blue to yellow at the spotted area indicated the presence of siderophore formation (Singh et al., 2020).

6.2.2.5. Assay for organic acid production

Pure rhizospheric bacterial cultures were cultivated for 24 hours at 37 °C in MRVP broth (pH 6.9). 2–3 drops of methyl red indicator were applied to an aliquot, and red colour was instantly detected for positive tests and yellow for negative tests. (Fahad et al., 2022).

6.2.2.6. Assay for Phosphate solubilization

To assess phosphate solubilization activity, isolated pure rhizospheric bacterial strains were spot-inoculated on Pikovskaya's agar plates with a metal loop and kept in incubator for 3 days at 30 °C. Clearing of zone formation surrounding the spotted colony was noted (Teymouri et al., 2016)

6.2.2.7. Assay for Potassium solubilization

To assess potassium solubilization, isolated rhizospheric bacterial strains were spot-inoculated on Aleksandrow agar plates with a metal loop and kept in incubator for 3 days at 30 °C. Clearing of zone formation surrounding the spotted colony was noted (Ashfaq et al., 2020).

6.2.2.8. *Assay for Zinc solubilization*

The isolates were inoculated into agar medium containing 0.1% insoluble zinc compounds by ZnO. Zinc solubilization potential of the bacterial isolates were evaluated by determining the zone diameter in plate assay and incubated at 30ºC for 48 hours (Costerousse et al., 2018).

6.2.2.9. Rhizospheric bacterial strain identification and phylogenetic analysis

At first stage, isolation of genomic DNA was performed by inoculating isolated bacterial strain in nutrient broth (NB) at 30°C in temperature controlled shaker at 220 rpm for 24 hours. After incubation the bacterial strain was centrifuged at 4000 rpm for 10 minutes at 24°C. The supernatant was discarded, and the cell pellet was used to extract genomic DNA using the Bacterial Genomic DNA Isolation Kit (HiMedia) according to the manufacturer's instructions. The 16s ribosomal RNA genes were targeted using the primers P027F and 1378R, which were used to amplify a sequence of approximately 1500 bp from genomic DNA. One microliter of template DNA, 0.2 microliters of primers UNI-16-GT-F (AGAGTTTGATCCTGGCTCAG) and UNI-16-GT-R (GGTTACCTTGTTACGACTT), 200 mL of each dNTP, 10X buffer, and 2 mM MgSO4, and one unit of High-Fidelity KOD Taq DNA Polymerase were used in a 25 mL PCR reaction. The following parameters were used during the cycle: Initial denaturation at 94°C for 4 minutes; 30 cycles of 30s denaturation at 94°C, 1 minute annealing at 63°C, and 1 minute extension at 68°C; and a final overall extension of 7 minutes at 68°C. The

PCR product was purified using the PCR Purification Kit (Norgen Biotek, Canada), and Biokart India Pvt. Ltd. perform the sequencing. To compare sequences to homologous bacterial 16S ribosomal RNA sequences, NCBI (www.ncbi.nlm.nih.gov/BLAST) was used. CLUSTAL W was used to align the sequences, and the MEGA 11 software using the maximum likelihood (ML) technique was used to create a phylogenetic tree (Lakhani et al., 2017)

6.3. Results and discussion

6.3.1. Indoleacetic acid (IAA) production

Among the eight isolates from *Phragmites communis* i.e. PHRCD1, PHRCD2, PHRCD3, PHRCD4, PHRCD5, PHRCD6, PHRCD7 and PHRCD8 all the isolates showed IAA production. Pink coloration was detected in their cell-free supernatant with inclusion of tryptophan in culture broth; maximum IAA production was recorded in strain PHRCD2, PHRCD5 and PHRCD6 (17 μg ml−1) broth culture (Figure 6.2a and Table 6.1.).

Additionally, eight isolates from *Typha spp*.i.e. TRCD1, TRCD2, TRCD3, TRCD4, TRCD5, TRCD6, TRCD7, and TRCD8. All bacterial were found potential for production of IAA production. Maximum IAA production was recorded in strain TRCD1, TRCD3, TRCD4, TRCD5, TRCD6, and TRCD7 (17 μg ml−1) broth culture (Figure 6.2b and Table 6.2.).

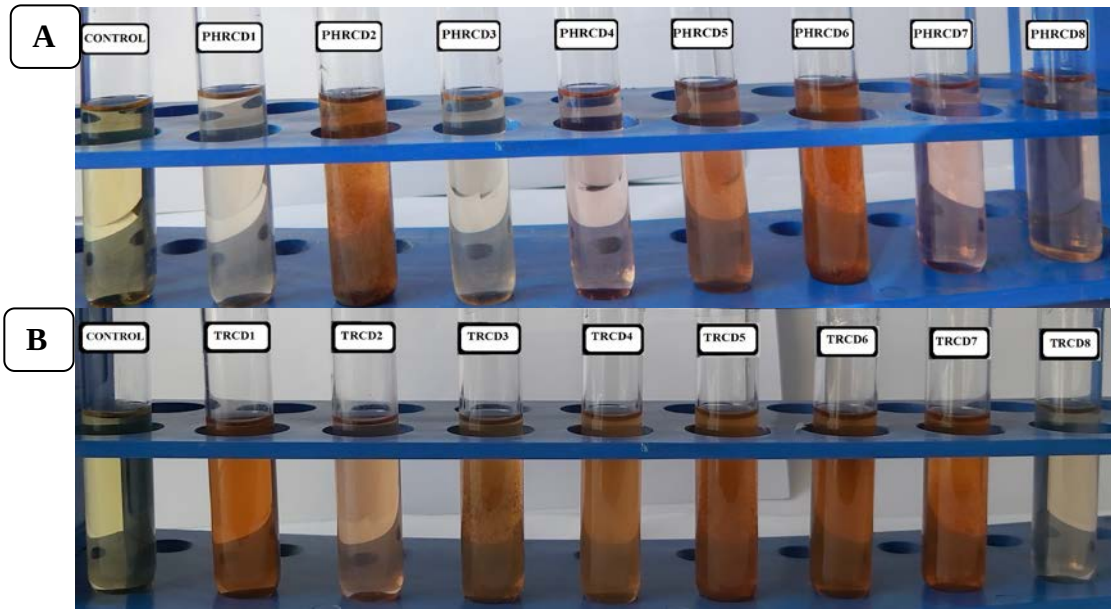


Fig. 6.2 Showing IAA production by isolated PGPR strains. (a) Rhizospheric strain from *Phragmites communis* (b) Rhizospheric strain from *Typha spp.*

6.3.2. NH_3 production

All the isolates showed NH_3 production from *Phragmites communis*. The transition of brown to yellow colour was a positive indicator of ammonia generation. Maximum production of NH_3 was recorded in strain PHRCD1, PHRCD3, PHRCD4, PHRCD5, and PHRCD6 (Figure 6.3a and Table 6.1.).

Additionally, eight isolates from *Typha spp.*i.e. TRCD1, TRCD2, TRCD3, TRCD4, TRCD5, TRCD6, TRCD7, and TRCD8. All bacterial were found potential for production of IAA production. Maximum IAA production was recorded in strain TRCD1, TRCD3, and TRCD6 (17 µg ml−1) broth culture (Figure 6.2b and Table 6.2.).

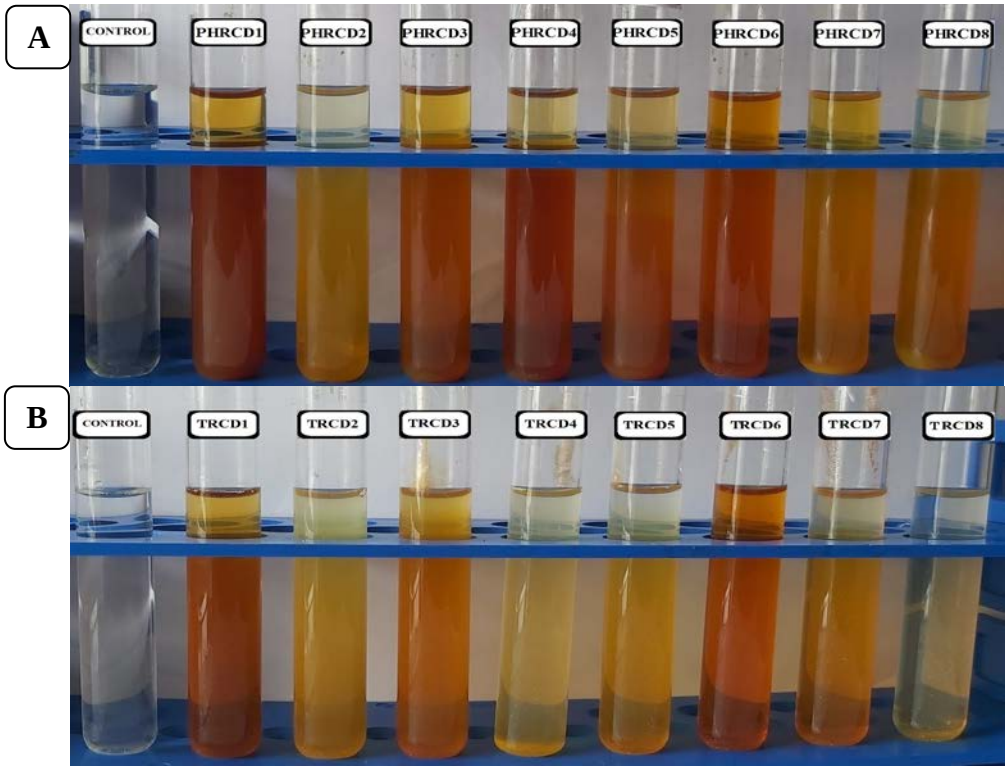


Fig. 6.3 Showing NH_3 production by isolated PGPR strains. (a) Rhizospheric strain from *Phragmites communis* (b) Rhizospheric strain from *Typha spp.*

6.3.3. Siderophore production

Among the eight isolates from *Typha spp.* i.e. TRCD1, TRCD2, TRCD3, TRCD4, TRCD5, TRCD6, TRCD7 and TRCD8, only strains TRCD1, TRCD2 and TRCD7 showed positive result for siderophore production (Fig.6.4a and Table 6.2). Subsequently, strain PHRCD1, PHRCD2, PHRCD3, PHRCD4, PHRCD6, PHRCD7 and PHRCD8 showed positive result isolated from *Phragmites communis* (Fig. 6.4b and 6.1).

All the isolates showed IAA production. Pink coloration was detected in their cell-free supernatant with inclusion of tryptophan in culture broth; maximum IAA production was recorded in strain PHRCD2, PHRCD5 and PHRCD6 (17 µg ml−1) broth culture (Figure 6.2a and Table 6.1.).

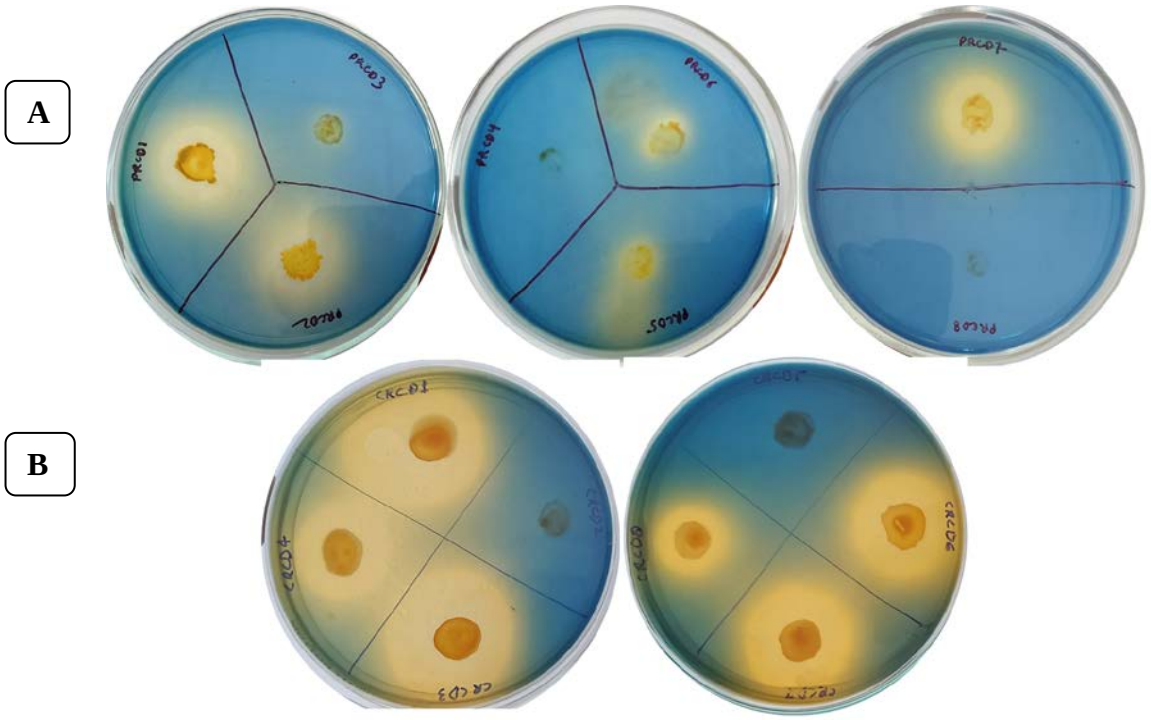


Fig. 6.4. Siderophore production test. (a) Siderophore production by rhizobacteria isolated from *Typha spp.* (b) Siderophore production by rhizobacteria isolated from *Phragmites communis*

6.3.4. N_2 production

Rhizospheric bacterial isolated strains were screened by changing the color of bromothymol blue (BTB) containing nitrogen free media, and these strains showed a color change in BTB containing media, indicating excretion of ammonia. Only strain PHRCD2, PHRCD3, PHRCD4, PHRCD6 and PHRCD8 were found positive for nitrogen fixing isolated from *Phragmites communis* (Fig. 6.4a. and Table 6.1) strain PRCD5, PRCD6, and PRCD8 were found positive for isolated rhizospheric strains from *Typha spp.*. (Fig. 6.4b. and Table 6.2)

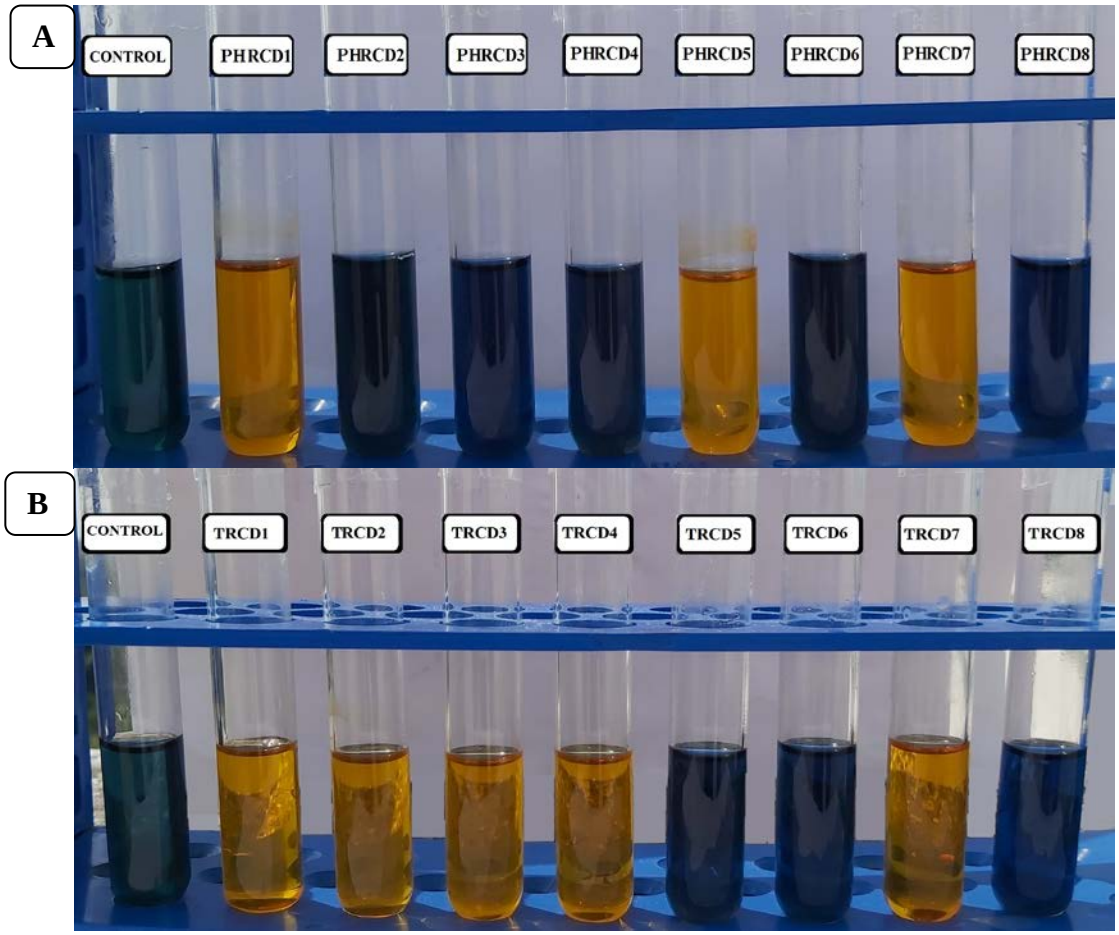


Fig. 6.5. Showing nitrogen production by isolated PGPR strains. (a) Rhizospheric strain from *Phragmites communis* (b) Rhizospheric strain from *Typha spp.*

6.3.5. Phosphate solubilization

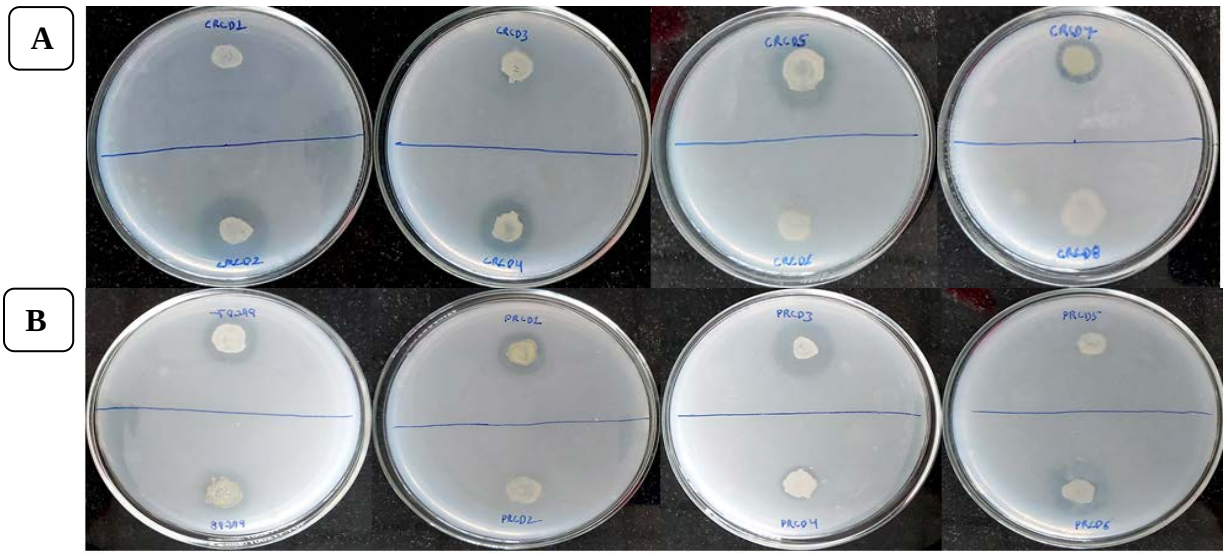
Among the eight strains only PHRCD2, PHRCD4, PHRCD5, and PHRCD7 showed significantly clearing of zone around the colonies (Fig. 6.5a. and Table 6.1.) isolated from rhizosphere of *Phragmites communis*. Whereas, strain TRCD1, TRCD2, TRCD3, TRCD5 and TRCD8 showed zone formation isolated from *Typha spp.*

At 3 days of incubation, the Phosphate Solubilization Index (PSI) of phosphate solubilizing bacterial strains was measured by the following equation:

$$PSI = \frac{\text{Colony Diameter + Total diameter of halo zone}}{\text{Colony diameter}}.$$

The sequence of PSI was as follows

PHRCD2 (3.1±0.2) > PHRCD4 (2.8 ± 0.4) > PHRCD5 (2.5 ± 0.1) > PHRCD7 (1.5 ± 0.2)



TRCD5 (3.2 ± 0.2) >TRCD1 (2.9) > TRCD3 (2.4) > TRCD2 (1.4) > TRCD8 (1.3)
Fig.6.6. Phosphate solubilization (a) Showing the phosphate solubilization of the isolated bacteria from the rhizosphere of *Phragmites communis*. (b) Showing the phosphate solubilization of the isolated bacteria from the rhizosphere of *Typha spp.*

6.3.6. *Zinc solubilization*

Zinc solubilization bacteria improve the plant growth and development by colonizing the rhizosphere and by solubilizing complex zinc compounds into simpler ones, thus making zinc available to the plants. Rhizospheric strain PHRCD2, PHRCD4, PHRCD6, PHRCD8, showed zinc solubilization isolated from *Phragmites communis* (Fig. 6.5a and Table 6.1). Additionally, rhizobacteria isolated from Typha spp. i.e. TRCD2, TRCD4, TRCD6, and TRCD8 showed zinc solubilization ((Fig. 6.5b and Table 6.1).

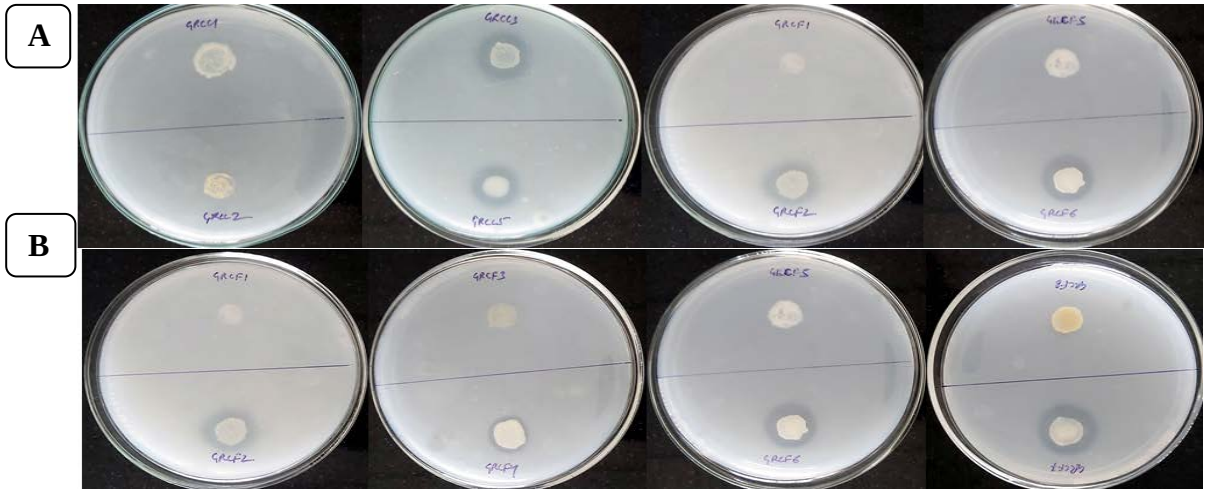


Fig.6.7. Zinc solubilization (a) Showing the zinc solubilization of the isolated bacteria from the rhizosphere of *Phragmites communis*. (b) Showing the zinc solubilization of the isolated bacteria from the rhizosphere of *Typha spp.*

6.3.7. *Potassium solubilization*

Potassium solubilization bacteria help in solubilizing the insoluble and inaccessible potassium (K) to accessible forms of potassium for plant uptake and transport. The strains isolated from *Phragmites communis* i.e. PHRCD2, PHRCD4, PHRCD5 and PHRCD7 showed potential for potassium solubilization (Fig. 6.5a and Table 6.1). Subsequently, rhizobacterial strains isolated from *Typha spp.* i.e. TRCD1, TRCD4, TRCD6 and TRCD7 showed potential for potassium solubilization (Fig. 6.5b and Table 6.2).

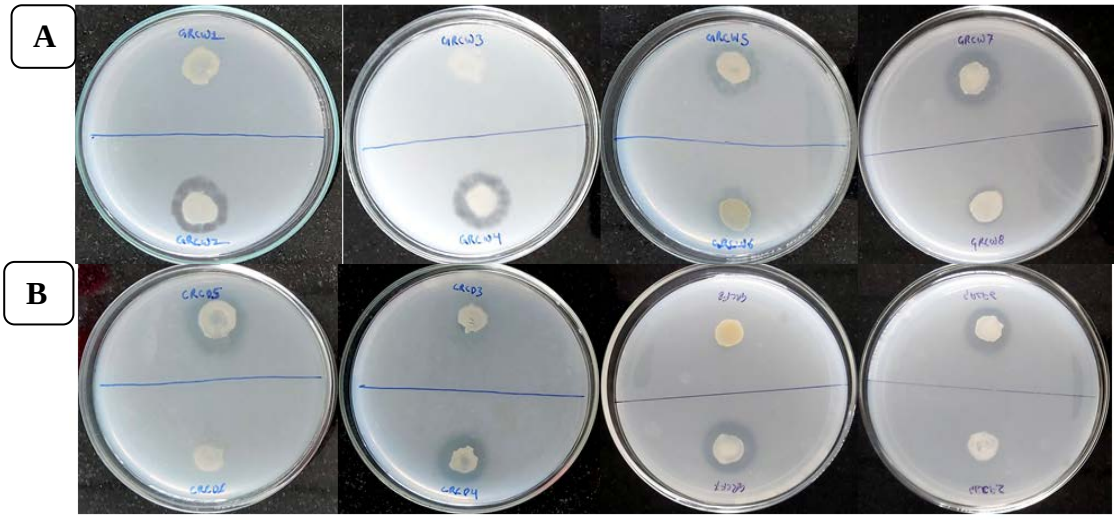


Fig. 6.8. Potassium solubilization (a) Showing the potassium solubilization of the isolated bacteria from the rhizosphere of *Phragmites communis*. (b) Showing the potassium solubilization of the isolated bacteria from the rhizosphere of *Typha spp.*

Table 6.1.: Detection of plant growth promoting parameter of rhizospheric bacteria isolated from *Phragmites communis* rhizosphere

ISOLATES	IAA	NH_3	Siderophore production	N_2 Fixation	Phosphate Solubilization	Zinc Solubilization	Potassium Solubilization
PHRCD1	+	++	+++	-	+	+	+
PHRCD2	++	+	+++	+	++	+	+
PHRCD3	-	++	+	+	-	-	-
PHRCD4	-	+++	-	+	+	+	++
PHRCD5	++	++	++	-	+	-	+
PHRCD6	++	++	++	+	-	++	-
PHRCD7	-	+	+++	-	+	-	++
PHRCD8	-	+	-	+	-	++	-

6.3.8. 16S rRNA gene analysis of rhizosphere bacteria and phylogenetic analysis

Purified isolated rhizosphere bacterial strains from *Phragmites communis* were identified on the basis of 16S rRNA sequencing. Among the seven rhizospheric bacterial strains i.e., PHRCD1, PHRCD2, PHRCD3, PHRCD4, PHRCD5, PHRCD6, PHRCD7 and PHRCD8, only four bacterial strains i.e. PHRCD1, PHRCD2, PHRCD6 and PHRCD7 could show most of the positive PGPR test. 16s rRNA sequencing data, the isolated strains PHRCD1, PHRCD2, PHRCD6 and PHRCD7 showed nearest relationships with *Pantoea agglomerans, Pseudomonas putida, Bacillus coagulans* and, Bacillus subtilis. The nucleotide sequences of these potentially diverse rhizospheric bacteria were deposited in the National Center for Biotechnology Information (NCBI) database under accession numbers ON740649, ON746677, ON740650 and, ON740653 respectively. Additionally, purified isolated rhizosphere bacterial strains from *Typha spp.* were also identified from TRCD1, TRCD2, TRCD3, TRCD4, TRCD5, TRCD6, TRCD7 and TRCD8, only four bacterial strains i.e. TRCD1, TRCD3, TRCD4 and TRCD6 could show most of the positive PGPR test. 16s rRNA sequencing data, the isolated strains TRCD1, TRCD3, TRCD4 and TRCD7 showed nearest relationships with *Pseudomonas putida, Bacillus Edaphicus, Streptomonas maltophilia,* and Enterobacter cloacae. The nucleotide sequences of these potentially diverse rhizospheric bacteria were deposited in the National Center for Biotechnology Information (NCBI) database under accession numbers ON740655, ON740654, ON740658 and ON740657 respectively. Using the MEGA 11 software, a phylogenetic tree was generated that illustrated the relationships between bacterial species and the most closely related genera deduced from sequences of the 16S rRNA gene (Fig.6.7) (Kumar and Chandra, 2018).

Table 6.2.: Detection of plant growth promoting parameter of rhizospheric bacteria isolated from *Typha spp* rhizosphere

ISOLATES	IAA	NH_3	Siderophore production	N_2 Fixation	Phosphate Solubilization	Zinc Solubilization	Potassium Solubilization
TRCD1	++	++	+++	-	++	-	+
TRCD2	-	+	++	-	+	+	-
TRCD3	++	++	+++	-	++	+	-
TRCD4	++	+++	-	-	-	+	+
TRCD5	++	++	-	+	+++	-	-
TRCD6	++	++	+++	+	-	+	+
TRCD7	++	+	+++	-	-	-	+
TRCD8	-	+	+	+	++	+	-

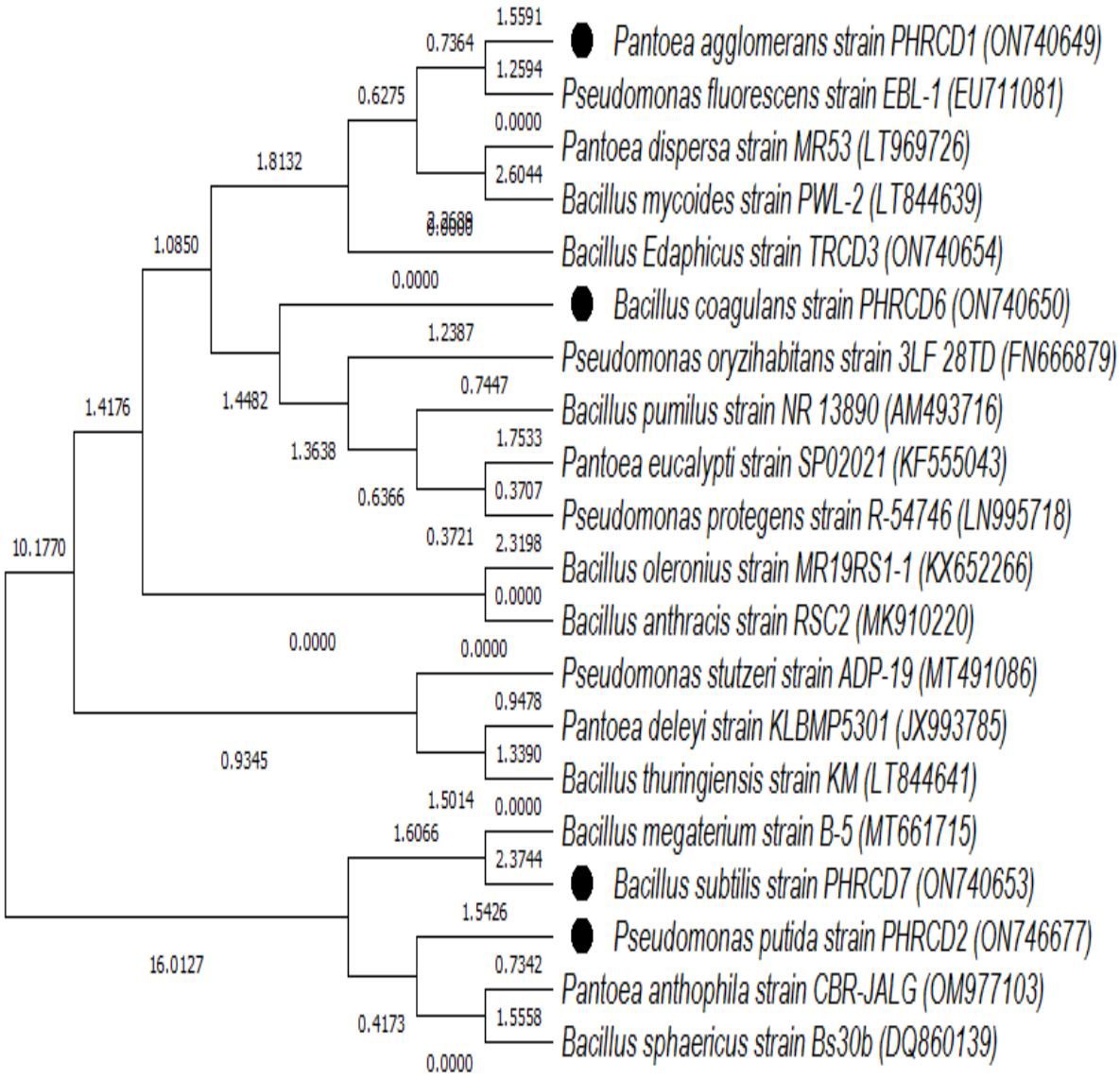


Fig. 6.9. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (-30498.27) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. This analysis involved 20 nucleotide sequences. There were a total of 1555 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021)

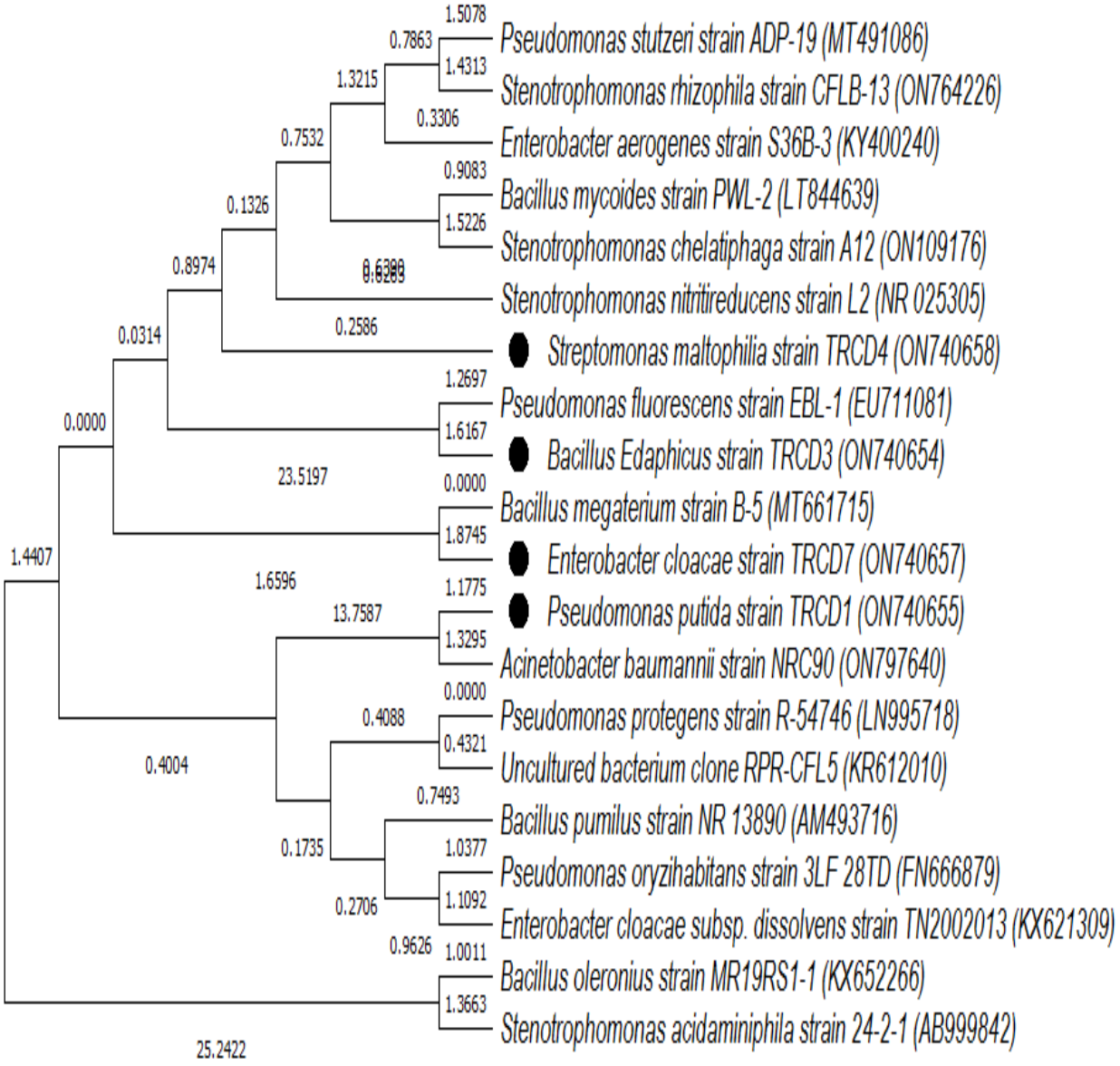


Fig. 6.9.1. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (-30498.27) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. This analysis involved 20 nucleotide sequences. There were a total of 1555 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021)

6.3. Conclusion

Phragmites communis and *Typha spp.* is a naturally robust and vigorous primary species in many wetland environments worldwide. This plant grows in different environmental conditions and can uptake, translocate, and accumulate a wide range of pollutants in both belowground and aboveground tissue. The ability of the plant to develop and grow in the polluted ecosystems allowed for the use of reeds in many types of sewage treatment plants. To increase the efficiency of phytoremediation of a polluted natural or artificial aquatic ecosystem and to estimate the required purification time and accelerate the rate of its reclamation, the interaction processes between common reeds and soil microbes, metal accumulation, and ionic homeostasis in the hydrophyte purification systems should be further tested. The researches of especially research carried out by interdisciplinary teams (plant physiologist, biochemist, geochemist, microbiologist, and agriculture and genetic engineer) in a short time can advance the efficiency of removing both metals and organic impurities.

Chapter 7

Correlation between bacterial community and organometallic pollutants during in-situ phytoremediation of distillery waste contaminated site

Chapter-7
Correlation between bacterial community and organometallic pollutants during in-situ phytoremediation of distillery waste contaminated site

7.1. Introduction

Soil organic matter is considered to be the single most important constituent which influence the sorption of various compounds in soil (Dhaliwal et al., 2019). The organic matter is added to soil from plant, animal and microbial residues, and, it is composed of many different organic molecules (Weisskopf et al., 2021). These are mainly cellulose, hemicelluloses and lignin compounds. They are easily degraded and stabilized, especially when forming complexes with mineral particles; or it may be potentially more accessible to microbial degradation as particulate organic matter (POM), the latter typically with characteristic features of plant residues (Rodrigues et al., 2022). Besides, the soil type also determines the biosorption properties of various organic compounds. Therefore the content of organic matter differs from place to place depending upon the nature of soil (Carter, 2020). Depending on the soil particle size fractions, which can be distinguished as clay (o2 mm), fine silt (2–20 mm), coarse silt (20–63 mm) and sand (63–2000 mm), the composition of their associated organic matter is also different (Hemkemeyer et al., 2018).

Soil quality is underpinned by a complex suite of below ground processes in both natural and agricultural ecosystems (Hermans et al., 2020). Soil quality is defined as the ability of soil to function as an ecosystem component capable of maintaining the quality of surrounding air and water while supporting plant and animal productivity. High quality soils are therefore crucial for sustaining agricultural and pastoral industries upon which both food security and financial stability depend. Soils harbour a rich collection of microbial life, which contribute to the cycling of important nutrients, impact plant growth and can act as, or protect other organisms from, pathogens. Macroorganisms interact with microorganisms to facilitate this and independently are important for processes such as decomposition. Despite the importance of living organisms for maintaining healthy soil ecosystems, most initiatives that directly monitor soil quality for applied purposes focus on changes in abiotic variables such as

soil nutrients, metal pollutants and soil structure. Where biological measures are included in monitoring efforts, they are often crude and generalized, such as microbial biomass or soil respiration, although some use more specific organisms, such as earthworms, as more sensitive indicators. As well as relaying important information about the biological functioning of the ecosystem, soil organisms only respond to bioavailable nutrients and contaminants, unlike chemical measures which reflect the total proportion present. Better incorporation of biological indicators in soil monitoring will provide a more sensitive, relevant and holistic insight into how anthropogenic activity impacts the soil environment. Soil bacterial communities are strongly impacted by changes in soil conditions. The diversity and composition of bacterial communities change with changing soil acidity (Wan et al., 2020). At national scales or larger, this is often observed to be the strongest explanatory variable for bacterial community richness to the extent where large-scale predictions of bacterial diversity are possible based on pH data alone. Additionally, plant diversity, nutrient concentrations, soil moisture and soil type have all been shown to correlate with changes in bacterial communities. Importantly, there is ample evidence that bacterial communities directly, or indirectly, respond to changes in the soil environment brought on by anthropogenic activity. Land use has been shown to correlate with changes in bacterial community composition, and heavily managed soils contain distinct bacterial communities compared to unmanaged soils (Praeg et al., 2020). More specifically, management practices such as fertilising, altering soil pH and creating monocultures of plants or animals have all been shown to influence soil microbial communities. Overall, the composition of bacterial communities appears to be heavily influenced by changes in the soil environment, many of which are the direct result of land use activities (Hermans et al., 2020). Given their ubiquitous nature, and sensitivity to environmental changes, bacterial communities are gaining recognition as useful indicators of environmental health. In stream ecosystems, bacterial communities have been shown capable of indicating the level of catchment disturbance, with results correlating with both abiotic water quality data and traditional macroinvertebrate community indicator data (Sagova-Mareckova et al., 2021). In soil ecosystems, similarly strong correlations between specific microbial taxa and soil variables have been reported, suggesting microbial community data can be used to indicate changes in physico-chemical conditions, serve as indicators of ecological restoration and even predict crop yields.

While progress has been made towards better understanding how bacterial communities can be indicative of environmental health, more effort needs to be made, and soil bacteria remain largely understudied in this regard. Investigating if soil bacterial communities respond in a predictable manner to human land use and soil physico-chemical changes across a wide variety of different soils, spatial gradients and climatic conditions will reveal their potential to serve widely as indicators of soil quality.

Pollution load is increasing at an alarming rate as a result of industrialization and population outburst. The industrialization has caused in the utilization and production of chemicals for hi-tech innovations which ensued the generation of non-biodegradable pollutants like xenobiotics, hydrocarbons, heavy metals, etc. (Chandran et al., 2020). These toxic pollutants remain persistent in the environment and pose a serious threat to living organisms. Increasing awareness has generated numerous approaches using advanced scientific technology to audit and curtail this arduous global issue.

Distilleries are among the most polluting industries because ethanol fermentation results in the discharge of large quantities of high-strength liquid effluents with high concentrations of organic matter and nitrogen compounds, low pH, high temperature, dark brown color, and high salinity (Mikucka and Zielińska, 2020). The wastewater discharged contains high biological oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS), sulfate, phosphate, phenolics, and toxic heavy metals. Unfortunately, if discharged into the environment without proper treatment, it causes serious environmental problems and health hazards in human and animals.

Organic and heavy metal pollution alters the soil microbial community composition, and the microorganisms that adapt to this stress increase in abundance (Huang et al., 2021). The remediation process of contaminated soil not only reduces the concentration of pollutants but also alters the bacterial communities (Dai et al., 2020). Soil bacterial communities are strongly impacted by changes in soil conditions. The diversity and composition of bacterial communities change with changing soil acidity. At national scales or larger, this is often observed to be the strongest explanatory variable for bacterial community richness to the extent where large-scale predictions of bacterial diversity are possible based on pH data alone. Additionally,

plant diversity, nutrient concentrations, soil moisture and soil type have all been shown to correlate with changes in bacterial communities. Importantly, there is ample evidence that bacterial communities directly, or indirectly, respond to changes in the soil environment brought on by anthropogenic activity (Dubey et al., 2019).

The present chapter has been focused to explore the correlation between bacterial community and organometallic pollutants during in-situ phytoremediation of distillery waste contaminated site, where the physiochemical properties of distillery sludge has been analysed to look the primary feature of sludge. Further, the organic content of distillery sludge also has been detected by using GC-MS after extraction by solid-liquid extraction (Kumar and Chandra, 2020). This will establish soil quality regarding organic parameters. In addition, the bacterial community from the rhizosphere of *Parthenium hysterophorus* and *Cannabis sativa* has been analysed through metagenomics to correlate with the organic pollutants present in the distillery sludges. The study will reveal the dominant bacterial growing with rhizosphere of *Parthenium hysterophorus* and *Cannabis sativa* which has been found potential for in-situ phytoremediation. This will explore the possibility of using these plants as tool for phytoremediation and eco-restoration of polluted site. Secondly, the degraded sludge can be used as high value organic manure. Besides, the plant biomass also has various commercial applications for the Indian farmers and sustainable development of industry.

7.2. Material and Methods

7.2.1. Site description

Sludge samples for laboratory analysis were collected from the sludge disposal site of the M/s United Spirits Limited, Rosa Shahjahanpur, located in Uttar Pradesh (27°49'25.1"N 79°54'56.5"E). Having a crushing capacity of about 4,200 tonnes of sugarcane per day. Estimated Molasses Produced 40500 Metric Tonnes Per Annum.

7.2.2. Sample collection

Sludges have much higher solids content than most wastewaters. Solids content and solids settling characteristics determine whether a given sludge will separate into different a fraction which increases the potential of obtaining a nonrepresentative

sample. In general, sludges of up to 20 percent solids may be conveyed by means of a pump. Sludge with a greater solids content, often referred to as sludge cake, must be conveyed by mechanical means. Increased solids content may require sample dilution and cause a corresponding increase in experimental error and detection limits. Anaerobically digested sludge is a thick slurry of dark-colored particles and entrained gases. When well digested, it dewaters easily and has a non-offensive odor. The addition of chemicals coagulates a digested sludge prior to- mechanical dewatering. The dry residue of digested sludge contains 30 to 50 percent volatile solids. Depending on the mode of digester operation, the percent solid of digested sludges ranges from 4 to 8 percent.

The fresh disposed dried distillery sludge cakes were collected in clean pre-sterilised polythene bags from the sludge dumping site of the distillery plant located inside the premises of the industry. Plants that were growing on the disposed distillery sludge i.e. *Parthenium hysterophorus* and *Cannabis sativa* were also uprooted for physiochemical analysis and its correlation with organometallic pollutants and its rhizospheric bacterial community.

Fig.7.1. Sample collection (a & c) Sludge sample collection from Rosa, Shahjahanpur, UP. (b) Collection of *Parthenium hysterophorus* with rhizospheric sludge (d) Collection of *Cannabis sativa* with rhizospheric sludge

7.2.2. Detection of organic pollutants

7.3.4.Physico-chemical analysis of distillery sludge

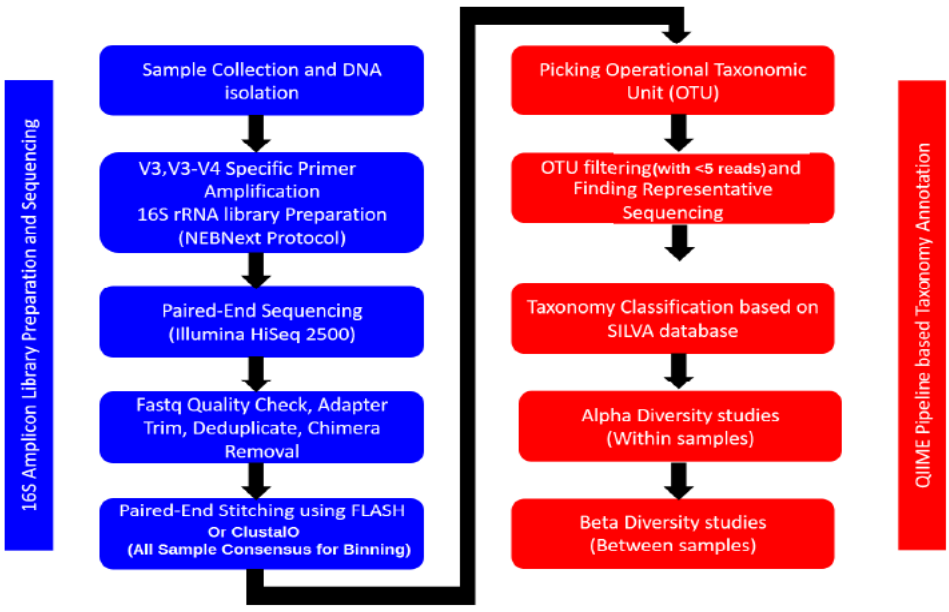
Samples of distillery sludge were analysed in accordance with the previously described method in chapter 2 for the determination of the pH, electrical conductivity, chloride (Cl^-), sodium (Na^+), nitrate and metals i.e. Fe, Zn, Cu, Mn, Ni, Pb, Cd and Cr (Dhote et al., 2020

7.3.5. *Solid liquid extraction of distillery and rhizospheric sludge*

Various organic compounds from the distillery sludge and rhizosphere soil were extracted using ethyl acetate as per the previously mentioned method (Chandra et al., 2018). The extraction was repeated three times. The organic solvent phase was dried over anhydrous Na_2SO_4, and the solvent was evaporated to dryness using a stream of nitrogen gas at room temperature. Dry organic filters were makeup in 1.5 ml ethyl acetate and filtered through 0.22 µm syringe filters (Millipore Ltd, Bedford, Massachusetts, USA) and used for GC–MS analysis (Kumar and Chandra, 2020).

7.3.6. Metagenomics analysis

The sample for metagenomics analysis was given to M/s AgriGenome Labs Pvt Ltd., India. The procedure followed is as below:



7.3.6.1. DNA isolation and 16s rRNA amplicon-based Illumina Library preparation

The DNA was isolated from soil samples using DNeasy PowerSoil kit (Qiagen, USA) and 2% CTAB conventional DNA extraction method for shoot and root samples as per the described protocol. The DNA concentration was estimated using Qubit Fluorimeter (V.3.0). Since, the V3-V4 region is highly conserved in bacteria. Therefore, the V3-V4 region of 16S rRNA was amplified using specific V3 Forward primer CCTACGGGNBGCASCAG and V4 Reverse primer GACTACNVGGGTATCTAATCC. The amplified product was checked on 2% agarose gel and gel purification was done to remove non-specific amplifications.5ng of amplified product was used for library preparation using NEBNext Ultra DNA library preparation kit. The 16S rRNA gene library was prepared in accordance with the Illumina MiSeq platform protocols (https://sapac.illumina.com/systems/sequencing-platf orms.html). The library quantification and quality estimation were done in Agilent 2200 TapeStation. The prepared library was sequenced in Illumina HiSeq 2500 with 2 x 250 cycles chemistry (Fahner et al., 2016).

7.3.6.2. Bioinformatics analysis

Rhizospheric bacterial community composition of *S. arundinaceum* was analyzed based on the raw V3–V4 16S rRNA sequencing data as prepared by Illumina MiSeq platform. Raw sequences obtained through paired-end sequencing were merged using standard procedure (Rochette et al., 2019). Furthermore, raw sequences were processed using the QIIME (Quantitative Insights Into Microbial Ecology) software package (version 1.7.0) (Zhang et al., 2019). Raw sequences were denoised and trimmed to remove barcodes, and primers and then clustered into operational-taxonomic units (OTUs) based on their sequence similarity at 97% using UCLUST program (version 1.2.22q). Phylogenetic relationship with diferent OTUs was analyzed using PyNAST (Python Nearest Alignment Space Termination) program (version 1.2) against “Core Set” dataset of Greengenes as referencing (Na et al., 2018). The taxonomic assignment was performed with a ribosomal database project (RDP) classifer (version 2.2) (Brandt et al., 2021) against the SILVA OTUs database (version 123) using a confdence threshold of 70% (Hung et al., 2022). Diference in diversity was calculated using Chao1, Shannon-D, and Observed-species indices estimation. The chao1 metric

estimates the species richness in environmental sample, while Shannon metric was evaluated to estimate observed OTU abundances, and accounts for both evenness and richness of species. Rarefaction curves were generated based on Shannon, Chao1, and Observed-species indices by QIIME (version 1.7.0) and displayed using R software (version 2.15.3).

7.3. Result and Discussion

7.3.1. Physico-chemical analysis of fresh sludge, sludge from rhizosphere of Parthenium hysterophorus and sludge from rhizosphere of Cannabis sativa

The physio-chemical parameter of fresh distillery sludge disposed from methane reactor, rhizospheric sludge of *Parthenium hysterophorus* and *Cannabis sativa* were analysed. The fresh sludge did not show any change of their property while the plant growing on disposed distillery sludge i.e. *Parthenium hysterophorus* and *Cannabis sativa* showed reduced value of analysed parameter as compared to fresh sludge, shown in Table 7.1.

The different parameters were analysed as per the previously described method (Chandra et al., 2018). The measured pH value, Electrical conductivity (EC), sodium, phosphate, chloride, nitrate, phenol sulfate showed higher in disposed fresh distillery sludge. Additionally, value of trace elements i.e. Fe, Zn, Pb, Cd, Mn, Ni, and Cu are found higher than permissible limit and toxic to animal and human health because of their long term persistence in the environment (Onakpa et al., 2018; Rehman et al., 2018).

Sludge has an alkaline pH of 8.1, which results from reactivity of numerous salts and oxides i.e. carbonates, bicarbonates, sulphides, and hydroxides. They are mostly applied in the process of fermentation to modify the pH level (Chandra et al., 2018). The value of EC has also been detected high due to various anions and cations present in sludge such as Na^{+}, Cl^{-}, NO^{-3}, PO_4^{3-} and SO_4^{2-}.

Table 7.1. Physico-chemical properties of disposed distillery sludge, sludge from rhizosphere of *Parthenium hysterophorus* and Sludge from rhizosphere of *Cannabis sativa*

S.No.	Parameter	Fresh distillery sludge	Sludge from rhizosphere of ***Parthenium hysterophorus***	Sludge from rhizosphere of ***Cannabis sativa***
1.	pH	8.1 ± 0.03	7.4 ± 0.01	7.6 ± 0.24
2.	EC (µS cm^{-1})	5.02 ± 0.11	5.09 ± 0.14	5.07 ± 0.67
3.	Sodium (mg kg^{-1})	43.17 ± 0.02	35.87± .057	36.06 ± 0.18
4.	Chloride (mg kg^{-1})	1101.11 ± 0.01	785.09 ± 0.84	792 ± 0.94
5.	Nitrate (mg kg^{-1})	89.02 ± 0.13	54.06 ± 0.67	38.95 ± 0.21
6.	Phosphate (mg kg^{-1})	2229.01 ± 0.03	1308.62 ± 0.17	1451 ± 0.36
7.	Sulfate (mg kg^{-1})	158.18 ± 0.11	98.02 ± 0.45	102.61 ± 0.98
8.	Phenol (mg kg^{-1})	506.13 ± 0.65	256.05 ± 0.84	276.84 ± 0.74
	Trace Elements (mg kg^{-1})			
a.	Fe	2264.01 ± 0.05	425.01 ± 0.60	512.65 ± 0.12
b.	Zn	53.02 ± 0.27	27.05 ± 0.51	22.06 ± 0.41
c.	Cu	352.01 ± 0.21	151.65 ± 0.84	132.85 ± 0.35
d.	Mn	171.04 ± 0.16	84.62 ± 0.74	71. 65 ± 0.74
e.	Ni	13.01 ± 0.02	7.62 ± 0.51	5.06 ± 0.84
f.	Pb	38.12 ± 0.10	16.32 ± 0.27	14.65 ± 0.12
g.	Cd	1.57 ± 0.13	0.24 ± 0.64	037 ± 0.84
h.	Cr	1.62 ± 0.14	0.27 ± 0.03	0.35± 0.08

7.3.2. Identification of organic pollutants

The GC-MS analysis of an ethylene acetate extracted sample from disposed distillery sludge revealed numerous significant peaks at 7.94, 10.57, 13.00, 18.57, 21.59, 26.16, 27.88, 29.70, 33.12, 37.56, 38.71, 41.03, 41.77, 44.33 and 50.26 as shown in Fig. (a). This indicated the presence of various complicated organic pollutants which are soluble in ethylene acetate. These compounds were identified by comparing the National Institute of Standards and Technology (NIST) library. These were identified as Diacetone alcohol, Pentasiloxane, Erythritol, 2,6-Bis(tert-butyl)phenol, 3-Chloropropionic acid, 2-Pyrrolidinone, Cyclic octaatomic sulphur, Cyclohexane, 1,3,5-Benzetriol, Lupan-3-ol, acetate, 2,4-Dihydroxybenzoic acid, 1-Octacosanol, 24-Ethyl-ë(22)-coprostenol, á-Sitosterol, Tris(2,4-di-tert-butylphenyl) phosphate (Table 5). Further, the organic compounds were also identified by GC-MS from same site where *Parthenium hysterophorus* was growing on the disposed distillery sludge. The identified compounds were as Thiazolo[4,5-f]quinoline, 2,7,9-trimethyl-, 2-Methyl-1-methyl (tetramethylene)silyloxypropane, Tartronic acid, Heptanoic acid, 4-Heptanol,

Phthalic acid, Silane, 1-Triethylsilyloxyheptadecane, Cannabinol, á-Sitosterol, Lanostan-18-oic acid, Quercetin, 11-hexavinylcyclo hexasiloxane, and 1,3,5,7,9,11,13-Heptaethylcycloheptasiloxane (Table 7.2), with altered RT and reduced peak (Fig.b). This indicated the biotransformation and removal of several organic compounds due to phytoremediation process by *Cannabis sativa*. The disappearance of some compounds revealed the degradation by rhizospheric microbial activities which facilitated for plant growth and accumulation of these pollutants as nutrient. While, most of the detected peaks at various RT were disappeared in the distillery sludge sample chromatogram of same site where the *Cannabis sativa* was growing (Fig.c). This result showed direct correlation of plant growth by using these organic compounds as nutrient (Sophia, and Shetty; 2020).

Table 7.2: Detection of comparative list of various organic compounds by GC-MS extracted from distillery waste at various stages (a) Sludge from rhizosphere of *Parthenium hysterophorus* (b) Sludge from rhizosphere of *Cannabis sativa*

S.No.	**Retention Time (RT)**	**Initial (Fresh)**	**Sludge from rhizosphere of *Parthenium hysterophorus***	**Sludge from rhizosphere of *Cannabis sativa***
1.	6.21	-	Thiazolo[4,5-f]quinoline, 2,7,9-trimethyl-	-
2.	7.90	-	-	Hexanoic acid
3.	7.93	-	2-Methyl-1-methyl (tetramethylene)silyloxypropane	-
4.	7.94	Diacetone alcohol	-	-
5.	10.57	Pentasiloxane	-	Pentasiloxane
6.	13.00	Erythritol	Tartronic acid	-
7.	13.01	-	-	Erythritol
8.	18.57	2,6-Bis(tert-butyl)phenol	-	-
9.	19.19	-	Heptanoic acid	-
10.	19.22	-	-	Undecanoic acid
11.	21.59	3-Chloropropionic acid,	4-Heptanol	-

12.	21.61	-	-	Dodecyl acrylate
13.	24.64	-	Phthalic acid,	-
14.	25.17	-	-	2-Fluoro-6-(4-thiomorpholinyl)benzonitrile
15.	26.16	2-Pyrrolidinone	-	-
16.	27.44	-	Silane	-
17.	27.88	Cyclic octaatomic sulphur	-	-
18.	27.96	-	-	Spiro(androstan-17,5'-furan-2'-one)
19.	29.70	Cyclohexane		-
20.	30.34	-	-	Bifenox
21.	30.45	-	1-Triethylsilyloxyheptadecane	-
22.	33.12	1,3,5-Benzetriol	-	-
23.	33.97	-	Cannabinol	-
24.	34.93	-	-	Ethanone
25.	37.56	Lupan-3-ol, acetate	-	4-Normethyl-9
26.	38.71	2,4-Dihydroxybenzoic acid	-	-
27.	41.03	1-Octacosanol	-	-
28.	41.77	24-Ethyl-ë(22)-coprostenol	-	-
29.	42.67	-	-	4'-(Salicylideneamino)acetanilide

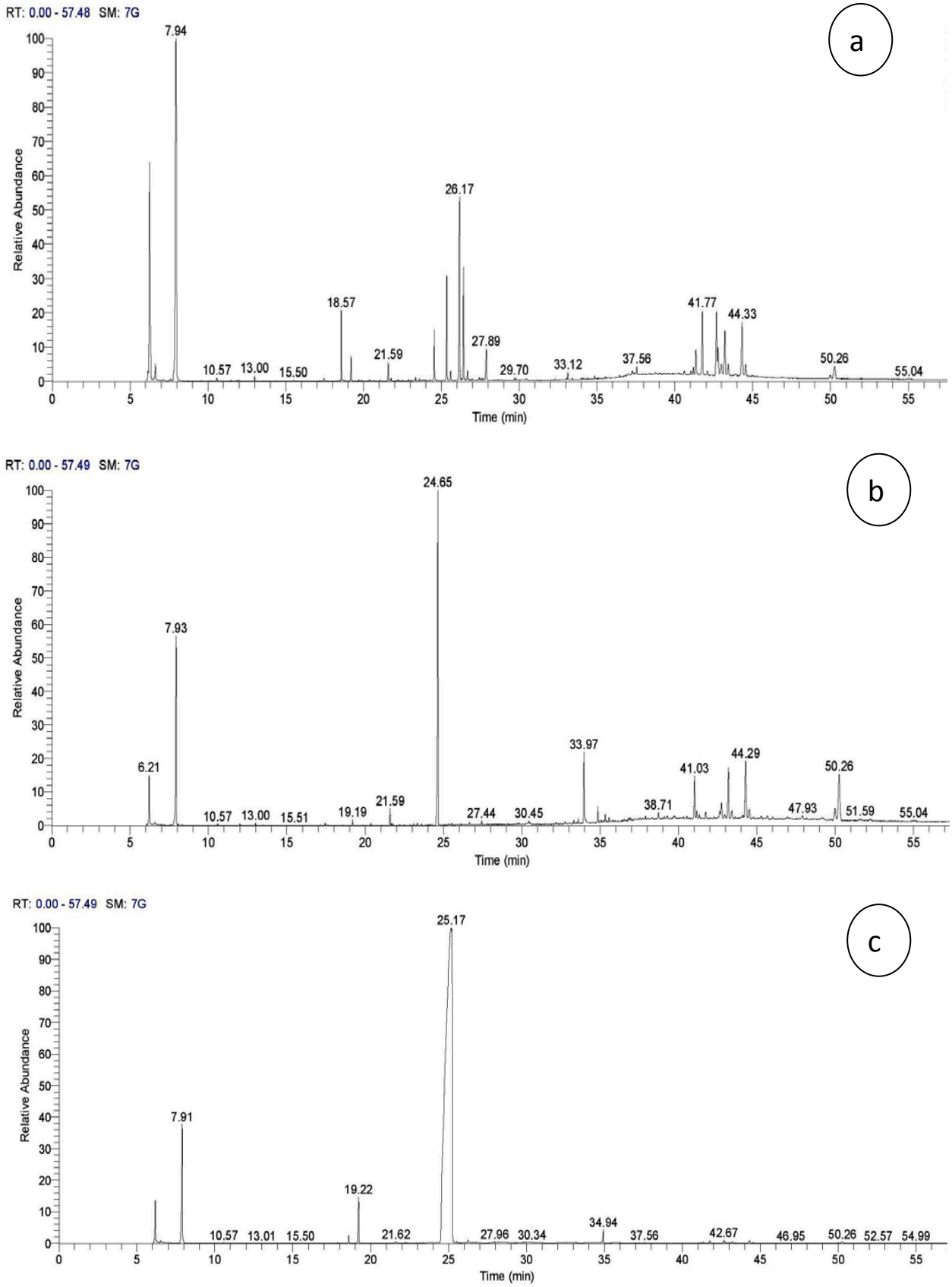


Fig. 7.2. Chromatogram of organic compounds extracted from distillery sludge (a) Fresh sludge without plant growth (b) Sludge from rhizosphere of *Parthenium hysterophorus* (c) Sludge from rhizosphere of *Cannabis sativa*

7.3.3. DNA isolation and 16s rRNA amplicon-based Illumina Library preparation

The DNA was isolated from soil samples using DNeasy PowerSoil kit (Qiagen, USA) and 2% CTAB conventional DNA extraction method for shoot and root samples as per the described protocol. The DNA concentration was estimated using Qubit Fluorimeter (V.3.0). The V3-V4 region of 16S rRNA was amplified using specific V3 Forward primer CCTACGGGNBGCASCAG and V4 Reverse primer GACTACNVGGGTATCTAATCC. The amplified product was checked on 2% agarose gel and gel purification was done to remove non-specific amplifications.5ng of amplified product was used for library preparation using NEBNext Ultra DNA library preparation kit. The library quantification and quality estimation were done in Agilent 2200 TapeStation. The prepared library was sequenced in Illumina HiSeq 2500 with 2*250 cycles chemistry. The library preparation steps are illustrated in Figure 7.2.

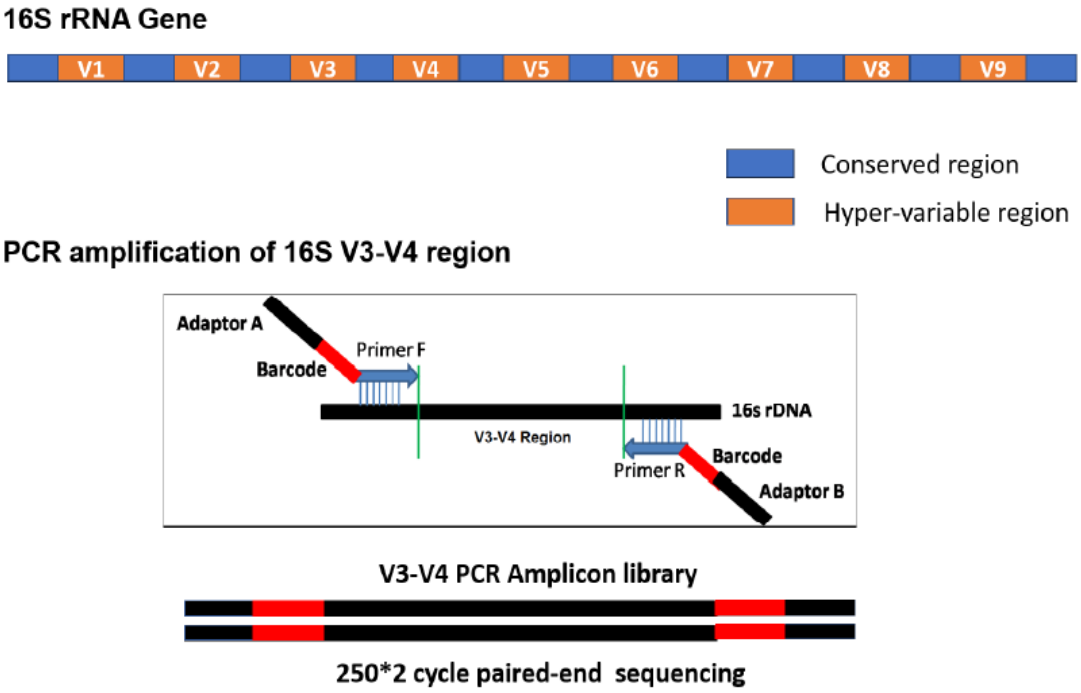


Fig. 7.3. Graphical representation of 16S rRNA V3-V4 library preparation and sequencing

7.3.4. GC distribution

The average GC content distribution of the sequenced read of the samples is shown in the figure below. The x-axis represents average GC content in the sequence and y-axis represents percentage of sequences. We observed that the reads have GC content in the range 30-60% (Auti et al., 2019).

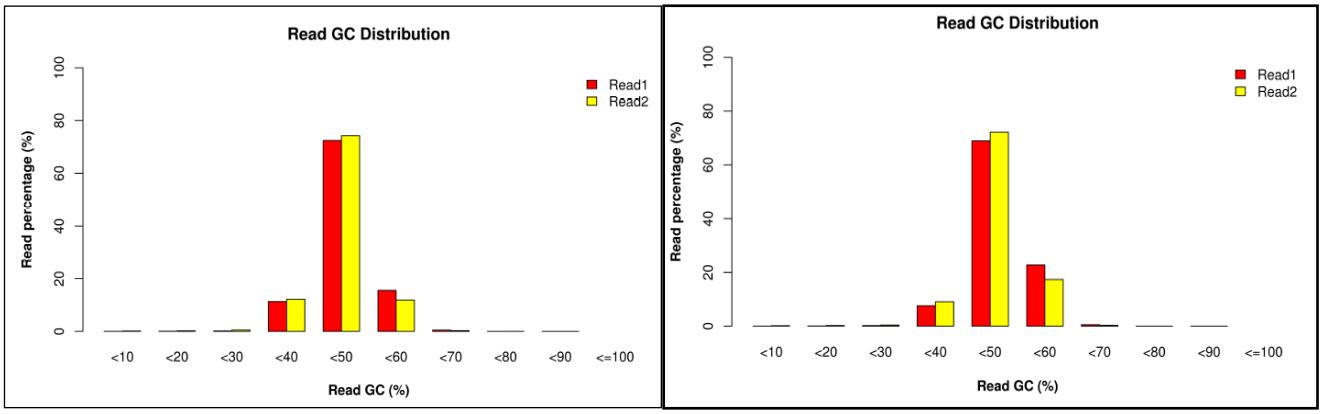


Fig. 7.4. GC distribution of samples (a) CS (b) PR

7.3.5. Identification of V3-V4 region from paired-end reads

Following steps were performed to extract V3-V4 region from Illumina paired-end sequences.

a) Trimming of sequencing primers

The forward V3 specific primer and reverse V4 specific primers were trimmed using In-house PERL script. The properly paired end reads with Phred score quality (Q>20) were considered for V3-V4 consensus generation.

b) Building consensus V3-V4 region from trimmed paired-end reads

The primer trimmed, high quality paired-end reads were pair-wisely allowed to merge/stitch to get the V3-V4 amplicon consensus FASTA sequences. The reads were merged using FLASH program (version 1.2.11) with minimum overlap of 10bp to maximum overlap of 240bp with Zero mismatches. While making consensus V3-V4 sequence all consensus reads formed with an average contig length of 350 to 450bp (Mohsen et al., 2019). A summary of trimmed consensus reads is shown in Table 7.3.

Table:7.3 Trimmed and Consensus Read Summary

Sample Name	**Total Paired end-Reads**	**Passed Reads with Primers**	**Total Consensus Sequences**
CS	230166	230166	195067
PR	277515	277515	235642

7.3.6. Pre-processing of reads: chimera filter

We have performed the following pre-processing steps before we start the analysis. Chimeras were removed using the de-novo chimera removal method UCHIME (version 11) implemented in the tool VSEARCH (Rognes et al., 2016). A detailed table of chimera filter based on individual sample is given in Table 2.

Table 7.4: Pre-processing reads statistics

Sample Name	Consensus Sequences	Chimeric Sequences	Pre-processed Consensus Sequences
CS	195067	63230	131837
PR	235642	62474	173168

7.3.7. Picking OTUs and Taxonomy classification

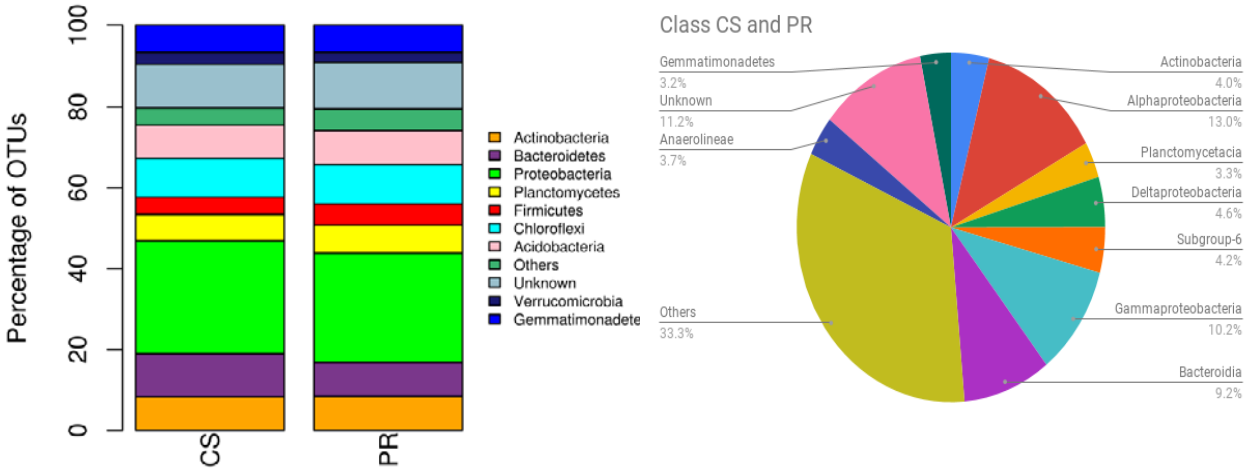
The Operational Taxonomic Units (OTU) picking and taxonomy classification were performed using the pre-processed consensus V3-V4 sequences. Pre-processed reads from all samples were pooled and clustered into OTUs based on their sequence similarity using Uclust program (similarity cuto. = 0.97) available in QIIME software. A total of 37418 OTUs were identified from 305005 reads. From 37418 total OTUs, 31822 OTUs with less than 5 reads were removed and 5596 OTUs were selected for further analysis (Yadav et al., 2019) (Table 7.5).

Table 7.5: Summary of OTUs

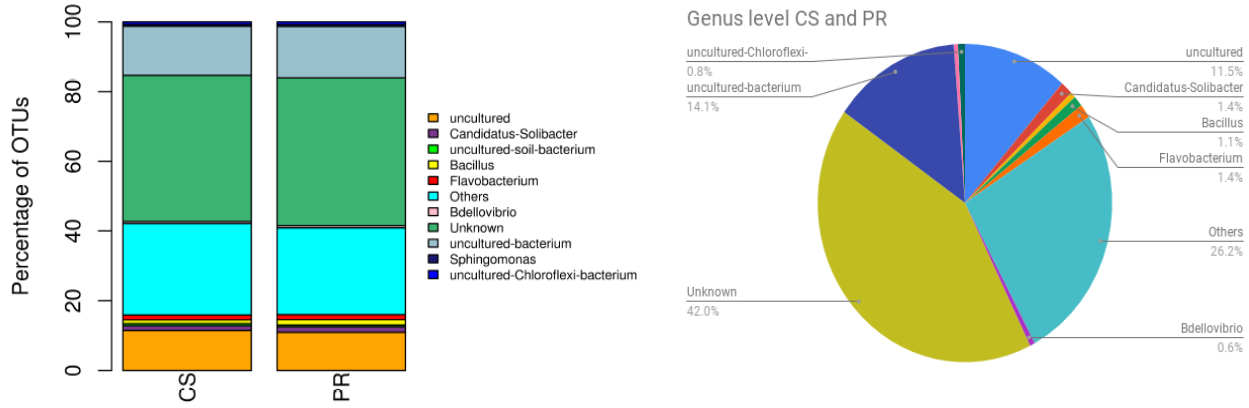
Total Pre-processed Consensus	305005
Total OTUs Picked	37418
Total Filtered OTUs(<5 reads)	31822
Total Filtered OTUs(after filtering <5 reads)	5596

7.3.8. Bacterial communities' analysis

Microbial community at class level in CS and PR were represented as, *Actinobacteria, Alphaproteobacteria, Planctomycetacia, Deltaproteobacteria, Subgroup-6, Gammaproteobacteria, Bacteroidia, Anaerolineae , Gemmatimonadetes* and others. Further, at the genus level in CS and PR most *abundant genus were candidatus-solibacter, uncultures soil bacterium, Bacillus, Flavobacteriaum, Bdellovibrio, uncultured bacterium, Sphingomonas, uncultured- chloroflexi bacterium* (Delgado-Baquerizo et al., 2017). The relative abundance plot at class and genus level based on OTU is shown in the below figure 7.5.



a. Relative abundance at Class level (OTUs)



b. Relative abundance at Genus level (OTUs)

Fig.7.5. The relative abundance plot at class and genus level based on OUT (a) At class level (b) At Genus level

7.3.9 Taxonomic classification of bacterial communities in rhizosphere of *Cannabis sativa* and *Parthenium hysterophorus* at Class and Genus level through heat map.

Heat map facilitated relative abundance of bacterial communities of *Cannabis sativa* and *Parthenium hysterophorus* at genus and species level . The top bacterial communities at species level of *Cannabis sativa* and *Parthenium hysterophorus* were *uncultured-Planctomycetaceae-bacterium, uncultured-Gemmatimonadales-bacterium, uncultured-Bacteroidetes-bacterium, uncultured- Acidobacterium-sp., uncultured planctomycete, Flavobacterium-sp.-L1I52, uncultured- archaeon, uncultured-soil bacterium, uncultured-gamma- proteobacterium, uncultured- Gemmatimonadetes bacterium, uncultured delta- proteobacterium, uncultured- prokaryote, uncultured bacterium, uncultured Actinomycetales- bacterium, uncultured- Rhizobiales-bacterium, uncultured-Chloroflexi- bacterium, uncultured- actinobacterium, uncultured- Acidobacteria-bacterium, Flavobacterium- daejeonense, uncultured-*

Acidimicrobidae- bacterium, uncultured- Gemmatimonas-sp., uncultured- bacterium, uncultured- Desulfuromonadales-bacterium, Unknown, uncultured-marine-bacterium.

The top bacterial communities at genus level of *Cannabis sativa* and *Parthenium hysterophorus* were *Hydrogenophaga, Cellvibrio, Nocardioides, uncultured, Geobacter, Candidatus- Solibacter, uncultured- soil- bacterium, Flavobacterium, Others Bdellovibrio, Ohtaekwangia, Nitrospira, Gemmatimonas, uncultured-bacterium, Mycobacterium, Sphingomonas, uncultured-Chloroflexi-bacterium, Pedobacter, Azoarcus, Flavisolibacter, Haliangium, Terrimonas, Bacillus, Unknown, Pseudomonas, Luteolibacter* (Hu et al., 2020)

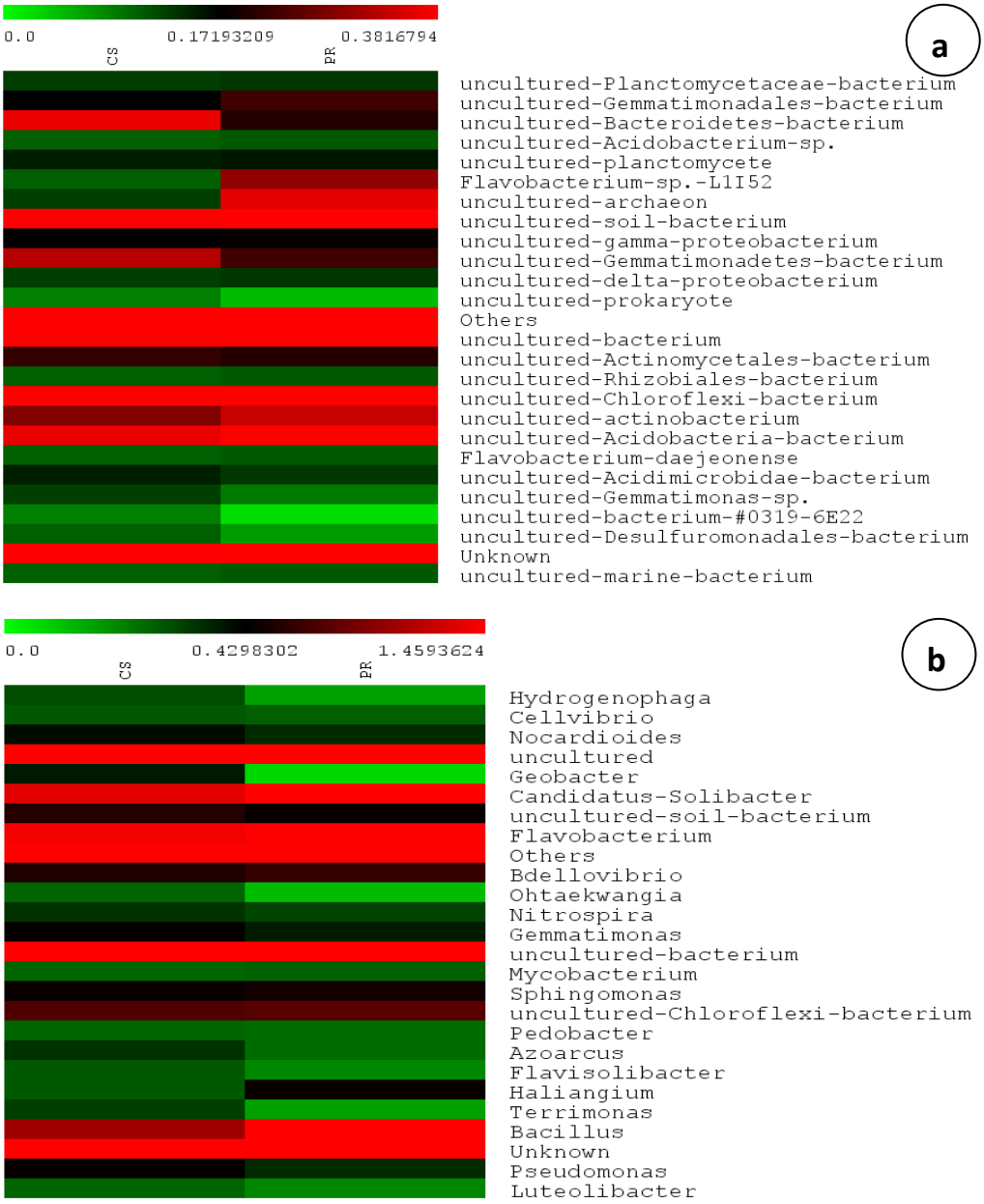


Figure 7.6: Heat map showing taxonomic classification of bacterial communities in rhizosphere of *Cannabis sativa* and *Parthenium hysterophorus* at (a) species level (b) Genus level

Conclusion

The present chapter aims to explore the rhizospheric bacterial communities associated with *Parthenium hysterophorus* and *Cannabis sativa* grown on organometallic pollutants-rich hazardous distillery sludge. The sequence analysis of 16S rRNA V3–V4 hypervariable region with Illumina MiSeq platform showed 230166 and 277515 OTUs derived from rhizospheric distillery sludge samples out of 305005 sequences read, respectively. The major genus detected in rhizospheric sludge sample were uncultured-*chloroflexi* (0.8%), *Candidatus-solibacter* (1.4%), *Bacillus* (1.4%), *Flavobacterium* (1.4%), others (26.2%), *Bdellovibrio (*0.6%) and unknown (42.0%). Our results suggested that rhizospheric bacterial communities associated with *Parthenium hysterophorus* and *Cannabis sativa* were substantially different in richness, diversity, and relative abundance of taxa compared to rhizospheric sludge. Further, the comparative organic pollutant analysis from non-rhizospheric and rhizospheric sludge samples through GC–MS analysis revealed the disappearance of several compounds and generation of some compounds as new metabolic products by the activity of rhizospheric bacterial communities. The results of this study will be helpful in understanding the role of rhizospheric bacterial communities responsible for degradation and detoxification of complex organometallic waste and, thus, can help in designing appropriate phytoremediation studies for eco-restoration of polluted sites.

Chapter 8

Characterization of rhizospheric bacterial communities of *Typha augustifolia* during rhizofilteration of distillery effluent in constructed wetland treatment system.

Chapter-8

Characterization of rhizospheric bacterial communities of *Typha aungustifolia* during rhizofilteration of distillery effluent in constructed wetland treatment system

8.1. Introduction

Rhizospheric bacteria play a very vital role in the plant growth. While due to their specific property of plant they also play very crucial and specific contribution for bioremediation of hazardous pollutants from soil and wetland ecosystem (Mani and Kumar, 2014). Some wetland plants have been reported for important role for hyperaccumulation and rhizofilteration of various toxic and recalcitrant compounds. The *Phragmites australis, Phalaris arundinacea, Typha domingensis, Typha latifolia, Cyperus rotundus, Phragmites karka, Juncus pallidus, Empodisma minus*, etc. have been reported as potential wetland plant for rhizofilteration of various pollutants from contaminated sites (Galal et al., 2018). Due to their unique property of their root morphology and potentiality to thrive in contaminated sites, they have been used in constructed wetland treatment system for the treatment of sewage and industrial waste both for safe disposal (Brix, 2020). The unique properties of wetland plant for rhizofilteration is due to presence of specific rhizospheric bacterial communities where they play an important role not only for plant growth but also prevent pathogen and they solubilise the nutrient for plant accumulation (Nihorimbere et al., 2011). However, these bacterial communities and plant are specific in particular environment.

A total dissolved solid (TDS) is one of the major factor for the growth of bacteria and degradation of pollutants in any aquatic environment. TDS are generally comprised of inorganic salts (such as chloride, calcium, magnesium, potassium, sodium, bicarbonates, and sulfates) and organic matters dissolved in water (Peng, 2020). This excess TDS can be toxic to many aquatic life such as fish, insects, amphibians, and macro-invertebrates. For example, TDS level above 250 mg/L can affect salmonid fertilization (Brix et al., 2010); increasing TDS from 270 to 1,170

mg/L can eliminate almost all coontail (*Ceratophyllus demersum*) from the ecosystem. TDS-related acute toxicity was also reported to the water flea *Ceriodaphnia dubia* and the fish *Pimephales promelas*, and as a matter of fact, these two aquatic animals have been used as test organism for TDS-related toxicity. Various methods such as physical adsorption, reverse osmosis (RO), distillation, precipitation, membrane filtration, and bacteria-based bioremediation have been developed to reduce/remove TDS from water streams. Most of those methods, however, are not cost-effective and/ or environmental friendly. For example, physical adsorption can only remove ions from water at certain capacity before the adsorbent reaches saturation and needs to be regenerated (Bilal et al., 2021). RO is not economical and requires high energy with the issue of filter fouling and brine disposal. Distillation produces low conductivity water but the process is very energy-intensive due to a large amount of latent heat required (Kesieme et al., 2013). Biological absorption avoids the addition of chemicals but the process is often limited by the low rate of absorption by microbial cells. Additionally some specific coagulants are also used reduce TDS ferric sulfate, polyaluminium chloride, aluminum sulfate, sodium aluminate, ferric chloride, ferric sulphate, classed as aluminum or iron salts, are common coagulants for water treatment (Sahu and Chaudhari, 2013).

The biological treatment system along with advanced oxidation and ozonation process has been reported as a potential, simple, low-cost wastewater tertiary treatment technique (Ribeiro et al., 2019). But the drawback is that these studies are reported with low organic load containing effluent in temperate region and different climate zone. These techniques are not reported with sugarcane molasses-based distillery effluent. The conventional treatment technology i.e., physical, chemical (filtration, coagulation) and activated sludge process is effective up to some extent but expensive and energy consuming at large scale (Rajasulochana and Preethy, 2016). Thus, constructed wetland systems planted with potential wetland plant have been optimized as an effective device at pilot scale. Combination of wetland treatment process with bacterial degradation at reduced total dissolve solid (TDS) of PMDE offers an excellent system for removal of colour from PMDE and further reduction of BOD, COD for safe disposal (Ahmed et al., 2017). The increased microbial population has been indicated for the degradation of organic chemical contaminated

soil. Hence, the combined application of bacteria and wetland plants would show maximum decolorization and degradation of all organic matters as a sequential bioreactor process (Kushwaha, 2015). Recently the biostimulation and bioaugmentation process has been reported for the detoxification and degradation of various residual organic pollutants present in industrial wastewater at tertiary stage treatment (Tripathi et al., 2021). The complexity of PMDE showed that high TDS as a key factor of effluent toxicity due to dissolved organic content in high concentration (Yadav and Chandra, 2019).

Therefore, to understand the mechanism of rhizospheric bacterial for the detoxification of Post Methanated Distillery Effluent (PMDE) in the designed constructed wetland treatment system is important to scale-up these unique properties of plant associated bacterial community for the treatment of various industrial wastewaters (Chowdhary and Bharagava, 2018). Since, this chapter has been focused on the integration of biodegradation of distillery waste by potential bacterial consortium with designed constructed wetland plant treatment system. Therefore, the dominant rhizospheric bacterial community has been identified. Moreover, the distillery effluent has very high TDS which do not support the bacterial growth in its original concentration (Tripathi et al., 2021). Therefore, the reductions of TDS by adequate method have to be optimized for the degradation of distillery effluent and bacterial growth in its original concentration for feasible and economical technology.

8.2. Materials and method

8.2.1. Physico-chemical analysis of PMDE

Electrical conductivity (EC) and pH values of distillery effluent samples were measured using Orion conductivity meter (Model-A322, Thermo Scientific, FL, USA) and Orion pH meter (Model-960, Thermo Scientific, FL, USA), respectively. For pH measurement sludge and distilled water was mixed in a ratio of 1:2.5 (weight: volume) (Chandra and Kumar 2017a). Cation exchange capacity (CEC) was quantified using the method as described by Gillman and Sumpter (1986). Total Kjeldahl nitrogen (TKN) and total ammoniacal nitrogen (NH4+-N) were measured by the methods reported by Ju et al. (2007). Total phosphorus (TP) was measured by the colorimetric method (Jackson 1973). Organic matter content was determined using

the Walkley and Black procedure (Nelson and Sommers 1982). Total oxygen and hydrogen was estimated by elemental analyzer (EuroVector EA 3000, University of AL-al-Bayt, Jordan). Concentration of Cl^-, Na^+, PO_4^{3-}, and SO_4^{2-} were estimated according to the method described by Kalra and Maynard (1991). Total phenol concentration in the sludge samples was determined using 4-aminoantipyrine reaction method as described by Ettinger et al. (1951). As outlined, the phenolic material was mixed with 4-aminoantipyrine in the presence of alkaline oxidizing agent, preferable potassium ferricyanide, at high pH, to yield a red chromogenic compound. The absorption of this red solution was measured at 500 nm by UV–Vis spectrophotometer. For heavy metal analyses, distillery sludge samples were first digested with nitric acid–hydrogen persoxide digestion method (3050B) (U.S. Environmental Protection Agency; USEPA 1996). Briefly, 1-g dried sludge sample was placed into a Teflon reaction vessel and digested with 10 mL of the HNO_3 (1:1, v/w) at 95 °C for 15 min. After cooling, 5 mL of concentrated HNO_3 was added into vessel and reflux at 95 °C until the solution becomes transparent. After cooling the solution, 2 mL of water and 3 mL of 30% H_2O_2 were added into Teflon vessel. The solution was allowed to evaporate by raising the temperature to 105 °C until the samples were digested and the solution becomes transparent. The acid–peroxide digestate was continually heating at 95 °C without boiling for 2 h. The volume of these samples was reduced to 5 mL by evaporating the acid–peroxide mixture. Thereafter, the samples were allowed to cool to room temperature before the vessel content was filtered with Whatman filter paper and diluted with double deionized water up to 100 mL. Three replicates of every sample were digested, together with the corresponding blanks. Subsequently, the total concentrations of different heavy metals (i.e., Fe, Zn, Cu, Ni, and Mn) in the digested solution were determined by inductively coupled plasma-mass spectrometry (ICP-MS; Agilent 7500Cx, USA). The instrument settings and operational conditions were done in accordance with the manufacturers ‘specifications.

8.2.2. Experimental setup and TDS reduction optimization

The TDS reduction experiments were carried out in a jar test apparatus (OSK-Japan) using coagulants. The wastewater after adding coagulants well mixing was allowed to settle for 3h and the supernatant was transferred to a clean container. The main

physico-chemical characteristics of the TDs reduced sample are shown in Table 8.1. Precise doses of coagulants including $Al_2(SO_4)3$, FeSO4, FeCl3and PACL, and coagulant aids including CaO, $Na2CO_3$, $CaCO_3$and $Na2SiO_3$, were added to 800-mL jars containing 200mL of settled wastewater with specified pH values. A series of jar tests were carried out as follows: first rapid mixing stage carried out on jars at 100rpm for 2min and then slow mixing stage carried out at 30rpm for 20min and finally the solutions were settled in 250-mL graduated cylinders for 30min. The produced supernatants were used for the measurement of remained physico-chemical analysis (Thamaraiselvi et al., 2019).

8.2.3. Bacterial growth in TDS reduced sample

Bacterial grown samples incubated at 6 and 12 days in the TDS reduction process were centrifuged at 6,500 × g for 20min. To remove the medium contents, the pellets were then washed three times with distilled water. The cells were then fixed in 0.1M phosphate buffer (pH 7.2) containing 1% glutaraldehyde for 2 h and washed twice with distilled water. The fixed cells were dehydrated using a series of acetone solutions (15, 30, 60, 90, and 100%) for 20min, as per the standard method described by Sangeeta et al. (2011). The dehydration process was carried out twice. The dried cells were then fixed on metal stubs, coated under vacuum with approximately 25 nm of high purity carbon, and examined by scanning electron microscopy (SEM; QUANTA FEG 450, FEI, Netherlands) (Akyon et al., 2015).

8.2.4. Optimize the reduction of TDS for bacterial growth of PMDE.

The TDS reduction experiments were carried out in a jar test apparatus (OSK-Japan) using coagulants. The wastewater after adding coagulants well mixing was allowed to settle for 3h and the supernatant was transferred to a clean container. The main physico-chemical characteristics of the TDs reduced sample are shown in Fig. Precise doses of coagulants including Al2(SO4)3, FeSO4, FeCl3and PACL, and coagulant aids including CaO, Na2CO3, CaCO3and Na2SiO3, were added to 800-mL jars containing 200mL of settled wastewater with specified pH values. A series of jar tests were carried out as follows: first rapid mixing stage carried out on jars at 100rpm for 2min and then slow mixing stage carried out at 30rpm for 20min and finally the solutions were settled in 250-mL graduated cylinders for 30min. The produced

supernatants were used for the measurement of remained physico-chemical analysis and bacterial growth (Chandra et al., 2008).

8.2.5. Bacterial growth using nutrient for detoxification

In presence of 1% glucose as additional carbon source 0.5% peptone as additional nitrogen source at 120 rpm to maintain oxygen concentration in the effluent medium at pH 8.0±0.5 for 96 hrs. incubation based on activated sludge process Same experimental were carried out at different temperatures (25-50 °C), pH (4-12), and shaking speeds (100-220 rpm) to see how different environmental parameters affected before and after TDS reduced sample for degradation and decolorization. Various other nutrient source are nitrogen sources, such as ammonium chloride, beef extract, sodium sulfate, yeast extract, peptone, and urea, were applied as trace element at a concentration of 0.5 percent (w/v) to determine the good condition for before and after TDS reduction process (Maiti et al., 2017).

8.2.6. Rhizofiltration through wetland plant.

The constructed wetland channel was in zigzag manner to maximize the retention time in minimum area having three vertical layers of gravel and concrete from upper to deeper side for each layer. The dimension of constructed wetland treatment system was 60x72x1000 cm (width x depth x length) having 0.88 % slopes. Retention time of wetland treatment was 10 days. Subsequently, the biomass was separated and supernatant was injected (passed through) with constructed wetland treatment system, upstream flow rate 2 L min-1 versus 1.5 L min-1 downstream having mixed vegetation of *Typha angustata* (Woraharn et al., 2021).

8.3. Result and discussion

8.3.1. Physico-chemical analysis before and after TDS reduction

The physico-chemical properties of the control (distillery wastewater), and TDS reduction samples are shown in Table 8.1. The control samples showed higher pH, TDS, BOD, COD, and EC values. The elevated pH of the control sample may be attributed to the binding with melanoidin with the distillery wastewater of high levels of soluble products and heavy metals. TDS reduced sample showed that after autoclave some chemical characteristics had been altered but the extend was not significant except for the chloride content. McGuire and Judd (2020) demonstrated

there were significant positive relationship between organic matter and Cl- retention, it was likely as a result of the thermal treatment chloride was released from the bound organic matter. Significant reduction in the pollution criteria in the wastewater was observed at the end of the bioaugmentation period (Table 8.1). The elevated values of COD and BOD of the untreated wastewater (control) indicated the existence of greater amounts of organic and inorganic compounds. Metals may be released during the fermentation and distillation processes of fermented sugarcane molasses in distilleries, resulting in post-methanation distillery effluent (PMDE) with an alkaline pH that is eventually discharged. TDS reduction sample, huge reduction in metal content was observed, probably as a result of passive adsorption due to the removed after the centrifugation and filtration process. In comparison, after TDS reduction of incubation, the treated showed a significant reduction in all of different pollution parameter except for Cl-. This suggested that the coagulants were actively biotransforming various organic and inorganic contents. The pH of the medium was initially reduced to 7.79 during melanoidin degradation, but after TDS reduction, the pH steadily decreased. The development of organic acids such as phosphoric acid, acetic acid, octadecanoic acid, and ethanedioic acid, can caused a reduction in pH during the early stages of bacterial growth. However, examination of BOD and COD values at different periods indicated a steady decline in BOD and COD values as the percentage of decolorization increased.

Table 8.1: Physico-chemical analysis before and after TDS reduction

S.No.	Parameters	Fresh PMDE	After TDS reduction	% change	Permissible limit (USEPA)
1.	Colour	139861	59641	57.35	-
2.	Ph	8.5	7.8	8.23	5.5 - 9.0
3.	T.D.S.	15748.0 mg/lit.	4456.0	71.70	2100
4.	T.S.S.	12154.0 mg/lit.	3368.0	72.28	100
5.	Sulphate	12651.7 mg/lit.	2987.65	76.38	1000
6.	T.Nitrogen	774.0 mg/lit.	198.75	74.32	100
7.	Phenol	330 mg/lit.	54.24	83.56	1.0
8.	C.O.D	47896	4364	90.88	250
9.	B.O.D.	24986	3456	86.16	30
10.	Phosphate	577.85	105.96	81.66	5.0
11.	Chloride	7.125	5.246	26.37	1000
12.	**Metal**				
	Cd	7.241	4.925	31.98	2.0
	Cu	4.610	3.127	32.16	3.0
	Fe	52.87	31.35	40.70	2.0
	Mn	14.52	9.75	32.85	0.20
	Ni	4.397	3.87	20.17	3.0
	Zn	8.658	6.89	20.42	5.0

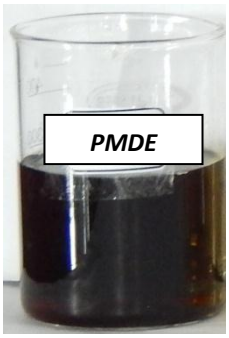
8.3.2. Optimized conditions for TDS reduction and bacterial growth

To optimize the condition for TDS reduction different pH and coagulants were optimized. It can be seen from the figure that $FeCl_3$ has more efficiency than the other coagulants, and increasing the coagulant dose increases TDS removal which may be due to an increasing in sweep coagulation shown in fig. The optimum pH for TDS removal was obtained at pH 10. Also, Na_2SiO_3 was found as the best coagulant aid type with 300 ppm as the best dose for TDS removal. The optimization of conditions for the removal of turbidity was done using residual turbidity as treatment goal. $FeCl_3$ was the best coagulant aid to remove the turbidity and the best levels for coagulant dose and coagulant aid dose were 800 and 600ppm, respectively (Hlordzi et al., 2020).

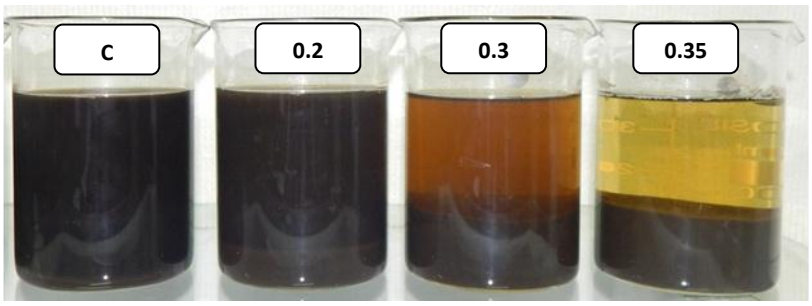
8.3.3. Optimization of bacterial growth after reduction of TDS

The bacterial consortium of four identified bacteria mention in the chapter 5 was inoculated in reduced TDS after extended aeration in presence of 1% glucose as additional carbon source and 0.5% peptone as nitrogen in 250ml flask at pH 8.0±0.5, 150±2 rpm and 35±2^{o}C temp. This showed ≈ 65% bacterial decolorization along with reduction of various pollution parameters (BOD, COD, TS, TDS, sulphate and heavy metals) up to 70-80% (Rane et al., 2018).

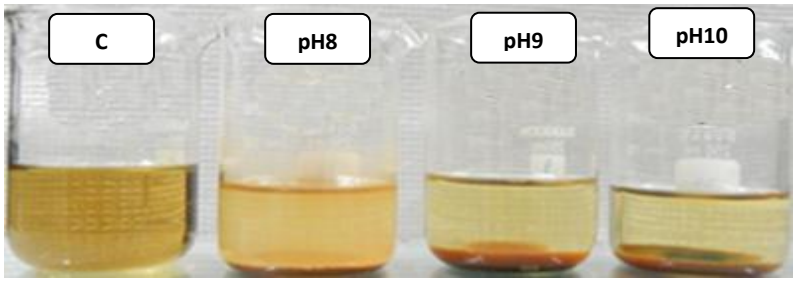
a. Sample collection of PMDE



b. PMDE in laboratory for further analysis



c.Optimized condition of PMDE with coagulants ($FeCl_3$)



d. Optimized condition of PMDE with various coagulants at various pH.

Fig. 8.1. Sample collection and reduction of TDS (a) Sample collection of PMDE (b) processing of sample in laboratory (c) Optimized condition of PMDE with coagulants (d) Optimized condition of PMDE with various coagulants at various pH.

8.3.4. T. angustifolia Optimization and rhizofilteration in constructed wetland

T. angustifolia growing in the distillery effluent contaminated site as potential wetland plant was collected and uprooted with associated sludge samples and carried in pre sterilized polythene bags (Chandra and Kumar, 2017). The uprooted plant with associated sludge was acclimatized in the square box pot with metal solution, sand layer and gravel for one month. The acclimatized plant was uprooted along with the complete root network and transferred to the constructed wetland system. The constructed wetland channel is in zigzag manner to maximize the retention time in minimum area having three vertical layers of gravel and concrete from upper to deeper side for each layer. The dimension of constructed wetland treatment system is 60x72x1000 cm (width x depth x length) having 0.88 % slopes. Retention time of wetland treatment is 10 days. The reduction of BOD and COD is observed up to 97 and 98%. While the color is reduced up to 98-99%, sulphate and heavy metals is also reduced up to 95% (Malyan et al., 2021).

8.3.5. Identification of bacteria from the rhizosphere of T. angustifolia

The method for bacterial identification was followed by as mention in chapter 5 and 6. The rhizospheric bacterial strains i.e TRCK1, TRCK2, TRCK3, TRCK4 16S rRNA sequencing data, the isolated strains KS20 showed nearest relationships with *Pseudomonas thivervalensis, Bacillus pumilus, Acinetobacter junii, Salmonella enterica.* The nucleotide sequences of these potentially diverse rhizospheric bacteria were deposited in the National Center for Biotechnology Information (NCBI) database under accession numbers OP093759, OP093755, OP093756 and, OP093758 respectively. Using the MEGA 11 software, a phylogenetic tree was generated that illustrated the relationships between bacterial species and the most closely related genera deduced from sequences of the 16S rRNA gene (Fig.8.3) (Kumar and Chandra, 2018).

Table 8.2: Physico-chemical analysis after bacterial decolorization and constructed wetland treatment

S.No.	Parameters	After bacterial decolorization	After constructed wetland treatment	% change	Permissible limit (CPCB)
1.	Colour	24321	13287	45.36	-
2.	pH	7.5	7.4	1.33	5.5 - 9.0
3.	T.D.S.	3235	1974	62.29	2100
4.	T.S.S.	1639	427	73.94	100
5.	Sulphate	2976.8	949.3	68.11	1000
6.	T.Nitrogen	107.67	89.38	16.98	100
7.	Phenol	39	23	27.77	1.0
8.	C.O.D	935	233	301.0	250
9.	B.O.D.	2563	903	103.0	30
10.	Phosphate	103.56	84.29	18.60	5.0
11.	Chloride	5.597	3.665	34.51	1000
12.	**Metal**				
	Cd	3.42	1.91	44.15	2.0
	Cu	2.50	1.70	32.00	3.0
	Fe	22.05	8.24	62.63	2.0
	Mn	4.18	3.95	31.66	0.20
	Ni	2.87	2.01	29.96	3.0
	Zn	6.21	4.71	24.15	5.0

Table 8.3: Overall physico-chemical analysis from fresh PMDE to constructed wetland treatment

S.No.	Parameters	Fresh PMDE	After TDS reduction	After bacterial decolorization	After constructed wetland treatment	% reduction	Permissible limit (USEPA)
1.	Colour	139861	59641	24321	13287	90.49	-
2.	Ph	8.5	7.8	7.5	7.4	12.94	5.5 - 9.0
3.	T.D.S.	15748.0 mg/lit.	4456.0	3235	1974	87.46	2100
4.	T.S.S.	12154.0 mg/lit.	3368.0	1639	427	96.48	100
5.	Sulphate	12651.7 mg/lit.	2987.65	2976.8	949.3	92.49	1000
6.	T.Nitrogen	774.0 mg/lit.	198.75	107.67	89.38	88.45	100
7.	Phenol	330 mg/lit.	54.24	39	23	93.03	1.0
8.	C.O.D	47896	4364	935	301	99.37	250
9.	B.O.D.	24986	3456	2563	903	96.38	30
10.	Phosphate	577.85	105.96	103.56	84.29	85.41	5.0
11.	Chloride	7.125	5.246	5.597	3.665	48.56	1000
12.	**Metal**						
	Cd	7.241	4.925	3.42	1.91	73.63	2.0
	Cu	4.610	3.127	2.50	1.70	63.12	3.0
	Fe	52.87	31.35	22.05	8.24	84.41	2.0
	Mn	14.52	9.75	4.18	3.95	72.79	0.20
	Ni	4.397	3.51	2.87	2.01	54.28	3.0
	Zn	8.658	6.89	6.21	4.71	45.81	5.0

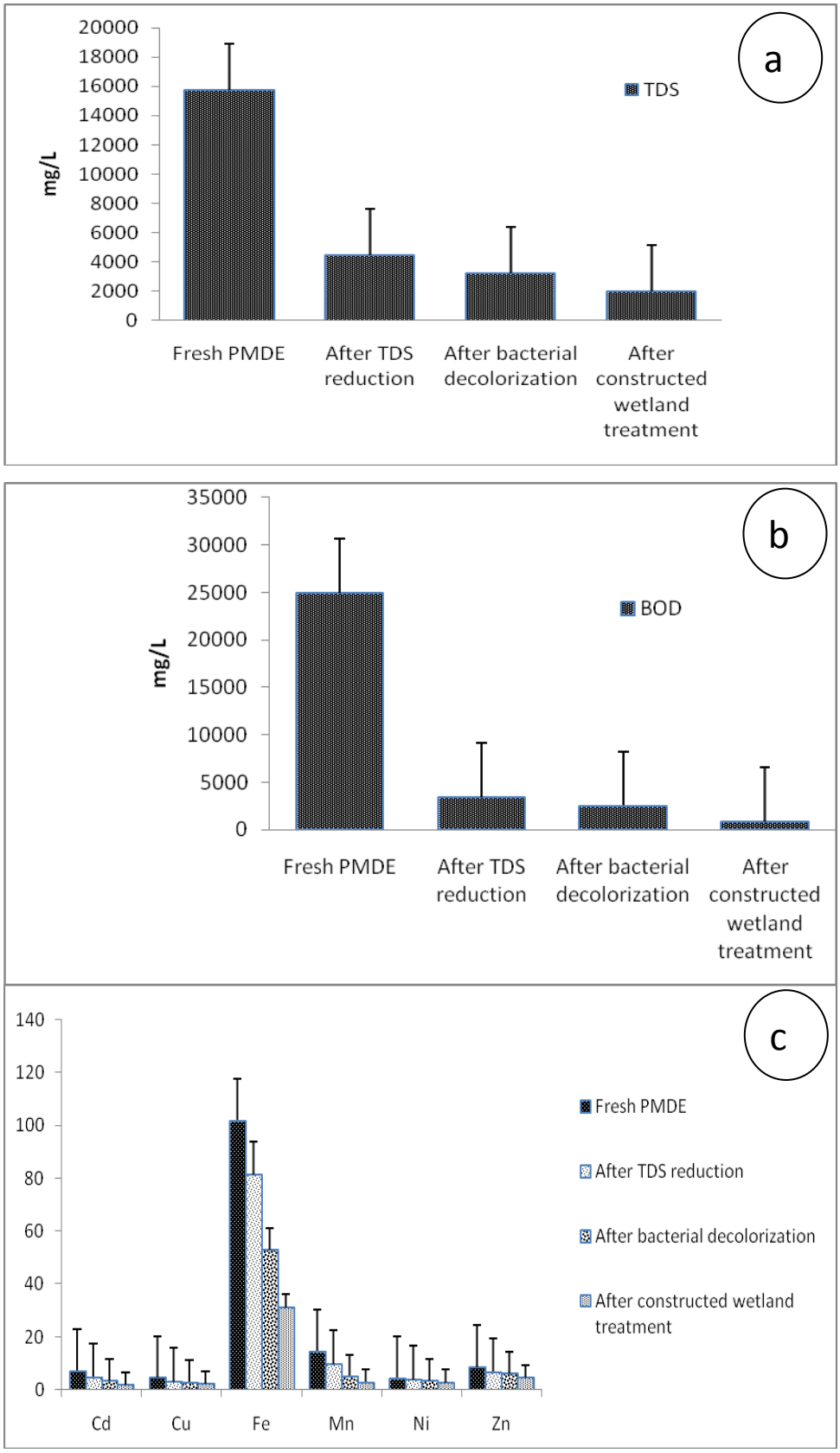
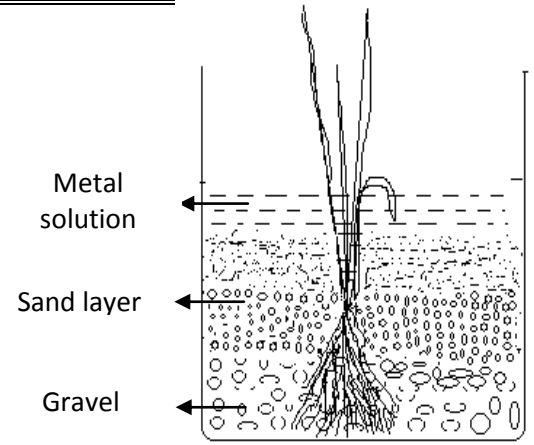


Table 8.2: Bar diagram ssof overall comparative physico-chemical analysis (a) TDS (b) BOD (C) Metal analysis

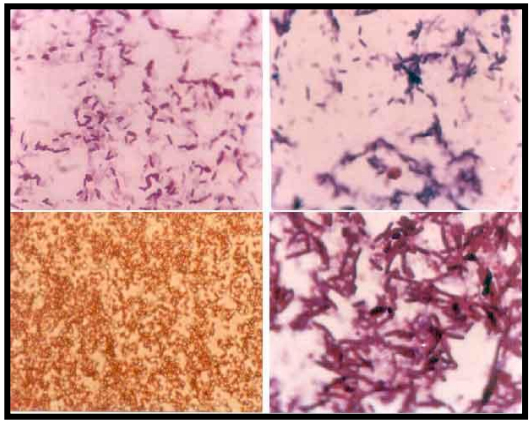
Typha angustata growing in the distillery effluent contaminated site as potential wetland plant



Acclimatization of *T. angustifolia* with distillery effluent

Biological decolourisation of post methanated distillery effluent (PMDE) in biphasic bacterial and wetland plant treatment system for environmental safety.

Uprooted *T. angustifolia* from constructed wetland



Isolated bacteria from rhizosphere of *T. angustifolia* from constructed wetland

Fig.8.3. Overall process of sample collection and Biological decolourisation of post methanated distillery effluent (PMDE) in biphasic bacterial and wetland plant treatment system for environmental safety and identification of potential bacteria

9. Conclusion

Post-methanated distillery effluent (PMDE) was found dark brown with very high BOD, COD, TDS and metallic content beyond its permissible limit. Hence, due to high TDS bacterial growth was not possible. Therefore, the TDS reduction was optimised for bacterial growth at variable range of pH and concentration. The optimum reduction of TDS was obtained with application of ferric chloride (0.34%) within 6-8hrs of incubation. The value of TDS and other pollution parameters are shown in table (Table 8.1 and 8.3). 71.70% decolorization was observed. The efficiency of decolorization and TDS reduction of PMDE was evaluated with rapid mixing along with the variable pH. The optimum TDS reduction resulted 80% supernatant and 20% precipitation of colloidal material. Further, the PMDE supernatant is assessed for bacterial growth in presence of variable nitrogen (0.5%, 1.0%, 1.5% & 2.0%) and carbon (0.5%, 1.0%, 1.5% & 2.0%) source to evaluate the bacterial growth for reduction of color from supernatant of PMDE. 79.45% decolorization was further observed. Subsequently, the biomass was separated and supernatant was passed through the constructed wetland treatment system with plantation of *Typha angustata*. The reduction of TDS was observed 87.46%, BOD 96.38% and COD 99.37%. While the color was reduced up to 90.49%, and the others physicochemical parameters also reduced up to 95%.

Therefore, the current chapter shows Biological decolourisation of post-methanated distillery effluent (PMDE) in biphasic bacterial and wetland plant treatment system for environmental safety and identification of potential bacteria.

Chapter 9

Summary

Chapter-9

Summary

Rhizospheric bacterial community has been reported primarily for the enhancement of plant growth and maintenance of soil fertility. The roles of plant growth promoting rhizobacteria (PGPR) are particularly revealed in agriculture sector for the crop production. Generally PGPR can be divided into two basic groups based upon their relation with plants. It may be symbiotic or free living bacteria. The PGPR influences to associated plant by three mode of action: (a) Releasing specific compounds required for plant growth (b) fascilitating the uptake of certain nutrients from the complex environment (c) inhibiting to pathogenic bacteria and preventing to the plant from diseases. Simultaneously, plant also releases the various root exudates i.e. organic acids, amino acids, amides, carbohydrates, enzymes and, phenolics, which work as a basic nutrient to the associated microbial communities. In response to the root exudates the associated microbial community contributes for solubilization of various micro and macronutrient for the bioavailability to the plant. Besides, the growing microbial community within the vicinity of the rhizosphere also releases out some specific chemicals which act as inhibitory effect upon pathogenic population in the rhizospheric zone. This mechanism of the PGPR is to protect the plant from the disease in field condition. Thus, this properties of PGPR in association to the plant root is a basic character for the adaptation and sustainable growth of plant communities and bacteria both in variable environment. However, the soil characteristic and geographical altitude also determines the growth of plant communities place to place.

But, the global rapid industrialization and deforestation created adverse effect on environment. This caused the environmental imbalance and immerged out as climate change and global warming. In India various agrobased industries i.e. distilleries, pulp paper industries, tanneries, textile etc. are major source of industrial waste discharge for environmental pollution. The conventional methods of wastewater treatment do not meet out the required standard of environment for waste disposal. Therefore, researcher has attempted from past few decades to develop the novel technologies for removal of hazardous and complex pollutants from discharge of industrial waste. But, yet the suitable

technologies are still warranted. However, In order to adapt the microbial population and plant communities in the environment, some potential plant species have been reported for phytoremediation of the polluted environment for the eco-restoration due to their specialized cellular structure and physiological adaptation. Keeping in view these pollutant characteristics phytoremediation has been reported as a green economical, feasible and novel technology for the eco-restoration of polluted site. Since the distillery waste is a complex and cumbersome waste with a mixture of organo-metallic compounds. Due to these properties, organo-metallic complex compounds of distillery waste persist for several years in environment and causes toxicity to flora and fauna. Moreover, the recent findings have revealed that the pollutants present in distillery waste have androgenic and mutagenic properties also (Chandra and Kumar, 2017). Since the phytoremediation potential plant is regulated by the specific bacterial community present in the rhizospheric zone. But, the detail nature of bacterial community growing around the root zone of plant at polluted site is not much known so far.

Hence, the present study has focused on the role of rhizospheric bacterial community during the phytoremediation of distillery waste. In this study, the potential non-edible plants have been evaluated for their phytoextraction potential of heavy metals from the complex sludge and effluent. These properties of bacterial communities with specific plants may be utilized for removal and detoxification of distillery waste for eco-restoration of polluted environment. The present thesis is divided into eleven chapters. The first chapter is Introduction of the topic. This chapter has imbibed the background information and rational of title of thesis, this information has been described in detail in this chapter. In the second chapter global and national states of art of the problems have been reviewed to understand the problem for development of new technologies. The chapter has also highlighted to understand the problem of distillery waste i.e. organic pollutants, heavy metals, Post Methanated distillery effluent (PMDE), and their environmental impact. Some bacterial species and consortium also have been reported for their capability to degrade the various pollutants present in the distillery waste. The dark brown colourant in the distillery wastewater is basically caramelized sugar which is known as melanoidins. The decolourizsation abilities of various bacteria have been reported in this chapter. The roles of some wetland plant for phytoremediation potential in different conditions are also mentioned. Though, the various attempts have been reported for decolourization and degradation of distillery wastewater at laboratory stages by using

different bacterial consortium but all these studies have been reported at very low concentration of effluent. Therefore, this restrict for pilot-scale up-gradation of technology. Therefore, in the present study, the study has been focused for decolourisation and degradation of distillery wastewater at high concentration for feasibility of technology. Moreover, the hyperaccumulator herbaceous plants are also screened out for heavy metal phytoextraction potential from complex organo-metallic distillery sludge for eco-restoration of polluted site.

Further, chapter three has focused on five objectives as per topic of thesis. Chapter four has focused on characterization of organic pollutants and heavy metals from Post-Methanated Distillery Effluent (PMDE) and their environmental effect. The study has focused on the characterization of organic pollutants by using GC-MS technique and heavy metals through Inductively Coupled Plasma (ICP) from Post-Methanated Distillery Effluent (PMDE) and their environmental effect. Various unknown complex recalcitrant organometallic complexes with EDC properties which are a major source of environmental pollutants and health hazards are identified. The absorption maxima of PMDE were noted between 200–350 nm in the UV–Vis spectral analysis also indicated polymerized-form of Maillard product. Hexadecanoic acid, butyl ester, octadecanoic acid, monopalmitin 2 TMS, effusanin E, 1-(benzyl) -2-fluoro-2-phenyl-3-(p-toluene's sulfonyl) propane, and 24-hydroxy-3,4-secolanost-4,(28),8- died-3-nitrile were prominent organic compounds in the category of mutagenic and androgenic compounds characteristics, detected in PMDE. In addition, the presence of various activity in germinating Zea mays seeds and gel imaging at different concentrations of PMDE heavy metals also indicated the contribution of toxic property of PMDE, as resulting in phototoxic effect on seed germination with Zea mays and *Heteropneustes fossilis* with the degradation of lamellae in gill. Thus, this affirmed the source of various unknown mutagenic, carcinogenic, and EDCs compounds with organometallic complexes.

The fifth chapter summarizes the characterization of rhizospheric bacterial communities from hyperaccumulator plant growing on organometallic sludge during phytoextraction for ecorestoration. The findings of study revealed that *P. hysterophorus* is as hyperaccumulator for various heavy metals from complex organometallic wastes during its growth on the disposed distillery sludge. The analysis of organic compounds showed degradation of pollutants present in sludge after growth of *P. hysterophorus*. Further, the histological observations of root by transmission electron microscopy (TEM) confirmed

the deposition of metal granules in their tissue after accumulation by plant from distillery sludge. Besides, identified rhizospheric bacteria i.e. *Alcaligenes faecalis* (ON024323), *Cytobacillus firmus* (ON024324), *Bacillus* subtilis (ON024325) and, *Niallia circulans* (ON024326), also showed potential for plant growth promoting rhizobacteria activities which has supported the bacterial assisted phytoextraction potential of heavy metals from complex organometallic sludge of distillery from polluted site. This plant may be used as biotechnological tools for eco-restoration of polluted site by industrial waste as a green technology.

The sixth chapter showed the comparative assessment of phosphate, zinc and potassium solubilization by rhizospheric bacterial communities in *Phragmites communis* and *Typha spp. Phragmites communis* and *Typha spp.* is a naturally robust and vigorous primary species in many wetland environments worldwide. This plant has characteristic capacity to grow in different environmental conditions and can uptake, translocate, and accumulate a wide range of pollutants in both belowground and aboveground tissue. The ability of the plant to develop and grow in the polluted ecosystems allowed for the use of *Phragmites communis* in many types of sewage treatment plants also. To increase the efficiency of phytoremediation of a polluted natural or artificial aquatic ecosystem and to estimate the required purification time and accelerate the rate of its reclamation, the interaction processes between common *Phragmites communis* and soil microbes, metal accumulation, and ionic homeostasis in the hydrophyte purification systems should be further tested. The research carried out by interdisciplinary teams (plant physiologist, biochemist, geochemist, microbiologist, and agriculture and genetic engineer) in a short time can advance the efficiency of removing both metals and organic impurities.

Furthermore, the seventh chapter correlates bacterial community and organometallic pollutants during in-situ phytoremediation by *Parthenium hysterophorus* and *Cannabis sativa* growing on organometallic pollutants-rich hazardous distillery sludge. The sequence analysis of 16S rRNA V3–V4 hypervariable region with Illumina MiSeq platform showed 230166 and 277515 OTUs derived from rhizospheric distillery sludge samples out of 305005 sequences read, respectively. The major genus detected in rhizospheric sludge sample were uncultured- *chloroflexi* (0.8%), *Candidatus-solibacter* (1.4%), *Bacillus* (1.4%), *Flavobacterium* (1.4%), others (26.2%), *Bdellovibrio (*0.6%) and unknown (42.0%). Our results suggested that rhizospheric bacterial communities associated with *Parthenium hysterophorus* and *Cannabis sativa* were substantially

different in richness, diversity, and relative abundance of taxa compared to rhizospheric sludge. Further, the comparative organic pollutant analysis from non-rhizospheric and rhizospheric sludge samples through GC–MS analysis revealed the disappearance of several compounds and generation of some compounds as new metabolic products by the activity of rhizospheric bacterial communities. The results of this study will be helpful in understanding the role of rhizospheric bacterial communities responsible for degradation and detoxification of complex organometallic waste and, thus, can help in designing appropriate phytoremediation studies for eco-restoration of polluted sites.

The eighth chapter has focused on characterization of rhizospheric bacterial communities of *Typha aungustifolia* during rhizofilteration of distillery effluent in constructed wetland treatment system by integrating bacterial degraded PMDE with constructed wetland treatment at pilot scale to evaluate the feasibility of technology based on the promising result. Post-methanated distillery effluent (PMDE) was found dark brown with very high BOD, COD, TDS and metallic content beyond its permissible limit. Hence, due to high TDS bacterial growth was not possible. Therefore, the TDS reduction was optimised for bacterial growth at variable range of pH and concentration. The optimum reduction of TDS was obtained with application of ferric chloride (0.34%) within 6-8hrs of incubation. The detail value of TDS and other pollution parameters are shown in table (Table 8.1 and 8.3). 71.70% decolorization was observed. The efficiency of decolorization and TDS reduction of PMDE was evaluated with rapid mixing along with the variable pH. The optimum TDS reduction resulted 80% supernatant and 20% precipitation of colloidal material. Further, the PMDE supernatant is assessed for bacterial growth in presence of variable nitrogen (0.5%, 1.0%, 1.5% & 2.0%) and carbon (0.5%, 1.0%, 1.5% & 2.0%) source to evaluate the bacterial growth for reduction of color from supernatant of PMDE. 79.45% decolorization was further observed. Subsequently, the biomass was separated and supernatant was passed through the constructed wetland treatment system with plantation of *Typha angustata*. The reduction of TDS was observed 87.46%, BOD 96.38% and COD 99.37%. While the color was reduced up to 90.49%, and the others physicochemical parameters also reduced up to 95%. Therefore, the current chapter showed Biological decolourisation of post-methanated distillery effluent (PMDE) in biphasic bacterial and wetland plant treatment system for environmental safety and identification of potential bacteria.

The chapter nine has summarized the whole thesis work and findings with research output in a systematic manner. The chapter ten has compiled the all cited references of thesis which are relevant to the topic and they are cited in each chapter. Each chapter has cited the recent references based on methodology and results. The available references are cited on synthesis of melanoidin, physico-chemical properties of distillery wastewater, impact of post methanated distillery wastewater and plant growth promoting rhizobacteria reported at polluted sites. The complete thesis has 200 cited references.

The last chapter has listed all the scientific output based on the thesis work. There is two original research paper published in peer reviewed journal of international repute with high impact factor. Two review paper are also published based on topic of thesis have been published in the international journal and three book chapter as invited chapter published CRC press. A certificate of participation and best oral presentation award has been provided in international conferences. One international patent also has been granted based on the work of thesis in credit of candidate.

References

References

Aalaei, K., Rayner, M., & Sjöholm, I. (2019). Chemical methods and techniques to monitor early Maillard reaction in milk products; A review. *Critical reviews in food science and nutrition*, *59*(12), 1829-1839.

Abbasi, M. K., Sharif, S., Kazmi, M., Sultan, T., & Aslam, M. (2011). Isolation of plant growth promoting rhizobacteria from wheat rhizosphere and their effect on improving growth, yield and nutrient uptake of plants. *Plant Biosystems*, *145*(1), 159-168.

Abdalla KZ, Hammam G (2014) Correlation between biochemical oxygen demand and chemical oxygen demand for various wastewater treatment plants in Egypt to obtain the biodegradability indices. *Int J Sci Basic Appl Res* 13(1):42–48

Abdullah, S. R. S., Al-Baldawi, I. A., Almansoory, A. F., Purwanti, I. F., Al-Sbani, N. H., & Sharuddin, S. S. N. (2020). Plant-assisted remediation of hydrocarbons in water and soil: Application, mechanisms, challenges and opportunities. *Chemosphere*, *247*, 125932.

Abo-Zaid, G. A., Soliman, N. A. M., Abdullah, A. S., El-Sharouny, E. E., Matar, S. M., & Sabry, S. A. F. (2020). Maximization of siderophores production from biocontrol agents, Pseudomonas aeruginosa f2 and Pseudomonas fluorescens JY3 using batch and exponential fed-batch fermentation. *Processes*, *8*(4), 455.

Adrian, J. (2019). The Maillard reaction. In *Handbook of nutritive value of processed food* (pp. 529-608). CRC Press.

Ahmed, M. B., Zhou, J. L., Ngo, H. H., Guo, W., Thomaidis, N. S., & Xu, J. (2017). Progress in the biological and chemical treatment technologies for emerging contaminant removal from wastewater: a critical review. *Journal of hazardous materials*, *323*, 274-298.

AIDA (All india distillers' associations) (2016) Annual report. AIDA, New Delhi. http:// www.aidai ndia.org/annua l-repor t.html

Akyon, B., Stachler, E., Wei, N., & Bibby, K. (2015). Microbial mats as a biological treatment approach for saline wastewaters: the case of produced water from hydraulic fracturing. *Environmental science & technology*, *49*(10), 6172-6180.

Alam MZ, Ahmad S, Malik A, Ahmad M (2010) Mutagenicity and genotoxicity of tannery effluents used for irrigation at Kanpur. India *Ecotoxicol Environ Saf* 73(7):1620–1628

Albretsen J (2006) The toxicity of iron, an essential element. Vet Med- Bonn Springs Edwardsville 101(2):82

Ali, H., & Khan, E. (2019). Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs—Concepts and implications for wildlife and human health. Human and Ecological Risk Assessment: *An International Journal*, 25(6), 1353-1376.

Ali, H., Khan, E., & Ilahi, I. (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of chemistry*, *2019*.

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals—concepts and applications. *Chemosphere*, 91(7), 869-881..

ALjahdali, N., & Carbonero, F. (2019). Impact of Maillard reaction products on nutrition and health: Current knowledge and need to understand their fate in the human digestive system. *Critical Reviews in Food Science and Nutrition*, *59*(3), 474-487.

Alkhatib, R., Bsoul, E., Blom, D. A., Ghoshroy, K., Creamer, R., & Ghoshroy, S. (2013). Microscopic analysis of lead accumulation in tobacco (Nicotiana tabacum var. Turkish) roots and leaves. *Journal of Microscopy and Ultrastructure*, *1*(1-2), 57-62.

Amaya-Farfan, J., & Rodriguez-Amaya, D. B. (2021). The Maillard Reactions. In *Chemical Changes During Processing and Storage of Foods* (pp. 215-263). Academic Press.

Ambatkar, M., & Mukundan, U. (2021). Modes of Enzyme Delivery in Treatment of Wastewater Containing Recalcitrant Pollutants. *Innovations in Microbiology and Biotechnology Vol. 2*, 1-14.

Amenorfenyo, D. K., Huang, X., Li, C., Li, F., Zeng, Q., Zhang, N., ... & Wang, P. (2020). A review of microalgae and other treatment methods of distillery wastewater. *Water and Environment Journal*, *34*, 988-1002.

American Public Health Association Vision/ Mission (2011) Available at http://www.apha.org/about /gov/execb oard/execu tiveb oardvision missi on.htm. Accessed 21 July 2011

Ammeri, R. W., Hassen, W., Hidri, Y., Di Rauso Simeone, G., & Hassen, A. (2022). Macrophyte and indigenous bacterial co-remediation process for pentachlorophenol removal from wastewater. *International Journal of Phytoremediation*, *24*(3), 271-282

Angassa, K., Leta, S., Mulat, W., Kloos, H., & Meers, E. (2019). Evaluation of pilot-scale constructed wetlands with Phragmites karka for phytoremediation

of municipal wastewater and biomass production in Ethiopia. *Environmental Processes*, *6*(1), 65-84.

Aniyikaiye TE, Oluseyi T, Odiyo JO, Edokpayi JN (2019) Physicochemical analysis of wastewater discharge from selected paint industries in Lagos, Nigeria. *Int J Environ Res Public Health* 16(7):1235

AOAC, I. (1990). AOAC: Official Methods of Analysis (Volume 1). K. Helriched.. Association of Official Analytical Chemists. *Inc., Arlington*.

Aoki KA, Harris CA, Katsiadaki I, Sumpter JP (2011) Evidence suggesting that di-n-butyl phthalate has antiandrogenic effects in fish. *Environ Toxicol Chem* 30(6):1338–1345

APHA (American Public Health Association), (2012). Standard Method for Examination of Water and Wastewater, 22nd ed. APHA, AWWA and WEF, Washigton, DC.

Appell, M., Hurst, W. J., Finley, J. W., & deMan, J. M. (2018). Amino acids and proteins. In *Principles of food chemistry* (pp. 117-164). Springer, Cham.

Aragón-Gastélum, J. L., Ramírez-Benítez, J. E., González-Durán, E., González-Salvatierra, C., Ramírez-Tobías, H. M., Flores, J., ... & Jarquín-Gálvez, R. (2020). Photochemical activity in early-developmental phases of Agave angustifolia subsp. tequilana under induced global warming: implications to temperature stress and tolerance. *Flora*, *263*, 151535.

Arimi, M. M., Zhang, Y., Götz, G., Kiriamiti, K., & Geißen, S. U. (2014). Antimicrobial colorants in molasses distillery wastewater and their removal technologies. *International Biodeterioration & Biodegradation*, 87, 34-43.

Aron, P. M., & Shellhammer, T. H. (2010). A discussion of polyphenols in beer physical and flavour stability. *Journal of the Institute of Brewing*, 116(4), 369-380.

Asberry HB, Kuo CY, Gung CH, Conte ED, Suen SY (2014) Characterization of water bamboo husk biosorbents and their application in heavy metal ion trapping. *Microchem J* 113:59–63

Ashfaq, M., Hassan, H. M., Ghazali, A. H. A., & Ahmad, M. (2020). Halotolerant potassium solubilizing plant growth promoting rhizobacteria may improve potassium availability under saline conditions. *Environmental Monitoring and Assessment*, *192*(11), 1-20.

Ashraf, S., Ali, Q., Zahir, Z. A., Ashraf, S., & Asghar, H. N. (2019). Phytoremediation: Environmentally sustainable way for reclamation of heavy metal polluted soils. *Ecotoxicology and environmental safety*, *174*, 714-727.

Auti, A. M., Narwade, N. P., Deshpande, N. M., & Dhotre, D. P. (2019). Microbiome and imputed metagenome study of crude and refined petroleum-

oil-contaminated soils: Potential for hydrocarbon degradation and plant-growth promotion. *Journal of Biosciences*, *44*(5), 1-16.

Ayaz, S. Ç., Aktaş, Ö., Akça, L., & Fındık, N. (2015). Effluent quality and reuse potential of domestic wastewater treated in a pilot-scale hybrid constructed wetland system. *Journal of environmental management*, *156*, 115-120.

Balapure, K., Aghera, P., Bhatt, N., & Madamwar, D. (2019). Community synergism: degradation of triazine dye reactive black 1 by mixed bacterial cultures KND_PR under microaerophilic and aerobic conditions. *Environmental Processes*, *6*(3), 713-739.

Balkrishna, A., Dabhade, N. R., Sharma, N., Sharma, J., Mishra, S., & Arya, V. (2022). Phytoremediation: Current Techniques and Futuristic Opportunities. *Indian Journal of Ecology*, *49*(3), 1057-1067.

Baptista, S. L., Costa, C. E., Cunha, J. T., Soares, P. O., & Domingues, L. (2021). Metabolic engineering of Saccharomyces cerevisiae for the production of top value chemicals from biorefinery carbohydrates. *Biotechnology Advances*, *47*, 107697.

Bashir, I., Lone, F. A., Bhat, R. A., Mir, S. A., Dar, Z. A., & Dar, S. A. (2020). Concerns and threats of contamination on aquatic ecosystems. In *Bioremediation and Biotechnology* (pp. 1-26). Springer, Cham.

Batista AH, Melo VF, Gilkes R, Roberts M (2018) Identification of heavy metals in crystals of sand and silt fractions of soils by scanning electron microscopy (SEM EDS/WD-EPMA). *Rev Bras Cienc Solo* 42

Becker, P. M., & Yu, P. (2013). What makes protein indigestible from tissue-related, cellular, and molecular aspects?. *Molecular nutrition & food research*, *57*(10), 1695-1707

Berger, R. G. (Ed.). (2007). *Flavours and fragrances: chemistry, bioprocessing and sustainability*. Springer Science & Business Media.

Beri V, Gupta R (2007) Acetylcholinesterase inhibitors neostigmine and physostigmine inhibit induction of alpha-amylase activity during seed germination in barley, Hordeum vulgare var. Jyoti. *Life Sci* 80(24–25):2386–2388

Bert, V., Seuntjens, P., Dejonghe, W., Lacherez, S., Thuy, H. T. T., & Vandecasteele, B. (2009). Phytoremediation as a management option for contaminated sediments in tidal marshes, flood control areas and dredged sediment landfill sites. *Environmental Science and Pollution Research*, 16(7), 745-764.

Bezuneh TT (2016) The role of microorganisms in distillery wastewater treatment: a review. *J Bioremediat Biodegrad* 7(6)

Bharagava, R. N., & Chandra, R. (2010a). Effect of bacteria treated and untreated post-methanated distillery effluent (PMDE) on seed germination, seedling

growth and amylase activity in Phaseolus mungo L. *Journal of Hazardous Materials*, *180*(1-3), 730-734.

Bharagava RN, Chandra R (2010b) Biodegradation of the major color containing compounds in distillery wastewater by an aerobic bacterial culture and characterization of their metabolites. *Biodegradation* 21(5):703–711

Bharagava RN, Chandra R, Rai V (2008) Phytoextraction of trace elements and physiological changes in Indian mustard plants (*Brassica nigra* L.) grown in post methanated distillery effluent (PMDE) irrigated soil. *Bioresour Technol* 99(17):8316–8324

Bharagava RN, Chandra R, Rai V (2009) Isolation and characterization of aerobic bacteria capable of the degradation of synthetic and natural melanoidins from distillery effluent. *World J Microbiol Biotechnol* 25(5):737–744

Bharagava, R. N. (Ed.). (2018). Recent advances in environmental management.

Bharagava, R. N., & Chandra, R. (2010). Effect of bacteria treated and untreated post-methanated distillery effluent (PMDE) on seed germination, seedling growth and amylase activity in *Phaseolus mungo* L. *Journal of Hazardous Materials*, 180(1-3), 730-734.

Bharagava, R. N., & Chowdhary, P. (Eds.). (2019). *Emerging and eco-friendly approaches for waste management*. *Springer Singapore.*

Bharagava, R. N., Chandra, R., & Rai, V. (2008). Phytoextraction of trace elements and physiological changes in Indian mustard plants (Brassica nigra L.) grown in post methanated distillery effluent (PMDE) irrigated soil. *Bioresource Technology*, 99(17), 8316-8324.

Bharagava, R. N., Chandra, R., & Rai, V. (2009). Isolation and characterization of aerobic bacteria capable of the degradation of synthetic and natural melanoidins from distillery effluent. *World Journal of Microbiology and Biotechnology*, *25*(5), 737-744.

Bharagava, R. N., Saxena, G., & Mulla, S. I. (2020). Introduction to industrial wastes containing organic and inorganic pollutants and bioremediation approaches for environmental management. In *Bioremediation of industrial waste for environmental safety* (pp. 1-18). Springer, Singapore.

Bhardwaj, S., Ruhela, M., Bhutiani, R., Ahamad, F., & Bhardwaj, R. (2019). Distellery spent wash (DSW) treatment methodogies and challenges with special reference to incineration: An overview. *Environment Conservation Journal*, *20*(3), 135-144.

Bhateria, R., & Jain, D. (2016). Water quality assessment of lake water: a review. *Sustainable Water Resources Management*, 2(2), 161-173.

Białkowska, A. M. (2016). Strategies for efficient and economical 2, 3-butanediol production: new trends in this field. *World Journal of Microbiology and Biotechnology*, *32*(12), 1-14.

Bilal, M., Ihsanullah, I., Younas, M., & Shah, M. U. H. (2021). Recent advances in applications of low-cost adsorbents for the removal of heavy metals from water: A critical review. *Separation and Purification Technology*, *278*, 119510.

Billaud C, Brun-Merimee S, Louarme L, Nicolas J (2004) Effect of glutathione and maillard reaction products prepared from glucose or fructose with glutathione on polyphenoloxidase from apple—I: enzymatic browning and enzyme activity inhibition. *Food Chem* 84(2):223–233

Billore, S. K., Singh, N., Ram, H. K., Sharma, J. K., Singh, V. P., Nelson, R. M., & Dass, P. (2001). Treatment of a molasses based distillery effluent in a constructed wetland in central India. *Water Science and Technology*, *44*(11-12), 441-448.

Bloch ME (1794) Der malabarische hecht. In: Naturgeschichte des auslandische fische Berlin, 149–150

Bortoletto AM, Silvello GC, Alcarde AR (2018) Good manufacturing practices, hazard analysis and critical control point plan proposal for distilleries of cachaca. *Sci Agricola* 75(5):432–443

Brandt, M. I., Trouche, B., Quintric, L., Günther, B., Wincker, P., Poulain, J., & Arnaud-Haond, S. (2021). Bioinformatic pipelines combining denoising and clustering tools allow for more comprehensive prokaryotic and eukaryotic metabarcoding. *Molecular Ecology Resources*, *21*(6), 1904-1921.

Brix, H. (2020). Wastewater treatment in constructed wetlands: system design, removal processes, and treatment performance. In *Constructed wetlands for water quality improvement* (pp. 9-22). CRC Press.

Brix, K. V., Gerdes, R., Curry, N., Kasper, A., & Grosell, M. (2010). The effects of total dissolved solids on egg fertilization and water hardening in two salmonids—Arctic Grayling (Thymallus arcticus) and Dolly Varden (Salvelinus malma). *Aquatic toxicology*, *97*(2), 109-115.

Cämmerer, B., Jalyschko, W., & Kroh, L. W. (2002). Intact carbohydrate structures as part of the melanoidin skeleton. *Journal of Agricultural and Food Chemistry*, *50*(7), 2083-2087.

Carter, M. R. (2020). Analysis of soil organic matter storage in agroecosystems. In *Structure and organic matter storage in agricultural soils* (pp. 3-11). CRC press.

Causin V, Casamassima R, Marega C, Maida P, Schiavone S, Marigo A, Villari A (2008) The discrimination potential of ultravioletvisible

spectrophotometry, thin layer chromatography, and Fourier transform infrared spectroscopy for the forensic analysis of black and blue ballpoint inks. *J Forensic Sci* 53(6):1468–1473

Central Pollution Control Board (CPCB) (2003) Environmental management in selected industrial sectors status and needs, CPCB, ministry of environment and forest, New Delhi. PROBES/97/ 2002–03

Chandra R, Bharagava RN, Kapley A, Purohit HJ (2012) Characterization of phragmites cummunis rhizosphere bacterial communities and metabolic products during the two stage sequential treatment of post methanated distillery effluent by bacteria and wetland plants. *Bioresour Technol* 103(1):78–86

Chandra R, Bharagava RN, Yadav S, Mohan D (2009) Accumulation and distribution of toxic metals in wheat (Triticum aestivum L.) and Indian mustard (Brassica campestris L.) irrigated with distillery and tannery effluents. *J Hazard Mater* 162(2–3):1514–1521

Chandra R, Castillo-Zacarias C, Delgado P, Parra-Saldivar R (2018) A biorefinery approach for dairy wastewater treatment and product recovery towards establishing a biorefinery complexity

Chandra R, Pandey PK, Srivastava A (2004) Comparative toxicological evaluation of untreated and treated tannery effluent with Nostoc muscorum L. (algal assay) and microtox bioassay. *Environ Monit Assess* 95(1):287–294

Chandra, R., Yadav, S., Bharagava, R. N., & Murthy, R. C. (2008). Bacterial pretreatment enhances removal of heavy metals during treatment of post-methanated distillery effluent by Typha angustata L. *Journal of environmental management*, *88*(4), 1016-1024.

Chandra R, Yadav S, Yadav S (2017) Phytoextraction potential of heavy metals by native wetland plants growing on chlorolignin containing sludge of pulp and paper industry. *Ecol Eng* 98:134–145

Chandra, R., & Kumar, V. (2017). Detection of Bacillus and Stenotrophomonas species growing in an organic acid and endocrine-disrupting chemical-rich environment of distillery spent wash and its phytotoxicity. *Environmental monitoring and assessment*, *189*(1), 1-19.

Chandra, R., & Kumar, V. (2017). Phytoextraction of heavy metals by potential native plants and their microscopic observation of root growing on stabilised distillery sludge as a prospective tool for in situ phytoremediation of industrial waste. *Environmental Science and Pollution Research*, *24*(3), 2605-2619.

Chandra, R., Bharagava, R. N., & Rai, V. (2008). Melanoidins as major colourant in sugarcane molasses based distillery effluent and its degradation. *Bioresource technology*, 99(11), 4648-4660.

Chandra, R., Bharagava, R. N., Kapley, A., & Purohit, H. J. (2012). Characterization of Phragmites cummunis rhizosphere bacterial communities and metabolic products during the two stage sequential treatment of post methanated distillery effluent by bacteria and wetland plants. *Bioresource Technology*, *103*(1), 78-86.

Chandra, R., Kumar, V., & Tripathi, S. (2018). Evaluation of molasses-melanoidin decolourisation by potential bacterial consortium discharged in distillery effluent. *3 Biotech*, *8* (4), 1-16.

Chandra, R., Kumar, V., Tripathi, S., & Sharma, P. (2018). Heavy metal phytoextraction potential of native weeds and grasses from endocrine-disrupting chemicals rich complex distillery sludge and their histological observations during in-situ phytoremediation. *Ecological Engineering*, 111, 143-156.

Chandra, R., Sharma, P., Yadav, S., & Tripathi, S. (2018). Biodegradation of endocrine-disrupting chemicals and residual organic pollutants of pulp and paper mill effluent by biostimulation. *Frontiers in Microbiology*, 9, 960.

Chandra, R., Yadav, S., & Mohan, D. (2008). Effect of distillery sludge on seed germination and growth parameters of green gram (Phaseolus mungo L.). *Journal of hazardous materials*, *152*(1), 431-439.

Chandrakala, C., Voleti, S. R., Bandeppa, S., Sunil Kumar, N., & Latha, P. C. (2019). Silicate solubilization and plant growth promoting potential of Rhizobium sp. isolated from rice rhizosphere. *Silicon*, *11*(6), 2895-2906.

Chandran, H., Meena, M., & Sharma, K. (2020). Microbial biodiversity and bioremediation assessment through omics approaches. *Frontiers in Environmental Chemistry*, *1*, 570326.

Chang TPY, Rangan C (2011) Iron poisoning: a literature-based review of epidemiology, diagnosis, and management. *Pediatr Emerg Care* 27(10):978–985

Chaturvedi, S., Chandra, R., & Rai, V. (2006). Isolation and characterization of Phragmites australis (L.) rhizosphere bacteria from contaminated site for bioremediation of colored distillery effluent. *ecological engineering*, *27*(3), 202-207.

Chaudhary R, Arora M (2011) Study on distillery effluent: chemical analysis and impact on environment. *Int J Adv Eng Technol* 2(2):352–356

Chen X, Xu S, Tan T, Lee ST, Cheng SH, Lee FWF, Xu SJL, Ho KC (2014) Toxicity and estrogenic endocrine disrupting activity of phthalates and their mixtures. *Int J Environ Res Public Health* 11(3):3156–3168

Chen, X., Yang, H., Chen, Y., Chen, W., Lei, T., Zhang, W., & Chen, H. (2017). Catalytic fast pyrolysis of biomass to produce furfural using heterogeneous catalysts. *Journal of Analytical and Applied Pyrolysis*, *127*, 292-298.

Chhonkar PK, Datta SP, Joshi HC, Pathak H (2000) Impact of industrial effluents on soil health and agriculture-Indian experience: part I-distillery and paper mill effluents

Chirakkara, R. A., Cameselle, C., & Reddy, K. R. (2016). Assessing the applicability of phytoremediation of soils with mixed organic and heavy metal contaminants. *Reviews in Environmental Science and Bio/Technology*, *15*(2), 299-326.

Choi YY, Baek SR, Kim JI, Choi JW, Hur J, Lee TU, Park CJ, Lee BJ (2017) Characteristics and biodegradability of wastewater organic matter in municipal wastewater treatment plants collecting domestic wastewater and industrial discharge. *Water* 9(6):409

Chowdhary P, Raj A, Bharagava RN (2018) Environmental pollution and health hazards from distillery wastewater and treatment approaches to combat the environmental threats: a review. *Chemosphere* 194:229–246

Chowdhary P, Yadav A, Kaithwas G, Bharagava RN (2017) Distillery wastewater: a major source of environmental pollution and its biological treatment for environmental safety. Green technologies and environmental sustainability. *Springer*, Cham, pp 409–435

Chowdhary, P., Bharagava, R. N., Mishra, S., & Khan, N. (2020). Role of industries in water scarcity and its adverse effects on environment and human health. In *Environmental concerns and sustainable development* (pp. 235-256). *Springer*, Singapore.

Chowdhary, P., Raj, A., & Bharagava, R. N. (2018). Environmental pollution and health hazards from distillery wastewater and treatment approaches to combat the environmental threats: a review. *Chemosphere*, 194, 229-246.

Chowdhary, P., Raj, A., & Bharagava, R. N. (2018). Environmental pollution and health hazards from distillery wastewater and treatment approaches to combat the environmental threats: a review. *Chemosphere*, *194*, 229-246.

Chowdhary, P., Yadav, A., Kaithwas, G., & Bharagava, R. N. (2017). Distillery wastewater: a major source of environmental pollution and its biological treatment for environmental safety. In *Green technologies and environmental sustainability* (pp. 409-435). Springer, Cham.

Clarkson PM, Tremblay ISABELLE (1988) Exercise-induced muscle damage, repair, and adaptation in humans. *J Appl Physiol* 65(1):1–6

Costerousse, B., Schönholzer-Mauclaire, L., Frossard, E., & Thonar, C. (2018). Identification of heterotrophic zinc mobilization processes among bacterial strains isolated from wheat rhizosphere (Triticum aestivum L.). *Applied and environmental microbiology*, *84*(1), e01715-17.

Cui, H., Yu, J., Zhai, Y., Feng, L., Chen, P., Hayat, K., ... & Ho, C. T. (2021). Formation and fate of Amadori rearrangement products in Maillard reaction. *Trends in Food Science & Technology*, *115*, 391-408.

Dai, Y., Liu, R., Zhou, Y., Li, N., Hou, L., Ma, Q., & Gao, B. (2020). Fire Phoenix facilitates phytoremediation of PAH-Cd co-contaminated soil through promotion of beneficial rhizosphere bacterial communities. *Environment international*, *136*, 105421.

Das, P. K., Rani, J., Rawat, S., & Kumar, S. (2022). Microalgal co-cultivation for biofuel production and bioremediation: Current status and benefits. *BioEnergy Research*, *15*(1), 1-26.

David Noel S, Rajan MR (2015) Phytotoxic effect of dyeing industry effluent on seed germination and early growth of lady's finger. *J Pollut Eff Contr* 2(126):10–4172

de Oliveira Silva J, Rodrigues Filho G, da Silva Meireles C, Ribeiro SD, Vieira JG, da Silva CV, Cerqueira DA (2012) Thermal analysis and FTIR studies of sewage sludge produced in treatment plants. The case of sludge in the city of Uberlandia-MG. Braz Thermochim Acta 528:72–75

Delgado, A., & Gómez, J. A. (2016). The soil. Physical, chemical and biological properties. In Principles of agronomy for sustainable agriculture (pp. 15-26). Springer, Cham.

Deng, L., Liu, Y., Zheng, D., Wang, L., Pu, X., Song, L., ... & Long, Y. (2017). Application and development of biogas technology for the treatment of waste in China. *Renewable and Sustainable Energy Reviews*, *70*, 845-851.

Dhaliwal, S. S., Naresh, R. K., Mandal, A., Singh, R., & Dhaliwal, M. K. (2019). Dynamics and transformations of micronutrients in agricultural soils as influenced by organic matter build-up: A review. *Environmental and Sustainability Indicators*, *1*, 100007.

Dhote, L., Kumar, S., Singh, L., & Kumar, R. (2021). A systematic review on options for sustainable treatment and resource recovery of distillery sludge. *Chemosphere*, *263*, 128225.

Dhote, L., Mandpe, A., Paliya, S., Kumar, S., Pandey, R. A., & Kumar, R. (2020). Characterization of distillery sludge for its thermal properties and

ascertaining its utilization as a low-cost fuel. *Journal of Cleaner Production*, *259*, 120872.

Di Benedetto, N. A., Corbo, M. R., Campaniello, D., Cataldi, M. P., Bevilacqua, A., Sinigaglia, M., & Flagella, Z. (2017). The role of plant growth promoting bacteria in improving nitrogen use efficiency for sustainable crop production: a focus on wheat. *AIMS microbiology*, 3(3), 413.

Diez-Simon, C., Mumm, R., & Hall, R. D. (2019). Mass spectrometry-based metabolomics of volatiles as a new tool for understanding aroma and flavour chemistry in processed food products. *Metabolomics*, *15*(3), 1-20.

Dubey, A., Malla, M. A., Khan, F., Chowdhary, K., Yadav, S., Kumar, A., ... & Khan, M. L. (2019). Soil microbiome: a key player for conservation of soil health under changing climate. *Biodiversity and Conservation*, *28*(8), 2405-2429

Echavarría, A. P., Pagán, J., & Ibarz, A. (2012). Melanoidins formed by Maillard reaction in food and their biological activity. *Food Engineering Reviews*, 4(4), 203-223.

Echavarria-Pinto, M., Escaned, J., Macías, E., Medina, M., Gonzalo, N., Petraco, R., ... & Macaya, C. (2013). Disturbed coronary hemodynamics in vessels with intermediate stenoses evaluated with fractional flow reserve: a combined analysis of epicardial and microcirculatory involvement in ischemic heart disease. *Circulation*, *128*(24), 2557-2566.

Eller, F., Skálová, H., Caplan, J. S., Bhattarai, G. P., Burger, M. K., Cronin, J. T., ... & Brix, H. (2017). Cosmopolitan species as models for ecophysiological responses to global change: the common reed Phragmites australis. *Frontiers in Plant Science*, *8*, 1833.

Erhirhie EO, Ihekwereme CP, Ilodigwe EE (2018) Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. *Interdiscip Toxicol* 11(1):5–12

Etesami, H., & Maheshwari, D. K. (2018). Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects. *Ecotoxicology and environmental safety*, 156, 225-246.

Ezati, P., Tajik, H., Moradi, M., & Molaei, R. (2019). Intelligent pH-sensitive indicator based on starch-cellulose and alizarin dye to track freshness of rainbow trout fillet. *International journal of biological macromolecules*, *132*, 157-165.

Fahad, S., Adnan, M., Saud, S., & Nie, L. (Eds.). (2022). *Climate Change and Ecosystems: Challenges to Sustainable Development*. CRC Press.

Fahner, N. A., Shokralla, S., Baird, D. J., & Hajibabaei, M. (2016). Large-scale monitoring of plants through environmental DNA metabarcoding of soil: recovery, resolution, and annotation of four DNA markers. *PloS one*, *11*(6), e0157505.

Faixo, S., Gehin, N., Balayssac, S., Gilard, V., Mazeghrane, S., Haddad, M., ... & Garrigues, J. C. (2021). Current trends and advances in analytical techniques for the characterization and quantification of biologically recalcitrant organic species in sludge and wastewater: A review. *Analytica Chimica Acta*, *1152*, 338284.

Fang, J., Dong, J., Li, C., Chen, H., Wang, L., Lyu, T., ... & Liu, J. (2021). Response of microbial community composition and function to emergent plant rhizosphere of a constructed wetland in northern China. *Applied Soil Ecology*, *168*, 104141.

Fei, Y., Huang, S., Zhang, H., Tong, Y., Wen, D., Xia, X., ... & Barceló, D. (2020). Response of soil enzyme activities and bacterial communities to the accumulation of microplastics in an acid cropped soil. *Science of The Total Environment*, *707*, 135634

Firestone, M., Kavlock, R., Zenick, H., Kramer, M., & the US EPA Working Group on the Future of Toxicity Testing. (2010). The US Environmental Protection Agency strategic plan for evaluating the toxicity of chemicals. *Journal of Toxicology and Environmental Health*, Part B, 13(2-4), 139-162.

Fito, J., Tefera, N. and Van Hulle, S.W., (2019). Sugarcane biorefineries wastewater: bioremediation technologies for environmental sustainability. *Chemical and Biological Technologies in Agriculture*, *6*(1), pp.1-13.

Fito, J., Tefera, N., Kloos, H., & Van Hulle, S. W. (2018). Anaerobic treatment of blended sugar industry and ethanol distillery wastewater through biphasic high rate reactor. *Journal of Environmental Science and Health, Part A*, *53*(7), 676-685.

Fuess, L. T., & Garcia, M. L. (2014). Implications of stillage land disposal: a critical review on the impacts of fertigation. *Journal of Environmental Management*, *145*, 210-229.

Galal, T. M., Eid, E. M., Dakhil, M. A., & Hassan, L. M. (2018). Bioaccumulation and rhizofiltration potential of Pistia stratiotes L. for mitigating water pollution in the Egyptian wetlands. *International journal of phytoremediation*, *20*(5), 440-447.

García, R., & Báez, A. P. (2012). Atomic absorption spectrometry (AAS). *Atomic absorption spectroscopy*, *1*, 1-13.

Ghazaryan, K. A., Movsesyan, H. S., Khachatryan, H. E., Ghazaryan, N. P., Minkina, T. M., Sushkova, S. N., ... & Rajput, V. D. (2019). Copper phytoextraction and phytostabilization potential of wild plant species growing in the mine polluted areas of Armenia. *Geochemistry: Exploration, Environment, Analysis*, *19*(2), 155-163.

Ghori, N. H., Ghori, T., Hayat, M. Q., Imadi, S. R., Gul, A., Altay, V., & Ozturk, M. (2019). Heavy metal stress and responses in plants. *International journal of environmental science and technology*, *16*(3), 1807-1828.

Ghosh Ray, S., & Ghangrekar, M. M. (2019). Comprehensive review on treatment of high-strength distillery wastewater in advanced physico-chemical and biological degradation pathways. *International Journal of Environmental Science and Technology*, *16*(1), 527-546.

Glick, B. R. (2020). Introduction to plant growth-promoting bacteria. In *Beneficial plant-bacterial interactions* (pp. 1-37). *Springer*, Cham.

Glösl, S., Wagner, K. H., Draxler, A., Kaniak, M., Lichtenecker, S., Sonnleitner, A., ... & Elmadfa, I. (2004). Genotoxicity and mutagenicity of melanoidins isolated from a roasted glucose–glycine model in human lymphocyte cultures, intestinal Caco-2 cells and in the Salmonella typhimurium strains TA98 and TA102 applying the AMES test. *Food and chemical toxicology*, 42(9), 1487-1495.

Gomes CEM, Sousa AKD, Araujo MEDSO, Ferreira SB, Fontanini P (2019) Mechanical and microstructural properties of redispersible polymer-gypsum composites. *Mater Res* 22(3)

Gouda, S., Kerry, R. G., Das, G., Paramithiotis, S., Shin, H. S., & Patra, J. K. (2018). Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. *Microbiological research*, 206, 131-140.

Gronwall, J., & Jonsson, A. C. (2017). Regulating effluents from India's textile sector: new commands and compliance monitoring for zero liquid discharge. *Law Env't & Dev. J.*, *13*, 13.

Gu FL, Kim JM, Abbas S, Zhang XM, Xia SQ, Chen ZX (2010) Structure and antioxidant activity of high molecular weight Maillard reaction products from casein–glucose. *Food Chem*120(2):505–511

Gum, X., & Gum, G. (2017). β-Glucan–156 Hemicellulose–159. *Mechanism and Theory in Food Chemistry*, *153*(3.10), 124.

Gunatilake, S. K. (2015). Methods of removing heavy metals from industrial wastewater. *Methods*, *1*(1), 14.

Gupta, A., Joia, J., Sood, A., Soo Gupta, A., Joia, J., Sood, A., Sood, R., Sidhu, C., & Kaur, G. (2016). Microbes as potential tool for remediation of heavy metals: a review. *J Microb Biochem Technol*, *8*(4), 364-372.

Gupta, R., Kumari, A., Sharma, S., Alzahrani, O. M., Noureldeen, A., & Darwish, H. (2022). Identification, characterization and optimization of phosphate solubilizing rhizobacteria (PSRB) from rice rhizosphere. *Saudi Journal of Biological Sciences*, *29*(1), 35-42.

Gupta, S. K., Singh, B., Mungray, A. K., Bharti, R., Nema, A. K., Pant, K. K., & Mulla, S. I. (2022). Bioelectrochemical technologies for removal of xenobiotics from wastewater. *Sustainable Energy Technologies and Assessments*, *49*, 101652.

Gupta, S., Pawar, S. B., & Pandey, R. A. (2019). Current practices and challenges in using microalgae for treatment of nutrient rich wastewater from agro-based industries. *Science of the total environment*, *687*, 1107-1126.

Hansen, A. P., Choudhary, D. K., Agrawal, P. K., & Varma, A. (Eds.). (2017). Rhizobium biology and biotechnology (Vol. 50). *Springer*.

Hassan, M. U., Chattha, M. U., Khan, I., Chattha, M. B., Aamer, M., Nawaz, M., ... & Khan, T. A. (2019). Nickel toxicity in plants: reasons, toxic effects, tolerance mechanisms, and remediation possibilities—a review. *Environmental Science and Pollution Research*, *26*(13), 12673-12688.

Hatano, K. I., & Yamatsu, T. (2018). Molasses melanoidin-like products enhance phytoextraction of lead through three Brassica species. *International journal of phytoremediation*, *20*(6), 552-559.

Hatano, K. I., Kanazawa, K., Tomura, H., Yamatsu, T., Tsunoda, K. I., & Kubota, K. (2016). Molasses melanoidin promotes copper uptake for radish sprouts: the potential for an accelerator of phytoextraction. *Environmental Science and Pollution Research*, *23*(17), 17656-17663.

Hayat, S., Faraz, A., & Faizan, M. (2017). Root exudates: composition and impact on plant–microbe interaction. *Biofilms in Plant and Soil Health. John Wiley & Sons Ltd*, 179-193.

Hemkemeyer, M., Dohrmann, A. B., Christensen, B. T., & Tebbe, C. C. (2018). Bacterial preferences for specific soil particle size fractions revealed by community analyses. *Frontiers in microbiology*, *9*, 149.

Hermans, S. M., Buckley, H. L., Case, B. S., Curran-Cournane, F., Taylor, M., & Lear, G. (2020). Using soil bacterial communities to predict physico-chemical variables and soil quality. *Microbiome*, *8*(1), 1-13.

Hidalgo, F. J., & Zamora, R. (2016). Amino acid degradations produced by lipid oxidation products. *Critical reviews in food science and nutrition*, *56*(8), 1242-1252.

Hidalgo, F. J., Delgado, R. M., & Zamora, R. (2013). Intermediate role of α-keto acids in the formation of Strecker aldehydes. *Food chemistry*, *141*(2), 1140-1146.

Hlordzi, V., Kuebutornye, F. K., Afriyie, G., Abarike, E. D., Lu, Y., Chi, S., & Anokyewaa, M. A. (2020). The use of Bacillus species in maintenance of water quality in aquaculture: A review. *Aquaculture Reports*, *18*, 100503.

Hoffmann GF, McKiernan P (2017) Liver disease. Inherited metabolic diseases. *Springer*, Berlin, Heidelberg, pp 203–226

Hou, J., Liu, W., Wang, B., Wang, Q., Luo, Y., & Franks, A. E. (2015). PGPR enhanced phytoremediation of petroleum contaminated soil and rhizosphere microbial community response. *Chemosphere*, 138, 592-598.

Hu, Y., Duan, C., Fu, D., Wu, X., Yan, K., Fernando, E., ... & Xu, J. (2020). Structure of bacterial communities in phosphorus-enriched rhizosphere soils. *Applied Sciences*, *10*(18), 6387.

Huang, C. C., Liang, C. M., Yang, T. I., Chen, J. L., & Wang, W. K. (2021). Shift of bacterial communities in heavy metal-contaminated agricultural land during a remediation process. *PLoS One*, *16*(7), e0255137.

Hung, Y. M., Lyu, W. N., Tsai, M. L., Liu, C. L., Lai, L. C., Tsai, M. H., & Chuang, E. Y. (2022). To compare the performance of prokaryotic taxonomy classifiers using curated 16S full-length rRNA sequences. *Computers in Biology and Medicine*, *145*, 105416.

Hussain, A., Rehman, F., Rafeeq, H., Waqas, M., Asghar, A., Afsheen, N., ... & Iqbal, H. M. (2022). In-situ, Ex-situ, and nano-remediation strategies to treat polluted soil, water, and air–A review. *Chemosphere*, *289*, 133252.

Ibanez B (2013) Disturbed coronary hemodynamics in vessels with intermediate stenoses evaluated with fractional flow reserve: a combined analysis of epicardial and microcirculatory involvement in ischemic heart disease. Circulation 128(24):2557–2566

Ijaz, A., Imran, A., Anwar ul Haq, M., Khan, Q. M., & Afzal, M. (2016). Phytoremediation: recent advances in plant-endophytic synergistic interactions. *Plant and Soil*, *405*(1), 179-195.

Inam, A., Adamowski, J., Halbe, J., & Prasher, S. (2015). Using causal loop diagrams for the initialization of stakeholder engagement in soil salinity management in agricultural watersheds in developing countries: A case study in the Rechna Doab watershed, Pakistan. *Journal of environmental management*, *152*, 251-267.

India (2010) Pollution control acts, rules and notifications issued there under. Central Pollution Control Board, New Delhi

Jain N, Bhatia A, Kaushik R, Kumar S, Joshi HC, Pathak H (2005) Impact of post-methanation distillery effluent irrigation on groundwater quality. *Environ Monit Assess* 110(1):243–255

Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN (2014) Toxicity, mechanism and health effects of some heavy metals. *Interdiscip Toxicol* 7(2):60–72

Javed, M. T., Tanwir, K., Akram, M. S., Shahid, M., Niazi, N. K., & Lindberg, S. (2019). Phytoremediation of cadmium-polluted water/sediment by aquatic macrophytes: role of plant-induced pH changes. In *Cadmium toxicity and tolerance in plants* (pp. 495-529). Academic Press

Jeelani, N., Yang, W., Qiao, Y., Li, J., An, S., & Leng, X. (2018). Individual and combined effects of cadmium and polycyclic aromatic hydrocarbons on the phytoremediation potential of Xanthium sibiricum in co-contaminated soil. *International journal of phytoremediation*, *20*(8), 773-779.

Jeelani, N., Yang, W., Zhu, H. L., & An, S. (2020). Phytoremediation for co-contaminated soils of cadmium and pyrene using Phragmites australis (common reed). *International Journal of Phytoremediation*, *22*(13), 1385-1395.

Jeyanthi, V., & Kanimozhi, S. (2018). Plant growth promoting rhizobacteria (PGPR)-prospective and mechanisms: a review. *J Pure Appl Microbiol*, 12(2), 733-749.

Jin, X., Yang, X., Islam, E., Liu, D., & Mahmood, Q. (2008). Effects of cadmium on ultrastructure and antioxidative defense system in hyperaccumulator and non-hyperaccumulator ecotypes of Sedum alfredii Hance. *Journal of hazardous materials*, *156*(1-3), 387-397.

Juliano BO, Varner JE (1969) Enzymic degradation of starch granules in the cotyledons of germinating peas. *Plant Physiol* 44(6):886–892

Kabir ER, Rahman MS, Rahman I (2015) A review on endocrine disruptors and their possible impacts on human health. Environ *Toxicol Pharmacol* 40(1):241–258

Kadam AA, Kamatkar JD, Khandare RV, Jadhav JP, Govindwar SP (2013) Solid-state fermentation: tool for bioremediation of adsorbed textile dyestuff on distillery industry waste-yeast biomass using isolated Bacillus cereus strain EBT1. *Environ Sci Pollut Res* 20(2):1009–1020

Kalam, S., Das, S. N., Basu, A., & Podile, A. R. (2017). Population densities of indigenous Acidobacteria change in the presence of plant growth promoting rhizobacteria (PGPR) in rhizosphere. *Journal of basic microbiology*, *57*(5), 376-385.

Kalavathi, D. F. (2021). Mineralization of Melanoidin by H_2O_2 Producing Enzymes from Marine Cyanobacteria Oscillatoria boryana BDU 92181. *European Journal of Biology and Biotechnology*, *2*(3), 28-32.

Kamle, M., Borah, R., Bora, H., Jaiswal, A. K., Singh, R. K., & Kumar, P. (2020). Systemic acquired resistance (SAR) and induced systemic resistance (ISR): role and mechanism of action against phytopathogens. In Fungal biotechnology and bioengineering (pp. 457-470). *Springer*, Cham.

Kannan A, Upreti RK (2008) Influence of distillery effluent on germination and growth of mung bean (Vigna radiata) seeds. *J Hazard Mater* 153(1–2):609–615

Kapoor, S. (2018). Microbial and Enzymatic Treatment for Decolorization of Distillery Spent-Wash (DSW). *International Journal of Environmental Sciences & Natural Resources*, *14*(2), 10-13.

Kataki, S., Chatterjee, S., Vairale, M. G., Dwivedi, S. K., & Gupta, D. K. (2021). Constructed wetland, an eco-technology for wastewater treatment: A review on types of wastewater treated and components of the technology (macrophyte, biolfilm and substrate). *Journal of Environmental Management*, *283*, 111986.

Kaur, J. (2021). PGPR in Management of Soil Toxicity. In *Rhizobiont in Bioremediation of Hazardous Waste* (pp. 317-344). *Springer*, Singapore.

Kaushik A, Nisha R, Jagjeeta K, Kaushik CP (2005) Impact of long and short term irrigation of a sodic soil with distillery effluent in combination with bioamendments. Biores Technol 96(17):1860–1866

Kavlock, R. J., Daston, G. P., DeRosa, C., Fenner-Crisp, P., Gray, L. E., Kaattari, S., ... & Tilson, H. A. (1996). Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the US EPA-sponsored workshop. *Environmental health perspectives*, *104*(suppl 4), 715-740.

Kaza, S., Yao, L., Bhada-Tata, P., & Van Woerden, F. (2018). *What a waste 2.0: a global snapshot of solid waste management to 2050*. World Bank Publications.

Kennen, K., & Kirkwood, N. (2015). *Phyto: principles and resources for site remediation and landscape design*. Routledge.

Keramati V, Jamili S, Ramin M (2010) Effect of diazinon on catalase antioxidant enzyme activity in liver tissue of Rutilus rutilus. *J Fish Aquat Sci* 5(5):368–376

Kesieme, U. K., Milne, N., Aral, H., Cheng, C. Y., & Duke, M. (2013). Economic analysis of desalination technologies in the context of carbon pricing, and opportunities for membrane distillation. *Desalination*, *323*, 66-74.

Khan, A. G. (2020). Promises and potential of in situ nano-phytoremediation strategy to mycorrhizo-remediate heavy metal contaminated soils using non-food bioenergy crops (Vetiver zizinoides & Cannabis sativa). *International Journal of Phytoremediation*, *22*(9), 900-915.

Khan, M. S., Zaidi, A., Wani, P. A., & Oves, M. (2009). Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils: a review. *Organic Farming, Pest Control and Remediation of Soil Pollutants*, 319-350.

Khan, N., & Bano, A. (2018). Role of PGPR in the phytoremediation of heavy metals and crop growth under municipal wastewater irrigation. In *Phytoremediation* (pp. 135-149). Springer,

Khazaleh, M., & Gopalan, B. (2018). Constructed wetland for wastewater treatment. *Journal of Modern Science and Technology*, *6*(1), 78-86.

Kim HY (2014) Analysis of variance (ANOVA) comparing means of more than two groups. *Restor Dent Endod* 39(1):74

Kimbrough, D. E., & Wakakuwa, J. R. (1989). Acid digestion for sediments, sludges, soils, and solid wastes. A proposed alternative to EPA SW 846 Method 3050. *Environmental science & technology*, *23*(7), 898-900.

Koottatep, T., Ferré, A., Chapagain, S., Fakkaew, K., Strande, L., Velkushanova, K., ... & Buckley, C. (2021). Faecal sludge sample collection and handling. *Methods for Faecal Sludge Analysis*.

Kostich MS, Batt AL, Lazorchak JM (2014) Concentrations of prioritized pharmaceuticals in effluents from 50 large wastewater treatment plants in the US and implications for risk estimation. *Environ Pollut* 184:354–359

Krieg, N. R., & Padgett, P. J. (2011). Phenotypic and physiological characterization methods. In *Methods in microbiology* (Vol. 38, pp. 15-60). Academic Press.

Kulkarni, S. P., Kakde, V. R., & Mane, K. A. (2016). Treatments to distillery spent wash by electro coagulation [ec] and adsorption: a review. *International Journal of Engineering Research and General Science*, *4*(3), 165-172.

Kumar S, Gopal K (2001) Impact of distillery effluent on physiological consequences in the freshwater teleost Channa punctatus. *Bull Environ Contam Toxicol* 66(5):617–622

Kumar V, Chandra R (2018) Characterisation of manganese peroxidase and laccase producing bacteria capable for degradation of sucrose glutamic acid-Maillard reaction products at different nutritional and environmental conditions. *World J Microbiol Biotechnol* 34(2):1–18

Kumar, A., Medhi, K., Fagodiya, R. K., Subrahmanyam, G., Mondal, R., Raja, P., ... & Pathak, H. (2020). Molecular and ecological perspectives of nitrous

oxide producing microbial communities in agro-ecosystems. *Reviews in Environmental Science and Bio/Technology*, *19*(4), 717-750.

Kumar, R., Mishra, R. K., Mishra, V., Qidwai, A., Pandey, A., Shukla, S. K., ... & Dikshit, A. (2016). Detoxification and tolerance of heavy metals in plants. *In Plant metal interaction* (pp. 335-359). Elsevier.

Kumar, V. (2021). Phytoremediation of distillery effluent: current progress, challenges, and future opportunities. *Bioremediation for Environmental Sustainability*, 349-374.

Kumar, V., & Chandra, R. (2019). Bacterial-Assisted Phytoextraction Mechanism of Heavy Metals by Native Hyperaccumulator Plants from Distillery Waste–-Contaminated Site for Eco-restoration. Microbes for Sustainable Development and Bioremediation.

Kumar, V., & Chandra, R. (2020). Bioremediation of melanoidins containing distillery waste for environmental safety. In *Bioremediation of industrial waste for environmental safety* (pp. 495-529). Springer, Singapore.

Kumar, V., & Chandra, R. (2020). Metagenomics analysis of rhizospheric bacterial communities of Saccharum arundinaceum growing on organometallic sludge of sugarcane molasses-based distillery. *3 Biotech*, *10*(7), 1-18.

Kumar, V., & Sharma, D. C. (2019). Distillery effluent: pollution profile, eco-friendly treatment strategies, challenges and future prospects. *Microbial Metabolism of Xenobiotic Compounds*, 337-357.

Kumar, V., Ameen, F., Islam, M. A., Agrawal, S., Motghare, A., Dey, A., ... & Ramamurthy, P. C. (2022). Evaluation of cytotoxicity and genotoxicity effects of refractory pollutants of untreated and biomethanated distillery effluent using Allium cepa. *Environmental Pollution*, *300*, 118975.

Kumar, V., Chandra, R., Thakur, I. S., Saxena, G., & Shah, M. P. (2020). Recent advances in physicochemical and biological treatment approaches for distillery wastewater. *Combined Application of Physico-Chemical & Microbiological Processes for Industrial Effluent Treatment Plant*, 79-118.

Kumar, V., Ferreira, L. F. R., Sonkar, M., & Singh, J. (2021). Phytoextraction of heavy metals and ultrastructural changes of Ricinus communis L. grown on complex organometallic sludge discharged from alcohol distillery. *Environmental Technology & Innovation*, *22*, 101382.

Kumaresan T, Begum KMS, Sivashanmugam P, Anantharaman N, Sundaram S (2003) Experimental studies on treatment of distillery effluent by liquid membrane extraction. *Chem Eng J* 95(1–3):199–204

Kumari, A., Lal, B., & Rai, U. N. (2016). Assessment of native plant species for phytoremediation of heavy metals growing in the vicinity of NTPC sites, Kahalgaon, India. International journal of phytoremediation, 18(6), 592-597.

Kundan, R., Raghuvanshi, S., Bhatt, A., Bhatt, M., & Agrawal, P. K. (2017). Toluene degrading bacteria from the rhizosphere of Solanum melongena contaminated with polycyclic aromatic hydrocarbon. *International Journal of Current Microbiology and Applied Sciences*, *6*(4), 2060-79.

Kunz, W., Henle, J. W. N. B., & Ninham, B. W. (2004). 'Zur Lehre von der Wirkung der Salze'(about the science of the effect of salts): Franz Hofmeister's historical papers. *Current Opinion in Colloid & Interface Science*, 9(1-2), 19-37.

Kurata, T., & Otsuka, Y. (2013). Amino-reductones. *Process-Induced Chemical Changes in Food*, *434*, 269.

Kushwaha, J. P. (2015). A review on sugar industry wastewater: sources, treatment technologies, and reuse. *Desalination and Water Treatment*, *53*(2), 309-318.

Kvesitadze, G., Khatisashvili, G., Sadunishvili, T., & Kvesitadze, E. (2015). Plants for remediation: Uptake, translocation and transformation of organic pollutants. In Plants, pollutants and remediation (pp. 241-308). *Springer*, Dordrecht.

L. J Environ Manag 88(4):1016–1024 Chandra R, Yadav S, Mohan D (2008) Effect of distillery sludge on seed germination and growth parameters of green gram (*Phaseolus mungo* L.). *J Hazard Mater 152*(1):431–439

Lakhani, J., Khuteta, A., Choudhary, A., & Harwani, D. (2017). Hierarchical Clustering-Based Algorithms and In Silico Techniques for Phylogenetic Analysis of Rhizobia. In *Rhizobium Biology and Biotechnology* (pp. 185-214). *Springer*, Cham.

Latt, Z. K., San S, Y., Kyaw, E. P., Lynn, T. M., Nwe, M. T., Mon, W. W., & Aye, K. N. (2018). Using Cellulolytic Nitrogen Fixing Bacterium, Azomonas agilis for effective degradation of agricultural residues. *The Open Microbiology Journal*, *12*, 154.

Lebrazi, S., & Fikri-Benbrahim, K. (2018). Rhizobium-Legume Symbioses: Heavy metal effects and principal approaches for bioremediation of contaminated soil. In *Legumes for soil health and sustainable management* (pp. 205-233). *Springer*, Singapore.

Lee, J. Y., Naylor, R. L., Figueroa, A. J., & Gorelick, S. M. (2020). Water-Food-Energy challenges in India: Political economy of the sugar industry. *Environmental Research Letters*, *15*(8), 084020.

Li, Y., Xu, Z., Ma, H., & S. Hursthouse, A. (2019). Removal of manganese (II) from acid mine wastewater: a review of the challenges and opportunities with special emphasis on Mn-oxidizing bacteria and microalgae. *Water*, *11*(12), 2493.

Linu, M. S., Asok, A. K., Thampi, M., Sreekumar, J., & Jisha, M. S. (2019). Plant growth promoting traits of indigenous phosphate solubilizing Pseudomonas aeruginosa isolates from Chilli (Capsicum annuum L.) Rhizosphere. *Communications in Soil Science and Plant Analysis*, *50*(4), 444-457.

Liu, X., Xia, B., Hu, L. T., Ni, Z. J., Thakur, K., & Wei, Z. J. (2020). Maillard conjugates and their potential in food and nutritional industries: A review. *Food frontiers*, *1*(4), 382-397.

Lucier G, Luster M, Mac MJ, Maczka C, Miller R (1996) Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the US EPA-sponsored workshop. *Environ Health Perspect* 104(suppl 4):715–740

Lund, M. N., & Ray, C. A. (2017). Control of Maillard reactions in foods: Strategies and chemical mechanisms. *Journal of agricultural and food chemistry*, *65*(23), 4537-4552.

Ma, Y., Oliveira, R. S., Freitas, H., & Zhang, C. (2016). Biochemical and molecular mechanisms of plant-microbe-metal interactions: relevance for phytoremediation. *Frontiers in plant science*, *7*, 918.

Mahajan, P. and Kaushal, J., (2018). Role of phytoremediation in reducing cadmium toxicity in soil and water. *Journal of toxicology*, *2018*.

Mahimairaja S, Bolan NS (2004) Problems and prospects of agricultural use of distillery spentwash in India. Magnesium 1715:2100

Maintinguer, S. I., Lazaro, C. Z., Pachiega, R., Varesche, M. B. A., Sequinel, R., & de Oliveira, J. E. (2017). Hydrogen bioproduction with Enterobacter sp. isolated from brewery wastewater. *International Journal of Hydrogen Energy*, *42*(1), 152-160.

Maiti, S., Sinha, S. S., & Singh, M. (2017). Microbial decolorization and detoxification of emerging environmental pollutant: cosmetic hair dyes. *Journal of hazardous materials*, *338*, 356-363.

Malyan, S. K., Yadav, S., Sonkar, V., Goyal, V. C., Singh, O., & Singh, R. (2021). Mechanistic understanding of the pollutant removal and transformation processes in the constructed wetland system. *Water Environment Research*, *93*(10), 1882-1909.

Mani, D., & Kumar, C. (2014). Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: an overview with special reference to phytoremediation. *International journal of environmental science and technology*, *11*(3), 843-872.

Manoj, S. R., Karthik, C., Kadirvelu, K., Arulselvi, P. I., Shanmugasundaram, T., Bruno, B., & Rajkumar, M. (2020). Understanding the molecular

mechanisms for the enhanced phytoremediation of heavy metals through plant growth promoting rhizobacteria: A review. *Journal of environmental management*, *254*, 109779.

Manzoor, M., Gul, I., Ahmed, I., Zeeshan, M., Hashmi, I., Amin, B. A. Z., ... & Arshad, M. (2019). Metal tolerant bacteria enhanced phytoextraction of lead by two accumulator ornamental species. *Chemosphere*, *227*, 561-569.

Marques, J. M., da Silva, T. F., Vollu, R. E., de Lacerda, J. R. M., Blank, A. F., Smalla, K., & Seldin, L. (2015). Bacterial endophytes of sweet potato tuberous roots affected by the plant genotype and growth stage. *Applied Soil Ecology*, 96, 273-281.

Martin, B. C., George, S. J., Price, C. A., Ryan, M. H., & Tibbett, M. (2014). The role of root exuded low molecular weight organic anions in facilitating petroleum hydrocarbon degradation: current knowledge and future directions. *Science of the Total Environment*, 472, 642-653.

Martins SI, van Boekel MA (2003) Melanoidins extinction coefficient in the glucose/glycine Maillard reaction. *Food Chem* 83(1):135–142

Marwa, N., Mishra, N., Singh, N., Mishra, A., Saxena, G., Pandey, V., & Singh, N. (2020). Effect of rhizospheric inoculation of isolated arsenic (As) tolerant strains on growth, As-uptake and bacterial communities in association with Adiantum capillus-veneris. *Ecotoxicology and environmental safety*, *196*, 110498.

Meena, M., Swapnil, P., Divyanshu, K., Kumar, S., Tripathi, Y. N., Zehra, A., ... & Upadhyay, R. S. (2020). PGPR-mediated induction of systemic resistance and physiochemical alterations in plants against the pathogens: Current perspectives. *Journal of Basic Microbiology*, 60(10), 828-861.

Migo, V. P., Del Rosario, E. J., & Matsumura, M. (1997). Flocculation of melanoidins induced by inorganic ions. *Journal of fermentation and bioengineering*, *83*(3), 287-291.

Mikucka, W., & Zielińska, M. (2020). Distillery stillage: characteristics, treatment, and valorization. *Applied Biochemistry and Biotechnology*, *192*(3), 770-793.

Mishra S, Chaube R (2017) Distribution and localization of 3β-hydroxysteroid dehydrogenase (3β-HSD) in the brain and its regions of the catfish *Heteropneustes fossilis*. *Gen Comp Endocrinol* 241:80–88

MOEF (**Ministry of Environment, Forest and Climate Change)** (2016) Environment (protection) amendment rules, 2015. Ministry of environment, forest, and climate change. The Gazette of India. New Delhi. Regd. No. D. L.-33004/99

Moh, Y. (2017). Solid waste management transformation and future challenges of source separation and recycling practice in Malaysia. *Resources, Conservation and Recycling*, *116*, 1-14.

Mohana, S., Acharya, B. K., & Madamwar, D. (2009). Distillery spent wash: treatment technologies and potential applications. *Journal of Hazardous Materials*, *163*(1), 12-25.

Mohsen, A., Park, J., Chen, Y. A., Kawashima, H., & Mizuguchi, K. (2019). Impact of quality trimming on the efficiency of reads joining and diversity analysis of Illumina paired-end reads in the context of QIIME1 and QIIME2 microbiome analysis frameworks. *BMC bioinformatics*, *20*(1), 1-10.

Montpetit, É., & Lachapelle, E. (2017). New environmental technology uptake and bias toward the status quo: The case of phytoremediation. *Environmental Technology & Innovation*, 7, 102-109.

Morel, J. L., Echevarria, G., & Goncharova, N. (Eds.). (2006). Phytoremediation of metal-contaminated soils (Vol. 68). *Springer Science* & Business Media.

Morin-Crini, N., Lichtfouse, E., Fourmentin, M., Ribeiro, A. R. L., Noutsopoulos, C., Mapelli, F., ... & Crini, G. (2022). Removal of emerging contaminants from wastewater using advanced treatments. A review. *Environmental Chemistry Letters*, 1-43.

Mulidzi, A. R. (2010). Winery and distillery wastewater treatment by constructed wetland with shorter retention time. *Water Science and Technology*, *61*(10), 2611-2615.

Na, X., Xu, T., Li, M., Zhou, Z., Ma, S., Wang, J., ... & Ma, F. (2018). Variations of bacterial community diversity within the rhizosphere of three phylogenetically related perennial shrub plant species across environmental gradients. *Frontiers in microbiology*, *9*, 709.

Nakajima-Kambe, T., Shimomura, M., Nomura, N., Chanpornpong, T., & Nakahara, T. (1999). Decolorization of molasses wastewater by Bacillus sp. under thermophilic and anaerobic conditions. *Journal of bioscience and bioengineering*, *87*(1), 119-121.

Navazas, A., Thijs, S., Feito, I., Vangronsveld, J., Peláez, A. I., Cuypers, A., & González, A. (2021). Arsenate-reducing bacteria affect As accumulation and tolerance in Salix atrocinerea. *Science of The Total Environment*, *769*, 144648.

Naveed, M., Bukhari, S. S., Mustafa, A., Ditta, A., Alamri, S., El-Esawi, M. A., ... & Siddiqui, M. H. (2020). Mitigation of nickel toxicity and growth promotion in sesame through the application of a bacterial endophyte and zeolite in nickel contaminated soil. *International journal of environmental research and public health*, *17*(23), 8859.

Nedjimi, B. (2021). Phytoremediation: a sustainable environmental technology for heavy metals decontamination. *SN Applied Sciences*, *3*(3), 1-19.

Nguyen TA, Juang RS (2013) Treatment of waters and wastewaters containing sulfur dyes: a review. *Chem Eng J* 219:109–117

Nihorimbere, V., Ongena, M., Smargiassi, M., & Thonart, P. (2011). Beneficial effect of the rhizosphere microbial community for plant growth and health. *Biotechnologie, Agronomie, Société et Environnement*, *15*(2).

Nikolov, P. (2013). Isotope labelling studies on the reactivity of n-alpha and n-epsilon of lysine in the presence of glucose and its degredation products.

Nivya, R. M. (2015). A study on plant growth promoting activity of the endophytic bacteria isolated from the root nodules of Mimosa pudica plant. *Int J Innov Res Sci Eng Technol*, *4*(8), 6959-6968.

Ntakirutimana, S., Xu, T., Liu, H., Zong, Q. J., Cui, J. Q., Liu, Z. H., ... & Yuan, Y. J. (2022). Amine-based pretreatments for lignocellulose fractionation and lignin valorization: a review. *Green Chemistry*.

Nurzhanova, A., Mukasheva, T., Berzhanova, R., Kalugin, S., Omirbekova, A., & Mikolasch, A. (2021). Optimization of microbial assisted phytoremediation of soils contaminated with pesticides. *International Journal of Phytoremediation*, *23*(5), 482-491.

Nzeteu, C. O., Trego, A. C., Abram, F., & O'Flaherty, V. (2018). Reproducible, high-yielding, biological caproate production from food waste using a single-phase anaerobic reactor system. *Biotechnology for biofuels*, *11*(1), 1-14.

OECD (2003) Guideline for testing of chemicals (2003 draft). Terrestrial plant tests: 208: seedling emergence and seedling growth test, pp 1–19

Ohmomo, S., Daengsubha, W., Yoshikawa, H., Yui, M., Nozaki, K., Nakajima, T., & Nakamura, I. (1988). Screening of anaerobic bacteria with the ability to decolorize molasses melanoidin. *Agricultural and biological chemistry*, *52*(10), 2429-2435.

Ojuederie, O. B., & Babalola, O. O. (2017). Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. *International journal of environmental research and public health*, *14*(12), 1504.

Olaniran, A. O., Hiralal, L., Mokoena, M. P., & Pillay, B. (2017). Flavour-active volatile compounds in beer: production, regulation and control. *Journal of the Institute of Brewing*, *123*(1), 13-23.

Oleńska, E., Małek, W., Wójcik, M., Swiecicka, I., Thijs, S., & Vangronsveld, J. (2020). Beneficial features of plant growth-promoting rhizobacteria for improving plant growth and health in challenging conditions: A methodical review. *Science of the Total Environment*, 743, 140682.

Oleszczuk P, Rycaj M, Lehmann J, Cornelissen G (2012) Influence of activated carbon and biochar on phytotoxicity of air-dried sewage sludges to *Lepidium sativum*. *Ecotoxicol Environ Saf* 80:321–326

Olguín, D. O., Waber, B. N., Kim, T., Mohan, A., Ara, K., & Pentland, A. (2008). Sensible organizations: Technology and methodology for automatically measuring organizational behavior. *IEEE Transactions on Systems, Man, and Cybernetics, Part B (Cybernetics)*, *39*(1), 43-55.

Onakpa, M. M., Njan, A. A., & Kalu, O. C. (2018). A review of heavy metal contamination of food crops in Nigeria. *Annals of global health*, *84*(3), 488.

Oo, K. T., Win, T. T., Khai, A. A., & Fu, P. (2020). Isolation, screening and molecular characterization of multifunctional plant growth promoting rhizobacteria for a sustainable agriculture. *American Journal of Plant Sciences*, *11*(6), 773-792.

Osweiler GD, Carson TL, Buck WB, Van Gelder GA (1985) Clinical and diagnostic veterinary toxicology. Kendall/Hunt Publishing Company

Pande, V., Pandey, S. C., Sati, D., Pande, V., & Samant, M. (2020). Bioremediation: an emerging effective approach towards environment restoration. *Environmental Sustainability*, *3*(1), 91-103.

Pandey, S., Gupta, S., & Ramawat, N. (2019). Unravelling the potential of microbes isolated from rhizospheric soil of chickpea (Cicer arietinum) as plant growth promoter. *3 Biotech*, *9*(7), 1-9.

Pant, D., & Adholeya, A. (2007). Biological approaches for treatment of distillery wastewater: a review. *Bioresource technology*, *98*(12), 2321-2334.

Pant, D., & Adholeya, A. (2009). Nitrogen removal from biomethanated spentwash using hydroponic treatment followed by fungal decolorization. *Environmental engineering science*, *26*(3), 559-565.

Papadopoulos, K. P., Economou, C. N., Dailianis, S., Charalampous, N., Stefanidou, N., Moustaka-Gouni, M., ... & Vayenas, D. V. (2020). Brewery wastewater treatment using cyanobacterial-bacterial settleable aggregates. *Algal Research*, *49*, 101957.

Parker, J. K. (2015). Thermal generation or aroma. In *Flavour development, analysis and perception in food and beverages* (pp. 151-185). Woodhead Publishing.

Patel, T. S., & Desai, P. B. (2015). Isolation and screening of PGPR from rhizospheric and non rhizospheric soil of Bt-cotton. *Indo-American Journal of Agricultural and Veterinary Sciences*, *3*, B1110-B1120.

Patil, S. N., & Prasad, S. R. (2020). An Impact of Nationwide Lockdown on Physico-chemical Parameters of Bhogavati River Water. ES Energy & Environment, 11, 28-39.

Patil, V., & Ghole, V. (2010). Comparative toxicological studies of distillery effluent treatments such as UASB reactor followed by an oxidizer unit using *Cyprinus carpio* fish. Environmental technology, 31(2), 185-191.

Pavlenko-Badnaoui, M., Protska, V., & Zhuravel, I. (2019). The study of the mineral composition of Heliopsis helianthoides. *Norwegian Journal of development of the International Science*, (31-1), 50-53.

Peng, J., Kumar, K., Gross, M., Kunetz, T., & Wen, Z. (2020). Removal of total dissolved solids from wastewater using a revolving algal biofilm reactor. *Water Environment Research*, *92*(5), 766-778.

Phisut N, Jiraporn B (2013) Characteristics and antioxidant activity of Maillard reaction products derived from chitosan-sugar solution. *Int Food Res* J 20(3):1077

Phisut, N., & Jiraporn, B. (2013). Characteristics and antioxidant activity of Maillard reaction products derived from chitosan-sugar solution. *International Food Research Journal*, *20*(3), 1077.

Potter, B. B., & Wimsatt, J. C. (2012). USEPA method 415.3: Quantifying TOC, DOC, and SUVA. Journal-American Water Works Association, 104(6), E358-E369.

Pourzarandian, A., Watanabe, H., Aoki, A., Ichinose, S., Sasaki, K. M., Nitta, H., & Ishikawa, I. (2004). Histological and TEM examination of early stages of bone healing after Er: YAG laser irradiation. *Photomedicine and Laser Therapy*, *22*(4), 342-350.

Praeg, N., Seeber, J., Leitinger, G., Tasser, E., Newesely, C., Tappeiner, U., & Illmer, P. (2020). The role of land management and elevation in shaping soil microbial communities: Insights from the Central European Alps. *Soil Biology and Biochemistry*, *150*, 107951.

Prajapati, A. K., & Chaudhari, P. K. (2015). Physicochemical treatment of distillery wastewater—a review. *Chemical Engineering Communications*, *202*(8), 1098-1117.

Pratap, B., Kumar, S., Purchase, D., Bharagava, R. N., & Dutta, V. (2021). Practice of wastewater irrigation and its impacts on human health and environment: a state of the art. *International Journal of Environmental Science and Technology*, 1-16.

Prica, M., Andrejić, G., Šinžar-Sekulić, J., Rakić, T., & Dželetović, Ž. (2019). Bioaccumulation of heavy metals in common reed (Phragmites australis) growing spontaneously on highly contaminated mine tailing ponds in Serbia and potential use of this species in phytoremediation. *Botanica Serbica*, *43*(1), 85-95.

Prijambada, I. D., Widada, J., Kabirun, S., & Widianto, D. (2009). Secretion of organic acids by phosphate solubilizing bacteria. *Journal of tropical soils*, *14*(3), 245-251.

Provost, J. (2019). The Maillard Reaction. In *Food Aroma Evolution* (pp. 281-291). CRC Press.

Quaratino D, D'Annibale A, Federici F, Cereti CF, Rossini F, Fenice M'(2007) Enzyme and fungal treatments and a combination thereof reduce olive mill wastewater phytotoxicity on Zea mays L. seeds. *Chemosphere* 66(9):1627–1633

Rai PK, Lee SS, Zhang M, Tsang YF, Kim KH (2019) Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environ Int* 125:365–385

Rajasulochana, P., & Preethy, V. (2016). Comparison on efficiency of various techniques in treatment of waste and sewage water–A comprehensive review. *Resource-Efficient Technologies*, *2*(4), 175-184.

Ramakritinan CM, Kumaraguru AK, Balasubramanian MP (2005) Impact of distillery effluent on carbohydrate metabolism of freshwater fish. *Cyprinus Carpio Ecotoxicol* 14(7):693–707

RRamana S, Biswas AK, Singh AB (2002) Effect of distillery effluents on some physiological aspects in maize. *Bioresour Technol* 84(3):295–297

Ramana, S., Tripathi, A.K., Kumar, A., Dey, P., Saha, J.K. and Patra, A.K., 2021. Phytoremediation of soils contaminated with cadmium by Agave americana. *Journal of Natural Fibers*, pp.1-9.

Rana, R. S., Singh, P., Kandari, V., Singh, R., Dobhal, R., & Gupta, S. (2017). A review on characterization and bioremediation of pharmaceutical industries' wastewater: an Indian perspective. *Applied water science*, 7(1), 1-12.

Rana, V., Bandyopadhyay, S., & Maiti, S. K. (2022). Potential and prospects of weed plants in phytoremediation and eco-restoration of heavy metals polluted sites. In *Phytoremediation Technology for the Removal of Heavy Metals and Other Contaminants from Soil and Water* (pp. 187-205). Elsevier.

Rane, M. D., Rathi, P., & Tambe, R. R. (2018). Microbial decolorization and bioremediation of melanoidin containing molasses spent wash.

Rasheed, T., Shafi, S., Bilal, M., Hussain, T., Sher, F., & Rizwan, K. (2020). Surfactants-based remediation as an effective approach for removal of environmental pollutants—A review. *Journal of Molecular Liquids*, *318*, 113960.

Rath, S., Paul, M., Behera, H.K. and Thatoi, H., 2022. Response surface methodology mediated optimization of Lignin peroxidase from Bacillus mycoides isolated from Simlipal Biosphere Reserve, Odisha, India. *Journal of Genetic Engineering and Biotechnology*, *20*(1), pp.1-20.

Ratna, S., Rastogi, S., & Kumar, R. (2021). Current trends for distillery wastewater management and its emerging applications for sustainable environment. *Journal of Environmental Management*, *290*, 112544.

Ratna, S., Rastogi, S., & Kumar, R. (2021). Phytoremediation: A synergistic interaction between plants and microbes for removal of unwanted chemicals/contaminants. In *Microbes and Signaling Biomolecules Against Plant Stress* (pp. 199-222). *Springer*, Singapore.

Raval, V. H., & Saraf, M. (2020). Biosynthesis and purification of indole-3-acetic acid by halotolerant rhizobacteria isolated from Little Runn of Kachchh. *Biocatalysis and Agricultural Biotechnology*, *23*, 101435.

Ravikumar, Y., Razack, S. A., Yun, J., Zhang, G., Zabed, H. M., & Qi, X. (2021). Recent advances in Microalgae-based distillery wastewater treatment. *Environmental Technology & Innovation*, *24*, 101839.

Ribeiro, A. R. L., Moreira, N. F., Puma, G. L., & Silva, A. M. (2019). Impact of water matrix on the removal of micropollutants by advanced oxidation technologies. *Chemical Engineering Journal*, *363*, 155-173.

Rocha OP, De Oliveira DP (2017) Investigation of a Brazilian tannery effluent by means of zebra fish (Danio rerio) embryo acute toxicity (FET) test. *J Toxicol Environ Health* A 80(19–21):1078–1085

Rochette, N. C., Rivera-Colón, A. G., & Catchen, J. M. (2019). Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. *Molecular ecology*, *28*(21), 4737-4754.

Rodrigues, L. A. T., Giacomini, S. J., Dieckow, J., Cherubin, M. R., Ottonelli, A. S., & Bayer, C. (2022). Carbon saturation deficit and litter quality drive the stabilization of litter-derived C in mineral-associated organic matter in long-term no-till soil. *Catena*, *219*, 106590.

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ*, *4*, e2584.

Saeed, M. U., Hussain, N., Sumrin, A., Shahbaz, A., Noor, S., Bilal, M., ... & Iqbal, H. M. (2021). Microbial bioremediation strategies with wastewater treatment potentialities–A review. *Science of The Total Environment*, 151754.

Saeed, Q., Xiukang, W., Haider, F. U., Kučerik, J., Mumtaz, M. Z., Holatko, J., ... & Mustafa, A. (2021). Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: a comprehensive review of effects and mechanisms. *International Journal of Molecular Sciences*, *22*(19), 10529.

Sagova-Mareckova, M., Boenigk, J., Bouchez, A., Cermakova, K., Chonova, T., Cordier, T., ... & Stoeck, T. (2021). Expanding ecological assessment by

integrating microorganisms into routine freshwater biomonitoring. *Water research*, *191*, 116767.

Saha NK, Balakrishnan M, Batra VS (2005) Improving industrial water use: case study for an Indian distillery. *Resour Conserv Recycl* 43(2):163–174

Sahu, O. P., & Chaudhari, P. K. (2013). Review on chemical treatment of industrial waste water. *Journal of Applied Sciences and Environmental Management*, *17*(2), 241-257.

Saleem, K., Asghar, M. A., Saleem, M. H., Raza, A., Kocsy, G., Iqbal, N., ... & Bhat, E. A. (2022). Chrysotile-Asbestos-Induced Damage in Panicum virgatum and Phleum pretense Species and Its Alleviation by Organic-Soil Amendment. *Sustainability*, *14*(17), 10824.

Saleem, M.H., Ali, S., Rehman, M., Hasanuzzaman, M., Rizwan, M., Irshad, S., Shafiq, F., Iqbal, M., Alharbi, B.M., Alnusaire, T.S. and Qari, S.H., 2020. Jute: A potential candidate for phytoremediation of metals—A review. *Plants*, *9*(2), p.258.

Saleh, H. M., Moussa, H. R., Mahmoud, H. H., El-Saied, F. A., Dawoud, M., & Wahed, R. S. A. (2020). Potential of the submerged plant Myriophyllum spicatum for treatment of aquatic environments contaminated with stable or radioactive cobalt and cesium. *Progress in Nuclear Energy*, *118*, 103147.

Sánchez-Galván, G., & Bolaños-Santiago, Y. (2018). Phytofiltration of anaerobically digested sugarcane ethanol stillage using a macrophyte with high potential for biofuel production. *International journal of phytoremediation*, *20*(8), 805-812.

Sangave PC, Pandit AB (2006) Enhancement in biodegradability of distillery wastewater using enzymatic pretreatment. *J Environ Manag* 78(1):77–85

Sankaran K, Premalatha M, Vijayasekaran M, Somasundaram VT (2014) DEPHY project: distillery wastewater treatment through anaerobic digestion and phycoremediation—a green industrial approach. *Renew Sustain Energy Rev* 37:634–643

Sankaran, K., Pisharody, L., Narayanan, G. S., & Premalatha, M. (2015). Bacterial assisted treatment of anaerobically digested distillery wastewater. *RSC advances*, *5*(87), 70977-70984.

Santal, A. R., & Singh, N. (2013). Biodegradation of melanoidin from distillery effluent: role of microbes and their potential enzymes. *Biodegradation of Hazardous and Special Products*, *5*, 71-100.

Saxena G, Chandra R, Bharagava RN (2016) Environmental pollution, toxicity profile and treatment approaches for tannery wastewater and its chemical pollutants. *Rev Environ Contam Toxicol* 240:31–69

Saxena, G., Purchase, D., Mulla, S. I., Saratale, G. D., & Bharagava, R. N. (2019). Phytoremediation of heavy metal-contaminated sites: eco-environmental concerns, field studies, sustainability issues, and future prospects. *Reviews of Environmental Contamination and Toxicology Volume 249*, 71-131.

Seshadri, B., Bolan, N. S., & Naidu, R. (2015). Rhizosphere-induced heavy metal (loid) transformation in relation to bioavailability and remediation. *Journal of soil science and plant nutrition*, *15*(2), 524-548.

Shaheen, S., Shorbagi, M., Lorenzo, J. M., & Farag, M. A. (2021). Dissecting dietary melanoidins: formation mechanisms, gut interactions and functional properties. *Critical Reviews in Food Science and Nutrition*, 1-18.

Shahid, M., Khalid, S., Bibi, I., Bundschuh, J., Niazi, N.K. and Dumat, C., 2020. A critical review of mercury speciation, bioavailability, toxicity and detoxification in soil-plant environment: Ecotoxicology and health risk assessment. *Science of the total environment*, *711*, p.134749.

Rastegari, A. A., Yadav, A. N., & Yadav, N. (Eds.). (2020). *New and future developments in microbial biotechnology and bioengineering: trends of microbial biotechnology for sustainable agriculture and biomedicine systems: diversity and functional perspectives*. Elsevier.

Sharma, P. (2021). Efficiency of bacteria and bacterial assisted phytoremediation of heavy metals: an update. *Bioresource Technology*, *328*, 124835.

Sharma, P., Bakshi, P., Khanna, K., Kour, J., Kapoor, D., Singh, A. D., ... & Bhardwaj, R. (2021). Plant and microbe association for degradation of xenobiotics focusing transgenic plants. Handbook of Assisted and Amendment: Enhanced Sustainable Remediation Technology, 501-516.

Sharma, P., Chaturvedi, P., Chandra, R., & Kumar, S. (2022). Identification of heavy metals tolerant Brevundimonas sp. from rhizospheric zone of Saccharum munja L. and their efficacy in in-situ phytoremediation. *Chemosphere*, *295*, 133823.

Sharma, P., Pandey, A. K., Kim, S. H., Singh, S. P., Chaturvedi, P., & Varjani, S. (2021). Critical review on microbial community during in-situ bioremediation of heavy metals from industrial wastewater. *Environmental Technology & Innovation*, *24*, 101826.

Sharma, P., Tripathi, S., & Chandra, R. (2020). Phytoremediation potential of heavy metal accumulator plants for waste management in the pulp and paper industry. *Heliyon*, *6*(7), e04559.

Shelake, R. M., Waghunde, R. R., Morita, E. H., & Hayashi, H. (2018). Plant-microbe-metal interactions: basics, recent advances, and future trends. Plant microbiome: stress response, 283-305.

Shon HK, Vigneswaran S, Snyder SA (2006) Effluent organic matter (EfOM) in wastewater: constituents, effects, and treatment. *Crit Rev Environ Sci Technol* 36(4):327–374

Shrestha, R., Ban, S., Devkota, S., Sharma, S., Joshi, R., Tiwari, A. P., ... & Joshi, M. K. (2021). Technological trends in heavy metals removal from industrial wastewater: A review. *Journal of Environmental Chemical Engineering*, *9*(4), 105688.

Shukla, S. K., Tripathi, V. K., & Mishra, P. K. (2020). Bioremediation of distillery effluent: present status and future prospects. *Bioremediation of Industrial Waste for Environmental Safety*, 77-97.

Singh, A. K., & Raj, A. (2020). Emerging and eco-friendly approaches for waste management: a book review.

Singh, K., Chandra, R., & Purchase, D. (2022). Unraveling the secrets of rhizobacteria signaling in rhizosphere. *Rhizosphere*, 100484.

Singh, K., Tripathi, S., & Chandra, R. (2021). Maillard reaction product and its complexation with environmental pollutants: A comprehensive review of their synthesis and impact. *Bioresource Technology Reports*, 15, 100779.

Singh, K., Tripathi, S., & Chandra, R. (2021). Maillard reaction product and its complexation with environmental pollutants: A comprehensive review of their synthesis and impact. *Bioresource Technology Reports*, *15*, 100779.

Singh, P. K., Tripathi, M., Singh, R. P., & Singh, P. (2019). Treatment and recycling of wastewater from sugar mill. In *Advances in biological treatment of industrial waste water and their recycling for a sustainable future* (pp. 199-223). Springer, Singapore.

Singh, R., Singh, T. A., Singh, T., Gaur, R., Pandey, P. K., Jamal, F., ... & Patel, R. (2019). Origin and Remediation of Melanoidin Contamination in Water Sources. *Int. J. Curr. Microbiol. App. Sci*, *8*(2), 1399-1415.

Singh, T. B., Sahai, V., Ali, A., Prasad, M., Yadav, A., Shrivastav, P., ... & Dantu, P. K. (2020). Screening and evaluation of PGPR strains having multiple PGP traits from hilly terrain. *Journal of Applied Biology and Biotechnology*, *8*(4), 3-4.

Sneddon, K. A. (2016). *A Comparative Aroma Analysis of Intermediate Wheatgrass and Whole Wheat Bread Crusts* (Doctoral dissertation, University of Minnesota).

Solovchenko, A., Pogosyan, S., Chivkunova, O., Selyakh, I., Semenova, L., Voronova, E., ... & Lobakova, E. (2014). Phycoremediation of alcohol distillery wastewater with a novel Chlorella sorokiniana strain cultivated in a photobioreactor monitored on-line via chlorophyll fluorescence. *Algal Research*, *6*, 234-241.

Song, Y., Jin, L., & Wang, X. (2017). Cadmium absorption and transportation pathways in plants. *International journal of phytoremediation*, *19*(2), 133-141.

Song, Y. Q., Shahir, S., & Abd Manan, F. (2021). Bacterial inoculant-assisted phytoremediation of heavy metal-contaminated soil: Inoculant development and the inoculation effects. *Biologia*, *76*(9), 2675-2685.

Sophia, S., & Shetty Kodialbail, V. (2020). Phytoremediation of soil for metal and organic pollutant removal. *Bioprocess Engineering for Bioremediation*, 45-66.

Sorrentino, M. C., Capozzi, F., Amitrano, C., Giordano, S., Arena, C., & Spagnuolo, V. (2018). Performance of three cardoon cultivars in an industrial heavy metal-contaminated soil: Effects on morphology, cytology and photosynthesis. *Journal of Hazardous Materials*, *351*, 131-137.

Srivastava, J., Kalra, S. J., & Naraian, R. (2014). Environmental perspectives of Phragmites australis (Cav.) Trin. Ex. Steudel. *Applied Water Science*, *4*(3), 193-202.

Sultana, B. (2017). *Degradation of dyes used in textile Industries by bacteria isolated from local textile effluents* (Doctoral dissertation, BRAC University).

Sun, Q., Li, Y., Shi, L., Hussain, R., Mehmood, K., Tang, Z., & Zhang, H. (2022). Heavy metals induced mitochondrial dysfunction in animals: molecular mechanism of toxicity. *Toxicology*, 153136.

Susilowati, P. E., Rajiani, N. A., Hermawan, H., Zaeni, A., & Sudiana, I. N. (2019, July). The use Immobilized Bacteria-Alginate-Chitin for crack remediation. In *IOP Conference Series: Earth and Environmental Science* (Vol. 299, No. 1, p. 012010). IOP Publishing.

Suthar, V., Memon, K. S., & Mahmood-ul-Hassan, M. (2014). EDTA-enhanced phytoremediation of contaminated calcareous soils: heavy metal bioavailability, extractability, and uptake by maize and sesbania. *Environmental monitoring and assessment*, *186*(6), 3957-3968.

Sutherland, D. L., & Ralph, P. J. (2019). Microalgal bioremediation of emerging contaminants-Opportunities and challenges. *Water research*, *164*, 114921.

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular biology and evolution*, *10*(3), 512-526.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular biology and evolution*, *38*(7), 3022-3027.

Tariq, M., Noman, M., Ahmed, T., Hameed, A., Manzoor, N., & Zafar, M. (2017). Antagonistic features displayed by plant growth promoting rhizobacteria (PGPR): a review. *J Plant Sci Phytopathol*, 1(1), 038-43.

Taylor, J. L., Demyttenaere, J. C., Abbaspour Tehrani, K., Olave, C. A., Regniers, L., Verschaeve, L., ... & De Kimpe, N. (2004). Genotoxicity of melanoidin fractions derived from a standard glucose/glycine model. *Journal of agricultural and food chemistry*, 52(2), 318-323.

Teymouri, M., Akhtari, J., Karkhane, M., & Marzban, A. (2016). Assessment of phosphate solubilization activity of Rhizobacteria in mangrove forest. *Biocatalysis and Agricultural Biotechnology*, *5*, 168-172.

Thakare, M., Sarma, H., Datar, S., Roy, A., Pawar, P., Gupta, K., ... & Prasad, R. (2021). Understanding the holistic approach to plant-microbe remediation technologies for removing heavy metals and radionuclides from soil. *Current Research in Biotechnology*, *3*, 84-98

Thamaraiselvi, C., Ancy Jenifer, A., & Vasanthy, M. (2019). Coagulation performance evaluation of natural and synthetic coagulants for the treatment of sugar wash. In *Waste Water Recycling and Management* (pp. 53-64). Springer, Singapore.

Tiwari, S. K., & Prakash, S. (2021). Impact of distillery effluent on aquatic environment: a review. *Indian Journal of Scientific Research*, *11*(2), 85-93.

Tiwari, S., & Gaur, R. (2019). The Application of Microbial Enzymes in Distillery Spentwash Decolorization. *Microbial Treatment Strategies for Waste Management*, 19.

Tonelli, F. C. P., Tonelli, F. M. P., Lemos, M. S., & de Melo Nunes, N. A. (2022). Mechanisms of phytoremediation. In *Phytoremediation* (pp. 37-64). Academic Press.

Touliabah, H. E. S., El-Sheekh, M. M., Ismail, M. M., & El-Kassas, H. (2022). A review of microalgae-and cyanobacteria-based biodegradation of organic pollutants. *Molecules*, *27*(3), 1141.

Traveset, A., & Richardson, D. M. (2014). Mutualistic interactions and biological invasions.

Travieso, L., Benítez, F., Sánchez, E., Borja, R., Leon, M., Raposo, F., & Rincon, B. (2008). Performance of a laboratory-scale microalgae pond for secondary treatment of distillery wastewaters. *Chemical and Biochemical Engineering Quarterly*, *22*(4), 467-473.

Tripathi, S., Purchase, D., Chandra, R., Nadda, A. and Bhargava, P.C., (2022). Mitigation of hazards and risks of emerging pollutants through innovative treatment techniques of post methanated distillery effluent-A review. *Chemosphere*, p.134586.

Tripathi, S., Sharma, P., Purchase, D., & Chandra, R. (2021). Distillery wastewater detoxification and management through phytoremediation employing Ricinus communis L. *Bioresource Technology*, *333*, 125192.

Tripathi, S., Sharma, P., Purchase, D., Tiwari, M., Chakrabarty, D., & Chandra, R. (2021). Biodegradation of organo-metallic pollutants in distillery wastewater employing a bioaugmentation process. *Environmental Technology & Innovation*, *23*, 101774.

Tripathi, S., Sharma, P., Singh, K., Purchase, D., & Chandra, R. (2021). Translocation of heavy metals in medicinally important herbal plants growing on complex organometallic sludge of sugarcane molasses-based distillery waste. Environmental Technology & Innovation, 22, 101434.

Tripathi, S., Singh, K., & Chandra, R. (2021). Adaptation of bacterial communities and plant strategies for amelioration and eco-restoration of an organometallic industrial waste polluted site. In *Microbes in Land Use Change Management* (pp. 45-90). Elsevier.

Tripathi, S., Singh, K., Singh, A., Mishra, A., & Chandra, R. (2022). Organo-metallic pollutants of distillery effluent and their toxicity on freshwater fish and germinating Zea mays seeds. *International Journal of Environmental Science and Technology*, *19*(3), 2025-2038.

Tripathi, S., Singh, V. K., Srivastava, P., Singh, R., Devi, R. S., Kumar, A., & Bhadouria, R. (2020). Phytoremediation of organic pollutants: current status and future directions. *Abatement of environmental pollutants*, 81-105.

Tripathi, S., Yadav, S., Sharma, P., Purchase, D., Syed, A., & Chandra, R. (2022). Plant growth promoting strain Bacillus cereus (RCS-4 MZ520573. 1) enhances phytoremediation potential of Cynodon dactylon L. in distillery sludge. Environmental Research, 208, 112709.

Turan, M., Kıtır, N., Alkaya, Ü., Günes, A., Tüfenkçi, Ş., Yıldırım, E., & Nikerel, E. (2016). Making soil more accessible to plants: the case of plant growth promoting rhizobacteria. Plant Growth, 1, 61-69.

Uddin, M. D., & Robinson, R. W. (2017). Responses of plant species diversity and soil physical-chemical-microbial properties to Phragmites australis invasion along a density gradient. *Scientific reports*, *7*(1), 1-13.

ur Rehman, I., Ishaq, M., Ali, L., Khan, S., Ahmad, I., Din, I. U., & Ullah, H. (2018). Enrichment, spatial distribution of potential ecological and human health risk assessment via toxic metals in soil and surface water ingestion in the vicinity of Sewakht mines, district Chitral, Northern Pakistan. *Ecotoxicology and Environmental Safety*, *154*, 127-136.

USEPA (2002) The environment protection rules, 3A, Schedule- II, III. U.S. Environmental Protection Agency, Office of research and development, Cincinnati

USEPA, US Environmental Protection Agency, (2012). Endocrine Disruptor Screening Program, Universe of Chemicals for Potential Endocrine Disrupt or Screening and Testing. Jointly developed by the Office of Chemical Safety & Pollution Prevention, the Office of Water and the Office of Research and Development, pp 1–176

Valderrama, L. T., Del Campo, C. M., Rodriguez, C. M., de-Bashan, L. E., & Bashan, Y. (2002). Treatment of recalcitrant wastewater from ethanol and citric acid production using the microalga Chlorella vulgaris and the macrophyte Lemna minuscula. *Water research*, *36*(17), 4185-4192.

Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., & Nasrulhaq Boyce, A. (2016). Role of plant growth promoting rhizobacteria in agricultural sustainability—a review. *Molecules*, *21*(5), 573.

Verma, M., & Ekka, A. (2015). Kraft lignin degradation through bacterial strain isolated from soils of timber areas. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, *1*(6), 28-32.

Viehweger, K., (2014). How plants cope with heavy metals. *Botanical Studies*, *55*(1), pp.1-12.

Viswanaathan, S., Perumal, P. K., & Sundaram, S. (2022). Integrated Approach for Carbon Sequestration and Wastewater Treatment Using Algal–Bacterial Consortia: Opportunities and Challenges. *Sustainability*, *14*(3), 1075.

Vuori KM (1995) Direct and indirect effects of iron on river ecosystems. In: Annales zoologici fennici. Finnish Zoological and Botanical Publishing Board, pp 317–329

Wan, W., Tan, J., Wang, Y., Qin, Y., He, H., Wu, H., ... & He, D. (2020). Responses of the rhizosphere bacterial community in acidic crop soil to pH: Changes in diversity, composition, interaction, and function. *Science of the Total Environment*, *700*, 134418.

Wang, H. Y., Qian, H., & Yao, W. R. (2011). Melanoidins produced by the Maillard reaction: Structure and biological activity. *Food chemistry*, *128*(3), 573-584.

Wang, P., Tyndall, S., Rahman, T., Roy, P., Jahromi, H., Adhikari, S., & Boersma, M. (2022). Sorption and recovery of phenolic compounds from aqueous phase of sewage sludge hydrothermal liquefaction using bio-char. *Chemosphere*, *287*, 131934.

Wang, Q., Sun, Q., Wang, W., Liu, X., Song, L., & Hou, L. (2022). Effects of Different Native Plants on Soil Remediation and Microbial Diversity in Jiulong Iron Tailings Area, Jiangxi. Forests, 13(7), 1106

Wang, X. Q., Zhao, D. L., Shen, L. L., Jing, C. L., & Zhang, C. S. (2018). Application and mechanisms of Bacillus subtilis in biological control of plant disease. In Role of rhizospheric microbes in soil (pp. 225-250). Springer, Singapore.

Wang, Y., Wang, H., Wang, X., Xiao, Y., Zhou, Y., Su, X., & Sun, F. (2020). Resuscitation, isolation and immobilization of bacterial species for efficient textile wastewater treatment: a critical review and update. *Science of the Total Environment*, *730*, 139034.

Weisskopf, L., Schulz, S., & Garbeva, P. (2021). Microbial volatile organic compounds in intra-kingdom and inter-kingdom interactions. *Nature Reviews Microbiology*, *19*(6), 391-404.

Wen, Q., & Zhang, T. (2022). Economic policy uncertainty and industrial pollution: The role of environmental supervision by local governments. *China Economic Review*, *71*, 101723.

Weselowski, B., Nathoo, N., Eastman, A. W., MacDonald, J., & Yuan, Z. C. (2016). Isolation, identification and characterization of Paenibacillus polymyxa CR1 with potentials for biopesticide, biofertilization, biomass degradation and biofuel production. *BMC microbiology*, *16*(1), 1-10.

Wibowo, S., Grauwet, T., Santiago, J. S., Tomic, J., Vervoort, L., Hendrickx, M., & Van Loey, A. (2015). Quality changes of pasteurised orange juice during storage: A kinetic study of specific parameters and their relation to colour instability. *Food Chemistry*, *187*, 140-151.

Woraharn, S., Meeinkuirt, W., Phusantisampan, T., & Chayapan, P. (2021). Rhizofiltration of cadmium and zinc in hydroponic systems. *Water, Air, & Soil Pollution*, *232*(5), 1-17.

Xu, C., Paone, E., Rodríguez-Padrón, D., Luque, R., & Mauriello, F. (2020). Recent catalytic routes for the preparation and the upgrading of biomass derived furfural and 5-hydroxymethylfurfural. *Chemical Society Reviews*, *49*(13), 4273-4306.

Yaashikaa, P. R., Kumar, P. S., Jeevanantham, S., & Saravanan, R. (2022). A review on bioremediation approach for heavy metal detoxification and accumulation in plants. *Environmental Pollution*, 119035.

Yadav, S., & Chandra, R. (2019). Environmental health hazards of post-methanated distillery effluent and its biodegradation and decolorization. In *Environmental biotechnology: for sustainable future* (pp. 73-101). Springer, Singapore.

Yadav, D., Dutta, A., & Mande, S. S. (2019). OTUX: V-region specific OTU database for improved 16S rRNA OTU picking and efficient cross-study taxonomic comparison of microbiomes. *DNA Research*, *26*(2), 147-156.

Yadav, K. K., Gupta, N., Kumar, A., Reece, L. M., Singh, N., Rezania, S., & Khan, S. A. (2018). Mechanistic understanding and holistic approach of phytoremediation: a review on application and future prospects. Ecological engineering, 120, 274-298.

Yadav, S., & Chandra, R. (2011). Heavy metals accumulation and ecophysiological effect on Typha angustifolia L. and Cyperus esculentus L. growing in distillery and tannery effluent polluted natural wetland site, Unnao, India. *Environmental Earth Sciences*, *62*(6), 1235-1243.

Yadav, S., & Chandra, R. (2019). Environmental health hazards of post-methanated distillery effluent and its biodegradation and decolorization. In Environmental biotechnology: for sustainable future (pp. 73-101). Springer, Singapore.

Yadav, S., Singh, K., & Chandra, R. (2019). Plant Growth–Promoting Rhizobacteria (PGPR) and Bioremediation of Industrial Waste. In Microbes for Sustainable Development and Bioremediation (pp. 207-241). CRC Press.

Yan N, Marschner P, Cao W, Zuo C, Qin W (2015) Influence of salinity and water content on soil microorganisms. Int Soil Water Conserv Res 3(4):316–323

Yan, A., Wang, Y., Tan, S. N., Mohd Yusof, M. L., Ghosh, S., & Chen, Z. (2020). Phytoremediation: a promising approach for revegetation of heavy metal-polluted land. *Frontiers in Plant Science*, *11*, 359.

Yan, J., Wang, L., Hu, Y., Tsang, Y. F., Zhang, Y., Wu, J., ... & Sun, Y. (2018). Plant litter composition selects different soil microbial structures and in turn drives different litter decomposition pattern and soil carbon sequestration capability. *Geoderma*, *319*, 194-203.

Yu, H., Zhang, R., Yang, F., Xie, Y., Guo, Y., Yao, W., & Zhou, W. (2021). Control strategies of pyrazines generation from Maillard reaction. *Trends in Food Science & Technology*, *112*, 795-807.

Zandi, P., Yang, J., Darma, A., Bloem, E., Xia, X., Wang, Y., ... & Schnug, E. (2022). Iron plaque formation, characteristics, and its role as a barrier and/or facilitator to heavy metal uptake in hydrophyte rice (Oryza sativa L.). *Environmental Geochemistry and Health*, 1-35.

Zayneb C, Lamia K, Olfa E, Naima J, Grubb CD, Bassem K, Hafedh M, Amine E (2015) Morphological, physiological and biochemical impact of ink industry effluent on germination of maize (Zea mays), Barley (Hordeum vulgare) and Sorghum (Sorghum bicolor). Bull Environ Contam Toxicol 95(5):687–693

Zhang, H., Feng, J., Chen, S., Zhao, Z., Li, B., Wang, Y., ... & Hao, H. (2019). Geographical patterns of nirS gene abundance and nirS-type denitrifying

bacterial community associated with activated sludge from different wastewater treatment plants. *Microbial ecology*, *77*(2), 304-316.

Zhang, H., Zhang, L. L., Li, J., Chen, M., & An, R. D. (2020). Comparative study on the bioaccumulation of lead, cadmium and nickel and their toxic effects on the growth and enzyme defence strategies of a heavy metal accumulator, Hydrilla verticillata (Lf) Royle. *Environmental Science and Pollution Research*, *27*(9), 9853-9865.

Zhang, M., Wang, Z., Li, P., Zhang, H., & Xie, L. (2017). Bio-refractory dissolved organic matter and colorants in cassava distillery wastewater: characterization, coagulation treatment and mechanisms. *Chemosphere*, *178*, 259-267.

Zhu, L. D., & Hiltunen, E. (2016). Application of livestock waste compost to cultivate microalgae for bioproducts production: A feasible framework. *Renewable and Sustainable Energy Reviews*, *54*, 1285-1290.

Publications and Achievments

Publications and Achievements

Scientific Publications and Achievements:

(A) Research/ Review Papers published high impact Journals of International Repute: *Four*

(1) Singh, K., Chandra, R. and Purchase, D., 2022. Unraveling the secrets of rhizobacteria signaling in rhizosphere. *Rhizosphere*, p.100484.

(2) Singh, K., Tripathi, S. and Chandra, R., 2021. Maillard reaction product and its complexation with environmental pollutants: A comprehensive review of their synthesis and impact. *Bioresource Technology Reports*, *15*, p.100779.

(3) Tripathi, S., **Singh, K.**, Singh, A., Mishra, A. and Chandra, R., 2021. Organo-metallic pollutants of distillery effluent and their toxicity on freshwater fish and germinating Zea mays seeds. *International Journal of Environmental Science and Technology*, pp.1-14.

(4) Tripathi, S., Sharma, P., **Singh, K**., Purchase, D. and Chandra, R., 2021. Translocation of heavy metals in medicinally important herbal plants growing on complex organometallic sludge of sugarcane molasses-based distillery waste. *Environmental Technology & Innovation*, *22*, p.101434.

(B) Research Paper Communicated: Two

(1) Singh, K. and Chandra, R., 2022. Bacterial assisted phytoremediation of heavy metals and organic pollutants by *Cannabis sativa* as hyperaccumulator plants growing on distillery waste disposal site for ecorestoration of polluted site.

(2) Singh, K. and Chandra, R., 2022. Bacterial-aided in-situ phytoremediation of heavy metals and organic pollutants by *Parthenium hysterophorus* growing natively on disposed distillery sludge: A prospective tool for ecorestoration of polluted site.

(C) Book Chapters: Four

(1) Tripathi, S., **Singh, K.,** & Chandra, R. (2021). Adaptation of bacterial communities and plant strategies for amelioration and eco-restoration of an organometallic industrial waste polluted site. In *Microbes in Land Use Change Management* (pp. 45-90). Elsevier.

(2) Yadav, S., **Singh, K**., & Chandra, R. (2019). Plant Growth–Promoting Rhizobacteria (PGPR) and Bioremediation of Industrial Waste. In *Microbes for Sustainable Development and Bioremediation* (pp. 207-241). CRC Press.

(3) Chandra, R., & **Singh, K.** (2017). Endophytic Bacterial Diversity in Roots of Wetland Plants and Their Potential for Enhancing Phytoremediation of Environmental Pollutants. In *Phytoremediation of Environmental Pollutants* (pp. 285-326). CRC Press.

(4) Chandra, R., Kumar, V., & **Singh, K.** (2017). Hyperaccumulator versus nonhyperaccumulator plants for environmental waste management. In Phytoremediation of environmental pollutants (pp. 43-80). CRC Press.

(D) Patent

(1) Australian patent granted on entitled "*Decolorization and Detoxification of Distillery Wastewater with Combined Use of Bacteria and Constructed Wetland Treatment*" (Application number :2021101010)

(E) Awards

(1) Awarded **first prize** in **oral presentation** entitled "**Bacterial-aided in-situ phytoremediation of heavy metals and organic pollutants by *Parthenium hysterophorus* growing natively on disposed distillery sludge: A prospective tool for ecorestoration of polluted site"** in the Global Conference on "**Lucknow Climate Change Conference on Control of Green House Gasses at the Source by Physical and Chemical Technologies_2k22 (LCCCCGGSPCT_2k22)"**on the occasion of "Earth Day (22 April)", , jointly organized by the **Department of Chemistry, Babasaheb Bhimrao Ambedkar University (A Central University) Lucknow-226 025**, **The Asian Association of Sugar Cane Technologists' (AASCT) Lucknow** and **Uttrakhand Mahaparishad (UMP)** Lucknow**.**

(F) Research Paper Presented in National /International symposium and conferences

(1) Kshitij Singh presented a paper entitled ""**Bacterial-aided in-situ phytoremediation of heavy metals and organic pollutants by *Parthenium***

hysterophorus **growing natively on disposed distillery sludge: A prospective tool for ecorestoration of polluted site"** in the Global Conference on "**Lucknow Climate Change Conference on Control of Green House Gasses at the Source by Physical and Chemical Technologies_2k22 (LCCCCGGSPCT_2k22)"**

(2) Kshitij Singh presented a paper entitled "**Bacterial assisted phytoremediation of heavy metals and organic pollutants by *Cannabis sativa* as hyperaccumulator plants growing on distillery waste disposal site for ecorestoration of polluted site"** in the International Conference on Biotechnology for Resource Efficiency, Energy, Environment, Chemicals and Health, jointly organized by CSIR-Indian Institute of Petroleum, Dehradun and The Biotech Research Society, India at Dehradun, India during December 01-04, 2021

(G) Conferences/workshop participation and poster presentations

(1) Kshitij Singh **participated** in '**Workshop on Good Laboratory Practice (GLP)**' organized by 'Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow' under the aegis of Indian Pharmacological Society (IPS) on 16th April, 2022.

(2) Kshitij Singh presented a paper entitled "**Bacterial assisted phytoremediation of heavy metals and organic pollutants by *Cannabis sativa* as hyperaccumulator plants growing on distillery waste disposal site for ecorestoration of polluted site"** in the International Conference on Biotechnology for Resource Efficiency, Energy, Environment, Chemicals and Health, jointly organized by CSIR-Indian Institute of Petroleum, Dehradun and The Biotech Research Society, India at Dehradun, India during December 01-04, 2021.

(3) Kshitij Singh presented a **poster** entitled "**Unraveling the Secrets of rhizobacteria in rhizosphere"** on the National Seminar on "**Horticulture: A Boon for Indian Economy**" during October 31, 2019 at Babasaheb Bhimrao Ambedkar University (A Central University) , Lucknow, Uttar Pradesh.

(4) Kshitij Singh presented a **poster** entitled "**Endophytic Bacterial Diversity in Roots of Wetland Plants and Their Potential for Enhancing Phytoremediation of Environmental Pollutants"** under the theme " Recent

Advances in Agricultural, Environmental and Applied Sciences for Global Development" (RAAEASGD-2019) held during September 27-29, 2019 at Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, H.P., India.

(5) Kshitij Singh **participated** in two day national seminar on **"Environment Protection: An Interdisciplinary Trend For Sustainable Development"** organized by **Departments of Environment Science and Geography, under quality improvement programme of Savitribai Phule Pune University, Pune held on 11th and 12th January, 2019.**

(6) Kshitij Singh **participated** in the **"106th Indian Science Congress"** held at **Lovely Professional University, Phagwara, Jalandhar** from **January 03 to 07, 2019.**

(7) Kshitij Singh **participated** in the **core organizing committee** of **58th Annual Conference of Association of Microbiologists of India & International Symposium on Microbes for Sustainable Development: Scope & Applications (MSDSA -2017) during November 16-19,2017,** at Babasaheb Bhimrao Ambedkar University(A Central University) , Lucknow, Uttar Pradesh.

(8) Kshitij Singh presented a **poster** entitled "**Plant Growth Promoting Rhizobacteria (PGPR) and bioremediation of industrial waste**" **in the of 58th Annual Conference of Association of Microbiologists of India & International Symposium on Microbes for Sustainable Development: Scope & Applications (MSDSA -2017)** during **November 16-19,2017,** at Babasaheb Bhimrao Ambedkar University(A Central University) , Lucknow, Uttar Pradesh.

- Kshitij Singh **participated** in the National Symposium on "**IPRs in Agricultural Research**" during August 30-31, 2017 at Babasaheb Bhimrao Ambedkar University (A Central University) **,** Lucknow, Uttar Pradesh.
- Kshitij Singh **attended and participated** in the **"IndoUS Workshop & International Symposium on Biological Timing and Health Issues in the 21st Century"** during **February 22-24 2017,** at Department of Zoology, University of Delhi, India.

Reprints

Rhizosphere 21 (2022) 100484

Contents lists available at ScienceDirect

Rhizosphere

journal homepage: www.elsevier.com/locate/rhisph

Unraveling the secrets of rhizobacteria signaling in rhizosphere

Kshitij Singh [a], Ram Chandra [a,*], Diane Purchase [b]

[a] Department of Environmental Microbiology, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, 226025, Uttar Pradesh, 226025, India

[b] Department of Natural Sciences, Faculty of Science and Technology, Middlesex University, The Burroughs, Hendon, London, NW4 4BT, England, UK

ARTICLE INFO

Keywords:
Rhizobacterium
Root exudate
Signaling molecules
Quorum sensing
AHL

ABSTRACT

Signaling among rhizobacteria and other soil microorganisms is an important mechanism to ensure a successful symbiotic relationship with their phytobionts. Quorum-sensing involves signaling molecules that provide essential networks for communication in the rhizosphere. These signaling molecules relay inter-and-intra-species information that coordinates and controls behavior in mixed communities, and the expression of these signaling molecules changes in response to the chemical cues. The diverse signaling molecules released in the rhizospheric zone affect the structural and physical heterogeneity of the soil and the quantity and identity of rhizobacteria. In general, rhizospheric signaling mechanisms can be categorized into three major types (i) plant to microorganisms signaling through low molecular weight molecules secreted by plants; (ii) interspecies and intraspecies microbial signaling, chiefly through quorum-sensing molecules; and (iii) microorganisms to plant signaling by microbially produced compounds. This review presents knowledge on the signaling molecules of the rhizosphere based on the above three mechanisms. The chemical nature of root exudates and their roles in attracting metabolically active rhizobacteria; the chemical properties of autoinducers secreted by rhizobacteria and their functions in intra- and inter-species interactions, including biofilm formation in the rhizosphere; and influence of quorum sensing on the root architecture, plant defense and biotic and abiotic stress responses, and gene expression are examined. The review provides a thorough understanding of rhizobacteria signaling and will help to develop novel strategies for agriculture, such as the novel use of plant growth-promoting rhizobacteria to enhance crops growth and quorum quenching technique to fight against plant pathogens.

1. Introduction

The rhizosphere is a narrow yet highly complex and dynamic zone consisting of nutrient-rich soil. It is influenced by plant roots and its diverse microbial community, such as plant growth-promoting rhizobacteria (PGPR), plant growth-promoting fungi (PGPF), nematodes, mycorrhizal fungi, mycoparasitic fungi, and protozoa. Through their diverse communications as symbionts, these microorganisms have acquired an evolutionary relationship with their phytobionts (Venturi and Keel, 2016; Mondal and Baksi, 2019; Mathesius and Costa, 2021). Plants roots exude a variety of primary metabolites (sugars, nucleic acids, amino acids, carbohydrates, lipids, and organic acids) and secondary metabolites (phenolics, alkaloids, sterols, steroids, terpenoids, lignin, and essential oils). The release of these metabolites in the rhizosphere constitutes a significant amount of nitrogen and reduced carbon that benefits the plant by attracting and encouraging the growth of beneficial microbes while inhibiting pathogenic ones (Olanrewaju et al., 2019). Thus, these root-derived materials play an important role in shaping and establishing a mutualistic association between the symbiotic partners as well as inducing plant defense mechanisms (Canarini et al., 2019).

The rhizospheric signaling mechanisms can be categorized into three major types (i) plant to microorganisms signaling through low molecular weight molecules secreted by plants, which are involved in numerous complex plant-microbes interactions; (ii) interspecies and intraspecies microbial signaling, which takes place chiefly through quorum-sensing (QS) molecules permitting microorganisms to shape and contemporize their behavior; and (iii) microorganisms to plant signaling by microbially produced compounds that influence the root architecture, plant defense and biotic and abiotic stress responses, and gene expression (Altaf et al., 2017; Venturi and Keel, 2016). The communication of rhizobacteria with the host plant is a dynamic and interdependent relationship that involves both interacting partners and biotic-abiotic factors of the rhizosphere (Hassan et al., 2019). However, the abiotic factors, particularly environmental factors that modulate

* Corresponding author.
E-mail address: prof.chandrabbau@gmail.com (R. Chandra).

https://doi.org/10.1016/j.rhisph.2022.100484
Received 5 August 2021; Received in revised form 7 February 2022; Accepted 7 February 2022
Available online 8 February 2022

rhizospheric structure, are relatively unclear. It has beenhypothesized that various abiotic elements may play a crucial role in modulating soil microbes' variability, including those colonizing in the rhizosphere (Santoyo et al., 2017).Indeed, in several cases, the soil or rhizosphere microbiome is affected by two or more abiotic factors, thereby complicating the analysis of the specific effects of single factors. Some abiotic modulating factors are pH, type of soil, soil moisture, soil water content, dissolved organic matter, temperature, atmospheric CO_2, etc. (Santoyo et al., 2017).

A diverse variety of QS networks have been identified among soil bacterial species involving a number of signaling molecules, including acylated homoserine lactones (AHLs), diketopiperazines (DKPs), diffusible signaling factor (DSF), secondary metabolites, phytohormone-like molecules, and other small organic compounds (Venturi, and Keel 2016; Mondal and Baksi, 2019). Among the signaling molecules, AHLs are the most extensively studied in bacteria (Ostroumova et al., 2015a, b), cyclodipepetides and tehir derivative diketopiperazines have also been shown to modulates auxin signaling inplants (Ortiz-Castro et al., 2011). In general, to recognize its rhizospheric associated microbes, plants use pattern recognition receptors (PRRs) which are membrane-associated receptor-like kinases or receptor-like proteins, reflecting the prevalence of apoplastic colonization of plant-infecting microbes. In addition, there are various precise microbe-associated molecular patterns (MAMPs) which are molecular signatures that are highly conserved in whole classes of microbes, butthey are yet to be fully characterized (Rashid et al., 2017). The signaling molecules also play a vital role in signal transduction both in inter- and intra-species interactions. They evoke a range of effects on different plant growth phases and other local and systemic immune responses, namely induced systemic resistance (ISR) and systemic acquired resistance (SAR) (Mhlongo et al., 2018; Shrestha et al., 2020).QS is also involved in the production of biofilm in the rhizosphere that facilitates colonization of the plant roots (Noirot-Gros et al., 2018; Zboralski and Filion, 2020). While several rhizobacteria can develop biofilms as multicellular communities, some develop into distinct morphotypes (Olanrewaju et al., 2017; Backer et al., 2018). These synchronized developmental dynamics are often controlled by cell-to-cell contact mediated by QS signals, as various strains isolated from the rhizosphere have been reported to produce andsecrete small molecules that act as autoinducers (AIs). For example, N-acyl homoserine lactones (AHLs) are used as AIs by Gram-negative bacteria, although AHLs are not exclusive to Gram-negative bacteria (Schikora et al., 2016).

QS plays an essential role in maintaining and improving crop production in agriculture. Several commercial products, such as Sudoz-P, Early green *Pseudomonas fluorescens*, Abtec, ROM, PMCS, Pangoo Plant grower, Green dual, Panther-PF, etc. are marketed as biofertilizer inocula, consisting chiefly of *Serratia* spp. or *Pseudomonas*, *Bacillus,* and other specialized fertility enhancer bacteria that are responsiblefor enhancing nitrogen fixation, colonization, and the transmission of phytopathogenic response (Jnawali et al., 2015). However, recently it has been realized that some microorganisms used as biofertilizers behave as opportunistic pathogens and belong to the biosafety level 2 (BSL-2) classification. Evidence presented in various scientific forums is increasingly favoring the merits of using BSL-2 microorganisms as biofertilizers (Keswani et al., 2019).

This review provides updated knowledge on the chemical nature and significance of the signaling compounds involved in plant-microbe interactions; it will help to develop further understanding of rhizobacteria communications and their applications in agricultural practices.

2. Plant to microorganisms: root exudates chemical compositions and influence of soil physico-chemical properties

The ability of plants to select specific bacteria may ensue directly from its chemical signals and their effects on its colonizing rhizobacteria (Doornbos et al., 2012). Root exudates secreted by plant roots serve as chemical attractants for numerous metabolically active microbial communities (Wang et al., 2021; Massalha et al., 2017). Several researchers found that plants establish a favorable environment by recruiting specific rhizospheric bacteria via root exudates (Dennis et al., 2010; Rudrappa et al., 2008; Loyola-Vargas et al., 2007; Fang et al., 2013). The stability of colonization is greater for specific regions of the root, presumably because these sites release abundant root exudates (Scharf et al., 2016). The rhizospheric bacterial community residing within the root vicinity use these soluble materials to mediate positive or negativeinteractions between plants and microbes. These signaling molecules can act as substrates for rhizobacteria, as plant pheromones to stimulate or antagonise other microorganisms. Once the rhizobacteria sense the signaling molecules, communication and interaction commence (Bhattacharya et al., 2010). The constituents of root exudates can modify microbial dynamics, favoring PGPR growth and preventing microbe growth considered hazardous to the associated plant (Dutta et al., 2013; De Coninck et al., 2015). Root exudates can also mediate tripartite signaling in the rhizosphere, among the host plant, pathogens, and the beneficial rhizobacteria (Liu et al., 2014).

2.1. Chemical composition of root exudates

Root exudates are rich in carbon and nitrogen sources, and therefore, are highly complex and difficult to classify. Although several constituents of root exudates have been identified and characterized (Ahmad et al., 2018; Scharf et al., 2016), but still a large proportion of root exudates are unidentified till date. The structural compositions of these root exudates (Table 1) alter soil physico-chemical properties to favor the colonization of rhizospheric bacteria (Godheja et al., 2017; Kumar et al., 2006; Dakora and Phillips, 2002). The compositions of the root exudates depend upon the physiological status of microorganisms, plants and soil (Hassan et al., 2019). For example, alterations in plant exudation of amino acids are regulated by microbial metabolites such as zearalenone, 2,4-diacetylphloroglucinol (DAPG, 5), and phenazine (Phillips et al., 2004).

Basically, root exudates can be categorized into low and high molecular weight signaling molecules. Low-molecular-weight exudates comprise various signaling molecules, i.e., sugars, organic acids, phenolics, amino acids, and other secondary metabolites. High molecular weight signaling molecules primarily include proteins and mucilages (as mucopolysaccharides). They are not diverse, but they constitute a substantial proportion of root exudates (Chapparo et al., 2014). Several compound types are exuded by plant roots, particularly amino acids and carbohydrates; they serve as general pheromones for various microbes, while others connect to more specific interactions (More et al., 2019). In non-pathogenic, i.e., a mutualistic or commensal interaction, the host is likely to distinguish analogous non-self, non-cultivar-dependent determinants known as SAMPs (Symbiont Associated Molecular Patterns). Hosts in commensal and mutualistic relationships are just as likely to recognize non-self as are hosts in pathogenic/parasitic interactions (McFall-Ngai, 2002).

Protein molecules released as root exudates are essential for antagonism against and rapport with bacteria (Pascale et al., 2020). Lectins are the most studied protein because of their role in biological recognition; they assist in both defense and symbiosis responses (Reyes-Montaño and Vega-Castro, 2018). Plants also exude several protein molecules with consistent enzymatic defense activities (Prasannath, 2017). Many mucopolysaccharides released into the rhizosphere from the plant's root apex contain arabinogalactans that interact between plant roots and the rhizospheric microbes, attracting PGPR and repelling pathogens (Nguema-Ona et al., 2013; Cannesan et al., 2012; Xie et al., 2012).

The exact mechanisms utilized by plants to form/regulate their microbiome are still not fully understood. However, hormones involved in provoking immunity within plants, especially salicylic acid, are believed toplay a role in shaping the rhizospheric microbiome (Lebeis

Table 1
Various root exudates released by plants in rhizosphere and their functions.

Root Exudates	Compounds	Functions
Enzymes	Amylase, proteases, invertase, peroxidase, phenolase, acid phosphatases, alkaline phosphatase, polygalacturonase, lectins, hydrolases, lipase	catalysts for P release from organic molecules, biocatalysts for organic matter transformation in soil
Organic acids	Acetic, succinic, laspartic, l-glutamic, salicylic, shikimic, isocitric, chorismic, sinapic, *p*-hydroxybenzoic, gallic, tartaric, protocatacheuic, *p*-coumaric, mugineic, oxalic, citric, piscidicaconitic, ascorbic, butyric, caffeic, citric, *p*-coumaric, ferulic, fumaric, glutaric, glycolic, glyoxilic, malic, malonic, oxalacetic, oxalic, *p*-hydroxy-benzoic, propionic, succinin, syringic, tartaric, valeric, vanillic	nutrient source, chemoattractant signals to microbes, chelators of poorly soluble mineral nutrients, acidifiers of soil, detoxifiers of Al, nod gene inducers
Phenolic acid and coumarin	Caffeic acid, benzoic acid, cinnamic acid, coumarin, ferulic acid, salycilic acid, syringic acid, vanillic acid	act as defense elements, antioxidant properties
Amino acids	α-Alanine, l-hydroxyproline, homoserine, mugineic acid β-alanine,γ-aminobutyric, arginine, aspartic, citrulline, cystathionine, cysteine, cystine, deoxymugineic, 3-epi-hydroxymugineic, aminobutyricacid, glutamine, glutamic, glycine, homoserine, isoleucine, leucine, lysine, methionine, mugineic, ornithine, phenylalanine, proline, serine, threonine, tryptophane, tyrosine, valine and all proteinogenic amino acids	nutrient source, nutrient acquisition from the rhizosphere
Vitamins	Thiamine, riboflavin, niacin, pantothenate, biotin	promoters of plant and microbial growth nutrient source
Polysaccharides and sugars	sucrose, pentose, arabinose, fructose, galactose, glucose, maltose, mucilages of various compositions, oligosaccharides, raffinose, rhamnose, ribose, sucrose, xylose, mannitol	Enhance root colonization
Lignin derivatives	Catechol, nicotinic acid, benzoic acid, phloroglucinol	Increases rhizospheric soil fertility
Nucleosides (purines)	Adenine, guanine, cytidine, uridine	nutrient source
Growth factors	*p*-Amino benzoic acid, biotin, choline, *N*-methyl nicotinic acid, niacin, pantothenic, vitamins B1 (thiamine), B2 (riboflavin) and B6 (pyridoxine)	coenzyme in metabolic pathways and central metabolism
Fatty acids	Linoleic, palmitic, stearic, linolenic, oleic	transport activities across the plasma membrane
Sterols	sitosterol, stigmasterol, Campesterol, cholesterol	regulate the fluidity and the permeability of phospholipid bilayers
Flavonoids and flavonols	Kaempferol, quercetin, rutin, naringenin, myricetin, strigolactone, genistein	inhibit root pathogens, stimulate mycorrhizal spore germination and hyphal branching, mediate allelopathic interactions between plants, affect quorum sensing, and chelate soil nutrients,
Inorganic ions and Volatile molecules	H^+, OH^-, HCO_3^-, CO_2, H_2	influences many of the chemical and biological activities of soil
Mescellaneous	Auxins, scopoletin, hydrocyanic acid, glucosides, unidentified ninhydrin-positive compounds, unidentified soluble proteins, reducing compounds, ethanol, glycinebetaine, inositol and myo-inositol-like compounds, Al-induced polypeptides, dihydroquinone, sorgoleone	responsible for several functions like regulating growth and development of plant while some act as negatively for both plant and soil biota.

Compiled from Godheja et al. (2017); Kumar et al., 2006; Dakora and Phillips (2002).

et al., 2015). Similarly, when the plant's jasmonic acid defense pathway is induced, the rhizospheric bacterial community leading to enrichment in microbes enhances plant defense mechanisms (Carvalhais et al., 2013). Phytochemical secretion occurred through active and passive processes by utilizing various transport mechanisms in plants (Loyola-Vargas et al., 2007). Passive exudation generally releases low-molecular-weight signaling molecules. Direct passive diffusion depends upon multiple factors such as permeability of the membrane, pH of the cytosol, and compound polarity (Badri and Vivanco, 2009). Other root exudates, such as secondary metabolites, proteins, and mucopolysaccharides, are exuded by active mechanisms that involve proteins embedded in a membrane (Huang et al., 2014). For instance, ATP-binding cassette transporters (ABC transporters) are linked with several transport mechanisms in plants, including root exudation (Wilkens, 2015; Hwang et al., 2016; Kang et al., 2011). The major facilitator superfamily (MFS) and the multidrug and toxic compound extrusion family (MATE) export several compounds across the plant cell membranes (Reddy et al., 2012). Moreover, proteins from the aluminum-activated malate transporter family (ALMT) have also been reported to act as key molecular actors in GABA (γ-aminobutyric acid) signaling that functions as a signal transducer in plants (Palmer et al., 2016).

Chemotaxis is a crucial mechanism for recruiting soil bacteria to the rhizospheric zone; it establishes the bacterial association with the plant's root (Parales and Ditty, 2018; Feng et al., 2021). The amount and concentrations of root exudates are dependent on the microbial activities in the rhizosphere which affect the root distribution/pattern and the availability of nutrients for uptake by the plant. The exudates that adhere to root surfaces induce chemotactic responses via methyl-accepting chemotaxis proteins (MCP), for example, McpA, McpB, McpC, TlpA, TlpB, TlpC, HemAT, YfmS, YvaQ, and YoaH (Feng et al., 2018). The MCP are bound to chemoreceptors (e.g., Tar, Tsr, Tap, Trg, and Aer in *E. coli*; Sourjik, 2004)in response to concentration gradients of amino acids, dipeptides, and sugars. The chemoreceptors are composed of a methyl-accepting (MA) domain, a cytosolic signaling domain, a HAMP (histidine kinase, adenyl cyclase, methyl-accepting chemotaxis protein [MCP] and phosphatase) linker (Aravind and Ponting 1999), and a ligand-binding domain (LBD), which is frequently located in the extracytoplasmic space and is responsible for extracellular binding compounds (Baker et al., 2006). The binding of the ligand to the LBD modulates the autophosphorylation of the histidine kinase CheA, which in turn transfers the phosphoryl groups to the response regulator CheY. The resulting CheY-Pinteracts with the flagellar motor to control cell swimming or tumbling that ultimately responsible for chemotaxis (Sourjik and Wingreen 2012).

2.2. Effect of soil physiological status on rhizobacteria and root exudates

Root exudate includes primary and secondary metabolites, enzymes, water, free oxygen, polysaccharides, and ions (Hayat et al., 2017). The nature and composition of root exudates are determined by various factors, including other micro-biota in plant roots, environmental factors, plant species, and their developmental stage (Doornbos et al., 2012; Hassan et al., 2019). Biotic factors, such asthe uptake of root exudate by the microbes, influence the soil's physiological status. Plant roots are constantly exposed to an array of microbes and must interact and defend according to the type of biotic stress.It has also been demonstrated that plants release host-specific flavonoids in response to compatible rhizobia strains. Research evidence showed that microbes can modulate plant root exudation of proteins (De-la-Peña and Loyola-Vargas, 2014). The study of De-la-Peña and Loyola-Vargas (2014)establishedthe compositions of proteins present in the root exudates varieddepanding on its microbial neighbor, and that the exudation of proteins by a given bacterium is modulated by the presence of a specific plant neighbor. The signaling between soil bacteria and plant roots is frequently based on root exudates eliciting variable responses from specific receptors. Chemical constituents of signaling molecules may reduce levels of one type of microorganism while attracting another. In fact, biological processes and nutrient availability to rhizobacteria are strongly affected by changes in soil chemistry. This could be a direct effect of signaling molecules, which for instance, could lead to resource competition (McNear, 2013). Competition among the subtypes in response to variation in chemotaxis to signaling molecules has been an essential factor in the colonization of roots. Rhizosphere acidification also plays a crucial role in determining the microbial environment. Bacteria are among the single-celled organisms most able to adapt to and thrive under harsh environmental pH conditions. The most sensitive component of the cell to pH changes is the protein (Hyyryläinen et al., 2001). Acidic soils are dominated by *Acidobacteria* and *Aphaproteobacteria* (Shen et al., 2019), while *Actinobacteria* abundance increases toward alkalinity (Jeanbille et al., 2016). Low rhizosphere pH increases phosphorus as well as those of micronutrients such as zinc, iron, and manganeseavailability in calcareous soils (Lei et al., 2015; Rengel, 2015). These alterations in nutrient content and the physical condition of the rhizosphere influence the habitat of PGPR.

3. Microorganisms to microorganisms: chemical properties of quorum sensing signaling molecules in rhizobacteria and their functions

3.1. Chemical properties of QS signaling molecules

Quorum sensing provides a significant competitive benefit to bacteria, increasing their probability of surviving while exploring difficult habitats. In bacterial communication, QS is related to the generation and release of signaling molecules into its rhizosphere, referred to as autoinducers (AIs). There are many QS network types; regulatory mechanisms result in the synthesis and expression of several signaling molecules, and their gene expressions have been reported by various workers (Papenfort and Bassler 2016; Rosier et al., 2018). The homoserine lactones signaling molecules range from those with short (C4-, C6-, and C8-) carbohydrate side chains to long (C12-, C14-, or even longer) side chains and can contain un-substituted groups in addition to OH- and oxo-C3-replaced compounds. Although AHLs are the ubiquitous AIs for Gram-negative bacteria, arrangements like AI-2 (furanosyl borate diester; alternative autoinducer), AI3, and quinolones (PQS) and a range of additional small compounds are recognized as signaling molecules (Effmert et al., 2012). A new group of Gram-negative bacteria signals (e.g., dialkylresorcinols and pyrones) that are mediatedby LuxR proteins werefound to be closely linked to the AHL-responsive LuxR family (Brameyer et al., 2015); however, it is not clear whether rhizobacteria release these signaling molecules.

Several bacteria identified from the rhizosphere are known to secrete QS signal molecules and interact withvolatile organic compounds (VOCs). For example, species of *Sinorhizobium, Pseudomonas, Burkholderia,* and *Rhizobium* are shown in Table 2, and the effect of QS on plant-microbe interactions is shown in Fig. 1. VOCs are presumed to play an important role in long-distance interaction within microbial populations, microbe-microbe, and plant-microbe associations within the rhizosphere (Bitas et al., 2013). VOCs have also been known to function as inter-and intra-species signals by affecting microbial function such as biofilm formation, stress tolerance, virulence, and gene expression (Audrain et al., 2015).

Thediffusible signal factor (DSF) family (cis-2unsaturated fatty acids) is a group of QS signals in Gram-negative rhizobacterial species such as *Stenotrophomonasmaltophilia* and *Burkholderia* spp. (Ryan et al., 2015). Interestingly, rhizobacterial DSF signaling molecules have also been shown to generate innate immunity in plants, thereby acting as inter-kingdom signal molecules (Kakkar et al., 2015). Furthermore, cyclic compounds such as diketopiperazines (DKZ) and 2-heptyl-3-hydroxy-4-quinolone (PQS) also have been reported as QS signaling molecules of pseudomonads (McKnight et al., 2000).

Various Gram-positive rhizobacterial root colonizers use peptides as QS signaling molecules; possibly, these molecules take part in interactions regulating inter- and intra-species functions (Monnet et al., 2016). Lipid compounds, namely 3-hydroxy-palmitate methyl ester (3OH-PAME) and cis-11-methyl- 2-dodecenoic acid (also referred to as diffusible signal factor or DSF), have been detected as QS-mediating molecules (Achari and Ramesh 2015). In Gram-positive bacteria, several incomplete cyclic peptides, butyrolactone, and AI-2, control various cellular activities and functions by perceiving the cell quantity. AI-2 was anticipated to be a "ubiquitous" QS signal in bacteria, but it might just be an exuded product of a common metabolic network of signaling (Winzer et al., 2002; Lyon and Novick 2004).

3.2. Functions and effect of bacterial signaling compounds in the rhizosphere

QS signaling molecules help rhizobacteria colonize plant-root surfaces or rhizospheric associated regions via QS-mediated gene expression (Rutherford et al., 2012; Nazzaro et al., 2013). The bacterial subfamily proteins LuxRs interact with small compounds and molecules but not with QS. The response of LuxRs to various plant signals involves a complex communal signaling mechanism (Venturi and Fuqua 2013; Gonzalez and Venturi 2013).

The interactions take place both outside and inside the plants (van der Burgh and Joosten, 2019). Quorum-imitating AHLs are synthesized and released in the vicinity by various plant species ranging from seedlings to mature plants (Teplitski et al., 2011). Based on differential proteome analysis, Mathesius et al. (2003) found that protein-related defense, stress, flavonoid metabolism, hormones, and several regulatory proteins were differentially expressed in plants treated with AHLs, indicating that AHLs modulate plant physiology. A recent study suggests that plants have the enzymatic machinery to degrade AHLs (Aziz and Chapman, 2020; Billot et al., 2020).

Researchers have reported the genes responsible for upregulation of auxin and downregulation of cytokinin and the ratio of auxin and cytokinin in a model plant inoculated with C6-homoserine lactone (C6-HSL) (von Rad et al., 2008); several workers have proposed the dual functions of AHL signaling molecules in *Arabidopsisthaliana* (Hartmann and Schikora, 2012; Schenk et al., 2012). The first study reporting the impact of AHLs on root biology was published ten years ago (Ortiz--Castro et al.2008). Short acyl chain AHLs, such as C4 or C6, was demonstrated to enhance plant growth rate, primarily in elongating the roots (Bai et al., 2012; Schenk et al., 2012), in contrast to signaling molecules with longer acyl chains (e.g., C12 or C14). Hartmann et al. (2014) examined the effect of AHLs on plant growth and found it was more complicated as the interactions may be very specific. For example,

Table 2
PGPR and its signaling molecules.

PGPR	Signaling molecules	References
S. meliloti Rm1021	C14-HSL	Chen et al., 2003
	3-Oxo-C14-HSL	Teplitski et al., 2003
	C16:1–9 cis-(L)-HSL	Marketon et al., 2002
	C18-HSL	Marketon et al., 2002
	C12-HSL	Marketon et al., 2002
S. meliloti AK 631	C10-HSL	Teplitski et al., 2003
S. meliloti Rm41	C8-HSL	Marketon et al., 2002
	3-Oxo-C8-HSL	Teplitski et al., 2003
	C16-HSL	Teplitski et al., 2003
Agrobacterium vitis	C14:1-9-cis-(L)-HSL	Li et al., 2005
Agrobacterium vitis	3-Oxo-C16:1–11cis- (L)-HSL, long-chain AHLs	Hao and Burr, 2006
Pseudomonas aeruginosa	C4-HSL	Pearson et al., 1995
P. aeruginosa	3-Oxo-C12-HSL	Pearson et al., 1994
Sinorhizobium meliloti AK 631	C6-HSL	Teplitski et al., 2003
Ochrobactrum sp. Pv2Z2	3O–C7-HSL; 3OHC7-HSL	Imran et al. (2014)
Burkholderia cepacia	C8HSL	Antunes et al. (2010)
Burkholderia pseudomallei	C8HSL, C10HSL, OHC8HSL, OHC10HSL,3OC14HSL, C8HSL, 3OHC8HSL, 3OHC10HSL	Antunes et al. (2010)
Sinorhizobium meliloti	C14HSL to C18HSL	Marketon et al. (2002)
Rhizobium leguminosarum	OHC6HSL, 3OC6HSL, C7HSL, 3OHC14HSL	Antunes et al. (2010)
Erwinia carotovora	3OC6HSL	Antunes et al. (2010)
Bacillus cereus	Peptide PapR	Van Delden et al. (1998)
Staphylococcus aureus	Autoinducing peptide	Grenha et al. (2013)
Burkholderia sp.	cis-11-Methyl-2-dodecenoic acid diffusible signal factor (DSF); N-acyl-L-HSL; N-(3-oxoacyl)-LHSL; N-(3-hydroxyacyl)-L-HSL	Schmid et al. (2012), Chapalain et al. (2013), Suppiger et al. (2013), and Ryan et al., (2015)
Mesorhizobium Huakuii	C8-HSL	Wang et al. (2004) and Braeken et al. (2008)
Stenotrophomonas Maltophilia	cis-11-Methyl-2-dodecenoic acid diffusible signal factor (DSF)	Alavi et al. (2013); Ryan et al. (2015)
Bacillus subtilis	Peptides	Duanis-Assaf et al. (2016)
Ochrobactrum sp. Pv2Z2	3O–C7-HSL; 3OHC7-HSL	Imran et al. (2014)
Pseudomonas aeruginosa	N-(3-Oxododecanoyl)-homoserine lactone (OdDHL); N-butyrylhomoserine lactone (BHL); 2-heptyl-3-hydroxy-4-quinolone (PQS); 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde (IQS)	Lee and Zhang (2015)
Pantoea agglomerans YS19	N-3-Oxooctanoyl-L-homoserine lactone	Jiang et al. (2015)
Pseudomonas sp.	N-Acyl-L-HSL; N-(3-oxoacyl)-LHSL; N-(3 hydroxyacyl) - L-HSL; 2-heptyl-3-hydroxy-4-quinolone (PQS)	Williams and Cámara (2009) and Hartmann and Schikora (2012)
Acinetobacter sp.	N-Acyl-L-HSL; N-(3-oxoacyl)-LHSL; N-(3-hydroxyacyl)-L-HSL	Atkinson and Williams (2009)
Pseudomonas fluorescens	3-OH-C6-HSL; 3-OH-C7-HSL; 3-OH-C8-HSL; 3-OH-C10-HSL, C 6-HSL, C8-HS	Khan et al. (2005)
Rhodopseudomonas sp.	N-(p-Coumaroyl)-HSL; R = OH (pC-HSL)	Atkinson and Williams (2009)
Rhizobium sp.	N-Acyl-L-HSL; N-(3-oxoacyl)-LHSL; N-(3-hydroxyacyl)-L-HSL	Sanchez-Contreras et al. (2007)
Bradyrhizobium sp.	N-(p-Coumaroyl)-HSL; R = OH (pC-HSL)	Sanchez-Contreras et al. (2007)
Sinorhizobium Meliloti	C14HSL to C18HSL, 3-Oxo-C16	Marketon et al. (2002); Mathesius et al., (2003) and Hartmann et al. (2014)
	C8-HSL	

Table 2 (*continued*)

PGPR	Signaling molecules	References
Mesorhizobium huakuii		Wang et al. (2004) and Braeken et al. (2008)
Bacillus subtilis	Peptides	Duanis-Assaf et al. (2016)
Pantoea agglomerans YS19	N-3-Oxooctanoyl-L-homoserine lactone	Jiang et al. (2015)
Serratia plymuthica HRO-C48	3-Oxo-C6	Pang et al. (2009)
Gluconacetobacter diazotrophicus PAL5	C6-, C8-, C10-, C12-, C14-HSL; 3-oxo-C10-, C12-, C14-HSL	Nieto-Penalver et al. (2012)
Prevotella intermedia	AI-2	Frias et al. (2001)
Pseudomonas aeruginosa	OdDHLg	Wilder et al. (2009)
Vibrio campbellii	AI-2, OH-BHLg, CAI-1	Defoirdt et al., 2008
Vibrio harveyi	CAI-1, AI-2, OH-BHLg	Defoirdt et al., 2008
Vibrio salmonicida	AHL	Bruhn et al. (2005)
Vibrio vulnificus	AI-2	Kim et al. (2003)
Porphyromonas gingivalis	AI-2	Frias et al. (2001)
Photobacterium Phosphoreum	OH-OHLg	Flodgaard et al., 2005
Fusobacterium Nucleatum	AI-2	Frias et al. (2001)
Erwinia amylovora	AI-2	Mohammadi and Geider (2007)
Burkholderia vietnamiensis	HHLg	Poonguzhali et al. (2007)
Aeromonas salmonicida	BHL and HHLg	Bruhn et al. (2005)

root nodulation in *Medicago truncatula* was increased by the production of long-chain 3-oxo-C14-HSL by *Sinorhizobium meliloti* (Veliz-Vallejos et al., 2020). Interestingly, the increased nodule number occurred only after a treatment with 3-oxo-C14-HSL, which is the main AHL component of *S. meliloti*. but when treated with other AHLs, there was no effect. Whereas, in *Vigna radiate* only 3-oxo-C10-HSL induced adventitious roots but not the unsubstituted C10-HSL or C12-HSL (Bai et al., 2012).

Several plant genes were upregulated, related to secondary metabolism (e.g., phenols), which resulted in a pathogen challenge. Consequently, those plants amass phenolic and callose compounds (Schenk and Schikora 2015), indicating the effect of QS molecules of the AHL group on plant physiology and its importance in developing stress tolerance regulations in plants against stressors (Fig. 1).

3.3. Inter- and intra-species signaling and signal interference

Upon identifying the signaling molecules at a particular concentration, transcription of specified genes directed by this mechanism is stimulated in the rhizobacteria. Several rhizobacterial mechanisms are controlled by the QS signals, which involve DNA transferase by siderophore production, biofilm formation, bioluminescence, conjugation, and stimulating potential of some rhizobacteria, often known as "swarming" (Barriuso et al., 2008b; Bramhachari, 2019). These signals can also be engineered in plants on trans-kingdom level in order to control gene expression in rhizospheric bacteria (Geddes et al., 2019).

The production of AHLs by diazotrophs such as *Rhizobium leguminosarum* suggests QS promotes signaling in the mature nodule (Daniels et al., 2002). Moreover, it is hypothesized that QS also affects rhizobacterial flow linked with host plants. For example, flavonoids produced by legumes enhance synthetic AHL gene' expression in rhizobia (Pérez-Montaño et al., 2011). As anticipated, signaling among various microbes also occurs and certainly has consequences for plant-microbe interactions. During interspecies interaction, AIs secreted by any species can alter the behavior of its competitors. AHLs are also reported to act as interkingdom signaling molecules that affect plant gene expression, initiate systemic plant resistance, and influence plant growth (Venturi and Fuqua 2013) (Fig. 2).

The process of hindrance in QS signaling is termed quorum

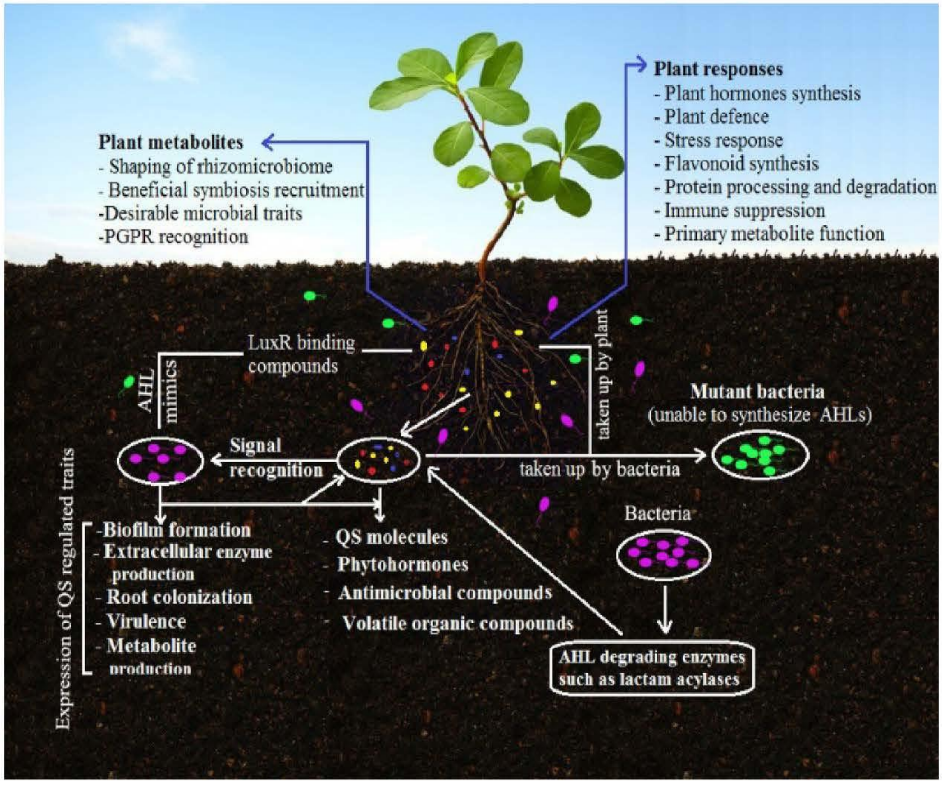


Fig. 1. Role of quorum sensing in plant-microbe interaction: Their impact and importance in developing stress tolerance in plants against stressors.

quenching (QQ) (Lareen et al., 2016a,b). Plants can use several approaches to avert bacterial QS signaling, including the inhibition of AI biosynthesis and/or secretion, enzymatic degradation of these molecules, and disruption of their binding, receptors, and regulator site (Koh et al., 2013) (Fig. 2). For instance, plants produce cytokinins and IAA that affect QS. Beneficial bacteria can also protect plants by interfering with QS that disrupts pathogen signaling and plant colonization.For instance, microbes may utilize enzymes like acylase and lactonase, which degrade AHLs or release VOCs that impede bacterial AHL production (Hong et al., 2012; Aziz and Chapman, 2020).

3.4. Biofilm formation through signaling by rhizobacteria

Biofilm formation under various stress conditions is an effective technique used by bacterial strains to ensure their survival in the plant rhizosphere. Rhizospheric biofilms can be both beneficial and pathogenic. The natural QS-driven beneficial biofilms that occur in rhizosphere suppress the plant pathogens and can be used as biocontrol agents, an alternative to chemicals (Harjai and Sabharwal, 2017). Pérez-Montaño et al. (2014) examined the bacterial surface components, especially mucopolysaccharides, and revealed that the formation of biofilm allows rhizobacteria to colonize their vicinity and remain vital under ecological stresses such as nutrient limitation and drought. This condition is often crucial for regular existence in bacteria of the genera *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium*. For example, biofilm production makes the conditions more favorable for root inhabitation and symbiosis among *Glycine* max cv Osumi and *Sinorhizobium fredii* SMH12. In *S. fredii*, nod gene-stimulating flavonoids and the NodD1 protein are responsible for developing the biofilm configuration from monolayer to microcolony. The expression of the *NodD1*gene has been linked to flavonoids production, which induced specific QS regulations of the symbiotic biofilm (Pérez-Montaño et al., 2014). Paungfoo-Lonhienne et al. (2016) established the function of QS in the inhabitation and formation of biofilm by *Burkholderia* Q208, a PGPR of Australian sugarcane; it was found to have an extremely conserved QS regulation, designated BraI/R, programmed by three genes (*braI*, *rsaL* and *braR*) for synthesis of, and reaction to, N-AHLs. In the biofilm formation by *Burkholderia*, *rsaL* and *braR* are downregulated while *braI* is upregulated (twofold).

4. Microorganisms to plants signaling: quorum sensing in plant growth-promoting rhizobacteria

AHLs have been reported to contribute as signaling molecules in inter-species communication among rhizobacteria (Bukhat et al., 2020). Nevertheless, there is a dearth of knowledge regarding inter-species communication in the natural habitat of microbes. Recent studies also found that these bacteria produce the cyclodipeptides cyclo (L-Pro-LVal), cyclo (L-Pro-L-Phe), and cyclo (L-Pro-L-Tyr), which modulates auxin-responsive gene expression in roots (Ortiz-Castro et al., 2020). Mathesius et al. (2003) reported the root exudates of *Medicago truncatula*contained substances that mimic a AHL function that regulate AHL-regulated response in bacteria. The chemical constituent of such active QS-acting signaling molecules is not yet characterized and requires further study (Chen et al., 2002; Podile et al., 2013). A recent hypothesis reveals that alkamides and*N*-acylethanolamines (NAEs) are excellent candidates to act as AHL mimics, regarding their structural, chemical identity (Aziz and Chapman, 2020). Expression of QS appeared

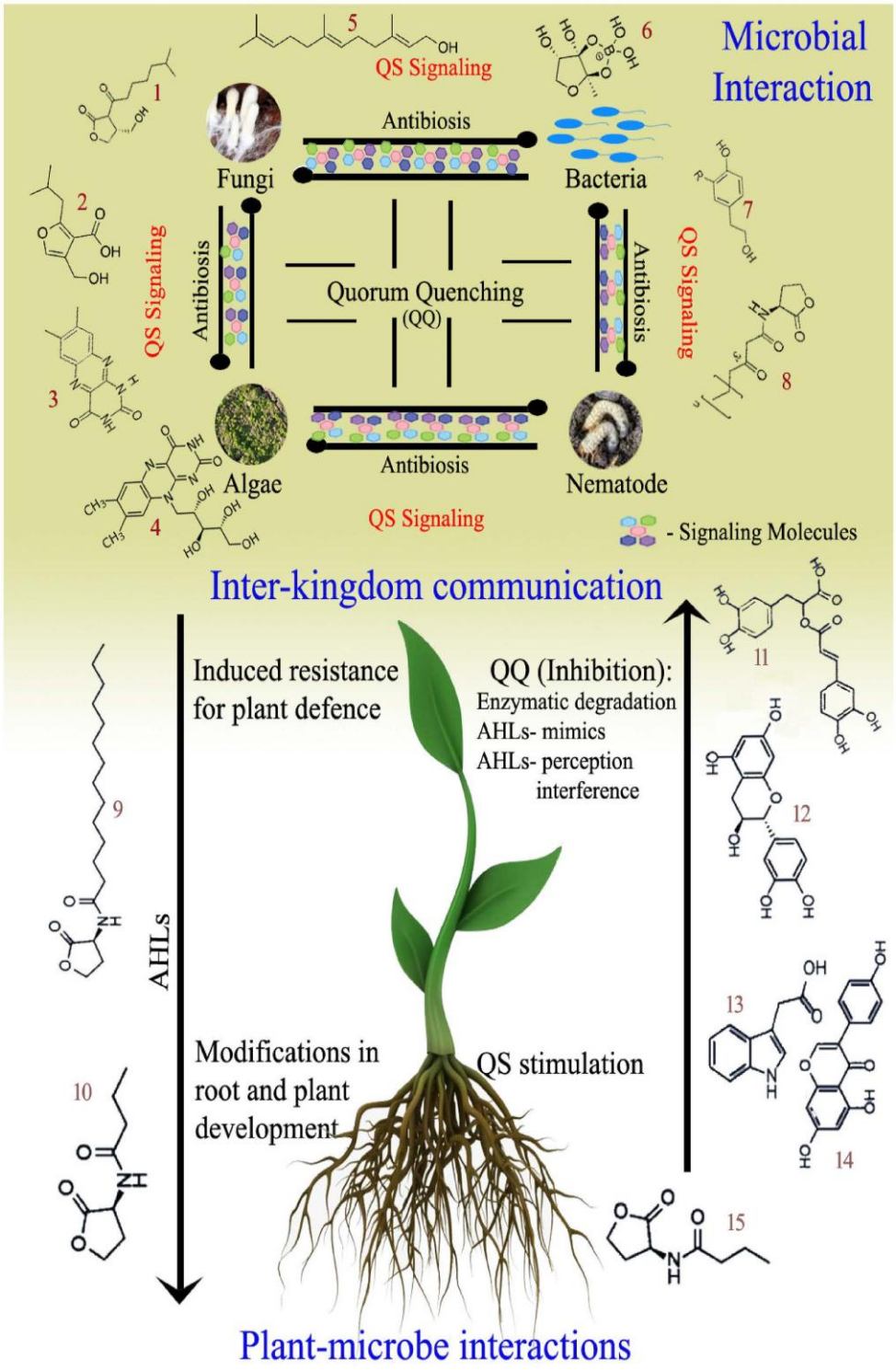


Fig. 2. Interkingdom communication between microbe-microbe and Plant-microbe interactions. Various microbes dwelling in the soil like fungi, bacteria, algae, and nematodes, etc. releases several quora sensing molecules (**1**-A-factor; **2**- MMF1; **3**- lumichrome; **4**- riboflavin; **5**- farnesol; **6**-AI-2; **7**-tyrosol; **8**–3′-O-C6-HSL = AI-1) between species including antagonist molecules to surpass in a competitive situation. Moreover, microbes also show interference between competitors via the quorum quenching (QQ) mechanism. Molecules like N-acyl homoserine lactones (AHLs) are used for both intraspecies and interkingdom communication (**9**- C14-HSL6, for plant defense; **10**- C14-HSL1, for plant development). Sometimes, plants produce molecules for interfering with bacterial QS signaling by reducing AHL interactions (**12**- genistein) or by mimicking AHL (**11**- rosmarinic acid). Plants may also activate microbial QS (**13**- IAA; **14**- D-(+)-catechin).

to be based not only on signaling molecules but also on the bacterial population density (Papenfort and Bassler, 2016).

As QS is used in the communication between a plant with its rhizobacteria, only a low concentration of the primary inoculum of PGPR is needed to promote plant growth (Persello-Cartieaux et al., 2003). The most effective PGPR inoculation was expected in organic and mineral poor soils or stressed soils, when development of the resident microflora was inhibited. This reseacrh was evaluated by using mathematical modeling and computer-based simulations (Strigul and Kravchenko et al., 2006). Particularly under field conditions, inoculation is not always successful due to the low survival rate of the inoculum. The balancing mechanism of QS and AIs may permit endophytic isolates to communicate with other linked endophytes and plant hosts, thus protecting mutualistic associations and habitat inside the plant tissues. PGPR can respond to QS-similar signaling molecules released by plants and other rhizospheric bacteria and even inactivate the QS signaling molecules released by other rhizobacterial species (Dong et al., 2002). For example, *Bacillus* releases various enzymes to inactivate or cleave the AHL produced by other Gram-negative bacteria. The AHL-cleaving enzyme, *aiiA*, allows *Bacillus* strains to break the lactone bond of AHLs through hydrolysis, expressing a procedure for an autoinducer-cleaving activity that permits these bacteria to co-exist with other Gram-negative bacteria (Lee et al., 2002). Therefore, the rhizobacteria's role can be directly or indirectly altered by plants and other microbial community members via QS signaling molecules (Podile et al., 2013).

4.1. QS and its role in regulating plant response

Besides regulating microbial functions, the QS molecules secreted by PGPR also evoke plant responsesthatarebeneficial to those host plants (Kusari et al., 2015). For instance, plants may respond more adeptly to biotic alterations when exposed to QS molecules (Lareen et al., 2016a,b; Schenk and Schikora et al., 2016). AHLs can alter plant physiology; when treated with N-(butanoyl)-L-homoserine lactone (C4-HSL) and N-(hexanoyl)-L-homoserine lactone (C6-HSL), the auxin/cytokinin ratio in *Arabidopsis thaliana*was altered which affected the root growth (von Rad et al., 2008).The dual function of AHLs in *Arabidopsis thaliana* has been examined; the short acyl chain AHLs enhance the growth rate and primary root elongation, whereas the long acyl chain AHLs, such as N-(tetradecanoyl)- L-homoserine lactone (C14-HSL) or N-(dodecanoyl)-L-homoserine lactone (C12-HSL) stimulate pathogen resistance (Palmer et al., 2014). This process is called the AHL-priming or AHL-induced resistance method (Fig. 2). Additionally, the effect of AHLs may vary among plant species. The response of plants to AHLs depends on AHL structures, the amount released and plant species. As already indicated, AHLs can stimulate plant pathogen resistance, and jasmonic acid/salicylic acid-dependent pathways were suggested as part of this response (Schenk and Schikora, 2015). In addition to causing changes in plant's physiological and stimulation of pathogen resistance, QS bacterial compounds also evoke the formation and secretion of compounds that imitate bacterial QS molecules in their effects on plants (Fig. 2) (Teplitski et al., 2000; Corral-Lugo et al., 2016; Hartmann et al., 2014; Pérez-Montaño et al., 2013). AHL imitates and regulates QS at several levels, particularly inducing or repressing the AHL receptor of bacteria involved in symbiotic or pathogenic associations with their host plants (Rasmussen and Givskov, 2006). A study also concluded that a plant secreted rosmarinic acid when infected by *Pseudomonas aeruginosa*. The natural ligand of the bacterial QS activator RhlR induces QS-mediated biofilm formation and secretion of virulence factors. Thus, rosmarinic acid was released as a plant defense mechanism to impede QS by *P. aeruginosa* and thus reduced its pathogenicity (Corral-Lugo et al., 2016). In addition, D-(+)-catechin impedes the recognition of C4-HSL negatively via RhlR of *P. aeruginosa*, leading to a reduction in the secretion of QS signals and virulence factors (Vandeputte et al., 2010).

4.2. Examples of QS in endophytes

Endophytes are often documented in enhancing plant defense mechanisms by quorum-averting methods. Intriguingly, endophytes are frequently observed to have a QS mechanism that facilitates their colonization of host plants and thwartsplant pathogens (Khare et al., 2018). For example, *Burkholderia phytofirmans*strain PsJNT is reported to enhance endophytic association with various plants and promote a favorable plant-rooting structure with enhanced vascular arrangements, an improved quantity of phytohormones and chlorophyll, and provides resistance to phytopathogens. It was documented to exude a quorum autoinducer, i.e., 3-hydroxy-C8-homoserine lactone (Sessitsch et al., 2005). *Serratia plymuthica*, with extensive biological control ability, was reported as retaining copious amounts of homoserine lactone (HSL), videlicet, C4-HSL, C5-HSL, C6-HSL, C7-HSL, C8-HSL, and 3-hydroxy derivatives (3-hydroxy-C6-HSL, 3-hydroxy-C8- HSL), and 3-oxo derivatives (3-oxo-C6-HSL, 3-oxo-C7-HSL, 3-oxo-C8 HSL) (Liu et al., 2011). *Pantoea agglomerans*, the olive plant epiphyte, and an endophyte (*Erwinia toletana*) associated with olive knot infection were found to secret signals similar to AHLs. This study is an example of tripartite associations among plants and associated microbes (Hosni et al., 2011). The genome sequencing of endophytic *Gluconacetobacter diazotrophicus* PAL5 associated with *Saccharum officinarum* showed the mechanism of QS and recognition of five AHLs, namely C6, C8, C10, C12 and C14-HSL (Nieto-Penalver et al., 2012). Dourado et al. (2013) described the misuse of quorum signaling molecules for *Methylobacterium* interaction with plants. A gene sequence was modified in plants and *Methylobacterium*, simultaneously initiating symbiotic interaction and colonization, showing the mutualistic association on QS mechanisms.

5. The role of quorum sensing signaling molecules in agriculture

The growing demand for food and the concern related to food quality are leading to the demand for novel and effective sustainable agricultural practices. PGPR is one of the most promising tools used in the agricultural industry. These beneficial microorganisms can act as biofertilizers to promote plant growth and stress tolerance, as well as combat plant pathogens. They boost plant resistance to infections via ISR and SAR. PGPR are an effective, sustainable, and environmentally friendly alternative to chemical fertilizersto be used in agriculture.For example, the use of nitrogen-fixing rhizobia (e.g., *Sinorhizobium meliloti*), with enhanced release of specific AHLs, might increase bacterial benefits and improve plant species effects not linked with specific strains (Zarkani et al., 2013; Hernandez-Reyes et al. 2014).

During the infection period, QS molecules direct the bacterial potential to produce biofilms and other density-regulated traits. Those molecules participate in the crucial role of QS among plant and bacterial cells. Several researchers have reported the role of QS molecules in controlling plant disease and the transmission of phytopathogens. Barriuso et al. (2008a) documented AHLs QS signaling compounds' functions in promoting plant growth and enhancing defense against salt stress. Johnson and Walcott (2013) documented that *Acidovorax citrulli* transformed from saprobic to pathogenic growth, causing bacterial fruit blotch (BFB) from seed-to-seedling in cucurbits; they concluded that QS was involved in the regulation of this transition. The role of QS in regulating genes was involved in the transmission of BFB to seedlings. Alavi et al. (2013) documented the DSF QS system's function in regulating the progressive impact of *Stenotrophomonas maltophilia* on plants. Oilseed rape seeds modified with the wild-type strain showed a significant improvement in growth rate compared to those coated with the mutant that lacks the gene *rpfF* for the regulation of DSF. They concluded that the QS molecule DSF is responsible for directing phenotypes in pathogenic *Stenotrophomonas*.Zúñiga et al. (2013) reported the importance of AHLs in mediating QS in well-organized colonization of *Arabidopsis thaliana* and the coordination of beneficial interactions using suitable mutant strains of *Burkholderia phytofirmans* PsJN. These

researchers also concluded that rhizobacterial reduction of the auxin indole-3-acetic acid (IAA) participates in promoting plant growth characters and is important for successful root inhabitation. A recent study supports that the critical role of auxin degradation by enzymes encoded in bacterial operons to modulate the behavior of synthetic bacterial communities and affect root growth have been examined (Finkel et al., 2020). Moreover, Jiménez-Vázquez (2020) demonstrated that an Achromobacter strain drives discrete root movements interfering with auxin transport and gravitropism.In addition, the interruption of QS via quorum quenching offers an interesting strategy to combat bacterial pathogens in agriculture. The use of enzymatic degradation of AHL have been reported to reduce the virulence of several plant pathogens (Rodríguez et al., 2020; Helman and Chernin, 2015; Faure and Dessaux, 2007).

6. Conclusions

Significant advances in understanding chemical signaling associated with plant-microbe interactions have contributed to clarifying the mechanisms involved in their associations. In this review, we have summarized the chemical signaling in the rhizosphere, the role of QS molecules in plant-microbe and microbe-microbe interactions, and their potentialapplications in agriculture. Investigation of symbiotic relationships provided valuable knowledge for the understanding of plant and microbe chemical signaling. However, in order to provide functional knowledge regarding the role of plant-microbe interaction, molecular entities involved in both plant and microbe signals must be interconnected. Moreover, each biological system's distinctiveness needs to be examined in depth as the interactions between specific signaling molecule and their targets are highly complex and difficult to generalize. There isknowledgegap in rhizospheric signaling that require further research and scientific attention. A better understanding in the metagenomic of the rhizosphere would provide further insight into the interactions of rhizobacteria and the host plants. Currently, only a small percentage of the genomes of rhizobacteria have been sequenced.A better understanding of QS molecules and the QQ process would alsohelp to develop novel strategies for agriculture in future.

Funding

The financial assistance from Department of Biotechnology (DBT), New Delhi, India is highly acknowledged as Grant-in-aid project vide letter No. BT/PR13922/BCE/8/1129/2015.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Achari, G.A., Ramesh, R., 2015. Characterization of bacteria degrading 3-hydroxy palmitic acid methyl ester (3 OH-PAME), a quorum sensing molecule of *Ralstonia solanacearum*. Lett. Appl. Microbiol. 60 (5), 447–455.

Alavi, P., Müller, H., Cardinale, M., Zachow, C., Sánchez, M.B., Martínez, J.L., Berg, G., 2013. The DSF quorum sensing system controls the positive influence of *Stenotrophomonas maltophilia* on plants. PLoS One 8 (7), e67103.

Altaf, M.M., Khan, M.S.A., Abulreesh, H.H., Ahmad, I., 2017. Quorum sensing in plant growth-promoting rhizobacteria and its impact on plant-microbe interaction. In: Plant-Microbe Interactions in Agro-Ecological Perspectives. Springer, Singapore, pp. 311–331.

Antunes, L.C.M., Ferreira, R.B., Buckner, M.M., Finlay, B.B., 2010. Quorum sensing in bacterial virulence. Microbiology 156 (8), 2271–2282.

Aravind, L., Ponting, C.P., 1999. The cytoplasmic helical linker domain of receptor histidine kinase and methyl-accepting proteins is common to many prokaryotic signalling proteins. FEMS Microbiol. Lett. 176 (1), 111–116.

Atkinson, S., Williams, P., 2009. Quorum sensing and social networking in the microbial world. J. R. Soc. Interface 6 (40), 959–978.

Audrain, B., Farag, M.A., Ryu, C.M., Ghigo, J.M., 2015. Role of bacterial volatile compounds in bacterial biology. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Rev. 39 (2), 222–233.

Aziz, M., Chapman, K.D., 2020. Fatty acid amide hydrolases: an expanded capacity for chemical communication? Trends Plant Sci. 25 (3), 236–249.

Backer, R., Rokem, J.S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., Smith, D.L., 2018. Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front. Plant Sci. 9, 1473.

Badri, D.V., Vivanco, J.M., 2009. Regulation and function of root exudates. Plant Cell Environ. 32 (6), 666–681.

Bai, X., Todd, C.D., Desikan, R., Yang, Y., Hu, X., 2012. N-3-oxo-decanoyl-L-homoserine-lactone activates auxin-induced adventitious root formation via hydrogen peroxide- and nitric oxide-dependent cyclic GMP signaling in mung bean. Plant Physiol. 158 (2), 725–736.

Baker, M.D., Wolanin, P.M., Stock, J.B., 2006. Signal transduction in bacterial chemotaxis. Bioessays 28 (1), 9–22.

Bartiuso, J., Solano, B.R., Gutiérrez Mañero, F.J., 2008a. Protection against pathogen and salt stress by four plant growth-promoting rhizobacteria isolated from Pinus sp. on Arabidopsis thaliana. Phytopathology 98 (6), 666–672.

Bartiuso, J., Solano, B.R., Lucas, J.A., Lobo, A.P., García-Villaraco, A., Mañero, F.J.G., 2008b. Ecology, genetic diversity and screening strategies of plant growth promoting rhizobacteria (PGPR). J. Plant Nutr. 4, 1–17.

Bhattacharya, A., Sood, P., Citovsky, V., 2010. The roles of plant phenolics in defence and communication during Agrobacterium and Rhizobium infection. Mol. Plant Pathol. 11 (5), 705–719.

Billot, R., Plener, L., Jacquet, P., Elias, M., Chabrière, E., Daudé, D., 2020. Engineering acyl-homoserine lactone-interfering enzymes toward bacterial control. J. Biol. Chem. 295 (37), 12993–13007.

Bitas, V., Kim, H.S., Bennett, J.W., Kang, S., 2013. Sniffing on microbes: diverse roles of microbial volatile organic compounds in plant health. Mol. Plant Microbe Interact. 26 (8), 835–843.

Braeken, K., Daniels, R., Ndayizeye, M., Vanderleyden, J., Michiels, J., 2008. Quorum sensing in bacteria-plant interactions. In: Molecular Mechanisms of Plant and Microbe Coexistence. Springer, Berlin, Heidelberg, pp. 265–289.

Brameyer, S., Bode, H.B., Heermann, R., 2015. Languages and dialects: bacterial communication beyond homoserine lactones. Trends Microbiol. 23 (9), 521–523.

Bramhachari, P.V. (Ed.), 2019. Implication of Quorum Sensing System in Biofilm Formation and Virulence. Springer.

Bruhn, J.B., Dalsgaard, I., Nielsen, K.F., Buchholtz, C., Larsen, J.L., Gram, L., 2005. Quorum sensing signal molecules (acylated homoserine lactones) in gram-negative fish pathogenic bacteria. Dis. Aquat. Org. 65 (1), 43–52.

Bukhat, S., Imran, A., Javaid, S., Shahid, M., Majeed, A., Naqqash, T., 2020. Communication of plants with microbial world: exploring the regulatory networks for PGPR mediated defense signaling. Microbiol. Res. 238, 126486.

Canarini, A., Wanek, W., Merchant, A., Richter, A., Kaiser, C., 2019. Root exudation of primary metabolites: mechanisms and their roles in plant responses to environmental stimuli. Front. Plant Sci. 10, 157.

Cannesan, M.A., Durand, C., Burel, C., Gangneux, C., Lerouge, P., Ishii, T., Laval, K., Follet-Gueye, M.L., Driouich, A., Vicré-Gibouin, M., 2012. Effect of arabinogalactan proteins from the root caps of pea and *Brassica napus* on *Aphanomyces euteiches* zoospore chemotaxis and germination. Plant Physiol. 159 (4), 1658–1670.

Carvalhais, L.C., Dennis, P.G., Badri, D.V., Tyson, G.W., Vivanco, J.M., Schenk, P.M., 2013. Activation of the jasmonic acid plant defence pathway alters the composition of rhizosphere bacterial communities. PLoS One 8 (2), e56457.

Chapalain, A., Vial, L., Laprade, N., Dekimpe, V., Perreault, J., Déziel, E., 2013. Identification of quorum sensing-controlled genes in *Burkholderia ambifaria*. Microbiol. 2 (2), 226–242.

Chen, H., Teplitski, M., Robinson, J.B., Rolfe, B.G., Bauer, W.D., 2003. Proteomic analysis of wild-type *Sinorhizobium meliloti* responses to N-acyl homoserine lactone quorum-sensing signals and the transition to stationary phase. J. Bacteriol. 185 (17), 5029–5036.

Chen, X., Schauder, S., Potier, N., Van Dorsselaer, A., Pelczer, I., Bassler, B.L., Hughson, F.M., 2002. Structural identification of a bacterial quorum-sensing signal containing boron. Nature 415 (6871), 545–549.

Corral-Lugo, A., Daddaoua, A., Ortega, A., Espinosa-Urgel, M., Krell, T., 2016. Rosmarinic acid is a homoserine lactone mimic produced by plants that activates a bacterial quorum-sensing regulator. Sci. Signal. 9 (409), ra1.

Dakora, F.D., Phillips, D.A., 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. In: Food Security in Nutrient-Stressed Environments: Exploiting Plants' Genetic Capabilities. Springer, Dordrecht, pp. 201–213.

Daniels, R., De Vos, D.E., Desair, J., Raedschelders, G., Luyten, E., Rosemeyer, V., Verreth, C., Schoeters, E., Vanderleyden, J., Michiels, J., 2002. The cin quorum sensing locus of Rhizobium etli CNPAF512 affects growth and symbiotic nitrogen fixation. J. Biol. Chem. 277 (1), 462–468.

De Coninck, B., Timmermans, P., Vos, C., Cammue, B.P., Kazan, K., 2015. What lies beneath: belowground defense strategies in plants. Trends Plant Sci. 20 (2), 91–101.

De-la-Peña, C., Loyola-Vargas, V.M., 2014. Biotic interactions in the rhizosphere: a diverse cooperative enterprise for plant productivity. Plant Physiol. 166 (2), 701–719.

Dennis, P.G., Miller, A.J., Hirsch, P.R., 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol. Ecol. 72 (3), 313–327.

Dong, Y.H., Gusti, A.R., Zhang, Q., Xu, J.L., Zhang, L.H., 2002. Identification of quorum-quenching N-acyl homoserine lactonases from *Bacillus* species. Appl. Environ. Microbiol. 68 (4), 1754–1759.

Mhlongo, M.I., Piater, L.A., Madala, N.E., Labuschagne, N., Dubery, I.A., 2018. The chemistry of plant–microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. Front. Plant Sci. 9, 112.

Mohammadi, M., Geider, K., 2007. Autoinducer-2 of the fire blight pathogen Erwinia amylovora and other plant-associated bacteria. FEMS Microbiol. Lett. 266 (1), 34–41.

Mondal, S., Baksi, S., 2019. Signalling of Rhizosphere Micobiomes: Benign and Malign Borders.

Monnet, V., Juillard, V., Gardan, R., 2016. Peptide conversations in Gram-positive bacteria. Crit. Rev. Microbiol. 42 (3), 339–351.

More, S.S., Shinde, S.E., Kasture, M.C., 2019. Root Exudates a Key Factor for Soil and Plant: an Overview.

Nazzaro, F., Fratianni, F., Coppola, R., 2013. Quorum sensing and phytochemicals. Int. J. Mol. Sci. 14 (6), 12607–12619.

Nguema-Ona, E., Vicré-Gibouin, M., Cannesan, M.A., Driouich, A., 2013. Arabinogalactan proteins in root–microbe interactions. Trends Plant Sci. 18 (8), 440–449.

Nieto-Penalver, C.G., Bertini, E.V., de Figueroa, L.I., 2012. Identification of N-acyl homoserine lactones produced by *Gluconacetobacter diazotrophicus* PAL5 cultured in complex and synthetic media. Arch. Microbiol. 194 (7), 615–622.

Noirot-Gros, M.F., Shinde, S., Larsen, P.E., Zerbs, S., Korajczyk, P.J., Kemner, K.M., Noirot, P.H., 2018. Dynamics of aspen roots colonization by Pseudomonads reveals strain-specific and mycorrhizal-specific patterns of biofilm formation. Front. Microbiol. 9, 853.

Olanrewaju, O.S., Ayangbenro, A.S., Glick, B.R., Babalola, O.O., 2019. Plant health: feedback effect of root exudates-rhizobiome interactions. Appl. Microbiol. Biotechnol. 103 (3), 1155–1166.

Olanrewaju, O.S., Glick, B.R., Babalola, O.O., 2017. Mechanisms of action of plant growth promoting bacteria. World J. Microbiol. Biotechnol. 33 (11), 197.

Ortiz-Castro, R., Campos-García, J., López-Bucio, J., 2020. Pseudomonas putida and Pseudomonas fluorescens influence Arabidopsis root system architecture through an auxin response mediated by bioactive cyclodipeptides. J. Plant Growth Regul. 39 (1), 254–265.

Ortiz-Castro, R., Díaz-Pérez, C., Martínez-Trujillo, M., del Río, R.E., Campos-García, J., López-Bucio, J., 2011. Transkingdom signaling based on bacterial cyclodipeptides with auxin activity in plants. Proc. Natl. Acad. Sci. U. S. A 108 (17), 7253–7258. https://doi.org/10.1073/pnas.1006740108.

Ostroumova, O.S., Efimova, S.S., Malev, V.V., 2015a. Modifiers of membrane dipole potentials as tools for investigating ion channel formation and functioning. International review of cell and molecular biology 315, 245–297 (Academic Press).

Ostroumova, O.S., Efimova, S.S., Malev, V.V., 2015b. Modifiers of membrane dipole potentials as tools for investigating ion channel formation and functioning. International review of cell and molecular biology 315, 245–297 (Academic Press).

Palmer, A.G., Senechal, A.C., Mukherjee, A., Ané, J.M., Blackwell, H.E., 2014. Plant responses to bacterial N-acyl L-homoserine lactones are dependent on enzymatic degradation to L-homoserine. ACS Chem. Biol. 9 (8), 1834–1845.

Palmer, A.J., Baker, A., Muench, S.P., 2016. The varied functions of aluminium-activated malate transporters–much more than aluminium resistance. Biochem. Soc. Trans. 44 (3), 856–862.

Pang, Y., Liu, X., Ma, Y., Chernin, L., Berg, G., Gao, K., 2009. Induction of systemic resistance, root colonisation and biocontrol activities of the rhizospheric strain of Serratia plymuthica are dependent on N-acyl homoserine lactones. Eur. J. Plant Pathol. 124 (2), 261–268.

Papenfort, K., Bassler, B.L., 2016. Quorum sensing signal–response systems in Gram-negative bacteria. Nat. Rev. Microbiol. 14 (9), 576.

Parales, R.E., Ditty, J.L., 2018. Chemotaxis to atypical chemoattractants by soil bacteria. In: Bacterial Chemosensing. Humana Press, New York, NY, pp. 255–280.

Pascale, A., Proietti, S., Pantelides, I.S., Stringlis, I.A., 2020. Modulation of the root microbiome by plant molecules: the basis for targeted disease suppression and plant growth promotion. Front. Plant Sci. 10, 1741.

Paungfoo-Lonhienne, C., Lonhienne, T.G., Yeoh, Y.K., Donose, B.C., Webb, R.I., Parsons, J., Liao, W., Sagulenko, E., Lakshmanan, P., Hugenholtz, P., Schmidt, S., 2016. Crosstalk between sugarcane and a plant-growth promoting Burkholderia species. Sci. Rep. 6 (1), 1–14.

Pearson, J.P., Gray, K.M., Passador, L., Tucker, K.D., Eberhard, A., Iglewski, B.H., Greenberg, E.P., 1994. Structure of the autoinducer required for expression of *Pseudomonas aeruginosa* virulence genes. Proc. Natl. Acad. Sci. Unit. States Am. 91 (1), 197–201.

Pearson, J.P., Passador, L., Iglewski, B.H., Greenberg, E.P., 1995. A second N-acylhomoserine lactone signal produced by *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. Unit. States Am. 92 (5), 1490–1494.

Pérez-Montaño, F., Guasch-Vidal, B., González-Barroso, S., López-Baena, F.J., Cubo, T., Ollero, F.J., Gil-Serrano, A.M., Rodríguez-Carvajal, M.Á., Bellogín, R.A., Espuny, M. R., 2011. Nodulation-gene-inducing flavonoids increase overall production of autoinducers and expression of N-acyl homoserine lactone synthesis genes in rhizobia. Res. Microbiol. 162 (7), 715–723.

Pérez-Montaño, F., Jiménez-Guerrero, I., Del Cerro, P., Baena-Ropero, I., López-Baena, F. J., Ollero, F.J., Bellogín, R., Lloret, J., Espuny, R., 2014. The symbiotic biofilm of Sinorhizobium fredii SMH12, necessary for successful colonization and symbiosis of Glycine max cv Osumi, is regulated by quorum sensing systems and inducing flavonoids via NodD1. PLoS One 9 (8), e105901.

Pérez-Montaño, F., Jiménez-Guerrero, I., Sánchez-Matamoros, R.C., López-Baena, F.J., Ollero, F.J., Rodríguez-Carvajal, M.A., Bellogín, R.A., Espuny, M.R., 2013. Rice and bean AHL-mimic quorum-sensing signals specifically interfere with the capacity to form biofilms by plant-associated bacteria. Res. Microbiol. 164 (7), 749–760.

Persello-Cartieaux, F., Nussaume, L., Robaglia, C., 2003. Tales from the underground: molecular plant–rhizobacteria interactions. Plant Cell Environ. 26 (2), 189–199.

Phillips, D.A., Fox, T.C., King, M.D., Bhuvaneswari, T.V., Teuber, L.R., 2004. Microbial products trigger amino acid exudation from plant roots. Plant Physiol. 136 (1), 2887–2894.

Podile, A.R., Vukanti, R.V.N.R., Sravani, A., Kalam, S., Dutta, S., Durgeshwar, P., Rao, V. P., 2013. Root colonization and quorum sensing are the driving forces of plant growth promoting rhizobacteria (PGPR) for growth promotion. Proc. Natl. Acad. Sci. India B Biol. Sci. 80, 407–413.

Poonguzhali, S., Madhaiyan, M., Sa, T., 2007. Quorum-sensing signals produced by plant-growth promoting *Burkholderia* strains under in vitro and in planta conditions. Res. Microbiol. 158 (3), 287–294.

Prasannath, Kandeeparoopan, 2017. Plant defense-related enzymes against pathogens: a review. AGRIEAST. J. Agric. Sci. 11, 38. https://doi.org/10.4038/agrieast.v11i1.33.

Rasmussen, T.B., Givskov, M., 2006. Quorum sensing inhibitors: a bargain of effects. Microbiology 152 (4), 895–904.

Reddy, V.S., Shlykov, M.A., Castillo, R., Sun, E.I., Saier Jr., M.H., 2012. The major facilitator superfamily (MFS) revisited. FEBS J. 279 (11), 2022–2035.

Rengel, Z., 2015. Availability of Mn, Zn and Fe in the rhizosphere. J. Soil Sci. Plant Nutr. 15 (2), 397–409.

Reyes-Montaño, E.A., Vega-Castro, N.A., 2018. Plant lectins with insecticidal and insectistatic activities. In: Insecticides-Agriculture and Toxicology [Prieiga Per Internetą 2018-11-03]. InTech, pp. 17–41.

Rosier, A., Medeiros, F.H., Bais, H.P., 2018. Defining plant growth promoting rhizobacteria molecular and biochemical networks in beneficial plant-microbe interactions. Plant Soil 428 (1–2), 35–55.

Rudrappa, T., Czymmek, K.J., Paré, P.W., Bais, H.P., 2008. Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiol. 148 (3), 1547–1556.

Ryan, R.P., An, S.Q., Allan, J.H., McCarthy, Y., Dow, J.M., 2015. The DSF family of cell–cell signals: an expanding class of bacterial virulence regulators. PLoS Pathog. 11 (7), e1004986.

Sanchez-Contreras, M., Bauer, W.D., Gao, M., Robinson, J.B., Allan Downie, J., 2007. Quorum-sensing regulation in rhizobia and its role in symbiotic interactions with legumes. Phil. Trans. Biol. Sci. 362 (1483), 1149–1163.

Santoyo, G., Pacheco, C.H., Salmerón, J.H., León, R.H., 2017. The role of abiotic factors modulating the plant-microbe-soil interactions: toward sustainable agriculture. A review. Spanish J. Agric. Res. 15 (1), 13.

Scharf, B.E., Hynes, M.F., Alexandre, G.M., 2016. Chemotaxis signaling systems in model beneficial plant–bacteria associations. Plant Mol. Biol. 90 (6), 549–559.

Schenk, S.T., Schikora, A., 2015. AHL-priming functions via oxylipin and salicylic acid. Front. Plant Sci. 5, 784.

Schenk, S.T., Stein, E., Kogel, K.H., Schikora, A., 2012. Arabidopsis growth and defense are modulated by bacterial quorum sensing molecules. Plant Signal. Behav. 7 (2), 178–181.

Schikora, A., Schenk, S.T., Hartmann, A., 2016. Beneficial effects of bacteria-plant communication based on quorum sensing molecules of theN-acyl homoserine lactone group. Plant Mol. Biol. 90 (6), 605–612.

Schmid, N., Pessi, G., Deng, Y., Aguilar, C., Carlier, A.L., Grunau, A., Omasits, U., Zhang, L.H., Ahrens, C.H., Eberl, L., 2012. The AHL-and BDSF-dependent quorum sensing systems control specific and overlapping sets of genes in *Burkholderia cenocepacia* H111. PLoS One 7 (11), e49966.

Sessitsch, A., Coenye, T., Sturz, A.V., Vandamme, P., Barka, E.A., Salles, J.F., Van Elsas, J.D., Faure, D., Reiter, B., Glick, B.R., Wang-Pruski, G., 2005. Burkholderia phytofirmans sp. nov., a novel plant-associated bacterium with plant-beneficial properties. Int. J. Syst. Evol. Microbiol. 55 (3), 1187–1192.

Shen, C., Shi, Y., Fan, K., He, J.S., Adams, J.M., Ge, Y., Chu, H., 2019. Soil pH dominates elevational diversity pattern for bacteria in high elevation alkaline soils on the Tibetan Plateau. FEMS Microbiol. Ecol. 95 (2), fiz003.

Shrestha, A., Grimm, M., Ojiro, I., Krumwiede, J., Schikora, A., 2020. Impact of quorum sensing molecules on plant growth and immune system. Front. Microbiol. 11, 1545.

Sourjik, V., Wingreen, N.S., 2012. Responding to chemical gradients: bacterial chemotaxis. Curr. Opin. Cell Biol. 24 (2), 262–268.

Sourjik, V., 2004. Receptor clustering and signal processing in E. coli chemotaxis. Trends Microbiol. 12 (12), 569–576.

Suppiger, A., Schmid, N., Aguilar, C., Pessi, G., Eberl, L., 2013. Two quorum sensing systems control biofilm formation and virulence in members of the Burkholderia cepacia complex. Virulence 4 (5), 400–409.

Teplitski, M., Robinson, J.B., Bauer, W.D., 2000. Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. Mol. Plant Microbe Interact. 13 (6), 637–648.

Van Delden, C., Pesci, E.C., Pearson, J.P., Iglewski, B.H., 1998. Starvation selection restores elastase and rhamnolipid production in a *Pseudomonas aeruginosa* quorum-sensing mutant. Infect. Immun. 66 (9), 4499–4502.

van der Burgh, A.M., Joosten, M.H., 2019. Plant immunity: thinking outside and inside the box. Trends Plant Sci. 24 (7), 587–601.

Vandeputte, O.M., Kiendrebeogo, M., Rajaonson, S., Diallo, B., Mol, A., El Jaziri, M., Baucher, M., 2010. Identification of catechin as one of the flavonoids from *Combretum albiflorum* bark extract that reduces the production of quorum-sensing-controlled virulence factors in *Pseudomonas aeruginosa* PAO1. Appl. Environ. Microbiol. 76 (1), 243–253.

Veliz-Vallejos, D.F., Kawasaki, A., Mathesius, U., 2020. The presence of plant-asociated bacteria alters responses to *N*-acyl homoserine lactone quorum sensing signals that modulate nodulation in *Medicago Truncatula*. Plants 9, 777.

Venturi, V., Fuqua, C., 2013. Chemical signaling between plants and plant-pathogenic bacteria. Annu. Rev. Phytopathol. 51, 17–37.

Venturi, V., Keel, C., 2016. Signaling in the rhizosphere. Trends Plant Sci. 21 (3), 187–198.

von Rad, U., Klein, I., Dobrev, P.I., Kottova, J., Zazimalova, E., Fekete, A., Hartmann, A., Schmitt-Kopplin, P., Durner, J., 2008. Response of Arabidopsis thaliana to N-hexanoyl-DL-homoserine-lactone, a bacterial quorum sensing molecule produced in the rhizosphere. Planta 229 (1), 73–85.

Wang, H., Zhong, Z., Cai, T., Li, S., Zhu, J., 2004. Heterologous overexpression of quorum-sensing regulators to study cell-density-dependent phenotypes in a symbiotic plant bacterium *Mesorhizobium huakuii*. Arch. Microbiol. 182 (6), 520–525.

Wang, N.Q., Kong, C.H., Wang, P., Meiners, S.J., 2021. Root exudate signals in plant–plant interactions. Plant Cell Environ. 44 (4), 1044–1058.

Wilder, C.N., Allada, G., Schuster, M., 2009. Instantaneous within-patient diversity of *Pseudomonas aeruginosa* quorum-sensing populations from cystic fibrosis lung infections. Infect. Immun. 77 (12), 5631–5639.

Wilkens, S., 2015. Structure and mechanism of ABC transporters. F1000Prime Rep 7, 14.

Williams, P., Cámara, M., 2009. Quorum sensing and environmental adaptation in Pseudomonas aeruginosa: a tale of regulatory networks and multifunctional signal molecules. Curr. Opin. Microbiol. 12 (2), 182–191.

Winzer, K., Hardie, K.R., Williams, P., 2002. Bacterial cell-to-cell communication: sorry, can't talk now—gone to lunch. Curr. Opin. Microbiol. 5 (2), 216–222.

Xie, F., Williams, A., Edwards, A., Downie, J.A., 2012. A plant arabinogalactan-like glycoprotein promotes a novel type of polar surface attachment by *Rhizobium leguminosarum*. Molecular plant-microbe interactions 25 (2), 250–258.

Zarkani, A.A., Stein, E., Röhrich, C.R., Schikora, M., Evguenieva-Hackenberg, E., Degenkolb, T., Vilcinskas, A., Klug, G., Kogel, K.H., Schikora, A., 2013. Homoserine lactones influence the reaction of plants to rhizobia. Int. J. Mol. Sci. 14 (8), 17122–17146.

Zboralski, A., Filion, M., 2020. Genetic factors involved in rhizosphere colonization by phytobeneficial Pseudomonas spp. Comput. Struct. Biotechnol. J. 3539–3554.

Zúñiga, A., Poupin, M.J., Donoso, R., Ledger, T., Guiliani, N., Gutiérrez, R.A., González, B., 2013. Quorum sensing and indole-3-acetic acid degradation play a role in colonization and plant growth promotion of Arabidopsis thaliana by Burkholderia phytofirmans PsJN. Mol. Plant Microbe Interact. 26 (5), 546–553.

Environmental Technology & Innovation 22 (2021) 101434

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Translocation of heavy metals in medicinally important herbal plants growing on complex organometallic sludge of sugarcane molasses-based distillery waste

Sonam Tripathi [a], Pooja Sharma [a], Kshitij Singh [a], Diane Purchase [b], Ram Chandra [a,*]

[a] Department of Environmental Microbiology, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar Central University, Vidya Vihar, Raebareli Road, Lucknow, Uttar Pradesh 226025, India
[b] Department of Natural Sciences, School of Science and Technology, Middlesex University, The Burrough, London NW4 4BT, UK

ARTICLE INFO

Article history:
Received 6 January 2021
Received in revised form 10 February 2021
Accepted 11 February 2021
Available online 16 February 2021

Keywords:
Medicinal plants
Heavy metals
Androgenic waste
Phytoremediation
Detoxification

ABSTRACT

This study aimed to assess the heavy metals accumulation patterns by some native plants such as *Achyranthus aspera* L., *Amaranthus viridis*, *Basella alba* L., *Sesbania bispinosa*, *Pedalium murex* L., and *Momordica doica*, which have been traditionally employed for medicinal and food purposes. The plants were grown on complex distillery waste containing a mixture of organometallic compounds. Results revealed bioaccumulation of Mn, Cd, Fe, Cr, Cu, As, Se, Mo, and Co in their roots, shoots, and leaves in levels higher than the surrounding sludge. *A. aspera* was noted as root accumulator for Mn (16.95 mg kg^{-1}), Zn (30.12 mg kg^{-1}), Fe (240.40 mg kg^{-1}), Co (3.19 mg kg^{-1}), while Se (4.07 mg kg^{-1}), Mo (4.36 mg kg^{-1}), was accumulated selectively in the shoot of the plant. Similarly, *S. bispinosa*, *P. murex*, and *M. doica* were found as root accumulators for Mn, Fe, and Ni. *A. viridis* accumulated Cd, Zn, and Cu in the shoot and leaves of the plant. The high bioconcentration factors (BCF) and translocation factors (TF) observed in these native plants (>1) suggested their tendency to hyperaccumulate heavy metals. The findings highlighted that these plants as a potential metal accumulator may pose health hazards and deteriorate the medicinal property if grown on such wastes.

1. Introduction

Heavy metal pollution in soil, water, and food material is a major threat to human health. Industrial wastes and geogenic activities are the major sources of heavy metals in the environment (Annan et al., 2013; Shammi et al., 2016; Sharma et al., 2021b). In India, several industries like distilleries, tanneries, pulp paper industries, electroplating industries, steel, and iron industries discharge a mixture of heavy metals along with various complex organic wastes into the environment. Due to the complexity in the matrix and non-degradability of the heavy metals, such discharge poses significant challenges for its remediation (Gupta and Sinha, 2007; Chandra et al., 2017; Kumar et al., 2013; Sushil and Batra, 2006). Heavy metals tend to accumulate in the soil, water, and plants in the environment, which could contaminate the food chain subsequently (Chandra et al., 2009; Sharma et al., 2020b; Sharma and Rath, 2020; Singh et al., 2012). For example, Indian mustard grown in distillery effluent irrigated soil showed significant accumulation of heavy metals (Cd, Cu, Fe, Mn, Ni, and Zn) in their

* Corresponding author.
E-mail address: prof.chandrabbau@gmail.com (R. Chandra).

https://doi.org/10.1016/j.eti.2021.101434
2352-1864/

edible parts (i.e. seeds and leaves) and could pose a health risk to humans (Bharagava et al., 2008). It is noteworthy that at <10% (v/v), the effluent showed an inducible effect on the growth of the mustard plant; while at >10% (v/v), the effluent showed an inhibitory effect on the various physiological parameters of the plants.

Various physicochemical methods i.e. filtration, flocculation, reverse osmosis, and chemical precipitation have been used for remediation of heavy metals from the terrestrial and aquatic environment, but they are energy-intensive and not cost-effective (Blöcher et al., 2003; Dialynas and Diamadopoulos, 2009; Bratskaya et al., 2009). Phytoremediation has emerged as a promising green technology for the decontamination of metals in polluted sites (Ghassemzadeh et al., 2008; Singh et al., 2017; Sharma et al., 2020a). Recent studies have highlighted the phytoextraction potential of some native plants such as weeds and grasses from metal-contaminated sources and organometallic industrial sludge disposed sites (Gupta and Sinha, 2007; Chandra et al., 2018; Franchi et al., 2017; Sharma et al., 2021a). Among the potential metal accumulators, several are known as food and medicinal plants (Shammi et al., 2016; Singh and Prasad, 2014). According to the World Health Organization (WHO), more than 70% of the modern world's population rely on medicines of herbal origin for their health care (Jaison and Muthukumar, 2017; Annan et al., 2013). The herbal preparation is presumed to be safe from all the contaminants, but studies have shown that they may be contaminated with various heavy metals like cadmium, chromium, lead, mercury, zinc, copper, nickel and manganese, etc. Several metals are intentionally added to the herbal preparation in the form of traditional medicine i.e. Ayurveda, and in many instances, they are artifacts of the manufacturing process. Various leafy herbal plants are known for their applications in medicine and have the inherent ability to bioaccumulate heavy metals, up to several folds higher than the surrounding soil. However, the capacity of plants to accumulate heavy metal may vary within and among the plant species (Jaison and Muthukumar, 2017; Sharma et al., 2020c). The heavy metal contamination in food and medicine could become health hazards and may also change the chemical properties of medicine. Ignorance of heavy metal accumulation in the raw material used in herbal preparation can lead to serious and long-term side effects (WHO, 1978).

Sugarcane molasses-based distilleries are sources of complex environmental pollutants due to the presence of various heavy metal-containing organic pollutants. In India, there are more than 317 sugarcane molasses-based distilleries in operation that generate on average 1500 tonnes of sludge per day during the anaerobic digestion of spent wash (Kansal et al., 1998). This reflects the magnitude of environmental pollution caused by solid waste generated from the distillery sector all over India. These sludges contained high iron (Fe), zinc (Zn), copper (Cu), chromium (Cr), cadmium (Cd), manganese (Mn), nickel (Ni), and lead (Pb) content, all present in concentrations above the prescribed limit in the environment recommended by US Environmental Protection Agency (USEPA). Recent studies highlighted that distilleries sludge contains several persistent organic compounds e.g. dodecanoic acid, octadecanoic acid, n-pentadecanoic acid, hexadecanoic acid, β-sitosterol, stigmasterol, β-sitosterol trimethyl ether, heptacosane, dotricontane, lanosta-8, 24-dien-3-one, 1-methylene-3-methyl butanol, and 1-phenyl-1-propanol as androgenic and mutagenic compounds. These compounds are listed by the USEPA as endocrine-disrupting chemicals (EDCs) (USEPA, 2012). Due to the complexation of metals with organic compounds, these organometallic compounds restricted the bioavailability of metals to plants. Hence, the generated sludge remains alkaline with a high pollution load of carcinogenic and mutagenic compounds that further impede biodegradation and subsequently damaged the vegetation at the dumping site. Many common native medicinal plants are found to grow well on disposed sludge of distillery waste, however, the accumulation of heavy metals by these plants in the environment from the multi-metal contaminated site is not well studied (Jaison and Muthukumar, 2017; Shammi et al., 2016). To understand the impact of complex industrial wastes in plants and explore their potential for phytoremediation, there is a need to identify the mutagenic and carcinogenic constituents of the distillery sludge and the metal-accumulation patterns in these native plants. Therefore, the main aim of this study is to assess the different metal accumulation patterns by these native plants which are well known for their medicinal use. Recognizing the distribution pattern of heavy metals in various parts or plant tissue will help to promote the correct usage of the plant materials and prevent accidental intake of heavy metals. This study seeks to determine heavy metal accumulation by different leafy medicinal plants from the sugarcane molasses-based distillery waste.

2. Material and methods

2.1. Sample collection and site description

The collection of plants and distillery sludge were carried out in month April to October (2019) from M/s Unnao distillery and breweries, situated in Uttar Pradesh, Unnao, India (26°320" N, 80°30′0"E). The distillery unit produces 9000 kilo litre alcohol and generates approx. 800 tonnes of sludge annually (AIDA, 2004). Freshly disposed dried distillery sludges cakes (approx. 5.0 kg) were collected in clean pre-sterilized polythene bags from the sludge dumping site of the distillery plant located inside the premises of the industry. It was treated as the control. Five healthy plants of each species growing on the sludge were collected from the site intermittently with a gap of one month to investigate changes in their properties. The abundant luxuriant growth of six plant species was identified as *Achyranthus aspera* L., *Amaranthus viridis*, *Basella alba* L., *Sesbania bispinosa*, *Pedalium murex* L. and *Momordica doica* with a standard method according to Duthie flora of Indo-Gangetic plain (Duthie et al., 1903).

2.2. Physico-chemical analysis of sludge before and after plant growth

The physico-chemical parameters e.g. pH, electrical conductivity (EC), chloride (Cl^-), sodium (Na^+), and nitrate (NO_3^-) of the distillery sludge was analyzed and estimated according to the method described by Chandra et al. (2018). The dried sludge cake was treated as a control while fresh sludge samples from the poluuted site were used for metal accumulation study. The phenol contents in sludge were determined as per standard methods described by the American Public Health Association (APHA, 2012). The pH and EC values (sludge: water = 1:2.5 w/v) of sludge samples were measured using an Orion pH meter (Model-960, Thermo Scientific, FL, USA) and Orion conductivity meter, respectively (Chandra et al., 2008). The total content of Fe, Zn, Cu, Mn, Ni, and Pb was measured using atomic absorption spectrophotometry (AAS) (ZEEnit 700, Analytic Jena, Germany) after acid digestion (APHA, 2012). While the BOD, COD was measured in prepared leachate from distillery sludge as described previously (Sharma et al., 2020c).

2.3. Scanning electron microscopy (SEM) and Fourier transform-infrared spectrophotometry (FTIR) analysis of sludge

One gram of distillery sludge was dried in a hot air oven (Evolution-201, Thermo Scientific, USA) at 50 °C overnight till constant weight. The sample was crushed into powder form in a porcelain mortar (Evolution-201, Thermo Scientific, USA) as previously described (Yadav and Chandra, 2018). Further, the sludge sample was treated in 2.5% glutaraldehyde for 3–4 h and postfixed with 1% Osmium tetroxide for 2 h before sputter coated in a gold film and examined under SEM and EDAX (SEM, QUANTA FEG 450, FEI, and the Netherlands) (Yadav and Chandra, 2018). The sample was further analyzed using Fourier transform-infrared spectrophotometry (FTIR) using a spectrophotometer (Nexus-890, Thermo Electron Co., Yokohama, Japan). For elemental analysis of the sludge sample, an area was selected, and the elements in the sediment were examined by a high-resolution SEM equipped with an EDX system (SEM, QUANTA FEG 450, FEI, and the Netherlands). Fourier transform-infrared spectrophotometry (FTIR) analysis of the sludge sample was also performed using a spectrophotometer (Nexus-890, Thermo Electron Co., Yokohama, Japan). The sample was dispersed in spectral-grade KBr (Merck, Darmstadt, Germany) and made into pellets by applying 5–6 tons cm^{-2} of pressure for 10 min using hydraulic pressure (Specac, United Kingdom) instrument. The spectrum was generated in the range of 400 to 4000 cm^{-2} with a resolution of 4 cm^{-1} for all samples (fresh sludge, and a plant grown sample).

2.4. UV–Vis spectral and high-performance liquid chromatography (HPLC) analysis of leachate

The collected sludge samples were pooled, mixed, and then air-dried before ground with a pestle mortar to crush the entire available particle; the crushed samples were sieved through a 63 μm pore size sieve to get a homogeneous powder. Further, the solvent (ethyl acetate) extraction was carried out to obtain 10% leachate from the sludge as described earlier (Chandra et al., 2008). Briefly, 100 g of sludge was added to 1000 ml of ethyl acetate (w/v) and the mixture was shaken continuously for 3–4 hr at room temperature (25 ± 2 °C), and the suspension was filtered through a 0.22 μm syringe filter. Furthermore, organic pollutants in the sludge sample were measured using a UV–Vis spectrophotometer (Thermo Fisher Scientific Shanghai spectrophotometer Evolution 2001, China) at a wavelength between 200–700 nm at ambient room temperature (25 °C) an HPLC equipped with a 2487 UV–Vis detector and using Millennium software (Waters 515). The samples (20 μl) were injected at a rate of 1 ml min^{-1} using an acetonitrile: water ratio of 70:30. To analyze the compounds at 320 nm by HPLC, a reverse-phase C-18 column (250 × 4.6 mm, particle size 5 μm) at 27 °C was used (Chandra et al., 2018).

2.5. Extraction and characterization of residual organic pollutants through GC–MS analysis

2.5.1. Solid–liquid extraction

Various organic compounds from the distillery sludge were extracted using ethyl acetate as per the previously mentioned method (Chandra and Kumar, 2017). The extraction was repeated three times. The organic solvent phase was dried over anhydrous Na_2SO_4, and the solvent was evaporated to dryness using a stream of nitrogen gas at room temperature. Dry organic filters were makeup in 1.5 ml ethyl acetate and filtered through 0.22 μm syringe filters (Millipore Ltd, Bedford, Massachusetts, USA) and used for GC–MS analysis.

2.5.2. Characterization of organic pollutants

The extracted samples were derivatized with trimethylsilyl (TMS) as described earlier (Chandra and Kumar, 2017). An aliquot (2.0 μl) of the derivatized sample was injected in a GC–MS instrument (Trace GC Ultra Gas Chromatograph; Thermo Scientific, FL, USA) equipped with a TriPlus auto-sampler coupled to TSQ Quantum XLS triple quadrupole mass spectrometer (Thermo Scientific, FL, USA). Separation of organic compounds occurred in a DB-5MS capillary column. The temperature of the GC oven started from 65 °C (hold for 2.0 min), increased to 230 °C at a rate of 6 °C min^{-1}, and finally reached 290 °C (hold for 20 min) at the rate of 10 °C min^{-1}. Helium was used as carrier gas at a flow rate of 1.1 ml min^{-1}. The mass spectrum (MS) was operated in the positive electron ionization (+EI) mode at 70 eV. The detected organic compounds extracted from fresh and plant grown samples were identified by matching with the MS library NIST version 1.0.0.12 available with the instrument.

2.6. Digestion of plants for metals estimation

To estimate the metal content accumulated in the potential plant tissue of native plants, the uprooted plants were washed thoroughly with deionized water to remove all the sludge particles from the roots, followed by rinsing with a 10 Mm solution of calcium chloride. Subsequently, the plant's roots, shoots, and leaves were separated and chopped into small pieces, and the resulting biomass was oven-dried at 70 °C for 5 days till constant weight. The dried plant parts were washed in a muffle furnace at 460 °C for 6 h. The weighted ash from these samples was digested in 2% nitric acid (HNO_3) and filtered through a 0.45 μM glass fiber filter (AOAC, 2002). One gram (1.0 g) of dried and sieved sediments was digested with 10 ml of HNO_3. If brown fumes appeared, additional 5 ml of HNO_3 was added and digestion continued till no generation of brown fumes. The concentrations of Cr, Zn, Mn, Ni, Cu, Fe, Cd, and Pb were determined by AAS (Chandra et al., 2017).

2.7. Metals accumulation efficiency

To evaluate the metal accumulation efficiency in plants, the bioaccumulation coefficient factor (BCF) and translocation factor (TF) were calculated. BCF is defined as the ratio of metal concentration in the root to the soil, and TF is the ratio of metal concentration in the shoot to the root. BCF and TF were calculated according to the formulae below which have also been reported earlier by Yoon et al. (2006).

$$\mathbf{BCF} = \mathbf{C_{root}}/\mathbf{C_{sludge}} \quad (1)$$

$$\mathbf{TF} = \mathbf{C_{shoot}}/\mathbf{C_{root}} \quad (2)$$

Where, C_{root} = concentration of metal in plant root (mg kg^{-1}), C_{sludge} = concentration of metal in distillery sludge (mg kg^{-1}), and C_{shoot}=concentration of metal in mg kg^{-1} as per the dry weight of plant shoot. Both BCF and TF have to be considered for evaluating whether a plant is a metal hyperaccumulator.

2.8. Histological observations of root tissues by transmission electron microscopy

Root segments (2.0 mm in length) of selected plants were immersed in H_2S saturated water as pre-treatment for 30 min at room temperature to precipitate trace elements. The root sample was washed with 0.1M SCB (sodium cacodylate buffer, pH 7.2) and fixed in 2.5% glutaraldehyde (v/v) prepared in sodium cacodylate (Ladd Research Industries, Williston, USA) buffer (pH 7.2) for 2 h at 4 °C. The root tissue was washed three times with 0.1M SCB with a 10 min interval between each washing and postfixed in 1% OsO_4overnight. The fixed tissue was washed with SCB, dehydrated in graded acetone series, and embedded in the Araldite-DDSA mixture (Ladd Research Industries, Williston, USA). After backing at 60 °C, blocks were cut (60–80 nm thick) by an ultra-microtome (Cryo Leica EM UC7, Leica Microsystem, India), and the sections were stained by uranyl acetate and lead citrate. Analysis of the section was done under transmission electron microscopy (TEM) (FEI Tecnai^{TM} G2 Spirit Twin, Hillsboro, USA) at an accelerating voltage of 80 kV (Chandra et al., 2018).

2.9. Statistical data analysis

In-situ phytoremediation process and metal accumulation compared with the original distillery sludge (pre-treatment) and Student's t-test ($p < 0.001$) was carried out. To confirm the variability of data obtained and the validity of results, the mean concentration of heavy metals in the root, shoot, and leaves were subjected for the ANOVA analyses (Ott, 1984). All statistical analyses were carried out using the SPSS statistical software (version 17.0; SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Physico–chemical characterization of fresh and after plant growth sample of sugarcane molasses sludge

All the physico–chemical parameters in leachate and sludge of the distillery waste were above the permissible limits as shown in Table 1. The BOD/COD ratio indicated low degradability due to the presence of recalcitrant compounds (Kumar et al., 2010). Different metals (i.e. Mn, Cr, Zn, Cu, Fe, Pb, Cd, and Ni) were also found in concentrations higher than the USEPA guideline (2012). The high content of heavy metals in distillery sludge might be due to the corrosive effect of sugarcane juice in sugar manufacturing industries and the boiling of the juice during molasses separation, fermentation, and distillation process of sugar cane molasses in the distilleries. In the subsequent step, the metals might be leached into the spent wash from the drainage pipe due to high acidic conditions. The leaching of heavy metal in the acidic medium in the industrial process is well documented in a previous study (Noor and Al-Moubaraki, 2008).

While after plant growth, various pollution parameters, phenol, and potassium, phosphate, nitrogen contents, and the metal contents were significantly reduced from fresh sludge (Table 1). These data provided a strong indication of the phytoextraction capability of heavy metals and the phytoremediation potential of organic pollutants of the native plants. The repeated application of metal-containing industrial effluent in irrigation also showed the accumulation of toxic metals in the edible parts of crop plants (Chandra et al., 2009). The high content of heavy metal in distillery sludge and their

Table 1
Physico-chemical characteristics of discharged distillery fresh sludge before and after plant growth collected from M/s Unnao Distillery Pvt. Ltd. *Unnao*, Uttar Pradesh, India.

Parameters	Sludge before plant growth	Sludge after plant growth	Reduction %	Permissible limit (USEPA, 2012)	CPCB (2017)
Color appearance	Blackish Brown	Brown	–	–	–
Odor	like molasses	Like molasses	–	–	–
pH	8.67 ± 0.16	7.95 ± 0.22[a]	94.5%	8.00 ± 0.01	7.54 ± 0.01
Biological oxygen demand (BOD) (mg l^{-1})	4166.82 ± 88.22	2500.11 ± 86.60[a]	60.0%	40.00	47.00 ± 0.00
Chemical oxygen demand (COD) (mg l^{-1})	12527.18 ± 182.22	6850.84 ± 128.11[a]	54.68%	121.00	79.00 ± 0.01
Electrical conductivity (EC)	1916.66 ± 60.09	1003.33 ± 31.79[a]	50%	1000	950
Total Dissolve solid (TDS) (mg l^{-1})	10720.78 ± 260.44	5521.99 ± 151.29[a]	51.3%	50–70	70 ± 0.00
VS (mg l^{-1})	1214.84 ± 36.01	515.84 ± 14.60[b]	41.16%		20 ± 0.01
Chloride (mg l^{-1})	2935.33 ± 70.95	1193.33 ± 52.06[b]	40.45%	750.00	11.82 ± 0.01
Total Nitrogen (TN) (mg l^{-1})	228.51 ± 8.78	141.81 ± 5.87[a]	57.7%	–	9.90 ± 0.00
Phenol (mg l^{-1})	8015 ± 73.99	2702.33 ± 35.89[c]	33.33%	0.50	–
Sulfate(mg l^{-1})	15,616.95 ± 606.47	8557.15 ± 83.36[b]	55.31%	750.00	
Phosphate (mg l^{-1})	48.04 ± 1.52	21.90 ± 0.92[b]	51.97%	–	3.40 ± 0.01
Heavy and alkali metals					
Mn (mg l^{-1})	8.75 ± 0.52	3.58 ± 0.22[b]	40.98%	0.20	0.15
Cr (mg l^{-1})	3.73 ± 0.38	1.06 ± 0.12[c]	26.0%	0.05	0.01
Zn (mg l^{-1})	18.84 ± 0.89	8.23 ± 0.67	35.10%	2.00	1.28
Cu (mg l^{-1})	3.91 ± 0.21	0.50 ± 0.08[NS]	12.56%	0.50	0.19
Fe (mg l^{-1})	423.88 ± 7.33	186.44 ± 8.89	41.46%	2.00	1.45
Pb (mg l^{-1})	5.08 ± 0.27	1.86 ± 0.25[c]	32.81%	0.05	0.02
Cd (mg l^{-1})	BDL	BDL	BDL	BDL	BDL
Ni (mg l^{-1})	8.78 ± 0.64	3.91 ± 0.58[a]	40.14%	0.10	0.04
Na (mg l^{-1})	488.28 ± 6.68	63.25 ± 3.70[NS]	20.49%	0.04	0.01
K (mg l^{-1})	245.15 ± 10.30	136.99 ± 1.99	60.86%	0.09	0.02

All the values are Mean ± SE. (n = 3); Unit of all parameters is in (mg l^{-1}) except pH, color (Co-Pt. Unit) and EC (μmhos cm^{-1}); Students *t* test (two tailed as compared to pre-treated sludge); BDL: below detection limit.
[a]Highly significant at $p < 0.001$.
[b]Significant at $p < 0.01$.
[c]Less significant at $p < 0.05$.
[NS]Non-significant at $p > 0.05$.

adverse effect on seed germination and growth parameter of green gram *Phaseolus mungo* L. were reported in a study by Chandra et al. (2008). However, in the current study, the native medicinal plants did not appear to be affected negatively by the elevated metal contents, further suggesting their tolerance to heavy metals. The high affinities of metallic ions with organic pollutants are known to restrict the bioavailability of metals to plants (Migo et al., 1997). The ability of the plants to accumulate heavy metals might be due to increased solubility and availability of metal resulting from the plant microbe's interaction (Rajkumar et al., 2012).

The increase of electric conductivity after plant growth on the distillery sludge indicated the function of ions (cations/anions) present in sludge (Bose and Bhattacharyya, 2008; Sinha et al., 2007). The reduction of total nitrogen suggested nitrogen was taken by the plant as a nutrient for growth; it could also be attributed to the conversion of nitrate to ammonium with a mixed reaction of microbes and plants. This finding collaborated with previous observations of plant growth on the sludge of distilleries (Chandra and Kumar, 2017). Similarly, the BOD, COD values in the sludge leachate also reduced after plant growth. This result clearly showed phytoremediation along with the phytoextraction of heavy metals from sludge.

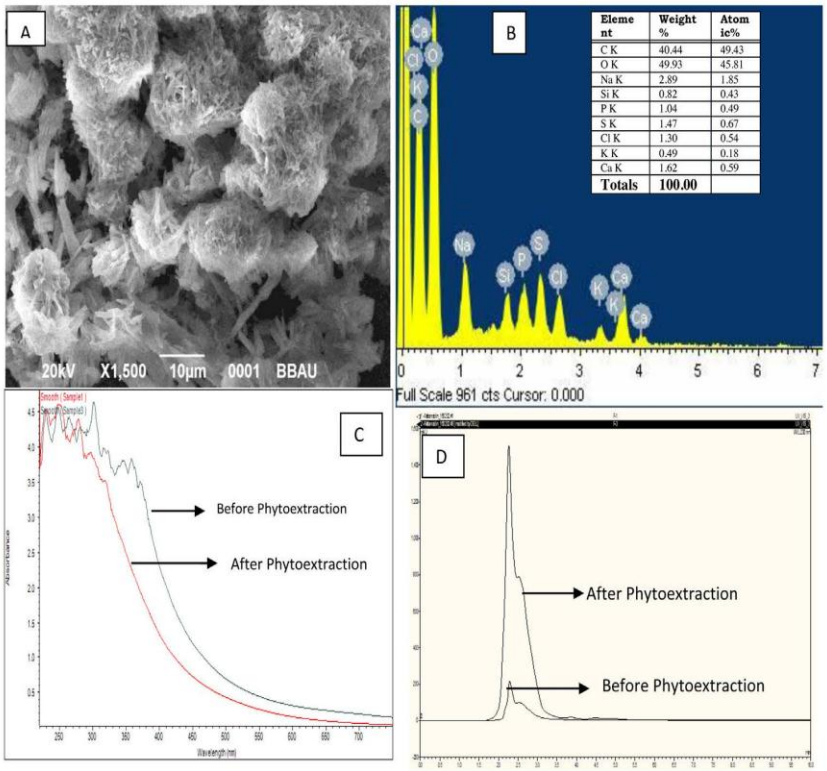


Fig. 1. A. Scanning Electron Microscopy analysis of distillery waste sludge sample; 1B: EDAX of distillery waste sludge sample; 1C: UV–Vis spectral analysis of before and after phytoextraction at various time intervals; 1D: HPLC analysis of before and after phytoextraction.

3.2. Morphological view, element components, and functional groups analysis of sludge

The SEM image of sludge showed fine crystals in needle shape. Some scattered elongated crystals were noted as well as the glomerated needle-shaped fine crystals as shown in Fig. 1A. The needle-like crystal properties have been reported as organic polymers and metallic crystals (Liu et al., 2013). The elemental analysis in EDX showed the presence of carbon, oxygen, sodium, potassium, calcium, and surface in different percentages as shown in Fig. 1B. The IR spectra of the sludge pre-plant growth showed medium and strong bonds of the compounds with the different stretching group at 3426.40 cm^{-1} (N–H), 2967.1 (C–H), 2470.5 (O=C=O), 1644.3 (C=N), 1559.7 (N–O), 1446.4 (C–H), 1411.6 (S=O), 1196.1 (C–O), 1048.3 (CO–O–CO), 866.7 (C–H)(Fig.S_1A& Table.S_1B). While the post-plant growth FTIR data showed maximum strong bond stretching frequency of compounds 3415.5 (O–H), 2967.1 (C–H), 2188.4 (C≡C), 1564.6(N–O), 1415.5 (S=O), 1211.0 (C–O), 1049.3(CO–O–CO), 1021.1(C=C), 654.4(C–Br). This suggested that the presence of various compound classes, such as carbon dioxide, sulfonyl chloride, ester, and anhydride. After plant growth, most of the medium bonds of detected compounds were broken; in contrast, compounds with strong bonds remained intact (Table S1.A& Fig.S1.B).

3.3. UV–Vis spectral and HPLC analysis of leachate

Analysis of the UV–Vis absorption spectra at wavelength 250–700 nm showed the presence of variable peaks in the UV-range due to the dissolved organic compounds in the sugarcane molasses-based distilleries sludge before phytoremediation; these peaks were diminished after plant growth as shown in Fig. 1C. The presence of soluble organic matter in the UV–Vis range was noted. The sludge sample before plant growth showed various mixed peaks which indicated a mixture of pollutants present in the leachate with absorption maxima at 320 nm. While after the plant growth, many of the absorption peaks disappeared signifying the degradation of various organic pollutants. In this analysis, the integrated UV absorption reflected the overall volume of aromatic or unsaturated compounds and their double bond absorption i.e. C=C, C=O, and N=N. The spectra (Fig. 1C) were produced by calculating the absorption of monochromatic radiation through a spectrum of wavelengths in the pre-and post-phytoextraction solution; it was one of the most common techniques for monitoring pollutants from sludge (Chandra et al., 2018). The comparative chromatograms of HPLC before and after phytoextraction were shown in Fig. 1D. The reduction in the major pollutant suggested the

degradation of organic pollutants in the sludge and phytoextraction process by plants, which removes complex organic compounds and most of the heavy metals that are toxic to the biota of the environment even at low concentrations. A similar pattern on the phytoextraction process from distillery sludge has been shown by other plants in previous studies (Chandra et al., 2018).

3.4. Identification of organic pollutants

The GC–MS analysis in ethyl acetate extract of distillery sludge of control sample (bulk sludge) and rhizospheric soil after plant growth on sludge was analyzed to investigate the alteration of organic compounds.

3.4.1. Identification of organic pollutants (bulk sludge)

The major peaks of an extracted sample (bulk sample) were observed at RT 6.77, 13.17, 13.56, 19.12, 20.50, 23.99, 25.00, 27.28, 30.16, 31.18, 35.04, 48.22 as shown in Fig. S2 A. These compounds were characterized as acetamide, 2,2,2-trifluoro-N-methyl, benzene acetic acid, TMS ester, butanedioic acid, bis(TMS)ester, hexadecane, dodecanoic acid, TMS ester, anthracene, dotricontane, ethanol, 2-(octadecycloxy), octadecanoic acid, TMS ester, hahnefett, nonacosane as described by the NIST library available with the instrument. Moreover, other minor peaks were also noted at RT values of 8.05, 17.20, 35.37, 36.69, 39.66, 40.52, 42.64, 49.02, and 51.12 as shown in Fig.S2. These compounds were characterized as butane, 2,3-bis(TMS oxy), α-ketoglutamic acid, bis(TMS)ester, nonacosane, stigmata-5,22-dien-3-ol (3β, 22E), stigmasterol, lanosta-8,24-dien-one, β-sitosterol, silane, [[(3β)-cholest-5-en-3-yl]oxy]TM, methylene bis (2,4,6-triisopropyl phenyl phosphine). Most of the identified compounds were classified either under the category of plant fatty acids or chemical reaction by-products of the distillation process (Chandra et al., 2018). The toxicities of detected pollutants were reported in Table 2. The source of complex phenolic compounds in sludge might be from the extract of sugarcane molasses after fermentation. Different forms of detected aromatic compounds could be the result of carbonyl and amine group reaction at elevated temperature in the sugar industry, which remains as complex in cane molasses known as melanoidin (Chandra et al., 2018). The presence of several fatty acids in the sludge might contribute to the reduced rate of metal accumulation processes and biotransformation processes of complex organometallic compounds in plants. When these organic pollutants reach the aquatic ecosystem, they can cause toxic effects on flora and fauna of water bodies. Recently, some of the detected plant organic residues (phytosterols) such asstigmata-5, 22-dien-3-ol (3β, 22E), stigmasterol, lanosta-8, 24-dien-one, β-sitosterol, and silane have been demonstrated to be toxic to the aquatic ecosystem. Other organic acids like octadecanoic acid, dodecanoic acid, and butanedioic acid are also listed as endocrine disruptors. The presence of EDCs compounds in distillery sludge has given strong evidence for the complex nature of sludge with various toxic compounds. These compounds are either generated at the time of fermentation or during the anaerobic treatment of distillery sludge at the disposed site (Chandra et al., 2018). Many of these detected compounds caused adverse effects in environmental flora and fauna of soil as well as the aquatic ecosystem.

3.4.2. Identification of organic pollutants from distillery sludge after plant growth

Detection of organic compounds from phytoextracted distillery sludge was analyzed in detail (Table 2). The number of organic compounds and peaks were reduced in the sludge after plant growth as compared to the control sludge sample. The major peaks of the phytoextracted sample were noted at RT 8.66, 12.82, 13.78, 16.01, 20.99, 21.91, 22.18, 22.81, and 22.90 as shown in Fig. S2B. These compounds were characterized as 1,3-propanediol, TMS ether, 5-methyl-2-(1-methyl ethyl) cyclohexanol, butanedioic acid, bis(TMS) ester, 2,3-butanediol, bis-O-(TMS), dodecanoic acid, TMS ester, butanedioic acid, bis(TMS) ester, cyclooctene, 1,2-bis(TMS), tricarboxylic Acid TMS. Similarly, other minor peaks were also detected at RT values of 9.88, 10.27, 11.54, 13.95, 14.98, 17.53, 17.72, 19.10, 20.08, 24.88, 25.96, 26.49 and 27.84. These compounds were characterized as propanoic acid, 3-[(TMS) oxy, 1, 3 propanediol, TMS ether, pentanoic acid, malic acid (O-(TMS)-bis (TMS ester), tert-butyl hydroquinone, bis (TMS), vanillin propionic acid, bis (TMS), tricarballylic acid 3 TMS, benzene acetic acid according to the NIST library. The result showed that original organic compounds present in distillery sludge before plant growth were either degraded or biotransformed into new compounds as shown in Table 2. This confirmed the medicinal plants harvested from the contaminated were able to bioremediate the distillery sludge. The bioremediation along with the phytoextraction of heavy metals by the other native weeds has shown similar observation and it was found as potential metal accumulation from complex organometallic wastes (Chandra et al., 2018).

3.5. Accumulation of total heavy metals in selected plants

To assess the potential of using native plants for phytoremediation of distillery sludge, the effect of metal accumulation by six native medicinal plants were further investigated. Six native medicinal plants (i.e. *Achyranthus aspera* L., *Amaranthus viridis, Basella alba* L., *Sesbania bispinosa, Pedalium murex* L., and *Momordica doica*) growing at the discharge site of distillery sludge were analyzed for their accumulation of twelve heavy metals (Mn, Pb, Cd, Zn, Cr, Fe, Cu, Ni, As, Se, Mo, and Co). The variable pattern of metal accumulation to the various parts of the plant species indicated different capacities of metal uptake as shown in Table 3 and Fig. S3 and S4. A previous study showed that the metal contents along with organic compounds in distillery sludge inhibited the development of the roots of various crops and stunted the growth of their shoot (Mazumdar and Das, 2015; Gupta and Sinha, 2007). However, the luxuriant growth of plants along with

Table 2
Detection of residual organic pollutants by GC–MS analysis from distillery sludge waste before and after phytoextraction.
*RT-retention time (in minutes), + present, − absent, (TMS) trimethylsilyl.

RT:	Name of Compound before extraction	Molecular Formula	Abundance (%)	Nature of compounds	% similarity (NIST Library)	Toxicity
6.77	Acetamide, 2,2,2-trifluoro-N-methyl	$C_6H_{16}O_2Si$	98	Organic Nature	75.95	Slight skin irritant, strong eye irritant,
7.10	2-(2-Hexyloxyethoxy)ethanol	$C_{13}H_{32}O_4Si$	76	Sulfonic benzoic	90.23	vomiting and diarrhea
8.05	Butane, 2,3-bis(TMSoxy)	$C_{12}H_{28}O_3Si$	68	Alkane Nature	87.18	Gastrointestinal (Digestive), Hepatic (Liver)
11.85	Hexanoic acid, 2-[(TMS)oxy]	$C_{12}H_{22}O_2Si_2$	70	Fatty acid	78.01	Hematological (Blood Forming),
13.17	Benzene acetic acid, TMS ester	$C_{10}H_{26}O_2Si$	52	Sulfonic benzoic	93.11	Aquatic Toxicology
13.56	Butanedioic acid, bis(TMS)ester	$C_{13}H_{30}O_5Si_3$	45	Fatty acid	93.12	Sore throat. Skin & Eye Redness.
15.49	Pentanedioic acid, bis(TMS)ester	$C_{14}H_{24}O_3Si_2$	61	Fatty acid	57.01	Skin, Eye, and Respiratory Irritations
16.64	Decanoic acid, TMS ester	$C_9H_{22}O_3Si_2$	98	Fatty acid	98.23	Irritation when applied to human skin
17.20	α-Ketoglutamic acid, bis(TMS)ester	$C_{19}H_{40}O_2Si$	59	Metabolic product	90.02	Neurological brain disorder
19.12	Hexadecane	$C_{21}H_{44}O_2Si$	73	Alkane Nature	97.13	Dizziness, headache and vomiting
20.50	Dodecanoic acid, TMS ester	$C_{19}H_{36}O_5Si$	68	Fatty acid	92.05	Ingestion, inhalation & skin absorption
21.40	Querecitin 7,3',4'-Trimethoxy	$C_{10}H_{24}OSi$	94	Saturated FA	74.35	Chronic diseases
23.99	Anthracene	$C_{11}H_{28}O_2Si$	93	Organic Nature	34.54	Melanoma, respiration hazard
25.00	Dotricontane	$C_{12}H_{36}O_4Si_5$	80	Saturated FA	95.14	Sensitization, Skin
27.28	Ethanol, 2-(octadecycloxy)	$C_{12}H_{32}O_3Si_3$	81	Organic Nature	71.23	Reproductive toxicity.
30.16	Octadecanoic acid, TMS ester	$C_{21}H_{44}O_2Si$	31	Fatty acid	65.00	Carcinogenicity
32.01	Tetracosane	$C_{27}H_{58}O_4Si$	54	Organic Nature	67.98	Aquatic environment, long-term hazard
32.40	Eicosanoic acid, TMS ester	$C_{31}H_{56}OSi$	68	Acyclic Nature	97.24	Headaches, drowsiness, confusion
35.04	Hahnefett	$C_{32}H_{56}OSi$	42	Alkane Nature	93.11	Seizures, and life-threatening complications
35.57	Nonacosane	$C_{32}H_{58}OSi$	55	Alkane Nature	89.74	Breakdown of the hemoglobin
(RT)	Name of Compound after extraction	Molecular Formula	Abundance (%)	Nature of compounds	% similarity (NIST Library)	Toxicity
8.66	1,3-Propanediol, TMS ether	$C_6H_{16}O_2Si$	53	Acyclic	76.34	Chronic poisoning, weakness, muscle aches
9.88	Propanoic acid, 3-[(TMS)oxy	$C_9H_{22}O_3Si_2$	97	Fatty acid	96.03	Skin and nail symptoms
10.27	1,3 Propanediol, TMS ether	$C_6H_{16}O_2Si$	78	Alkane Nature	89.02	Hyper pigmentation
11.54	Pentanoic acid	$C_{12}H_{28}O_3Si$	76	Fatty acid	24.22	Inflammation of sensory and motor nerve
13.78	Butanedioic acid, bis(TMS) ester	$C_{10}H_{22}O_4Si2$	98	Fatty acid	75.74	Hemolysis, anemia, hypotension
15.52	Resorcinol, 0-bis(TMS)	$C_{12}H_{22}O_2Si_2$	76	Saturated FA	98.54	Low level of iron in the red blood cells
16.01	2,3-Butandiol, bis-0-(TMS)	$C_{10}H_{26}O_2Si$	68	Sulfonic benzoic	90.75	Low blood pressure
17.53	Malic acid (0-(TMS)-bis(TMS ester)	$C_{13}H_{30}O_5Si_3$	79	Saturated FA	98.23	Abdominal pain; fever; and diarrhea
21.91	Cyclooctene, 1,2-bis(TMS)	$C_{14}H_{30}O_2Si$	68	Acyclic Nature	95.64	Encephalopathy
22.18	Tricarballylic Acid TMS	$C_{15}H_{32}O_6Si_3$	69	Organic Nature	78.35	Nerve disease of the extremities
23.54	Benzoic acid,	$C_{16}H_{30}O_4Si_3$	79	Acyclic Nature	86.18	Loss or deficiency of the fatty coverings
24.88	Tert-butyl hydroquinone, bis (TMS)	$C_{16}H_{30}O_2Si_2$	58	Sulfonic benzoic	73.65	Stomach tumors and damage
26.49	Vanil propionic acid, bis (TMS)	$C_{17}H_{32}O_3Si_2$	65	Fatty acid	95.12	Skin corrosion/irritation
27.83	Tricarballylic acid 3 TMS	$C_{15}H_{32}O_6Si_3$	76	Fatty acid	93.16	Specific organ toxicity, Respiratory tract irritation
30.61	Benzene-acetic acid	$C_{15}H_{26}O_4Si_2$	99	Saturated FA	99.00	Cough sore throat. Skin redness. Eyes pain

a well-developed root system indicated the inherent potential of the native plants due to their genetic property. As the distillery sludge had a complex matrix, the presence of various organic compounds could reduce the bioavailability of the multi-metals to the native plants.

The overall accumulation pattern of different metals in various plants was noted as below:

The accumulation of various heavy metals by *A. aspera* L. has been reported from a separate study (Saraf and Samant, 2013). Similarly, another study showed Fe and other metals accumulation in plants from the lignocellulosic waste containing multi-metal complex (Chandra et al., 2017). Excess Fe was observed in this study in the root of the plants. From a toxicological point of view, although Fe is an essential element, excess level could cause phytotoxicity, the ability of *A. viridis* to accumulate up to 830 mg kg^{-1} Fe indicated the presence of metal detoxifying mechanisms and the potential of the plant as a phytoremediation candidate. A similar pattern for the accumulation of Mn and Fe has been reported in the root of *A. aspera* L. in a previous study (Saraf and Samant, 2013). The study highlighted the accumulation of high content of Fe, Cu, Co, and Au from metal contaminated soil. *S. Bispinosa* was also reported to be able to accumulate a high concentration of lead (Sahi et al., 2002). Overall, the six tested plant species showed a high concentration of metal accumulation as compared to the normal uptake of metal, this indicated that these plants were capable to grow at the contaminated site of distillery sludge and could play a vital role in phyto-accumulation of heavy metals.

The accumulation of heavy metal depends upon the plant species, distribution, and variability of the microbial community, the chemical property of organic pollutants, and the pH of a substrate (Laghlimi et al., 2015; Kumar et al., 2013; Gupta and Sinha, 2006; Sharma et al., 2020b). The disposed distillery sludge after anaerobic digestion became slightly alkaline (pH 8.67 ± 0.16) which could restrict metal availability to plant. But the metal accumulation by growing plant on sludge showed the potentiality of plant and there was a gradual decrease of pH in the rhizospheric soil. This might be due to the plant microbes' interaction in the rhizospheric zone, there is an abundant release of acids from root hairs like gluconate, 2-ketogluconate, oxalate, citrate, acetate, malate, and succinate (Ma et al., 2011). Our result indicated that the distillery sludge as waste comprised various organic compounds and metals that formed organo-metallic complexes. Most of the compounds in sludge were anionic which has a strong cationic metal-binding tendency (Migo et al., 1997). Improved bioavailabilities of metals to plant by growing rhizospheric bacterial communities have also been reported previously (Sessitsch et al., 2013; Rajkumar et al., 2012).

Table 3
Heavy metal accumulation (mg kg^{-1} DW) in the root, shoots, and leaves of various hyperaccumulator plant species growing contaminated site of distillery waste site. All the values are mean of three replicates (n = 3) ± standard deviation (SD), BDL: below detection limit, R: Root, S: Shoot, L: Leaf.

Plant	Plant part	Mn	Pb	Cd	Zn	Cr	Fe	Cu	Ni	As	Se	Mo	Co
A. aspera L.	*Root*	16.95 ± 0.1	2.45 ± 0.4	4.76 ± 0.2	30.12 ± 0.3	3.65 ± 0.2	240.4 ± 0.1	23.25 ± 0.3	3.87 ± 0.5	4.02 ± 0.1	3.67 ± 0.1	2.57 ± 0.2	3.19 ± 0.2
	Shoot	13.35 ± 0.4	Nil	Nil	17.87 ± 0.1	15.54 ± 0.6	345.9 ± 0.5	35.45 ± 0.4	1.83 ± 0.1	1.98 ± 0.5	4.07 ± 0.3	4.36 ± 0.2	2.34 ± 0.3
	Leaves	12.25 ± 0.3	5.54 ± 0.3	3.32 ± 0.3	19.34 ± 0.2	6.76 ± 0.5	538.7 ± 0.1	17.52 ± 0.6	4.59 ± 0.6	3.03 ± 0.1	1.87 ± 0.3	2.87 ± 0.2	2.98 ± 0.2
	Total	42.55 ± 0.8	7.99 ± 0.7	8.08 ± 0.5	67.33 ± 0.6	25.9 ± 1.3	1125.0 ± 0.7	76.22 ± 1.3	10.29 ± 1.2	9.03 ± 0.7	9.61 ± 0.7	9.80 ± 0.6	8.51 ± 0.7
Accumulation pattern		***R> S> L***	***L > R> S***	***L> R> S***	***R> L >S***	***S> L> R***	***R> S> L***	***S > R > L***	***L> R> S***	***S> R > L***	***S> L> R***	***S> R> L***	***R > L> S***
A viridis	*Root*	56.02 ± 0.2	4.65 ± 0.5	1.87 ± 0.4	23.38 ± 0.7	6.78 ± 0.5	497.6 ± 0.3	9.56 ± 0.9	5.78 ± 0.7	5.70 ± 0.7	6.21 ± 0.8	5.45 ± 0.5	6.25 ± 0.2
	Shoot	Nil	3.65 ± 0.6	1.54 ± 0.7	35.99 ± 0.6	7.98 ± 0.5	117.5 ± 0.5	16.12 ± 0.7	3.76 ± 0.6	4.87 ± 0.7	7.28 ± 0.7	4.65 ± 0.4	5.34 ± 0.3
	Leaves	9.98 ± 0.5	Nil	2.43 ± 0.5	34.87 ± 0.4	5.93 ± 0.1	215.3 ± 0.2	20.76 ± 0.4	5.54 ± 0.5	3.56 ± 0.8	5.44 ± 0.3	5.74 ± 0.3	6.44 ± 0.6
	Total	66.00 ± 0.7	8.30 ± 1.1	5.84 ± 1.6	94.24 ± 1.7	20.69 ± 1.1	830.4 ± 1.0	46.44 ± 2.0	15.08 ± 1.8	14.8 ± 2.2	18.93 ± 1.8	15.84 ± 1.2	18.03 ± 1.1
Accumulation pattern		***R> L***	***R> S***	***L> R> S***	***L> S> R***	***S> R> L***	***R> L > S***	***L >S > R***	***R> L> S***	***R> S> L***	***R> S> L***	***R> S> L***	***R> L>S***
B. alba L.	*Root*	48.96 ± 0.3	0.98 ± 0.7	1.23 ± 0.7	42.65 ± 0.3	5.67 ± 0.2	375.4 ± 0.3	57.61 ± 0.4	5.08 ± 0.5	47.9 ± 0.4	7.67 ± 0.4	9.99 ± 0.5	7.47 ± 0.7
	Shoot	46.07 ± 0.2	Nil	2.35 ± 0.3	33.54 ± 0.3	4.78 ± 0.2	Nil	23.08 ± 0.3	5.29 ± 0.3	50.01 ± 0.6	0 ± 0.9	4.76 ± 0.4	33.65 ± 0.6
	Leaves	20.29 ± 0.3	Nil	6.12 ± 0.4	25.32 ± 0.7	4.99 ± 0.2	214.4 ± 0.3	15.09 ± 0.3	3.65 ± 0.2	24.25 ± 0.3	0 ± 0.9	5.54 ± 0.3	24.54 ± 0.3
	Total	115.32 ± 0.8	0.98 ± 0.7	9.70 ± 1.4	101.51 ± 1.0	15.44 ± 0.6	589.8 ± 0.6	95.78 ± 1.0	14.02 ± 1.0	122.16 ± 1.3	7.67 ± 2.2	20.29 ± 1.2	65.70 ± 1.6
Accumulation pattern		***R> S> L***	***BLD***	***L>S> R***	***R> S> L***	***R> L>S***	***R>S***	***R >S> L***	***S> R> L***	***S> R> L***	***BLD***	***R> L>S***	***S> L>R***
S.bispinosa	*Root*	17.03 ± 0.5	7.00 ± 0.0	Nil	16.65 ± 0.6	0.57 ± 0.4	477.2 ± 0.6	10.23 ± 0.8	25.46 ± 0.8	8.76 ± 0.7	5..67 0.7	20.35 ± 0.3	4.65 ± 0.1
	Shoot	14.07 ± 0.2	Nil	Nil	45.76 ± 0.5	Nil ± 0.3	156.3 ± 0.4	17.76 ± 0.7	7.76 ± 0.7	15.07 ± 0.5	Nil	Nil	45.76 ± 0.7
	Leaves	9.66 ± 0.1	18.98 ± 0.3	Nil	14.65 ± 0.5	Nil ± 0.3	245.5 ± 0.5	25.45 ± 0.4	3.45 ± 0.5	7.98 ± 0.1	20.98 ± 0.9	Nil	15.54 ± 0.8
	Total	40.76 ± 0.8	25.98 ± 0.3	Nil	77.06 ± 1.6	0.57 ± 1.0	879.0 ± 1.5	53.44 ± 1.9	36.67 ± 2.0	31.81 ± 1.3	26.65 ± 1.6	20.35 ± 0.3	65.95 ± 1.6
Accumulation pattern		***R> S> L***	***L> R***	***BLD***	***S> R> L***	***BLD***	***R> L>S***	***L > S> R***	***R> S> L***	***S> L> R***	***L> R>***	***BLD***	***S>L>R***
P. murex L.	*Root*	20.98 ± 0.4	10.76 ± 0.5	2.43 ± 0.7	18.21 ± 0.2	8.43 ± 0.7	45.25 ± 0.5	37.98 ± 0.4	22.98 ± 0.7	25.65 ± 0.5	20.54 ± 0.9	15.92 ± 0.8	4.34 ± 0.5
	Shoot	18.74 ± 0.5	2.32 ± 0.7	2.54 ± 0.4	22.54 ± 0.5	13.65 ± 0.7	29.33 ± 1.4	18.76 ± 0.5	17.78 ± 0.7	4.50 ± 0.6	15.87 ± 0.5	15.94 ± 0.9	6.74 ± 0.5
	Leaves	22.43 ± 0.5	9.98 ± 0.6	5.66 ± 0.6	6.97 ± 0.3	9.35 ± 0.7	23.78 ± 0.5	20.34 ± 0.8	9.65 ± 0.7	18.56 ± 0.6	14.71 ± 0.7	15.44 ± 0.5	7.76 ± 0.5
	Total	62.15 ± 1.4	23.06 ± 1.8	10.63 ± 1.7	47.72 ± 1.0	31.43 ± 2.1	98.36 ± 2.4	77.08 ± 1.7	50.41 ± 2.1	49.71 ± 1.7	51.12 ± 2.1	47.30 ± 2.2	18.84 ± 1.5
Accumulation pattern		***R> S> L***	***L> R> S***	***L> R> S***	***S> R> L***	***S> L> R***	***R> S> L***	***R > L>S***	***R> S> L***	***R> S> L***	***R> S> L***	***S> L> R***	***L>S> R***
M. dioica	*Root*	20.97 ± 0.5	9.56 ± 0.3	2.23 ± 0.2	14.43 ± 0.4	8.23 ± 0.3	45.67 ± 0.5	8.76 ± 0.4	19.76 ± 0.4	7.99 ± 0.5	15.87 ± 0.5	17.34 ± 0.6	13.54 ± 0.5
	Shoot	14.38 ± 0.5	2.89 ± 0.2	2.54 ± 0.3	23.45 ± 0.6	14.56 ± 0.5	33.98 ± 0.4	19.65 ± 0.5	5.23 ± 0.5	6.99 ± 0 .7	13.98 ± 0.5	15.98 ± 0.4	12.76 ± 0.4
	Leaves	20.09 ± 0.5	8.43 ± 0.3	8.56 ± 0.4	9.98 ± 0.2	9.98 ± 0.4	21.76 ± 0.5	9.07 ± 0.5	9.54 ± 0.3	5.76 ± 0.4	13.76 ± 0.2	13.43 ± 0.4	12.43 ± 0.4
	Total	55.44 ± 1.5	20.88 ± 0.8	13.35 ± 0.9	47.86 ± 1.2	32.77 ± 1.2	101.41 ± 1.4	37.48 ± 1.4	34.53 ± 1.2	20.74 ± 1.6	43.61 ± 1.2	46.75 ± 1.4	38.73 ± 1.3
Accumulation pattern		***R> L> S***	***R> L> S***	***L> R> S***	***S> L> R***	***S> L> R***	***R> S> L***	***S> L> R***	***R> S> L***	***R> S> L***	***R> S> L***	***R> S> L***	***R> L>S***

Table 4
BCF and TF of different Heavy metal accumulation (mg kg^{-1} DW) by various hyperaccumulator plant of a different part in the root, shoot and leaves on distillery sludge bed.

Native hyperaccumulator plants	Bioconcentration factor										
	Mn	Pb	Zn	Cr	Fe	Cu	Ni	As	Se	Mo	Co
Achyranthus aspera L.	81,33	17,45	17,65	18,72	15,65	94,34	1,698	1,534	15,768	15,376	12,375
Amaranthus viridis	13,75	15,75	8,021	19,75	5,675	57,92	6,254	6,124	12,751	7,101	09,995
Basella alba L.	55,4	48,96	8,765	15,06	6,111	46,98	5,121	9,123	17,232	51,121	7,100
Sesbania bispinosa	47,76	38,10	5,212	24,10	4,989	24,98	5,090	2,457	7,369	12,355	11,846
Pedalium murex L.	14,96	10,91	5,986	18,98	3,987	22,10	6,987	5,139	9,246	7,615	10,041
Momordica dioica	21,05	18,04	11,05	23,55	9,098	55,20	8,989	7,410	8,789	8,355	3,356
	Translocation factor										
	Mn	Pb	Zn	Cr	Fe	Cu	Ni	As	Se	Mo	Co
Achyranthus aspera L.	11,55	6,355	8,543	9,871	15,64	37,15	0,009	3,33	3,335	6,324	5,435
Amaranthus viridis	3,908	3,432	8,654	7,324	14,99	42,55	1,111	6,23	4,463	7,369	7,765
Basella alba L.	3,999	9,123	9,987	3,776	14,90	28,39	1,123	3,98	7,714	7,451	13,43
Sesbania bispinosa	5,001	8,798	5,111	7,324	13,17	18,43	2,232	9,23	9,865	8,254	58,65
Pedalium murex L.	6,012	3,432	7,213	8,654	12,44	13,44	1,989	12,45	11,345	10,23	15,65
Momordica dioica	9,21	7,987	9,111	9,266	19,23	20,45	0,009	7,23	14,665	9,654	16,87

3.6. Bioconcentration factor and translocation factor

The ability of heavy metals accumulation for plants can be evaluated through the BCF and TF (Yoon et al., 2006). The BCF of the plants grown in distillery sludge indicated that all the plants had a high ability for phytoextraction of metals in their root. In general, the bioavailability of heavy metals in sludge is very poor but all the tested plants shown very high metal accumulation in their different parts which indicated the hyperaccumulation tendency of these plants (Table 4).

Metal concentration-dependent accumulation in plant parts has been reported by several studies in aquatic and terrestrial soil (Yoon et al., 2006; Bharagava et al., 2008). In this study, the metal accumulation pattern was in order roots> shoots>leaves. This indicated the concentration-dependent mobility of metals in plant tissues (Gupta and Sinha, 2006). *Achyranthus aspera* L. exhibited high potential for the accumulation Cu (76.22 $\pm$ 1.3 mg kg^{-1}), Mn (9.80 $\pm$ 0.6 mg kg^{-1}), Cr (25.9 $\pm$ 1.3 mg kg^{-1}), Pb (7.99 $\pm$ 0.7 mg kg^{-1}), and Zn (67.33 $\pm$ 0.6 mg kg^{-1}) with the highest BCF in five out of eleven metals (Table 3). *Amaranthus viridis* displayed maximum BCF for Cu followed by Cr and Pb. Different heavy metals accumulation potential of *A. aspera* L. had been reported from the soil with different characteristic properties (Vijaya Kumar et al., 2009). These studies showed that *A. aspera* L. has a propensity to the accumulation of Fe, Cu, Mn, Na, in higher amount. To our knowledge, this is the first report on the metal accumulation pattern of *A. aspera* L. from organometallic sludge. *Basella alba* L. also showed a high accumulation of Fe, Cu, and As in root, but higher arsenic concentration was noted in the shoot (50.01 mg kg^{-1}). The higher accumulation tendency for Na, Cu, and Mn of *B. alba* L. has been reported in other studies (Shammi et al., 2016). However, the metal accumulation pattern from industrial sludge and its impact on medicinal quality is still not clear. The higher accumulation of Mn (40.76 $\pm$ 0.8 mg kg^{-1}), Zn (77.06 $\pm$ 1.6 mg kg^{-1}), Fe (879.0 $\pm$ 1.5 mg kg^{-1}), As (31.81 $\pm$ 1.3 mg kg^{-1}) and Mo (20.35 $\pm$ 0.3 mg kg^{-1}) in root and shoot of *S. bispinosa*. *Pedalium murex* L. showed high accumulation of all the tested metals in their root, shoot, and leaves from sludge this indicated most potential plant for phytoextraction of metal from the organometallic complex shown in Table 3. This might be due to root nodule forming bacteria of the plant which may be favorable for metal bioavailability in sludge to plant.

High BCF and metal accumulation have been reported in the previous study from the metal-contaminated site (Saraf and Samant, 2013). *Basella alba* L. showed a high BCF for Mn & Mo followed by Pb and Cu which indicated the potentiality of the plant for metal accumulation from any contaminated site (Table 4) (Shammi et al., 2016). The deep and strong growth of roots also supported the potentiality of the plant. A similar pattern was observed in *S. bispinosa*, *P. murex* L., and *M. doica*. The maximum translocation factor of Cu and Co was found in *Amaranthus* and *Sesbania*, respectively. The minimum TF was noted in *Achyranthus* and *Momordica* for a nickel. The TF >1 indicated the high physiological and transpiration rate of the plant. Concomitantly, these plants have shown their adaptability to accumulate a higher concentration of metals from distillery sludge. The accumulation of metals by plants from sludge to root depends on the chemical nature of the element, pH, and other co-pollutants of sludge that inhibits the mobility of metals in plants (Gupta and Sinha, 2008).

The high translocation and bioconcentration of metals in different parts of the medicinal plants is a health hazard as metals accumulate in shoots and leaves that are traditionally harvested for medicinal purposes. When textile effluent was reused as irrigation water, *B. alba* L. was found to pose a health risk due to the accumulation of high levels of heavy metals (Shammi et al., 2016).

3.7. Observation of metal accumulation in root tissue of selected plants

The root tissue analysis of collected indigenous hyperaccumulator plants by transmission electron microscope shown metal accumulation in vesicle near the cell wall and cytoplasm (Fig. 2A-F). The metal granules were deposited near the

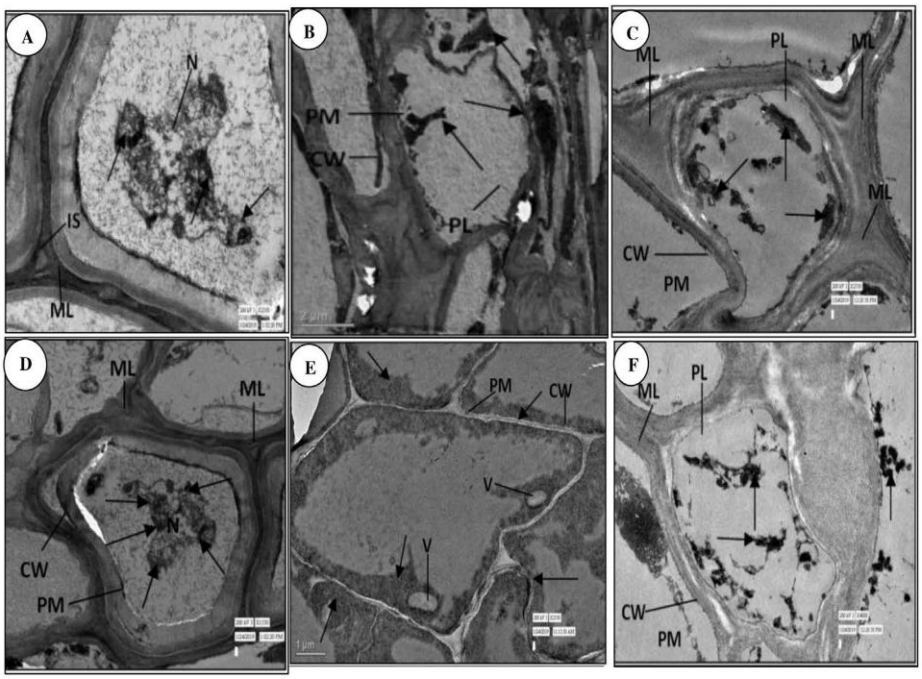


Fig. 2. Electron micrographs of transverse section of plants root after phytoextraction of heavy metals (A–F), V: Vacuole; PM: Plasma membrane; P: Peroxisomes; CW: Cell wall; CM: Cell membrane; ML: Middle lamella; N: Nucleus; Arrow (→) indicated metals deposition; IS: Intercellular space. PL:- Plasmalema.

nucleus in *A. aspera* L. and *S. bispinosa*; while big granules were observed in the cytoplasm and near the cell walls in *A. viridis, B. alba* L. *and M. doica* (Fig. 2). The continuous deposition of fine metal granules at the cell wall regions was noted in *P. murex* L. This study concluded that the mechanism of plant detoxification of heavy metals was based on vacuole sequestration and cell wall deposition (Fig. 2). *Basella alba* L. and *S. bispinosa* root tissues showed the presence of multi-nucleolus, multi-vacuoles, thickened in the center, and round the lamellae with metal accumulation on the cell wall, cytoplasm, and middle lamella. The development and deposition of metal granules in cell walls indicated a plant resistance mechanism for metal aggregation and detoxification with a larger volume of cellular tissues (Tong et al., 2004).

The phytoextraction potential of some native plants growing on distillery sludge reported in a previous study also showed a close resemblance to the multi-vacuoles development and deposition of granules, as an adaptive mechanism of the plants in the presence of organic pollutants and metal accumulation. But the pattern of a multi-metal accumulation from industrial waste by medicinal plants has not been reported. The ultra-structural observation of root tissue of *P. murex* L., *M. doica* at low and high magnification showed deposition of metal granules inside the cell walls, cell membranes, cytoplasms, and nucleoplasm (Najeeb et al., 2011). The whole plant root also showed cell wall thickening. Increased development of nucleolus and vacuoles at high heavy metal concentration increased the output of the ribosome and mRNA, which finally increased the development of fresh proteins engaged in the tolerance of heavy metals in plants (Najeeb et al., 2011). In our research, all species of plants were noted with extravagant growth on disposed distillery sludge without any biochemical deformities in their aerial parts. These plants showed good adaptability to survive and develop in organo-metallic and EDCs containing distillery sludge and are suitable for in situ phytoremediation. They are good candidates for the monitoring of heavy metals in intricate and dangerous industrial wastes and eco-restoration polluted areas.

4. Conclusion

The analysis of sugarcane molasses-based distillery waste revealed the presence of various organic compounds as residual pollutants; many of them compounds were known as mutagenic and carcinogenic compounds. The presence of various metals could increase the vulnerability of the flora due to the high binding tendency of metals with organic polymers. But the luxuriant growth of the native plants indicated potentiality for metal accumulation and phytoremediation. The BCF and TF analyses of these plants showed hyperaccumulation properties. Various heavy metals i.e. Pb, Cd, Ni, As, Cr, and Mo, were accumulated in leaves and shoots of *A. aspera* L., *B. alba* L. and *M. doica*, which are well known diurnal plants.

Further, the transmission electron microscopy (TEM) analysis of root tissues revealed the deposition of metal granules in the root cytoplasm and cell wall of all the tested plants, which further confirmed their hyperaccumulation properties. Despite their potential in phytoremediation of organometallic pollutants, arbitrary application and subsequent harvesting of these plants are not recommended due to their functions as medicine or food crops. However, these plants can be used for the eco-restoration of sites polluted by organometallic industrial waste.

CRediT authorship contribution statement

Sonam Tripathi: Experiment designed, Writing - original draft, Sample collection, Analysis of data. **Pooja Sharma:** Experiment designed, Writing - original draft, Sample collection, Analysis of data. **Kshitij Singh:** Sample collection, Update, Review manuscript. **Diane Purchase:** Conceptualization, Writing - review & editing. **Ram Chandra:** Conceptualization, Experiment design, Visualization, Project administration, Funding acquisition, Reviewing of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research is financially supported by the Department of Biotechnology (DBT), Govt of India. Vide Letter No. BT/PR13922/BCE/8/1129/2015. Instrumentation facilities for SEM, FTIR, and EDX analysis from USIC, BBAU Lucknow, Uttar Pradesh, India are gratefully acknowledged. While the HPLC and GC–MS analysis from CSIR-Indian institute of toxicology research (IITR), Lucknow are highly acknowledged. TEM analysis have been conducted at SAIF facilities, AIIMS, New Delhi. The authors gratefully acknowledge all the anonymous reviewers and Editor-In-Chief for their valuable comments on the earlier version of this paper. All authors have read and agreed to the final version of the manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.eti.2021.101434.

References

AIDA, 2004. All India Distillers Association and Sugar Technology Association of India. Annual Report, New Delhi.
American Public Health Association (APHA), 2012. Standard methods for the examination of water and wastewater, method 9221 B.
Annan, K., Dickson, R.A., Amponsah, I.K., Nooni, I.K., 2013. The heavy metal contents of some selected medicinal plants were sampled from different geographical locations. Pharmacogn. Res. 5 (2), 103.
AOAC, 2002. Official methods of analysis of AOAC international. In: AOAC International, Gaithersburg.
Bharagava, R.N., Chandra, R., Rai, V., 2008. Phytoextraction of trace elements and physiological changes in Indian mustard plants (Brassica nigra l.) grown in post methanated distillery effluent (PMDE) irrigated soil. Bioresour. Technol. 99 (17), 8316–8324.
Blöcher, C., Dorda, J., Mavrov, V., Chmiel, H., Lazaridis, N.K., Matis, K.A., 2003. Hybrid flotation-membrane filtration process for the removal of heavy metal ions from wastewater. Water Res. 37 (16), 4018–4026.
Bose, S., Bhattacharyya, A.K., 2008. Heavy metal accumulation in wheat plants grown in soil amended with industrial sludge. Chemosphere 70 (7), 1264–1272.
Bratskaya, S.Y., Pestov, A.V., Yatluk, Y.G., Avramenko, V.A., 2009. Heavy metals removal by flocculation/precipitation using N-(2-carboxyethyl) chitosans. Colloids Surf. A 339 (1–3), 140–144.
Chandra, R., Bharagava, R.N., Rai, V., 2008. Melanoidins as a major colorant in sugarcane molasses-based distillery effluent and its degradation. Bioresour. Technol. 99 (11), 4648–4660.
Chandra, R., Bharagava, R.N., Yadav, S., Mohan, D., 2009. Accumulation and distribution of toxic metals in wheat (Triticum aestivum L.) and Indian mustard (Brassica campestris L.) irrigated with distillery and tannery effluents. J. Hard Mater. 162 (2–3), 1514–1521.
Chandra, R., Kumar, V., Tripathi, S., Sharma, P., 2018. Heavy metal phytoextraction potential of native weeds and grasses from endocrine-disrupting chemicals rich complex distillery sludge and their histological observations during in-situ phytoremediation. Ecol. Eng. 111, 143–156.
Chandra, R., Yadav, S., Yadav, S., 2017. Phytoextraction potential of heavy metals by native wetland plants growing on chlorolignin containing sludge of pulp and paper industry. Ecol. Eng. 98, 134–145.
Dialynas, E., Diamadopoulos, E., 2009. Integration of a membrane bioreactor coupled with reverse osmosis for advanced treatment of municipal wastewater. Desalination 238 (1–3), 302–311.
Duthie, J.F., Parker, R.N., Turrill, W.B., 1903. Flora of the upper Gangetic plain, and the adjacent Siwalik and sub-Himalayan tracts (Vol. 2). In: Office of the Superintendent of Government Print.
Franchi, E., Rolli, E., Marasco, R., Agazzi, G., Borin, S., Cosmina, P., Petruzzelli, G., 2017. Phytoremediation of a multi contaminated soil: mercury and arsenic phytoextraction assisted by mobilizing agent and plant growth-promoting bacteria. J. Soil. Sediment. 17 (5), 1224–1236.
Ghassemzadeh, F., Yousefzadeh, H., Arbab-Zavar, M.H., 2008. Arsenic phytoremediation by Phragmites australis: green technology. Int. J. Environ. Studies 65 (4), 587–594.
Gupta, A.K., Sinha, S., 2006. Chemical fractionation and heavy metal accumulation in the plant of Sesamum indicum (L.) var. T55 was grown on soil amended with tannery sludge: Selection of single extractants. Chemosphere 64 (1), 161–173.
Gupta, A.K., Sinha, S., 2007. Phytoextraction capacity of the plants growing on tannery sludge dumping sites. Bioresour. Technol. 98 (9), 1788–1794.
Gupta, A.K., Sinha, S., 2008. Decontamination and/or revegetation of fly ash dykes through naturally growing plants. J. Hazard. Mater. 153 (3), 1078–1087.

Jaison, S., Muthukumar, T., 2017. Chromium accumulation in medicinal plants growing naturally on tannery contaminated and non-contaminated soils. Biol. Trace Elem. Res. 175 (1), 223–235.
Kansal, A., Rajeshwari, K.V., Balakrishnan, M., Lata, K., Kishore, V.V.N., 1998. Anaerobic digestion technologies for energy recovery from an industrial wastewater-a study in the Indian context. TERI Inf. Monit. Environ. Sci. 3 (2), 67–75.
Kumar, N., Bauddh, K., Kumar, S., Dwivedi, N., Singh, D.P., Barman, S.C., 2013. Accumulation of metals in weed species grown on the soil contaminated with industrial waste and their phytoremediation potential. Ecol. Eng. 61, 491–495.
Kumar, A., Dhall, P., Kumar, R., 2010. Redefining BOD: COD ratio of pulp mill industrial wastewaters in BOD analysis by formulating a specific microbial seed. Int. Biodeterioration Biodegrad. 64 (3), 197–202.
Laghlimi, M., Baghdad, B., El Hadi, H., Moussadak, R., Taoufik, M., 2015. Assessment of heavy metals soil contamination in the abandoned pb-mine of zaida (Morocco). Eur. J. Sci. Res. 129 (2), 167–178.
Liu, X., Zhu, H., Qin, C., Zhou, J., Zhao, J.R., Wang, S., 2013. Adsorption of heavy metal ion from aqueous single metal solution by aminated epoxy-lignin. BioResources 8 (2), 2257–2269.
Ma, Y., Prasad, M.N.V., Rajkumar, M., Freitas, H., 2011. Plant growth-promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotech. Adv. 29 (2), 248–258.
Mazumdar, K., Das, S., 2015. Phytoremediation of Pb, Zn, Fe, and Mg with 25 wetland plant species from a paper mill contaminated site in North East India. Environ. Sci. Pollut. Res. 22 (1), 701–710.
Migo, V.P., Del Rosario, E.J., Matsumura, M., 1997. Flocculation of melanoidins induced by inorganic ions. J. Ferment. Bioengineer. 83 (3), 287–291.
Najeeb, U., Jilani, G., Ali, S., Sarwar, M., Xu, L., Zhou, W., 2011. Insights into cadmium induced physiological and ultra-structural disorders in Juncus effusus L. and its remediation through exogenous citric acid. J. Hard Mater. 186 (1), 565–574.
Noor, E.A., Al-Moubaraki, A.H., 2008. Thermodynamic study of metal corrosion and inhibitor adsorption processes in mild steel/1-methyl-4 [4′(-x)-styryl pyridinium iodides/hydrochloric acid systems. Mater. Chem. Phys. 110 (1), 145–154.
Ott, L., 1984. An Introduction to Statistical Methods and Data Analysis, second ed. PWS, Boston, MA.
Rajkumar, M., Sandhya, S., Prasad, M.N.V., Freitas, H., 2012. Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotech. Adv. 30 (6), 1562–1574.
Sahi, S.V., Bryant, N.L., Sharma, N.C., Singh, S.R., 2002. Characterization of a lead hyperaccumulator shrub, Sesbania drummondii. Environ. Sci. Technol. 36 (21), 4676–4680.
Saraf, A., Samant, A., 2013. Evaluation of some minerals and trace elements in Achyranthes aspera Linn. Int. J. Pharma Sci. 3 (3), 229–233.
Sessitsch, A., Kuffner, M., Kidd, P., Vangronsveld, J., Wenzel, W.W., Fallmann, K., Puschenreiter, M., 2013. The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. Soil Biol. Biochem. 60, 182–194.
Shammi, M., Kashem, M.A., Rahman, M.M., Hossain, M.D., Rahman, R., Uddin, M.K., 2016. Health risk assessment of textile effluent reuses as irrigation water in leafy vegetable Basella alba. Int. J. Recycl. Org. Waste Agric. 5 (2), 113–123.
Sharma, P., Pandey, A.K., Udayan, A., Kumar, S., 2021a. Role of microbial community and metal-binding proteins in phytoremediation of heavy metals from industrial wastewater. Bioresour. Technol. 124750.
Sharma, P., Purchase, D., Chandra, R., 2021b. Residual pollutants in treated pulp paper mill wastewater and their phytotoxicity and cytotoxicity in Allium cepa. Environ. Geochem. Health 1–22.
Sharma, P., Rath, S.K., 2020. Potential applications of fungi in the remediation of toxic effluents from pulp and paper industries. In: Fungi Bio-Prospects in Sustainable Agriculture Environment and Nanotechnology. Academic Press, pp. 193–211.
Sharma, P., Singh, S.P., Pandey, S., Thanki, A., Singh, N.K., 2020a. Role of potential native weeds and grasses for phytoremediation of endocrine-disrupting pollutants discharged from pulp paper industry waste. Bioremediation Pollut. 17–37.
Sharma, P., Tripathi, S., Chandra, R., 2020b. Metagenomic analysis for profiling of microbial communities and tolerance in metal-polluted pulp and paper industry wastewater. Bioresour. Technol. 124681.
Sharma, P., Tripathi, S., Vadakedath, N., Chandra, R., 2020c. In-situ toxicity assessment of pulp and paper industry wastewater on Trigonella foenum-graecum L: Potential source of cytotoxicity and chromosomal damage. Environ. Technol. Innov. 101251.
Singh, A., Prasad, S.M., 2014. Effect of an agro-industrial waste amendment on cd uptake in Amaranthus caudatus grown under contaminated soil: an oxidative biomarker response. Ecotoxicol. Environ. Safety 100, 105–113.
Singh, H., Verma, A., Kumar, M., Sharma, R., Gupta, R., Kaur, M., Sharma, S.K., 2017. Phytoremediation: a green technology to clean up the sites with low and moderate levels of heavy metals. Austin. Biochem. 2 (2), 1012.
Singh, S., Zacharias, M., Kalpana, S., Mishra, S., 2012. Heavy metals accumulation and distribution patterns in different vegetable crops. J. Enviro. Chem. Ecotoxicol. 4 (10), 170–177.
Sinha, S., Gupta, A.K., Bhatt, K., 2007. Uptake and translocation of metals in fenugreek grown on soil amended with tannery sludge; involvement of antioxidants. Ecotoxicol. Environ. Safety 67 (2), 267–277.
Sushil, S., Batra, V.S., 2006. Analysis of fly ash heavy metal content and disposal in three thermal power plants in India. Fuel 85 (17–18), 2676–2679.
Tong, Y.P., Kneer, R., Zhu, Y.G., 2004. Vacuolar compartmentalization: a second-generation approach to engineering plants for phytoremediation. Trends Plant Sci. 9 (1), 7–9.
USEPA, 2012. Endocrine Disruptor Screening Program. Universe of Chemicals.
Vijaya Kumar, S., Sankar, P., Varatharajan, R., 2009. Anti-inflammatory activity of roots of Achyranthes aspera. Pharm. Biol. 47 (10), 973–975.
WHO, World Health Organization, 1978. International Classification of Procedures in Medicine (Vol. 2). World Health Organization.
Yadav, S., Chandra, R., 2018. Detection and assessment of the phytotoxicity of residual organic pollutants in sediment contaminated with pulp and paper mill effluent. Environ. Monit. Assess. 190 (10), 581.
Yoon, J., Cao, X., Zhou, Q., Ma, L.Q., 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci. Total Environ. 368 (2–3), 456–464.

Bioresource Technology Reports 15 (2021) 100779

Contents lists available at ScienceDirect

Bioresource Technology Reports

journal homepage: www.sciencedirect.com/journal/bioresource-technology-reports

Maillard reaction product and its complexation with environmental pollutants: A comprehensive review of their synthesis and impact

Kshitij Singh, Sonam Tripathi, Ram Chandra *

Department of Environmental Microbiology, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow-226025, Uttar Pradesh 226025, India

ARTICLE INFO

Keywords:
Melanoidin
Maillard reaction
Sulfur compound
Heavy metals
Organic compound

ABSTRACT

Melanoidins are the randomly polymerized condensed anionic, non-enzymatic product of maillard reaction between amino and carbonyl group at elevated temperature. It occurs particularly during the food processing and wastewater released from distilleries and various fermented industries. Their synthesis is influenced by several factors because they alter the reaction path and change the product composition. Moreover, melanoidins have been known to be beneficial at low concentrations in food products due to their several properties in a biological system. But, when they are released into the environment at a high concentration from fermentation industries they cause a serious threat to land and aquatic ecosystem. This review encompasses the detailed chemical reaction of melanoidin synthesis and its functional properties to various substances present in nature with implications to its impact on the environment and their role in maintaining the nutritional and flavoring properties in food products.

1. Introduction

Melanoidins are nitrogenous, heterocyclic brown pigments polymer generated during the chemical reaction between amino and carbonyl compounds at elevated temperatures (James, 2019; Wang et al., 2011). These compounds are synthesized by the reaction occurring between the amino groups of amino acids, peptides, or proteins and the carbonyl groups of reducing sugars. A previous study also has revealed in a chemical reaction that carbohydrates and amino acids are the basic skeleton of the melanoidin compound (Cämmerer et al., 2002; Sharma et al., 2021a, 2021b). The formation of melanoidin involves three significant steps. The initial stage includes the condensation of sugar-amine. In contrast, the second stage involves dehydration and fragmentation of sugar along with degradation of the amino acid via the Strecker degradation mechanism, preferably at elevated temperatures. At the final stage, highly brown color heterocyclic nitrogenous compounds are produced through aldehyde-amine condensation with the by-products formed in the second stage of maillard reaction. The compounds formed in this stage are of high molecular weight and are known as melanoidins (Liu et al., 2020). Most studies have been done on melanoidin food products like bakery products, cooked meat, roasted coffee, honey, sweet wine, etc. as an antioxidant at low concentration (Shaheen et al., 2021). Their formation occurs during the thermal processing of food material in cooking, baking, roasting, and storage, due to reducing sugar and free amino groups (Lund and Ray, 2017). However, melanoidin at high concentration in the environment has been reported as pollutant by several researchers when it is discharged from industrial activities and makes complexation with other environmental pollutants along with heavy metals due to its anionic properties (Chandra et al., 2017; Tripathi et al., 2021a, 2021b, 2021c, 2021d, 2021e). Various researchers have reported melanoidin pollution when it enters into the environment as a waste product. The major source of melanoidin in the environment is the sugarcane molasses-based distilleries waste water, where melanoidin makes complexation along with various mutagenic and carcinogenic compounds (Chowdhary et al., 2018a, 2018b; Tripathi et al., 2021a). The effect of melanoidin has been reported for severe aquatic and soil pollution in the environment. Due to severe threats to the environment, various attempts have been made globally by the researchers using the chemical and biological method, for degradation and decolorization of molasses-based melanoidin of distillery wastewater for environmental protection and its safe disposal (Tripathi et al., 2021a, 2021b, 2021c, 2021d, 2021e; Sharma et al., 2021a, 2021b). Therefore, in the chemical analysis of distillery waste, there is a mixture of compounds with a variable absorption peak has been noted in the absorption

* Corresponding author.
E-mail addresses: rc_microitrc@yahoo.co.in, prof.chandrabbau@gmail.com (R. Chandra).

https://doi.org/10.1016/j.biteb.2021.100779
Received 28 May 2021; Received in revised form 15 July 2021; Accepted 15 July 2021
Available online 21 July 2021

International Journal of Environmental Science and Technology (2022) 19:2025–2038
https://doi.org/10.1007/s13762-021-03233-1

ORIGINAL PAPER

Organo-metallic pollutants of distillery effluent and their toxicity on freshwater fish and germinating *Zea mays* seeds

S. Tripathi[1] · K. Singh[1] · A. Singh[2] · A. Mishra[2] · Ram Chandra[1]

Received: 13 July 2020 / Revised: 28 January 2021 / Accepted: 16 February 2021 / Published online: 13 March 2021

Abstract
Anaerobically treated molasses-based distilleries spent wash is a major source of terrestrial and aquatic pollution due to the mixture of the various unknown organometallic compounds in discharged waste. The nature of pollutants present in effluent has to be characterized prior to the evaluation of their fate in the environment at various tropic levels. The absorption maxima (λ_{max}) were obtained at 295 nm of pollutants present in spent wash which indicated that melanoidins are a major colorant along with other organic compounds. Abundantly identified compounds with GC–MS in effluent were found: 1,2,4,5-tetrahydro-2-methyl-3H-2-benzazepine-3-one, 6-(trifluoromethyl)-1H-imidazole [4,5-c] pyridine, 4,6-di-t-butyl-1H, 3H-thieno[3,4-c] thiophene, butanoic acid, trimethylsilyl ester, silane, (dodecyloxy)trimethylsilyl ester, 4-styryl [2,2] paracyclophane, 4-(p-cumylphenoxy) phthalonitrile, hexadecanoic acid, trimethylsilyl ester, butyl ester, and squalene. Some of these compounds are known with mutagenic and androgenic properties. The toxicity test of post-methanated distillery effluent (PMDE) showed direct toxic effects on catfish (*Heteropneustes fossilis*) even at 1% (v/v) concentration and caused degeneration of primary and secondary lamellae of the gill and the epithelial layer. Further, higher concentrations between 5 and 10% of PMDE showed loss of primary and secondary lamellae of gills, and liver catalase activity was also increased dramatically in *H. fossilis*, indicating the generation of oxidative free radicals. Similarly, inhibition of α-amylase activities in germinating *Zea mays L.* (Maize) seeds was also noted at 10% PMDE. This study confirmed the environmental toxicity of effluent with freshwater fish and *Zea mays* seeds.

Keywords Distillery waste · Endocrine-disrupting chemicals · Fish toxicity · *Heteropneustes fossilis* · Organic compounds · Seed germination

Introduction

Discharged sugarcane molasses-based distillery effluent, also known as post-methanated distillery effluent (PMDE), is a dark-brown and highly complex chemically recalcitrant organometallic compound as a source of environmental pollutants (Yadav and Chandra, 2019). The PMDE has pH of 8.5, total suspended solids (21,000–40,700 mg L^{-1}), total dissolved solids (29,810 mg L^{-1}), biological oxygen demand (8000–12,000 mg $L^{-1)}$, chemical oxygen demand (35,000–52,000 mg L^{-1}), nitrogenous compounds (15,284–28,696 mg L^{-1}), and total sulfate (3875–4096 mg L^{-1}). In addition, there are phosphate (1625 mg L^{-1}), potassium at 537 mg L^{-1}, and chloride (7842–7997 mg L^{-1}) as salts and phenolic compounds (6893–7202 mg L^{-1}) (Bhargava and Chandra, 2010a, b). The dark color of PMDE is reported due to the thermal processing of various water-soluble compounds in sugarcane juice, such as cane pigments, phenolics, and different aminocarbonyl compounds, which are likely to form a complex polymer known as melanoidin at elevated temperature (Yadav and Chandra 2019). Melanoidin is a known nonenzymatic product, separated with sugarcane molasses as a by-product during the clarification of sugarcane juice (Chowdhary et al. 2018). In various studies, the BOD/COD ratio has been described as

Editorial responsibility: S. Mirkia.

✉ Ram Chandra
prof.chandrabbau@gmail.com

[1] Department of Environmental Microbiology, School of Environmental Sciences, Babasaheb Bhimrao Ambedkar University (A Central University) Vidya Vihar, Raebareli Road, Lucknow, Uttar Pradesh 226025, India

[2] Department of Zoology, School of Life Sciences, Babasaheb Bhimrao Ambedkar University (A Central University) Vidya Vihar, Raebareli Road, Lucknow, Uttar Pradesh 226025, India

Environmental Technology & Innovation 22 (2021) 101434

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Translocation of heavy metals in medicinally important herbal plants growing on complex organometallic sludge of sugarcane molasses-based distillery waste

Sonam Tripathi [a], Pooja Sharma [a], Kshitij Singh [a], Diane Purchase [b], Ram Chandra [a,*]

[a] Department of Environmental Microbiology, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar Central University, Vidya Vihar, Raebareli Road, Lucknow, Uttar Pradesh 226025, India
[b] Department of Natural Sciences, School of Science and Technology, Middlesex University, The Burrough, London NW4 4BT, UK

ARTICLE INFO

Article history:
Received 6 January 2021
Received in revised form 10 February 2021
Accepted 11 February 2021
Available online 16 February 2021

Keywords:
Medicinal plants
Heavy metals
Androgenic waste
Phytoremediation
Detoxification

ABSTRACT

This study aimed to assess the heavy metals accumulation patterns by some native plants such as *Achyranthus aspera* L., *Amaranthus viridis*, *Basella alba* L., *Sesbania bispinosa*, *Pedalium murex* L., and *Momordica doica*, which have been traditionally employed for medicinal and food purposes. The plants were grown on complex distillery waste containing a mixture of organometallic compounds. Results revealed bioaccumulation of Mn, Cd, Fe, Cr, Cu, As, Se, Mo, and Co in their roots, shoots, and leaves in levels higher than the surrounding sludge. *A. aspera* was noted as root accumulator for Mn (16.95 mg kg^{-1}), Zn (30.12 mg kg^{-1}), Fe (240.40 mg kg^{-1}), Co (3.19 mg kg^{-1}), while Se (4.07 mg kg^{-1}), Mo (4.36 mg kg^{-1}), was accumulated selectively in the shoot of the plant. Similarly, *S. bispinosa*, *P. murex*, and *M. doica* were found as root accumulators for Mn, Fe, and Ni. *A. viridis* accumulated Cd, Zn, and Cu in the shoot and leaves of the plant. The high bioconcentration factors (BCF) and translocation factors (TF) observed in these native plants (>1) suggested their tendency to hyperaccumulate heavy metals. The findings highlighted that these plants as a potential metal accumulator may pose health hazards and deteriorate the medicinal property if grown on such wastes.

1. Introduction

Heavy metal pollution in soil, water, and food material is a major threat to human health. Industrial wastes and geogenic activities are the major sources of heavy metals in the environment (Annan et al., 2013; Shammi et al., 2016; Sharma et al., 2021b). In India, several industries like distilleries, tanneries, pulp paper industries, electroplating industries, steel, and iron industries discharge a mixture of heavy metals along with various complex organic wastes into the environment. Due to the complexity in the matrix and non-degradability of the heavy metals, such discharge poses significant challenges for its remediation (Gupta and Sinha, 2007; Chandra et al., 2017; Kumar et al., 2013; Sushil and Batra, 2006). Heavy metals tend to accumulate in the soil, water, and plants in the environment, which could contaminate the food chain subsequently (Chandra et al., 2009; Sharma et al., 2020b; Sharma and Rath, 2020; Singh et al., 2012). For example, Indian mustard grown in distillery effluent irrigated soil showed significant accumulation of heavy metals (Cd, Cu, Fe, Mn, Ni, and Zn) in their

* Corresponding author.
E-mail address: prof.chandrabbau@gmail.com (R. Chandra).

https://doi.org/10.1016/j.eti.2021.101434
2352-1864/

12

Endophytic Bacterial Diversity in Roots of Wetland Plants and Their Potential for Enhancing Phytoremediation of Environmental Pollutants

Ram Chandra and Kshitij Singh

CONTENTS

2

Hyperaccumulator versus Nonhyperaccumulator Plants for Environmental Waste Management

Ram Chandra, Vineet Kumar, and Kshitij Singh

CONTENTS

2.1 Introduction

Environmental problems posed by industrial and municipal wastes are well documented. Worldwide, every day, enormous quantities of waste are generated; at present, these wastes are in need of attention. Environmental waste management promotes the proper management and utilization of industrial waste, delivering in-depth, state-of-the-art information on the physico-chemical properties, chemical composition, and environmental risks

Australian Government
IP Australia

CERTIFICATE OF GRANT
INNOVATION PATENT

Patent number: 2021101010

The Commissioner of Patents has granted the above patent on 21 April 2021, and certifies that the below particulars have been registered in the Register of Patents.

Name and address of patentee(s):

RAM CHANDRA of Professor and PI Department of, Environmental Microbiology, B.B. Ambedkar university (A Central University) , Lucknow 226025 India

SONAM TRIPATHI of Research Scholar Department of, Environmental Microbiology, B.B. Ambedkar university (A Central University) Lucknow India

KSHITIJ SINGH of Research Scholar Department of, Environmental Microbiology, B.B. Ambedkar university (A Central University) Lucknow India

SANGEETA YADAV of Post Doctoral Fellow, Department of, Environmental Microbiology, B.B. Ambedkar university (A Central University) Lucknow India

Title of invention:

Decolorization and Detoxification of Distillery Wastewater with Combined Use of Bacteria and Constructed Wetland Treatment

Name of inventor(s):

CHANDRA, RAM; TRIPATHI, SONAM; SINGH, KSHITIJ and YADAV, SANGEETA

Term of Patent:

Eight years from 24 February 2021

NOTE: This Innovation Patent cannot be enforced unless and until it has been examined by the Commissioner of Patents and a Certificate of Examination has been issued. See sections 120(1A) and 129A of the Patents Act 1990, set out on the reverse of this document.

COMMONWEALTH OF AUSTRALIA
AUSTRALIA
PATENT OFFICE

Dated this 21st day of April 2021

Commissioner of Patents

PATENTS ACT 1990

The Australian Patents Register is the official record and should be referred to for the full details pertaining to this IP Right.

International Conference on Biotechnology for
Resource Efficiency, Energy, Environment, Chemicals and Health

CERTIFICATE

This is to certify that

Kshitij Singh

Department of Environmental Microbiology, School for Environmental

has

PARTICIPATED AND PRESENTATED PAPER/POSTER

entitled **Bacterial assisted phytoremediation of heavy metals and organic pollutants by Cannabis sativa as hyperaccumulator plants growing on distillery waste disposal site for ecorestoration of polluted site** authored by **Kshitij Singh** in the International Conference on Biotechnology for Resource Efficiency, Energy, Environment, Chemicals and Health, jointly organized by CSIR-Indian Institute of Petroleum, Dehradun and The Biotech Research Society, India at Dehradun, India during December 01-04, 2021.

Prof. Sudhir K Sopory	Prof. Thallada Bhaskar	Dr. Debashish Ghosh	Prof. Ashok Pandey
President, BRSI	Chairman, Org Com, BRE3CH2021	Convenor, BRE3CH2021	General Chair, BRE3CH2021

This event has been proposed on the occasion of the
WORLD EARTH DAY-22 April 2k19 By
Department of Chemistry, School of Physical & Decision Sciences (SPDS)
Babasaheb Bhimrao Ambedkar University (A Central University)
Vidya Vihar, Raebareli Road, Lucknow - 226025, India
In Association With **The Asian Association of Sugarcane Technologists' (AASCT)**

GCCHGSPCT-2k19
GLOBAL CONFERENCE ON THE CONTROL OF GREENHOUSE GASES AT THE SOURCE BY PHYSICAL AND CHEMICAL TECHNOLOGY

Global Conference On The Control Of Green House Gases At The Source By Physical And Chemical Technology

22 - 24, April - 2019

Certificate of Participation/Presentation

This is to certify that Prof./ Dr./ Mr./ Ms. Kshitij Singh
from Deptt. of Environmental Microbiology, CSEES, BBAU, Lucknow
has participated and contributed in the conference as

Chairman of Session/Plenary Speaker/Distinguished Speaker/Invited Speaker/
Oral/ Poster Presentor/Volunteer

(Dr. Anjani K. Tiwari)
Organizing Secretary

(Prof. Kaman Singh)
Chairman/Convener

Organized by

AEDS
Agro Environmental Development Society,
Majhra Ghat, Rampur, U.P. India

Co-Organized by

Dr. Yashwant Singh Parmar University
of Horticulture and Forestry,
Nauni, Solan, H.P., India.

2nd International Conference
on
Recent Advances in Agricultural, Environmental & Applied Sciences for Global Development
(RAAEASGD-2019)

27-29, September, 2019

In Association With

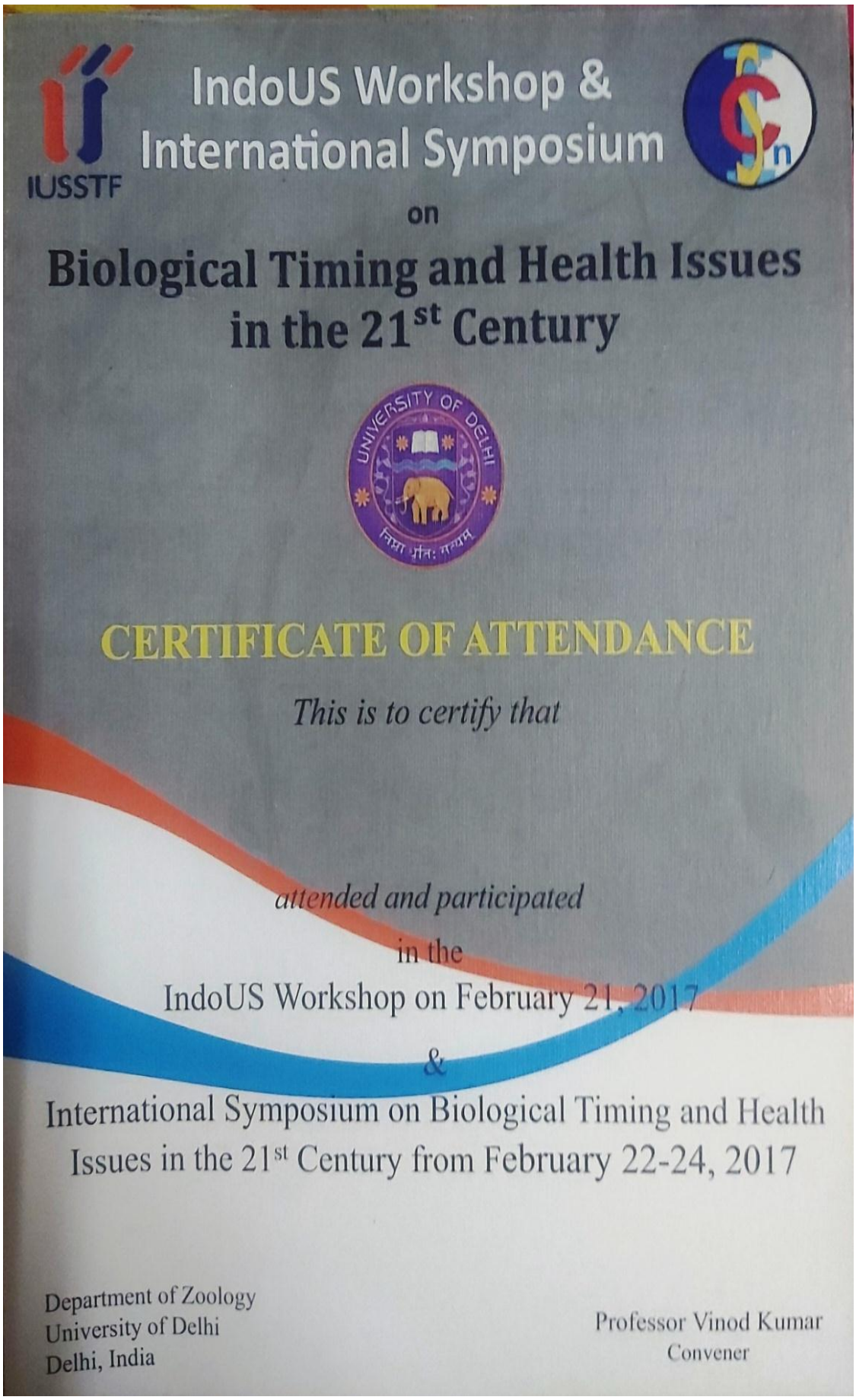
Plant Pathology Research Institute,
ARC, Cairo University, Giza, Egypt

Tribhuvan University, Institute of
Forestry, Pokhra Campus, Nepal

Certificate of Award

This is to certify that Prof./Dr./Mr./Ms. Kshitij Singh **has been participated / presented a paper from** Dept. of Environmental Microbiology **entitled** Correlation between bacterial community ... contaminated ... **authored by** Kshitij Singh and Ram Chandra **in the oral/poster session under the theme** ..... **during the 2nd International conference on "Recent Advances in Agricultural, Environmental and Applied Sciences for Global Development" (RAAEASGD-2019) held during September 27-29, 2019 at Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, H. P., India. The presentation has been adjudged** 3rd.

Co-Chairman **Organising Secretary** **Convener**



IUSSTF

IndoUS Workshop & International Symposium

on

Biological Timing and Health Issues in the 21st Century

UNIVERSITY OF DELHI

CERTIFICATE OF ATTENDANCE

This is to certify that

attended and participated

in the

IndoUS Workshop on February 21, 2017

&

International Symposium on Biological Timing and Health Issues in the 21st Century from February 22-24, 2017

Department of Zoology
University of Delhi
Delhi, India

Professor Vinod Kumar
Convener

Ouriginal by Turnitin

Document Information

Analyzed document	Final Thesis_kshitij03101991@gmail.com.pdf (D144580752)
Submitted	2022-09-22 10:49:00
Submitted by	O. P. Saini
Submitter email	gbl.bbau@gmail.com
Similarity	2%
Analysis address	gbl.bbau.bbau@analysis.urkund.com

Sources included in the report

W	URL: https://open.library.ubc.ca/media/stream/pdf/24/1.0395350/3 Fetched: 2021-12-14 05:01:54	1
J	**07_chapter-1.pdf** URL: 17a3563d-320f-42c8-b328-dd74e38e88ad Fetched: 2022-08-20 16:51:35	20
J	**17_bibliography.pdf** URL: d0ad42f9-3874-4aad-8714-d93d08bcb6c4 Fetched: 2022-08-20 16:51:37	41

Entire Document

Study the role of rhizospheric bacterial community during the phytoremediation of distillery waste THESIS SUBMITTED TO BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY (A CENTRAL UNIVERSITY) LUCKNOW FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ENVIRONMENTAL MICROBIOLOGY SUBMITTED BY KSHITIJ SINGH Enrolment No. 1082/17 UNDER SUPERVISION OF PROF. RAM CHANDRA HEAD, DEPARTMENT OF ENVIRONMENTAL MICROBIOLOGY SCHOOL FOR ENVIRONMENTAL SCIENCES BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY (A CENTRAL UNIVERSITY) VIDYA VIHAR, RAE BARELI ROAD, LUCKNOW- 226025 (U.P.) INDIA AUGUST 2022 Pre-Ph.D. Thesis
Study the role of rhizospheric bacterial community during the phytoremediation of distillery waste THESIS SUBMITTED TO BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY (A CENTRAL UNIVERSITY) LUCKNOW FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ENVIRONMENTAL MICROBIOLOGY SUBMITTED BY KSHITIJ SINGH Enrolment No. 1082/17 UNDER SUPERVISION OF PROF. RAM CHANDRA HEAD, DEPARTMENT OF ENVIRONMENTAL MICROBIOLOGY SCHOOL FOR ENVIRONMENTAL SCIENCES BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY (A CENTRAL UNIVERSITY) VIDYA VIHAR, RAE BARELI ROAD, LUCKNOW- 226025 (U.P.) INDIA August, 2022 Pre-Ph.D. Thesis