Study the Degradation Mechanism of Distillery Waste by Potential Bacterial Consortium for its safe Disposal and Re-cycling in Environment

THESIS

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

FOR THE DEGREE OF

Doctor of Philosophy

IN

ENVIRONMENTAL MICROBIOLOGY

SUBMITTED BY

Sonam Tripathi

(M.Sc., Microbiology)
Enrolment No. 1083/17

UNDER SUPERVISION OF

Prof. Ram Chandra

DEPARTMENT OF ENVIRONMENTAL MICROBIOLOGY
SCHOOL FOR ENVIRONMENTAL SCIENCES
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VIDYA VIHAR, RAE BARELI ROAD,
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CERTIFICATE

This is to certify that the thesis entitled **"Study the Degradation Mechanism of Distillery Waste by Potential Bacterial Consortium for its safe Disposal and Re-cycling in Environment"** submitted by **Ms. Sonam Tripathi** 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-1999 as amended in 2008/2010/2013/2017* and it is fit for submission and evaluation for the award of the degree of **Doctor of Philosophy** of the University.

Date:

Supervisor

Head of Department

DECLARATION

I, **Sonam Tripathi** hereby declare that the work which is being presented in the thesis entitled **"Study the Degradation Mechanism of Distillery Waste by Potential Bacterial Consortium for its safe Disposal and Re-cycling in Environment"** in 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, Lucknow, Uttar Pradesh is an authentic record of my own work carried out during the period from February 2018 to September 2021 under the supervision of **PROF. RAM CHANDRA**, *Professor & Head*, Department of Environmental Microbiology, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar 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.

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Dedicated

to

My Parents

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(SONAM TRIPATHI)

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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

Chapter-One

Introduction

1. Introduction

Alcohol distilleries in India are one of the most agro-based polluting agro-industries; which are uses have high quantity of fresh water in processing of raw materials. Most distilleries co-exist with sugar mills and use cane sugar molasses as a starting material to produce alcohol. The process of alcohol production and effluent generation has been illustrated in Fig.1.1. The distillation process is the major source of sludge and effluent generation. For each liter of ethanol produced during the distillation process, approximately 12-15L sludge and effluent is generated. In India, there are more than 319 sugarcane molasses-based distilleries releasing approximately 3.5×10^{13} kl Distillery waste (spent wash) annually (Bharagava et al. 2009). Spent wash is generally characterized with unpleasant odor, deep brown colour, high level of biological oxygen demand (BOD; 42,000-46,666 mg L^{-1}), chemical oxygen demand (COD; 90,000-104,130 mg L^{-1}), total dissolved solids (TDS; 77,000-81,733 mg L^{-1}), total nitrogen (TN; 1635-2,800 mg L^{-1}), sulphate (1738-5760 mg L^{-1}) and the presence of heavy metals (i.e. Fe, Zn, Cu, Ni, Mn), and nitrogen containing complex organic pollutants with low pH (Chandra et al. 2004a; Tiwari et al. 2013). Spent wash properties are depending on the diversity of alcohol-based substrates such as sugarcane molasses and grapes (rice, barely, wheat and maize). The Ministry of the Environment and Forest (MoEF) has designated the alcoholic industry as the top industry under 'Red Category' due to the obvious contamination of the distillery spent wash after distillation (Tewari et al., 200 7). In 1976, the Indian government issued laws and regulations on water pollution and amended it again in 1983 (Suneeth Kumar 1998). Bureau of Indian Standard (BIS) offers guidance to State and Central Government agencies, which will assist to determine the acceptable limit for effluent disposals, the appropriate site and the level of treatment necessary for effluents before disposal and the appropriate technology for disposing them. In 2003, the Central Pollution Control Board (CPCB) provided for zero distillation of distilleries in inland surface water runs by the end of 2005.Therefore, distillery waste should be implemented properly before being released to the environment (Tiwari et al. 2007). Three ways in the industry usually carried out the distillery spent wash process; (i) the concentration followed by the incineration; (ii) the direct oxidation of the air by

aerobic treatment at a high temperature; and (iii), biogas regeneration and aerobic polishing. Anaerobic digestion has been regarded as the best first step approach in the treatment of distillery effluent across three pathways due to its reputation as a low cost and eco-friendly technology in addition to its bio-methane production potential (Satyawali and Balakrishnan 2008a,b; Oller et al. 2011). After anaerobic treatment, the effluent obtained is known as post-methanated distillery effluent (PMDE). In addition, Indian distilleries produce ~1500 tonnes of post methanated distillery sludge (PMDS) per day during anaerobic digestion of spent wash (Kansal et al. 1998) which is characterized by high organic matter, TDS, phenol, sulphate and heavy metals along with melanoidins. Anaerobic digestion is recommended since a substantial part of the spentwash might be biodegradable. Since it has been observed that anaerobic digestion removes around 40-50% COD and 60-65% BOD (Arimi et al. 2015). This indicates PMDE still retains some organic pollutants after anaerobic digestion and is not acceptable for environmental disposal. After anaerobic treatment PMDE becomes darker with greater TDS because of the intercalation of organic and inorganic contaminants together in spent wash. Extensive distillery-effluent aeration hence does not affect its physiochemical attributes even after recirculation. The conventional anaerobic-aerobic treatment processes can accomplish the degradation of melanoidins only upto 6 % or 7% (Ojijo et al. 2010; Blonskaja and Zub 2009). The anaerobic digestion procedure did not lead to efficient for colour-removal has been observed by Satyawali and Balakrishnan (2008a). The dark pigmentation of PMDE hampers the passage of sunlight into the water by releasing untreated or partially treated waste water to surface water resources, reducing photosynthesis activity and ultimately harming the existence of aquatic microbiota.

Therefore, further colour removal techniques for molasses-based distillery effluent before or after treatment must be studied. The complexity of waste water makes it difficult to create efficient solutions before being disposed of in the environment for tertiary treatment or colour removal. For example, MoEF has instructed Indian Distilleries to achieve a zero liquid discharge from December 2005 into the surface water body. Many industries dilute PMDE by mixing with crude water before discharge to comply with the requirement for disposal of waste. Although this dilution is tolerated in certain areas, it does not diminish absolute contamination of the PMDE and hence constitutes a major environmental issue (Chaudhary et al. 2007). Various physico-chemical methods such as column filtration

(Satyawali and Balakrishnan 2008b), coagulation/flocculation (Fan et al. 2011; Liang et al. 2009a), adsorption (Onyango et al. 2011), chemical precipitation/coagulation (Chandra and Singh 1999; Liang et al. 2009b), UV/H_2O_2 treatment (Dwyer and Lant 2008) and ozone oxidation (Kim et al. 1985; Pena et al. 2003) has been reported for removal of COD and decolourisation of PMDE, but these techniques are not feasible at large scale due to high cost, blockage of filtration devices and sensitivity to variable water input. Moreover, due to high TDS it generates huge amount of toxic sludge and other secondary pollutants (Liang et al. 2009b; Chandra et al. 2008). In the last several years, a number of microorganisms, such as bacteria, funguses, yeast and cynobacteria, have been postulated as autonomous function for microorganisms in degradation and decoloration in PMDE. The growing acceptance of microorganisms for decolourisation and detoxification of melanoidins containing distillery effluent are due to facts that some microorganisms have a specific extracellular ligninolytic enzymatic system known as manganese peroxidase (MnP), laccase and lignin peroxidase (LiP) capable of breaking a large number of C═C, C=O and C≡N bonds present in melanoidins (Miyata et al. 2000). Melanoidins have been more effectively degraded and decolorized by fungal substances as a consequence of the prominence of ligninolytic enzymes that metabolise melanoidins as sole sources for carbon and nitrogen (Miyata et al., 2000; González et al., 2008). However, because fungal growth was limited in low pH aquatic circumstances, its wide use for the development of effluent process was constrained. In comparison, due to their enormous environmental adaptability and their multifaceted metabolism for the catabolism of complicated resistant substances, a bacterial consortium has proved more prospectful. Some researchers have demonstrated a bacterial removal efficiency and deterioration of melanoidin sugarcane and melanoidin models both but the decolourisation was achieved only up to certain limit (Kumar and Chandra 2006; Bharagava and Chandra 2010a,b; Bharagava et al. 2009). In addition, the majority of molasses-melanoidins decolorization and degradation are described exclusively as pure melanoidins by dialysis processes with particular molecular weight at 475 nm whereas molasses melanoidin comprises mixed maillard reaction products (MRPs) (i.e. initial, intermediate and advanced stages with variable molecular weight). Moreover, it is necessary for an understanding of the decolorization and degradation mechanism of melanometric products, due to the complicated character of molasses-melanoidins at varied absorption spectrums in various wavelengths. However, no investigation has

been carried out to examine the original form of molasses-melanoidin degradation for the viability of any possible bacterial consortia. Therefore, the degradation of molasses melanoidins with mixture of complex MRPs should be investigated in order to degrade the effluent from a distillery before attempting to degrade and decolourize it. In addition, it will be important to optimize the process of degrading the PMDE by possible bacterial consortia at varied concentrations and TDS before a two-stage method is developed. Constructed wetland (CW) treatment systems have been built to use natural processes including the vegetation of wetlands, soils, and related microbial assemblies to help the processing of wastewater. The systems have low cost, easy to operate and have been successful in removing a range of pollutants from municipal and industrial waste water globally during the recent six decades (Vymazal 2014; 2011; Zhang et al. 2015a,b). CW planned system with possible wetland installations would therefore be a useful biofilters tool for the pilot or large-scale system. In the built-in humidity treatment system in tropical climates, many types of wetland plants like Phragmites karka and Phragmites australis are employed (Vymazal et al. 2009; Sochacki et al. 2015; Badejo et al. 2015). Plants can be advantageous for symbiotic colonising rhizosphere and detoxification of contaminants for microbial colonisation (Stottmeister et al. 2003; Doran 2009; Kadlec and Wallance 2009). Plants have extremely sophisticated, effective, prominent and diverse methods of metabolism that might breakdown and also mineralize highly poisonous xenobiotic substances. The diverse microbial collectivities that arise from contact with waste water play a significant role in wetland biogeochemical cycle, principally responsible for aerobic and anaerobic degradation of target organic pollutants (Stottmeister et al. 2003; Ibekwe et al. 2007; Ligi et al. 2014). The combination of bacterial-based wetland treatment technology offers sophisticated and novel treatment systems for removal of colour from PMDE and further reduction of safe disposal BOD, COD and organic pollutants. For the treatment of contaminants and ecological stability, the microbial community structure and diversity of wetland plants are extremely relevant (Hallberg and Johnsonb, 2005; Sirivedhim and Gray 2006). Too far, unfortunately, only a small number of studies have concentrated on research by the microbial community in full-fledged laboratories and CW under particular PMDE treatment conditions (Chandra et al. 2012; Chaturvedi et al. 2006). Hybrid techniques that combine partial processes with two or more stages have been shown to support the further treatment of this wastewater in recent times (Thompi 2000).No single

technique can be utilized for the whole treatment of PMDE in view of the advantages and limitations of several treatment technologies. A complete strategy to treatment incorporating successive technologies must be developed. Therefore, integrated biological techniques for treating PMDE have been used by biological processes. A new and promising bioremediation solution for PMDE is a two-stage treatment with integrated bacteria and a designed wetland treatment. The low operating costs and the effectiveness of integrated biological systems in pollution disposal have defined and which can be applied for the management of contaminated areas. The combined usage of bacteria and designed constructed treatment plants for the degradation and detoxification of PMDE has been proposed in a sequential way. Phytoremediation by utilizing CWs is a plants mediated techniques and their root associated microbes haves capability to improve the removal of pollutants.

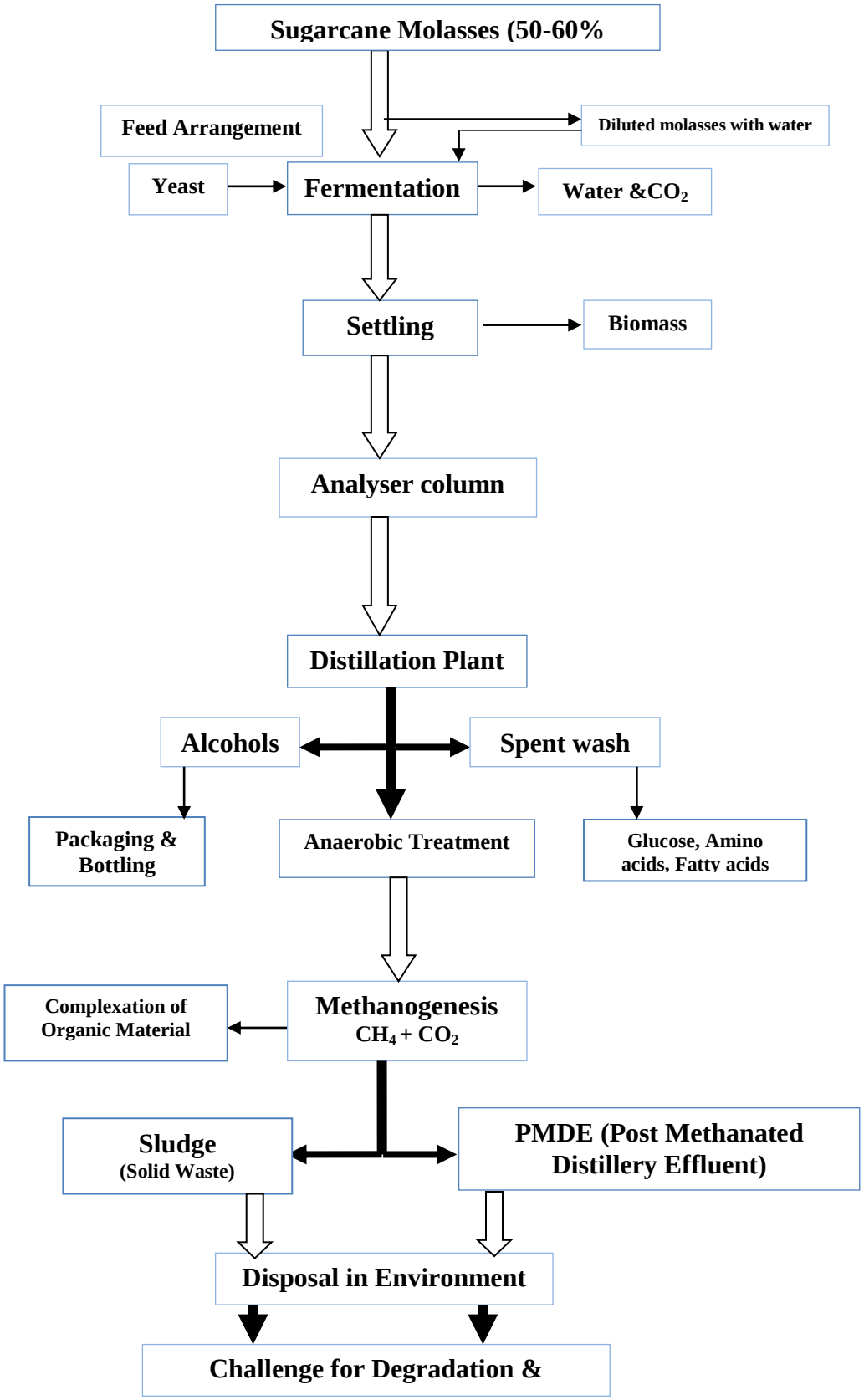


Fig. 1.1: Flow chart presentation of ethanol production and effluent generation.

The maillard reaction (MR) is in fact a complicated chain of reactions; the first phase includes initial sugar condensation with a protein and isomerization into Amadori or Heyns metabolites of the resultant products. The product of Amadori and its derivatives can be subjected simultaneously to retro-aldol reactions creating more reactive sugar fragments, including C2, C3, C4 and C5, such as hydroxyacetone derivatives, glyceraldehydes and diketones. This process is called a degradation of stretchers and is characterized by CO2 generation (Arimi et al. 2015). As auxiliary flavor chemicals, the produced aldehyde can be significant and also contribute to the synthesis of melanoidin. Melanoidins in their structure have combined two carbon-carbon bonds – C=C – responsible for their brown colour (Kim et al. 1985). In general, melanoidins are considered to be heterogeneous, with a high molecular weight, acidic and highly distributed polymers because carboxylic acid and phenol groups with comparable chemical characteristics are dissociated from humic chemicals (Migo et al. 1993). The melanoidin empirical formulation C17-18 H 26-27O10N and molecular weight between 5kDa and 40kDa have been proposed. During the purification and evaporation process melanoidins are produced during sugar processing. The basic and the chemical structure of melanoidins are highly dependent on the reactant and reaction circumstances' nature and molecular concentration (pH, temperature, heating time and solvent). The fundamental structure of the melanoidins pigment composed of 3-deoxyhexosuloses and amadori reaction products has been suggested by Cammerer et al. (2002). Caramel is a brownish, black, viscous, caramel-formed liquid. In the absence of nitrogen molecules the reaction of caramelisation happens when the sugar is warmed and the process can be catalyzed by acid and bases. The sugar undergoes first dehydrating, then condensing or polymerizing in complex molecules during a caramelisation reaction. Color creation in caramelisation involves first intermolecular rearrangement of sugar, typically the monosaccharide's structure. Yellow or brown solution is produced depending on time and temperature. Osuloses that are believed to be the intermediate of caramelisation are produced in the sugar degradation process. Osulosis leads to the production of characteristic caramel and caramel flavour components.

1.1. Alkaline degradation products of hexose and presented polyphenols

The ADPH and melanoidins represent up to 80% of the colour of PMDE. ADPH is produced through degradation in alkaline environments of hexoses (glucose or fructose) at high temperatures (> 80 0C). Sucrose is a non-reducing sugar which is decayed at high temperatures only by alkaline (Yang and Montgomery 1996). Sugarcane molasses contain large sucrose content, and certain glucose and fructose are subject to reversible and irreversible aqueous and generated ADPH reactions. The distillery produces significant quantities of waste water with phenolic chemicals that give this residue a high degree of inhibitory and antibacterial activity, therefore slowing down the anaerobic digestion process. Naturally occurring polyphenols are antioxidant chemicals mostly found in fruits, vegetables, cereals and alcohol. It is divided into 3 major categories: phenolic acid, flavonoids and tannins. Phenolic acids, including Benzoic Acid (e.g. gentisic acid, gallic acid), Cinnamic Acid (Senate Acid and Ferric Acid) and its derivatives (p-coumaric acid, caffeic acid, chlorogenic acid, and ferulic acid) have been found to exhibit significant levels of anti-bacterial action (Payer et al. 2005, 2006; Incedayi et al.2010) (Borja et al. 1993; Keyser et al. 2003). In PMDE many chemicals are manufactured and extracted in PMDE having this highly reactive phenol group and are responsible for its pigmentation. Phenolic acids are first transparent but can be oxidized with an oxygen exposure to brownish-yellow colour.

1.2.Health hazards and environmental impact of PMDE and PMDS

The ecosystem is dangerous to dispose of untreated and partially treated distillery effluent because of the high contamination potential and the existence of numerous undiscovered recalcitrant contaminants. In their high organic content, PMDE is slightly alkaline in nature and causes significant aquatic and soil pollution. Intensive amounts of recalcitration organic compounds and metal sulphides are present in the molasses base distillery effluent as dark colored organic pollutants. The strong colour of PMDE is attributable due to a dark brown, acidic pigment in melanoidins. The standard biotreated treatment technique, namely anaerobic digestion (biomethanation), anaerobic lagoons and activated sludge processes cannot be readily destroyed by these antioxidant and refractory polymers, because of their complex structure and the xenobiotic nature, and they may also be both carcinogenic and mutagenic. Heavy metals such as Fe, Cu, Zn, Ni, Mn, and Pb, are inorganic ions such

as $Ca2^{+}$, K^{+}, and Na^{+} (Harada et al. 1996; Ramana et al. 2002; Natarajan et al. 2006) and sulphates have been identified in PMDE (Harada et al., 1996; Ramana et al., 2002). In addition to other nutrients such as nitrate and phosphate, higher levels of these components (Ramana et al. 2002; Eusébio et al. 2004), make prospective PMDE emission into water bodies problematic since this leads to eutrophication and other undesirable environmental consequences (Borja et al. 1993; Collins et al. 2005). Melanoidins have been shown to have net negative charges, therefore several heavy metals (CU2+, Cr3 c+, Fe3+, Zn2+ and Pb2+) are firmly bound into organic-metallic complexes with melanoidins in Effluent (Migo et al. 1997). The increased propensity to bind melanoidins also increases the sensitivity of organo-metallic complex to their environmental toxicity. In soil, seed Germination is inhibited by reduced alkalinity and accessibility to manganese and eventually affects crops. Jagdale and Savant (1979) reported that the unfavorable management of spent wash might have a negative effect on crop development and land characteristics by increasing soil salinity. The effects of hydraulic conductivity, water stable aggregates and water-based water retention were also seen in Jadhav and Savant (1975). Constant disposal/irrigation of soil with distillery effluent severely impacts soil micro-organisms that regulate the several nutrient recycling processes in the soil. The effects on the retention of water, hydraulic conductance and water resilient soiled aggregates were also identified by Jadhav and Savant (1975) with significant levels of use of discarded effluent. Constant elimination/irrigation of the soil with the effluence of the distillery adversely affects the soil microorganisms which regulate the different nutrient recycling processes in soil. Bharagava and Chandra (2010a) also observed detrimental consequences in Phaseolus mungo L. for the germination of seed and for seedling growth parameters. The inhibition in seed germination at higher PMDE content might be attributed to high salt concentration and TDS, which increases high osmotic pressure (OP) and anaerobic conditions, respectively. Environmental circumstances impact several biochemistry and physiology functions such as solvent transport, breathing and the enzyme activity of sprouting of seed. Excessive PMDE concentration has also been found to be an antagonist to plant growth hormones (auxins and gibberlin), which play a pivotal role in plant expansion (Subramani et al. 1997). Moreover, Bharagava et al. (2008a) 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. In addition, the sulphide has a high metal binding propensity which makes a significant contribution to the effluent's toxicity and coloration. The disposal of largely or untreated wastewater from distilleries also impacts marine life (Kumar and Gopal, 2001; Saxena and Chauhan, 2003; Matkar and Gangotri, 2003; Ramakritinan et al. 2005). It decreases sunlight penetration into water bodies by reducing photosynthesis and dissolving oxygen content, which causes oxygen deficit killing aquatic life (Raghukumar et al. 2004; Kumar and Chandra 2006). Dissolved wastewater is harmful to fish and other benthic organisms. The LC_{50} for distillery effluent has been estimated at 0.5% using a bio-toxicity study on fresh water fish Cyprinus carpio var. communis. Impact of distillery effluent on carbohydrate metabolism of freshwater fish, C. carpio has also been studied . In the event of a sub lethal inxication, the respiratory mechanism in C. carpio is disrupted by increased fluid stress leading in anaerobiosis at organ level. Ion laxation influences groundwater quality by changing colour, pH and electrical conductivity physicochemical proprieties (Jain et al. 2005). PMDE contains phenolic substances, including gallic acid, p-coumaric acid, and gentissic acid, which confer strong antibacterial activity in addition to COD and BOD pollution (Borja et al., 1993; Seghezzo et al., 1998; Keyser et al. 2003). Phenol acid constitutes a very minor proportion of PMDE but, if untreated, has a major detrimental effect on treatment systems as well as on the environment. The inhibition of the biological treatment mechanism for waste distilling has been affected by these chemicals. Many phenolic compounds are not biodegradable easily and even at low quantities are harmful to micro-organisms. Phenol can even block microbial growths that are able to use just carbon and energy from aromatic molecules. During the biological treatment of alcohol distillery waste waters have become highly poisonous and biodegradable owing to the presence of polyphenol compounds (Goodwin et al. 2001) which showed that the previous literature claimed antibacterial activities (Borja et al. 1993). In certain distillery effluent, polyphenol contents are significantly different and vary between 29 and 474 mg L-1 (Bustamante et al. 2005). Polyphenols have significant inhibitor effects on microbial activity. Due to their environmental and public health concerns, polyphenols must be eliminated during wastewater treatment. Human exposure to phenol is significantly increased at concentrations of 1300 mg L-1 in diarrhea, black urine, mouth sores and mouth burning (Collins et al. 2005).

Distillery industry is a major source of water pollution and produces pollutants which harm people and the environment enormously. The most common type of water pollution in India and many others around the world is water pollution through these industries. In the same way other areas with problems lead to water pollution, the method through which industries contaminate water. Industries produce something toxic, regardless of whether it's the actual product they want to produce, a by- product and their production process of waste and legally not dump it into water bodies or on land. Sometimes the toxic substance is stored in places that are unsafe. Both the surface or groundwater eventually gets into the substance and pollution is not far behind. The seriousness of water pollution in industries around the world depends on several different reasons. What the industry really is doesn't matter. Ultimately, if not many of these problems will arise, any industry contributing to water pollution. Pollutants from pesticides and fertilizers are the major problems in the agricultural lands. Pesticides consist of incredibly severe chemical substances, while fertilizers can be packed with nitrates, even when made of natural substances. It is made from extremely harsh chemical ingredients, whereas fertilizers can be packaged with nitrates even if made of natural substances. However, chemicals are far worse than natural fertilizers. A pesticide pollutant is often used to keep the plants healthy and free of insects and other pesticides. Although it is not the use of harsh chemicals that involve many natural ways to do this, it is often more expensive and many companies are uncomfortable with it. In order to help crops grow, fertilizers are also used. Practices in the use and disposal of fertilizers are, however, not very healthy. As part of the toxic runoff that is created at many agricultural sites, pesticides are washed into soil and surface water sources. It takes not long for them to get into the soil or to find a route to nearby rivers or lakes when they are sprayed on crops. The same applies to fertilizers, even though they percolate much more often into the ground water than they are transported as rivers. Groundwater is the pollution from agricultural sites that has the greatest impact. But farms that are close to the sources of freshwater can also contaminate these surface water bodies. Groundwater pollution mainly originates from farming sites which can be transported over long periods of time through soil water, before it reaches human populations with potable water. Pollution from fertilizers leads to the growth of nitrates in the environment, in particular in surface water. Fish life is choked out when this happens and plants begin to die off. Mammals and birds that rely on fish to supply food are rapidly put at risk. Pesticide pollution

can cause serious harm to humans and animals that consume this polluted water and sometimes even to humans. Without complicated processes, serious cases of pesticidal pollution cannot be removed. The water treatment of nitrate pollution has to be regulated.

Metal storage capacity in plants is regulated by soil pH, the agility of metals, plant growth and also the natural environment, i.e. temperature, sunlight and air flow (Ma et al. 2011; Sinha et al. 2007). In addition, bioaccumulation of heavy metals is also influenced by the presence of organic substances and other pollutants. In addition the role of rhizospheric bacteria in metal mobilization and permeability of organic molecules heavy metals seems to be well-observed (Kuffner et al. 2008; Huang et al. 2013; Rajkumar et al. 2012). Two important features are generally used to determine plant Phyto-extraction efficiency: metal hypercapacity and biomass production (McGrath and Zhao 2003), can be accelerated by adding agents such as dissolve by chemical mobilizing ethylene diaminetriacetic acid (EDTA), N-(2-hydroxyethyl)-ethylene diaminetriacetic acid (HEDTA) and diethylenetriamine-pentaacetic acid (DTPA) can significantly improve phytoextraction utilization. (Nowack et al. 2006). However, the risk of soil pollution is prevalent. Conversely, plant biomass production capacity is also a popular, feasible and huge potential method of plant recovery process through fast plant growth (Bharagava et al. 2008). However, few recent studies have shown PMDE's became general health hazards as heavy metals are accumulated in edible plants during arbitrary recurred irrigation use of effluent (Chandra et al. 2008b; Bharagava et al. 2008). Various potential wetland plants cultivated in natural conditions in distillery waste sites also demonstrated the potential for phytoremediation of those polluted site plants as the natural accumulators of heavy metals from complex organic waste (Mazumdar and Das 2015). The large proportion of phytoscopic experimentation and laborational hydroponic solution studies were carried out (Solhi et al. 2005; Wenzel et al. 2003), while very few studies were carried out in order to evaluate the potential of natural high biomass accumulators of plants for field conditions for Phytoextraction. (McGrath et al. 2006; Hammer and Keller 2003). Although certain studies for heavy metal accumulation and tolerance have been reported, (Yoon et al. 2006; Shu et al. 2002; McGrath and Zhao 2003). However, before application of these plants in the field of distillery residue-contaminated site, the details of the magnitude and pattern of heavy metal accumulation in different plants are not known; The Phytoextraction potential for the

accumulation and translocation of heavy metal in its diverse parts of these native plants is urgently needed to study. In addition, the threshold limits for the accumulation of these potential growing native plants should be investigated, without showing any adverse impacts on plants for sustainable use. In addition, in situ cellular damage in simulated condition was also revealed by the long-term accumulator of heavy metals by potential wetland plants (Yadav and Chandra 2011). Moreover, recent reports have also been published on the accumulation of various heavy metals in wetland processing systems from metals contaminated waste by wetland plants as effective tools for processing and managing industrial waste A. fumigatus (Liu et al. 2007; Deng et al. 2004; Yoon et al. 2006; Zhuang et al. 2007; Galfati et al. 2011; Lorestani et al. 2011).

1.3. Biodegradation challenges of PMDE and PMDS.

PMDE's main problems are the elimination of TDS and color oxidation adding chemicals, such as melanoidins, phenolics, sugar, ADPH and related metabolites and inorganic compounds before being disposed of safely in the environment. Inconsistent structures of the different colorants can result in different feedstocks, fermenting processes and biological treatments (Chandra et al., 2018). Polymerization reacts in varying amounts to different polysaccharides, organic molecules and other aromatic substances, and is possibly complicated and persists in the various concentrations. Thus, characterization of these pigments is not straightforward. It can also be emphasized that several colors in place of just one kind of pigment may provide the undesired color. The decoloration and organic removal of PMDE will then become more complex. Caramels and ADPH are recorded in the aerobic stage to be 70 percent reduced. However, the antimicrobial and antioxidants are high in melanoidins and plant polyphenols (Arimi et al. 2014). Conventionally biological processes are able, but the small pigments are possibly re-polymerized in biological processes, to achieve melanoidin degradation by only 67 per cent (Mohana et al., 2009). In addition to the color components, during chlorine disinfection of wastewater effluents, melanoidins and polyphenols can also induce aromatic halogenated disinfection sub-products (DSP). Recent experiments have shown that, overall, the developmental toxicity and growth inhibition of aromas halogenated DBPs have considerably been higher than the aliphatic ones (Jiang et al. 2017; Liu and Zhang 2014; Meng et al. 2016). Since chlorine disinfection has occurred so far, the effluent distilling molasses was mainly studied in associated studies, where melanoidins were considered the leading or even

specific form of pigments (Hatano et al. 2008; Liang et al. 2009a; Liakos and Lazaridis 2014). Therefore, state-of-the-art technique are required for organic matter and colorants to be disposed of in the organically treated distillery wastewater (Arimi et al. 2014; Prajapati and Chaudhari 2015; Tsioptsias et al. 2015). The portrayal by bacteria and a constructed wetland plant treatment system of organic pollutants present in the wastewater and PMDE is essential for their care. For a microbiological understanding of distillery waste, the bacterial communities grown in this adverse setting are very important. Furthermore it will be a primary step for the advancement of two-stage method therapy for PMDE to evaluate molasses melanoidin by a potentially bacterial consortium. The colorization of PMDE is nevertheless a crucial issue, and the development of an effective method scheme for removal involves an understanding of the structure and features of melanoidins. The sugarcane molasses used for alcohol manufacture in distilleries have large quantities of sulphate and aromatic alcohol moieties, such as sugar products. Moreover, in the distillery effluent, sulphate and heavy metals become blackened sulphides precipitate and are a competitive barrier for bacteria reduction by sulphate and bacteria reduce non-sulfate (McCartney and Oleszkiewicz 1991) leading to inhibition of methanogenesis or sulphate reduction and giving toxicity to PMDE.

Recently two phases/sequences have been shown to be very promising for detoxification of bacteria and wetland plants, but with the comprehensive microbiological methods of wetland plant, plant rhizosphere and detoxification process, this needs to be optimized. It is not clear how melanoidins bind with other toxic compounds in PMDE under various environmental conditions. In PMDE, the chemical composition of various melanoidin groups is difficult and must be investigated. In addition, the existence and degree to which melanoidins, phenolics, sulphates, phosphates and heavy metals are applied to the atmosphere must become known in recalcitrant toxic compositions. Bearing all details in mind, my theme of this research is PMDE oxidation and decolouration in a two-step bacterial and wetland treatment scheme for the protection of the ecosystem. The thesis based in the review of GC-MS on the characterisation of organic and permanent toxins in waste distillery waste. The evaluation of the degradability of natural and synthetic melanoidins and the impact of co-pollutants in the distillery effluent is warranted, until the creation of a two-tier treatment scheme for PMDE deterioration and decolourization. But the large scale application of decolourization and detoxification

not yet developed. He preliminary challenge for development of any feasible technology is due to complexicity of PMDE with various organometallic pollutants and high TDS. Due to complex pollutant concentrated amountno microbial growth reported in effluent. Consequently it remains unchanged in environment for several years.

Keeping in view of these above problems-

The thesis of study has been compiled into following 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.

The aims of this study have been outlined in Chapter two and a new approach for industrial scale applications is also described to examine novel methods and the empirical results on pilot scale.

Chapter three of this thesis has described the comprehensive existing information on various physico-chemicals, biological (i.e. aerobic and anaerobic), enzymatic treatment as well as emerging techniques for the treatment of Distillery wastewater.

Chapter four has described anaerobically treated post methanated distillery effluent is a major source of terrestrial and aquatic pollution due to the mixture of the various unknown organo-mettalic 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 (λmax) were obtained at 295 nm of pollutants present in spent wash which indicated that melanoidins are a major colorant along with other organic compounds. The new solvents (ethyl acetate, DCM, methanol) has been used for extraction of unknown compound which has not been reported sofar. Abundantly identified compounds with GC–MS in effluent were found 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.

Chapter five of this study comprised detoxification and degradation of organo-mettalic pollutants from distillery wastewater by an indigenous bacterial community with biostimulation and bioaugumentation process. Discharged wastewater showed high total phenol, chemical oxygen demand, total suspended solids, total dissolved solids, sulfate, phosphorus, and biological oxygen demand, value along with heavy metals were high. The results showed that after the biostimulation and bioaugumentation processes, the reduction in Physico-chemical parameters of pollutants was greater than 50-70 %. Scanning electron microscopy analysis image showed bacterial community and their relationship with complex organo-mettalic pollutants during biostimulation and bioaugumentation processes. Further, the major organic pollutants i.e., Acetic acid, Oxo-,trimethylsilyl ester [CAS], Hydrocinnamic acid, p-[Trimethylsiloxy]-trimethylsilyl ester and Tetradecanoic acid, trimethylsilyl ester [CAS] have been observed in control while some, new metabolic products generated as a by-product. Therefore, study showed that biostimulation and bioaugmentation are a successful bioremediation strategy for detoxification and distillery wastewater.

Chapter six of this study comprised optimization of color reduction and detoxification process of Distillery effluent by developed bacterial consortium at reduced TDS of Effluent. The degradation and decolorization of PMDE by potential bacterial consortium and optimization of nutritional and environmental parameters for the maximum degradation and decolourisation of melanoidins by bacterial consortium. This chapter also deals with the complete physico-chemical analysis of TDS reduction before and after bacterial decolourisation. Further, the change in absorption peaks (200-700 nm) by UV-Vis spectrophotometric analysis has been correlated for their structural changes and reduction in color by investigation through FT-IR, HPLC, and GC-MSanalysis. Furthermore, the toxicity assessment has been done in pre and post degradation of TDS reduction by using P. mungo L. seeds.

In Chapter seven, this study deals has imbibed the process and approach to reveal the unknown microbial community during in-situ biodegradation of sludge at various stages of sludge degradation. Therefore, the metagenomics analysis has been

done for fresh, intermediate and degraded sludge sample.To accomplish the identification of chemical nature of distillery waste, we employed several organic solvents to extract the broad range organic pollutants under acidic conditions using the liquid–liquid extraction method. Further, the extracted organic compounds present in this waste has been detected and characterized by GC-MS analysis. Simultaneously, the dominant autochthonous bacterial communities were investigated by the RFLP method using a metagenomics approach to reveal the microbial niche in this polluted environment.

Chapter eight has described the heavy metal accumulation by potential weeds, herbaceous traditional medicinal and food plants (Achyranthus aspera, Amaranthus viridis, Basella alba, Sesbania bispinosa, Pedalium murex, and Momordica doica) grown on complex distillery waste containing a mixture of organo-mettalic compounds. The result revealed that the growing weeds are showing as potential source for phytoextraction of heavy metal from the complex organo-metallic sludge. In addition, some medicinal and vegetative plants have shown their potential also for phytoextraction of heavy metal from PMDS. The bioaccumulation of Mn, Cd, Fe, Cr, Cu, As, Se, Mo, and Co in their root, shoots and leaves in levels higher than the surrounding sludge.

Chapter nine of this study deal with designing detail of pilot scale constructed wetland plant treatment system and the optimization for enhancement of distillery wastewater decolourisation for development of a feasible technology after bacterial treatment in constructed wetland treatment system. The detail physico-chemical characteristics of Distillery wastewater collected from the effluent treatment plant of M/s Unnao distillery and Breweries, Unnao (U.P.), India before and after bacteria and wetland plants rhizosphere treatment has also described. Further, this chapter has also shown the complete emphasis of constructed wetland plant treatment technology. This technique has been optimized by using two stage treatment primary i.r. bacterial degradation followed by integradtion with rhizofiltration technique by using potential wetland plant i.e Phragmities communis and Typha angustofolia.

This Chapter ten has highlighted the major finding of my study described in the thesis which has focused on the new organic pollutant detected in study and the toxicity profile with flora and fauna similarly other achievement of microbial and phytoremediation potential has been documented. At the end, I have mentioned the technology for recycling of distillery wastewater in environment for safe disposal.

Chapter eleven listed the sources in question quoted throughout the study. The section of reference was written in a traditional format and all relevant topical sources are also included.

Chapter twelve has been annexed with the title page of published papers and other scientific output of work. The cover page of each published original research papers in National and International journal has also been attached. Two Published patent related with this PhD work.

Chapter-Two

Objectives

Objectives

The objectives of study were as below-

1. **Detection of complex Organo-metallic Compounds and other EDC related compounds of Distillery waste & their Environmental effect.**

2. **Isolation and characterization of potential bacterial community responsible for bio-stimulation and bio-augmentation process during detoxification of distillery waste.**

3. **Optimization of color reduction and detoxification process of distillery effluent by developed bacterial consortium at reduced TDS of effluent.**

4. **Profiling of bacterial community of distillery waste degradation through next generation sequencing (NGS).**

5. **Assessment of bacterial assisted phytoremediation technique by native wetland plant growing in distillery waste polluted site.**

6. **Development and optimization of a novel technique for decolorization and detoxification of PMDE at high TDS for safe disposal and re-use.**

Chapter-Three

Review of literature

3. Review of Literature

As expressed in the decreased BOD and COD values of the PMDE, anaerobic treatment eliminates much of the biodegradable organic matter. Without eliminating pigment, anaerobic digestion using activated sludge process breaks down these biodegradable polymers into low molecular weight compounds and thus coloring content persists in the form of high COD amounts in the PMDE. However, since these bodies are bio-refractory, PMDE colorants such as lignin, caramel, ADPH, and melanoidins remain. Present treatment systems used for the treatment of PMDE include, prior to disposal, physical, chemical and biological processes. The choice of treatment approaches depends on different considerations, such as the effectiveness of treatment, the cost of treatment, geographic location, environment, land use, legislative restrictions and public approval of treatment.

3.1. Physico-chemical treatment

The techniques of physico-chemical application are a mixture of physical and chemical technologies. The separation of suspended solids from the PMDE is a physical procedure, while a chemical process is the reduction of the dissolved solid. By adding additives, all functions are conducted on PMDE. Several physico-chemical techniques have also been shown to reduce the distillery effluent's contaminant load. Coagulation/flocculation, hydroid oxide and radiation, hydrogen peroxide and adsorption of products such as chitosan and activated carbon are used in this treatment.

3.1.1. Adsorption

Adsorption is a wastewater purification technique for removing a wide range of compounds from industrial wastewater. Adsorption is most commonly implemented for the removal or low concentrations of non-degradable organic compounds from groundwater, drinking water preparation, process water or as tertiary cleansing after, for example, biological water purification. Adsorption takes place when molecules in a liquid bind themselves to the surface of a solid substance. Adsorbents have a very high internal surface area that permits adsorption. Active carbon is by far the most commonly used adsorbent and is particularly suited to the removal of polar

compounds. Adsorption is a surface phenomenon with common mechanism for organic and inorganic pollutants removal (Akmehmet and Otker, 2004) (Amat et al., 2007; Shu and Chang, 2005; Yonar et al., 2005; Wu and Chang, 2006; Hsing et al., 2007). When a solution containing absorbable solute comes into contact with a solid with a highly porous surface structure, liquid–solid intermolecular forces of attraction cause some of the solute molecules from the solution to be concentrated or deposited at the solid surface. The solute retained (on the solid surface) in adsorption processes is called adsorbet, whereas, the solid on which it is retained is called as an adsorbent. This surface accumulation of adsorbate on adsorbent is called adsorption This creation of an adsorbed phase having a composition different from that of the bulk fluid phase forms the basis of separation by adsorption technology. In a bulk material, all the bonding requirements (be they ionic, covalent, or metallic) of the constituent atoms of the material are filled by other atoms in the material. However, atoms on the surface of the adsorbent are not wholly surrounded by other adsorbent atoms and therefore can attract adsorbateThis method has also been successfully eliminated from distillery effluent unhealthy criteria such as COD and colour (Swamy et al. 1997).Different researchers have documented decolorization of distillery effluent involving adsorption on both commercial and indigenous activated carbons(Satyawali and Balakrishnan 2009; Chandra and Pandey 2000).Satyawali and Balakrishnan (2007) investigated the discoloured use of various agro-residue-based, active coals and commercially processed activated carbons to treat anaerobically treated distillery effluent. For both agro-residue-based carbons, colour removal was 50% in 5g/100 mL of highest doses tested, whereas industrial activated carbons increased at 80% colour extraction at almost the same maximum concentration. For both agro-residue-based carbons, colour removal was 50% in 5g/100 mL of highest doses tested, whereas industrial ACs increased at 80% colour extraction at almost the same maximum concentration. The use of chemically modified bagasse using 2-diethylaminoethyl (DEAE) chloride hydrochloride and 3-chloro-2-hydroxypropyltrimethyl ammonium chloride (CHPTAC) was found to be capable of decolorizing diluted spent wash (Mane et al. 2009).2-diiethylaminoethyl (DEAE) and 3-chloro-2-hydroxypropyl ammonium chloride (CHPTAC) chemically engineered bagasse has been found to use for decolorization of diluted spent wash.0.6 g of synthetically produced sugarcane in contact with 100 mL of effluent reduced by 50% of the colour in 4 h. Decolonization of biomethane distillery effluent in packed beds using commercially available

activated carbon reduces the colour of 99 per cent and BOD by 90%. Comparative colour removal studies with commercially activated carbon and bagasse fly-ash were carried out. The 58% bagasse fly ash colour deletion is reported with 30 g/dm3, and the 80% colour deleting is reported with 20g/dm3 commercial AC. Few studies with natural carbon dioxide chitosan have been carried out. The (Lalov et al. 2000) research in dilute waste distillery water using chitosan as an anion exchanger gave 10 g/dm3 and 30 minutes of contact time 98 per cent colour and 99 per cent COD elimination. It has been stated that with large indigenous dose powder and granular pyrochar in comparison with commercial ACs they are attaining the same degree of colour removal.The ACs are not cheap materials; thus, their use can often be limited by economic considerations despite their strong quality and applicability for adsorption of a variety of materials.

3.1.2. Membrane filtration

The Membrane Filter (MF) Technique was introduced in the late 1950s as an alternative to the Most Probable Number (MPN) procedure for microbiological analysis of waste water samples. The Membrane Filter Technique offers the advantage of isolating discrete colonies of bacteria, whereas the MPN procedure only indicates the presence or absence of an approximate number or organisms (indicated by turbidity in test tubes). Industrial waste water treatment plants monitoring, waste, and surface water for the presence of coliform bacteria by the Membrane Filter Technique. The key organism monitored in water treatment facilities is E. coli. In the membrane filter technique, water is drawn through a thin filter. Filters with a variety of pore sizes are available. Pores of 0.45 µm are used for filtering out most bacteria. Bacteria are retained on the filter, which is then placed on a suitable nutrient medium. In field situations, nutrients are added to a thick absorbent pad on which the filter is placed. Nutrients that diffuse through the filter can be metabolized by bacteria trapped on the filter. Each bacterium that is trapped on the filter will develop into a colony. Bacterial colonies growing on the medium can then be counted. When a selective or differential medium is used, desired colonies will have a distinctive appearance. In addition to its use by government labs for monitoring drinking water, the MF Technique is also used for microbial monitoring in the pharmaceutical, cosmetics, electronics, and food and beverage industries. The Membrane Filter Technique is used in these industrial labs to monitor the presence of microorganisms in process waters and final product. Most modern laboratories use a refinement of total plate count in

which serial dilutions of the sample are vacuum filtered through purpose made membrane filters and these filters are themselves laid on nutrient medium within sealed plates. The methodology is otherwise similar to conventional total plate counts. Membranes have a printed millimeter grid printed on and can be reliably used to count the number of colonies under a binocular microscope. The MF Technique is an effective, accepted technique for testing fluid samples for microbiological contamination. It involves less preparation than many traditional methods, and is one of a few methods that will allow the isolation and enumeration of microorganisms. The MF Technique also provides presence or absence information within 24 hours.

- Permits testing of large sample volumes.
- Reduces preparation time as compared to many traditional methods.
- Allows isolation and enumeration of discrete colonies of bacteria.
- Provides presence or absence information within 24 hours.
- Effective and acceptable technique. Used to monitor drinking water in government laboratories.
- Useful for bacterial monitoring in the pharmaceutical, cosmetics, electronics, and food and beverage industries.
- Allows for removal of bacteriostatic or cidal agents that would not be removed in Pour Plate, Spread Plate, or MPN techniques.

3.1.3. Coagulation and flocculation

A coagulant (typically a metallic salt) with the opposite charge is added to the water to overcome the repulsive charge and "destabilize" the suspension. For example, the colloidal particles are negatively charged and alum is added as a coagulant to create positively charged ions. Once the repulsive charges have been neutralized (since opposite charges attract), the Vander Waals force will cause the particles to cling together (agglomerate) and form micro Floc. Conversely, flocculation involves the addition of polymers that clump the small, destabilized particles together into larger aggregates so that they can be more easily separated from the water. Flocculation is a physical process and does not involve the neutralization of charge. Coagulation may be used in conjunction with flocculation to assist with water clarification. Coagulation and flocculation are an essential part of drinking water treatment as well

as wastewater treatment. This article provides an overview of the processes and looks at the latest thinking. Material for this article was largely taken from reference1. Coagulation and flocculation are essential processes in various disciplines. In potable water treatment, clarification of water using coagulating agents has been practiced from ancient times. As early as 2000 BC the Egyptians used almonds smeared around vessels to clarify river water. The use of alum as a coagulant by the Romans was mentioned in around 77 AD. By 1757, alum was being used for coagulation in municipal water treatment in England. In modern water treatment, coagulation and flocculation are still essential components of the overall suite of treatment processes – understandably, because since 1989 the regulatory limit in the US for treated water turbidity has progressively reduced from 1.0 NTU in 1989 to 0.3 NTU today. Many water utilities are committed to consistently producing treated water turbidities of less than 0.1 NTU to guard against pathogen contamination. It is also important in several wastewater treatment operations. A common example is chemical phosphorus removal and another, in overloaded waste water treatment plants, is the practice of chemically enhancing primary treatment to reduce suspended solids and organic loads from primary clarifiers. Coagulation-flocculation is a chemical water treatment technique typically applied prior to sedimentation and filtration (e.g. rapid sand filtration) to enhance the ability of a treatment process to remove particles. Coagulations a process used to neutralize charges and form a gelatinous mass to trap (or bridge) particles thus forming a mass large enough to settle or be trapped in the filter. Flocculation is gentle stirring or agitation to encourage the particles thus formed to agglomerate into masses large enough to settle or be filtered from solution.

3.1.4. Sedimentation

Sedimentation is a physical water treatment process using gravity to remove suspended solids from water. Solid particles entrained by the turbulence of moving water may be removed naturally by sedimentation in the still water of lakes and oceans. Settling basins are ponds constructed for the purpose of removing entrained solids by sedimentation. Clarifiers are tanks built with mechanical means for continuous removal of solids being deposited by sedimentation.

- Settling of discrete particles
- Settlement of flocculent particles

- Zone-settling behaviour
- Compression settling
- Horizontal flow tanks
- Radial flow tanks
- Inclined settling
- Ballasted sedimentation
- Floc blanket sedimentation

3.1.5. Electromagnetic separation

Magnetic treatments for water and wastewater attract a special attention due to their safety, ecological purity, simplicity and low operating costs. Thus this study was carried out in order to determine the feasibility and effectiveness of applying magnetic technology for a better understanding of the effluent characteristics. The main objectives of this research are to investigate the feasibility of magnetic technology in assisting sedimentation of suspended particles and to understand the mechanism and impact of magnetic application in sewage. The effects of various parameters, magnetic field strength, and flow rate, usage of pin-jet and magnetic orientations are used to investigate their effectiveness on the suspended solids removal(Akmehmet and Otker, 2004), surfactants (Amat et al., 2007), dyes (Shu and Chang, 2005; Yonar et al., 2005; Wu and Chang, 2006; Hsing et al., 2007). A series of electromagnets magnets was used as a reactor in this study and the sewage was taken from Taman Sri Pulai, Johor with estimated PE of 10,300. Experiments indicate that suspended solids removal increases as magnetic field strength and exposure time are increased and flow rate is decreased. It was found out that magnetic field increases the suspended solids removal by 41 percent to 49 percent at 670 gauss compared to untreated raw sewage. Besides that usage of pin-jet in the magnetically treatment reactors also help to increase another 6 percent of the suspended solids removal. Study carried out also shows that magnetic field enhances the suspended solids removal by accelerating the settling of sludge (settlement time) as well as increasing the sludge density. Hence this technology is definitely beneficial in reducing the volume of sedimentation tank as well as increasing the treatment plant efficiency. Magnetic technology has been shown in the past to be a promising treatment process that can be used to enhance water and wastewater quality. Magnetic water treatment is a process of water that does not require any chemical and filtration substitutes. The scientific explanation of

magnetic water treatment has been the subject of investigation by many researchers. These studies involved the formation of scale and methods for its prevention (Florenstano, 1996). Magnetic treatment of water is an attractively simple approach by which the water to be treated flows through a magnetic field and consequently changes some of its physicochemical properties. Some researchers use particle of higher magnetic susceptibility to flocculate with particles (weakly magnetic and nonmagnetic particles) in the suspension and subsequently form paramagnetic flock that can be removed by a magnetic filter. (Ying et al., 1999). This process is called magnetically seeded filtration and has been widely applied in the industrial wastewater treatment such as filtration of nuclear reactor coolant (Heitmann, 1979), removal of phosphate from water (Sheikh & Dixit, 1992), recovery of hematite and chromites fines and ultra-fines (Wang & Forsberg, 1994) and separation of dissolved heavy metals from wastewater (Terashima et al., 1986). Usages of magnetite slurry, magnetic particles and magnetic powder in treating wastewater have becoming the main interest of many researchers. Common wealth of Australia Scientific and Industrial Research Organization (CSIRO) has conducted a research onto the application of magnetic particle technology to wastewater treatment with respect to absorption and coagulation process (Bolto, 1990). This study was made on various numbers of wastewater applications such as metal recovery from electroplating rinse water, sewage sludge and hydrometallurgical effluents. Magnets had been used as the core element of a complete system to eliminate phosphate, heavy metals and other pollutants from wastewater. This system is based on attachment of wastewater pollutants to a magnetic carrier material (magnetite). After separation the magnetic is recovered and reused in the process. On the similar approach Sakai (1994) has studied the submerged filter system consisting of magnetically an isotropic tubular support media for sewage treatment with biofilm system. Activated sludge was supplemented with ferromagnetic powder for the preparation of the biofilm. The biofilm was formed within 15 min on magnetic support media by magnetic attraction. The magnetic support media were able to treat sewage containing 0.2 g/l COD removing 72-94% COD with a retention time of 8h. When a magnetic field is applied across the column, the magnetic filling dehomogenizes the magnetic field in the column, producing large field gradients around the magnetic matrix that attract magnetic particles to their surfaces and trap them there. The collection of particles strongly depends on the creation of these large magnetic field gradients, as well as on the particle size and

magnetic properties. For successful collection of magnetic particles by HGMS, the magnetic force attracting particles toward the magnetic matrix must dominate the fluid drag, gravitational, inertial, and diffusion forces as the particle suspension flows through the separator (Ambashta and Sillanpaa, 2010).

3.1.6. Drying and evaporation

Water removal from industrial effluent streams constitutes an important step in wastewater and sludge treatment. The purpose is to concentrate, separate, dispose, or utilize wastes and pollutants and to regenerate and return clean water to the environment. Drying and evaporation consist of a combination of mass and heat transfer processes common to all dryer designs for a given sludge. Evaporation is the process by which water changes from a liquid to a gas or vapor (Akmehmet and Otker, 2004), (Amat et al., 2007), (Shu and Chang, 2005; Yonar et al., 2005; Wu and Chang, 2006; Hsing et al., 2007). Evaporation is the primary pathway that water moves from the liquid state back into the water cycle as atmospheric water vapor. Studies have shown that the oceans, seas, lakes, and rivers provide nearly 90 percent of the moisture in the atmosphere via evaporation, with the remaining 10 percent being contributed by plant transpiration. Removing water from effluent constitutes a significant portion of the processing activity for persons working in the industries. Two major moisture removal methods are drying (or dehydration) to produce a solid product and evaporation to produce a more concentrated liquid. The words drying and dehydration are often used interchangeably, especially when referring to industrial effluent. Although certain basic factors are involved in all drying processes, the equipment and techniques vary greatly depending upon the product and other factors. In this unit, we will consider some basic factors affecting drying and briefly examine some drying methods. Evaporation is the removal of some water from a liquid product to produce a more concentrated liquid... Most evaporation systems are large-scale commercial operations, although small-scale farm operations still exist for production of maple, sorghum, and sugar cane syrups. The governing principles of evaporator operation will be briefly examined in this unit.

3.1.7. Oxidation process (Ozonation)

In ozonolysis, ozone is sparged into a mixture of biomass and water at room temperature and specific time periods leading to the solubilization of lignin and

hemicelluloses. The focus of attack is on the aromatic ring of lignin and the process is affected by ozone concentration, biomass type and moisture content, and air/ozone flow rate. Though the process is relatively expensive due to large requirements of ozone, the process comes with benefits as follows: high dry matter concentrations (45–60%), effective removal of lignin, very low production of inhibitory products, and reactions performed at atmospheric conditions (Akmehmet and Otker, 2004), surfactants (Amat et al., 2007), dyes (Shu and Chang, 2005; Yonar et al., 2005; Wu and Chang, 2006; Hsing et al., 2007),. Ozone, thus, barely attacks carbohydrates. Lignin degradation products such as carboxylic acids that may form can be eliminated by washing with water at room temperature even though it comes at the expense of some carbohydrates losses.

In some instances, ozone application to specific biomass resulted in low sugar yields. In basic medium, ozone application was found to be inefficient on wheat and rye straw. Ozone treatment of cotton stalk (10% w/v) at 4°C for 30–90 minutes reduced lignin by 11.97–16.6%, at xylan and glucansolubilisation of 1.9–16.7% and 7.2–16.6%, respectively; comparatively, NaOH treatment achieved higher delignification of 65.63%. In another work, high delignification and low carbohydrate loss were observed when a two-step method comprising ozone and ethanosolv was applied to Sweet gum, Miscanthus, and Loblolly pine. In addition, combined ozonolysis and auto hydrolysis offer benefits such as high hemicellulose solubilisation, high glucose and ethanol yields, low use of chemicals, and low waste production.

Recently, the use of plasma-generated ozone (from air or oxygen-enriched air) at atmospheric conditions has attracted interest among researchers including Schultz-Jensen and team at the RisøNational Laboratory for Sustainable Energy in Denmark. Employing a fixed-bed reactor, a CO_2 detector, and a technique for continuous determination of ozone consumption, lignin degradation of ozone pretreatment of wheat straw was monitored in real time with respect to ozone consumption and CO_2 emission. Lignin degradation of 1 mm particles was found to be almost complete while that of 2 mm particles was less than 80%, leaving a solid fraction mainly composed of carbohydrates. Maximum glucose and ethanol yields of 78% and 52% were observed after enzymatic hydrolysis and SSF (based on glucan), respectively, based on optimal ozonisation for 1 h. The ethanol yield was relatively low and the process economics has the potential to be improved via the recovery and use of lignin

byproducts and hemicellulose, as well as developing schemes to reduce ozone consumption for similar yields.

3.2. Biological processes

The organisms that operate in the absence of oxygen are used to treat high-servicing waste water and are generally treated to a degree that enables a wastewater to be released into a municipal sewage system. Here, when compared with aerobic treatments, the amount of sludge produced is very low. This process is accepted because the water with low biomass production can be stabilized. The bacteria are manufactured as a feed in the anaerobic process of the biodegradable material. In total, around 40% to 60% of organic solids are methane (CH_4) and carbon dioxide (CO_2) translated into methane.

3.2.1. Conventional method

Conventional treatment systems are used where space is very limited (sometimes referred to as mechanical systems). They are more expensive than natural therapy systems which require more space, including lagoons, because they are mechanized. Traditional processes for the removal of heavy metals from waste water include many methods, including chemical precipitation, flotation, adsorption and the exchange of ions. Different kinds of conventional treatment systems are used most frequently for heavy metal removal from inorganic effluent. However, some of the most common systems are listed below:-

3.2.1.1. Activated sludge process

The activated sludge process is a very modular, affordable and environmentally friendly biological wastewater treatment system. PMDE wastewater is aerated in aerobic form by a flocculent and then is known as the activated sludge process by the conventional method as the biological oxidation method by using mixed bacterial culture. Air tank in which the activated sludge and sludge and inbound wastewater have been thoroughly mixed up and the abundant supply of oxygen dissolved, a final settler tank for removing the activated sludge from the treated effluent and a return-sludge system for recycling the activated sludge solid are key elements in this process. Compared with all the other above processes, the unique feature of activated sludge processes is that the retention time of active sludge solids and liquid effluent is controlled separately and positively. It is therefore easier than other processes to

monitor it much closer. In this method, the organic matter-containing sewage is aerated in an aerating tank by a mechanical aerator. This process accelerates the breakdown of waste. Aeration is based on pumping air into a tank which promotes a microbial development in a wastewater process in a sludge process. In a settling tank, sometimes referred to as a secondary settler or a clarifier, the effluvium from the air tank contains the flocculent microbial mass, known as the sludge.

Activated sludge has commonly been used worldwide to treat large volumes of wastewater. By definition, activated sludge refers to "the active population of micro-organisms that are used to treat wastewater." Through a series of controls, pumps, and tanks, the sewage undergoes various processes in separate basins or tanks; these processes include: primary settling, aerobic biological treatment, secondary settling, disinfection, and discharge.

Applying the appropriate mass balances, the correlations derived for microbes (D) and food (A) under the persuing suppositions:

1. Influent and effluent microbes clustering are slight.
2. Food and microorganisms are clumping in the aeration tank.

$$A = \frac{P_s(1 + p_d\theta_c)}{\theta_c(\mu_m - p_d) - 1} \qquad D = \frac{\theta_c Z(A_o - A)}{\theta(1 + p_d\theta_c)} \qquad \theta = \frac{M}{N}$$

$$\theta_c = \frac{MD}{N_w D_r}$$

A = BOD concentration in activated sludge (S = BOD_5 allowed – BOD_5 in SS) [mg/L]

D = microorganism concentration in activated sludge (2,000 to 3,500 mg/L) [mg/L of MLVSS]

D_r = microorganism concentration in recycle [mg/L of VSS]

A_O= influent BOD_5 [mg/L]

θ_c = mean cell resident time in the aeration tank [d]

θ = hydraulic detention time [d]

M = aeration tank volume [m^3]

N = flow rate [m^3/d]

N_w = flow rate of waste sludge [m^3/d]

μ_m =maximum specific substrate utilization rate [d^{-1}]

P_S= half-maximum rate concentration [mg/L]

Pd = endogenous-decay rate coefficient [d^{-1}]

Z = yield coefficient [mg/L MLVSS/mg/L]

Process Optimization of Activated sludge Process:-

Oxygen Requirements: - In the Activated sludge consume large amount of oxygen in the formation of sludge and the consumption of energy. However, oxygen is produced during microbe's movement from right to left as per the following equation:

$$C_5H_7O_2N + 5O_2 \longleftrightarrow 5CO_2 + 2H_2O + NH_3 + \text{energy}$$

$$C_6H_{12}O_6 + 6O_2 \longleftrightarrow 6CO_2 + 6H_2O$$

In particular, three main phenomena consist of the elimination or stabilization of organic matter in waste water:

- **Decomposition of organic matter**

At this stage, oxygen dissolved in the water is used to generate the energy required for live processes from the biochemical reactions:

$$CxHyO_2 + O_2 \text{ microorganisms } CO_2 + H_2O$$

Organic matter is oxidized to produce CO_2 and H_2O (cellular respiration). CATABOLISM.

- **Construction of cell mass**

It is used for the synthesis of new cell mass using dissolved oxygen to generate the necessary energy:

$(CxHyO_2) + NH_4 + + PO_4^{3-} + O_2$ microorganisms New Microorganisms $+CO_2 + H_2O$

It generates new cell material (anabolic route) ANABOLISM starting with the organic matter, and using ammonium and phosphate as sources of nitrogen and phosphorus.

• Cellular excretion

The microorganisms finally undergo a gradual auto oxidation of their cell mass.

$$(\text{Microorganisms}) + O_2 \text{ microorganisms } CO_2 + H_2O + NH_4 + + PO_4^{3-}$$

3.2.1.1. Trickling filter

A filtration system for slowly creeping seems to be a PMDE wastewater treatment system used by Dibden and Clowes for the first time. It is composed of a fixed rock bed, a lava bath, a coke, a slag or a plastic media with a microbial slime adhesive (biofilms) that is also flowing freely up rather than down through wastewater or other waste water. Aerobic conditions are kept when the filter medium is porous by splattering, diffusion and either by the forced air simply passing through the

mattresses or by the natural air evaporation. For a trickle filter, the term trickle filters, biofilters, biological filters and bio-filters are often used. These systems were also described as rough mechanisms of productivity growth and resolved film mechanisms. Filters, intermittent filters, media bed packaged filters, alternate treatment plants, filters for stirring, affixed. Simple devices that can run without electricity are trickling filters. The power of PMDE wastewater is generally everything necessary for the dispensing arm to move. The pre- treated waste water may also be supplied by a fixed spray device. The processing of PMDE wastewater is very efficient. Although primary settlement reservoirs, clarification and disinfection systems are still needed, they use less energy than active sludge.

The treatment of industrial PMDE wastewater may involve specialized tricking filters which use plastic media and high flow rates. PMDE from a variety of industrial processes have been treated in trickling filters.

- Large tanks or concrete containers of plastic or other forms of media.
- Plastic beams or other mainstream media filled with vertical spires.

3.2.2. Innovative method

To comply with technology-based treatment standards, innovative processes for industrial wastewater which contains heavy metals often involve toxicity reduction technologies. Recent developments and the technical applicability of various treatments for heavy metal deletion in industrial waste water are discussed in this article. The most dangerous industries of chemical-intensive industries include the release of large quantities of metal-contaminated wastewater, such as Cd, Cr, Cu, Ni, As, Pb and Zn, . Heavy metals can be absorbed by living organisms due to their high solubility in the aquatic environment. High levels of heavy metals may accumulate in the human body when entering the food chain. If metals are taken beyond the allowed concentration, serious health conditions may occur. (Babel and Kurniawan, 2004). Consequently, before discharge into the environment, metal contaminated wastewater must be treated. Innovative methods of treatment such as:-TDS reduction, enzyme pre-treatment, bacterial and fungal treatment processes, etc. are used to remove heavy metal from inorganic effluent.

3.2.2.1. TDS Reduction

In recent decades, huge growth has happened in the manufacturing industries of developing nations. These manufacturing industries drain PMDE effluent that carries higher concentrations of contaminants and metabolic oxygen supply .These harmful by-products should also be actually treated for completely safe contested possessions that act in accordance with the regulations imposed on the manufacturing sectors. PMDE wastewaters also have higher concentrations of total dissolved solids (TDS), which was a daunting task for technologists to eliminate them from industrial manufacturing wastewaters.

3.2.2.2. Fungal treatment

In Bioremediation Process, fungi are used in the degradation and detoxification process is known as Mycoremediation. The primary role of fungi in the ecosystem is decomposition carried out by fungal mycelium. In microorganisms, fungi are unique in that they secrete a variety of extracellular enzymes. Lignocellulose decomposition is classified as the most important degrading event in the earth's carbon cycle (Bennett and Faison, 1997). It determines the right fungal species to target a particular pollutant. The importance of fungi in the environment is that organic and inorganic substrates are decomposed and processed e.g. P. chrysosporium, F. flavus, A. fumigatus etc. Various researchers have studied nutrient removal using fungal species i.e. Cooke (1976) advocated the use of fungi in wastewater treatment because fungi showed higher levels of organic matter degradation.

3.2.2.3. Bacterial treatment

Bacteria perform a major part in bioremediation technique and have been confirmed that it is a powerful, cost effective and eco- friendly substitute to physicochemical approaches. Various bacterial sps.e.g.Pseudomonas sp., B. cereus, B. thuringiensis, X. fragariae have been reported for their capacity in to degradation and Decolor
ization of toxic chemical pollutants that are present in PMDE. Free or immobilized cells are studied wide for bioremediation of PMDE waste product. Immobilization of bacteria on inert support material as well as alginate, polyacrylamide, agar, styrene, and polymer is a lot of advantageous compared thereto of free-cell. A number of the benefits embrace compact body of carrier pellets, high biomass retention, reusability of culture and easier separation method. The accessibility of Bacteria in PMDE

wastewater treatment is much more vulnerable on the type of chemical composition of wastewater, nutrient, pH, temperature, oxygen and Inoculum size.

3.3. Recent approaches for degradation and decolorization of PMDE

3.3.1. Phytoremediation approach and constructed wetland approach in distillery effluent

Phytoremediation using wetland plants is an environmentally beneficial, appealing, affordable, energy conversion, passive technology helpful to remove pollutants with low to moderate pollution levels (USEPA 2000). Comparatively phytoremediation is a novel technique, and until now, only a small amount of plant species have been studied to address melanoidin degradation; i.e. Phragmites karka, P. australis, P. communis, Typha angustifolia, Cyperus esculentus (Chandra and Yadav 2010, 2011; Tripathi et al. 2021). Aquatic macrophyte Potamogeton Pectinatus was recently utilised in heavy metals bioaccumulated (Fe, Cu, Zn and Mn) and the distillery effluent efficiently cleaned (Sharma et al., 2021). Billore et al., 1999 study on horizontal subsurface gravity bed created in Central India using P. karka has done considerable work on the elimination of industrial pollution. Billore et al. (2001) published another research at Khodigram Village on the surroundings of the town of Baraha, India, on the use of horizontal wetland to process secondary-treating waste distillery from a private distillery. BOD5 and COD values were respectively 4850 and 22,322 mg/L^{-1} respectively in the distillery effluent even after standard secondary treatments, which prompted to additional treatment. The method produced a 64% reduction in the COD, BOD5, TKN, and TP, 84%, 59% and 79%. The study showed that built wetlands might be an appropriate tertiary treatment option. Trivedy and Nakate (2000) in another research used T. latipholia to clean wastewater from distilleries in a designed wetland. In a 10-day period, the system reduced COD and BOD by 78%, and 47%, respectively. Increased effluent concentration drastically decreased biomass production with the highest accumulation of Fe reported in 100 % pure effluent-growing plants. Chaturvedi et al. (2006) recently revealed the phytoremedial ability of P. australis, cultivated at a polluted distillery effluent site, also characterising various bacterial species from the P. australis rhizospheric zone. Bacterial culture proved advantageous for the breakdown of the hazardous substances in the distillery waste. They noticed that the colour decreased by 75.0% by the same bacteria and concomitantly reduced results for COD, BOD, phenol, sulphate and

heavy metals. The chelating properties of melanoidin like product (MLP) are investigated by Hatano et al. (2016) and the facilitative effect on the phytostatic efficacite of Raphanus sativus longipinnatus in Japan is assessed. It demonstrated that MLP binds to all metal ions under examination and that maillard product binding ability towards Cu2+ appears to be the greatest of all. MLP metal reduction was carried out in accordance with an order Pb2+> Zn2+> Ni2+> Cu2+> Fe2+> Cd2+> CO2+. Moreover, the application of maillard product approach in the phytoextraction experiment with copper sulphate. In addition, the use of maillard product increased copper detoxification and bioavailability in radish sprouts in the phytoextraction experiment with copper sulphate. The facilitatory impact on the phyto-extraction of maillard product on cadmium or lead, the amounts of which have been adjusted according to the Soil Contamination Contraction Act regulatory values in Japan, has also been assessed in another study (2009). Their rapid growth and high biomass production, and heavy metal absorption have been used to control area of Brassica species. The addition of maillard product greatly enhanced the use of biomass and plumage in the nutritional medium of 1 mM plum nitrate and virtually all plumage in the root tissue accumulated. Thus the detoxification of lead ions and the improvement of their bioavailability in Brassica species were possible for maillard product.

3.3.2. Enzymatic pre-treatment integrated with bacteria/fungi

These micro-organism exhibit extensive bioremediation activities that are mainly based upon their capabilities to produce one or more extracellular lignin-modifying enzymes (Wesenberg et al. 2003). Lignin peroxidases (LiP), manganese-dependent peroxidases (MnP) and laccase are the three major lignin-degrading enzymes with great potential in industrial applications (D'Souza et al. 2006). Production of these enzymes from bacteria and fungi has been well documented. However, in recent years, there are several reports of these ligninolytic enzymes being produced from other fungi like Phylosticta, Aspergillus, Fusarium and Penicillium (Sahoo and Gupta 2005; Shah et al. 2005; Kumari et al. 2002) and Klebsiella, Pseudomonas, Bacillus sps. Etc. Recently, laccase, lignin peroxidase, xylanase, endo-1,4-β-d-glucanase and exo-1,4-β-d-glucanase production by Aspergillus sp. on agricultural waste of banana under solid state fermentation (SSF) condition was reported by (Shah et al. 2005). Extracellular MnP production under alkaline conditions has been reported in Aspergillus terreus (Kanayama et al. 2002). Laccase production in Fusarium proliferated cultures, using wheat bran as a natural lignin–carbon source

and benzyl alcohol as laccase inducer has been reported by (Fernaud et al. 2006). Bacteria are use for biodegradation of ligninolytic enzyme in Chandra et.al (2017).

Table: 3.1: Different Treatment Techniques, their Preference/Advantage and Limitation for PMDE wastewater

Treatment	Merits	Demerits
Conventional		
Activated Sludge Process	Cost Effective requires small area.	If there is a sudden increase in the volume of sewage there are adverse effects on the working of the process.
Trickling Filter	Flexible in operation, Filter loading is high, required less land areas and smaller quantities of filter for installations.	The beds loss through these filters is high, Fly nuisance and odour nuisance may prevail.
UASB Bioreactor	Flexibility, Simplicity, Low Sludge Production, low space required	Long Start-up, Necessity of post- Treatment
Innovative		
TDS Reduction	Low cast, remove alkalinity & Conductivity	Requires Dilution (More than 5%), pH sensitive.
Fungal Treatment	Acceptable naturalness, Effectiveness and potentially high specificity, Protection of biodiversity in managed ecosystems	Can be very costly, They can have a short shelf life
Bacterial treatment	Bacteria are beneficial to clean up oil spills by the process of bioremediation, bacteria are used for production of pure chemicals, Bacteria re bioengineered for the production of proteins like insulin, growth hormones and also antibodies	Cause diseases and infection, cause death, bacterial disease caused by sexual contact.
Enzymatic		
Lignin peroxidise Maganese peroxidise Laccase	Uses in Bio-refinery, textile, Energy, bioremediation, cosmetology, and dermatology industries. do not need pre-conditioning to a particular pollutant tolerate a wide range of environmental conditions	Hyper pigmentation, skin-lightening, complex hetero-polymer.
Chemical Treatment		
Alkaline	Powerful Antioxidant Properties, Optimizes the pH Levels from Our Bodies, Boosts our Immunity, Helps with Weight Loss	The BOD/COD ratios increased. Maintaining pH value
Wet Oxidation	Lighter particles removed, Lighter particles removed	More complex/more maintenance,
Acid	Lighter particles removed,	Much Sensitive for human body, More complex/more maintenance

Source: - (Pant D&Adholeya A., 2006), (Bhargava et.al 2017)

3.4. Future prospects of Bioremediation in distillery wastewater.

Mostly with the incredibly quick industrial progress, environmental conservation is still one of the world's major concerns. In the manufacture and distribution of alcohol, the distillery industry uses also huge quantities of water and various types of chemical products, which generate high-risk resistance to waste This wastewater is classified as an environmental hazard because of the very high BOD and COD. The limitations of existing methods need therefore urgently be addressed and integrated treatment processes developed which offer a complete solution for the handling of wastewater in PMDE distillery. Microbial techniques give an environmentally friendly approach to the treatment of wastewater; however in situ management of pollutants these technologies are not easily handled. Different types of contaminants do not decolorize complicated industrial wastewater readily by a single step treatment procedure. Therefore, a new two-stage treatment/phase separation technique employing bacteria and a built-in wetland system might be a new, more viable approach to industrial wastewater bioremediation for high-strength complicated wastewater. The integration of the bacterial treated effluents (biodegradative bacteria) into the constructed wetland treatment system is generally effective in helping wastewater to breakdown contaminants and increasing the bioavailability of contaminants in plants. Two stage treatments have been found to be more efficient than single bacteria and plant treatment. Innovative approaches such as bacteria, fungus, yeast and their synergistic networks have shown efficient for distillery effluent decolorization. Combination of bacterial wetland processing technology gives a superb colour removing system from industrial wastewater and additional reductions in BOD, COD, TDS and heavy metals for safe disposal.. The effluent distillery has been effectively treated by Kumar and Chandra (2004), in two stages of treatment processes, comprising transformation of the resistant colouring components of the effluent by an Aerobic Bacterium B. thuringiensis. Similar biphasic effluent treatment was done by Chandra et al., (2008c), which resulted in a decrease of BOD, COD (98-99 percent) and of colour after 7 days, in a built wetland using B. thuringiensis and T. angustata L. The results indicate that the combined bacterial pretreatment of PMDE with phytoremediation will improve the process of PMDE treatment and promote a safer disposal of this waste. Kaushik et al. (2010) examined distillery treatment by use of fungus and bacteria, which wash in the three-stage bioreactor. Emiricella nidulans varlata first conducted treatment.

Intermediate Neurospora followed by Bacillus sp. After 30 hours, the treated wash exhibited a substantial decline in colour (82%) and COD (93%). In two phases in sequence treatments for PMDEs by bacteria and Phragmites communis, Chandra et al. (2012) found a 94.5% and 96.0% decrease in BOD/COD levels. During the two phases of sequential PMDE processing by bacteria and wetlands plants, he also characterised P. communis rhizosphere bacterial populations and metabolism products. Pant and Adholeya have described two-stage sequences for anaerobic digested wastewater from a cane molasses distillery (2009a). In the first stage, the effluent has been treated with the hydroponic system employing two kinds of plants (Vetiveria zizanioides and Phragmites karka). These plant roots demonstrated abundant effluent discharge. The elimination of nitrogen by 84% has been achieved. In this hydroponically treated effluent, the Aspergillus flavus (74.67 percent) was exposed to 86.33 percent decoloration, with considerable COD decreases. Therefore, the proposed technique is significant from the point of view of handling waste distillery without high dilution and inclusion of additional carbon sources. The effectiveness of the two-step treatment method has been shown at pilot level. This method has proven effective in the field and will likely be widely used for organic contaminants in waste water during the next 5 to 10 years.

Chapter-Four

Detection of complex Organo-metallic Compounds and other EDC related compounds of Distillery waste & their Environmental effect

Chapter-4

Detection of complex Organo-metallic Compounds and other EDC related compounds of Distillery waste and their Environmental effect

4.1. 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 (8,000-12,000 mg $L^{-1)}$, chemical oxygen demand (35,000-52,000 mg L^{-1}), nitrogenous compounds (15,284-28,696 mg L^{-1}), and total sulphate (3,875-4,096 mg L^{-1}). In addition, there is phosphate (1,625 mg L^{-1}), potassium at 537 mg L^{-1}, and chloride (7,842-7,997 mg/ L^{-1}) as salts and phenolic compounds (6,893-7,202 mg L^{-1}) (Bhargava and Chandra, 2010a, b). The dark color of PMDE is known due to the thermal processing of various water-soluble compounds in sugarcane juice, such as cane pigments, phenolics, and different amino–carbonyl compounds, which are likely to form a complex polymer known as melanoidin at elevated temperature (Yadav and Chandra, 2019). Melanoidin is a known non-enzymatic 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 a measurement for the presence of organic pollutant and their 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 sulphur compounds (Nguyen et al. 2013). The BOD/COD ratio (0.35) of an anaerobically degraded spent wash of distillery has been reported in a previous study. This indicated the non-degradability of waste with the presence of various complex compounds. Therefore, due to the high concentration of pollutants in the effluent. The bacterial community does not show any bioconversion (Chandra et al., 2008). Besides, the complex organic compounds there are substantial amounts of heavy metals reported in the PMDE, which increases its toxicity because of their strong binding tendency with the organic polymer component and their solubility under acidic conditions (Chandra et al., 2008). These organometallic wastes along

with the salts, phenolics, and chlorides, the effluent 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 presence of a significant amount of N and P as a source of nutrients to the microbial communities (Chowdhary et al., 2018). In India and various other countries use this highly toxic wastewater for agricultural irrigation due to lack of irrigation water. But, Irrigation with distillery wastewater in agricultural fields contributes to soil pollution, as a result, increases the heavy metal uptake by crop plants, 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, anaemia, 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 diarrhoea (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). Nickel, is also found in substantial amounts in distillery waste, can accumulate in plants and enter humans through the food chain. 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, have been reported for stunted stem growth and a reduced root system with inhibition of fruiting and flowering in *Phaseolus mungo* L. (Chandra et al., 2008). However, the toxicity data of PMDE with the aquatic organism are less known. Besides, recent studies have reported the presence of various recalcitrant

organic pollutants which are listed under the screening list of endocrine-disrupting chemicals (EDCs) by the (USEPA, 2012). The prominent compounds characterized by GC-MS in PMDE are hexanoic acid, butanoic acid, benzene propanoic acid, trimethylsilyl ester, monopalmitin 2-trimethylsilyl, effusion E, squalene, di-thioerythritol 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). Recently, the compounds with androgenic, mutagenic activity with EDCs properties have been reported in other industrial wastewater also including sewage and biomedical wastewater (Kostich et al., 2014; Chandra et al., 2017). However, detailed characteristics regarding such hazardous compounds from various sources of industrial waste for environmental risk assessment have not been yet much known. Moreover, Distilleries discharge an average of 12-15 L of the spent wash per litre of alcohol produced by Indian distilleries (Chandra et al., 2018). In India, more than 397 distilleries generate approx 28×10^{15}L of effluent annually, reflecting the magnitude of the environmental problem (AIDA, 2016). Therefore, this study will not reveal 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. This study was conducted in Bioremediation and Metagenomics Laboratory, Department of Environmental Microbiology, School of Environmental Sciences, Babasaheb Bhimrao Ambedkar University, (A Central University) Vidya Vihar, Raebareli Road, Lucknow-226025, UP., India during October 2018- November 2019.

4.2. Material and Methods

4.2.1. Sample collection

The PMDE sample was collected from M/s Unnao Distillers, Unnao, Uttar Pradesh, India (26°32'N, 80°30'E). The industry produces 225×10^3/L of ethanol per year, which generates approximately 3.0×10^9 L PMDE annually after anaerobic treatment (methanogenesis) (Chandra et al., 2018; Chandra and Kumar, 2018). The PMDE sample was collected in pre-sterilized 10.0 L plastic jerricans (Tarsons Products Pvt. Ltd.) from the distillery effluent discharge site. The sample was 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 hours for Physico-chemical analysis.

Fig. 4.1: (a) View of distillery effluent and their impact in environmental (b) Industrial contamination view (c)post methanated distillery effluent discharged after anaerobic treatment (d) collection of PMDE.

4.2.2. Physico-chemical analysis of PMDE

The Physico-chemical properties of color, pH, BOD, COD, TDS, TSS, chloride, total N, ammonium N, phenolics, and sulphate were analyzed as per standard methods for the examination of water and wastewater by the American Public Health Association (APHA, 2012). After nitric acid–perchloric acid digestion method no. 3030H heavy metals were analyzed by atomic absorption spectrophotometry (ZEEnit 700, Analytic Jena, Germany) as mentioned previously (Echavarría et al., 2013).

4.2.2.1. Color

Color in water may be resulted due to the presence of natural metallic ions (Fe and Mn), humus and peat materials, plankton, weeds and industrial wastes. Color is removed to make water suitable for general and industrial application. Colored

industrial waste water may require color removal before discharge into water courses. In some highly colored industrial wastewaters like distillery effluent, color is principally contributed by melanoidins.

(a) Principle

Color was determined by the visual comparison method of the sample with known concentration of colored samples. Comparison may also be made with special, properly calibrated, colored glass disks. The platinum-cobalt method of color measurement is the standard method, the unit of color being that produced by 1.0 mg platinum/l in the form of chloroplatinate ion. The ratio of cobalt to platinum may be varied to match the hue in special cases.

(b) Apparatus

a. Nessler tubes: Matched, 50 mL, tall form.

b. pH meter: for determining sample pH

(c) Reagents

a. Potassium chloroplatinate (K_2PtCl_6)

b. Cobaltous chloride ($CoCl_6.6H_2O$)

c. Hydrochloric acid (HCl)

(d) Procedure

a. Preparation of standards: Dissolved 1.246 g potassium chloroplatinate, K_2PtCl_6 (equivalent to 500 mg metallic Pt) and 1.0 g crystallized cobaltous chloride, $CoCl_2.6H_2O$ (equivalent to about 250 mg metallic Co) in distilled water containing 100 ml of concentrated HCl and diluted to 1000 ml with distilled water. This stock standard has a colour of 500 units. Then, prepared standards having colour of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, and 70 by diluting 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, and 7.0 mL stock standard with distilled water to 50 ml in Nessler tubes and protected these standards against evaporation and contamination when not in use.

(e) Calculation

Calculated the color units by the following equation

$$\text{Colour units} = \frac{A \times 50}{B}$$

Where, A = Estimation colour of a diluted sample B = mL sample taken for dilution.

4.2.2.2. Biological oxygen demand (5-Day BOD Test)

(a) Principle

The method consisted of filling with samples to overflowing an air tight bottle of the specified size and incubating it at the specified size and incubating it at the specified temperature for 5 days. Dissolved oxygen (DO) is measured initially and after incubation, and BOD is computed from the difference between initial and final D.O. Because the initial D.O. is determined immediately after the dilution is made, all oxygen uptake, including that occurred during the first 15 min is included in BOD measurement.

(b) Apparatus

a. Incubation bottles: 300 mL capacity.

b. Air incubator or water bath: Thermostatically controlled at 20±1 ºC exclude all light to prevent the possibility of photosynthetic production of DO.

(c) Reagents

a. Phosphate buffer solution: Dissolved 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4.7H_2O$ and 1.7 g NH_4Cl in about 500 mL distilled water and diluted to 1000 mL. The pH should be 7.2 without further adjustment.

b. Magnesium sulfate solution: Dissolved 22.5 g $MgSO_4.7H_2O$ in distilled water and diluted to 1000 mL.

c. Calcium chloride solution: Dissolved 27.5 g $CaCl_2$ in distilled water and diluted to 1000 ml.

d. Ferric chloride solution: Dissolved 0.25 g $FeCl_3.6H_2O$ in distilled water and diluted to 1000 mL.

e. Sodium sulfite solution: Dissolved 1.575 g Na_2SO_3 in 1000 mL distilled water.

f. Manganous sulphate solution: Dissolved 480 g $MnSO_4.4H_2O$ in 1000 mL distilled water.

g. Alkai-iodide azide reagent: Dissolved 500 g NaOH and 135 g NaI in 1000 mL distilled water.

h. Starch: Dissolved 2 g starch in 1000 mL distilled water.

i. Standard sodium thiosulphate: Dissolved 6.025 g $Na_2S_2O_3.5H_2O$ and 0.4 g NaOH in 1000 mL distilled water.

(d) Procedure

a. Preparation of dilution water: Placed 300 mL sample water in a suitable bottle and added 1ml each of phosphate buffer, $MgSO_4$, $CaCl_2$ and $FeCl_3$ solutions/l of water, seeded dilution water 1mL L^{-1}. Temperature was maintained at 20±1 ºC.

b. Determination of initial D.O.: To the sample collected in a 300 mL bottle, added 1.0 mL $MnSO_4$ solution followed by addition of 1.0 mL alkaline-iodide-azide reagent, mixed by inverting the bottle a few times. Let the precipitate to settle sufficiently and added 1.0 mL concentrated H_2SO_4 and mixed again by inverting bottle several times until dissolution was completed, titrated with 0.025 M $Na_2S_2O_3$ solution to a pale straw colour. Added few drops of starch solution and continued titration till disappearance of blue colour.

c. Incubation: Incubated at 20±1 ºC BOD bottles containing 1000 × dilution, seed controls and dilution water blanks.

d. Determination of final D.O.: After 5 day incubation period determined dissolved oxygen in sample dilution as described above.

(e) Calculation

$$BOD_5 \text{ (mg } L^{-1}) = \frac{(D_1-D_2)-(B_1-B_2)\,f}{P}$$

Where,

D_1 = D.O. of diluted sample immediately after preparation in mg L^{-1}

D_2 = D.O. of diluted sample after 5-day incubation at 20 ºC in mg L^{-1}

P = Decimal volumetric fraction of sample used

B_1 = D.O. of seed control before incubation in mg L^{-1}

B_2 = DO of seed control after incubation in mg L^{-1}

f = Ratio of seed in diluted sample to seed in seed control

4.2.2.3. Chemical oxygen demand (Open Reflux Method) (COD)

(a) Principle

Majority of organic matter are oxidized by a boiling mixture of chromic and sulfuric acids. A sample is refluxed in strongly acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion, the remaining unreduced ($K_2Cr_2O_7$) is titrated with ferrous ammonium sulfate to determine the amount of ($K_2Cr_2O_7$)

consumed and the oxidizable organic matter is calculated in terms of oxygen equivalent.

(b) Apparatus

a. Reflux apparatus: Consisting of 250 mL Erlenmeyer flasks with ground-glass 24/40 neck and 300 mm jacket Liebig, west or equivalent condenser with 24/40 ground-glass joint.

b. Hot plate: Having sufficient power to produce at least 1.4 w/cm^2 of heating surface.

(c) Reagents

a. Standard potassium dichromate solution, 0.0417 M: Dissolved 12.259 g $K_2Cr_2O_7$, previously dried at 103 ^oC for 2 h, in distilled water and diluted to1000 mL.

b. Sulphuric acid reagent: Added 5.5 g Ag_2SO_4 in 1000 g sulphuric acid (H_2SO_4). Let stood for 1 to 2 days to dissolve Ag_2SO_4.

c. Ferroin indicator solution: Dissolved 1.484 g phenonthroline monohydrate and 695 mg $FeSO_4.7H_2O$ in distilled water and diluted to 100 mL.

d. Standard ferrous ammonium sulphate (FAS), titrant, 0.25N: Dissolved 98 g ferrous ammonium sulphate [$Fe(NH_4)_2(SO_4)_2.6H_2O$] in distilled water. Added 20 mL of concentrated sulphuric acid (H_2SO_4), and diluted to 100 mL. Standardized the solution against standard $K_2Cr_2O_7$ solution as follows: Diluted 10 mL standard $K_2Cr_2O_7$ up to 100 ml. Added 30 mL concentrated hydrogen sulfides and cooled and titrated with FAS titrant using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator.

$$\text{Molarity of FAS solution} = \frac{\text{Volume of 0.0417 M } K_2Cr_2O_7 \text{ solution titrated (mL)} \times 0.25}{\text{Volume of FAS used in titration (mL)}}$$

e. Mercuric sulphate: Powdered mercuric sulphate ($HgSO_4$).

(d) Procedure

Taken 1.0 mL of sample and diluted to 50 mL in a 250 mL refluxing flask, added 1.0 g $HgSO_4$, some glass beads and 5 mL sulphuric acid reagent, (added very slowly), with mixing to dissolve $HgSO_4$. Cooled while mixing to avoid the loss of volatile materials and added 25 mL of 0.0417 M $K_2Cr_2O_7$ solution and mixed. Attached the flask to a condenser and turned on cooling water. Remaining sulphuric acid reagent (70 mL) was added through open end of condenser, continued swirling and mixing while adding sulphuric acid reagent. Open end of condenser was covered with a small beaker to prevent foreign material from entering refluxing mixture and refluxed for 2 h. Cooled and washed down condenser with distilled water. Disconnected reflux

condenser and diluted mixture to about twice its volume with distilled water. Cooled to room temperature and titrated excess $K_2Cr_2O_7$ with FAS using 2-3 drops (0.10 to 0.15 mL) ferroin indicator. The first sharp change from blue-green to reddish brown was taken as end point of the titration. In same manner, refluxed and titrated a blank containing the reagents and a volume of distilled water equal to that of sample.

(e) Calculation

$$\text{COD (mg } O_2\text{ /l)} = \frac{(A-B) \times M \times 8000}{\text{mL of sample}}$$

Where,

A = mL FAS used for blank

B = mL FAS used for sample

M = Molarity of FAS

4.2.2.4. Total Nitrogen (Macro-Kjeldhal Method)

The Kjeldhal method determines nitrogen in the trinegative state. It fails to account nitrogen in the form of azide, azime, azo, hydrazone, nitrate, nitroso, oxime and semi-carbazone. This method is used for samples having NH_3-N concentration greater than 5 mg L^{-1}. Since, distillery effluent is waste water and contains large quantities of nitrogen, hence we employed this method. This method also gives least relative error as compared to other methods.

(a) Principle

In presence of sulphuric acid (H_2SO_4), potassium sulphate (K_2SO_4) and mercuric sulphate ($HgSO_4$) as catalyst, amino nitrogen of many organic materials are get converted into ammonium sulphate [$(NH_4)_2SO_4$]. Free ammonia and ammonium-nitrogen also are converted to $NH_4)_2SO_4$. During sample digestion, a mercury ammonium complex is formed and then decomposed by sodium thiosulphate ($Na_2S_2O_3$). After decomposition, ammonia is distilled from an alkaline medium and absorbed in boric or sulphuric acid. The ammonia is determined colorimetrically or titration with a standard mineral acid.

(b) Apparatus

a. Digestion apparatus: Kjeldhal flask with a total capacity of 800 ml, a heating device that can provide the temperature range of 365 to 370 ºC for effective digestion.

b. Distillation apparatus: A borosilicate glass of 800 to 2000 ml capacity attached to a vertical condenser so that the outlet tip may be submerged below the surface of the receiving acid solution.

(c) Reagents

Ammonia free water was used in making all of reagents.

a. Digestion reagent: Dissolved 134 g K_2SO_4 in 650 mL water containing 200 ml of concentrated H_2SO_4. Added with stirring 25 mL $HgSO_4$ solution and diluted the combined solution to 1000 ml with distilled water. Temperature was kept close to 20 ^oC to prevent crystallization.

b. Sodium hydroxide-sodium thiosulphate reagent: Dissolved 500 g NaOH and 25 g $Na_2S_2O_3.5H_2O$ in distilled water and diluted to 1000 mL.

c. Mixed indicator solution: Dissolved 200 mg methyl red indicator in 100 ml 95% ethyl alcohol. Combined the solution and mixed well.

d. Boric acid solution: Dissolved 20 g H_3BO_4 in distilled water, added 10 ml mixed indicator solution and diluted to 1000 mL.

e. Standard sulfuric acid titrant, 0.02 N: Diluted 200 ml 0.1 N standard acid to 1000 ml with distilled water and standardized by potentiometric titration of 15 mL 0.05 N Na_2CO_3.

(d) Procedure

A measured volume of sample (25 mL of distillery effluent) was taken in 800 mL Kjeldhal flask to which 50 mL digestion mixture was added carefully. After mixing, the contents were heated gently under a hood to remove acid fumes. Then, it was boiled briskly until the volume was greatly reduced (to about 25 mL) and copious white fumes emanated. The digestion was continued for an additional 30 min or up to charred ash. After digestion, flask and contents were allowed to cool and diluted up to 300 mL with distilled water and mixed well. Tilted the flask and added carefully 35 mL sodium hydroxide-thiosulphate reagent to form an alkaline layer at the flask bottom. Connected flask to steamed-out distillation apparatus and shaken the flasks to insure complete mixing. A black precipitate, HgS was formed. The contents were then distilled and 300 mL distillate was collected in other flask containing 50 mL boric acid (absorbent) solution. This purple coloured indicator solution turns green, indicating the presence of nitrogen. Now titrated the solution against 0.02 N H_2SO_4 as

titrant. The end point was indicated by the reappearance of purple color. A blank was run simultaneously throughout the process.

(e) Calculation

$$N_{org}\ (mg\ L^{-1}) = \frac{(A-B) \times 280}{mL\ of\ sample}$$

Where,

A = Volume of H_2SO_4 titrated for sample (mL)

B = Volume of H_2SO_4 titrated for blank (mL)

4.2.2.5. Total dissolved solids (dried at 180 °C)

(a) Principle

A well mixed sample is filtered through a standard glass fiber filter, and the filtrate is evaporated to dryness in a weighed dish and dried to constant weight at 180 °C. The increase in dish weight represents the total dissolved solids. This procedure may be used for drying at other temperatures.

(b) Apparatus

a. Evaporating dishes: Dishes of 100 mL capacity made of one of the following materials:

1) Porcelain, 90 mm diam.

2) Platinum-Generally satisfactory for all purposes.

3) High-silica glass.

b. Muffle furnance for operation at 550 °C.

c. Steam bath

d. Desiccator, provided with a desiccant containing a color indicator of moisture concentration or an instrument indicator.

e. Drying oven, for operating at 103 to 105 °C.

f. Analytical balance, capable of weighing to 0.1 mg.

g. Magnetic stirrer with TFE stirring bar.

h. Wide-bore pipets

i. Graduated cylinder

j. Low form beaker

k. Glass-fiber filter disks without organic binder

l. Filtration apparatus with reservoir and coarse (40 to 60 µm) fritted disk as filter support.

Suction flask of sufficient capacity for sample size selected.

4) Drying oven, for operation at 180±2 °C.

(c) Procedure

Heated clean dish to 103 to 105 ºC for 1 h. Cooled in desiccator and weighed immediately. Now 50 ml of well mixed sample was taken in a pre-weighed dish and evaporated to dryness in a drying oven at approximately 2 ºC below boiling to prevent splattering. Dried evaporated sample for at least 1 h in an oven at 103 to 105 ºC, cooled dish in desiccators to balance temperature and weighed. Repeated cycles of drying, cooling, desiccating and weighing till a constant weight was obtained, or until weight change was less than 4% of previous weight or 0.5 mg.

(e) Calculation

$$\text{mg total dissolved solids/L} = \frac{(A-B) \times 1000}{\text{sample volume, mL}}$$

Where, A= weight of dried residue + dish, mg, and B= weight of dish, mg

4.2.2.6. Total solids (Dried at 103-105 °C)

(a) Principle

A well mixed sample is evaporated in a weighed dish and dried to constant weight in an oven at 103 to 105 °C. The increase in weight over that of the empty dish represents the total solids.

(b) Apparatus

a. Evaporating dishes: Dishes of 100 mL capacity made of one of the following materials:

1) Porcelain, 90 mm diam

2) Platinum-Generally satisfactory for all purposes.

3) High-silica glass.

b. Muffle furnace for operation at 550 °C.

c. Steam bath.

d. Desiccator, provided with a desiccant containing a color indicator of moisture concentration or an instrumental indicator.

e. Drying oven, for operating at 103 to 105 °C.

f. Analytical balance, capable of weighing to 0.1 mg.

g. Magnetic stirrer with TFE stirring bar.

h. Wide-bore pipets.

i. Graduated cylinder

j. Low-form beaker

(c) Procedure

Heated clean dish to 103 to 105 ºC for 1 h. Cooled in desiccator and weighed immediately. Now 50 ml of well mixed sample was taken in a pre-weighed dish and evaporated to dryness in a drying oven at approximately 2 ºC below boiling to prevent splattering. Dried evaporated sample for at least 1 h in an oven at 103 to 105 ºC, cooled dish in desiccator to balance temperature and weighed. Repeated cycles of drying, cooling, desiccating and weighing till a constant weight was obtained, or until weight change was less than 4% of previous weight or 0.5 mg.

(e) Calculation

$$\text{mg total solids/L} = \frac{(A-B) \times 1000}{\text{Sample volume, mL}}$$

Where, A= weight of dried residue + dish, mg, and B= weight of dish, mg

4.2.2.7. Estimation of phenolics

(a) Principle

Phenols, defined as hydroxy derivatives of benzene and its condensed nuclei may occur in domestic and industrial waste waters. Steam-distillable phenols react with 4-amino antipyrine at pH 7.9±0.1 in presence of potassium ferricyanide to form a coloured antipyrine dye. This dye is extracted from aqueous solution with $CHCl_3$ and the absorbance is measured at 460 nm. This method covers the phenol concentration ranging from 1.0 µg L^{-1} to over 250 µg L^{-1} with a sensitivity of 1 µg L^{-1}.

(b) Apparatus

a. Photometric equipment: A spectrophotometer.

b. Filter funnels: Buchner type with fritted disk.

c. Filter paper: alternatively, an appropriate 11 cm filter paper for filtering chloroform ($CHCl_3$) extracts instead of the buchner-type funnels and anhydrous Na_2SO_4 can be used.

d. pH meter.

e. Separatory funnels: 1000 mL Squibb form, without ground-glass stoppers and TFE stopcocks.

(c) Reagents

Prepared all the reagents in distilled water, it should be free of phenols and chlorines.

a. Stock phenol solution: Dissolved 100 mg phenol in freshly boiled and cooled distilled water and diluted to 100 mL.

b. Intermediate phenol solution: Diluted stock phenol solution (1 mL) in freshly boiled and cooled distilled water to 100 ml; [1 mL = 10 µg phenol, Prepared daily].

c. Standard phenol solution: Diluted 50 ml intermediate phenol solution to 500 ml with freshly boiled and cooled distilled water. [1 mL = 1 µg phenol, Prepared with in 2 h of use].

d. Bromate-bromide solution: Dissolved 2.784 g anhydrous potassium bromo-oxide ($KBrO_3$) in distilled water. Then, added 10 g potassium bromide (KBr) crystals, dissolved and diluted to 1000 mL.

e. Hydrochloric acid: Concentrated hydrochloric acid (HCl)

f. Ammonium hydroxide (NH_4OH), 0.5 N: Diluted 35 mL fresh concentrated NH_4OH to 1000 ml with distilled water.

g. Standard sodium thiosulphate titrant, 0.025 M: Dissolved 6.025 g $Na_2S_2O_3.5H_2O$ and 0.4 g NaOH in 1000 mL distilled water.

h. Starch solution: Dissolved 2.0 g laboratory-grade soluble starch and 0.2 g salicylic acid, as a preservative, in 1000 ml hot distilled water.

i. Phosphate buffer solution: Dissolved 104.5 g K_2HPO_4 and 72.3 g KH_2PO_4 in water and dilute to 1000 mL distilled water (pH=6.8).

j. 4-aminoantipyrine solution: Dissolved 2.0.g 4-aminoantipyrine in water and diluted to 100 mL. Prepared daily.

k. Potassium ferricyanide solution: Dissolved 8.0 g $K_3Fe(CN)_6$ in water and diluted to 100 ml. Filtered, if necessary, it was stored in brown glass bottle or prepared freshly.

l. Chloroform: Laboratory grade chloroform ($CHCl_3$).

m. Sodium sulphate: Anhydrous sodium sulphate (Na_2SO_4).

n. Potassium iodide: Laboratory grade potassium iodide (KI) crystals.

(d) Procedure

A measured volume (25 mL) of sample was taken in a distillation flask and its pH was adjusted to 2-3 using 1N hydrochloric acid and removed oil and grease from the sample by transferring it in a separatory funnel and extracted oil and grease with 25 mL of chloroform. Repeated this process twice to ensure complete removal of oil and grease, then added four drops of orthophosphoric acid and three drops methyl orange indicator to the sample. Distilled the solution and placed 500 mL distillate in a 1000 mL flask. Prepared a 500 ml blank and a series of 500 mL phenol standards 5, 10, 20, 30, 40, and 50 µg phenol. The treated samples, blank and standards as follows: added 12.0 mL 0.5 N NH_4OH and immediately adjusted pH to 7.9±0.1 with phosphate buffer (10 mL) and transferred it to a 1000 mL separating funnel. Added 3 mL of amino antipyrine solution, mixed well followed by addition of 3 ml $K_3Fe(CN)_6$ solution. Mixed the solution well and let colour to develop for 15 min. Extracted immediately with 50 ml of chloroform each time. Shaken the separating funnel many times (10 times), let $CHCl_3$ to settle, shaken again and let the $CHCl_3$ to settle again. Filtered each $CHCl_3$ extract through filter paper or fritted glass funnels containing a 5 g layer of anhydrous Na_2SO_4. The dried extract was collected in clean test tubes or cells for absorbance measurements. Read absorbance of samples and standard against the blank at 460 nm. Constructed calibration curve by plotting absorbance against the micrograms of phenol concentration and calculated the amount of phenol in samples using this curve.

(e) Calculation

$$\text{Phenol } (\mu g\ L^{-1}) = \frac{A \times 100}{B}$$

Where,

A = µg phenol in sample, from calibration curve

B = mL original sample

4.2.2.8 Total organic carbon (High temperature combustion method)

(a) Principle

The sample is homogenized and diluted as necessary and microportion is injected into a heated reaction chamber packed with an oxidative catalyst such as cobalt oxide, platinum group metals, or barium chromate. The water is vaporized and the organic

carbon is oxidized to CO_2 and H_2O. The CO_2 from oxidation or organic and inorganic carbon is transported in the carrier gas streams and is measured by means of a nondispersive infrared analyzer, or titrated coulometrically. Because total organic carbon is measured, inorganic carbon must be removed by acidification and sparging or measured separately and TOC obtained by difference.

(b) Apparatus

a. Total organic carbon analyser

b. Sampling, injection, and sample preparation accessories, as prescribed by instrument manufacture.

c. Sample blender or homogenizer.

d. Magnetic stirrer and TFE-coated stirring bars.

e. Filtering apparatus and 0.45 µm filter.

(c) Reagents

a. Reagent water: Prepare reagents, blanks, and standard solution from reagent water with TOC value less than 2× the MDL.

b. Acid: Phosphoric acid, H_3PO_4

c. Organic carbon stock solution: Dissolve 2.1254 g anhydrous primary standard grade potassium biphthalate, $C_8H_5KO_4$, in carbon free water and dilute to 1000 mL; 1.0 mL=1.00 mg carbon. Prepare laboratory control standards using any other appropriate organic-carbon containing compound adequate purity, stability, and water solubility. Preserve by acidifying with H_3PO_4 or H_2SO_4 to pH≤2, and store at 4 ^oC.

d. Inorganic carbon stock solution: Dissolve 4.4122 g anhydrous sodium carbonate in water, add 3.497 g anhydrous sodium bicarbonate and dilute to 1000 mL; 1.00 mL=1.00 mg carbon.

Carrier gas: Purified oxygen or air, CO_2 free and containing less than 1 ppm hydrocarbons (as methane)

f. Purging gas: Any gas free of CO_2 and hydrocarbons.

(d) Procedure

a. Instrument operation: Follow manufacturer’s instructions for analyzer assembly, testing, calibration, and operation. Adjust to optimum combustion temperature before using instrument; monitor temperature to insure stability.

b. Sample treatment: If a sample contains gross solids or insoluble matter, homogenize until satisfactory replication is obtained. Analyze a homogenizing blank consisting of reagent water carried through the homogenizing treatment.

If inorganic carbon must be removed before analysis, transfer a representative portion (10 to 15 mL) to a 30-mL beaker, add acid to reduce pH to 2 or less, and purge with gas for 10 min. Inorganic carbon also may be removed by stirring the acidified sample in a beaker while directing a stream of purified gas into the beaker. Because volatile organic carbon will be lost during purging of the acidified solution, report organic carbon as total nonpurgeable organic carbon. Check efficiency of inorganic carbon removal for each sample matrix by splitting a sample into two portions and adding to one portion an inorganic carbon level similar to that of the sample. The TOC values should agree; if they do not, adjust sample container, sample volume, pH, purge gas flow rate, and purge time to obtain complete removal of inorganic carbon. If the available instrument provides for a separate determination of inorganic carbon (carbonate, bicarbonate, free CO_2) and total carbon, omit decarbonation and determine TOC by difference between TC and inorganic carbon. If dissolved organic carbon is to be determined, filter sample through 0.45 µm pore-diam filter; analyze a filtering blank.

c. Sample injection: Withdraw a portion of prepared sample using a syringe fitted with a blunt-tipped needle. Select sample volume according to manufacturer's direction. Stir samples containing particulates with a magnetic stirrer. Select needle size consistent with sample particulate size. Repeat injections until consecutive measurements are obtained that are reproducible to within ±10%

d. Preparation of standard curve: Prepare standard organic and inorganic carbon series by diluting stock solutions to cover the expected range in samples within the linear range of the instrument. Dilute samples higher than the linear range of the instrument in reagent water. Inject and record peak height or area of these standards and a dilution water blank. Plot carbon concentration in mg L^{-1} against corrected peak height or area on rectangular coordinate paper.

(e) Calculation

Calculate corrected instrument response of standards and samples by subtracting the reagent-water blank instrument response from that of the standard and sample. Prepare a standard curve of corrected instrument response vs. TOC concentration. Subtract procedural blank from each sample instrument response and compare to

standard curve to determine carbon content. Apply appropriate dilution factor when necessary. Subtract inorganic carbon from total carbon when TOC is determined by difference.

4.2.2.9. Chloride (Cl^-) (Argentometric Method)

(a) Principle

In a neutral or slightly alkaline solution, potassium chromate can indicate the end point of the silver nitrate titration of chloride. Silver chloride is precipitated quantitatively before red silver chromate is formed.

(b) Apparatus

a. Erlenmeyer flask, 250 mL.

b. Buret, 50 mL.

(c) Reagents

a. Potassium chromate indicator solution: Dissolve 50 g K_2CrO_4 in a little distilled water.

Add $AgNO_3$ solution until a definite red precipitate is formed. Let stand 12 h, filter, and dilute to 1 L with distilled water.

b. Standard silver nitrate titrant, 0.0141M (0.0141N): Dissolve 2.395 g $AgNO_3$ in distilled water and dilute to 1000 ml. Standardize against NaCl by the procedure described in 4b below; 1.00 mL = 500 µg Cl^-. Store in a brown bottle.

c. Standard sodium chloride, 0.0141M (0.0141N): Dissolve 824.0 mg NaCl (dried at 140°C) in distilled water and dilute to 1000 mL; 1.00 mL = 500 µg Cl^-.

d. Special reagents for removal of interference:

1) Aluminum hydroxide suspension: Dissolve 125 g aluminum potassium sulfate or aluminum ammonium sulfate, $AlK(SO_4)_2 \cdot 12H_2O$ or $AlNH_4(SO_4)_2.12H_2O$, in 1 L distilled water. Warm to 60 °C and add 55 mL conc. ammonium hydroxide (NH_4OH) slowly with stirring. Let stand about 1 h, transfer to a large bottle, and wash precipitate by successive additions, with thorough mixing and decanting with distilled water, until free from chloride. When freshly prepared, the suspension occupies a volume of approximately 1 L.

2) Phenolphthalein indicator solution.

3) Sodium hydroxide, NaOH, 1N.

4) Sulfuric acid, H_2SO_4, 1N.

5) Hydrogen peroxide, H_2O_2, 30%.

(d) Procedure

a. Sample preparation: Use a 100-mL sample or a suitable portion diluted to 100 mL. If the sample is highly colored, add 3 mL $Al(OH)_3$ suspension, mix, let settle, and filter. If sulfide, sulfite, or thiosulfate is present, add 1 mL H_2O_2 and stir for 1 min.

b. Titration: Directly titrate samples in the pH range 7 to 10. Adjust sample pH to 7 to 10 with H_2SO_4 or NaOH if it is not in this range. For adjustment, preferably use a pH meter with a non-chloride-type reference electrode. (If only a chloride-type electrode is available, determine amount of acid or alkali needed for adjustment and discard this sample portion. Treat a separate portion with required acid or alkali and continue analysis.) Add 1.0 mL K_2CrO_4 indicator solution. Titrate with standard $AgNO_3$ titrant to a pinkish yellow end point. Be consistent in end-point recognition. Standardize $AgNO_3$ titrant and establish reagent blank value by the titration method outlined above. A blank of 0.2 to 0.3 mL is usual.

(e) Calculation

$$\text{Mg } Cl^-/L = \frac{(A-B) \times N \times 35450}{\text{Sample volume, mL}}$$

Where,

A= mL titration for sample

B= mL titration for blank, and

N= normality of AgNO3

mg NaCl/L = (mg Cl^-/L) × 1.65

4.2.2.10. Sulfate (SO_4^{2-}) (Gravimetric Method with Drying of Residue)

(a) Principle

Sulfate is precipitated in a hydrochloric acid (HCl) solution as barium sulfate ($BaSO_4$) by the addition of barium chloride ($BaCl_2$). The precipitation is carried out near the boiling temperature, and after a period of digestion the precipitate is filtered, washed with water until free of Cl^-, ignited or dried, and weighed as $BaSO_4$.

(b) Apparatus

a. Steam bath.

b. Drying oven, equipped with thermostatic control.

c. Muffle furnace, with temperature indicator.

d. Desiccator.

e. Analytical balance, capable of weighing to 0.1 mg.

f. Filter: Use one of the following:

1) Fritted-glass filter, fine (‘‘F’’) porosity, with a maximum pore size of 5 μm.

2) Membrane filter, with a pore size of about 0.45 μm.

b. Vacuum oven.

(c) Reagents

a. Methyl red indicator solution: Dissolve 100 mg methyl red sodium salt in distilled water and dilute to 100 mL.

b. Hydrochloric acid, HCl, 1 + 1.

c. Barium chloride solution: Dissolve 100 g BaCl2· 2H2O in 1 L distilled water. Filter through a membrane filter or hard- finish filter paper before use; 1 mL is capable of precipitating approximately 40 mg SO_4^{2-}.

d. Silver nitrate-nitric acid reagent: Dissolve 8.5 g AgNO3 and 0.5 mL conc. HNO_3 in 500 mL distilled water.

(c) Procedure

a. Precipitation of barium sulfate: A measured volume of sample (50 mL) was taken in a flask and pH was adjusted to 4.5 to 5.0 with HCl using a pH meter or the orange color of methyl red indicator. Added 1 to 2 mL HCl and heated to boiling and while stirring gently, slowly added warm $BaCl_2$ solution until precipitation appeared to be completed, then added about 2 mL in excess, digested precipitate overnight at 80 to 90 ºC.

b. Filtration and Weighing: Filtered $BaSO_4$ through a pre-weighed membrane filter at room temperature. Washed precipitate with several small portions distilled water until washings are free of Cl^- as indicated by testing with $AgNO_3$-HNO_3 reagent. Added a few drops of silicone fluid to the suspension before filtering, to prevent adherence of precipitate to holder. Dried filter and precipitated in a conventional oven at a temperature of 103 to 105 ºC. Cooled in desiccator and weighed.

(e) Calculation

$$\text{mg } SO_4^{2-}/L = \frac{\text{mg } BaSO_4 \times 411.5}{\text{mL sample}}$$

4.2.2.11. Phosphate

(a) Principle

Organic phosphates are formed primarily by biological processes. They are contributed to sewage by body wastes and food residues and also may be formed from

orthophosphates in biological treatment processes or by receiving water biota. Molybdophosphoric acid is formed and reduced by stannous chloride to intensely coloured molybdenum blue. The minimum detectable concentration is about 3 µg phosphate/l. The sensitivity at 0.3% absorbance is about 10 µg P/l for an absorbance change of 0.009.

(b) Apparatus

a. Colorimetric equipment: Spectrophotometer (400-490 nm).

b. Acid washed glassware: Cleaned all glassware with hot diluted HCl and rinsed well with distilled water,

c. Filtration apparatus and filter paper: Whatman No. 42 or equivalent.

(c) Reagents

a. Phenolphthalein indicator aqueous solution.

b. Strong-acid solution: Slowly added 300 ml of concentrated H_2SO_4 to about 600 mL distilled water. When cold, added 4.0 ml concentrated HNO_3 and diluted to 1000 mL.

c. Ammonium molybdate reagent I: Dissolved 25 g $(NH_4)_6MO_7O_{24}.4H_2O$ in 175 ml distilled water. Cautiously added 280 ml concentrated H_2SO_4 to 400 ml distilled water. Cooled, added molybdate solution and diluted to 1000 mL.

d. Stannous chloride reagent I: Dissolved 2.5 g fresh $SnCl_2.2H_2O$ in 100 mL glycerol. Heated in a water bath and stirred with a glass-rod to hasten dissolution. The reagent was stable and required neither preservative nor special storage.

e. Standard phosphate solution: Dissolved 219.5 mg anhydrous KH_2PO_4 in distilled water and diluted to 1000 mL; 1.00 mL= 50.0 µg PO_4^{3-}- P.

f. Reagents for extraction:

1. Benzene-isobutanol solvent: Mixed equal volumes of benzene and isobutyl alcohol.

2. Ammonium molybdate reagent II: Dissolved 40.1g $(NH_4)_6MO_7O_{24}.4H_2O$ in approximately 500 ml distilled water. Slowly added 396 mL ammonium molybdate reagent I. Cooled and diluted to 1000 mL

3. Alcoholic sulphuric acid solution: Cautiously added 20 mL conc. H_2SO_4 to 980 ml methyl alcohol with continuous mixing.

4. Diluted stannous chloride reagent II: Mixed 8 mL stannous chloride reagent I with 50 ml glycerol. The reagent was stable for at least 6 months.

(d) Procedure

A measured volume (20 mL) of sample was taken and diluted to 100 ml with distilled water and added 4.0 mL molybdate reagent I and 0.5 mL (10 drops) stannous chloride reagent I. After 10 min, but before 12 min, measured colour photometrically at 690 nm and compared with a calibration curve using distilled water blank.

(e) Calculation

$$P\ (mg\ L^{-1}) = \frac{mg\ P\ (in\ approximately\ 104.5\ ml\ final\ volume)}{mL\ of\ sample} \times 1000$$

4. The sample after digestion was treated with acid water mixture and filtered through Whatman No. 42 filter paper.

5. The filtrate was analyse for total Fe, Mn, Ni, Zn, Cu, Pb and Cd by using atomic absorption spectrophotometer.

(e) Calculations

Concentration of element (mg L^{-1}) = (Observed concentration – Blank) × Dilution factor. Results were calculated up to three significant figures.

4.2.3. Extraction and identification of organic pollutants

4.2.3.1. 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 the same volume (10 mL) of ethyl acetate in a separating funnel (100 mL). The mixture was continuously shaken for 2- 4 h to mix the solvent, with liquid-liquid extraction at irregular time intervals. After proper shaking, the solution was kept to separate the organic solvent. Thereafter, the organic component was further separated in a clean beaker; this process was repeated thrice to completely extract complete organic pollutants. Finally, all the extracted organic solvent was pooled in a clean beaker. Then, it was dehydrated by passing it through anhydrous sodium sulfate. The extracted organic solvent with organic pollutants was dried by using a rotavapor (Rotavapor RE 120; Buchi, Flawil, Sweden) at low pressure. Then, the dried residue was dissolved in 1 mL of ethyl acetate, filtered using

a 0.2 µm syringe filter (Millipore Ltd., Bedford, MA, USA), and further, it was used in FTIR and Gas Chromatography-Mass Spectrometry (GC–MS) analysis.

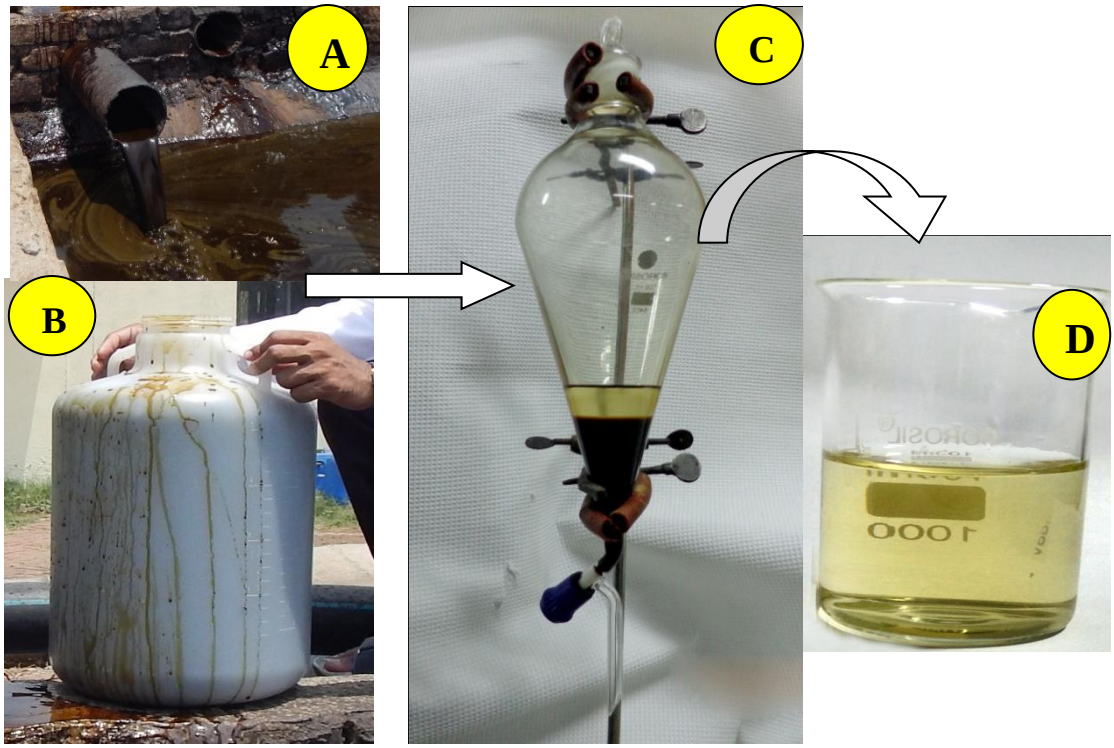


Fig. 4.2: Extraction of organic pollutants by using liquid-liquid extraction procedure (a) PMDE mixed with organic solvent (b) Collection of PMDE in Jeri cane (c) Extraction of PMDE using solvent (d) After extraction solvent separation for further analysis

4.2.3.2 Scanning electron microscopy and energy-dispersive X-ray spectroscopy

Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) was performed to assess the surface morphology of dissolved pollutants in PMDE. The sample was kept into an aluminium foil for drying in the oven for 4 to 5 hours at 600C followed by keeping Desiccator for 2 hours and the sample was 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 micro pore 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).

4.2.3.3. Ultraviolet-visible and Fourier transform-infrared spectroscopic analyses

To evaluate the absorption pattern of the various pollutants 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 from 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 previously described (Chandra et al., 2018). The FTIR spectrum for the analysis of PMDE samples was done in the range from 400 - 4,000 cm−1 (Causin et al., 2008).

4.2.3.4. Derivatization of the extracted sample

A sample of PMDE (300µL) was transferred to GC vials and evaporated to dryness by nitrogen gas. Then, Derivatisation was performed by adding 50µl pyridine and 80µl trimethylsilyl BSTFA and TMCS were added to 300µl samples. Lastly, the mixture was heated to 700C 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).

4.2.3.5. Gas chromatography–Mass spectrometry analysis

The extracted PMDE and sludge leachate samples were derivatized with trimethylsilyl (TMS), as defined in GC–MS analysis (Chandra et al., 2017). In this process, 50µl pyridine and 80µl trimethylsilyl BSTFA and TMCS were added to 300µl samples. Further, the sample was heated to 700C 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 the compounds available in the National Institute of Standards and Technology (https://www.nist.gov/).

4.2.4. Phototoxicity assay with post-methanated distillery effluent

4.2.4.1. Seed germination experiment

The phytotoxicity test of the PMDE was used seed germination test of Zea mays L. Seed germination was evaluated at different concentrations of effluent using a Petri dish method as described previously (David and Rajan, 2015). Ten sterilized seeds were put in different Petri dishes at the same spacing. The dishes contained the PMDE concentrations of 10%, 20%, 40%, 60%, 80%, and 100% (v/v), while tap water was

used as the control in the same condition. The dishes were incubated at 28°C till germination (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):

$$\text{Percentage of Phytotoxicity (\%)} = \frac{\text{Radicle length of control- Radicle length of the test}}{\text{Radicle length of the control sample}} \times 100 \ldots\ldots (1)$$

$$\text{Index of Tolerance (\%)} = \frac{\text{Mean Length of the longest root in the treatment}}{\text{Mean length of the longest root in the control sample}} \times 100 \ldots\ldots\ldots (2)$$

4.2.4.2. Enzyme extracts preparation and screening of α-amylase activity

Seeds of each treatment were homogenized in 0.1 M sodium acetate buffer to prepare the enzyme extract (pH 4.8). Large particles in the solution were filtered through two layers of cheese cloth, and the supernatant was centrifuged at 15,000×g for 20 min. All experiments were performed at 4^0C to maintain 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).

4.2.4.3. Enzyme concentration and purification

The supernatant with crude α-amylase enzyme was further concentrated by the addition of cold acetone (−200C) 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 millilitres of protein fraction was eluted at the flow rate of 0.5 mL min−1 and then stored at −200C until further analysis (Bhargava and Chandra, 2010a).

4.2.4.4 Determination of enzyme molecular weight using SDS-PAGE

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)

4.2.5. Toxicity of post-methanated distillery effluent to a fish

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

4.2.5.1. Animal collection and maintenance

Live and healthy adult Heteropneustes fossilis (35-45 g) were collected from the local fish market during the pre-spawning 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 ± 20C). During the acclimatization period, fish were fed ad libitum with goat liver daily.

4.2.5.2. Experimental Set-up

The PMDE toxicity test was performed to determine the effect in terms of the LC50 (Erhirhie et al., 2018) in a 24-h exposure. Fishes were divided into four groups in duplicates (n=2) with 10 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 control in plant fresh water. the control in plain water. After 24 h, mortality data were recorded to calculate the LC50. Fish were sacrificed 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 −200C until catalase analysis. Catalase activity was used to check the stress level caused by exposure to the effluent.

4.2.5.3 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 620C). 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.

4.2.5.4 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).

4.3. Results and Discussion

4.3.1. Physico-chemical 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, in addition to other pollution parameters, were very high as shown in Table 1. In addition, the PMDE has been 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 non-degradability of discharged effluent as reported in earlier studies regarding various wastewater (Chou 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 TDS and other organic compounds corroborated with the previous analysis reported anaerobically treated distillery spent wash (Chandra et al., 2008; Sankaran et al., 2014). The detailed mechanism for the formation of complicated colouring compounds in anaerobically treated distillery effluent has been also reported by various studies (Sankaran et al., 2014). The alkaline pH of PMDE favored 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

S.No.	Parameters	Values	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 $CaCo_3$	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

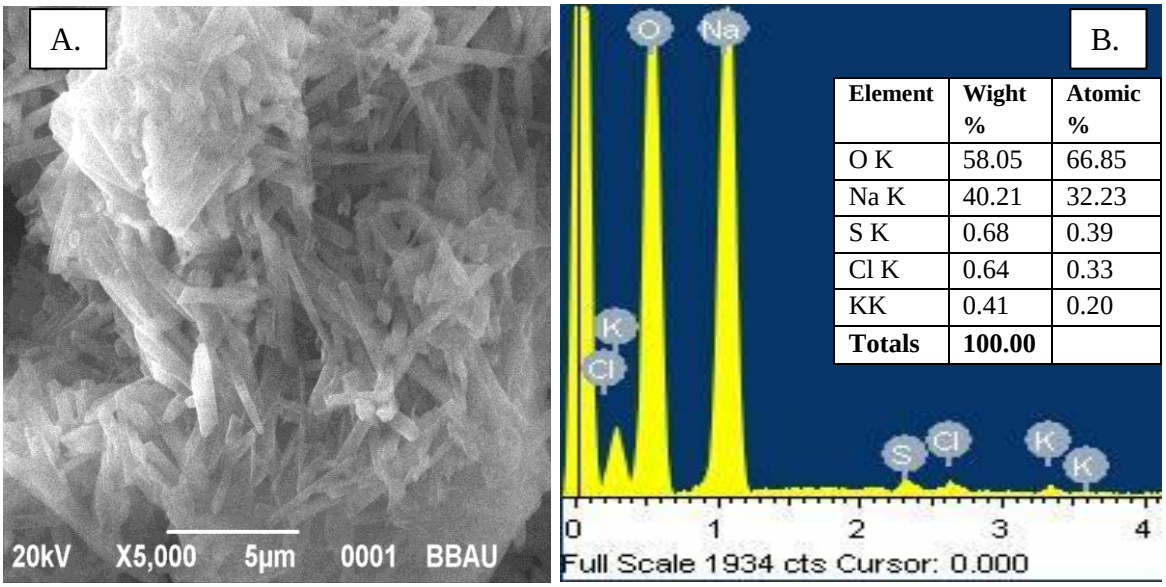
All values are mean (n=3) ±SD in mg l^{-1} except color intensity (Co-Pt unit), pH, and temperature (^{0}C), (BOD): - at $p < 0.001$, [b]Less significant at $p < 0.0$

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 5,000× (fig 1a). This indicated the presence of melanoidin, the crystalline rod structure of polymer has been reported in previous studies (Gomes et. al., 2019). These findings strongly supported our current observations, this also corroborated

with amorphous organometallic pollutants of the environment under scanning electron microscopy (Batista et al., 2017) as shown in Fig. 1a & b, the elemental analysis by SEM-EDX with their percent composition along with organic pollutants indicated the presence of multi-metals complex along 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 sulphur (0.68%), Cl (0.64%), and K (0.14%). The EDX-elemental analysis to confirm the elemental composition in the effluent. The high concentrations of various salts in the effluent indicated that it could be a major source of pollution in aquatic and terrestrial ecosystems.



Element	Wight %	Atomic %
O K	58.05	66.85
Na K	40.21	32.23
S K	0.68	0.39
Cl K	0.64	0.33
KK	0.41	0.20
Totals	**100.00**	

Fig: 4.3: A-Surface topology of PMDE by SEM images **B-** Elemental analysis of the PMDE

4.3.2.2. 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 from 200- 400 nm has been 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. The absorption peaks were at 290 nm, 350 nm, and 400 nm, but the absorption-maxima of the spectral analysis was at λ_{max}= 295 nm. This maxima 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 (Echavarría et al., 2013; Chandra et al., 2018). The absorption spectra pattern of the PMDE sample at 50% concentration showed a similar absorption pattern, but some absorption peaks disappeared between 350-400 nm. This result explained the diminishing of organic compounds along with dilution. However, the absorption maxima at 295 nm remained constant, this indicated as the major product of PMDE shown in fig 1.c. 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. During the early stage in the production of melanoidin, various organic compounds, such as hydroxymethyl furfurals, reductones, and furanones, are also reported in the chain of polymerization process shown the absorption spectrum in the range of UV region (Echavarría 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 (500-4,000 cm^{-1}) indicated several functional groups. The peak values at 3,610.8, 2,923.8, and 2,854.0 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 3,434.7 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 1,737.5 & 1,638.4 cm^{-1}, the C=O stretching of amides was indicated. The spectrum region with strong intensity at 1,576.9 and 1,462.6 cm^{-1} indicated compounds with a benzene ring. 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.

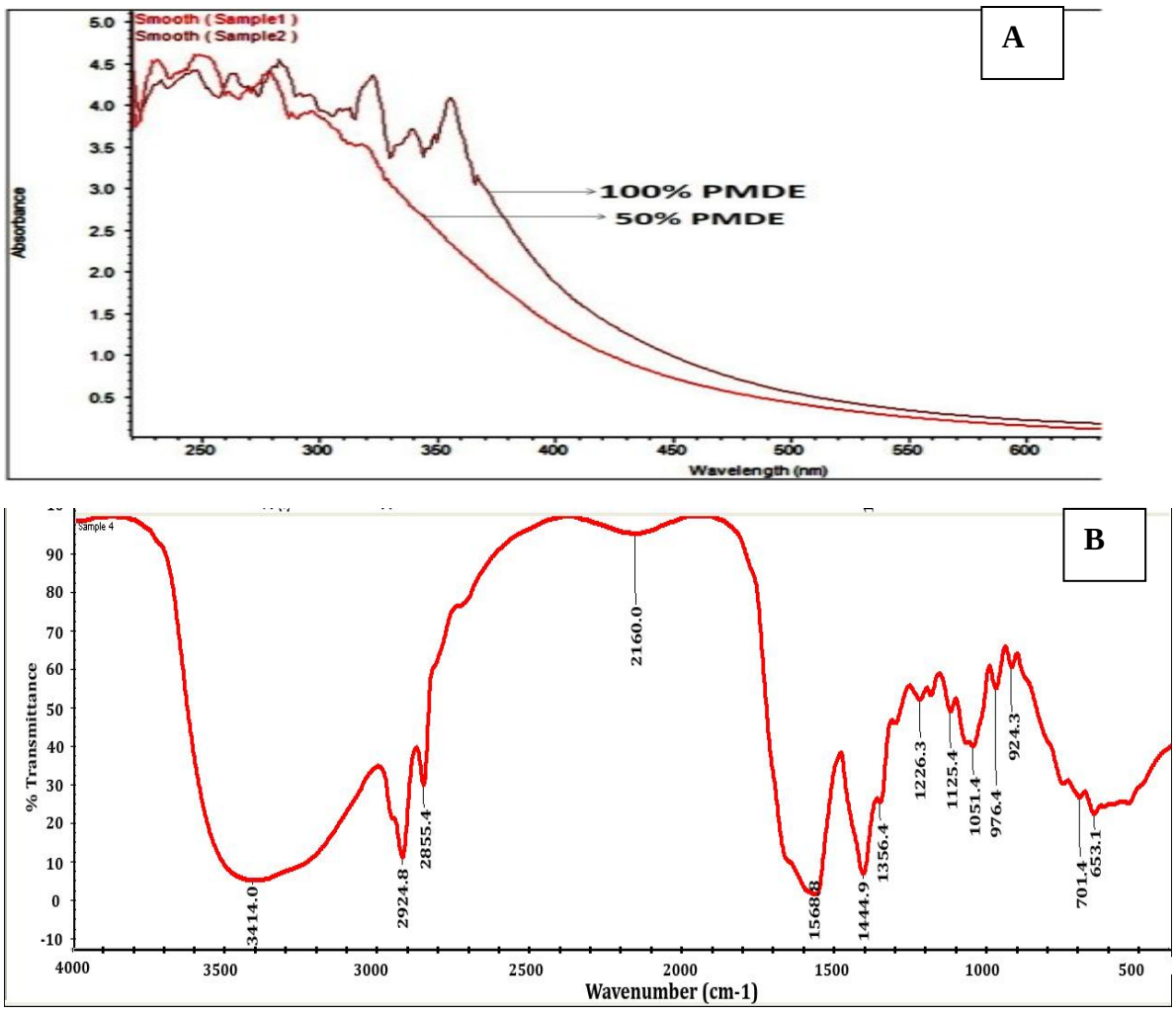


Fig: 4.4: A.UV-Vis spectral analysis **B-** Fourier transforms infrared (FTIR) spectra of PMDE sample.

4.3.2.3. 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 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, these compounds are also listed under endocrine-disrupting chemicals (EDCs) as per the USEPA screening program list (USEPA, 2012). Their toxicity parameters known in the literature are also listed in table 2. However, the EDCs properties of various pollutants discharged from industries are still unknown. 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, in addition to metals and other metalloids in discharged effluent.

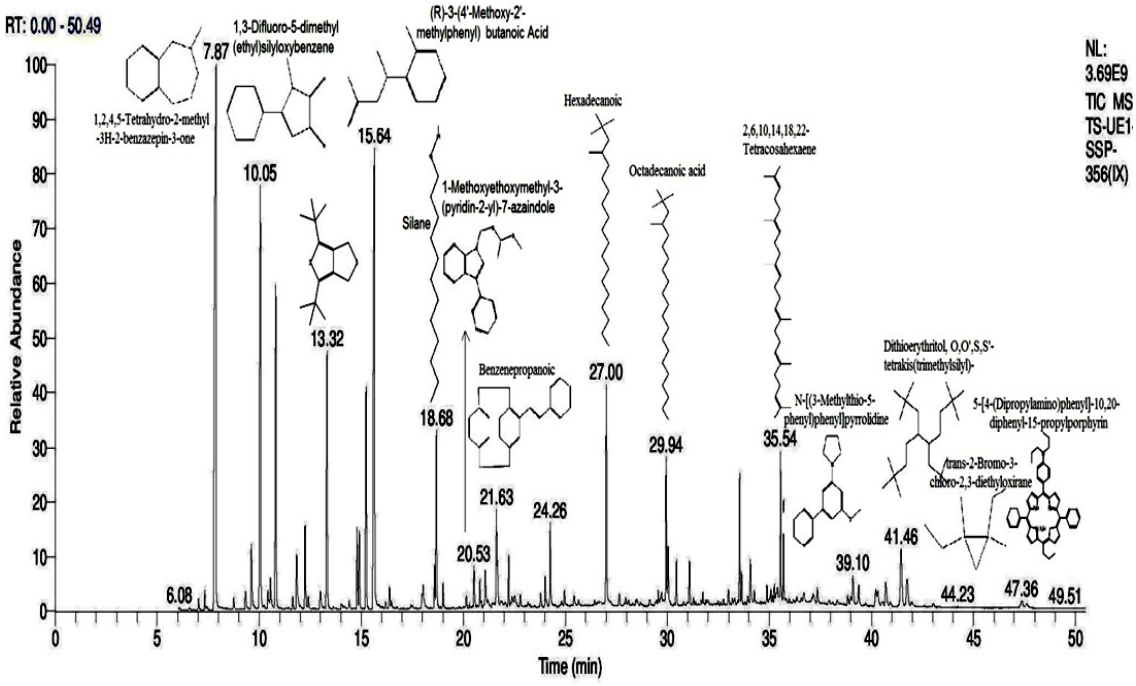


Fig: - 4.5: GC–MS chromatogram of TMS derivatized organic compounds extracted from PMDE .

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

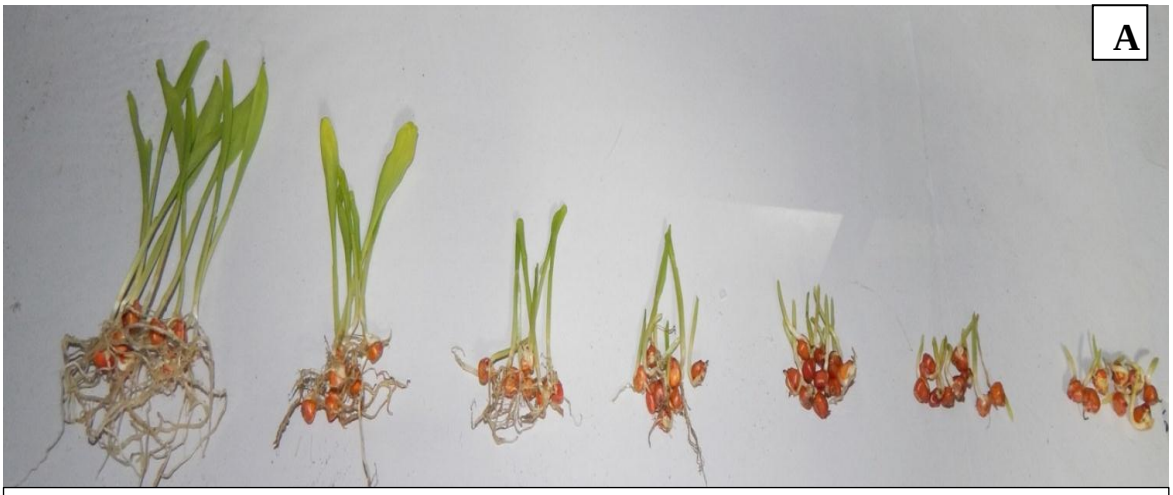
S.No.	RT	COMPOUND	Toxicity
1.	6.08	ZINC(II)-à-ACETOXY-á FORMYLOCTAETHYLPORPHRIN	Bloody vomiting, liver failure, dilate the blood vessels.
2.	7.87	3,7-Dioxa-2,8-disilanonane, 2,2,8,8-tetramethyl	Endocrine disruption, central nervous system damage,
3.	8.47	Hexanoic acid, TMS ester	Endocrine disruption.
4.	10.05	HEPTANOIC ACID TMS	Acute renal failure.
5.	11.54	Cyclohexanecarboxylic acid,	Carcinogenic, allergy reaction.
6.	13.32	3,7-Dioxa-2,8-disilanonane,2,2,8,8-tetramethyl-5-[(TMS)oxy]- (CAS)	Hypertension, Stress, Osteoporosis, urinary stones,
7.	13.71	Benzeneacetic acid, TMS ester	Gastrointestinal, Hematological, Respiratory (Nose to Lungs).
8.	15.64	Nonanoic acid, TMS ester	strong eye irritant, vomiting
9.	16.35	Benzenepropanoic acid, TMS ester	DNA damage, carcinogenic, allergy reaction, irritant dermatitis,
10.	18.68	Decanoic acid, TMS ester	Inflammation, Fibrosis, Necrosis
11.	19.49	4-Hydroxyphenylethanol, di-TMS	Parageusia, pink disease. Diarrhea Fever, Vomiting
12.	20.53	Pentanedioic acid, 2-[(TMS)oxy]-,bis(TMS) ester	Carcinogenic, allergy reaction.
13.	21.63	Dodecanoic acid, TMS ester (CAS)	inflammation and irritation
14.	23.81	Octadecane	Cough. Sore throat. Skin & Eye Redness.
15.	24.26	1,4-Benzenedicarboxylic acid,	Neurologic manifestations headache, nausea, vomiting,
16.	27.00	1-(Benzyloxy)-2-fluoro-2-phenyl-3-(p-toluenesulsulfonyloxy)propane	Vision loss, Movement disorders , Prognosis
17.	27.84	Hexadecanoic acid, trimethylsilyl ester	Degenerative bone disease, lung cancer,
18.	29.94	Hexadecanoic acid, butyl ester	Difficulty Breathing Stomach Upset.
19.	30.17	Nonacosane (CAS)	epigastria pain
20.	30.35	Oleic acid, TMS ester	Carcinogens, Cardiovascular
21.	30.70	Octadecanoic acid, TMS ester	Hypertension, Stress, Premenstrual syndrome, Osteoporosis,
22.	34.28	MONOPALMITIN 2TMS	Fatty Liver Disease (NAFLD), effect of Pancreas.
23.	35.54	Effusanin E	Toxicity to humans, including carcinogenicity
24.	36.35	Enterolactone(2,3-bis(3-hydroxybenzyl)butyrolactone-di(TMS)	worsen asthma attacks, and aggravate existing heart disease
25.	42.65	Stigmasta-5,22-diene, 3-methoxy-, (3á,22E)-	Chest or Abdominal Pain Shock, Difficulty Breathing

RT- Retention time (in minutes), **C**-control, **+** present, **−** absent, **TMS**-trimethylsilyl

4.3.3. Phytotoxicity assay

4.3.3.1. 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 shrunk and turned black, as shown in Fig. 3a and b. The data of the different tests 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 that is converted 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, this activity was decreased at higher concentrations (>10%). The inhibition inactivity might have been noted due to the penetration of organic pollutants along with the combined toxic effect of metallic compounds on the seed during the osmotic effect. This observation corroborated with previous observation noted 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 drew a conclusion that the inhibition of plant growth hormones at a high concentration of effluent which has been indicated in other plant growth (Chandra et al., 2008). The concentrations of heavy metals 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)



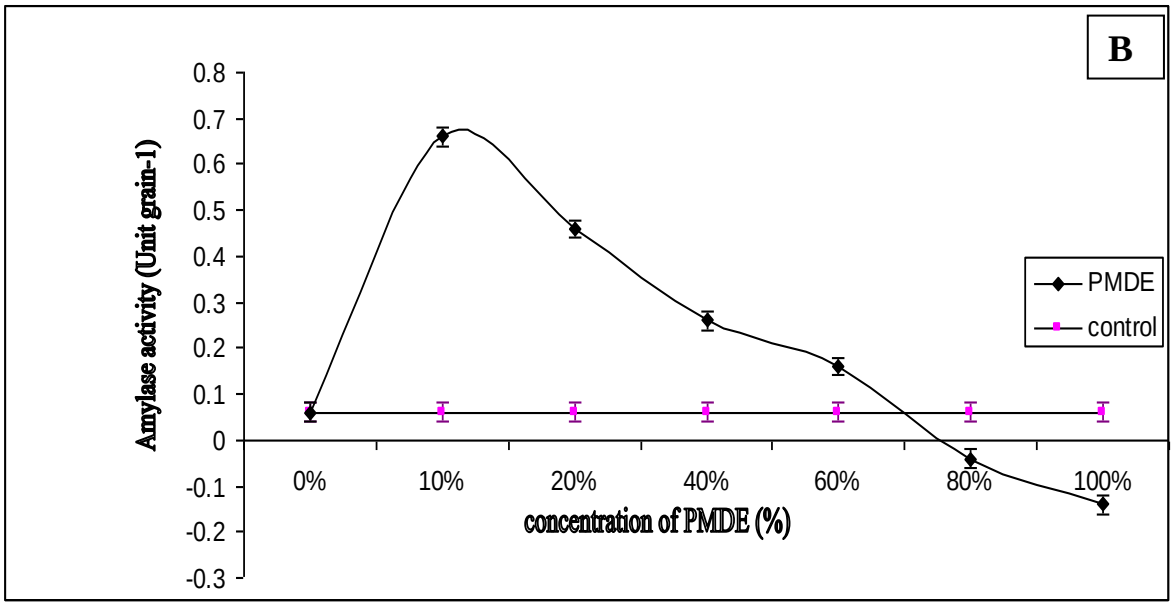


Fig: - 4.6: A. Effect of toxicity of PMDE on seedling growth of *Zea-mays L.* against PMDE sample at various concentrations. **(1-** control**., 2-**10%**., 3-**20%., **4-**40%., **5-**60%., **6-**80%., **7-**100%)

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*

No. of seed plated	Concentration (%)	Germination (%)	Reduction in germination (%)	Radical length (cm)	Phytotoxicity (%)
10	Control	100 ± 0.00	00 ± 0.00	95.12 ± 0.82	00 ± 0.00
10	10	80 ± 0.00	20 ± 0.00*	79.21 ± 0.07*	05.62 ± 0.23 ^{ns}
10	20	60± 0.00	40± 0.00*	63.35 ± 0.05 ^{ns}	16.61 ± 0.52*
10	40	40± 0.00	60± 0.00 ^{ns}	35.51 ± 0.09 ^{ns}	67.90 ± 8.96*
10	60	10± 0.00	70 ± 0.00 ^{ns}	27.76 ± 0.08	82.68 ± 3.64
10	80	05± 0.00	85± 0.00	12.21 ± 0.00	89.80 ± 3.99
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 wate

4.3.3.2. Fish toxicity

The PMDE was noted very toxic to *H. fossilis*, though this is a known sturdy freshwater fish (Fig. 4a and b). The percentage of mortality significantly increased with an increase in the concentration of PMDE during 24h incubation. Based on the mortality percentage, the estimated LC_{50} for 24 h was 3.663% PMDE. The toxicity of distillery effluent has been also reported in a previous study (Chowdhary et al., 2017). The photomicrograph of gills, the level of effluent toxicity was well expressed after 24h, compared with the control (Fig. 4a). 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 indicated by a significant increase in CAT activity in the liver with increasing concentrations of PMDE after 24h (Fig. 4b). This result also indicated the activation of oxidative defence mechanisms against the generation of free radicals caused by the exposure of effluent. The present study also reported the toxicity of distillery effluent in terms of lethal concentration, nutritional biochemistry, and haematological 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 LC_{50} 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 (Bloch et al., 1974).

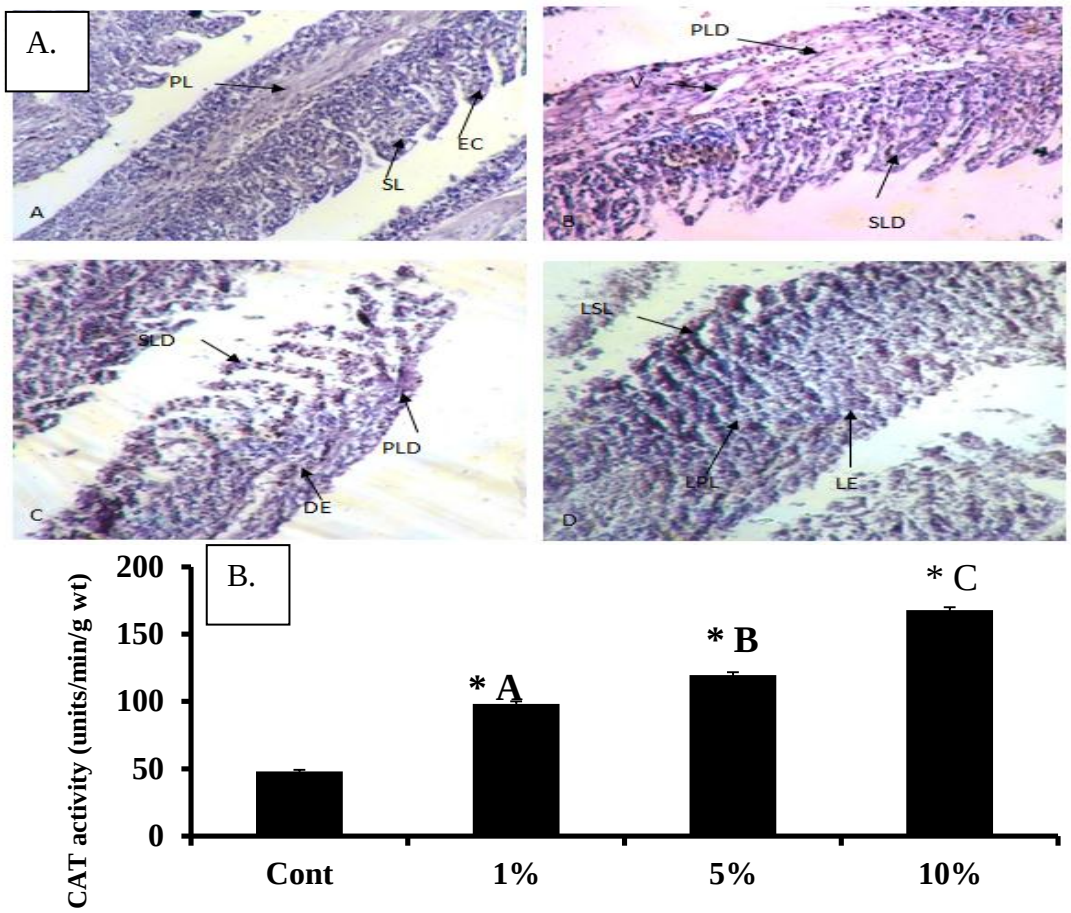


Fig.4.7: A. Concentration effect of post-methanated distillery effluent (PMDE; 1%, 5 %, 10 %) on gill histopathology of freshwater catfish, *Heteropneustes fossilis*, after 24 hr; (A) Control; (B) PMDE (1 %); (C) PMDE (5 %); (D) PMDE (10 %). Where PL: Primary lamellae; SL: Secondary lamellae; EC: Epithelial 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.

Conclusions:

The study concluded that the PMDE retained a complex mixture of organic and inorganic compounds with EDCs properties that is a major source of environmental pollution and a health hazard. The absorption maxima of PMDE between 200-350 nm in the UV–Vis spectral analysis indicated polymerized 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 as prominent organic compounds in the category of mutagenic and androgenic compounds, presented in PMDE. In addition, the various heavy metals contributed to the toxic properties and resulted in as organometallic complex in the PMDE, as resulted by the phytotoxic effect on seed germination with *Zea mays* and *Heteropneustes fossilis* with the degradation of lamellae in gill. Thus, this effluent showed the source of various unknown mutagenic, carcinogenic and EDCs compounds with organometallic complex.

Chapter-Five

Isolation & characterization of potential bacterial community responsible for bio-stimulation and bio-augmentation process during detoxification of Distillery waste

Chapter-5

Isolation & characterization of potential bacterial community responsible for bio-stimulation and bio-augmentation process during detoxification of Distillery wastewater

5.1 Introduction

Industries have become the important players in many developed countries' national economies; unfortunately, they are still the main environmental sources of pollution. Industrial wastewater discharged from different industries is referred to as the primary source of environmental pollution among various threats to the environment (Goutam et al., 2018; Jiang et al., 2019; Sharma et al., 2021a). There are several organic and inorganic pollutants in treated waste which can cause severe groundwater pollution and health risks (Arora et al., 2018). The toxicity of toxic organic and inorganic pollutants was reported on human and plant life by various researchers (Chandra et al., 2018; Sharma et al., 2021b & c). As a result of their high toxicity, many of them have been classified as priority pollutants by the US Environmental Protection Agency (USEPA), the World Health Organization (WHO) the Agency for Hazardous Substances and Disease Registry (ATSDR). Distillery wastewater is a significant source of economic activity, but it is also one of the most significant sources of pollution due to the discharge of large volumes of black wastewater. Such dark-colored wastewater contains a very high total solids (TS), phosphate, sulfate, phenolics, biological oxygen demand (BOD), chemical oxygen demand (COD), and heavy metals (Fe, Ni, Cu, Cr, Pb, Cd, Zn), respectively. Melanoidins, di-n-octyl phthalate, di-butyl phthalate, Benzenepropanoic acid, and 2-hydroxysocaproic acid are present in distillery wastewater and have been well identified as carcinogenic, mutagenic, genotoxic, and endocrine disruptors in the environment (Chaudhary et al., 2018). There are 319 distilleries in India, producing $3.25x10^9$ liters of alcohol and $40.4x10^{10}$ liters of wastewater annually (Tripathi et al., 2021b). Eutrophication of polluted water resources is induced only by high BOD, COD, sulfate, phosphate, and nitrogen content (Mahimaraja and Bolan, 2004). Microorganisms have the potential to tolerate a high concentration of different pollution parameters along with toxic metals and help to reduce the environmental toxicity and plants' health (Sharma and Singh, 2021; Sharma and Rath, 2021; Sharma, 2021). Even low concentrations of heavy

metals cause a high range of toxicity to humans and plants. Some metals store in the lungs and other organs of living animals via contaminated food (Benson et al., 2018; Huang et al., 2018; Ngo et al., 2020). Many other microbial species have been investigated for heavy metals, like *Micrococcus* sp. *Flavobacterium, Pseudomonas, Bacillus,* and *Enterobacter.* Bacteria have excellent biosorbent ability due to the high surface ratio and active chemisorption sites in their cell wall (Delil et al., 2020).

Melanoidins are the primary Colourants in distillery wastewater, presenting serious environmental and health risks to both animals and humans (Tamanna and Mahmood, 2015; Arimi et al., 2015). Many toxic substances found in distillery wastewater include di-n-octyl phthalate, di-butyl phthalate, benzenepropanoic acid, and 2-hydroxysocaproic acid. Maillard reactions are a series of non-enzymatic chemical reactions that result in Maillard reaction products (MRPs). In addition to MRPs, distillery wastewater contains a number of mutagenic, cytotoxic, carcinogenic, and endocrine-disrupting chemicals (EDCs) (Tripathi et al., 2021a; Dixit et al., 2015). Some hormonal activities, such as metabolism, sexual growth, hormone output and utilisation in development, stress response, sexual activity, and replication functions, may be altered by such EDCs compounds (Kabir et al., 2016).

In recent years, bioremediation via biostimulation and bioaugmentation has become a more relevant technique (Alessandrello et al., 2017). Moreover, using them as defined microbial consortia is better for bioremediation than using them as pure cultures, since the diversity of the consortia increases the number of catabolic pathways available for biodegradation, which enhances toxin removal efficiency (Fuentes et al., 2014; 2016). Biological agents are considered to be a more efficient alternative to physicochemical treatment approaches, which are environmentally toxic and produce secondary pollution while clean up the environment. The US Environmental Protection Agency has accepted it as an environmentally friendly waste management plan. Equally, other studies discovered that enzymatic activity levels were higher in changed soils than in non-amended soils during the process of bioremediation (Cuevas-Díaz et al., 2017). The use of various biological strategies in the design of an effective bioremediation procedure to achieve full pollutant removal (Raimondo et al., 2020a; b). Between them, bioaugmentation, consisting of the introduction of pollutant-degrading microbes to polluted sites and biostimulation, which means increasing the nutritional quality of soils to increase microbial growth and contaminant biodegradation (Adams et al., 2015; Alessandrello et al., 2017).

Bioremediation involves the processes of bio attenuation, biostimulation, and bioaugmentation (Maszenan et al., 2011).

In this Chapter, we summarize the recent developments in the bioremediation process through these techniques is useful for environmental concern in extreme habitats and so many diverse chemicals involved, they are most likely exerting their effects through multiple pathways.

5.2 Material and methods

5.2.1. Site study and sample collection

The collection of distillery wastewater was carried from M/s Unnao distillery industry, situated in Uttar Pradesh, Unnao, India (26°320″ N, 80°30′0″E). This industry uses sugarcane molasses as raw material spent wash methanogenesis process of distillation pursed by various steps for making the pure alcohol. Freshly discarded approx 20-liter distillery wastewater cakes were obtained in clean, pre-sterilized polythene bags from the distillery plant's dumping site, which was situated inside the industry's premises (Tripathi et al., 2021b).

5.2.2. Bacterial consortium preparation

This analysis used an aerobic bacterial consortium consisting of two bacterial strains in a stimulated sample (*Stenotrophomonas maltophilia* [RCS-1] &*Bacillus cereus* [RCS-2], as well as a bioaugumented strain *Enterobacter cloacae* (IITRCS10) purified from wastewater sample in our lab to degrade synthetic distillery industry wastewater.

5.2.3. Experiment setup and observations

In the wastewater sample adding nutrient is treat as biostimulation process and nutrient with isolated bacteria is treated as bioaugumentation process. In the biostimulation process firstly wastewater sample was taken at 250 ml conical flask & pour 100 ml of wastewater into each flask by the accurate amount of nutrient added (1.0 gm of glucose as carbon source and 0.5 gm nitrogen source) respectively. The flasks were autoclaved at 121^0C. In a shaker, the sterilised flask samples were incubated at 37°C for 10 minutes (New Brunswick scientific excellent E24 incubator shaker series) at the 150-rpm speed for 48 h. after the incubation period when the growth may occur and produce biomass than its measured by taking optimum density (OD) at 610 nm at per 24h interval up to 48h (Incubation period). Further, after full completion of the biostimulation process and bacterial growth then added isolated bacteria strain for the bioaugmentation process. The biostimulation and

bioaugmentation process samples were centrifuged at 10000 xg for 20 minutes and OD at 460 nm in the Ultra-violet range in a spectrophotometer (Evaluation 20, Thermo Fisher Scientific, India) at one-day intervals up to fully exponential growth (144 h) (Chandra et al., 2018).

5.2.4. Assessment of environmental and nutrition condition

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 biostimulation and bioaugumented sample 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 biostimulated and bioaugumented process.

5.2.5. Physico-chemical and heavy metals analysis

Distillery wastewater was analyzed using conventional techniques for wastewater analysis. The pH of the obtained wastewater sample will be calculated using an Orion ion meter (Model 960), COD will be determined using an open reflux method, BOD by using a 5-day method, total nitrogen (TN) was determined using a drying system, total organic carbon (TOC), and total solid (TS) was calculated by using a Vcsnanalyzer from Shimadzu, Japan. The amounts of phosphorus and sulfate will be calculated using the vanadomolybdo– phosphoric acid colorimetric system and the $BaCl_2$ precipitation method. The ions sodium, nitrate, chloride, and potassium can be measured using an ion meter (Orion ion meter - 960) and a selective electrode. To assess heavy metal concentrations in wastewater (Thermo Electron; Model IRIS Intrepid II XDL, USA).

5.2.6. Estimation of total proteins, colony-forming unit, and biomass

The total extracellular protein of both samples was calculated by using the standard method (Lowry, 1951). Count the total microbial load of microbes in the biostimulation and bioaugmentation process was analyzed. The plates were incubated at 37^0C for 24 h and then after the next day when well cleared, separated colonies become arise then counted colony forming units (CFU) (Chandra et al., 2018). 1 ml of a developed colonies sample in a pre-weighed Eppendorf tube was centrifuged and dried for the biomass approximation. Around 50^0C cells were fully dried. The bacterial biomass per ml before and after drying was known to be the source of the difference in tube masses. After centrifuging and drying 1 ml of bacterial growth

sample in a pre-weighed Eppendorf tube at 50°C, the post-weight of the Eppendorf post-weight minus the pre-weight of the Eppendorf tube equalled bacterial biomass per ml.

5.2.7. Estimation of enzyme assay

The estimation of enzymatic activity like MnP and laccase during biostimulation and bioaugmentation process, centrifugation the collected supernatant at 6500g for 10 minutes at 4 °C. The using of phenol red and guaiacol as a substrate as per reported by Arora et al., 2002.

5.2.8. Scanning electron microscopy and UV-Vis spectrophotometer analysis

A UV-Vis spectrophotometer was used to measure the decolorization of biostimulation and bioaugmentation process samples at 25 °C in the wavelength range 200-700 nm (Sharma et al., 2021d). After that, the fixed samples were dehydrated for 5 minutes in ethanol solutions containing 25, 50, 75, 95, and 100 percent ethanol. Before being analyzed using scanning electron microscopy (SEM), the samples were dried in a critical point dryer and sputter-coated with a thin gold conductive film (FEI Quanta 450, Hillsboro, USA).

5.2.9. High-performance liquid chromatography (HPLC) analysis

The high-performance liquid chromatography (HPLC) analysis was carried out with a diode detector unit (1100 type, Agilent Technology, USA) and a column reverse phase C18 (250= 4.6 mm, 5M size) with the gradient of solvent A (Milli-Q water) and solvent B, at a flow rate of 0.4ml/min1 for the 60s, using the solvent gradient (Acetonitrile with TFA 0.1 percent, Merck KGaA, Darmstadt, Germany). The detection was monitored at maximum absorption wavelength i.e. 295 nm to determine the degradation and decoloration in the biostimulation and bioaugmentation process sample.

5.2.10. Characterization of organic compounds

The optimal organic solvent for extracting organic contaminants was found to be ethyl acetate. The distillery wastewater sample (10.0 ml as control sample) was measuring and pouring 10 ml of ethyl acetate solvent into the Erlenmeyer (250 ml) flask and aggressively mixing and after the mixture had been constantly stirred for 5 h, an equivalent amount of ethyl acetate was applied, and the liquid-liquid extraction intermittently continued. The extraction of the organic pollutants was performed successively three times. After bioremediation, a related procedure was used to

remove metabolic products from the biostimulation and bioaugmentation phase samples used for GC-MS analysis.

5.2.11. Identification of indigenous and isolated bacterial strains

The probable indigenous and isolated bacterial strains from the incubated flask used for the biodegradation process were isolated and purified using serial dilution and the plate-streak method. The Cowan and Steels Manual for identifying medical bacteria was used to identify dominantly emerging purified autochthonous bacterial strains (Barrow and Feltham, 1993).

5.3 Results and discussion

5.3.1 Physico-chemical analysis

The Physico-chemical properties of the control, biostimulation, and bioaugmentation samples are shown in table 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. In the biostimulated sample the values are less degrade rather than the control sample and much degraded in the bioaugumented sample (Table 1). The elevated values of COD and BOD of the accessible wastewater solution indicate the existence of greater amounts of organic and inorganic compounds. Furthermore, the high concentration of metals wastewater may be attributed to the devastating impact of cane sugar during the sugar production process (Chandra et al., 2018). Furthermore, metals may be applied during the fermentation and distillation processes of fermented sugarcane molasses in distilleries, resulting in PMDE with an alkaline pH that is eventually discharged. In comparison to the sample obtained after 168 hours of incubation, the biostimulated and bioaugumented sample showed a reduced of different pollution parameter. This suggested that a possible bacterial consortium was biodegrading and bio transforming various organic and inorganic contents. The pH of the medium was initially reduced to 4.47 during melanoidin degradation, but after 168 hours of bacterial development, the pH was steadily increased. The development of organic acids such as phosphoric acid, acetic acid, octadecenoic acid, and ethanedioic acid, can cause 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 percent decolorization increased (76%).

Table. 5: Physico-chemical analysis of discharged distillery industry wastewater, biostimulation and bioaugmentation collected from M/s Unnao Distillery pvt. Ltd. *Unnao*, Uttar Pradesh, India

Parameters	Distillery wastewater	After biostimulation	After bioaugmentation	Permissible Limit (CPCB 2012)
pH	7.00 ± 0.20 l	7.20 ± 0.10	8.114 ± 0.21ˆa	7.54± 0.01
EC	4.7 ± 2.11 l	3.6 ± 1.14	2.8 ± 1.34ˆa	--
Ions				
Na^+	56 ± 1.31 l	55.108 ± 0.97	13.352 ± 0.80ˆa	--
Cl^-	1829 ±0.10 l	7038.6. ± 0.89	6880.674 ± 129.45ˆns	11.82 ± 0.01
NO^{3-}	110 ±3.14 l	119.916 ± 1.30	47.295 ±0.90ˆa	47.00± 0.00
Ammoniacal nitrogen	195 ± 1.24 l	188.794 ± 0.94	86.594 ± 0.71ˆa	9.90± 0.00
Total dissolve solid	–	16750.4 ± 2.07	2506 ± 89.19ˆa	152± 0.01
Biological oxygen demand	17400.47 ± 19.18	11050.2 ± 2.28	2455.698 ± 1.78ˆa	47.00± 0.00
Chemical oxygen demand	37193.68 ± 7.65	22551 ± 1.58	4518.934 ± 1.90ˆa	79.00± 0.01
Total organic carbon	18.318 ± 0.21 l	15.404 ± 0.28	7.407 ± 1.09ˆa	11.82± 0.01
Total nitrogen	2.473 ± 0.01 l	2.094 ±0.04	1.982 ± 0.05ˆc	9.90± 0.00
Heavy metals				
Iron (Fe)	2403 ± 3.11l	1401.22 ± 1.86	852.528 ± 2.05ˆa	----
Zinc (Zn)	210.15 ± 2.14 l	95.273 ± 0.68	21.463 ± 1.67ˆa	0.15
Copper (Cu)	73.62 ± 1.14 l	62.928 ± 1.20	11.526 ± 1.02ˆa	0.01
Chromium (Cr)	22.825 ± 0.41 l	19.447 ± 0.60	9.500 ±0.49ˆa	1.28
Cadmium (Cd)	2.440 ± 0.12l	1.786 ± 0.15	0.94 ± 0.05ˆc	0.19
Manganese (Mg)	127.30 ± 0.94 l	95.602 ± 1.13	45.140 ± 0.72ˆa	1.45
Nickel (Ni)	14.425 ± 0.21 l	9.302 ± 0.31	2.250 ± 0.20ˆa	0.02

All the values are Mean ±SE. (n=3); Unit of all parameters is in (mgL^{-1}) except pH, color (Co-Pt. Unit) and EC ($\mu mhoscm^{-1}$); Students *t* test (two tailed as compared to pre-treated sludge); [a]Highly significant at $p<0.001$; [b]Significant at $p<0.01$; [c]Less significant at $p<0.05$; [NS]Non-significant at $p>0.05$; BDL: Below detection Limit.

5.3.2 Effect of nutrition and environmental condition

For a 168-hour bacterial incubation period, distillery wastewater degradation was assessed using a 1% (w/v) concentration of various glucose sources. The findings demonstrate that the consortium's efficiency increased as glucose concentration increased from 0.1 to 1%, achieving maximum decolorization of 40-70 % in biostimulated samples and 70-90 % in bioaugumented samples. This indicates that the presence of glucose has been a consequence of the oxidation of organic contaminants and metals and that further increases in glucose levels do not encourage the growth or decolorization of melanoidins by bacteria. However, the efficiency of glucose and peptone was less than that of the trace element which showed melanoidin coloration up to 42-58 % in bioaugumented sample. This showed that the presence of glucose resulted from the oxidation of organic contaminants and metals, although the further rise in glucose concentrations does not help the bacterial growth and melanoidin decolorization. Glucose and peptone were however not as successful as glucose, where melanoidins decolorated by up to 50-60%. However, for the development of the bacterial consortia, the glucose present in the media was readily accessible. This showed that the isolation and oxidation of melanoidins containing effluent were more successful for our bacterial consortium. This may be due to high enzyme presence, making it easier for complex pollutant degradation processes. But when we added the isolated bacterial strain after the full growth of the biostimulation process then showed 70-89 % decolorization in the bioaugumentation process. Besides, increased peptone concentrations have caused the decoloration process to be gradually reduced in the degradation capacity of the evolved bacterial consortium (Sharma et al., 2020a,b& c). At pH 8.1, the bacterial consortium recorded far more decolorization (79%). The higher decolorization at alkaline pH (8.1) may be due to the combined action of the bacterial enzymes and the loosening of conjugated bonds of Maillard reaction products. The bacterial consortium depolymerized melanoidins at high pH, causing the color to fade. The optimized bacterial consortium also showed maximum decolorization (81%) at 37 °C, while increasing the temperature to 45 °C harmed the bacterial consortium's growth and decolorization capacity. Moreover, the impact of shaking speed revealed that 180 rpm resulted in the best degradation of organic pollutants by a potential bacterial consortium.

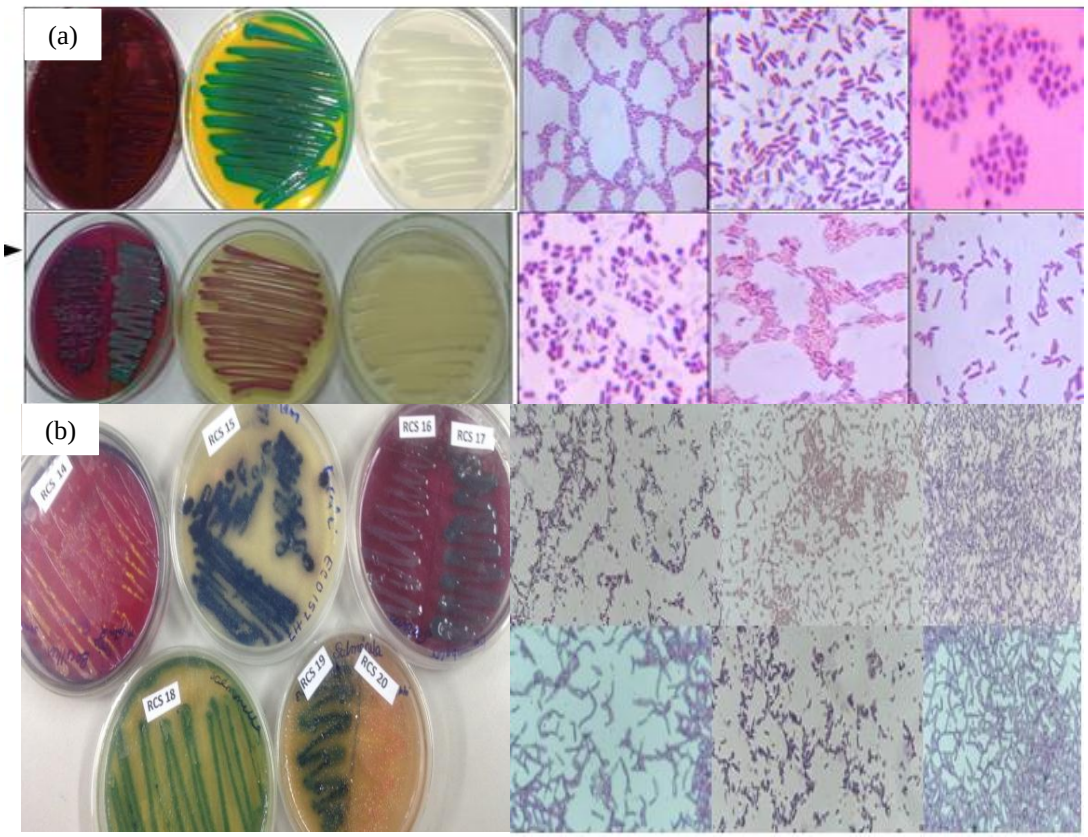


Fig: 5.2: (a) Autochthonous and (b) Isolated bacterial strain growing in separated molasses melanoidin from anaerobic treated distillery effluent and their morphological view

5.3.3 Performance of UV Vis, total proteins, CFU, and biomass analysis

At different incubation times, the increasing total protein content correlated positively with the trends in oxidative enzymes. The analyses of CFU and biomass revealed a constant increase in incubation time compared with the control sample. Moreover, when comparing the growing biostimulation and bioaugumentation samples to the control, periodic measurements of OD at 620 nm revealed that their values increased. Periodic SEM assessments of degrading samples often display an improvement in bacterial population and biodiversity in the biostimulation and bioaugumentation method. During the bacterial growth in the biostimulation and bioaugumentation process, there was also a reduction of color compared with that of the control. The decolorization was much in bioaugumentation in comparison with biostimulation and control sample.

Periodical bacterial growth curve and biomass testing demonstrated a median incubation time of 144 hours of OD-620 nm (2,49) and biomass (5.22 g of L−1; CFU/mL 27.5 alternate to 106). This has become the best way to reach optimal

decoloration (70-90% respectively) of melanoidins, with the fastest bacterial growth and production of biomass. Furthermore, denser bacterial cells have emerged from the SEM study of rising bacterial biomass. This showed rapid bacterial growth in medium-bearing melanoidins, leading to melanoidin degradation and discoloration. Moreover, bacterial consortium incubation did not improve the decolorization rate of melanoidins. The direct association of OD 620 with high biomass also showed that a bacterial consortium is directly involved in the melanoidin decoloration and degradation process.

5.3.4 Enzyme activity during degradation

In decolorization and color destruction, ligninolytic enzymes play a major role and promote the development of microorganisms in the use of mixed pollutants (Bonugli-Santos et al., 2012). The MnP is the dominant enzyme at the initial stage of melanoidin degradation during the biostimulation and bioaugumentation process. MnP activity was maximized at 168 h (3.8 mL^{-1} min^{-1}), where the laccase activity at 144 hours of incubation was detected at the highest level (2.39 U mL−1 min−1) and maximum melanoidin decolorisation (70-90 %). This may be attributed to MnP direct participation in degrade when the further incubation caused by the synthesis of phenolic compounds causes the bacterial laccase enzyme production. In the bioaugumentation process, during the decolorisation evaluation, laccase concentration was found to be higher at a later stage of the increasing bacterial consortium. Laccase and MnP are substrate oxidizing enzymes that can sever a wide variety of chemical bonds found in recalcitrant compounds, including phenolic and non-phenolic compounds. However, the MnP and laccase-enzyme activities of the proposed bacterial consortium were shown to be gradually reduced after 168 and 144 h incubation, respectively. MnP and laccase development may be attributable to the depolymerization of melanoidins.

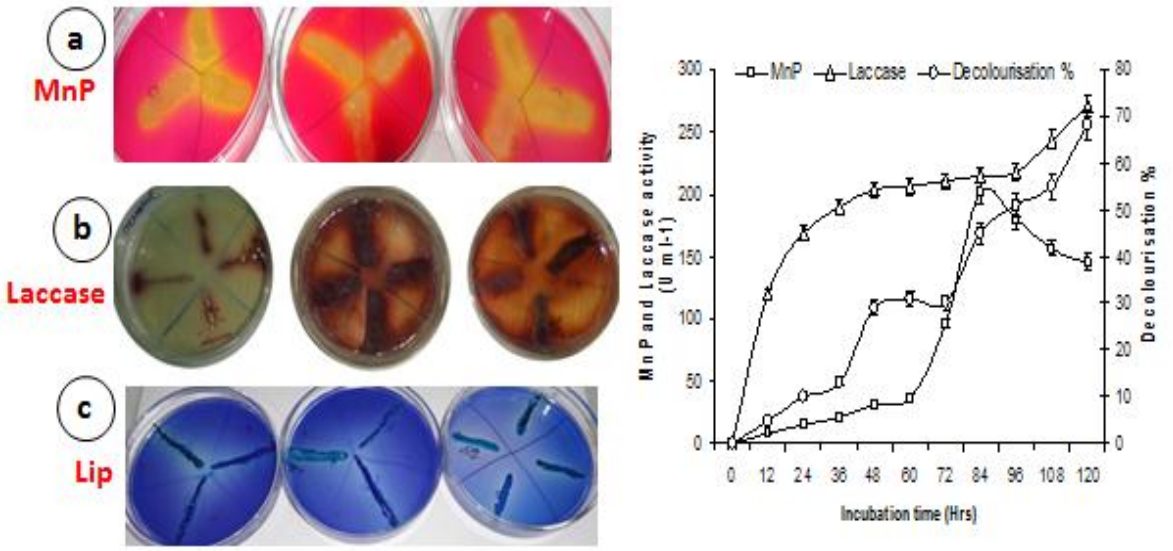


Fig. 5.3: Screening of ligninolytic enzymes producing potential bacterial strain by using different substrates (a) Phenol red for MnP (b) Guiacol for Laccase(c) azure B for Lip

5.3.5 Metabolites assessment by HPLC analysis and Fourier transform infrared spectroscopy analysis in biostimulation and bioaugumentation

In biostimulation and bioaugumentation bacterial treatment, the HPLC analytics report shows the location, height, and retention time of different compounds in distillery wastewater and confirms degradation (Fig. 1a). HPLC analysis of the biostimulated sample revealed a high and small peak area, while the stimulated sample was less in 96 hours compared to the control sample, demonstrating the consortium's ability to decolorize and degrade distillery wastewater through their enzymatic action. Furthermore, several additional peaks emerged in the enhanced and augmented samples, indicating the development of some new metabolites. Also, when compared to the control sample, the HPLC chromatogram showed peak shifting, reduced the height of peak, and the showed some new compounds after bioaugumentation treatment. It indicated that the developed bacterial consortium in both samples biotransformed and biodegraded distillery wastewater into various metabolites.To investigate the chemical structure of the control, biostimulation, and bioaugmentation process samples, an FT-IR study was performed in the range of 400–4000 cm1 using a spectrophotometer (NicoletTM 6700, Thermo Scientific, USA). To prepare the pellet for FT-IR analysis, KBr (potassium bromide) was mixed with the separated sample of biostimulation and bioaugmentation process.

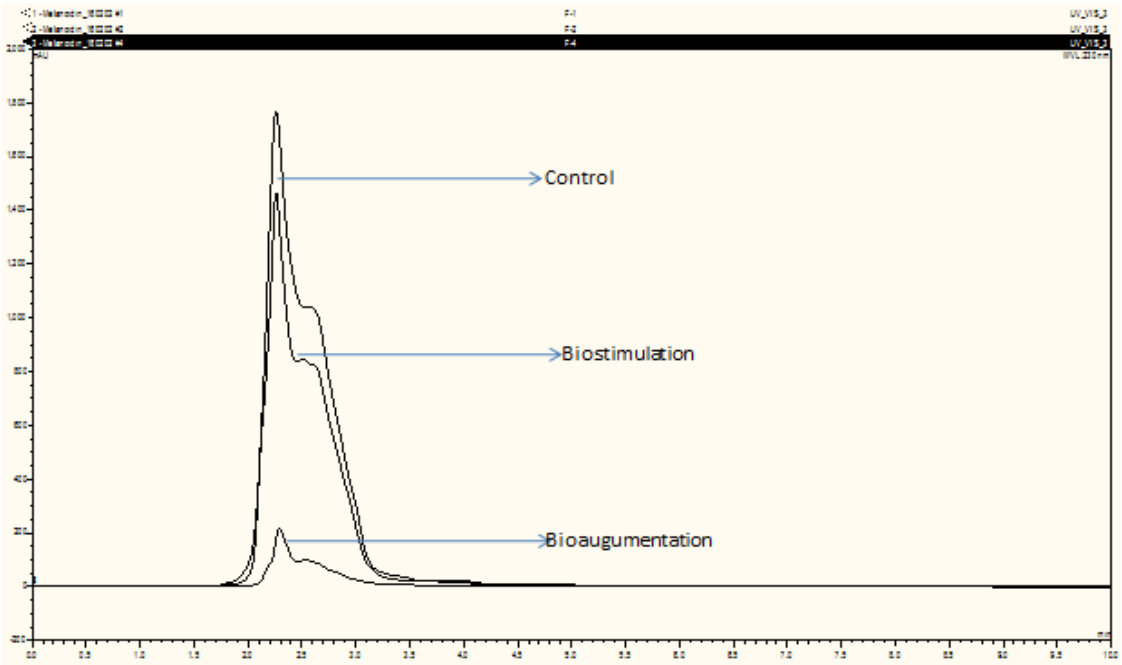


Fig: 5.4: HPLC analysis of disposed distillery Effluent during the Biostimulation and Bioaugmentation Process.

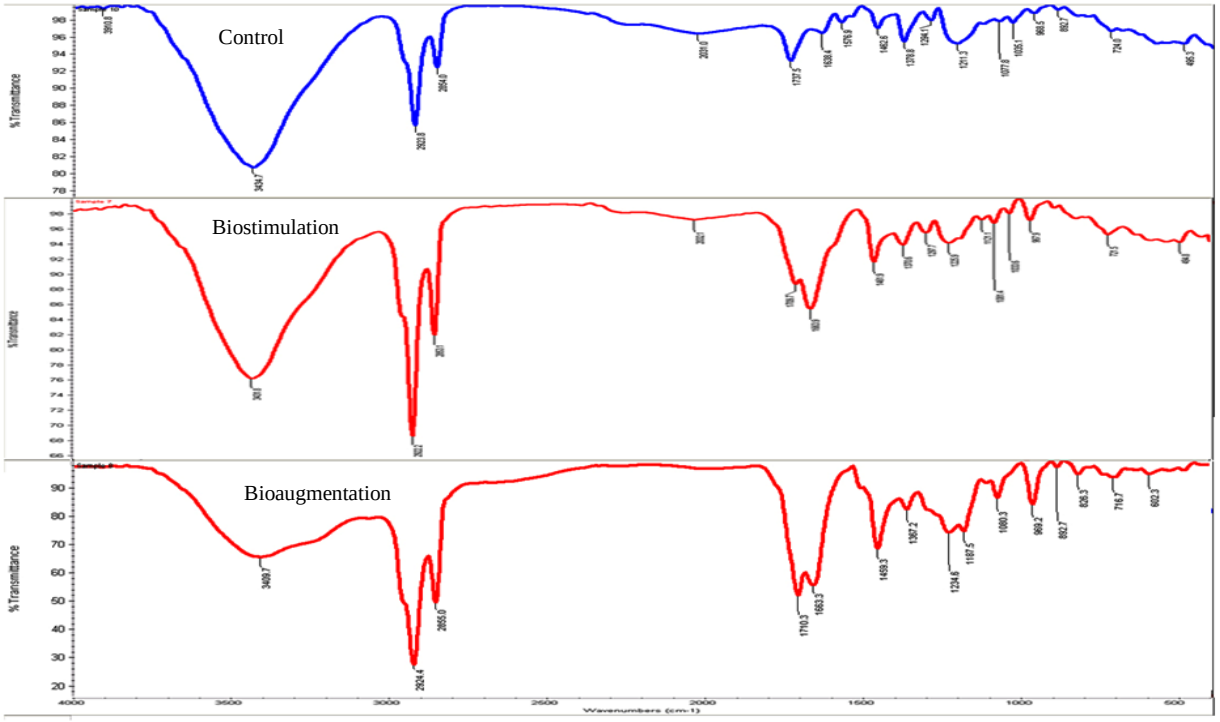


Fig: 5.5: FTIR analysis of disposed distillery Effluent during the Biostimulation and Bioaugmentation Process**.**

Table: - 5.2: Functional group and Intensity comparison of FTIR analysis of disposed distillery Effluent during the Biostimulation and Bioaugmentation Process

Control	Functional Group	Biostimulation	Functional Group	Bioaugmentation	Functional Group	Intensity
3610.8	O-H	3431.8	Amine, N-H	3409.7	Amine, N-H	Medium
3434.7	N-H	2922.2	Amine, N-H	2924.4	Amine, N-H	Medium
2923.8	Carboxylic Acid,O-H	2853.1	Carboxylic Acid,O-H	2855.0	Carboxylic Acid, O-H	Strong
2854.0	Carboxylic Acid,O-H	2032.1	Alkyne, C≡C	1710.3	Ketone C=O	Strong
2031.0	Alkyne, C≡C	1709.7	Ketone C=O	1663.3	Amide C=O	Strong
1737.5	Ketone C=O	1663.9	Amide C=O	1459.3	Benzene Ring C=C	Strong
1638.4	Amide C=O	1461.9	Benzene Ring C=C	1367.2	Alkane -C-H	Medium
1576.9	Benzene Ring C=C	1370.6	Alkane -C-H	1234.6	Acid C-O	Strong
1462.6	Benzene Ring C=C	1297.7	Acid C-O	1187.5	Acid C-O	Strong
1294.1	Acid C-O	1121.1	Ester, C-O	969.2	Alkene, =C-H	Medium
1211.3	Acid C-O	1081.4	Ester, C-O	892.7	Alkene, =C-H	Medium
1077.8	Ester, C-O	1033.6	Ester, C-O	826.3	Alkene, =C-H	Medium
1035.1	Ester, C-O	967.9	Alkene, =C-H	716.7	Alkyl Halide, C-Cl	Strong

5.3.6 Assessment of degradation after biostimulation and bioaugumentaion process

In table. 2 lists the main residual organic contaminants found in ethyl acetate under alkaline (pH 8.5) and acidic (pH 2.0) conditions, as well as the isolated compounds detected by GC-MS at various retention times (RT) (Sharma et al., 2021d & e). The peak at 13.45 was detected as Benzene acetic acid. Organo-metallic compounds present in the distillery industry but after degradation in the biostimulation and bioaugumentation process was not extracted under similar conditions to the control. The main metabolic products identified as phenol and phenolic compounds in the control and bacterially degraded samples were classified as 3-Hydroxy methyl-2-tri methyl silyl oxy pentane, Benzene Propanoic acid, tert-butyl dimethyl silyl ester, Hydrocinnamic acid, Tetradecanoic acid, trimethylsilyl ester [CAS], Octadecanoic acid, 2,3-bis[(trimethylsilyl)oxy, Hexadecanoic acid,4-[(trimethylsilyl)oxy] butyl ester [CAS] shown in (Figures 3A,B & C). Both these products demonstrated that the 2-methoxy phenol group was present in their structures but had some replacements on their aromatic ring. Most of these compounds are organometallic and some are disappeared after treatment by the biostimulation process, and the left are transformed in another form in bioaugumentation (Figures 3A, B & C). After 6 days of incubation, acetic acid, Oxo-, trimethylsilyl, was found in degraded effluent in the biostimulation phase, but not in the bioaugmentation process.

5.3.7 Identification isolated bacterial strains

16S rRNA sequencing was used to identify the purified isolated and autochthonous bacterial strains. The parameters of the Gamma model were determined to within 0.1000000000 logarithmic probability units of accuracy. Bio stimulated samples, such as *Stenotrophomonas maltophilia* (RCS1) and *Bacillus cereus* (RCS 2), as well as bioaugumented strain *Enterobacter cloacae* (IITRCS10), were deposited to the NCBI database based on 16S rRNA sequencing data.

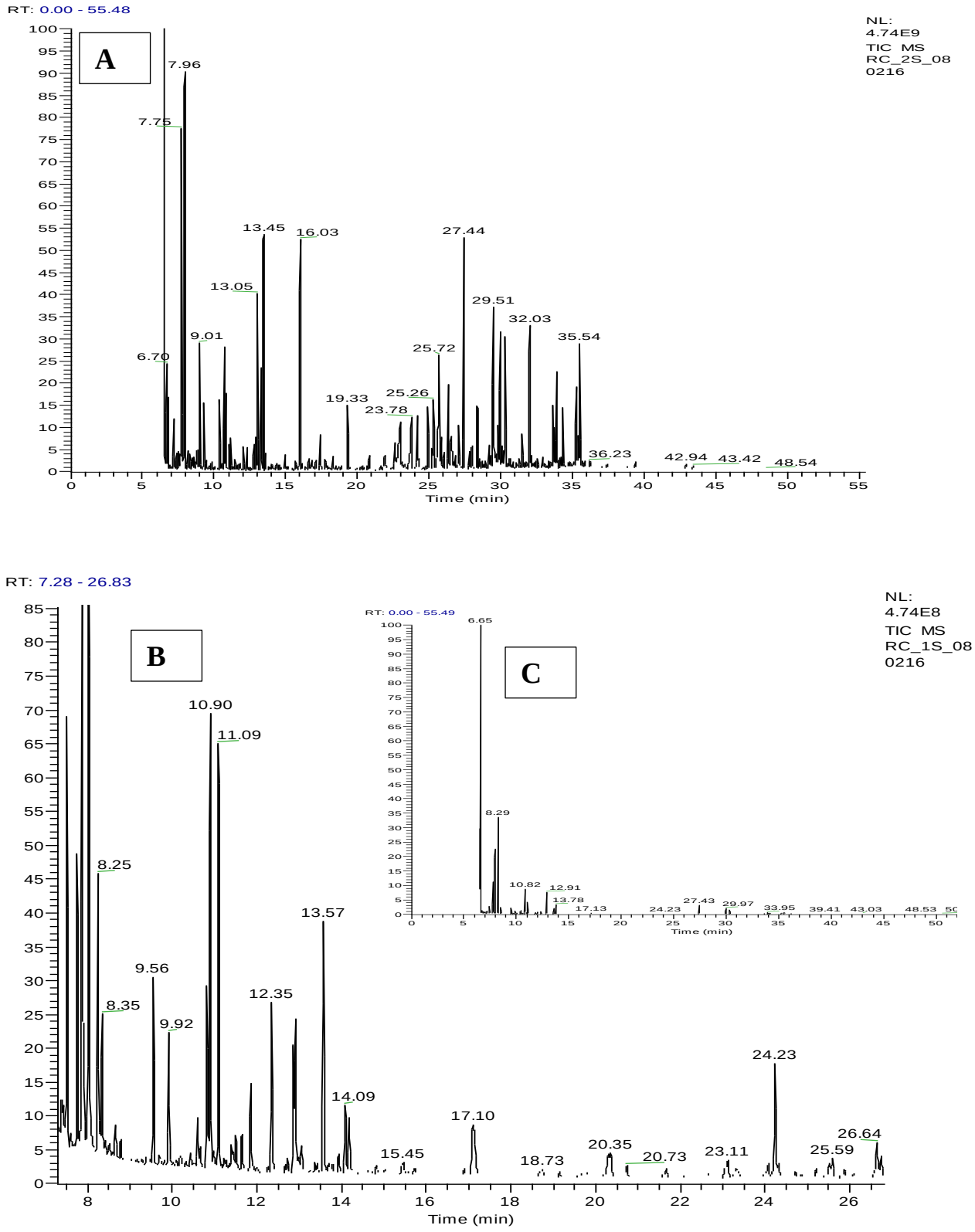


Fig.5.6: Gas chromatography mass spectroscopy analysis of distillery industry wastewater after biostimulation and bioaugmentation process (b-c) compare with control sample (a) (Control).

Table 5.3: Identification of residual organic pollutants by GC-MS analysis from distillery industry wastewater before and after biostimulation (BS) and bioaugmentation process (BAN)

RT	Compounds name	Control	BS	BAN
6.70	[2R,3R]-2 Methylallo threonine	+++++	++++	-----
6.80	2,5 Hexanedione,3,4-dihydroxy-3,4-dimethyl	+++++	-----	-----
6.87	Pentanoic acid, trimethylsilyl ester [CAS]	+++++	-----	-----
7.13	2-[2-Butoxyethoxy) ethoxy-tri methyl silane	+++++	-----	++
7.25	N-tri methyl silyl methyl (n-butyl) amine	+++++	++++	-----
7.75	Butane, 2,3-bis (Trimethylsiloxy)	+++++	-----	-----
7.82	3-Hydroxy methyl-2-tri methyl silyl oxy pentane	+++++	++++	-----
8.84	Hexane – 1,3,4-triol 3,5-dimethyl	+++++	-----	-----
9.01	Silane, trimethyl (1-methyl butoxy)	+++++	++++	-----
9.31	3-Hydroxy-2-butanone, trimethylsilyl ether	+++++	-----	-----
10.87	t- Butyldimethyl[2-styryl [1,3] dithian-2-yl]silane	+++++	++++	++
11.06	Butane-1,3diol, 1-methyl,bis [trimethylsilyl] ether+	+++++	-----	-----
11.18	3-Hydroxy- 2-butanone, trimethylsilyl ether	+++++	-----	-----
12.33	Silanol,trimethyl-,benzoate [CAS]	+++++	++++	-----
12.78	2-Methyl-2,3-dihydro-1Hbenz[g] indole	+++++	++++	++
13.32	2,4-Dimethyl-3-pentanol,trimethylsilyl ether	+++++	-----	-----
13.45	Benzene acetic acid, trimethylsilyl ester	+++++	-----	-----
16.03	1-[trimethylsilyl]-6-phenyl-1,3,5-hexa triyne	----	----	++
16.03	Benzene Propanoic acid, tert-butyl dimethyl silyl ester	+++++	++++	-----
17.42	Silanol, trimethyl, -2 –amino benzoate [CAS]	+++++	-----	-----
17.75	Acetic acid, Oxo-,trimethylsilyl ester [CAS]	-	++++	-----
20.83	3-[o-Azidophenyl] propenol	+++++	++++	++
23.79	5-chlorobenzimididazole-2-corboxlic acid	+++++	-----	++
24.22	Pyrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-[2-methylpropyl]	+++++	++++	++
24.22	Tetradecanoic acid, trimethylsilyl ester [CAS]	+++++	-----	-----
25.26	n-pentanedecanoic acid, trimethylsilyl ester	+++++	-----	-----
25.71	1,4-diazo-2,5-dioxo-3-isobutyl bicycle[4.3.0]nonane	+++++	-----	-----
26.38	2,Ethyl-3-methylnaphtho[2,3-b]thiophene-4,9-dione	+++++	++++	++
27.08	Cis-9-Hexadecanoic acid, trimethylsilyl ester	+++++	-----	-----
28.37	n-Hexadecycloxy[trimethylsilane]	+++++	-----	-----
29.87	9,2- Octadecanoic acid, [Z,Z]- trimethylsilyl [CAS]	+++++	++++	-----
29.97	Trans-9-Octadecanoic acid, trimethylsilyl ester	+++++	-----	-----
35.54	Octadecanoic acid, 2,3-bis[(trimethylsilyl)oxy	+++++	-----	-----

> AS7 consensus seq

GGGGGGGGGGGGGGGTAACAAAGCCCTTATCACCGCCCAGTAGTTGATCCTGGCTCAGATGAACGCTGGCGGCAGGCCTA
ACACATGCAAGTCGAGCGGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAAC
TGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAATGTCGCAAGACCAAAGTGGGGGACCTTCGG
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ATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCC
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GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTGG
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GAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTA
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GAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGA
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Phylogenetic Tree

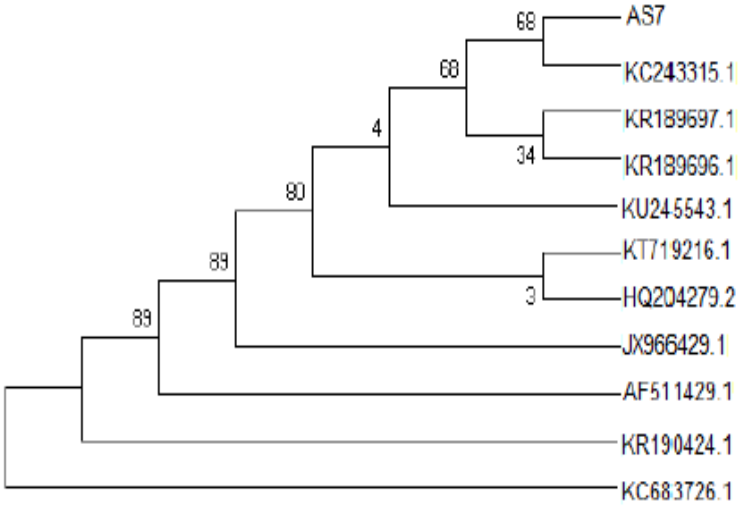

***Figure*. Evolutionary relationships of 11 taxa**

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 0.01036687 is shown. The confidence probability (multiplied by 100) that the interior branch length is greater than 0, as estimated using the bootstrap test (500 replicates is shown next to the branches [2, 3]. The evolutionary distances were computed using the Kimura 2-parameter method [4] and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 1306 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 [5].

Fig.57: 16sRNA sequencing & Phylogenetic tree of Thermo tolerant Autochthonous and Isolated bacterial strain during Biostimulation and Bioaugumentation process.

>AS1 Consensus data

AGTTTGATCTCAGGCTACGAGAACTGGAGAAGTAGAAGATGATTTTGAGACGGGTGAATAAGACTGGGAAACTGCCT
GATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGGACCTTCGGGC
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CCT

Phylogenetic Tree

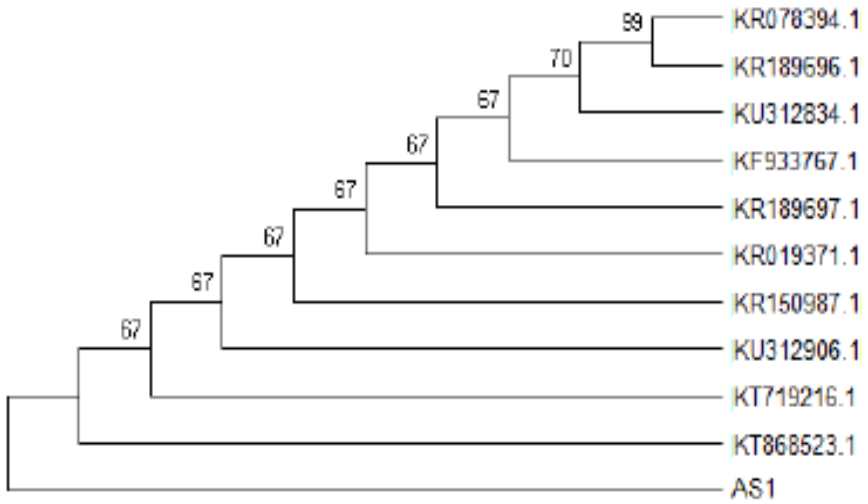

***Figure*. Evolutionary relationships of 11 taxa**

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 0.01950056 is shown. The confidence probability (multiplied by 100) that the interior branch length is greater than 0, as estimated using the bootstrap test (500 replicates is shown next to the branches [2, 3]. The evolutionary distances were computed using the Kimura 2-parameter method [4] and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 830 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 [5].

Fig.5.8: 16sRNA sequencing & Phylogenetic tree of Thermo tolerant Autochthonous and Isolated bacterial strain during Biostimulation and Bioaugmentation process.

5.3.8 Toxicity Assessment of Bacterially Treated Samples

Comparative toxicity assessment of effluent degraded by the biostimulation process and the control in the seed germination test showed a 60% reduction of toxicity with Triticum aestivation and *Phaseolus* mungo seeds (Figures 6A–C). P. mungo was found to be more sensitive than *T. aestivum*. Comparative cytotoxic and genotoxic effects of the effluent before and after detoxification by biostimulation were also tested with Allium cepa on the basis of chromosomal aberrations. Root cells of A. cepa treated with effluent sample before and after detoxification showed various chromosomal aberrations, as shown in Figure 7. Microscopic observation of onion root tips treated with control effluent (without biostimulation) showed abnormal and vagrant metaphase, diagonal anaphase, chromosome laggards at anaphase, ring chromosomes, and sticky anaphase, as shown in Figure 7, while normal prophase, metaphase, anaphase, and telophase were observed in A. cepa treated with effluent degraded by the autochthonous bacterial communities.

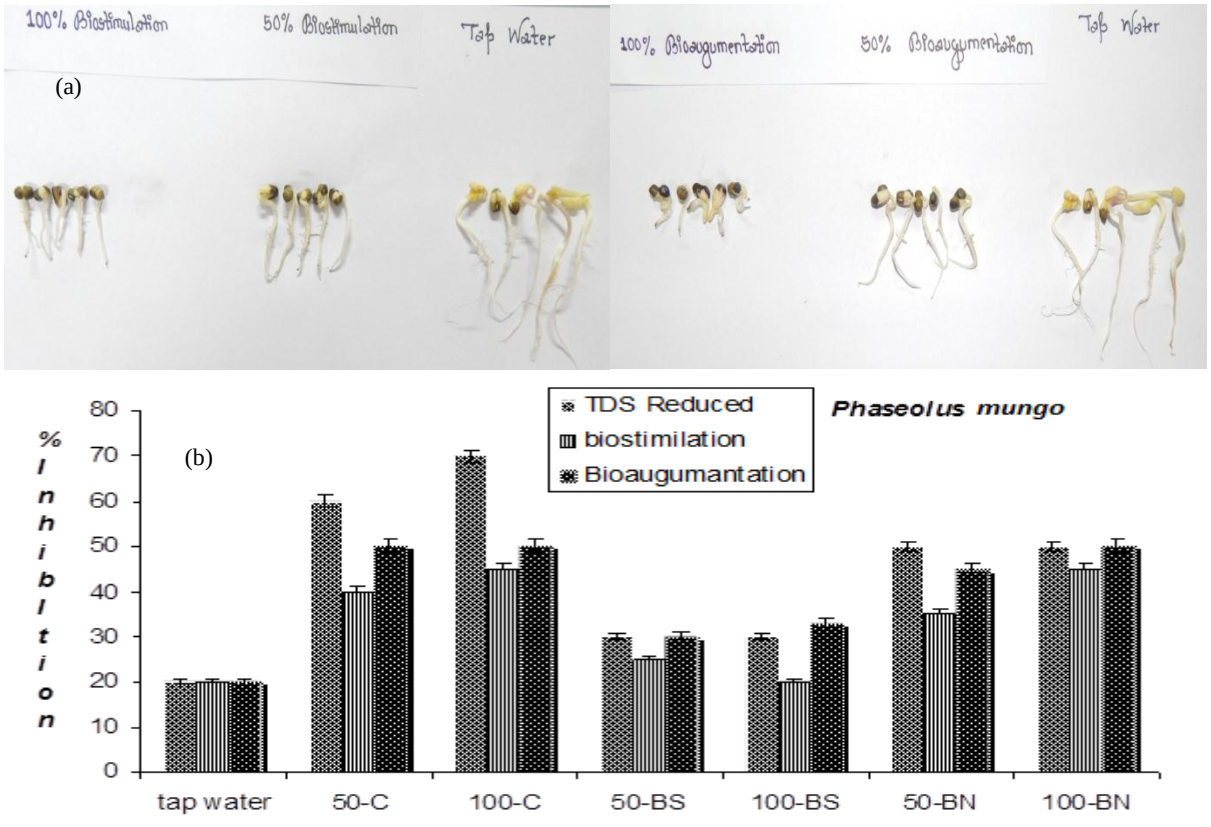

Fig. 5.9: (a) Seed Germination and (b) seedling growth effect of *Phaseolous Mungo L*. during Biostimulation and Bioaugumentation process**.**

Conclusion:

The findings revealed that distillery wastewater contains EDCs such as organometallic complex compounds, as well as other contamination parameters (BOD, COD, TSS, TDS), which can be degraded by an autochthonous bacterial consortium using a biostimulation and Bioaugumentation process with a supplementary source of nutrients under specific environmental conditions such as pH, shaking speed, and temperature. The production of different ligninolytic enzymes likes, Lip, MnP, and Laccase activities as bio-tools for detoxification and degradation of wastewater after secondary treatment. The assessment of degradation of organic compounds by GC-MS, UV-Vis and HPLC, analysis confirmed using of bacterial consortia *Stenotrophomonas maltophilia* (RCS1) *Bacillus cereus* (RCS 2), and *Enterobacter cloacae* (IITRCS10).

Chapter-Six

Optimization of Color reduction and detoxification process of Distillery effluent by developed bacterial consortium at reduced TDS of Effluent.

Optimization of Color reduction and detoxification process of Distillery effluent by developed bacterial consortium at reduced TDS of Effluent.

6.1 Introduction

Most of the distilleries co-exist with sugar mills and use cane sugar molasses as a raw material to produce alcohol. India is the greatest rice producing country, and the primary material after sugar cane molasses is rice grain. Alcohol distilleries in India are one of the most agro-based polluting agro-industries; they also have high raw water consumption. In India there are more than 317 sugarcane molasses-based distilleries industry pouring down their treated and untreated effluent at rate of approximately 3.5 X 10^{14} kilolitres annually to water bodies through their discharge system (AIDA, 2004). In the distillation process, ethanol ranges from 8 to 10% by volume, hence it follows that the amount of waste varies from 90% to 92% by volume of the alcohol distilled. The distillery effluent after anaerobic digestion converts dark black colour with high BOD and unpleasant odour. This is known as post methanated distillery effluent (PMDE). The major colour contributing complex biopolymer of distillery waste is known as melanoidin, which is generated during polymerization of amino group with reducing carbohydrate at the time of boiling of sugarcane juice concentration process for sugar crystallization in sugar industry. In India, there are number of large-scale distilleries integrated with sugar mills. The waste products of sugar mills comprise bagasse's (residue from the sugarcane crushing), press mud and molasses. The effluents from molasses-based distilleries contain large amounts of dark brown coloured molasses- spent-wash (MSW). This causes soil and aquatic pollution and threat to environmentalist and challenge for industries. Worldwide environment regulatory authorities have set strict norms for discharge of wastewaters from industries. It further says that till 100% utilization of PMDE should be achieved, controlled and restricted discharge of treated effluent from lined lagoons during rainy season will be allowed by SPCBs /CPCB in such a way that the perceptive colouring of river water bodies does not occur. The drawback is that ability of bacteria is reported only for synthetic melanoidin in laboratory condition. But a reference may be made to a publication wherein presence of other pollutants in effluents like phenolics, sulphate and heavy metals makes more toxic and recalcitrant nature of effluent.

However, the application of this technique for the effluent decolorization up to safe limit of environment at large scale is not feasible. Furthermore, the references may be made to the publication wherein demonstrated the ability of some aquatic macrophyte for reducing the level of toxic metals in polluted water. The drawback is that, these above reported plants showed the ability of bioaccumulation only in pure metals solution. The colour of post methanated distillery effluent (PMDE) is suspected to be due to high content of sulphur, phenol and other unknown organic compounds, which inhibits the microbial activity for further degradation. The major obstacle in the effluent treatment of molasses spent wash is the presence of high dark brown coloured natural polymer called melanoidins; due to its water-soluble properties causes water as well as soil pollution. The inhibitory nature of melanoidin has been reported in India and worldwide to many organisms, which cannot be removed by conventional wastewater treatment process due to its recalcitrant nature. Further, the in this study have been revealed that the bacterial pre-treatment of distillery effluent enhance the bio-remediation process. Due to lack of any adequate technique for the decolorization of PMDE after extended aeration at industrial scale still it is major source of environmental pollutants in India. Currently, no any feasible and economically viable technology is available for its safe disposal. Hence, the safe disposal for environmental potential and conservation of aquatic resources for sustainable development is warranted.

Recently, the biological treatment system along with advanced oxidation and ozonation process has been reported as a potential, simple, low-cost wastewater tertiary treatment technique. 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. 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. 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. 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. The complexity of PMDE showed that high TDS as a key factor of effluent toxicity due to dissolved organic content in high concentration. For precipitation of phenol colourant and other ionic salts for reduction of total dissolved solids (TDS) from various industrial wastewater to achieve the biodegradation with bacterial growth. Hence, the aim of this paper is development of novel technique for decolourisation and detoxification of distillery effluents after anaerobic digestion for its safe discharge and re-use in various applications for sustainable development. Lastly the decolorized effluent after toxicity reduction can be used either for effluent recycling or its application to aquaculture and ferti-irrigation/ green belt development around industries would prevent aquatic and soil pollution with safe disposal.

6.2 Materials and method

6.2.1 Sample collection

The experimental site for plant and distillery sludge sampling was selected in Unnao, Uttar Pradesh, India, situated at the north latitude of 26° 32′ 0″ and east longitude of 80° 30′ 0″, which had been polluted due to extensive and indiscriminate disposal of anaerobically digested sludge. This site is well known for high pollution with organic and inorganic pollutants as previously described by Tripathi et al., 2021. The climate in this area is classified as humid subtropical dry weather with an average annual rainfall of 850 mm (Rainy season: July–September). Annual temperature of is 25.6 °C, which varies between 15 and 40 °C from winter to summer, respectively. Distillery sludge samples were collected in October 2017 from the two different sludge disposed site located in premises of the M/s Unnao Distilleries & Breweries Limited. All the collected samples were transported to laboratory in ice-cold condition (~ 4 °C) for further analyses.

6.2.2 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 1.

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.

6.2.3 Physico-chemical analysis

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^{-3}_4 , and SO^{-2}_4 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 peroxide 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 HNO3 (1:1, v/w) at 95 °C for 15 min. After cooling, 5 mL of concentrated HNO3 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% H2O2 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.

6.2.4 SEM –EDAX of distillery effluent before after TDS reduced sample

Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) was performed to assess the surface morphology of dissolved pollutants at reduced TDS. The sample was kept into an aluminium foil for drying in the oven for 4 to 5 hours at 600C followed by keeping Desiccator for 2 hours and the sample was 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 micro pore 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).

6.2.5 FTIR and UV-Vis analysis

To observe the absorbance characteristics of different organic and inorganic pollutants, UV–visible (UV-Vis) spectroscopy and absorption spectra were obtained between 250 and 700 nm at 37 ◦C (Thermo Fisher Scientific Shanghai spectrophotometer Evolution 2001, China). Fourier-transform infrared spectroscopy (FT-IR) analyses of the wastewater samples before (WS-1) and after (WS-2) phytoremediation was recorded by a FT-IR Perkin Elmer Spectrum-II spectrometer. The sample was centrifuged at 6000 rpm and then dried for 6 h in an oven at 65 ◦C to remove the moisture content. To make the pellets, 100 mg of potassium bromide (KBr) and 2 mg of dried sample were mixed as per the previously mentioned method

[3]. The KBr based pellets were compressed into a thin disk using a hydraulic press (CAP-15 T) at 15 tons pressure. The disks were fixed in an FT-IR spectrometer (Thermo-Nicolet 6700) and analyzed in the spectral region of 4000–400 cm-1 with potassium bromite (KBr) pallets.

6.2.6 GC-MS analysis

The extracts obtained from rhizospheric and non-rhizospheric distillery sludge were firstly derivatized using TMCS, BSTFA and pyridine as per method described earlier (Chandra and Kumar 2017a) and analyzed by GC–MS (TRACE GC Ultra Gas Chromatographs, Thermo Fisher Scientific, USA) united with a TriPlus auto sampler coupled to TSQ Quantum XLS triple quadrupole mass spectrometer (Thermo Scientific, USA). Organic compound separation was carried out in a DB-5MS capillary column (No. 2713S18) by running with helium as the carrier gas at a flow rate of 1.1 mL min−1. The GC oven conditions were as follows: an initial temperature of 65 °C (hold time: 2 min), increased to 230 °C (flow rate of 6 °C min−1) and finally increased up to 290 °C (hold time: 20 min; flow rate of 10 °C min−1) (Chandra and Kumar 2017a). An aliquot (1.0 µl) of derivatized sample was injected in the GC column in the splitless mode. The injector temperature was operated at 250 °C while the mass detector was operated at 300 °C. The MS was operated in full-scan mode (45–800 m z−1) at electron energy of 70 eV with a solvent delay of 7 min. The organic compounds were detected and identified through spectral matching of mass spectra obtained at different retention times with mass spectra of standard compounds mentioned in National Institute of Standard and Technology (version 1.0.0.12, NIST, USA) mass spectra library existing with GC–MS.

6.2.7 Toxicity assessment

6.2.7.1 Seed germination test

The Phytotoxicity test of the PMDE before and after TDS reduction was used seed germination test of *Phaseolous mungo L.* Seed germination was evaluated at different concentrations of effluent using a Petri dish method as described previously (David and Rajan, 2015). Ten sterilized seeds were put in different Petri dishes at the same spacing. The dishes contained the PMDE concentrations of 10%, 20%, 40%, 60%, 80%, and 100% (v/v), while tap water was used as the control in the same condition. The dishes were incubated at 28°C till germination (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).

6.2.7.2 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).

6.3 Result and discussion

6.3.1 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 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 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 bio transforming 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.

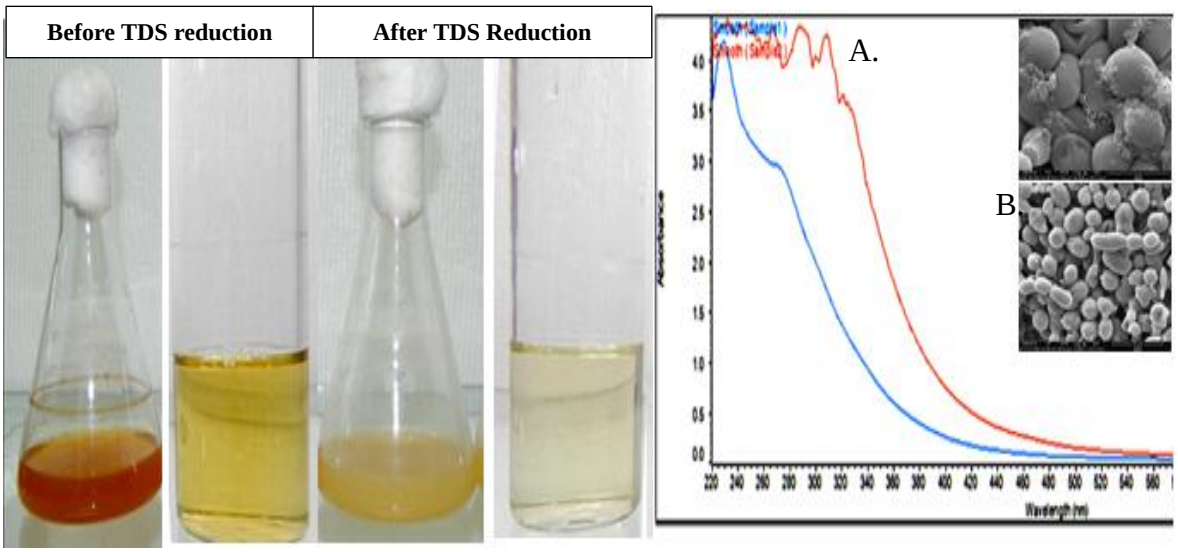

Fig. 6.1: Degradation of post methanated distillery effluent after TDS reduction (PMDE) through biodegradation process (a). UV-Vis spectroscopy of before and after TDS reduction (b). SEM structure of bacterial grown in After TDS reduction sample (c)

Table 6.1: Physico-chemical characteristics of before and after TDS reduction of Distillery effluent along with heavy metals content collected from *M/s Unnao Distillery Pvt. Ltd. Unnao, Lucknow-Uttar Pradesh, and India.*

S.No.	Parameters	PMDE	After TDS reduction	Permissible Limit	
				USEPA (2012)	CPCB (2017)
1.	Color appearance	Blackish Brown	Brown	--	--
2.	Odor	Like molasses	-----	--	--
3.	pH	8.67 ± 0.16	7.95 ± 0.22[a]	8.00± 0.01	7.54± 0.01
4.	Biological oxygen demand (BOD) (mg/L^{-1})	4800.45 ± 98.23	1500.55± 67.54[a]	40.00	47.00± 0.00
5.	Chemical oxygen demand (COD) (mg/L^{-1})	10111.15 ± 232.32	2345.65 ± 765.32a	121.00	79.00± 0.01
6.	Electrical conductivity (EC)	1008.65 ±40.03	1098.65±54.29[a]	1000	950
7.	Total Dissolve solid (TDS) (mg/L^{-1})	10720.78 ± 260.44	400.5 ± 323.49[a]	50-70	70± 0.00
8.	VS (mg/L^{-1})	1509.21 ± 98.07	321.12 ± 43.40[b]		20± 0.01
9.	Chloride (mg/L^{-1})	3402.76± 32.65	954.87 ± 34.00[b]	750.00	11.82± 0.01
10.	Total Nitrogen	398.23 ± 9.21	89.21 ± 6.67[a]		9.90± 0.00
11.	Phenol (mg/L^{-1})	10,123 ± 00.98	1999.45 ± 35.89[c]	0.50	-
12.	Sulphate(mg/L^{-1})	19,231.00 ± 23.09	4987.11 ± 12.12[b]	750.00	
13.	Phosphate (mg/L^{-1})	56.09 ± 2.589	17.54 ± 0.92[b]	-	3.40± 0.01
	Heavy metals				
a)	Mn(mg/L^{-1})	10.564 ± 0.76	2.32 ± 0.32[b]	0.20	0.15
b)	Cr (mg/L^{-1})	3.73 ± 0.38	0.78 ± 0.12[c]	0.05	0.01
c)	Zn (mg/L^{-1})	18.84 ± 0.89	2.12 ± 0.12	2.00	1.28
d)	Cu (mg/L^{-1})	3.91 ± 0.21	0.10 ± 0.02[NS]	0.50	0.19
e)	Fe (mg/L^{-1})	423.88 ± 7.33	87.32 ± 4.12	2.00	1.45
f)	Pb(mg/L^{-1})	5.08 ± 0.27	0.65± 0.432[c]	0.05	0.02
g)	Cd (mg/L^{-1})	BDL	BDL	BDL	BDL
h)	Ni (mg/L^{-1})	13.32± 0.34	2.12 ± 0.58[a]	0.10	0.04
i)	Na (mg/L^{-1})	500.34 ± 9.42	63.233 ±0.7[NS]	0.04	0.01
j)	K (mg/L^{-1})	298.67±65.43	99.12±1.12	0.09	0.02

All the values are Mean ±SE. (n=3); Unit of all parameters are in (mg/L^{-1}) except pH, color (Co-Pt. Unit) and EC ($\mu mhoscm^{-1}$); Students *t* test (two tailed as compared to pre-treated sludge); [a]Highly significant at $p<0.001$; [b]Significant at $p<0.01$; [c]Less significant at $p<0.05$; [NS]Non-significant at $p>0.05$

6.3.2 Optimized conditions for TDS removal

The efficiency of the different levels of studied factors on TDS removal. It can be seen from the figure that FeCl3 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 6.1. The optimum pH for TDS removal was obtained equal to 9. Also, Na2SiO3 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. Fecl3 3wasthe best coagulant aid to remove the turbidity and the best levels for coagulant dose and coagulant aid dose were 800 and 600ppm, respectively.

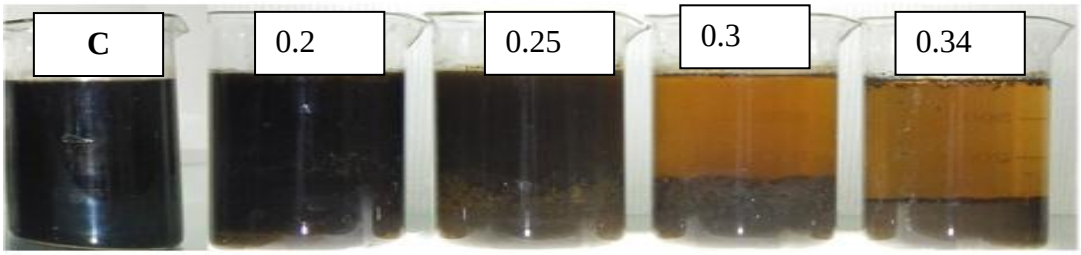

Fig. 6.2 Optimized condition of post methanated distillery effluent (PMDE) with coagulants (Fecl3)

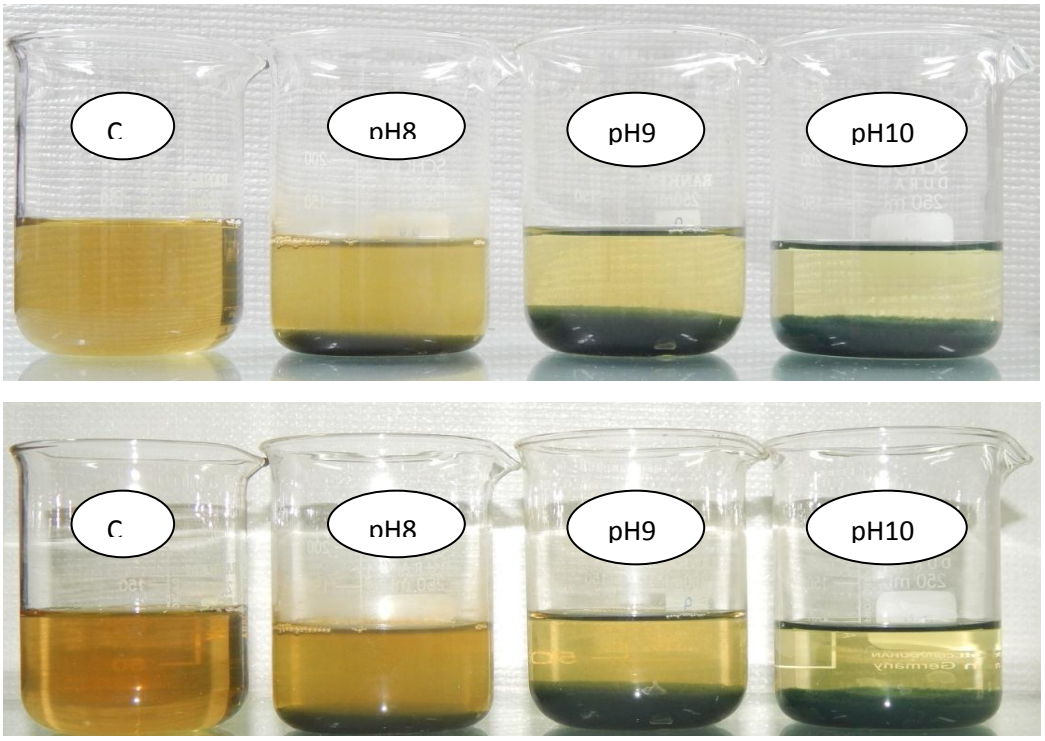

Fig. 6.3 Optimized condition of post methanated distillery effluent (PMDE) with various coagulants at various pH.

6.3.3 Metabolites assessment FTIR analysis and UV Vis-Scanning

The FTIR analysis confirmed degradation of the organo-metallic compounds in the Control and TDS reduced sample. FTIR analysis and UV-Vis spectrophotometry scanning of the sterilized sample revealed very similar pattern to the untreated control while the TDS reduction samples showed peak shifting, reduction in height of peaks, and the detection of new metabolites demonstrating the consortium's ability to decolorize and degrade distillery wastewater by biotransformation and biodegradation of distillery wastewater into various metabolites.

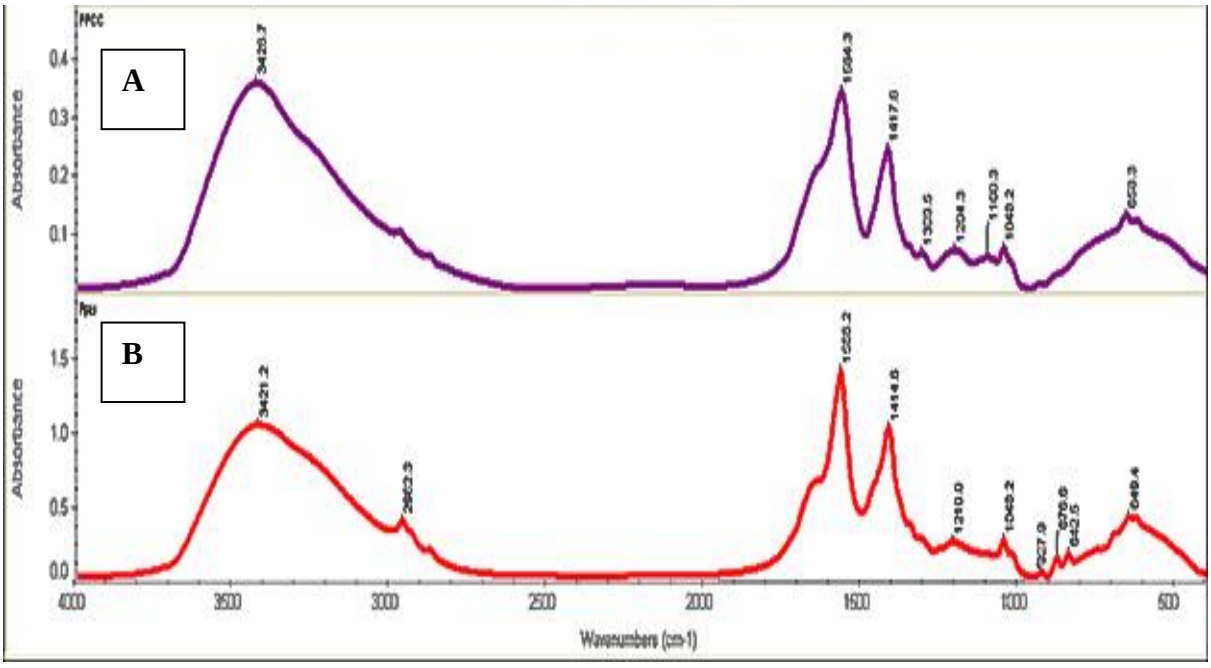

Fig: 6.4: FTIR analysis of Distillery Effluent A. Control, B. after TDS Reduction

6.3.4 Identification of organic pollutants

The metabolites produced by the constructed bacterial consortium were identified using GC-MS (Table 2). The untreated distillery wastewater containing a large number of organic compounds that were absent in the treated wastewater. The main metabolic products identified as phenol and phenolic compounds in the control were classified as Pyridine, 2-methyl-3-[TMS ester]-4,5-bis-[(TMS ester)methyl], 12-Methoxy-2 trimethylsilyloxy-19-nor-5β-podocarpa-1,3,8,11,13-pentane, 3-benzyl-1,4-diazo-2,5-dioxobicyclo[4,3,0] nonane, Hexadecanoic acid, 2,3-bis[(TMS)oxy]propyl ester[CAS], (Ss*,S*)-Phenyl[((2,4,6-TM phenyl) sulfinyl)phenyl) methanol, 2-Monostearin TMS ether, 1,,3-DIMETHYL-6- TMS OXYMETHYL-URACIL shown in (Figures 2a-c). Both of these products demonstrated that the 2-methoxyphenol group was present in their structures but had some replacements on

their aromatic ring. A number of the organo-metallic compounds were thermally degraded after autoclave but a number of thermal stable compounds remained (Table 2). However, after the TDS reduction, all these compounds were either degraded below the detection limit or greatly reduced in quantity, confirming again that active biotransformation and biodegradation had taken place. It was interesting to note that1-[trimethyl silyl]-6-phenyl-1, 3, 5-hexa triyne was detected in the bioaugumented samples only and was absent in the untreated and thermally treated samples, indicating the production of a new metabolite.

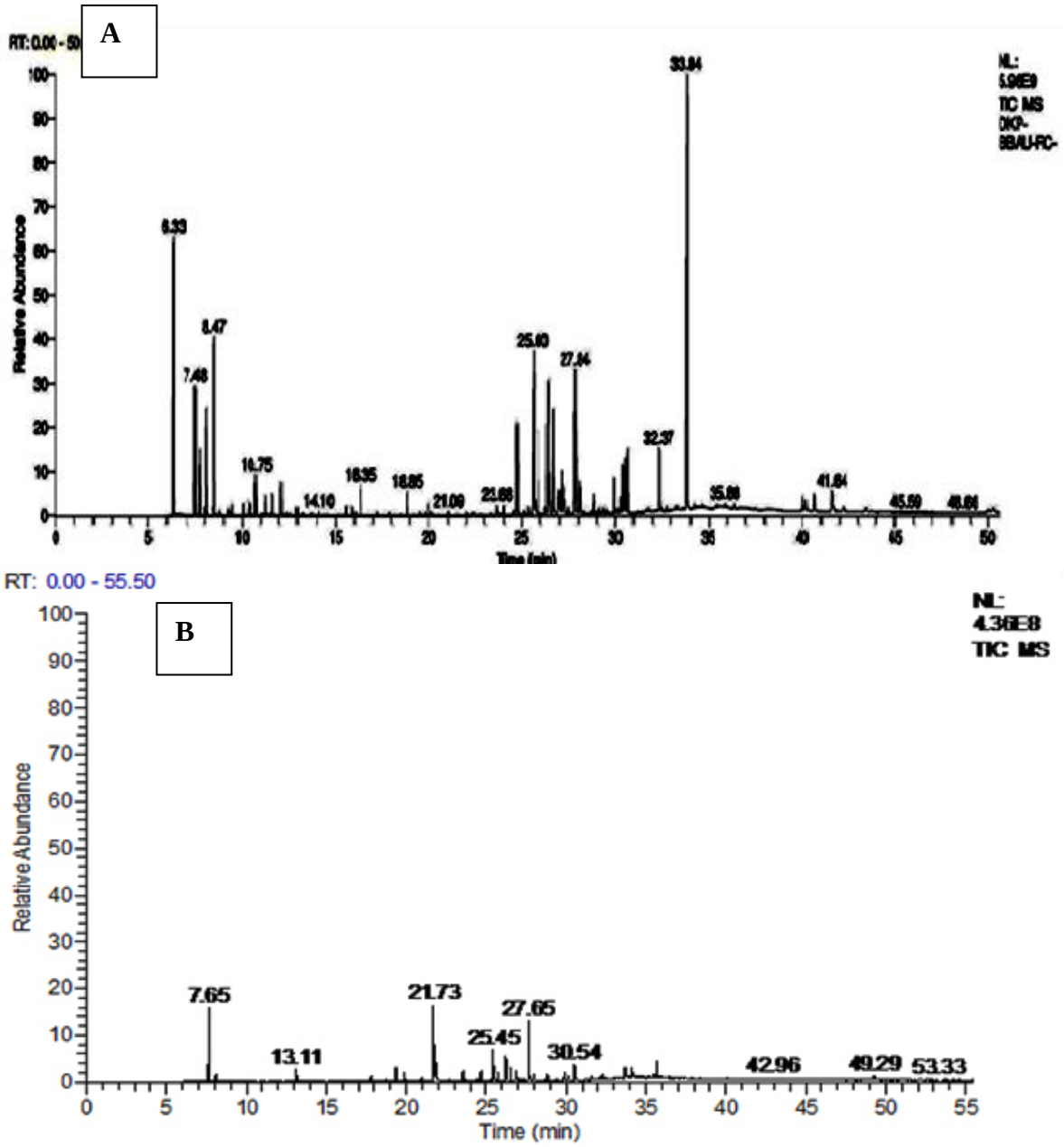

Fig: 6.5: GC-MS chromatogram of before and after TDS Reduction for pollutants analysis present in Distillery Effluent A. Control, B. after TDS Reduction

Table 2: Identification of residual organic pollutants in distillery industry wastewater before and after TDS reduction process by GC-MS analysis.

S.No.	RT	COMPOUND	Toxicity
1	6.33	O,O-tri(trimethylsilyl)-1-c(13)-N-carboxy-glycine	Bloody vomiting, liver failure, dilate the blood vessels.
2	7.48	ç-CHLORO-OCTAETHYL-à-OXOPHLORIN	Endocrine disruption,
3	8.47	Hexanoic acid, trimethylsilyl ester	Endocrine disruption,
4	10.75	2,2'-Methylenebis[4-t-butyl-6-(triphenylsilyl)phenol]	Acute renal failure.
5	11.54	Cyclohexane carboxylic acid, trimethylsilyl ester	Carcinogenic, allergy reaction.
6	13.21	3,7-Dioxa-2,8-disilanonane,2,2,8,8-tetramethyl-5-[(trimethylsilyl)oxy]- (CAS)	Hypertension, Stress, Osteoporosis, urinary stones,
7	14.10	Benzeneacetic acid, trimethylsilyl ester	Gastrointestinal, Hematological, Respiratory (Nose to Lungs).
8	15.16	Nonanoic acid, trimethylsilyl ester	strong eye irritant, vomiting
9	16.35	Benzenepropanoic acid, trimethylsilyl ester	DNA damage, carcinogenic, allergy reaction, irritant dermatitis,
10	17.23	Decanoic acid, trimethylsilyl ester	Inflammation, Fibrosis, Necrosis
11	19.49	4-Hydroxyphenylethanol, di-TMS	Parageusia, pink disease. Diarrhea Fever, Vomiting
12	19.59	Pentanedioic acid, 2-[(trimethylsilyl)oxy]-,bis(trimethylsilyl) ester	Carcinogenic, allergy reaction.
13	21.09	Dodecanoic acid, trimethylsilyl ester (CAS)	inflammation and irritation
14	23.81	Octadecane	Cough. Sore throat. Skin Redness. Eye Redness.
15	27.84	Hexadecanoic acid, trimethylsilyl ester	Degenerative bone disease, lung cancer,
16	29.95	Hexadecanoic acid, butyl ester	Difficulty Breathing Stomach Upset.
S.No.	**RT**	**COMPOUND**	**Toxicity**
1.	7.65	Octadecanoic acid, trimethylsilyl ester	Hypertension, Stress
2.	13.11	MONOPALMITIN 2TMS	Fatty Liver Disease (NAFLD), effect of Pancreas.
3.	21.73	Effusanin E	Toxicity to humans
4.	25.45	Enterolactone(2,3-bis(3-hydroxybenzyl)butyrolactone-di(trimethylsilyl)	worsen asthma attacks, and aggravate existing heart disease
5.	27.45	24-hydroxy-3,4-secolanost-4,(28),8-dien-3-nitrile	Endocrine disruption,
6.	30.54	7-(Trimethylsilyloxy)-3-[4-(trimethylsiloxy)phenyl]-4H-1-benzopyran-4-one	kidney and proximal tubule cells,
7.	42.96	ZINC(II)-à-ACETOXY-á FORMYLOCTAETHYLPORPHRIN	Chest Pain
8.	49.29	2-(1-Phenylsulfanyloctyl)cyclohex-2-enone	
9.	48.67	Benzeneacetic acid, à,4-bis[(trimethylsilyl)oxy]-,trimethylsilyl ester (CAS)	Diarrhea Fever, Vomiting
10.	53.33	(5,10,15,20-tetraphenyl[2-(2)H1]prophyrinato)zinc(II)	inflammation and irritation

6.3.5 Effect on seed germination and microbial community

Periodic SEM analysis of degrading samples in the TDS reduction process also showed an increase of bacterial population and diversity. During the bacterial growth, there was also reduction of color compared with that of the control in TDS reduction sample. There is no any bacterial growth possible s such in control sample but the result revealed that after TDS reduction the luxuriant growth are found after TDS reduction. Comparative toxicity assessment of effluent degraded by the TDS reduction process and the control in the seed germination test showed a 80-85% reduction of toxicity with Phaseolous mungo seeds. *P. mungo* was found to be more sensitive. The reduction of toxicity of the effluent supported the fact of removal of toxic compounds present in distillery wastewater by bacterial growth. The seed germination tests revealed that the toxic compounds that were inhibitory for the amylase enzyme responsible for seed germination of Phaseolous mungo L. showed apparent differences between the control and TDS reduction samples.

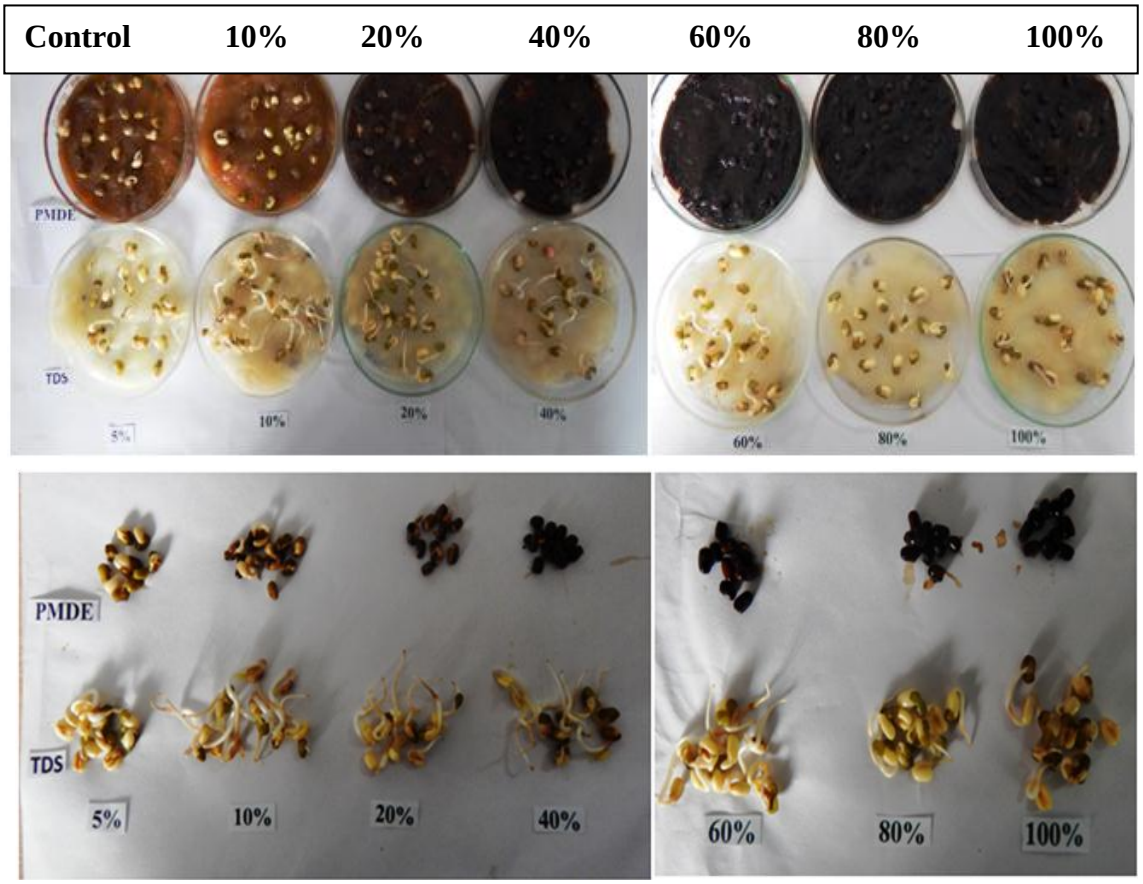

Fig: 6.6_*Phaseolus mungo L.* seed Germination_test_of before and after TDS Reduction to detect the seedling growth.

Conclusion

The present study showed that total dissolved solids (TDS) can be removed effectively from aquaculture wastewater with the use of ferric chloride (Fecl3) as coagulants. The process was found to be dependent on the dosage of coagulant, settling time, temperature and pH of the effluent. Optimum TDS removal of 82 % was obtained at pH of 8, dosage of 0.34 g/L, solution temperature of 303 K and settling time of 60min. This coagulant is environmentally friendly, readily available and cheap to obtain. Thus, the use of this material in wastewater treatment could be more suitable and advantageous when compared to other chemical coagulants that are normally expensive and also have a lot of undesirable environmental and health impacts. In addition, it can be concluded that TDS reduction method is suitable to find the optimum conditions of distillery wastewater treatment and reduces the cost and the time, which are necessary to find the optimum conditions. TDS reduction method using coagulants also can be used to determine the importance of each of studied factors in wastewater treatment.

Chapter-Seven

Profiling of bacterial community of Distillery waste Degradation through Next Generation Sequencing (NGS).

Chapter-7

Profiling of bacterial community of Distillery waste Degradation through Next Generation Sequencing (NGS)

7.1 Introduction

The distillery industry is a key economic actor in developing countries, including India (Annan et al., 2013; Shammi et al., 2016; Tripathi et al., 2021a). However, distilleries are also major sources of environmental pollution due to the presence of toxic organic and inorganic compounds in the discharged wastewater (Kumar and Chandra, 2020; Jiang et al., 2019; Tripathi et al., 2021b). According to the All India Distiller's Association (AIDA), there are approximately 397 sugarcane molasses-based distilleries in India and an average 3.5×10^{16}L of raw effluent is discharged into the receiving water per annum (AIDA, 2016).These wastewater and sludge contain 55-60% of organo-metallic compounds that are generated during the fermentation process (Valentino et al., 2019; Leng et al., 2020). Amino acids, fatty acids, pentadecanoic acids, organo-metallic compounds, amino carbonyl compounds, aldehyde-amines, metallic and non-metallic ions and elements, phenolic compounds, fungicides endocrine disruptors (EDCs) have been detected in distillery wastewater (Caizán-Juanarena, et al., 2020; Chowdhary et al., 2018,Canle et al., 2017; Campanale et. al., 2020; Varjani&Sudha, 2020)that are harmful to human health and the environment. Distillery sludge contains a large number of residual organic and inorganic contaminants but it contains relatively few carbon and nitrogen nutrient resources (Rashmi et al., 2020). Sludge-dwelling microorganisms mineralize organo-metallic compounds and utilise them as a source of energy for their metabolic processes (Srivastava et al., 2020). As a result, these endogenous bacterial species inhabiting the distillery sludge disposal sites could be developed and integrated into along-term waste management strategy (Duncan et al., 2017). Though the microorganisms in sugarcane molasses-based distillery sludge have been reported for the bioremediation and detoxification of the residual organic pollutants (Chandra et al., 2018a; Tripathi et al., 2021c), the impact of the diverse forms of pollutants on the bacterial communities are still unknown. The aim of this research is to explore the microbial diversity and interaction of the microbial community with residual organic pollutants using 16S rRNA-based next-generation sequencing (NGS), as well as their modes of actions and survival attributes. To our knowledge, this is the first

metagenomic analysis on microbial communities and their relationships with organo-metallic compounds in a polluted sugarcane molasses-based distillery sludge site. The results provide essential knowledge on the bacterial populations for sustainable ecological restoration of distillery sludge sites.

7.2 Material Method:

7.2.1. Site description and sampling

In this study samples were collected from M/s Unnao Distillers, Unnao, Uttar Pradesh, India (26°32'N, 80°30'E). This industry is generated wastewater, groundwater, and soil pollution with a large amount of organic and inorganic pollutants. The sludge samples were collected from three sites of discharged effluents. The site D1 was near the discharge of industrial effluent, site D2 was one km away from the industry and mixing point of industrial effluent and sewage wastewater, while the site D3 was degraded sludge 2 km away the industry, industry effluent with the sewage water. The collected sludge samples were immediately transferred to the polyethylene sterilized bags. The collected sludge samples were transported under the ice-cold condition (~ 4 °C) for laboratory analysis

Fig.7.1: Map of location of and sample collection sites located in Unnao near Kanpur, Uttar Pradesh, India where sludgewere generated in huge amount.

7.2.2. Physico-chemical characterization of collected samples

The distillery industry sludge sample was air-dried and crushed into powder form. Orion pH meter (Model-960, Thermo Scientific, FL, USA) and Orion conductivity meter (Model-A322, Thermo Scientific, FL, USA) were used to calculate the pH and electrical conductivity (EC) of sludge samples, respectively (Tripathi et al., 2021a). The colorimetric method was used for phosphate measurement. The total concentration of sodium (Na^+), chloride (Cl^-), and sulphate (SO_4^{2-}) were measured by the method (Tripathi et al., 2021c). Phenol concentrations present in the sludge sample were also determined (Kumar and Chandra, 2020). The metal concentration in the sludge sample of distillery effluent was determined after sample digestion with nitric, perchloric, and hydrofluoric acid according to the procedure followed by (Tripathi et al., 2021a and 2021b). Flame atomic absorption spectrophotometry (AAS) was used to calculate Cd, Cu, Mn, Cr, Fe, Ni, and Zn under optimal conditions.

7.2.3. Detection and characterization of various organic Pollutants

The GC-MS analysis has been done of all sludge samples (D1, D2, and D3) for identification of emerging pollutants. Ethyl acetate, an organic solvent with a pH less than 8.0, was used to remove contaminants from distillery sludge. The extraction method for extracting full organometallic contaminants was carried out three times. Further, the extracted solvent was dried and dissolved into the ethyl acetate (2.0 mL) and filtered by 0.22-µm syringe filters (Millipore Ltd., Bedford, USA) for the further analysis process. The extracted organic pollutants through ethyl acetate were subjected to GC-MS analysis for the characterization of pollutants in sludge samples (D1, D2, and D3). The detected pollutants were identified through the mass spectral comparing at retention times (RT) interval with the standard compounds, which are already mentioned in the National Institute of Standard and Technology (NIST) (version 1.0.0.12, NIST, USA) library.

7.2.4. Bacterial community's characterization of sludge

The pico green Victor 3 fluorometry procedure was used to determine the amount of Template DNA. The fluorescence-based quantification approach was used to implement the genomic method of quantification. Nanodrop was used to quantify the DNA samples. A 1% agarose TAE gel was used to verify the DNA consistency. V3-V4 PCR was set up using V3-V4 Forward and Reverse Primer and the PCR product was loaded on an agarose gel to screen for positive amplification using the 16S amplicon as a template (around 460 bp). Positive v3-v4 amplification was observed in

all D1, D2, and D3 samples, with a band size of 460 bp. The size distribution of the prototype was tested using an Agilent Technologies 2100 Bioanalyzer and a 1000 chip of DNA for the verification of the size of an enriched PCR fragment. On Illumina sequencing platforms, optimal cluster densities were created across every lane of every flow cell for achieving the highest data quality. It requires the accurate quantitation of the template DNA library. Further, the quantification of the prepared library was performed by qPCR as per the Illumina PCR Quantification Protocol Guide. Then, Roche's Rapid library standard Quantification was utilized for the calculation and standard curve reading in fluorescence as well as to calculate the concentration of library sample.

7.2.5. Bioinformatics analysis

The bacterial community of D1, D2, and D3 was analyzed as per the 16S V3-V4 PCR raw data. The raw sequences were merged and removed barcodes and primers via a trimmed and denoised process. It was clustered based on sequence similarities (97%) into the operational-taxonomic units (OTUs) using the UCLUST program (version 1.2.22q). The OTUs with phylogenetic relation were analyzed and ribosomal database project (RDP) classifier (version 2.2) taxonomic assignment was performed against the SILVA OTUs database (version 123) with a confidence threshold of 70% (Bokulich et al., 2013). Differences in the diversity among the samples were calculated by Shannon-D, Chao1, and Observed-species indices estimation. Chao1 metric estimation showed the presence of species richness in the sampling site of the environment (Wang et al., 2019). However, the Shannon metric evaluated the observed OTU abundance and evenness, and species richness in the samples D1, D2, and D3 (Fig. 5). Finally, Chao1, based on Shannon, and Observed-species indices, rarefactions curves were prepared via QIIME (version 1.7.0) and displayed by R software (version 2.15.3).

7.3 Result and discussion

7.3.1. Physiochemical properties of the sludge samples

The effluent sludge from the distillery was dark brown and smelled turbid and nasty. The dark brown color is attributed to the presence of melanoidin polymer in the effluent and sludge of D1, D2, and D3 (Tripathi et al., 2021c; Sharma et al., 2021a).Various researchers also reported that the melanoidin content of distillery sludge was biodegradable by bacterial communities and fungi. The bacterial population involved in melanoidin degradation has mainly been found in bacteria and

fungi. The melanoidin content of wastewater can be easily degraded and detoxified by bacteria. These bacteria have been characterized by α-Proteobacteria, β-Proteobacteria and Streptomyces and these bacterial groups have also been reported in our sample (Sharma et al., 2021b; Sharma et al., 2020). The dark colour of the effluent formed a blanket, which decreased the opacity of water and thus reduced the photo-synthetic behaviour of the water plants (Tripathi et al., 2021c; Chandra et al., 2018). However, the color was pH-dependent, and recorded alkaline with a pH of 8.29 ± 0.10, 7.29 ± 0.19*, and 8.01 ± 0.12**) for D1, D2, and D3, respectively. Bacteria retain an optimum functional and structural integrity of the cytoplasmic proteins that sustain growth, consistent with the cytoplasmic pH. The majority of non-extremophilic bacteria evolve from 5.5 to 9.0 over a wide range of pH levels, with a cytoplasmic pH. In this study, *Pseudomonas* genus under *Proteobacteria phylum* was present in D1, D2, and D3, which survive at extreme pH conditions due to bioenergetics cycles that are entirely Na+-coupled, and thus lacks active H+ extrusion or uptake systems that can support pH homeostasis. Consequently, the pH expands only within an optimum cytoplasmic pH within the small pH range of 6.3-7.7 (Valentino et al., 2019; Collins et al., 1994). The high pH of D1 was due to the bleaching process, D2 is increased and reduced in D1 it may be due to the mixing of domestic waste with D1 sample and the reduction of pH in D3 is due to the formation of organic acids by fungal and bacterial metabolic activities (Chandra et., al 2018). Besides, a high EC level in the effluent of D1, D2, and D3 indicates the presence of different cations and anions in the effluent i.e., sodium (Na+), chloride (Cl−), Sulfate (SO4 2−), and phosphate (PO4−) and these ions provide the key nutrient factor for the growth of microbial community (Table 1). The level of cations and anion is decreased continuously from D1, D2, and D3that might be due to the mixing of different domestic effluent with distillery effluent. During water flow, different organic and inorganic matter breaks down to form new chemical compounds, and resultantly, cations and anion are decreased in the effluent from the first site (D1) to the last site (D3) (Table 1). In our results, bacterial communities of distillery sludge include *Proteobacteria, Actinobacteria*, *Firmicutes,* and *Tenericutes* followed by *Patescibacteria* and *Cloacimonetes*.

Table.7.1: The physico-chemical characteristics of distillery sludge collected from Dumping site located in premises of Unnao Distilleries & Breweries Limited.

S. No.	Parameters	Sludge sample (D1)	Sludg sample (D2)	Sludge sample (D3)	Permissible Limit
1.	pH	8.29 ± 0.10	7.29 ± 0.19*	8.01 ± 0.12**	---
2.	EC	5.7 ± 0.00	6.8 ± 0.00**	7.9 ± 0.00*	---
3.	CEC	68.19 ± 0.34	58.19 ± 0.98**	72.19 ± 0.34**	---
4.	TOC	15.25 ± 0.19	13.25 ± 0.23***	19.54 ± 0.78**	---
5.	TKN	5.65 ± 0.08	4.65 ± 0.02**	7.43 ± 0.23*	---
6.	Ammonical Nitrogen	18.25 ± 0.89	16.34 ± 0.67**	20.89 ± 0.98**	1
7.	Total hydrogen	4.22 ± 0.08	5.67 ± 0.00	3.22 ± 0.08*	---
8.	Total oxygen	35.35 ± 0.09	38.35 ± 0.02	40.65 ± 0.01**	---
9.	Sodium (Na^{+})	35.15 ± 4.32	40.15 ± 7.32	31.30 ± 3.32	200
10.	Chloride (Cl^{-})	798.25 ± 15.12	734.23 ± 11.12*	676.43 ± 89.76*	1500
11.	Sulfate (SO_4^{2-})	165.08 ± 8.43	134.10 ± 6.43	199.09 ± 9.49	---
12.	Phenol	450.14 ± 1.22	398.14 ± 1.34**	399.1423 ± 7.00	---
13.	Phosphate (PO_4^{3-})	1267.23 ± 9.70	1190.23 ± 7.70	1150.34 ±2.90	---
Heavy metals					
a)	Iron (Fe)	1678 ± 45.28	1598 ± 34.54	1502 ± 32.45**	2.0
b)	Zinc (Zn)	88.88 ± 1.87	56.78 ± 3.87	95.23 ± 2.65*	2.0
c)	Copper (Cu)	71.56 ± 0.99	76.76 ± 1.99***	75.43 ± 4.77***	0.5
d)	Chromium (Cr)	20.628 ± 0.00	18.435 ± 2.00**	19.435 ± 1.00**	0.05
e)	Cadmium (Cd)	2.011 ± 0.00	1.013 ± 2.00*	2.009 ± 1.00***	0.01
f)	Manganese (Mn)	99.20 ± 0.19	88.18 ± 9.34	92.13 ± 0.21*	0.20
g)	Nickel (Ni)	13.123 ± 0.09	15.321 ± 0.09	11.311 ± 0.05**	0.1

All values are mean of three replicate ± SD and presented in mg kg−1 except electrical conductivity (μS cm−1), TOC (%), TKN (%), total hydrogen (%), and total oxygen (%); CEC (Cmol), *EC* electric conductivity, *CEC* cation exchange capacity, *TOC* total organic carbon, *TKN* total Kjeldahl nitrogen. Asterisks indicate significant differences between the non-rhizospheric and rhizospheric distillery sludge; Student's *t* test: *non-significant ($p < 0.05$), **less significant $p < 0.01$, ***highly significant ($p < 0.001$).

7.3.2. GC-MS analysis for detected organic pollutants

The isolated organic compounds of D1, D2, and D3 from distillery sludge revealed that each sample had a different set of compounds. The major peaks (D1, D2, and D3) are seen in Fig. 1, and the compounds are described in Tables 2. The majority of the molecules aren't the same in any study. The organic compounds found in sludge samples are hazardous, and the toxicity of each compound is described in Tables 2 (Mukherjee et al., 2017; Ngo et al., 2020; Ojuederie et al., 2017). Results found that most D1, D2 and D3 contaminants were biodegraded and bio-transformed by the fact that the infected site had been present in bacterial populations shown in Fig. 1 and Table 2 Mukherjee. Compounds detected by bacterial populations at the sample site are not identical in each sample due to the activity. The GC-MS technique was used to identify organic contaminants at the soil sites D1, D2 and D3. The volatile character of organic compounds in sludge's leads to harmful health and environmental effects (Tripathi et al., 2021a&b; Igiri et al., 2018; Selvi et al., 2019). Organic toxic compounds are released in the many stages of alcohol production from the industry i.e., acid digestion, fermentation, and methanogenesis. Some studies have earlier reported similar toxic compounds from the distillery industries sludge (Bhargava and Chandra, 2009; Kumar and Chandra, 2020). The highest growth in the sampling area of bacterial communities revealed that the resistance and ability of the bacterial communities could be caused by residual toxic organic contaminants degraded and intoxicated from distillery sludge (Tripathi et al., 2021a). The different organic compounds were detected from the different sites of contaminating sludge, but some compounds were found in all samples (D1, D2, and D3), like pentachlorophenol, hexadecane, quinone, and furanone. These compounds may be released during the fermentation process, multistage molasses melanoidin, and the use of organic and inorganic compounds for alcohol production. These are commonly detected toxic compounds in the distillery sludge and cannot be biodegraded and detoxified by bacterial communities. However, some of the compound listed in Tables 2 are might be due to the degradation and detoxification process and may also be formed by recombination of ionic bonding of pollutants during effluent run flow and mixing of domestic water at different sites of sludge (Table 2). The toxic profiles of these organic pollutants are listed in detail (Table 2) (USEPA, 2012). The bacterial communities present in the sludge (D1, D2, and D3) might degrade and detoxify most

of the residual organic compounds of distillery sludge via their metabolic, catabolic, and enzymatic properties.

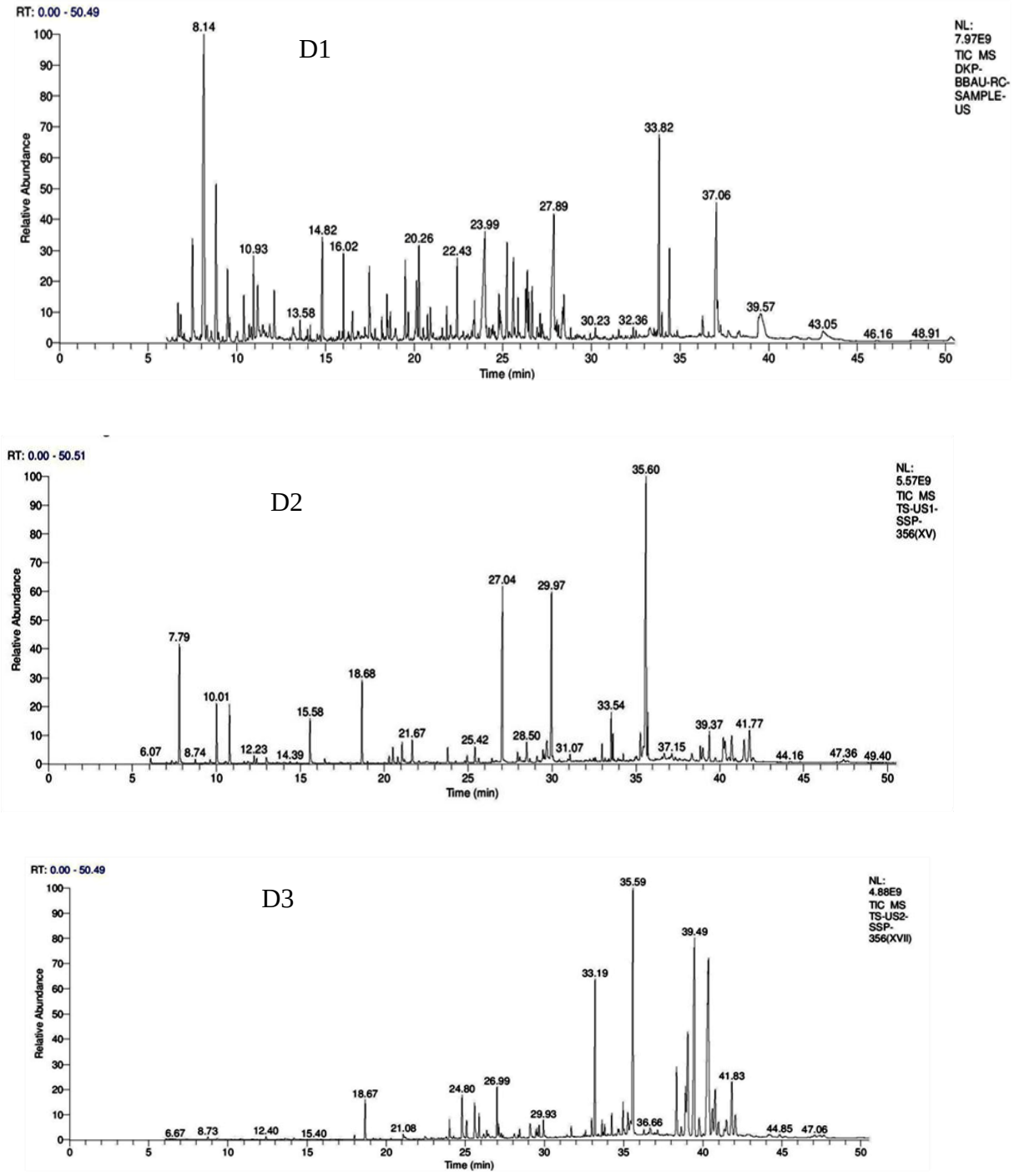

Fig 7.2: The GC-MS chromatogram of site D1, D2, and D3 of Distillery industry sludge contaminated site.

Table: 7.2: Organic compounds identified by GC–MS analysis extracted with ethyl acetate from distillery waste and their toxicity profile.

S.No.	RT	Compound	D1	D2	D3
1.	6.04	Arabinitol, pentaacetate (CAS)	+++++	-----	-----
2.	7.79	Hexanoic acid, trimethylsilyl ester (CAS)	-----	+++++	-----
3.	8.14	Pantolactone	+++++	-----	-----
4.	8.73	E-2-(4'-Tolyl)-1-(phenylsulfonyl)ethene	-----	-----	+++++
5.	10.01	HEPTANOIC ACID TMS	-----	+++++	-----
6.	10.93	(8S)-8-Hydroxypatchoulol	+++++	-----	-----
7.	12.23	Octanoic acid, trimethylsilyl ester	-----	+++++	-----
8.	12.40	Kadsulignan D	-----	-----	+++++
9.	13.58	Benzoic acid trimethylsilyl ester	+++++	-----	-----
10.	14.39	Nonanoic acid, trimethylsilyl ester	-----	+++++	-----
11.	14.82	2-(1-Hexynyl)benzeonitrile	+++++	-----	-----
12.	15.40	Lupane-3á,12à,28-triol	-----	-----	+++++
13.	15.58	Benzenepropanoic acid, trimethylsilyl ester	-----	+++++	-----
14.	16.02	Nonanoic acid, trimethylsilyl ester	+++++	-----	-----
15.	18.36	(3-Methoxyphenyl)trimethylstannane	+++++	+++++	+++++
16.	18.68	Decanoic acid, trimethylsilyl ester (CAS)	-----	+++++	-----
17.	20.26	1-Ethoxy-4-hexyl-5-(TMS)-1,4-pentadiene	+++++	-----	-----
18.	21.08	Dodecanoic acid, trimethylsilyl ester (CAS)	-----	-----	+++++
19.	21.67	n-Tridecanoic acid, trimethylsilyl ester	-----	+++++	-----
20.	23.99	Benzeneacetic acid	+++++	-----	-----
21.	24.80	Phthalic acid	-----	-----	+++++
22.	25.42	n-Pentadecanoic acid, trimethylsilyl ester	-----	+++++	-----
23.	26.99	Hexadecanoic acid, trimethylsilyl ester	-----	-----	+++++
24.	27.04	Sclerodin	-----	+++++	-----
25.	27.89	á,á,ç-triphenyl-benzenepropanol	+++++	-----	-----
26.	29.81	Lucenin 2	+++++	+++++	+++++
27.	29.93	Octadecanoic acid, trimethylsilyl ester	-----	-----	+++++
28.	29.94	Hexadecanoic acid, butyl ester	-----	+++++	-----
29.	29.97	9,12-Octadecadienoic acid (Z,Z)TMS	-----	+++++	-----
30.	30.23	3-(4-Ethoxybenzoyl)acrylic acid	+++++	-----	-----
31.	32.36	Octadecanoic acid, butyl ester	+++++	-----	-----
32.	33.19	Dehydrobenzo[20]annulene	-----	-----	+++++
33.	34.06	Dimethoxy[tri(trimethylsilyl)methyl]silane	-----	+++++	-----
34.	34.39	COMPACTONE	+++++	+++++	+++++
35.	35.59	5,10-dihydrobenzo[b][1,8]naphthyridine	-----	+++++	+++++

RT- Retention time (in minutes), **C**-control, **+** present, **−** absent, **TMS**-trimethylsilyl

7.3.3. Diversity analysis and richness OTUs of sludge samples

The structure of bacterial groups in the samples was analysed using the Next-Generation Sequence Technologies to gain an insight into bacterial diversity. The V3 and V4 hypervariableregions (16S rRNA) were selected for the taxonomic assignment using QIIME to examine, compare, and determine the composition of bacterial communities in contaminated sites, D1, D2, and D3. In this study, 240376; 288604, and 333147 raw reads were collected from the D1, D2, and D3 samples, respectively. The lengths of sequences were 250 bp, and GC contents were 50.44, and 36.67%, in D1, 250 bp, and GC contents were 51.99, and 36.27%, in D2, and 250 bp, and GC contents were 52.59, and 36.28%, in D3, respectively. 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 13033 OTUs were identified from 544497 reads. From 13033 total OTUs, 10054 OTUs with less than 5 reads were removed and 2979 OTUs were selected for further analysis. In D1, D2, and D3, estimates of alpha diversity indices revealed a higher phylogenetic diversity and evenness. The rarefaction based on Mothur v.1.21.1 was conducted to reveal the diversity indices D1, D2, and D3, including the sobs, Ace, Chao, jackknife, Shannon, and Simpson (Table 3). The alpha diversity was estimated via Chao1 and Shannon indices calculation using rarefaction sampling of D1, D2, and D3 samples (Fig 2a, b and c). *Spirocheaeta* is a genus of bacteria belonging to the *spirochaetaceae* family that was identified in the research. The organic compounds in the sludge are readily and quickly degraded by the *Allidiomarina* species. Besides, the most common genus *Brevibacillus* was also detected under phylum *Actinobacteria.* Some other identified genera in sites D1 (*Massilia, Aquicella, Fictibacillus and Unclassified*), D2 (*Ralstonia, Brevibacterium, Ochrobactrum, Dechloromonas, Hypomicrobium, Pleomorphomonas, Proteus, Rhodobacter,,Ruminococcus, and Unclassified*) and D3 (*Enterobacter, Branchiibius, Verrucomicrobium, Hymenobacter, Ezakiella, Fibrisoma, Rickettsia, Rudaeicoccus*). In all of the samples, certain bacterial genera were found to be normal. The *acholeplasma*and*Pseudomonas* bacterial communities have been reported for the biodegradation and detoxification of persistent organic pollutants of distillery sludge (Schneider et al., 2020; Kim et al., 2020; Tripathi et al., 2021a). Notably, D1 sample were distinct from D2 and D3

samples due to environmental conditions as per the result of PCA, which is based on the functional distribution in the various types of environments, suggesting the importance of biogeochemical measurements with integrating metagenomics analysis for the study of ecology The microbial community was characterized and analyzed in the three site sludge samples of distillery effluent. This is the first study report for the metagenomics full-scale analysis of distillery effluent discharge, middle and mixed site of the industry with the study of coordination between pollutants in the contaminated site and bacterial communities. In conclusion, the phyla *Actinobacteria, Proteobacteria, Spirochaetes, Sinergistetes, Tenericutes, Patescibacteria, Fermicutes, others, Bacteriodetes, Cloacimonetes and Euryechaeota*.in samples D1, D2, and D3 were the predominant and play important roles in the detoxification and biodegradation of persistent organic pollutants in distillery industry sludge. The findings suggested the significant variance for the relative abundance of the bacterial phyla of three sites of distillery sludge samples.

7.3.4. Comparative microbial diversity analysis of D1, D2, and D3

The differences in the taxonomic profiles produced by the MG-RAST technique were revealed by a comparative analysis of three metagenomics samples (D1, D2, and D3). The phylum level classification of predominant phyla in sample D1 microcosm are the *Euryechaeota* (6.00%), *Actinobacteria* (8.00%), *Bacteriodetes* (9.00%), *Patescibacteria* (2.00%), *Tenericutes* (24.00%),*Firmicutes* (5.00%), *Sinergistetes* (5.00%), (5.00%), *Proteobacteria* (24.00%), *Cloacimonetes 10%, Others* (2.00%). In contrast, the predominant phyla D2 microcosm are *Euryechaeota* (9.00%), *Actinobacteria* (11.00%), *Bacteriodetes* (13.00%), *Patescibacteria* (2.00)%, *Tenericutes* (4.00%), *Firmicutes* (5.00%), *Sinergistetes* (4.00%),*Spirochaetes 2%, Proteobacteria 31%, Cloacimonetes (0.00%), Others* (7.00%). The highest frequency of occurrence in class *Gammaproteobacteriawas* found in both D1 (21.00%), and D2 (21.00%), while D3 *Clostridia* has the highest frequency of occurrence (21.00%) in microcosm. In family delineation, the D1 and D2 microcosm showed predominance *Ruminococcaceae (6.00%, 2.00%), Spirochaetaceae (2.00%, 1.00%), Uncultured Bacterium (5.00%, 5.00%), Dysgonomonoadacae (4.00%, 0.00%), Achloeplasmataceae (4.00%, 0.00%), Unknown (11.00%, 15.00%), Burkholderiacae (6.00%, 10.00%), clostridiales (8.00%, 2.00%), Family (4.00%, 10.00%), Rikenellaceae (6.00%, 1.00%, others (44.00%, 58.00%).*, while D3 microcosm showed the major preponderance of *Ruminococcaceae (6.00%), Spirochaetaceae*

(1.00%), Uncultured Bacterium (8.00%), Dysgonomonoadacae (7.00%), Achloeplasmataceae (5.00%), Unknown (11.00%), Burkholderiacae (11.00%), clostridiales (0.00%), Family (10.00%), Rikenellaceae (1.00%), others (40.00%)(Fig. 6). In addition, an unknown bacterial genus predominates were found microcosms constituting (24.00, 26.00, and 25.00%) in D1, D2 and D3, respectively. The other predominance genus D1 and D2 microcosm showed i.e., *Fermentimonas (1.00%, 0.00%), Others (45%.00, 63.00%), Sphaerochaeta (2.00%, 2.00%) Sedimentibacter (2.00%, 0.00%), Christensenellaceae-R-7-group (1.00%, 4.00%), uncultured-bacterium (8.00%, 4.00%) Pseudomonas (1.00% 0.00%), uncultured (8.00%, 5.00%), Tissierella (1.00%, 0.00,* while the D3 microcosm showed the major preponderance, i.e.,*Fermentimonas (0.00%), Unknown(25.00%), Others (59.00%), Sphaerochaeta (0.00%), Sedimentibacter (0.00%), Christensenellaceae-R-7-group (0.00%), uncultured-bacterium (9.00%), Pseudomonas (1.00%), uncultured (6.00%), Tissierella (0.00%)* (Fig. 7).At the species level, the unclassified bacterial species predominates were found microcosms constituting (*Alidiomarina-sp), (Uncultured-Tissierella-sp.), (Uncultured- Bacteroidetes-bacterium), (Uncultured- Clostridiales-bacterium), (Uncultured-archaeon), (Uncultured- prokaryote), (Uncultured-Firmicutes-bacterium), (others), (Unknown), (Uncultured Bacterium), (Firmicutes-Bacterium-ADurb.Bin080)*D1, D2, and D3, respectively. The others predominance species D1, D2 microcosm showed i.e., *Sphingomonas*(0.08, 5.12%), Listeria-sp.(0.29, 0.38%), *Weissella-confusa*(0.03, 0.01%), Kocuria-flava(0.23, 0.002%), *Moryella*(0.004, 0.31%), *Micrococcus-luteus*(0.008, 0.01%), *Succinogenes*(0.18, 0.00%), *Loktanella*(0.008, 0.14%), *Fragi*(0.008, 0.025%), and other (0.78, 0.86%), while the D3 microcosm showed the major preponderance i.e. *Streptococcus-pneumoniae*(0.33%), *Caenorhabditis*(0.00%), *unidentified* (0.49%), *Lactobacillus* (0.12%), *Vabuuchiae* (0.01%), *Succinicans* (0.30%), *Succinogenes* (0.10%), and other (1.18%) (Fig. 8) (Sahu et al., 2018; Rashmi et al., 2020).The relative abundance of Principal component analysis, which accounts for 40.32% and 60.76 %t of the overall difference, is used to analyse bacterial genera. Important variations in the relative abundance of a bacterial phylum were discovered in the relative abundance of D1, D2, and D3sludge samples in this analysis (Fig. 2c). Metagenomics analysis of the sludge samples suggest that the bacterial phylum and organic pollutants at the contaminated site have relations between the bacterial communities and pollutants and

bacterial communities play a crucial role in the bioremediation of refractory organic pollutants in a contaminated site.

7.3.5. Mechanisms of bacterial communities at a polluted site

Every year, a large amount of industrial wastewater is emitted around the world, but only about 30% of it is managed by using current wastewater treatment techniques before being discharged into the aquatic system. Several researchers have reported a diverse group of bacterial species in distillery waste that could have an effect on the ecosystem's microbial communities. The awareness about bacterial diversity and structure of bacterial populations found in environmental samples D1, D2, and D3 is explored using next-generation sequencing technology. The V3-V4 hyper variable region was selected for 16S rRNA gene analysis using Illumina (MiSeq) sequencing, and conspicuous differences were observed in the bacterial communities of each sample D1, D2, and D3. Rarefaction curves confirmed that the OTU bacterial community abundance was found diverse in every sample D1, D2, and D3) (Kumar et al., 2020, Sharma et al., 2021b). The Shannon diversity indices and Chao1 species richness were calculated using rarefaction sampling for the estimation of alpha and beta diversity of D1, D2, and D3 sludge samples (Fig. 2a, b&c). The Shannon-D and Chao1 indexes, on the other hand, revealed the diversity of bacterial species in each sludge sample D1, D2, and D3. Due to variations in pollution parameters, the bacterial diversity and structure of D1, D2, and D3 microbial communities differed between organisms. In addition, there have been differences in microbial diversity in each sample, depending on the physico-chemical parameters of sludge, contaminant loads, chemical compounds or any of the pollutant environmental conditions (Chandra et al., 2018; Tripathi et al., 2021aAt the organometallic polluted site, the results showed that D1 and D2 had greater bacterial diversity than D3. At a species level, the most abundant top ten uncultured species were found i.e. *Sphingomonas*(0.08, 5.12%), Listeria-sp.(0.29, 0.38%), Weissella-confusa(0.03, 0.01%), Kocuria-flava(0.23, 0.002%), *Moryella*(0.004, 0.31%), *Micrococcus-luteus*(0.008, 0.01%), *Succinogenes*(0.18, 0.00%), *Loktanella*(0.008, 0.14%), *Fragi*(0.008, 0.025%), and other (0.78, 0.86%), The percent of these uncultured species were approximately the differ in each sample D1, D2, and D3 (Fig.8). In each sample D1, D2, and D3, the phylum Proteobacteria was found to be the most prevalent in comparison to the other phyla, and this trend continued throughout all samples. This ability of *Firmicutes*leads to in-situ bioremediation of industrial

pollutants and this finding was consistent with different studies reported on *Firmicutes* for the bioremediation of toxic pollutants of distillery sludge (Womersley, 2006). In our study, the third-largest phylum *Actinobacteria* was found in distillery sludge D1, D2, and D3 and members of this phylum have to colonize different ecological niches and they performed their different biological functions. Besides, *Bacteriodetes* are well known for the degradation of persistent organic pollutants present in distillery waste because of their hydrolytic capacities (Liang et al., 2017). This phylum was found in a higher percentage in sample D1 and D3 but half in D2 due to the mixing of domestic water with effluent at site D2.The class of *Gammaproteobacteria* was found most abundant in the sample D2>D3>D1. Moreover, *Anaerolineae* and *Alphaproteobacteria* grow dominantly in D2, D3, and D1 (Fig. 7.4). Besides, genus *Fermentimonas, Pseudomonas, Christensenellaceae-R-7-group,* and *Tissierella* were the most abundantly well-known genera in phylum *Proteobacteria* for the physiological and different metabolic activities in distillery effluent wastewater (Ahmed et al., 2018). Genus *spirochaeta* was found under the phylum *spirochaetes,* and has been found higher in D3 due to the presence of genus *spirochaeta* because of its rapid movement makes then easily observed in water samples and their aseptic capability. As a result, this spirit can provide a pathway for treating polluted sludge and water at low temperatures by their metabolism and enzymatic activity and can degrade organic sludge contaminants in all samples. (Ghribi et al., 2016). The *sedimentibacter* species, which are present at higher concentrations in the D1>D2>D3 samples, is in the family *Eubacteriales,* the classes *Clostridia*, and Phylum *Farmicutes*by combining a high content of domestic waste with extracting waste effluent, thereby promoting the bacterial growth and metabolism within the D1, D2 and D3. The condensed type of effluent released directly from the distillery cannot sustain *sedimentibacter* development in the D2 industrial site. Table 2 showed a list of the pollutants which affect the growth of the bacterial community at each site (Mukesh et al., 2020; Sharma et al., 2021b). This is the first time data on bacterial community and contaminants detected from the distillery industry have been identified at a different location than the one specified in the material method. This community supports the pollutants bioremediation and detoxification of organic and inorganic chemical and chlorinated compounds present in the sludge after the discharge of toxic chemicals from the distillery effluent during alcohol production.

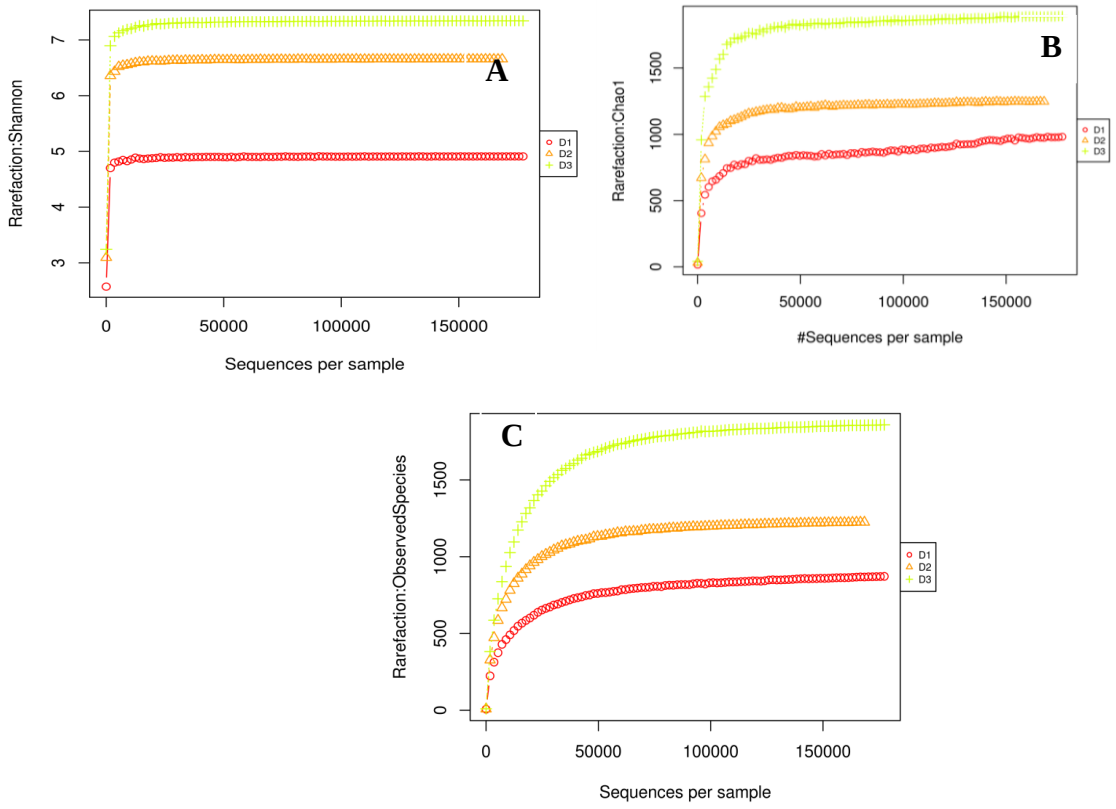

Figure 7.3: (A). Shannon curve obtained for the samples **(B).** Chao1 curve obtained for the samples **(C)**. Observed species curve obtained for the samples

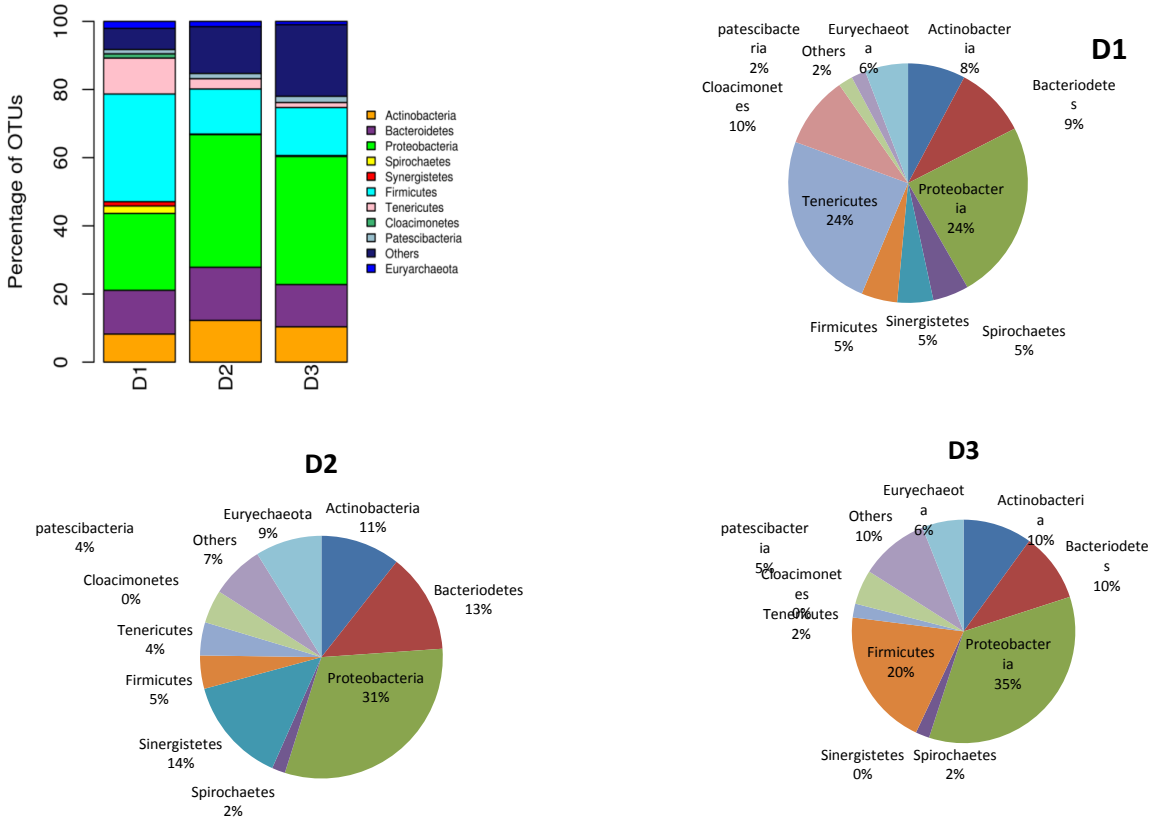

Fig 7.4: - Phylum level classification percent OTU Present in D1, D2, and D3.

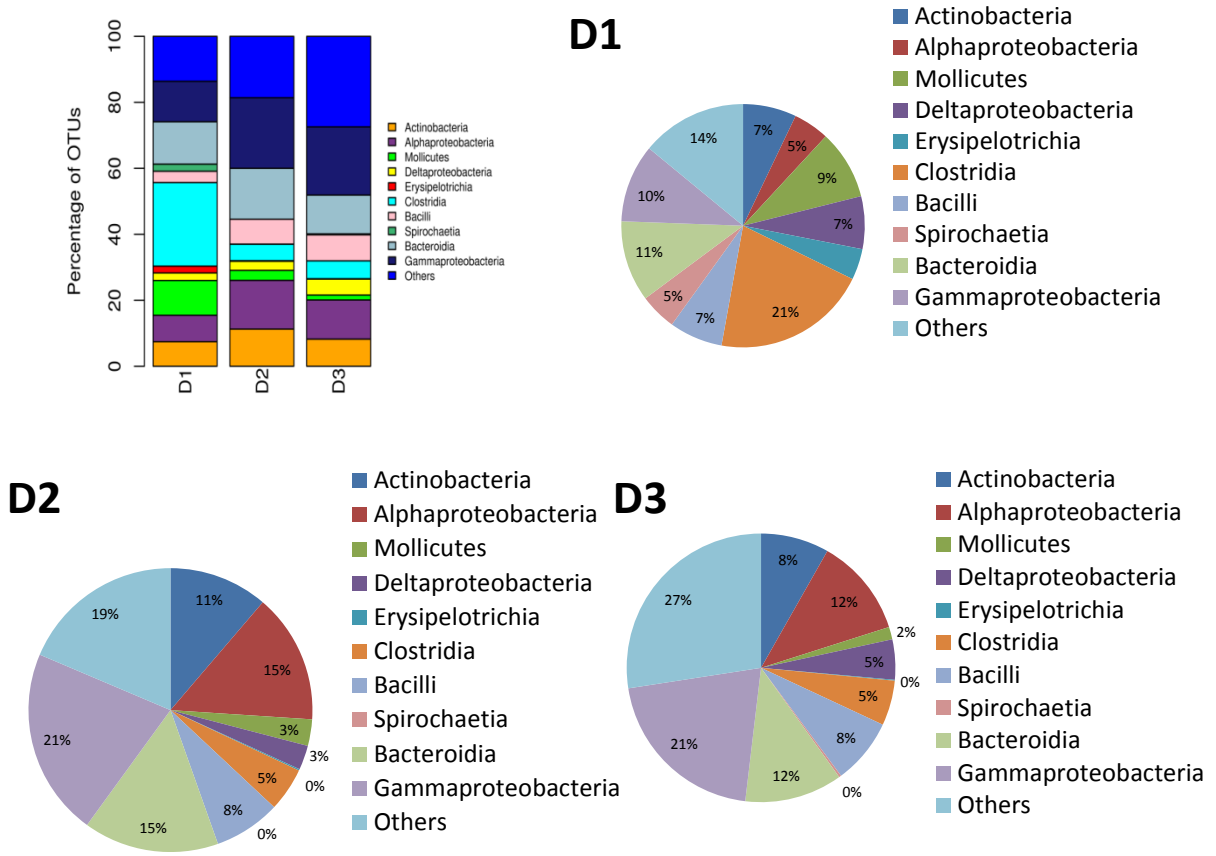

Fig 7.5: -Class level classification percent OTU Present in D1, D2, and D3.

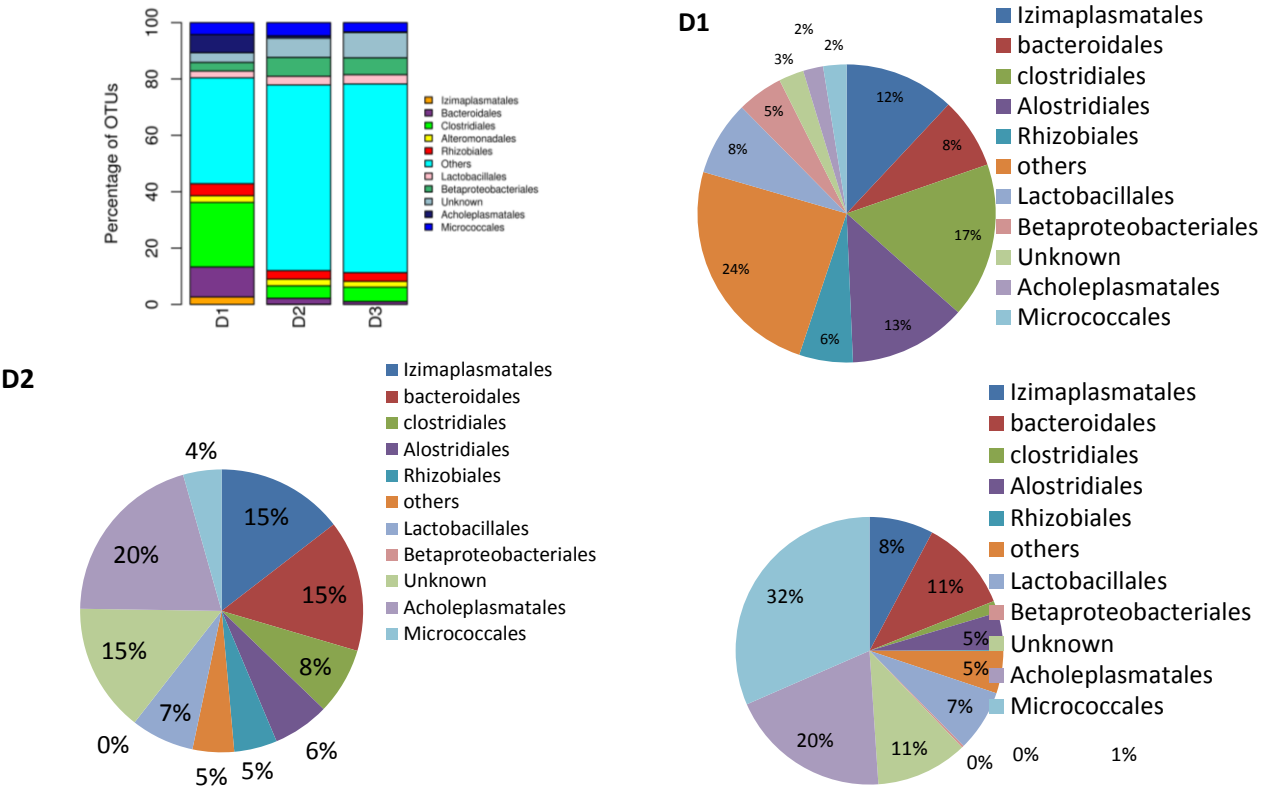

Fig 7.6: Order level classification percent OTU Present in D1, D2, and D3.

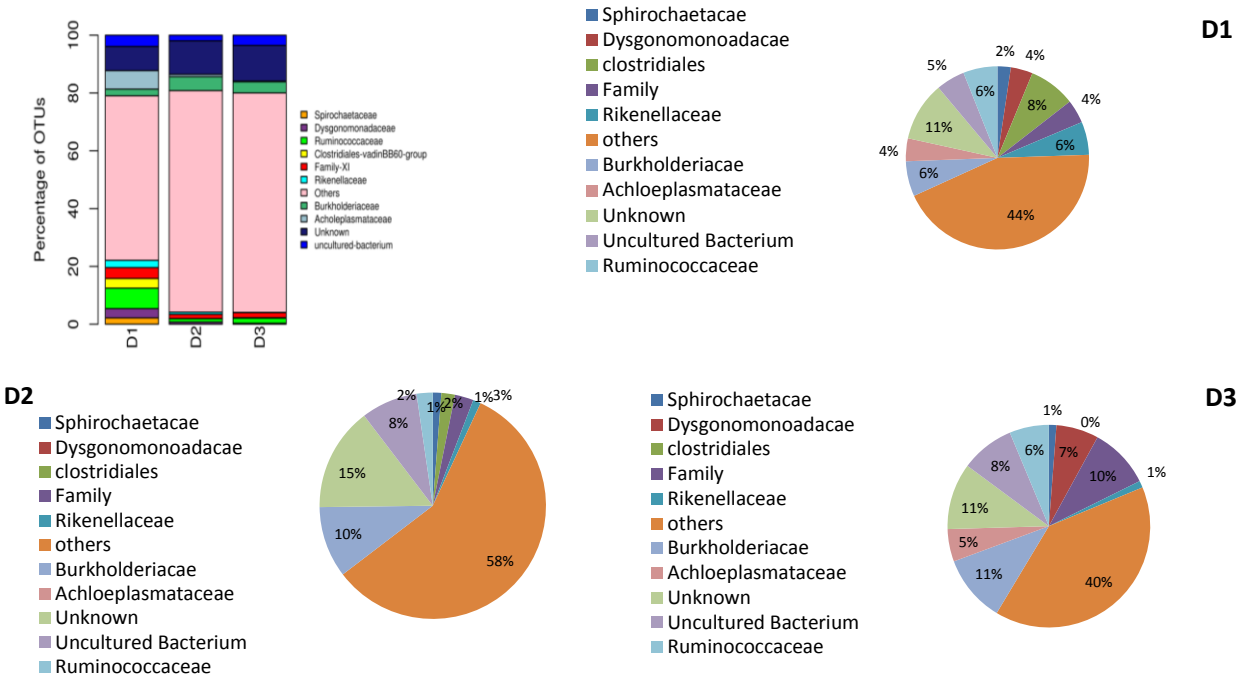

Fig 7.7: - Family level classification percent OTU Present in D1, D2, and D3.

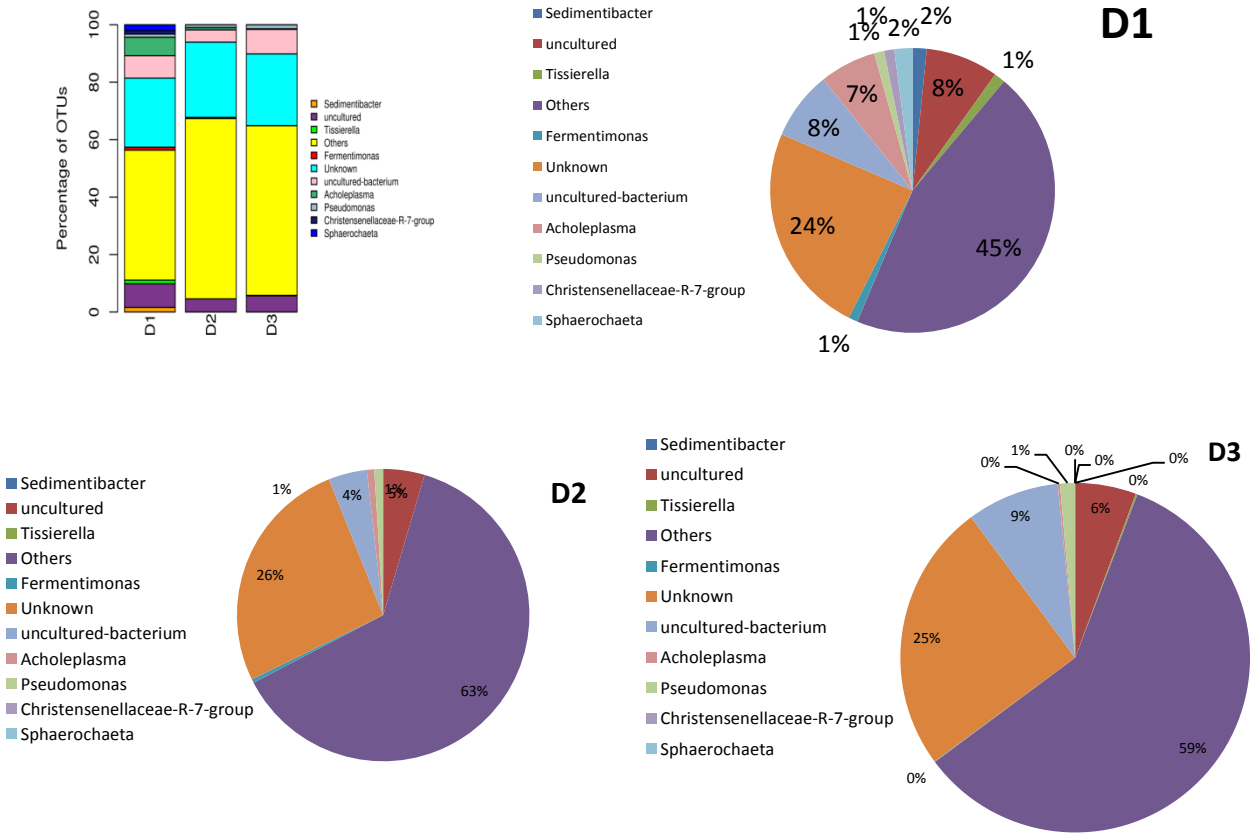

Fig 7.8: Genus level classification percent OTU Present in D1, D2, and D3.

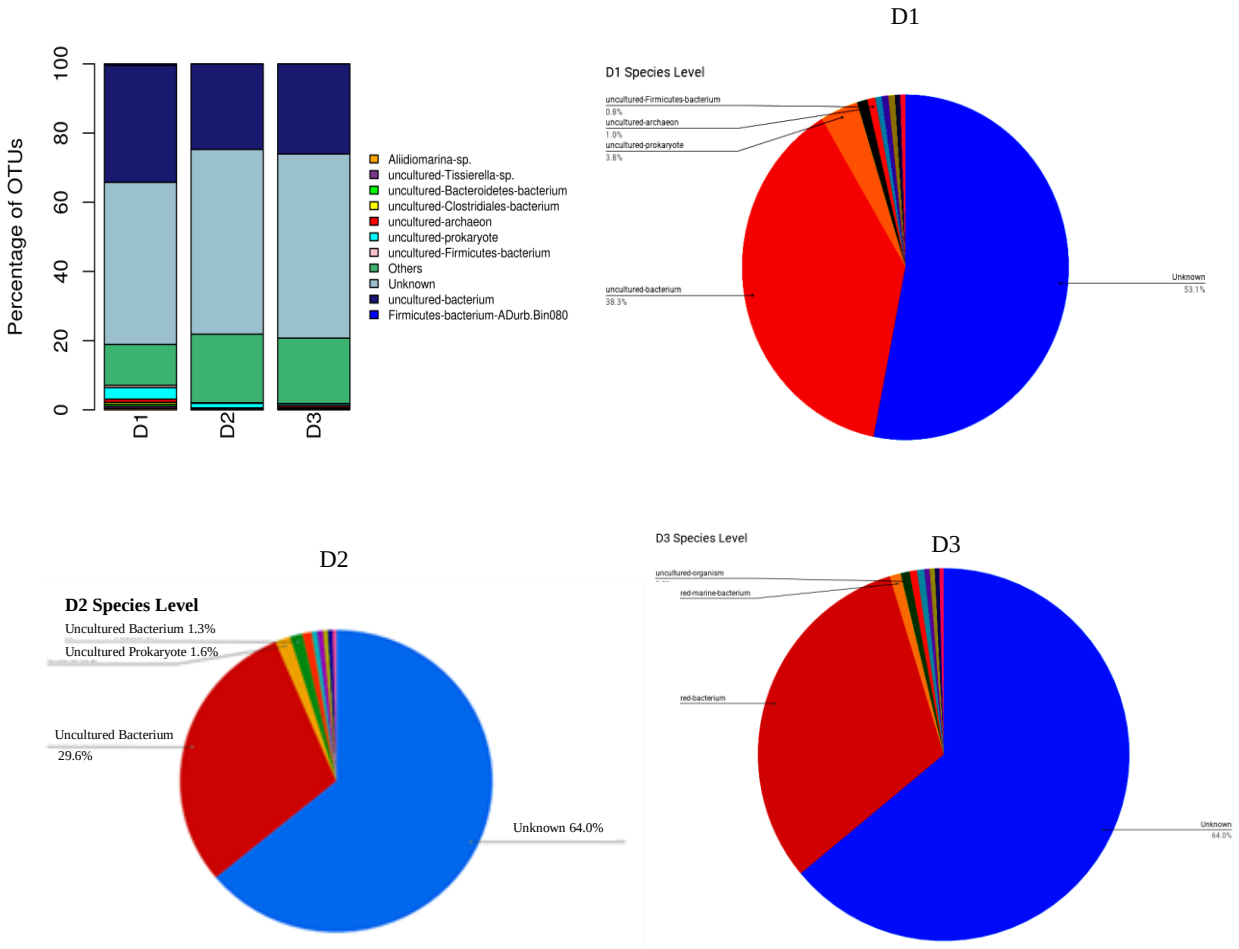

Fig 7.9: Species level classification percent OTU Present in D1, D2, and D3

Table: 7.3: Rarefaction indices show the diversity in each sample in distillery wastewater sludge.

Sample	Ace	Chao 1	Sobs	Jacknife	Simpson	Shannon
D1	45653.847317	36706.548099	40534	43786	0.001298	10.181837
D2	49655.893298	25987.712398	58935	45981	0.0000987	6.655499
D3	30654.883098	30345.534321	32560	30535	0.002098	8.311326

Conclusion:

The complex physico-chemical characteristics of distillery sludge have significant impact on the diversity and relative abundance of the bacterial community. Site D1 has the highest levels of organo-metallic pollutants, it showed a more diverse and higher species richness but with low percentage abundance. When the environmental pressure was reduced through mixing and dilution of the receiving water, the competitive advantage was removed and was reflected in reduced bacterial diversity. This is the first report of bacterial communities in the distillery sludge and their relationships with organic pollutants. The knowledge provides information on candidate for bioremediation and toxicity assessment on the bacterial communities.

Chapter-Eight

Assessment of bacterial assisted phytoremediation technique by native wetland plant growing in distillery waste polluted site

Chapter-8

Assessment of bacterial assisted phytoremediation technique by native wetland plant growing in distillery waste polluted site.

The aim of this chapter is to study the bacterial-assisted phytoremediation to demonstrate the importance of the bacterial mechanism in the phytoremediation process. The native plants growing at contaminated site showed high potentiality for phytoextraction of heavy metals from organometallic complex along with phytoremediation for the Eco restoration of polluted site. During my study I found diverse plant communities capable for phytoremediation as biotechnological tools for eco-restoration which can be divided in two groups: **A.** Medicinal and leafy vegetables **B.** *Ricinus communis* L. A diversity of microbial community from rhizosphere has been also detected as evidence for bacteria assisted phytoremediation. The efficiency of phytoremediation can be enhanced by the assistance of plant growth promoting (PGP) bacteria from the sugarcane molasses-based distillery waste.

PART-1

Translocation of heavy metals in medicinally important herbal plants growing on complex organometallic sludge of sugarcane molasses-based distillery waste

8.1 Introduction

Heavy metal pollution in soil water and food material is a major threat to human health and industrial waste and geo-genic activities are the major source of heavy metal in the environment (Annan et al., 2013; Shammi et al., 2016). In India, a number of industries (e.g. 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 matrices and non-degradability of heavy metals, it poses significant challenges for their remediation (Chandra and Kumar, 2017a &b; Gupta and Sinha 2007; Chandra et al., 2017; Kumar et al., 2013; Sushil and Batra, 2006). Heavy metals tend to accumulate in soil, water and plants in the environment, which subsequently contaminates the food chain (Chandra et al., 2009; Singh et al., 2012). Research in

health risks of food crops irrigated with sewage in China showed that there was substantial accumulation of heavy metals in the edible parts of the plants and further higher accumulation of heavy metals was noted in blood of human as health risk Index (HRI) (Khan et al., 2008). In another study, Indian mustard was grown on distillery effluent irrigated soil this showed accumulation of heavy metals in their edible parts i.e. seed and leaf as health risk to human (Bhargava et al., 2008). This study has revealed a significantly higher accumulation of Cd, Cu, Fe, Mn, Ni and Zn in their various parts. The effluent showed inducible effect on the growth of mustard plant <10% (v/v), while > 10% (v/v) the effluent showed inhibitory effect on the various physiological parameters of plants.

The various physicochemical methods i.e. filtration, flocculation, reverse osmosis, and chemical precipitation are reported for remediation of heavy metal from the terrestrial and aquatic environment, but they are energy-intensive and not cost-effective (Blocher et al., 2003; Dialynas & Diamadopoulos, 2009; Bratskaya et al., 2009). Phytoremediation of heavy metals from the contaminated sites has emerged out as green technology for the de-contamination of metals from the polluted site (Ghassemzadeh et al., 2008; Singh et al., 2017; Khan et al., 2008). Recent studies have highlighted the phytoextraction potential of some native plants such as weeds and grasses from the metal-contaminated sources and organometallic industrial sludge disposed site (Gupta and Sinha 2007; Chandra et al., 2018; Franchi et al., 2017). However, among the potential metal accumulators, a number of plants are known food and medicinal plants (Shammi et al., 2016; Mellem et al., 2009; 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 et al., 2017; Annan et al., 2013). The herbal preparation is presumed to be safe all the contaminants, but studies have shown that they may be contaminated with various heavy metals like cadmium, chromium, lead, mercury, zinc, copper, nickel & manganese, etc (Khan et al., 2008). Several metals are intentionally added to the herbal preparation in the form of traditional medicine i.e. Ayurveda, and in many instances they are artefacts of the manufacturing process. Various leafy herbal plants are known for their applications in medicine and have the inherent ability to bioaccumulate the heavy metals, up to several folds higher than the surround soil. But, the capacity of plants to accumulate heavy metal may vary within and among the plant species (Jaison and Muthukumar 2017). The heavy metal accumulation ability is

also regulated by environmental factor and soil characteristics. Detail knowledge of metal accumulation in medicinal and herbal plants from different environment are not widely investigated. Therefore, the heavy metal contamination in food and medicine has been highlighted as health hazards. Ignorance of heavy metal accumulation in the raw material used in herbal preparation can lead to serious and long-term side effects and change the chemical properties of medicine also (WHO, 1978).

Sugarcane molasses-based distilleries are sources of complex environmental pollutants due to the presence of various heavy metal-containing organic pollutants (Chandra and Kumar, 2017). In India, there are more than 397 sugarcane molasses-based distilleries are in operation that generate an average 1500 tones 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. Recent studies reported 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 (Chandra and Kumar, 2017). These compounds are listed by the US Environmental Protection Agency as endocrine-disrupting chemicals (EDCs) (USEPA, 2012). The study also revealed that these organic pollutants create a complex environment with organometallic compounds that restricted the bioavailability of metals to plants. The reported metal are iron (Fe), zinc (Zn), copper (Cu), chromium (Cr), cadmium (Cd), manganese (Mn), nickel (Ni), and lead (Pb), all present in concentration above the prescribed limit in the environment recommended by USEPA (Chandra and Kumar, 2017). The generated sludge is alkaline pH with a high pollution load of carcinogenic and mutagenic compounds that further impede biodegradation and subsequently damaged the vegetation at the dumping site (Chandra and Kumar, 2017a). 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 contaminated site is not investigated with multi metal contaminated are not well studied (Chandra and Kumar, 2017a; Jaison and Muthukumar, 2017; Shammi et al., 2016).Understanding to the distribution of heavy metals in various parts or plant tissue will help to promote correct usage of the plant materials and prevent accidental intake of heavy metals. Therefore, the present study seeks to

determine heavy metal accumulation by different leafy medicinal plants from the sugarcane molasses-based distillery waste.

8.1.1. Material and methods

8.1.1.1. Sample collection

The collection of plants and distillery sludge were carried out from M/s Unnao distillery and breweries, situated in Uttar Pradesh, Unnao, India (26°320″ N, 80°30′0″E). The distillery unit has capacity of 9000 kL alcohol production and generates approx. 800 tones of sludge annually (AIDA 2004). The fresh disposed of dried distillery sludges cakes were collected in clean pre-sterilized polythene bags from the sludge dumping site of the distillery plant located inside the premises of the industry. The healthy growth of some potential native plants was noted on the sludge without showing any adverse effect. This indicated potentiality of growing plant. After plant growth the sludge were collected periodically to investigate changes in their properties. The abundant luxuriant growth of six plant species indicated their potential for bioremediation of sludge as shown in fig 1. These plants were identified as*Achyranthus aspera, Amaranthus viridis, Basella alba, Sesbania bispinosa, Pedalium murex L.* and *Momordica doica* with standard method according to Duthie flora of Indo-Gangetic plain *(*Duthieet al., 1903).

Fig.8.1.1: Collection of sludge sample from M/s Unnao Distilleries & Breweries Ltd., Unnao

8.1.1.2. Physico-chemical analysis of collected sludge

The physico-chemical parameters of the distillery sludge sample from both site was analyzed i.e. pH, electrical conductivity (EC), chloride (Cl^-), sodium (Na^+), and nitrate was estimated according to the method described by Chandra et al.(2018). The phenol contents in sludge were analyzed 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 in the dry weight sample of sludge 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 (Chandra and Kumar, 2017).

8.1.1.3. 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 50^0C overnight to constant weight. The sample was then crushed into powder form in a porcelain mortar (Evolution-201, Thermo Scientific, USA) as the previously described (Yadav and Chandra, 2018). Further, the sludge sample was treated in 2.5% glutaraldehyde for 3-4 h and post fixed with 1% osmium tetroxide for 2h 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 analysed 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 scanning electron microscope equipped with an EDAX system (SEM, QUANTA FEG 450, FEI, and the Netherlands). Fourier transform-infrared spectrophotometry (FTIR) analysis of 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 4,000 cm^{-2} with a resolution of 4 cm^{-1} for all samples (fresh sludge, and a plant grown sample).

8.1.1.4. UV-Vis Spectral and High-performance liquid chromatography (HPLC) analysis of Leachate

Collected sludge samples were pooled mixed and it was air-dried, subsequently, it was ground with a pestle mortar to crush the entire available particle

and it was sieved through a 63µm Pore size sieve to get a homogenous powder. Further, the solvent (Ethyl acetate) extraction was carried out to obtain a 10% leachate of sludge as described earlier (Chandra et al., 2005). Briefly, 100g of sludge was added to 1000ml of Ethyl acetate (w/v) and the mixture was shaken continuously for 3-4 hr at room temperature (25± 2^0C) and the suspension was filtered through 0.22µm syringe filter. Furthermore, Organic pollutants in the sludge sample were measured using UV–Vis spectrophotometer (Thermo Fisher Scientific Shanghai spectrophotometer Evolution 2001, China) at a wavelength between 200-700 nm at ambient room temperature (25°C) (Chandra et. al. 2018) and HPLC equipped with a 2487 UV–Vis detector and using Millennium software (Waters 515). Twenty microliters of sample were injected at a rate of 1 ml/min^{-1} using acetonitrile: water ratio of 70:30. To analyse the compounds at 320 nm by HPLC, a reverse-phase C-18 column (250 × 4.6mm, particle size 5µm) at 27°C was used (Chandra et al., 2018).

8.1.1.5. Extraction and characterization of Residual Organic Pollutants for GC-MS analysis

8.1.1.5.1. Solid-liquid extraction

The various organic compounds present in fresh and plant have grown distillery sludge samples were extracted by using ethyl acetate due to wide range of solubility for organic compounds present in sludge as per the previously mentioned method (Chandra and Kumar, 2017). The method of extraction for organic compounds from sludge 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.

8.1.1.6. Characterization of organic pollutants by GC-MS from distillery sludge

Identification of organic compounds from distillery sludge i.e. fresh and plant grown sample was carried out by GC–MS analysis. 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 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). The separation of organic compounds occurred in DB-5MS capillary column. The temperature of GC oven was programmed; it was started

from 65°C (hold for 2.0 min), increased to 230 °C at a rate of 6°C min^{-1}, and finally reached to 290°C (hold for 20 min) at the rate of 10°C min^{-1}. Helium used as carrier gas at a flow rate of 1.1 ml min^{-1}. 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 instrument.

8.1.1.7. Digestion of plant tissues for metal accumulation

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 root, shoot, and leaves were separated and chopped into small pieces and the resulting biomass was oven-dried at 70^0C 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, 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).

8.1.1.8. Metal 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 as below mentioned formula which has also been reported earlier by (Yoon et al., 2006).

$$\mathbf{BCF = C_{root} / C_{sludge}}$$

$$\mathbf{TF = C_{shoot} / C_{root}}$$

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.

8.1.1.9. Histological observations of root tissues by TEM

Root segments (2.0mm in length) of selected plants were quickly 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 10 min interval between each washing and postfixed in 1% OsO_4 overnight. The fixed tissue was washed with SCB, dehydrated in graded acetone series (50, 60, 70, 80, 90, 95, and 100%) 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 ultramicrotome (Cryo Leica EM UC7, Leica Microsystem, India), and sections were stained by uranyl acetate and lead citrate. Analysis of the section was done under TEM (FEI Tecnai™ G2 Spirit Twin, Hillsboro, USA) at an accelerating voltage of 80 kV (Chandra et al., 2018).

8.1.3. Result and discussion

8.1.3.1. Physico-chemical characterization of fresh and after plant growth sample of sugarcane molasses distilleries.

The physico-chemical analysis of different pollutant parameters in leachate and sludge of distillery waste was noted above the permissible limit in fresh and after plant growth as shown in Table. 8.1. The analysis value of different metals i.e. Mn, Cr, Zn, Cu, Fe, Pb, Cd, Ni as also found in higher concentration as per guideline of (USEPA, 2012). The BOD/COD ratio indicated less degradability due to the presence of recalcitrant compounds (Kumar et al., 2010). While after plant growth the metal contents were significantly reduced from fresh sludge (Table.1). The high binding tendency of metallic ions with organic pollutants is known to restrict the bioavailability of metals to plants (Migo et al., 1997). The ability of the plants to accumulate might be due to increased solubility and availability of metal as a result of plant microbe's interaction (Rajkumar et al., 2012). The high conte nt 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 metal might be leached into spent wash from drainage pipe due to high acidic conditions (Chandra and Kumar, 2017). 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). The high contents of several heavy metals in industrial waste have been reported as health hazards through the food chain and source of various diseases (Barakat, 2011). In the an other study, the high content of heavy metal in distillery sludge and their adverse effect on seed germination and growth parameter of green gram *Phaseolus mungo L*. were reported (Chandra et al., 2008). 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).

Table 8.1.1: Physico-chemical characteristics of discharged distillery waste collected from M/s Unnao Distillery Pvt.Ltd. *Unnao*, Lucknow-Uttar Pradesh, and India.

Parameters	Fresh sludge	Sludge after plant growth	Reduction %	Permissible limit (USEPA, 2012)
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
Biological oxygen demand	4166.82 ± 88.22	2500.11± 86.60[a]	60.0%	40.00
Chemical oxygen demand	12527.18 ± 182.22	6850.84 ± 128.11[a]	54.68%	121.00
Electrical conductivity	1916.66 ±60.09	1003.33±31.79[a]	50%	1000
Total Dissolve solid	10720.78 ± 260.44	5521.99 ± 151.29[a]	51.3%	50-70
VS (mg/L^{-1})	1214.84 ± 36.01	515.84 ± 14.60[b]	41.16%	
Chloride (mg/L^{-1})	2935.33± 70.95	1193.33 ± 52.06[b]	40.45%	750.00
Total Nitrogen (TN) (mg/L^{-1})	228.51 ± 8.78	141.81 ± 5.87[a]	57.7%	
Phenol (mg/L^{-1})	8015 ± 73.99	2702.33 ± 35.89[c]	33.33%	0.50
Heavy metals				
Mn(mg/L^{-1})	8.75 ± 0.52	3.58 ± 0.22[b]	40.98%	0.20
Cr (mg/L^{-1})	3.73 ± 0.38	1.06 ± 0.12[c]	26.0%	0.05
Zn (mg/L^{-1})	18.84 ± 0.89	8.23 ± 0.67	35.10%	2.00
Cu (mg/L^{-1})	3.91 ± 0.21	0.50 ± 0.08[NS]	12.56%	0.50
Fe (mg/L^{-1})	423.88 ± 7.33	186.44 ± 8.89	41.46%	2.00
Na (mg/L^{-1})	488.28 ± 6.68	63.25 ±3.7[NS]	20.49%	0.04
K (mg/L^{-1})	245.15±10.30	136.99±1.99	60.86%	0.09

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 ($\mu mhoscm^{-1}$); Students *t* test (two tailed as compared to pre-treated sludge); [a]Highly significant at $p<0.001$; [b]Significant at $p<0.01$; [c]Less significant at $p<0.05$; [NS]Non-significant at $p>0.05$; BDL: Below detection Limit.

8.1.3.2. Scanning electron microscopy (SEM), Energy-Dispersive X-ray Spectroscopy (EDAX) and Fourier transform-infrared spectrophotometry (FTIR) Analysis of sludge.

The SEM image of sludge showed fine crystals in needle shape. Some scattered elongated crystals were noted while the glomerated needle-shaped fine crystals in SEM observations were also noted as shown in Fig. 8.1.2A. The needle-like crystal properties have been reported as organic polymers and metallic crystals by previous researchers (Liu et al., 2013). The elemental analysis in EDAX showed the presence of carbon, oxygen, sodium, potassium, calcium, and surface in different percentage as shown in Fig 8B. In the IR spectra of sludge in pre and post-plant growth and their stretching frequency 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) respectively in supplementary Fig.S_1A & Table. S_1, which showed the medium and strong bond appearance of the compound with the different stretching group. While the FTIR data of plant grown sludge sample showed maximum strong bond stretching frequency of compounds 3415.5 (O-H), 2967.1 (C), 2188.4 (CΞC), 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 suggests a compound of various classes such as carbon dioxide, sulfonyl chloride, ester, and anhydride. After plant growth, most of the medium bond of detected compounds was broken except for the strong bonds showing compounds that were detectable even after the plant growth. This confirmed the recalcitrant properties of the compounds.

8.1.3.3. UV-Vis Spectral and High-performance liquid chromatography (HPLC) analysis of Leachate

UV-Vis absorption spectra wavelength range of 250-700nm analysis showed the presence of variable peaks in UV- range due to the dissolved organic compounds in the sugarcane molasses-based distilleries sludge before phytoremediation; these peaks were diminished after plant growth. 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 320nm. 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 are produced by calculating the absorption of monochromatic radiation through a spectrum of wavelengths, through a solution during pre and post-phytoextraction and it is the most common technique for monitoring removal processes of some peak which is shown in Fig. 1C (Chandra et al., 2018).The comparative chromatogram of HPLC analysis before and after phytoextraction (Fig. 8D) showed the reduction and shifting of the peak in comparison to the control sample. This suggests the degradation of organic pollutants in sludge and phytoextraction process by plants, which removes complex organic compounds and most heavy metals that are toxic to the biota of the environment even at low concentration. Similar pattern on the phytoextraction process from distillery sludge have been shown by other plants previous study (Chandra et al., 2018).

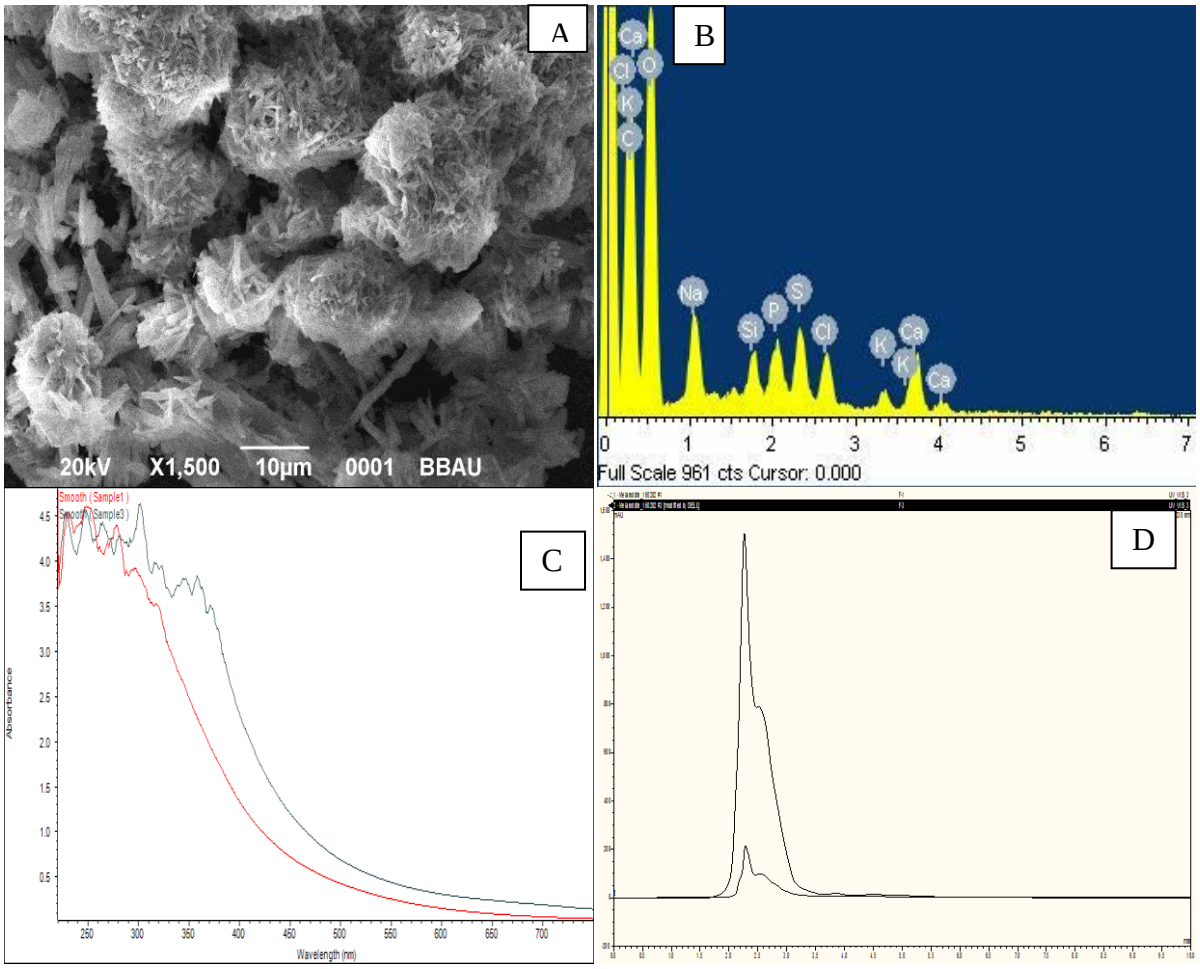

Fig.8.1.2. A. Scanning Electron Microscopy analysis of waste distillery waste sludge sample; 1B: EDAX of distillery waste sludge sample; 1C: UV–Vis spectral analysis of before and after phytoextraction at various time intervals; 1D: HPLC analysis of before and after phytoextraction

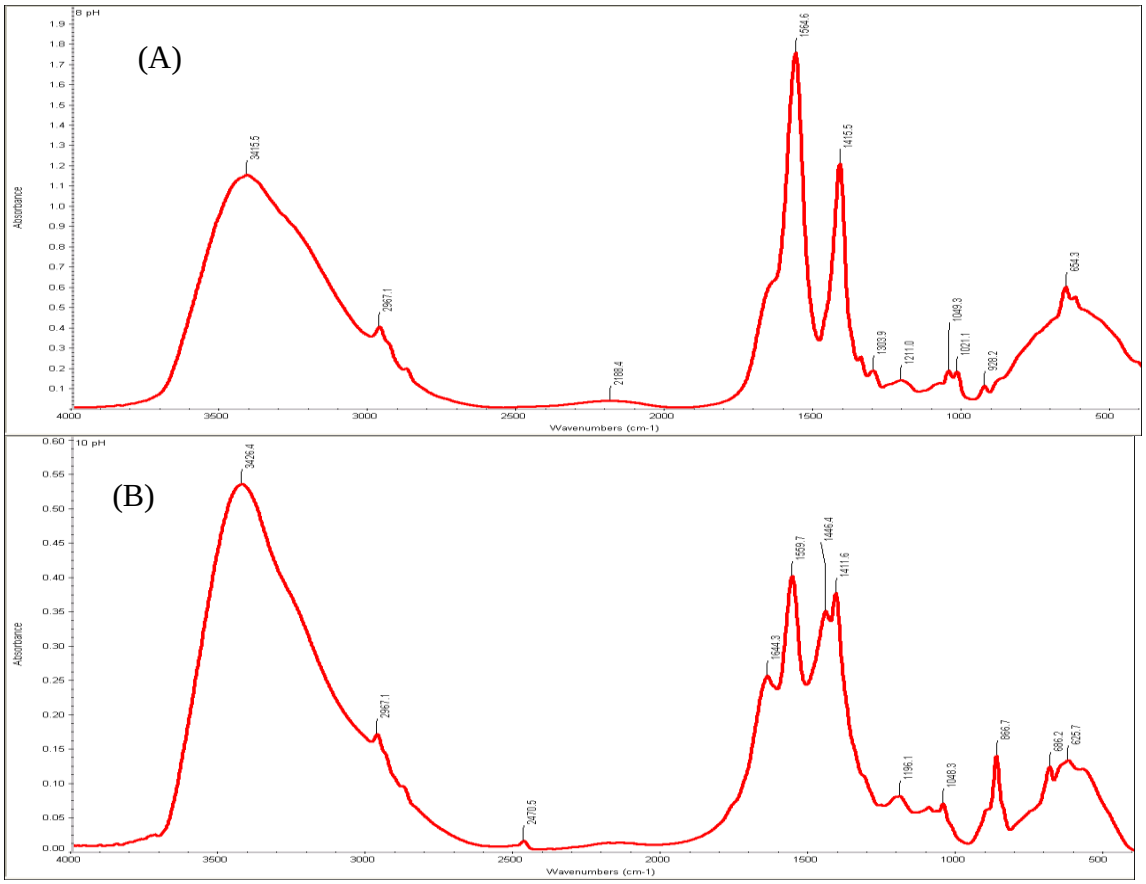

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Fig. 8.1.3: FTIR spectra; **A)** fresh distillery waste sludge**, B)** Phytoextracted distillery waste sludge

Table. 8.1.2: Showing detail of FTIR absorption band in the spectra of mollasses based distillery waste before and after phytoextraction

Before phytoextraction			**After phytoextraction**		
Wave number	**Group**	**Compound Class**	**Wave number**	**Group**	**Compound Class**
3426.4	N-H	primary amine	3415.5	O-H	alcohol
2967.1	C-H	alkene	2967.1	C-H	Alkane
2470.5	O=C=O	carbon dioxide	2188.4	C≡C	Alkyne
1644.3	C=N stretching	conjugated alkene	1564.6	N-O	nitro compound
1559.7	N-O stretching	nitro compound	1415.5	S=O	sulfate
1446.4	C-H bending	alkane	1303.9	S=O	sulfone
1411.6	S=O	sulfonyl chloride	1211.0	C-O	alkyl ether
1196.1	C-O	ester	1049.3	CO-O-CO	anhydride
1048.3	CO-O-CO	anhydride	1021.1	C=C	alkene
866.7	C-H	1,3-disubstituted	928.2	C=C	alkene
686.2	C-H	mono substituted	654.4	C-Br	halo compound
625.7	C-I stretching	halo compound			

8.1.3.4. 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.

8.1.3.4.1. Identification of Organic Pollutants (bulk sludge)

The major peaks of 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. These compounds were characterized as acetamide, 2,2,2-trifluoro-N-methyl, benzeneacetic 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, stigmasta-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 either under the category of plant fatty acids or the chemical reaction by product of the distillation process (Chandra et al., 2018; Chandra and Kumar, 2017). The toxicity of detected pollutants was also reported in the findings shown in Table 8.1.2. The presence of these phenolics compounds in sludge might have affected the soil microbiota and the nutrient availability to roots uptake. The presence of several fatty acids in the sludge might be responsible in reducing the rate of metal accumulation processes in plant and biotransformation processes of complex organometallic compounds. Additionally, when these organic pollutants reach the aquatic ecosystem it can cause toxic effect 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 demonstrated their toxicity to the aquatic ecosystem. Other organic acids like octadecanoic acid, Dodecanoic acid and butanedioic acid are listed as endocrine disruptors by the (USEPA, 2012). The presence of EDCs compounds in distillery sludge 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). The majority of these detected compounds pose adverse effect in environmental flora and fauna of soil as well as the aquatic ecosystem (Chandra and Kumar, 2017).

8.1.3.4.2. Identification of Organic Pollutants from distillery sludge after plant growth

Detection of organic compounds from phytoextracted distillery sludge was analyzed as the detail is shown in Table .8.1.2. The number of organic compounds and peaks were reduced in the sludge after plant growth as compared to 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.8.1.4. 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), tricarballylic 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, benzeneacetic acid according to the NIST library. This confirmed the bioremediation of sludge by growing medicinal and vegetable plants. 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 organo metallic wastes (Chandra et al., 2018).

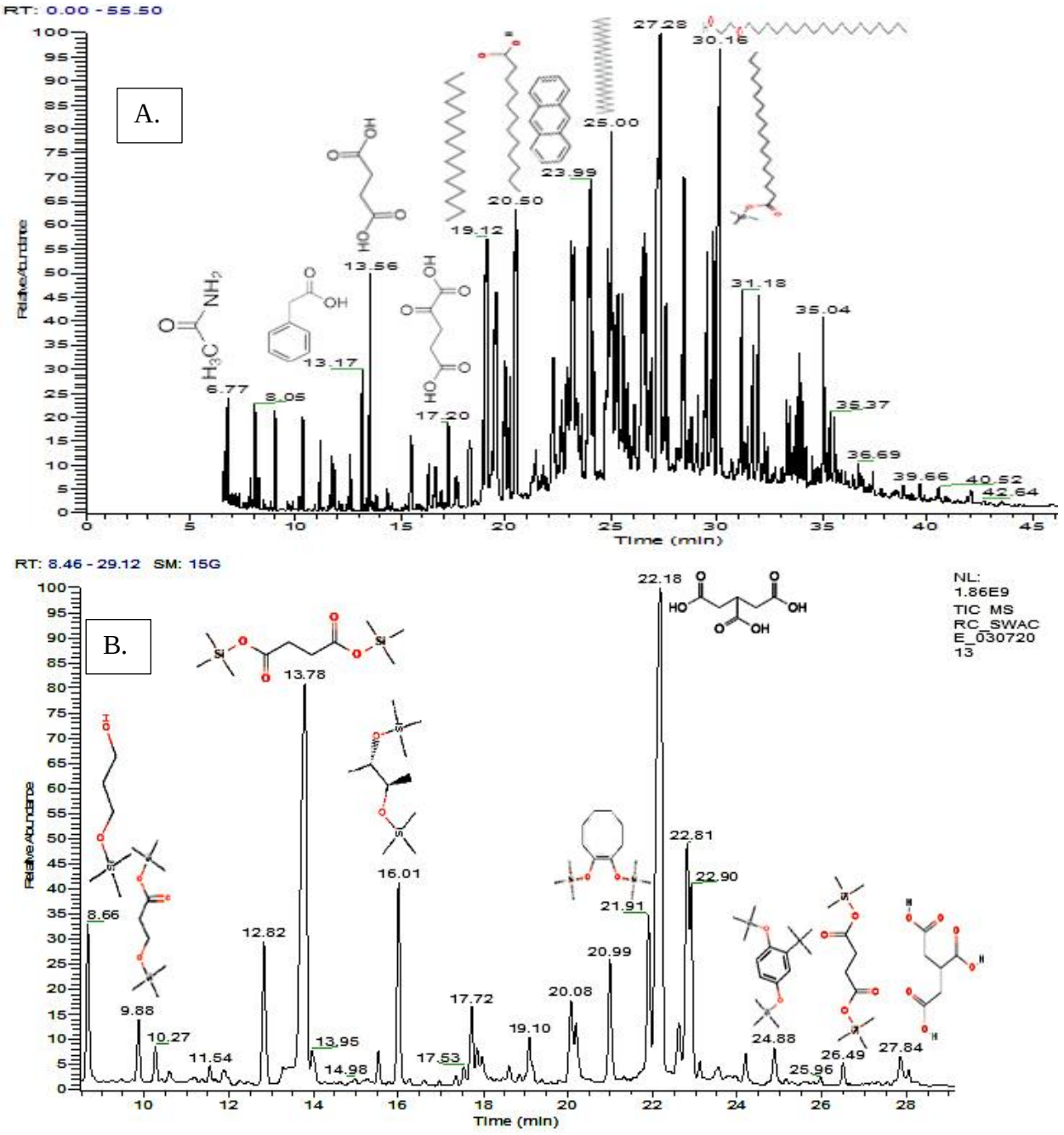

Fig 8.1.4: GC–MS chromatogram of organic compounds extracted from distillery waste sludge with ethyl acetate: A) non-phytoextracted sludge sample; B) phytoextracted distillery waste sludge sample.

Table 8.1.3: Detection of residual organic pollutants by GC–MS from distillery sludge waste before and after phytoextraction.

RT:	Name of Compound before extraction	Molecular Formula	Toxicity
6.77	Acetamide, 2,2,2-trifluoro-N-methyl	$C_6H_{16}O_2Si$	slight skin irritant, strong eye irritant,
7.10	2-(2-Hexyloxyethoxy)ethanol	$C_{13}H_{32}O_4Si$	vomiting and diarrhea
8.05	Butane, 2,3-bis(TMSoxy)	$C_{12}H_{28}O_3Si$	gastrointestinal (Digestive), Hepatic (Liver)
11.85	Hexanoic acid, 2-[(TMS)oxy]	$C_{12}H_{22}O_2Si_2$	Hematological (Blood Forming),
13.17	Benzene acetic acid, TMS ester	$C_{10}H_{26}O_2Si$	Aquatic Toxicology
13.56	Butanedioic acid, bis(TMS)ester	$C_{13}H_{30}O_5Si_3$	Sore throat. Skin & Eye Redness.
15.49	Pentanedioic acid, bis(TMS)ester	$C_{14}H_{24}O_3Si_2$	Skin, Eye, and Respiratory Irritations
16.64	Decanoic acid, TMS ester	$C_9H_{22}O_3Si_2$	Irritation when applied to human skin
17.20	α-Ketoglutamic acid, bis(TMS)ester	$C_{19}H_{40}O_2Si$	Neurological brain disorder
19.12	Hexadecane	$C_{21}H_{44}O_2Si$	Dizziness, headache and vomiting
20.50	Dodecanoic acid, TMS ester	$C_{19}H_{36}O_5Si$	Ingestion, inhalation & skin absorption
21.40	Querecitin 7,3',4'-Trimethoxy	$C_{10}H_{24}OSi$	chronic diseases
23.99	Anthracene	$C_{11}H_{28}O_2Si$	Melanoma, respiration hazard
25.00	Dotricontane	$C_{12}H_{36}O_4Si_5$	Sensitization, Skin
27.28	Ethanol, 2-(octadecycloxy)	$C_{12}H_{32}O_3Si_3$	Reproductive toxicity.
30.16	Octadecanoic acid, TMS ester	$C_{21}H_{44}O_2Si$	Carcinogenicity
32.40	Eicosanoic acid, TMS ester	$C_{31}H_{56}OSi$	headaches, drowsiness, confusion
35.04	Hahnefett	$C_{32}H_{56}OSi$	seizures, and
35.57	Nonacosane	$C_{32}H_{58}OSi$	breakdown of the hemoglobin
(RT)	**Name of Compound after extraction**	**Molecular Formula**	**Toxicity**
8.66	1,3-Propanediol, TMS ether	$C_6H_{16}O_2Si$	chronic poisoning, weakness,
9.88	Propanoic acid, 3-[(TMS)oxy	$C_9H_{22}O_3Si_2$	Skin and nail symptoms
10.27	1,3 Propanediol, TMS ether	$C_6H_{16}O_2Si$	hyper pigmentation
11.54	Pentanoic acid	$C_{12}H_{28}O_3Si$	inflammation
13.78	Butanedioic acid, bis(TMS) ester	$C_{10}H_{22}O_4Si2$	Hemolysis, anemia, hypotension
15.52	Resorcinol, O-bis(TMS)	$C_{12}H_{22}O_2Si_2$	low level of iron in the red blood cells
16.01	2,3-Butandiol, bis-O-(TMS)	$C_{10}H_{26}O_2Si$	low blood pressure
17.53	Malic acid (O-(TMS)-bis(TMS ester)	$C_{13}H_{30}O_5Si_3$	abdominal pain; fever; and diarrhea
21.91	Cyclooctene, 1,2-bis(TMS)	$C_{14}H_{30}O_2Si$	encephalopathy)
22.18	Tricarballylic Acid TMS	$C_{15}H_{32}O_6Si_3$	nerve disease of the extremities
23.54	Benzoic acid,	$C_{16}H_{30}O_4Si_3$	loss or deficiency of the fatty coverings
24.88	Tert-butyl hydroquinone, bis (TMS)	$C_{16}H_{30}O_2Si_2$	stomach tumors and damage
26.49	Vanil propionic acid, bis (TMS)	$C_{17}H_{32}O_3Si_2$	Skin corrosion/irritation
27.83	Tricarballylic acid 3 TMS	$C_{15}H_{32}O_6Si_3$	specific organ toxicity,

*RT-retention time (in minutes), + present, − absent, (TMS) trimethylsilyl

8.1.3.5. Accumulation of heavy metal in plant

The heavy metal analysis was performed for the accumulation of twelve metals (Mn, Pb, Cd, Zn, Cr, Fe, Cu, Ni, As, Se, Mo, and Co) by six potential plants i.e. (*Achyranthus aspera, Amaranthus viridis, Basella alba, S. bispinosa, P. murex* L. *and M. doica*) growing at the discharge site of distillery sludge. The variable pattern of metal accumulation to the various parts of the plant species indicated different capacities of metal uptake. The distillery sludge with multi metal along with various organic compounds make more complex with less bioavailability of plant. Previous study has also shown that the metal contents along with organic compounds in distillery sludge have shown inhibition of roots of various crops along with stunted growth of their shoot (Mazumdar and Das, 2015; Gupta and Sinha, 2007). But, the luxuriant growth of plants along with a well-developed root system indicated the inherent potential of plant due to their genetic property. The accumulation of various heavy metals by *A. aspera* has been reported as a medicinal plant from a separate study (Saraf and Samant, 2013). A similar pattern for the accumulation of Mn and Fe has been reported in the root of *A. aspera* in a previous study (Saraf and Samant, 2013). The study has highlighted the accumulation high content of Fe, Cu, Co, and Au from metal contaminated soil. A similar pattern of lead accumulation has been also reported in *S. bispinosa* (Sahi et al., 2002). The heavy metal accumulation in the different vegetable crops has been also reported in pot study, which indicated the health hazards and high translocation & Bioconcentration of metals in their different parts (Singh and Prasad, 2014). The health risk assessment of textile effluent reuses as irrigation waster in leafy vegetable *B. alba* has been reported due to the accumulation of heavy metals (Shammi et al., 2016). The accumulation of heavy metal depends upon the potentiality of 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). The disposed distillery sludge after anaerobic digestion become slightly alkaline (pH 8.67 ± 0.16) which restrict metal availability to plant. But the metal accumulation by growing plant on sludge showed the potentiality of plant and there was gradual decrease of pH in the rhizospheric soil. The accumulation of heavy metals of plant growing on sludge may be called as hyperaccumulator, as they have the potential to accumulate the metal from in such complex and an alkaline condition without showing any adverse effect (Chandra and Kumar, 2017). Our result indicated that the distillery sludge as waste consists of various organic compounds and metals that make organo-metallic complexes. Most of the compounds in sludge are anionic which has a strong cationic metal-binding tendency (Migo et al., 1997). The imbalanced bacterial assisted accumulation pf trace element and various heavy mettles have been reported due to improved bioavailability of metals to plant by growing rhizospheric bacterial communities (Sessitsch et al., 2013; Rajkumar et al., 2012). The metal accumulation

pattern was in order root> shoot>leaf. This indicated the concentration-dependent mobility of metals in plant tissues (Gupta and Sinha, 2006). Metal concentration dependent accumulation in plant parts has been reported by several studies in aquatic and terrestrial soil (Yoon et al., 2006; Bhargava et al., 2008). But, the presence of various co-pollutants and chelating agents present in soil also regulates the phyto-accumulation process. Moreover, the inherent potential of plant for metal accumulation from various environment also vary plant to plant (Kumar et al., 2013; Gupta and Sinha, 2006). All the tested plant species showed a high concentration of metal accumulation as compared to the normal uptake of metal, this indicated that these plants are highly potential therefore capable to grow at the contaminated site of distillery sludge and play a vital role in phyto-accumulation of heavy metal.

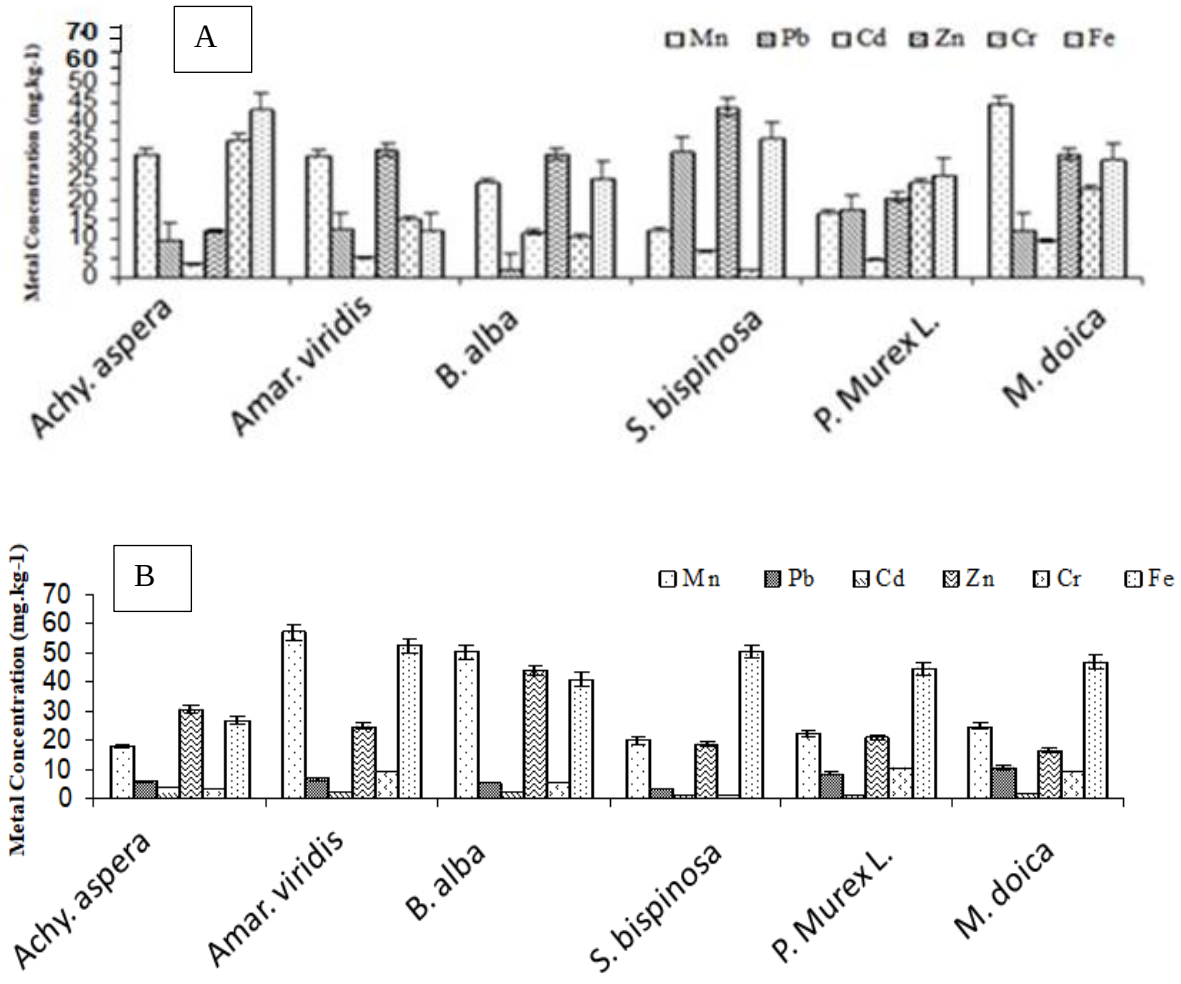

Fig. 8.1.5: Accumulation of Mn, Pb, Cd, Zn, Cr and Fe in different parts of native plants (A-B- = leaf, root,) collected from distillery sludge waste dumping site.

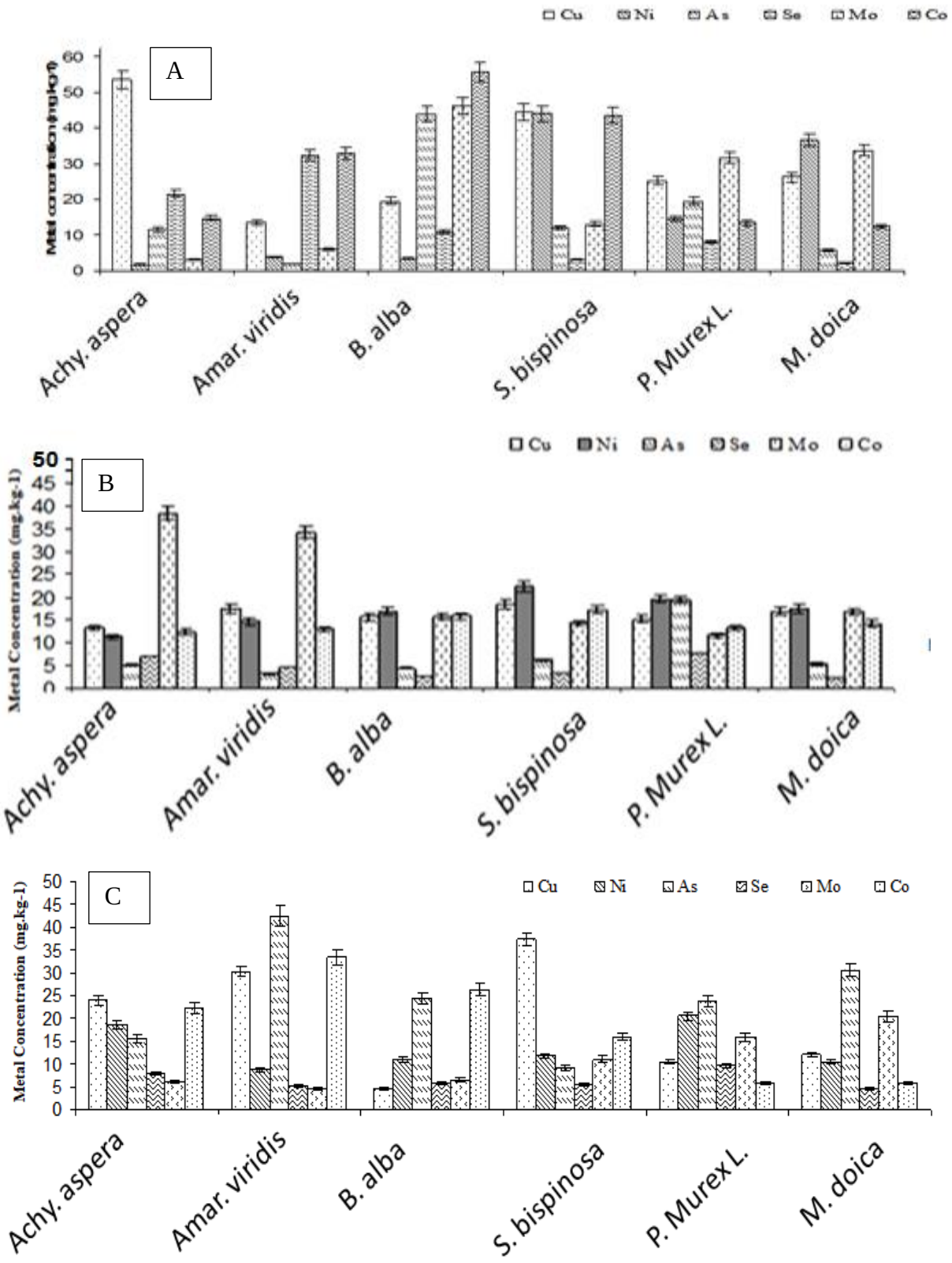

Fig. 8.1.6: Accumulation of Cu, Ni, As, Se, Mo and Co in different parts of native plants (A-B-C = leaf, root, shoot) collected from distillery sludge waste dumping site.

8.1.3.6. Bioconcentration factor and Translocation factor

The ability of heavy metals accumulation for these plants from distillery sludge can be evaluated through the Bioconcentration factor (BCF) and translocation factor (TF), (Yoon et al., 2006). The Bioconcentration factor of these plants has indicated that all the plants showed a high ability for phytoextraction of metals in their root from sludge. In general, the bioavailability of heavy metals in sludge is very poor but all the tested plants have shown very high metal accumulation in their different parts which indicated the hyperaccumulation tendency of these plants as shown in Table 8.1.4. *Achyranthus aspera* has high potential for the accumulation Cu (76.22±*1.3* mg/kg^{-1}), Mn (9.80±*0.6* mg/kg^{-1}), Cr (25.9±*1.3* mg/kg^{-1}), Pb (7.99±*0.7* mg/kg^{-1}), and Zn (67.33±*0.6* mg/kg^{-1}) with the highest BCF. *Amaranthus viridis* showed maximum BCF for Cu followed by Cr & Pb. Different heavy metal accumulation potential of *A. aspera* has been reported from soil as characteristic properties (Kumar et al., 2009). These studies have shown that *A. aspera* has propensity to accumulation of Fe, Cu, Mn, Na, in higher amount. The higher accumulation tendency for Na, Cu, Mn of *B. alba* has been indicated in other study (Shammi et al., 2016). But, the metal accumulation pattern from Industrial sludge and their impact on medicinal quality is still not clear. The higher accumulation of Mn (40.76±0.8 mg/kg^{-1}), Zn (77.06±1.6 mg/kg^{-1}), Fe (879.0±1.5 mg/kg^{-1}), As (31.81±1.3 mg/kg^{-1}) and Mo (20.35±0.3 mg/kg^{-1}) in root and shoot of *Sesbania bispinosa*. But *P. 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 organo metallic complex shown in Table 3. while the *Basella alba* has a high BCF for Mn & Mo followed by Pb & Cu as shown in Table 4. The deep & strong growth of root also supported the potentiality of the plant. The higher BCF and TF have been reported in *B. alba* which indicated the potentiality of the plant for metal accumulation from any contaminated site (Shammi et al., 2016). Similar pattern was observed in *Sesbania bispinosa, Pedalium murex,* and *Momordica doica*. 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 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).

Table 8.1.4: Showing BCF and TF of different Heavy metal accumulation (mg/kg^{-1} DW) by various hyperaccumulator plants of a different part in the root, shoot and leaves on distillery waste disposal sludge bed.

Native hyperaccumulator plants	**Bioconcentration Factor (BCF)**						
	Mn	**Pb**	**Zn**	**Cr**	**Fe**	**Cu**	**Ni**
Achy. aspera	81.33	17.45	17.65	18.72	15.65	94.34	1.698
Amar. viridis	13.75	15.75	8.021	19.75	5.675	57.92	6.254
Basella alba	55.4	48.96	8.765	15.06	6.111	46.98	5.121
Sesbania bispinosa	47.76	38.10	5.212	24.10	4.989	24.98	5.090
Pedalium murex L.	14.96	10.91	5.986	18.98	3.987	22.10	6.987
Momordica dioica	21.05	18.04	11.05	23.55	9.098	55.20	8.989
	Translocation Factor (TF)						
	Mn	**Pb**	**Zn**	**Cr**	**Fe**	**Cu**	**Ni**
Achy. aspera	**11.55**	6.355	8.543	**9.871**	15.64	37.15	0.009
Amar. viridis	3.908	3.432	8.654	7.324	14.99	**42.55**	1.111
Basella alba	3.999	9.123	9.987	3.776	14.90	28.39	1.123
Sesbania bispinosa	5.001	8.798	5.111	7.324	13.17	18.43	2.232
Pedalium murex L.	6.012	3.432	7.213	8.654	12.44	13.44	1.989
Momordica dioica	9.21	7.987	9.111	9.266	**19.23**	20.45	0.009

8.1.3.7. Histological observations by TEM analysis:

The root tissue analysis of collected indigenous hyperaccumulator plants by transmission electron microscope (TEM) has shown metal accumulation in vesicle near the cell wall and cytoplasm. The metal granules have been deposited near the nucleus in *Achyranthus aspera, Sesbania bispinosa* while big granule deposited in the cytoplasm and near to cell wall has been observed in *Amaranthus viridis, Basella alba and Momordica doica.* The continuous deposition of fine metal granule at the cell wall region has been noted in *Pedalium murex L.* This study concluded about the mechanism of plant detoxification of heavy metals based on vacuole sequestration and cell wall deposition. *Basella alba* root tissue, also *Sesbania bispinosa*, were shown to develop multi-nucleolus, multi-vacuoles, thickened in the centre and round lamellas 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 is reported in previous study (Chandra and Kumar, 2017a) showed a close resemblance to the multi-vacuoles development and deposition of granule, as an adaptive mechanism of the plant in the presence of organic pollutants, of metal accumulation. The whole plant root also showed cell wall thickening. Increased development of nucleolus and vacuoles at high heavy metal concentration increases the output of the ribosome and mRNA, which finally increases 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 extravagantly cultivated on disposable of distillery sludge without any biochemical deformities in their aerial parts. These crop species had powerful potential and adaptation for survival and development in organo-metallic and EDC containing distillery sludge for in situ phytoremediation. They have also provided powerful indications for the monitoring of heavy metals and phytoremediation of intricate and dangerous industrial wastes to eco-restoration of polluted areas.

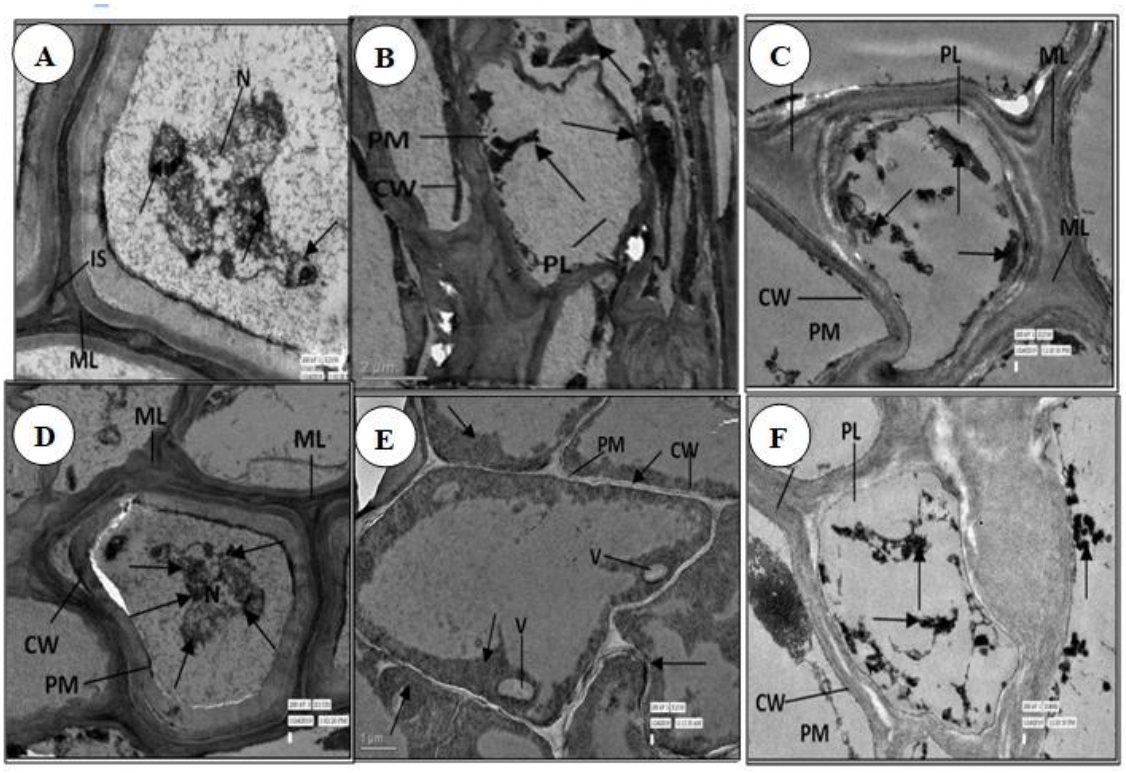

Fig.8.1.7: Electron micrographs of transverse section of plants root after phytoextraction of heavy metals (A–F), V: Vacuole; PM: Plasma membrane; P: Peroxisomes; CW: Cell wall; CM: Cell membrane; ML: Middle lamella; N: Nucleus; Arrow (→) indicated metals deposition; IS: Intercellular space. PL:-Plasmalemma.

Conclusion:

The physico-chemical analysis of molasses-based distillery sludge showed alkaline pH with a high concentration of (Fe: 2403.64), (Zn: 210.624), (Cu: 73.63), (Cr: 21.84), (Cd: 1.446), (Mn: 126.292), (Ni: 13.425), (Pb: 16.332 mg/kg^{-1}) this was above the permissible level as described in USEPA/WHO. The GC-MS analysis of sludge also revealed the abundant presence of Acetamide, 2,2,2-trifluoro-N-methyl, 2-(2-Hexyloxyethoxy)ethanol, Butane, 2,3-bis(TMSoxy), hexanoic acid, 2-[(TMS)oxy], benzeneacetic acid, TMS ester, butanedioic acid, bis(TMS)ester, pentanedioic acid, bis(TMS)ester, decanoic acid, TMS ester, α-ketoglutamic acid, bis(TMS)ester, octadecanoic acid, TMS ester, eicosanoic acid, TMS ester as persistent organic pollutants with androgenic and chromogenic properties, which is known for complex binding of metallic ions with organic component this condition restrict the bioavailability of metals to plant. But, the luxuriant growth of *Achyranthus aspera, Amaranthus viridis, Basella alba, Sesbania bispinosa, Pedalium murex,* and *Momordica doica*, without any adverse effect indicated the potentiality of these plants for phytoextraction of metals and utilization of organic content as their nutrient. Analysis of metal content in their root, shoot & leaves revealed that *A. aspera* and *A. viridis* were noted as root accumulator for Mn (16.95±0.1; 56.02±0.2), Fe (240.4±0.1; 497.6±0.3), Co (3.19± 0.2; 6.25±0.2). Similarly, *S. bispinosa, P. murex, M. doica* were found as root accumulator for Mn (17.03±0.5; 20.98±0.4; 20.97±0.5), Fe (477.2±0.6; 45.25±0.5; 45.67±0.5), Ni (25.46±0.8; 22.98±0.7; 19.76 ±0.4). Higher accumulation of metal by the plant in their various parts than the concentration available in sludge indicated the hyperaccumulation tendency of these plants along with the reduction of organic pollutants. Histological observation of root by TEM showed apparent deposition of metallic granules at the cell wall and vacuole as an adaptive feature of the plant. This study has given strong evidence of metal accumulation by medicinal plant from the contaminated site this may be avoided to use as medicine.

PART-2

Wastewater Detoxification and Management through Phytoremediation Employing *Ricinus communis* L.

8.2. Introduction

The development of the industry is a major reason for high environmental pollution (Esmaeili et al., 2015; Sharma and Rath, 2020). A huge amount of metals are release directly from the anthropogenic activity and mixed into groundwater and soil and after then cause the problem to human health (Jiang et al., 2019; Sharma et al., 2020a). More than 317 distillery industries are currently working in India which producing a total no. of 1500 tons of wastewater and sludge. The discharge into the environment and cause carcinogenic and androgenic pollutants like endocrine-disrupting chemicals, recalcitrant toxic compounds, phenolics, melanoidins, organic acids, and heavy metals without sufficient treatment techniques (Tripathi et al., 2021a). Some metals store in the lungs and other organs of living animals via contaminated food (Benson et al., 2018; Huang et al., 2018; Sharma et al., 2020c Ngo et al., 2020).

The role of bacterial communities like *Bacillus*, *Rhodococcus*, *Paenibacillus*, *Acidovorax*, *Alcaligenes*, *Mycobacterium*, and *Pseudomonas* in the phytoremediation process. By increasing metal availability for the processing of polysaccharides, polymers, by bacteria are significantly helping in the remediation process (Zhang et al., 2020). Subsequently, some factors like soil quality temperature, moisture, and light characteristics which influence plant root growth systems and development processes may affect the impact of phytoremediation (Liu et al., 2017). Biomass and plant development has a direct impact on the effects of microbe and phytoremediation, primarily species of *Pseudomonas* and *Bacillus* which may increase phytoremediation by promoting plant life (Girolkar et al., 2020; Sharma et al., 2020a). By adopting various strategies, including the production of antioxidant enzymes, subcellular localization, and organic acid exudation, *Ricinus communis* L. can accumulate a high amount of metals (Yeboah et al., 2020).

The toxicity of metals on sperm, gamete, and embryo on the reproductive system of the plant as well as on marine organisms, such as egg hatching, physical deformity in fish, and larval death (Fatima et al., 2014). Numerous fish species are

strongly affected by pollution, such as endocrine hormone deficiencies, genetic anomalies, and oxidative stress (Javed et al., 2016). The dark color of DWw affects aquatic organisms by affecting the photosynthesis process and reduces the oxygen contents in water life. Melanoidins are the most recalcitrant color pollutants in DWw, causing environmental and health concerns in humans and plants (Arimi et al., 2015; Chowdhary et al., 2017). *Caenorhabditis elegans* has been used as a model for the toxicity assessment of industrialization and urbanization wastewater (Jiang et al., 2016; Cadkova et al., 2020).According to the Environmental Protection Agency (EPA), heavy metals such as Cu, Cd, Pb, As, Cr, Ni, and Hg can be found in most environmental pollutants. The bioconcentration and translocation factor is very important for the assessment of the efficiency of metal accumulate in plants.

Even low concentrations of heavy metals cause a high range of toxicity to humans and plants (Sharma et al., 2020f; Sharma et al., 2021b). Remediation of metals enhanced by bacteria and pants has emerged as renewable and efficient wastewater treatment techniques (Ma et al., 2016). Cadmium, arsenic, and lead are the most toxic contaminants worldwide(Oconnor et al., 2019). Phytoremediation is an attractive and environmentally friendly strategy for the degradation of hazardous metals from wastewater used by native plant species (Ekperusi et al., 2019; Sharma et al., 2020c). The phytoremediation ability of species of *Salicaceae* was recently reported by Simiele et al., 2020. Several bacterial-promoting plant growths have been reported to help abiotic stress plants such as heavy metal toxicity and to enhance plant growth (Wang et al., 2019). Any plant species, with high tolerance, can thrive and reproduce in metallic soils i.e., hyper-resistance to usually toxic amounts of heavy metals in growing substrates. Many other microbial species have been investigated for heavy metals, like *Micrococcus* sp. *Flavobacterium, Pseudomonas, Bacillus,* and *enterobacter.* Bacteria have excellent biosorbent ability due to high surface ratio and active chemisorption sites in their cell wall (Tarekegn et al., 2020; Delil et al., 2020;). Some of the most important measures of phytoremediation efficiency are biological concentration factors (Hrynkiewicz et al., 2018). Because of their large biomass, metal-accumulating plants like *Cannabis sativa, Zea mays, Thelypteris palustris, Chenopodiaceae Typha latifolia, Nicotiana tabacum*, and *Helianthus annuus* L are helpful in metal absorption (Tozser et al., 2019; Chauhan et al., 2020; Hejna et al., 2020). Phytoremediation has arisen as a potential green metal decontamination technique in contaminated areas (Singh et al., 2017; Sharma et al., 2020c). There is an

urgent need for safe distillery wastewater management; technology must be economically viable, environmentally friendly, and socially acceptable. These works assess the possible relationship between metals and other co-pollutants biosorption by *Ricinus communis* to evaluate their phytoremediation potential.

8.2.1. Materials and methods

8.2.1.1. Sample collection and site description

The sample was collected from M/s Unnao distillery located at Unnao, Uttar Pradesh, India (26°320″ N, 80°30′0″E). Freshly disposed distillery wastewater samples were collected in the pre-sterilized tank (20 liters) near the disposal site (Tripathi et al., 2021b). Luxuriantly growing plant species *Ricinus communis* L. (*Spurges*) were collected based on their abundance. To eliminate the adherent soil particles, the extracted plants were vigorously cleaned with a calcium chloride solution.

8.2.1.2. Estimation of heavy metals and Physico-chemical parameters

The estimation of total metals (Cr, Mn, Fe, Cu, Ni, Zn, and Pb,) content in the DWw was measured using atomic absorption spectrophotometry (AAS) (ZEEnit 700, Analytic Jena, Germany) (APHA, 2012; Sharma et al., 2020). The Physico-chemical properties of the DWw sample from both locations (wastewater and near the rhizospheric zone of the plant), including pH (Orion pH meter-Model-960, Thermo Scientific, FL, USA), electrical conductivity (EC), chloride (Cl^-), sodium (Na^+), BOD, COD, and nitrate, were analyzed and estimated. The phenol content of DWw was measured using the American Public Health Association's standard methods (APHA, 2012; Chandra et al., 2018).

8.2.1.3. Scanning electron microscopy examination of DWw

The investigation of the microbial community, metals, and other organic polymer using Scanning electron microscopy (SEM, QUANTA FEG 450, FEI, and the Netherlands) analysis. For SEM analysis of metals and other carbonyl compounds in DWw, one gram of the DWw sample was put in a hot air oven till dry (Evolution-201, Thermo Science, USA) (Sharma et al., 2021a).

8.2.1.4. Organic pollutant analysis through gas chromatography-mass spectroscopy

To identify organic compounds in the DWw sample, analysts used gas chromatography-mass spectroscopy (GC-MS). Trimethylsilyl was used to derivatized the extracted samples (TMS) and prepared sample injected in GC-MS instrument

(Trace GC Ultra Gas Chromatograph; Thermo Scientific, FL, USA) (Sharma et al., 2020a).

8.2.1.5. Isolation and plant growth-promoting activity of isolates

8.2.1.5.1. Ligninolytic enzyme production

Ligninolytic enzymes are important for the breakdown and detoxifying of DWw pollutants. Various microbes have been reported which are capable of degrading the DWw, like bacteria, actinomycetes, cyanobacteria, and fungi. The production of Mnp and Lip revealed that bacteria can be secreting extracellular enzymes by degrading DWw during the early stages of incubation, suggesting the plant growth-promoting activity. Manganese peroxidase is a glycosylated heme protein with a molecular mass of 40-50 kDa.In *Enterobacter aerogenes* IITRCS-12 (KU726958.1) The existence of all three intracellularly ligninolytic enzymes is played a significant role in the removal of a wide variety of organometallic pollutants. Laccase (EC 1.10.3.2) is a copper-containing extracellular enzyme made up of monomeric, dimeric, and tetrameric glycoproteins. Janusz et al., (2017) found this mainly in microorganisms such as actinomycetes, fungi, and bacteria. Laccase is a multi-copper oxidase that, due to the presence of guaiacol, produces a dark zone for the related radical species by single-electron oxidation of organic compounds (Ladole et al., 2020).

Media composition

Luria Bertani (LB) agar

Casein enzymic hydrolysate	10g/L
Yeast extracts	5g/L
Sodium chloride	10g/L
Agar	15 g/L

Peptone - Peptides, Amino acids, Nitrogen

Skim milk (SM) powder

Skim milk powder	28.000
Tryptone	5.000
Yeast extract	2.500
Dextrose (Glucose)	1.000
Agar	15.000

Final pH (at 25°C) 7.0±0.2

8.2.1.5.2. Production of indole-3-acetic acid and siderophore

Near the rhizosphere of *Ricinus communis* L, the DWw bacterium *Enterobacter aerogenes* IITRCS-12 (KU726958.1) develops the rhizosphere signaling molecule IAA.IAA is produced by a wide range of bacteria, and its role in plant growth and pathogenesis is well understood. The role of IAA is in auxin for regulation of the physiological and morphological system of plant and use L-tryptophan as a substrate. IAA carried on a medium pink color and was put to the test for IAA quantitative estimation. Another important feature of rhizobacteria in plant development and growth which might enhance the plant development is siderophore positive isolated strains exhibiting the change of greenish-blue color to yellow color during the siderophore production. After incubation, the maximum yield of siderophores was observed at 36 hours and then began to decline as production. Furthermore, siderophores have a lower affinity for metal complexes than $Fecl_3$. Metals such as Cd_2, Hg_2, and Co_2 inhibited the formation and growth of siderophores due to metal binding behavior and increase the production of ROS in cells of bacteria. The production of siderophores in bacteria showed stomatal conduction and improve plant health during the accumulation of iron from DWw.

8.2.1.5.3. Hydrolytic enzyme assay

For evaluation, the activity of hydrolytic enzymes such as α-amylases and proteases by methods of Cappuccino and Sherman, (2005). In addition, determining the production of α-amylase and protease enzymes from bacteria, one loopful of bacterial cell suspension was streaked on peptone-containing starch agar plate media-5 g, beef extract- 3 g, soluble starch- 10g, agar-15 g, distilled water-1000 mL and skim milk agar plate containing skim milk- 100g, peptone-5g, agar -15g, water-1000 mL, respectively. The plate was observed for the clear area around the streak after 48 h of incubation at 28ºC. In addition, the media was prepared to determine the production of pectinase and cellulose by adding 1% pectin and cellulose to the basal medium containing NaNO3–1 g, K2HPO4–1 g, KCl–1 g, MgSO4-0.5 g, yeast extract-0.5 g, glucose–1 g, distilled water, 1000mL, and Agar-15 g. One loopful of the bacterial cell suspension was streaked on the medium and incubated for 5 days. Moreover, a gram iodine solution was poured in the pectin agar and a zone of clearance was observed against the dark blue background. The cellulose medium was flooded with 0.01% Congo red solution for 15 min and the plates were destained using a 1% NaCl

solution for 5 min. The clear zone against the red background indicated that the rhizobacteria were positive for pectinase and cellulase production.

8.2.1.5.4. Phosphate Solubilization

For determining of Phosphate Solubilization, the cultures were inoculated on to Pikovskaya's agar medium (Hu et al., 2006). The media composition of the Pikovsakya agar medium for 1000 ml i.e. calcium phosphate- 5 g, glucose-10 g, potassium chloride- 0.2 g, ammonium sulfate-0.5 g, magnesium sulfate-0.1 g, yeast extract-0.5 g, agar- 15 g, distilled water- 1000 mL. One loop full of the 24 h broth culture was spot inoculated on the Pikovskaya's culture plate. The plates were incubated at 28ºC for 96 hrs and were observed for the zone of clearance around the bacterial colony, which indicated the Solubilization of Phosphate. The Solubilization zone was determined by subtracting the diameter of the bacterial colony from the diameter of the total zone (Gaur, 1990).

8.2.1.5.5. Zinc Solubilization

Zinc Solubilization ability of the isolates was detected by spotting the log phase culture of bacterial strains on mineral salts agar (MSA) medium plates having zinc phosphate, and zinc carbonate as a source of insoluble inorganic zinc. Moreover, the inoculated plates were then incubated at 28±1ºC for 3 days and observed for the clearing zone around the colonies. Zn Solubilization was determined in MSA medium (Glucose-10 g, (NH4)2SO4-1.0 g, KCl – 0.2 g, K2HPO4-0.1 g, MgSO4- 0.2 g and H2O-1000 Ml with pH 7.0) amended with 0.1% of insoluble zinc oxide (ZnO) or zinc carbonate (ZnCO3). The actively growing cultures (5µL) were spot inoculated onto the medium, incubated at 28ºC and Solubilization zone was measured 15 days after inoculation and clearing zone was expressed as the area in cm2 (Venkatakrishnan et al., 2003).

8.2.1.5.6. Hydrogen cyanide determination

Hydrogen cyanide (HCN) production was determined by the modified method of Bakker and Schippers, (1987). Exponentially grown cultures (10-8 cells ml-1) of strains were streaked on solid agar plates supplemented with or without 4.4 g glycine l-1 with simultaneous addition of filter paper soaked in 0.5% picric acid in 1% Na2CO3 in the upper lids of plates along with inoculated control. The plates were sealed with parafilm and incubated at 28±1ºC, the development of color from yellow to light brown, moderate brown or strong brown was examined for putative HCN production.

8.2.1.5.7. Morphological characterization by gram staining

The isolated bacterial strains were identified using the standard procedure depending on phenotypic traits (Barrow and Feltham, 2003). The method is used as a tool for differentiating gram-positive and gram-negative bacteria to determine the identification of a specific bacterial sample as a first step.

8.2.1.6. DNA isolation and sequencing of the isolated strain

The bacteria were isolated by serial dilution and plate streak method. For the identification of isolated bacteria, the DNA was isolated from a fresh bacterial colony. Bacterial strains were identified using the Cowan and Steels Manual for Bacterial Identification (Barrow and Feltham, 1993). Also, the bacterial culture was inoculated in Luria Bertani broth for 16S rRNA sequencing (Himedia Pvt Ltd). Total DNA was extracted from an overnight grown bacterial culture using a genomic DNA extraction kit (real Biotech Corporation). Universal primers 16Sf (5' CAGCAGCCGCGGTAATAC 3') and 16 Sr (5' TACGGCTACCTTGTTACG 3') were used for amplification of the 16S rRNA gene. The PCR reaction mixture contained an assay buffer 5µl, forward primer 1 µl, reverse primer 1 µl, dNTP 1 µl, template 2 µl, tag polymerase 1 µl and the final total volume was made up 50 µl with Milli Q. Polymerase chain reaction was performed in a thermocycler (Sure Cycler 8800; Agilent Technologies, Malaysia) under the following conditions, denaturation at 94°C for 1 min, followed by annealing at 55°C for 1 min and extension at 72°C for 2 min, for 35 repeated cycles. Approximately 1500 bp region of the gene was amplified and the amplification product was gel purified using QIA gel extraction kit and sequenced. The sequence data were analyzed by BLAST and identified based on closet similarity with the reported sequenced data. All query sequences and other homologous sequences available online in the NCBI (National Centre for Biotechnology Information) nucleotide database were saved in a single FASTA file format after retrieval.

8.2.1.7. Assessment of metals and bioconcentration factors

In estimating the content of the metal deposited in the *Ricinus communis* L plant tissue of the root, the shoots and leaves were cut into small pieces and oven-dried for 5 days at 70°C until a consistent weight was reached (AOAC, 2002). The absorptions of Zn, Cr, Cu, Mn, Ni, Fe, Pb, and Cd in the plant were determined by atomic absorption spectrometry (AAS) (Sharma et al., 2020a). Bioaccumulation coefficient factors (BCFs) and translocation factors (TFs) were calculated to determine the

efficiency of metal accumulation. The calculation of BCFs as the metals in the root and wastewater, while TFs is defined as the metal concentrations in the root and shoot (Yoon et al., 2006). The statical analysis of results verifies data inconsistency and validity data will be interpreted standard deviation mean. Using program Graph Pad, all data were subjected to Tukey's Statistical Analysis Test (Ott, 1984) (Graph Pad Software, San Diego, CA.).

8.2.2. Results and discussion

8.2.2.1. Physico-chemical characteristic

The concentrations of heavy metals and Physico-chemical parameters in DWw were both above the permissible limit (Table 1). The pH of the DWw ranged from 8.40 and EC varied 1877.62 but samples near the rhizospheric zone of *Ricinus communis* L. reduced the pH (7.01) and EC (890.30). Similarly, the BOD (4234.96), and COD (11452.22) values near the rhizospheric zone of *Ricinus communis* L., also decreased as the plant developed. The combined action of hydroxides, carbonates, bicarbonates, calcium, and magnesium ions, which are used to adjust the pH of diluted molasses during the fermentation process, results in a higher pH value in distillery wastewater. The interaction of rhizospheric microorganisms with organometallic contaminants in distillery wastewater could explain the small decrease in in-situ phytoremediation wastewater pH. Furthermore, plant-microbe interactions in the rhizosphere can increase metal bioavailability by secreting amino acids, organic acids, protons, and enzymes, which reduces wastewater pH and enables plants to thrive in polluted environments. Results revealed that the selected plants can enhance the phytoremediation of wastewater. Eutrophication is caused by the accumulation of high concentrations of ions and other co-pollutants in distillery wastewater, posing a danger to aquatic life. Different metals like Mn (7.96), Cr (3.32), Zn (15.65), Cu (2.00), Fe (378.66), Pb (4.79), and Ni(7.89) was shown in high concentrations (USEPA, 2012). After the *Ricinus communis* L., plant growth the reduction of metals Mn (2.45), Cr (1.76), Zn (7.19), Cu (0.30), Fe (123.77), Pb (0.86), and Ni (2.50). The source of metals in DWw during the sugar can juice to sugar processes like separation, fermentation, and distillation phase at distillery industry.Various emission parameters are measured after plant growth like phenol (7000-233.09), sulfate (14543.76-7654.10), potassium (234.45-129.76), phosphate (40.03-20.40), and the sodium (455.98-59.34) contents were significantly reduced. These results gave a very good indicator of the ability of heavy metals to phytoremediation and the propensity

of *Ricinus communis* L to phytoremediator. The frequent use of wastewater containing metals in irrigation has also demonstrated that hazardous metals accumulate in the human diet of plant species. The elevated DWw metals content and its harmful effect on the growth of seed along with other parameters of *Phaseolus mungo* L. It is revealed that the strong affinity of metallic ions with organic contaminants reduces the bioavailability harmful influence on germinating seeds of metals in plants. Plants' ability to absorb metals may be attributed to enhanced bioavailability and metal production as a result of the plant-microbe interaction. Heavy metal concentrations are frequently monitored using plants as biomonitors (Sevik et al., 2019).

Table 8.2.1: Physico-chemical characteristics of discharged distillery fresh DWw before and after plant growth collected from M/s Unnao Distillery Pvt. Ltd. *Unnao*, Uttar Pradesh, India

Parameters	**Distillery wastewater**	**After bacterial growth**	**CPCB (2017)**
pH	8.40 ± 0.26	7.01 ± 0.18[a]	7.54 ± 0.01
Biological oxygen demand	4234.96± 77.76	1800.23± 65.40[a]	47.00 ± 0.00
Chemical oxygen demand	11452.22± 188.32	5980.54 ± 119.08[a]	79.00 ± 0.01
Electrical conductivity	1877.62± 45.03	890.30±28.70[a]	950
Total dissolve solid	9978.67 ± 230.32	5043.78 ± 100.87[a]	70 ± 0.00
Chloride	2578.54± 68.78	1076.43 ± 49.08[b]	11.82 ± 0.01
Total nitrogen	200.49 ± 7.68	123.61 ± 4.70[a]	9.90 ± 0.00
Phenol	7000 ± 63.90	233.09 ± 30.80[c]	-
Sulphate	14,543.76± 454.32	7654.10 ± 73.32[b]	
Phosphate	40.03 ± 1.30	20.40± 0.79[b]	3.40± 0.01
Na	455.98 ± 5.89	59.34 ±5.9[NS]	0.01
K	234.45 ± 09.19	129.76 ± 0.76	0.02
Heavy metals(mg L^{-1})			
Mn	7.96 ± 0.42	2.45 ± 0.34[b]	0.15
Cr	3.32 ± 0.78	1.76 ± 0.57[c]	0.01
Zn	15.65 ± 0.64	7.19 ± 0.34	1.28
Cu	2.00 ± 0.10	0.30 ± 0.05[NS]	0.19
Fe	378.66 ± 5.23	123.77 ± 6.67	1.45
Pb	4.79 ± 0.34	0.86 ± 0.32[c]	0.02
Ni	7.89 ± 0.87	2.50 ± 0.40[a]	0.04

All the values are Mean ±SE. (n=3); Unit of all parameters is in (mgL^{-1}) except pH, color (Co-Pt. Unit) and EC (µmhoscm^{-1}); Students *t* test (two tailed as compared to pre-treated sludge); [a]Highly significant at $p<0.001$; [b]Significant at $p<0.01$; [c]Less significant at $p<0.05$; [NS]Non-significant at $p>0.05$, Central pollution control board (CPCB).

8.2.2.2. Morphological view of DWw and rhizospheric wastewater

DWw SEM image revealed fine oval needle-shaped crystals, indicating the presence of metals and polymer. Organic polymers and metallic crystals have been documented to have needle-like crystal properties (Liu et al., 2013). The SEM images revealed that the DWw had irregular components that provided the adsorption of different contaminants along with heavy metals and organic polymers with a wide surface. The crystal forms revealed the existence of various heavy metals. SEM analysis study showed that most of the implant surface had intimate contact with the *Ricinus communis* L. root zone.

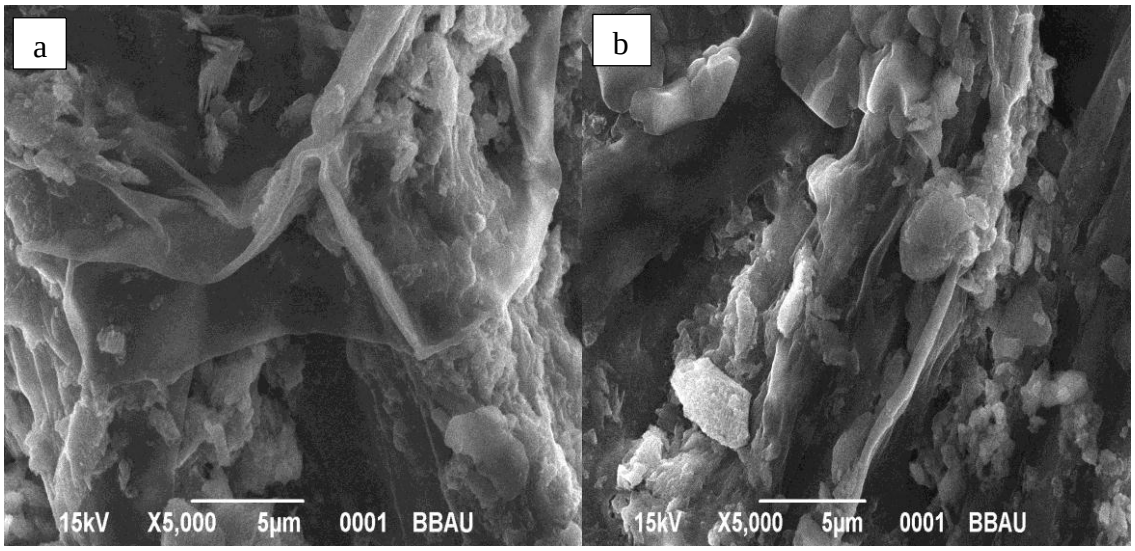

Fig 8.2.1: Scanning electron microscopy analysis of distillery wastewater sample showed different structure of pollutants (a-b)

8.2.2.3. Identification of organic pollutants

GC-MS chromatograms and the compound identifications showed figures S2a-b and Table 2. The major dominant peaks of a DWw were observed at RT 7.64, 13.21,19.36, 21.86, 26.84, 36.67, 43.20, and 49.32, respectively. This compound showed > 90% correlations with compounds described in the NIST library included with the program. The characterized of compounds 2-METHYL-4-KETO-PENTAN-2-OL 1TMS (RT-7.64), Octadecanoic acid (RT-13.21), Heptadecane, 2-methyl- (CAS) (RT-19.36), Heptadecane (CAS) (RT-21.36), 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS) (RT-26.84), Docosane (CAS) (RT-36.67), 2-Acetamido-3-(3-hydroxyphenyl) Propanoic acid (RT-43.20), and 3-t-Butyl-5-methyl-4-hexen-2-ol (RT-49.32), respectively. Identified compounds are showed a high level of toxicity like mutagenic, androgenic, and carcinogenic in aquatic systems and

humans. Table 2 summarises the toxicity of the pollutants identified. The characterized pollutants give the knowledge of toxicity and health problems which requires more analysis and assessment of environmental and health risk before the discharge. Plant fatty acids Heptadecane, 2-methyl- (CAS) (RT-19.36), and Heptadecane (CAS) (RT-21.36) are toxic to aquatic organisms. Other small peaks were also observed at RT values of 19.36, 21.72, 25.44, and 33.67 are not known or data not reported. Sugarcane molasses extract can be the main source of phenolic and carbon compounds after fermentation in DWw. Different types of detectable aromatic compounds can be the result of the carbonyl and amine group reaction at high temperatures in the sugar industry, which exists as a complex in cane molasses known as melanoidin. The involvement of several fatty acids in the DWw will slow down metal accumulation and bioremediation processes in complex plant organometallic.. All these compounds are generating during the anaerobic digestion of DWw or fermentation process. The results demonstrate that some recalcitrant organic pollutants present in DWw after the plant growth these pollutants remediate or bio-transformed some metabolic compounds or value-added products (Table 2). This indicated that the *Ricinus communis* L was able to bioremediate DWw. The bioremediation of the *Ricinus communis* L. showed similar findings and was discovered as a possible aggregation of metals from complex organometallic waste.

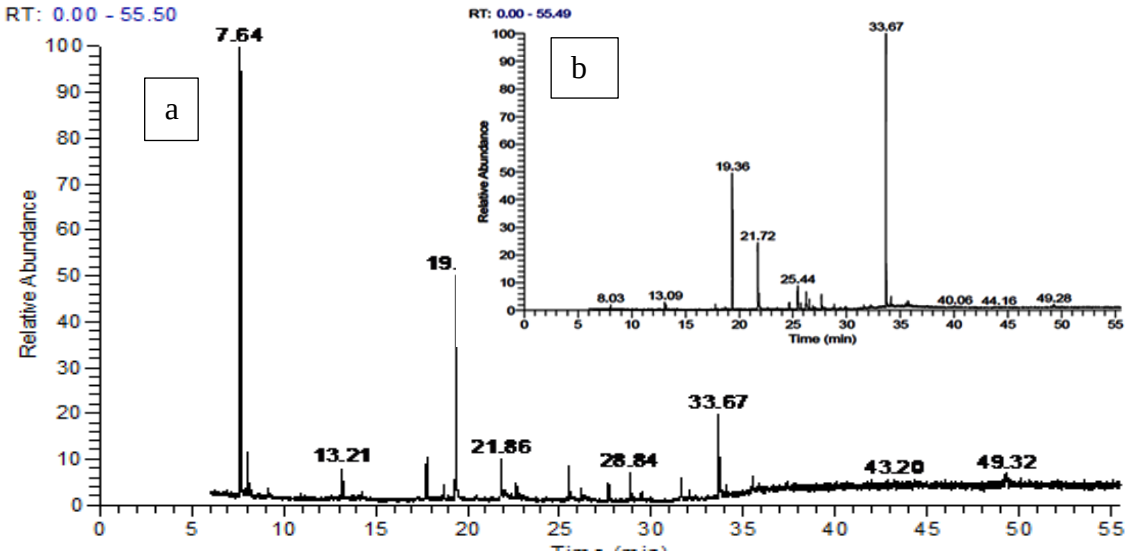

Fig. 8.2.2: Total ion chromatogram (TIC) of TMS derivatized detected organic pollutants from ethyl acetate extract of distillery waste contaminated site (a) Wastewater (b) Rhizospheric effluent**.**

Table 8.2.2: Detection of residual organic pollutants by GC-MS analysis from distillery sludge waste before and after phytoextraction.

Retention time	**Compound name in distillery effluent**	**Toxicity**
7.64	2-METHYL-4-KETO-PENTAN-2-OL 1TMS	Hypertension, Stress
8.07	Octacosane (CAS)	Irritation to mucous membranes
9.14	Ethyl 5,6-epoxyhexanoate	Acute toxicity
13.21	Octadecanoic acid	Developmental toxicity, neurotoxicity
14.03	(R,S)-1-decyn-3-ol	Carcinogenicity, reproductive
17.70	N-cetyl thiocyanate	Eye tearing, muscle weakness,
17.98	Dodecane, 4-methyl- (CAS)	nose and throat irritation
19.36	Heptadecane, 2-methyl- (CAS)	Chest pain or retrosternal burning
21.86	Heptadecane (CAS)	Nausea and vomiting
27.66	7-Methoxy-2,3-dihydro-2-phenyl-4-quinolone	Nausea and vomiting
34.11	Tricosane (CAS)	Acute toxicity
35.84	Undecane, 2-methyl- (CAS)	Abdominal discomfort, Nausea and vomiting
36.67	Docosane (CAS)	Bloody vomiting, liver failture
38.74	PROSTAGLANDIN F1A TMS ESTER TRI TMS ETHER	Dilate the blood vessels
38.86	1,1,1,3,5,5,5-Heptamethyltrisiloxane	Effect on eyes
42.94	(E)-6-(tert-Butyldimethylsiloxy)-2-ethyl-1-(trimethylsilyl)hex-1-ene	Abdominal discomfort, Nausea and vomiting
44.69	2,2'-di-O-methylstenosporic acid	Data not reported
45.85	6-(4'-Chlorobutyl)undecan-6-ol	Data not reported
47.78	2-NITRO-5-BROMOFURAN	Central nervous system damage,
Retention time	**Compounds name near rhizospheric zone**	**Toxicity**
8.03	Dotriacontane (CAS)	Data not reported
13.09	1,2-O-Isopropylidene-3-O-(p-tert-butylbenzyl)-á-D-fructopyranose	
19.36	Trimethylsilyl n-Dodecyl Ether	Data not reported
21.72	acrylic acid decyl ester	Unknown
25.44	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS)	Data not reported
33.67	1,2-Benzenedicarboxylic acid, dioctyl ester (CAS)	Unknown
40.06	Neopentyl 2,2-dimethylpropanoate	Data not reported
49.28	3-tert-Butyl-5-methyl-4-hexen-2-ol	

*RT-retention time (in minutes), (TMS) trimethylsilyl

8.2.2.4. Screening of PGPR activity

The total twelve bacteria are isolated from, rhizospheric soil of *R. communis* from the contaminated sites of the pulp paper industry.. Out of theses total isolates, seven isolates (RCS1- MZ490795- *Stenotrophomonas maltophilia*, RCS2- MZ490796 *Bacillus cereus*, RCS3- MZ490797 *Bacillus anthracis*, RCS4- MZ520573 *Bacillus cereus*, RCS5- MZ520574 *Bacillus cereus*, RCS6- MZ520575 *Bacillus thuringiensis*, RCS7- MZ520576 *Bacillus thuringiensis*, RCS-8- MZ520577 *Bacillus cereus*) are highly potential for producing ligninolytic enzyme and hydrolytic enzyme screening.

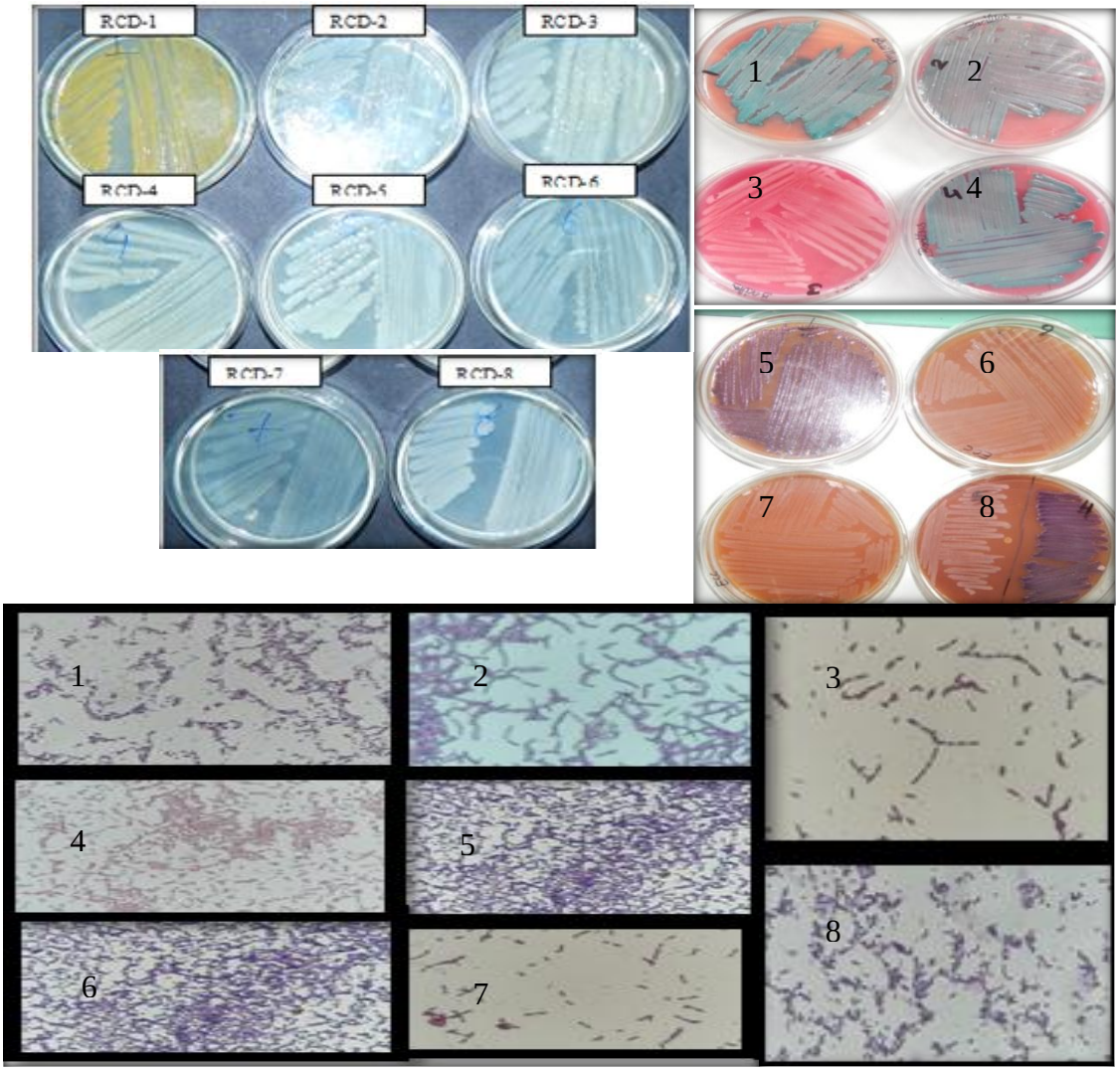

Fig 8.2.3: Purification of isolated PGPR bacterial strains RCS-1 to RCS-8 (1-8) on Hi-chrome specific media and their microscopic observation.

8.2.2.4.1. Lignolytic enzyme producing bacteria

Eight isolates out of the twelve showed positive results for the lignin degradation enzyme based on the selective agar qualitative screening and different indicator dye. It was evident from the good growth of agar plates supplemented with kraft lignin as a carbon source and the formation of clearance areas on phenol red, methylene blue, and guaiacol plates. Moreover, Laccase is multi-copper oxidases in which the single-electron oxidation of organic compounds for the respective radical species is formed a zone due to presence of guaiacol. The ability to produce manganese peroxidase enzymes of seven species of isolated bacterial strains was measured by comparing the diameter of the colony and yellow-brown circles. The results revealed that the yellow circle diameter percentage and bacterial colony diameter are the largest for 24 to 48 hrs.

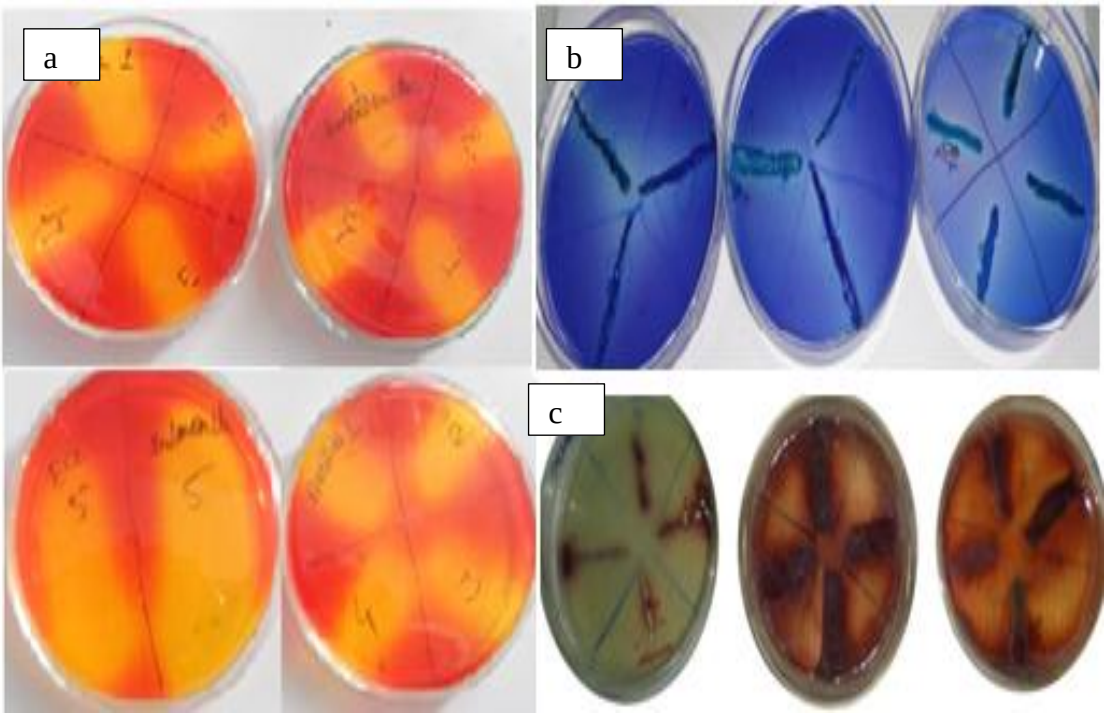

Fig. 8.2.4: screening of ligninolytic enzymes producing potential bacterial strain by using different substrates (a) Phenol red for MnP (b) Guaiacol for Laccase(c) azure B for Lip

8.2.2.4.2. Phosphate solubilization

The favoured ecological niche of plant rhizosphere is considered for different soil microorganisms due to wealthy nutrient availability. The result showed the growth of bacterial culture formation of the hallo zone around the culture inoculation. In addition, the Pikovskaya's media containing Bromo Thymol blue (BTB) changed the color from blue to yellow because of the decrease in the pH of the media. Moreover, phosphorus, the second essential nutrient to limit the growth of plants after nitrogen, is accessible organically as well as inorganically rich soils. After appropriate incubation, colonies showing clear halos around the colony as **P. solubilizes**

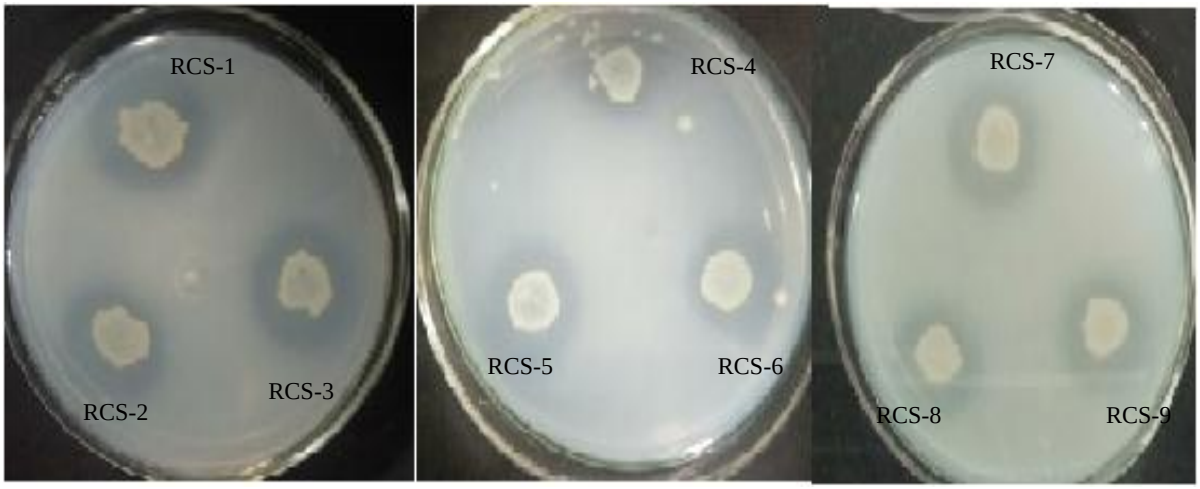

Fig. 8.2.5: Screening of phosphate solubilizing potential bacterial strain

8.2.2.4.3. Zinc solubilization

Plants might well take up Zinc as a divalent cation, but a very small quantity of complete zinc is available as a soluble form in soil solution (Kabata-Pendias and Pendias, 2001) PGPR have always been soil-borne, rhizocolonizing bacteria, multiplying and competing for plant growth with other bacteria. Detection of zinc solubilization by different rhizobacteria isolates were based upon the ability of solubilization zone formation.

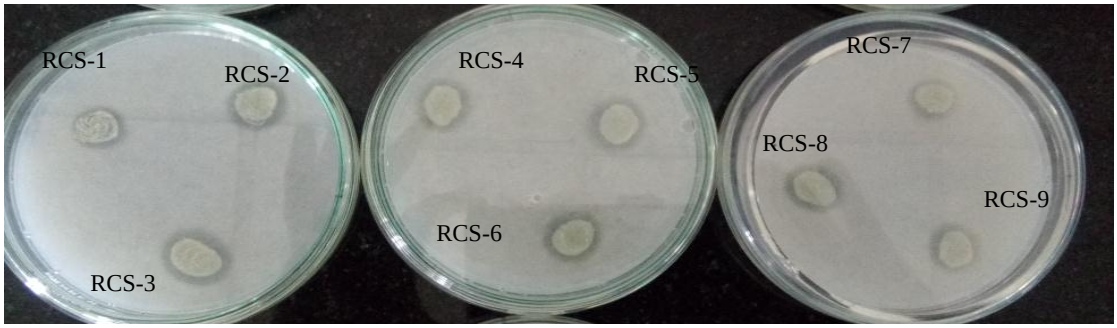

Fig. 8.2.6: Screening of Zinc Solubilizing potential bacterial strain

8.2.2.4.4. IAA production

All the total isolate showed the production of IAA developed pink color in medium Table. A total of four selected isolates i. e. RCS 1-8 were tested for the quantitative estimation of IAA. Moreover, color growth first became visible at the highest IAA concentration within minutes and continued to rise in frequency for a period of 30 min. In addition, IAA is the key member of the plant-produced auxins family as it plays a significant role in a variety of plant activities such as seed forming, embryo growth, root initiation, and production, abscission i.e. flower falling, phototropism, geotropism, fruit development, etc. Moreover, IAA helps to increase the root length by increasing the number of root branches root hairs and root laterals which continue to absorb the relevant nutrient.

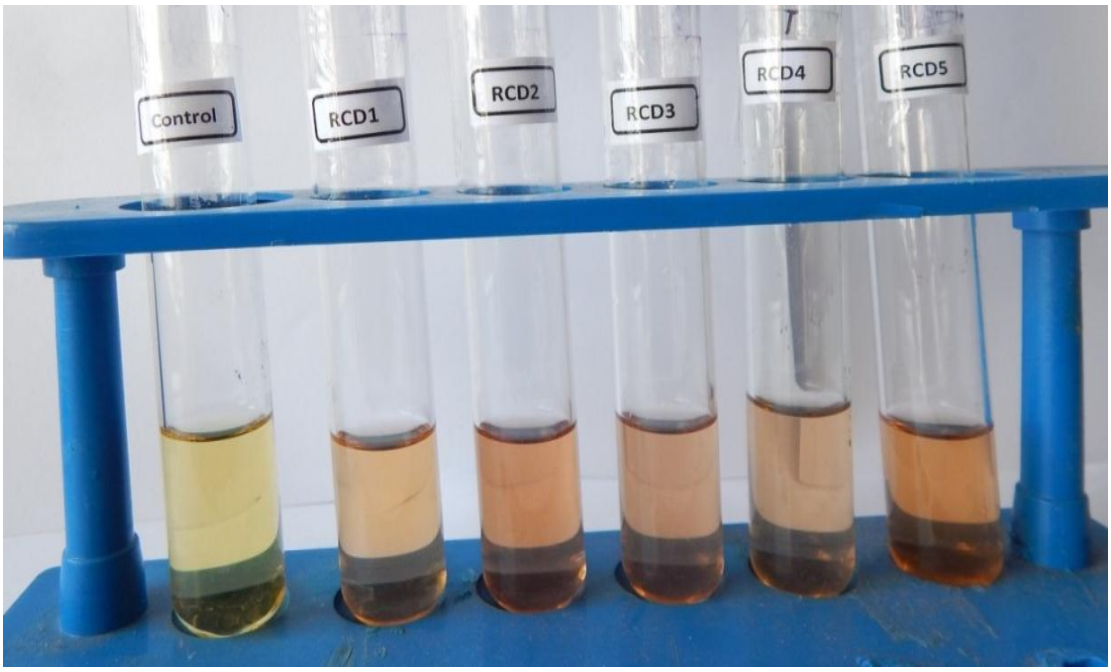

Fig. 8.2.7: Screening of IAA producing potential bacterial strain

8.2.2.4.5. Siderophore production

Four isolates were siderophores positive showing color change from greenish blue to yellow the production of siderophores is yet another significant feature of PGPR, which could influence plant growth. Optimum siderophores yield has been noted at 36 hrs after incubation and began to decline as production its stationary level. In addition, siderophores can form metal complexes with reduced affinity than Fecl3 Baysse et al., (2000). However, metals including Cd2 as, Hg2 as, and Co2 as revealed an inhibitory impact on both productivity and siderophores growth that might have

been owing to competitive metal binding, thus increasing oxidative stress in bacterial cells (Dimkpa et al., 2009). The improvement in development with an increase in iron content expressed a need for iron in metabolic processes by the strain.

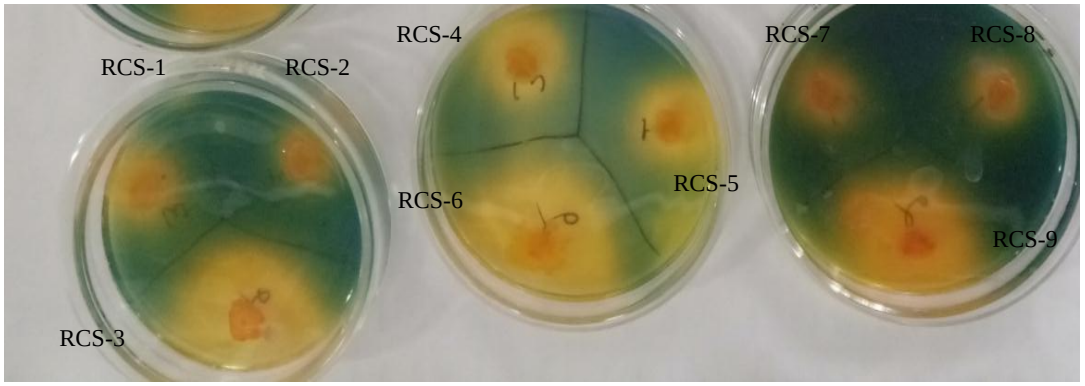

Fig. 8.2.8: Screening of siderophore producing potential bacterial strain

8.2.2.4.6. HCN Production

HCN production was detected for any of the strains and production of ammonia was checked for 71% of the total isolate isolates. The *Bacillus sp.* genus is studied extensively and the elevated frequency of *Bacillus sp.* identified in this experiment can be assigned to the potential to form endospores that allow bacteria to flourish in adverse circumstances such as heat, radiation, dehydration, and hunger. One of the original HCN studies found which HCN may be harmful to plant pathogens. The statistical technique used to calculate the (CN-) in the liquid media has been based on a modified colorimetric methemoglobin technique, which is used in the extracellular and dissolved free non-complex cyanide.

Fig. 8.2.8: Screening of HCN producing potential bacterial strain

8.2.2.5 16sRNA sequencing of bacteria and Construction of phylogenetic tree

The 16S rRNA gene sequences data showed that isolated bacteria i.e. RCS1-MZ490795- Stenotrophomonas maltophilia, RCS2- MZ490796 Bacillus cereus, RCS3- MZ490797 Bacillus anthracis , RCS4- MZ520573 Bacillus cereus, RCS5-MZ520574 Bacillus cereus, RCS6- MZ520575 Bacillus thuringiensis, RCS7-MZ520576 Bacillus thuringiensis, RCS8- MZ520577 Bacillus cereus, respectively. Constructed a phylogenetic tree by using Mega 6.0 software. Moreover, Phylogenetic analysis like morphological, physiological, and bionomic characteristics, allozyme and RFLP data were widely used to infer the evolutionary relationship between organisms.

8.2.2.6 Total metal accumulation and Bioconcentration factors

The impact of metal uptake by the Ricinus communis L plant was further examined to evaluate the ability for phytoremediation of DWw. *Ricinus communis* L. growing at the DWw assessed the accumulated metals (Cr, Zn, Cd, Ni, Pb, Cu, Fe, and Mn). As shown in Table 3, the parameter distribution of metal uptake in different parts of the plant suggested tolerance and ability of metal accumulation shown in (Fig. 1). To withstand metal stress and toxicity, plants employ a range of defense mechanisms, including the activation of various antioxidant enzyme systems, heavy metal binding to phytochelatins/metallothioneins, metal sequestration to vacuoles, and heavy metal absorption removal (Shahid et al., 2015). The previous study reported that the metal content of DWw, combined with organic compounds, disrupted the development of various plants' roots, and stunted their seed germination (Ha et al., 2011). The presence of various organic compounds in the DWw can reduce the bioavailability of the metals (Hu et al., 2019).

The presence of Fe in plant cells demonstrated the detoxication pathway of pollutants and help in the phytoremediation process. The results showed that the high concentration of accumulation of Fe (65.29 ± 0.40), Cu (59.40 ± 0.39), and Pb(40.09 ± 0.50) in the shoot of *Ricinus communis* L. This indicated that *Ricinus communis* L has the ability to resistant to the high concentration of metals in DWw and absorbed the metals in the root, shoot, and leaves. The highest concentration of Zn (34.15 ± 0.89) was observed in the shoot of *Ricinus communis* L. Zn is a micronutrient that is also a protein molecule that serves as a physical, structural, or controlling co-factor for a variety of enzymes. Zn is also important for DNA and RNA structures, the stabilization of DNA function for the enzymes generated, and the regulation of RNA

and gene expression regulation. The Ricinus communis L was capable of accumulating higher concentrations of Cu (22.64 ± 0.78) in the root, Cd (20.59 ± 0.20) in the root, Ni (29.09 ± 0.55) in the root, and As (20.75 ± 0.09) in the root, respectively. The microbial community near the rhizospheric zone of the plant influence the pH and other organic compounds along with metals accumulation in the plant(Sharma et al., 2020d). After anaerobic digestion, the disposal site of distillery wastewater became alkaline (pH 8.67 0.16), which can affect the metal absorption in plant cells. However, translocation of metals by increasing plants on wastewater demonstrated the potential of the plant, and the pH in the rhizospheric soil gradually decreased. It may be caused by the interaction of microorganisms and plants in the rhizosphere, which results in an excess of acids such as citrate, succinate, gluconate, oxalate, 2-ketogluconate, acetate, and malate being released from root hairs. According to our results, DWw as waste contains several metals and organic, fatty acids, and carbon-containing compounds. The majority of DWw pollutants are anionic, with a strong cationic metal-binding propensity. The rhizospheric microbial population has improved the bioavailability of metals in plants.

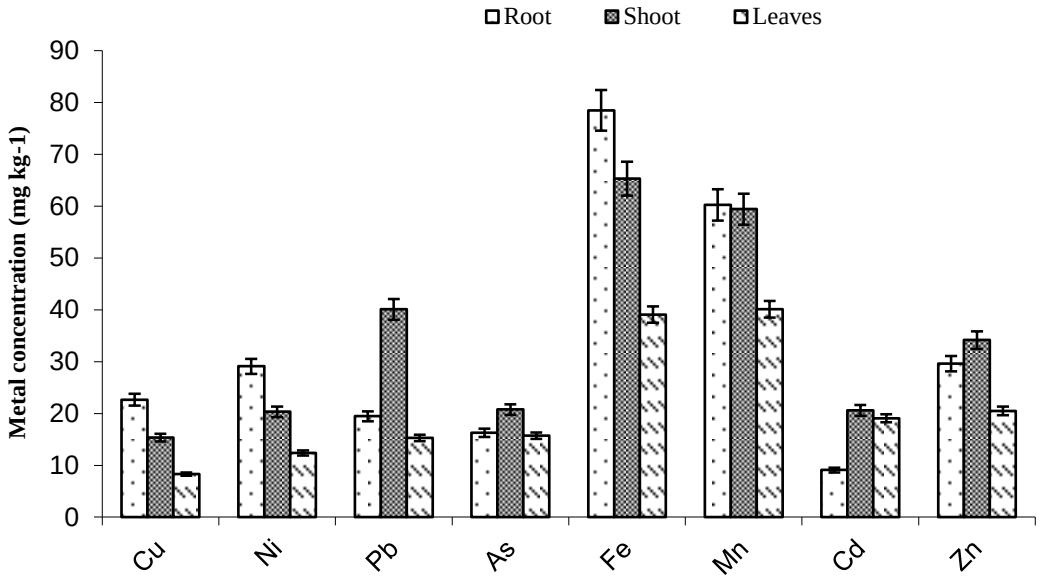

Fig. 8.2.10: Metals accumulation pattern in root, shoot and leaves *Ricinus communis* L growing on the distillery wastewater bed

The BCF and TF measure the capacity of plants to absorb heavy metals. The BCF values of metals in plants revealed that they have the potential to metal phytoremediation in the roots. The metal absorption in DWw is generally low, but *Ricinus communis* L had exceptionally high metal accumulation in different sections, indicating that these plants have a hyperaccumulation propensity. Besides, the deposit of metals from pollutants to roots by plants mainly depending on the properties of soil components, pH, and other complex or co-contaminants that may impede the movements of metals thus prevents its uptake and efflux in plants. Results revealed that the highest BCF for Ni (41.29), and Cu (9.246), were present in *Ricinus communis* L. As a removal process, ions can be sequestered in vacuole by binding to ligands, i.e., proteins, amino acids, and peptides, leading to an increased degree of metalicilous environments. This may be because the plant's root nodule-forming bacteria encourage metal bioavailability in DWw to the plant. The plant's high phytoremediation efficiency and stress tolerance capability rate were indicated by BCF and TF >1. Simultaneously, these plants have shown their capacity to absorb higher metal concentrations from DWw. Metal accumulation from DWw to root is affected by the nature of chemicals the element that inhibits metal mobility in plants. The translocation value has been used to assess the Phytostabilization of metals by plants developing at the wastewater storage site.

Table 8.2.3: Heavy metal accumulation ($mgkg^{-1}$ 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- Leaves

Metals (mg kg ^{1})	**Different parts of *Ricinus communis* L**		
	Root	**Shoot**	**Leaves**
Cu	22.64 ± 0.78	15.34 ± 0.50	8.30 ± 0.20
Ni	29.09 ± 0.55	20.34 ± 0.60	12.40 ± 0.50
Pb	19.45± 0.40	40.09 ± 0.50	15.30 ± 0.80
As	16.25 ± 0.70	20.75 ± 0.09	15.70 ± 0.19
Fe	78.46 ± 0.50	65.29 ± 0.40	39.08 ± 0.14
Mn	60.23 ± 0.68	59.40 ± 0.39	40.10 ± 0.08
Cd	09.09 ± 0.94	20.59 ± 0.20	19.06 ± 0.30

Bioconcentration factor							
	Cu	**Ni**	**Pb**	**As**	**Fe**	**Mn**	**Cd**
	30.65	50.98	19.09	17.05	94.90	1.78	1.00
Translocation factor							
	0.78	0.78	1.78	1.00	0.045	0.78	1.89

Conclusion :

The results revealed that DWw containing various organic compounds and metals were generated during the fermentation process of molasses. Moreover, the detected pollutants are persistent in nature and damage the quality of soil and water. The study showed that *Ricinus communis* L. potential phytoremediation of metals of DWw and reduced 50- 85 % of other pollution parameters. Hence, *Ricinus communis* L may be used on a wide scale in the field for the removal of metals from the polluted site. However, more research is needed to optimize their performance and integrate them with the existing wastewater treatment process.

Chapter-Nine

Development and optimization of a novel technique for decolorization and detoxification of PMDE at high TDS for safe disposal and re-use

Development and optimization of a novel technique for decolorization and detoxification of PMDE at high TDS for safe disposal and re-use.

9. Interoduction

In India there are more than 317 sugarcane molasses based distilleries industry pouring down their treated and untreated effluent at rate of approximately 3.5 X 10^{14} kiloliters' annually to water bodies through their discharge system (AIDA, 2004).The major color contributing complex biopolymer of distillery waste is known as melanoidin, which is generated during polymerization of amino group with reducing carbohydrate at the time of boiling of sugarcane juice concentration process for sugar crystallization in sugar industry.In India, there are number of large scale distilleries integrated with sugar mills. The waste products of sugar mills comprise bagasses (residue from the sugarcane crushing), press mud and molasses. The effluents from molasses based distilleries contain large amounts of dark brown colored molasses-spent-wash (MSW). This causes soil and aquatic pollution and threat to environmentalist and challenge for industries. Worldwide environment regulatory authorities have set strict norms for discharge of wastewaters from industries. In India, for instance, distillery industry had been told to achieve zero pollution discharge of spent wash by December 2005 according to the Charter of Central Pollution Control Board (CPCB) the apex pollution control authority (CPCB, 2003). It further says that till 100% utilization of spent wash should be achieved, controlled and restricted discharge of treated effluent from lined lagoons during rainy season will be allowed by SPCBs /CPCB in such a way that the perceptive coloring of river water bodies does not occur.

The present invention relates to a novel technique for biological decolorization of post methanated distillery effluent (PMDE) in biphasic bacterial and wetland plant treatment system for environmental safety. Using the bacterial pre-treatment followed by wetland plant treatment in constructed wetland treatment system as sequential bioreactor. The original distillery effluent after anaerobic digestion which is known as PMDE, retains high BOD (29000-32000 mg/l), COD(58000-70000 mg/l), color(150,000-180,000 Co-Pt),TS (34317 mg/l),TDS (50000 mg/l) , phenol (700-800 mg/l),nitrogen (600-650 mg/l), sulphate (20000-25000 mg/l) and some heavy

metals(i.e. Fe, Mn, Zn, Cu, Ni), pH (8.5 ±0.5) even after extended aeration of PMDE in industries with alkaline pH (8.0 ± 0.5). Due to recalcitrant nature of different pollutants of effluent present as colorant do not support microbial growth for its mineralization in its original concentration which causes the soil and aquatic pollution. However, in present technique could degrade and decolorized the PMDE up to 88% with developed bacterial consortium at optimized nutrient and reduced TDS within 4 days incubation at 120 rpm. Further integration with constructed wetland plant treatment system enhanced the decolorization and detoxification of PMDE up to 95-96% within 10 days retention times. Physico-chemical analysis of treated PMDE revealed that there was reduction in color along with reduction of other pollution parameters i.e. BOD, COD, TS, phenol, sulphate, nitrogen and heavy metals. In addition the absorbance analysis of colorant by HPLC showed the sequential reduction of absorbance peak of colorant which correlated with the physico-chemical data of decolorized PMDE. The effect of different season on the performance of this technique revealed that summer and monsoon season was very much conducive while there was comparatively slow process in winter due to slow biological process at low temperature. Further the toxicity evaluation of finally decolorized PMDE with bacteria and wetland plant treatment tested with *V.fabae* for environmental safety showed not only reduction of toxicity but supported the plant growth and yield compared with control irrigated with tap water. Hence, the wetland plant treated effluent for irrigation would not only solve the disposal problem but serve as additional source of liquid fertilizer. This does not degrade in environment for long time even 8-10 after year of its persistence. Therefore, it is a major source of aquatic and soil pollution due to its high discharge from various part of country. A reference may be made to a publication wherein the impacts of distillery effluent on carbohydrate metabolism of freshwater fish, *C. carpio* (Ramakritinan et. al., 2005) which showed stress resulting in a shift towards anaerobiosis at organ level during sublethalintoxification. Furthermore a reference may be made to a publication wherein the inhibitory effect on seed germination along with soil pollution is also observed during the distillery effluent irrigation (Chandra et. al., 2004). But, a reference may be made to a publication wherein presence of other pollutants in effluents like phenolics, sulphate and heavy metals makes more toxic and recalcitrant nature of effluent (Borja et. al., 1993). However, the application of this technique for

the effluent decolorization up to safe limit of environment at large scale was not feasible. Furthermore the references may be made to the publication wherein researches have demonstrated the ability of some aquatic macrophyte for reducing the level of toxic metals in polluted water (Munshi et. al., 1989; Selvapathi and Shrireedar, 1991; Chandra et. al., 1992; 2004; Shah et al., 2015). The drawback is that, these above reported plants showed the ability of bioaccumulation only in pure metals solution. The color of post methanated distillery effluent (PMDE) is suspected to be due to high content of sulphur, phenol and other unknown organic compounds, which inhibits the microbial activity for further degradation. The major obstacle in the effluent treatment of molasses spent wash is the presence of high dark brown colored natural polymer called melanoidins; due to its water soluble properties causes water as well as soil pollution. Further, the researchers have revealed that the bacterial pretreatment of distillery effluent enhance the phytoremediation process. In addition the enhanced heavy metal removal potential by *Typha angustata L.* from PMDE after bacterial treatment also indicated in the finding (Chandra et al., 2008). Due to lack of any adequate technique for the decolorization of PMDE after extended aeration at industrial scale still it is major source of environmental pollutants in India. Currently, no any feasible and economically viable technology is available for its safe disposal. Hence, the safe disposal for environmental potential and conservation of aquatic resources for sustainable development is warranted. 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 (Bari et al., 2018; Imran et al., 2012; Ali Shah et al., 2012). 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 TDS of PMDE offers an excellent system for removal of color from PMDE and further reduction of BOD, COD for safe disposal. A reference may be made to a publication wherein the increased microbial population has been indicated for the degradation of organic chemical contaminated soil (Nichols et. al., 1997). The role of rhizosphere bacteria for a degradation of industrial wastewater is yet not reported in India. The potential growth of many macrophytes has been observed in distillery effluent

contaminated site in Indian condition. A reference may be made to a publication wherein isolation and characterization of *Phragmites australis* (L.) rhizosphere bacteria from contaminated site for bioremediation of colored distillery effluent (Chaturvedi et. al., 2006). The drawback is that, these studies do not describe the systematic application of these macrophytes (i.e. *Typha sp*., *Cyperus sp.* and *Phragmites sp*.) for reduction of pollution load. This indicated the application of these macrophytes in wetland treatment system has tremendous opportunities for the absorption and accumulation of many heavy metals and pollution reduction as a process of biofilteration. Hence, the combined application of bacteria and wetland plants would show maximum decolorization and degradation of all organic matters as a sequential bioreactor process. 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 (Chandra et al., 2018). The complexicity of PMDE showed that high TDS as a key factor of effluent toxicity due to dissolved organic content in high concentration. The reference nay be made to the publication for precipitation of phenol colourant and other ionic salts for reduction of total dissolved solids (TDS) from various industrial wastewater to achieve the biodegradation with bacterial growth (Swamy et al., 2011; Isla, and Guha, 2013; Bari et al., 2013). Hence, the aim of this patent is development of novel technique for decolourisation and detoxification of distillery effluents after anaerobic digestion with the combined application of bacteria and potential wetland plant for its safe discharge and re-use in various applications for sustainable development. Lastly the decolorized effluent after toxicity reduction can be used either for effluent recycling or its application to aquaculture and ferti-irrigation/ green belt development around industries would prevent aquatic and soil pollution with safe disposal.

9.1Material and Method

9.1.1. Designing of Pilot plant

The biphasic bacterial and wetland plant treatment system for environmental safety 800L capacity bioreactor with working volume of 500L followed by 10 m zigzag structure of constructed wetland treatment system consisting cemented tank having the dimension of 150x150x150 cm with working capacity of 500 L as bioreactor and containing the dimension of constructed wetland treatment was 60x72x1000 cm (width x depth x length) having 0.88% slopes. With upstream flow rate 2.0 l/min

versus 1.5 l/min downstream, contains plant of *Phragmites cummunis*, *Typha angustata* and *Cyperus aloecupiroides*.

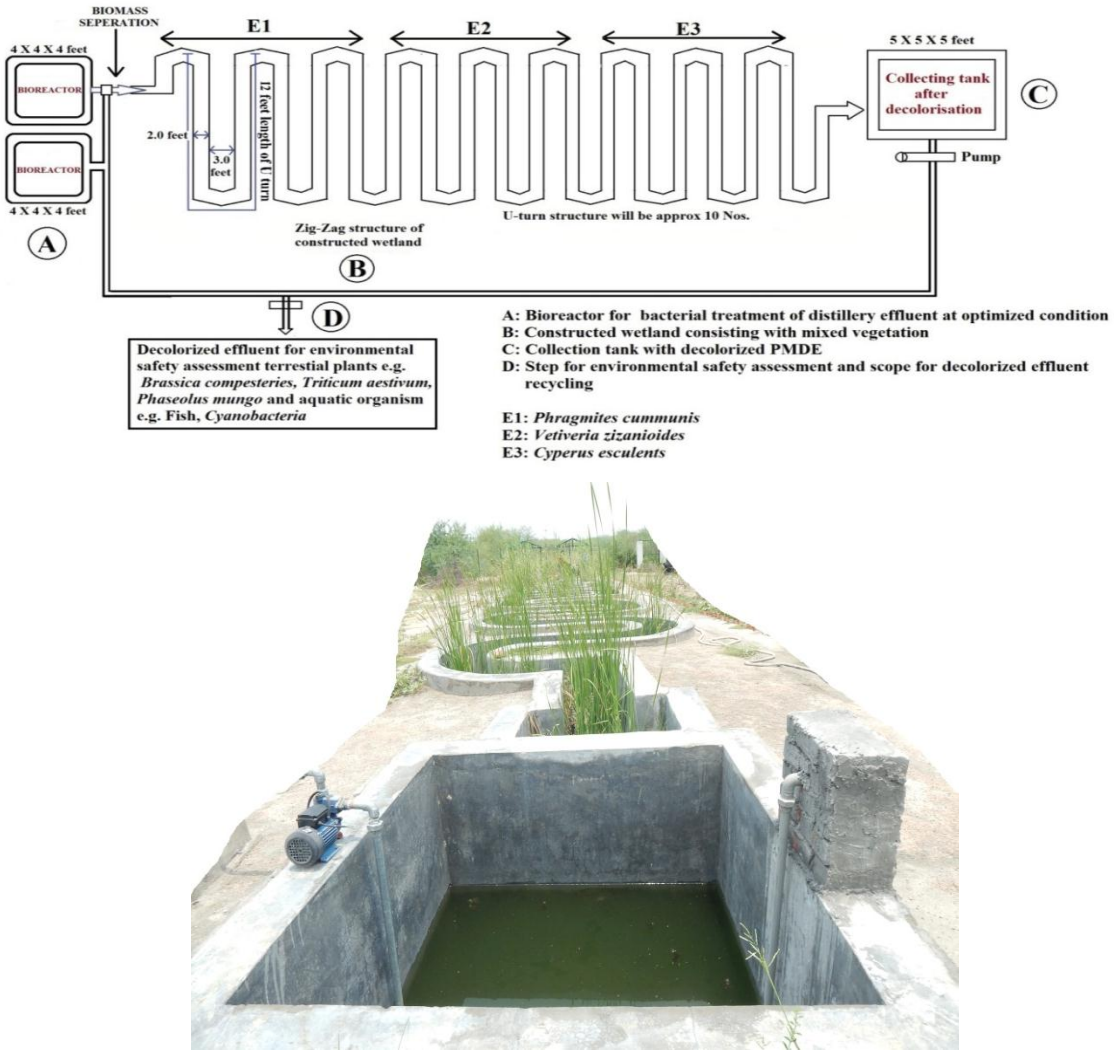

Fig. 9.1: A. Sketch diagram of constructed wetland treatment at pilot scale.

B: - Showing the view of constructed wetland treatment at pilot scale.

Fig 9.2: Designed pilot plant with constructed wetland plant treatment system with *Phragmites communis* and *Typha latifolia*

Fig 9.3: Luxuriant growth of *Phragmites communis* and *Typha latifolia* in designed constructed wetland treatment system for detoxification of Post Methanated Distillery Effluent.

9.1.2. 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 9.3. 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.

9.1.3. 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.

9.1.4. 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 *Phragmites cummunis*& *Typha angustata* for rhizofiltration.

9.2. Result and discussion

The Clear and fresh distillery effluent after anaerobic digestion was obtained in the clear plastic jerry cane. The total dissolved solid (TDS) was optimized with using various coagulants such as aluminum sulfate, aluminum chloride, sodium aluminate, ferric chloride, ferric sulfate, ferrous sulphate, lime etc, at variable concentration (10%, 20%, 40%,50% & 100%) of PMDE. But the ferric chloride showed optimum decolorization in presence of 0.34% within 6-8hrs. The (80%) decolorization period was also influence with 97% TDS reduction with mixing of ferric chloride in PMDE. Fast and prolong mixing enhanced the decolorization process. Further the efficiency of decolorization and TDS reduction was evaluated with rapid mixing variable pH and the coagulant dosage which determined the hydrolysis species for effective treatment of PMDE. The decolorized supernatant of PMDE was separated from optimized dose of coagulant. The optimum TDS reduction resulted 80% supernatant and 20% colloidal precipitated material. The PMDE supernatant was 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%) to evaluate the bacterial growth in biostimulation for reduction of colour from supernatant of PMDE. The optimum decolorization of PMDE was noted 96% in biostimulation process while the reduction of BOD & COD was noted 98% & 97% respectively. Further, the biomass separated supernatant was (96%). The bacterial consortium of four identified bacteria were inoculated in 30% PMDE collected after extended aeration in presence of 1% glucose as additional carbon source and 0.5% peptone as nitrogen in 800 L capacity bioreactor with working capacity of 500 L at pH 8.0±0.5, 120±2 rpm and 37±2^{o}C temp. This was incubated in tank upto 96 hrs. This showed 98% bacterial decolorization along with reduction of various pollution parameters (BOD, COD, TS, TDS, sulphate and heavy metals) upto 70-99%. 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 *Phragmites cummunis*&*Typha angustata*. 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. The reduction of BOD and COD was observed upto 97 and 98%. While the color was reduced upto 98-99%, sulphate and heavy metals was also reduced upto 95%.the color reduction was also validated with HPLC analysis which showed the comparative reduction of coloring peak. The Physico-chemical analysis of decolorized PMDE after biofilters with constructed wetland treatment was found environmentally safe with seed germination test using *Phaseolus mungo* and ferti-irrigation to *Viciafabae* (L.) as terrestrial test model approved by OECD,1995; USEPA, 1980.

Accordingly the present invention provides a novel technique for biological decolorization of post methanated distillery effluent (PMDE) in biphasic bacterial and wetland plant treatment system for environmental safety which comprises bacterial pretreatment of 30% PMDE in 800L capacity bioreactor with working volume of 500L followed by 10 m zig-zag structure of constructed wetland treatment system consisting the mixed vegetation of *Phragmites cummunis, Typha angustata* and *Cyperus aloecupiroides* . The bacterial treatment is accomplished with a consortium of *Klebsiella pneumoniae* (MF278771), *Klebsiella pneumoniae* (MF278772) and *Klebsiella pneumoniae* (MF278773), 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. While, the wetland treatment is accomplished within 10 days retention of bacterial pre-treated PMDE by circulating the effluent in constructed wetland treatment system. The environmental safety of decolorized PMDE was accessed by ferti-irrigation to *Vicia fabae* (L.) in a pot grown experiment. This technique pertains with biological decolorization and detoxification of PMDE after extended aeration for environmental safety using bacterial pretreatment followed by constructed wetland plant as sequential bioreactor and the concentration of PMDE after extended aeration was used in between 30-50% having the BOD and COD in order of 19125 - 21687 and 21297- 32154 mg/l respectively .

The bacterial consortium capable for decolorization and detoxification of PMDE comprises *Klebsiella pneumoniae* (MF278771), *Klebsiella pneumoniae* (MF278772) and, *Klebsiella pneumoniae* (MF278773) in biostimulation as autochthonous bacteria. The additional carbon source of bacterial growth is selected glucose having at least 1% concentration in initial stage. The required condition for

bacterial growth in 800 L capacity bioreactor was rpm 120-140,pH 8.0±0.5, temperature 37±1°C within 96 hrs to get maximum decolorization of effluent and the nitrogen source for bacterial growth is used peptone at low concentration (0.5%). The bacterial biomass is easily settled when aeration is stopped in bioreactor after 96 hrs incubation and the melanoidin pigment of PMDE are decolorized by bacterial biomass growth and the supernatant of decolorized effluent is released in constructed wetland treatment system. The dimension of constructed wetland treatment was 60x72x1000 cm (width x depth x length) having 0.88% slopes. With upstream flow rate 2.0 l/min versus 1.5 l/min downstream, contains plant of *Phragmites cummunis, Typha angustata* and *Cyperus aloecupiroides*. The retention time of effluent with wetland plant is 10 days by circulation in wetland system and decolorized and detoxified PMDE is collected in 2500 L capacity water reservoir which is at the end of wetland plant treatment system and the toxicity of the effluent was reduced about 98% within 14 days after combined system of bacterial and constructed wetland plant treatment system and the detoxification was established by looking the effect of decolorized effluent on *Vicia fabae* (L.) on seed germination and plant growth parameter as ferti-irrigation on terrestrial ecosystem and the bacterial consortium was inoculated in a cemented tank having the dimension of 150x150x150 cm with working capacity of 500 L as bioreactor containing the 30% diluted PMDE collected from M/S Unnao distillery, UP, India after extended aeration having the BOD,COD limit of 11200 and 22300 mg/l respectively. The pre-inoculation of bacterial consortium in reactor the 1% glucose and 0.3% peptone was added as carbon and nitrogen source for bacterial growth. While the pH was maintained 8.0±0.5 and rpm was given 120-150 for aerobic bacterial growth in distillery effluent. The carbon and nitrogen source supported the bacterial growth initially which subsequently degraded to melanoidin as result of co-metabolism. Further, no bacterial growth indicated the exhaust of bioavailable nutrient. Subsequently the flow of bacterial decolorized effluent was circulated through the constructed wetland plant system (*Phragmites cummunis, Typha angustata* and *Cyperus aloecupiroides).* The plant rhizospheric zone acted as biofilters which facilitated the bioaccumulation of heavy metal present in effluent and melanoidins through rhizospheric bacteria and plant. The bio-transformed decolorized effluent indicated the apparent bioavailable, dissolved constituent as nutrient in effluent. This process acted as sequential bioreactor using

bacteria at one step and constructed wetland treatment system at second step. The effect of decolorized PMDE to *Viciafabae* (L.) for seed germination and plant growth parameters in ferti-irrigation at pilot scale experiment also indicated the safe recycling of distillery effluent in environment.

The available source of carbon is completely utilized during the methanogenesis by methanogens. Consequently there is hardly any bacterial growth in aeration tank at industrial treatment process. In addition the pH of spent wash increase in methane reactor from 4 to 8.5 due to oxidation of organic acid to CO_2 and the reaction between CO_2 to basic compounds and generates carbonates and bicarbonates. The bacterial degradation of melanoidin from effluent is as result of co-metabolism in presence of 1% glucose as additional carbon source. Therefore, partial decolorization and biotransformation of various pollutants was noted. However, the bacterial consortium does not grow further this reveals the non-availability of nutrient. While injection of supernatant of decolorized PMDE was further rapidly decolorized and detoxified. This revealed the biofilteration of all available pollutants in effluent which is not easily degraded by bacterial treatment. The reduction of pathogenic bacteria in decolorized effluent indicated the utilization of organic compounds. In addition the reduction of coloring peak in effluent during the HPLC analysis revealed the detoxification of effluent and bioremediation of various pollutants. Further, the effect of decolorized effluent on the seed germination, plant growth parameter of *Viciafabae* (L.) also showed the supportive effect. This indicated the reduction of toxicants and colouring constituents for safe disposal in environment.

The following examples are given by way of illustration of the working of the invention in actual practice and therefore should not be constructed to limit the scope of the present invention in any way.

Table. 9.1:-Physico-chemical analysis of discharged distillery industry wastewater, after TDS reduction and after bacterial and rhizofiltration collected from M/s Unnao Distillery pvt. Ltd. *Unnao*, Uttar Pradesh, India

Parameters	**Distillery wastewater**	**Bacterial Treatment**	**After Rhizofiltration**	**Reduction (%)**	**Permissible Limit (CPCB 2012)**
pH	7.00 ± 0.20 l	7.20 ± 0.10	8.114 ± 0.21ˆa	09.96	
Electric conductivity	4.7 ± 2.11 l	2.6 ± 1.14	0.8 ± 1.34ˆa	<85%	--
Ions					
Na^+	56 ± 1.31 l	35.108 ± 0.97	09.352 ± 0.80ˆa	<95%	--
Cl^-	1829 ±0.10 l	560.6. ± 0.89	15.78±0.09	<98.76%	11.82 ± 0.01
NO^{3-}	110 ±3.14 l	76.916 ± 1.30	49.295 ±0.90ˆa	<95%	47.00± 0.00
Ammoniacal nitrogen	86.594 ± 0.71	32.004 ± 0.09	12. 40 ± 0.71	<90.55%	9.90± 0.00
Total dissolve solid	750.4 ± 2.07	253.0 ± 2.09	150 ± 89.19ˆa	<95.56%	152± 0.01
Biological oxygen demand	878.2 ± 2.28	298.5 ± 5.28	56.698 ± 1.78ˆa	<95.87%	47.00± 0.00
Chemical oxygen demand	551 ± 1.58	132.654± 1.58	18.934 ± 1.90ˆa	<90.87%	79.00± 0.01
Total organic carbon	18.318 ± 0.21 l	15.404 ± 0.28	7.407 ± 1.09ˆa	100%	11.82± 0.01
Total nitrogen	2.473 ± 0.01 l	2.094 ±0.04	1.982 ± 0.05ˆc	100%	9.90± 0.00
Heavy metals					
Iron (Fe)	403 ± 3.11l	101.22 ± 1.86	52.528 ± 2.05ˆa	<90.87%	----
Zinc (Zn)	10.15 ± 2.14 l	5.273 ± 0.68	1.463 ± 1.67ˆa	<95.56%	0.15
Copper (Cu)	3.62 ± 1.14 l	2.928 ± 1.20	1.526 ± 1.02ˆa	<97%	0.01
Chromium (Cr)	2.825 ± 0.41 l	1.447 ± 0.60	0.500 ±0.49ˆa	<99%	1.28
Cadmium (Cd)	2.440 ± 0.12l	1.786 ± 0.15	0.34 ± 0.05ˆc	<90%	0.19
Manganese (Mg)	7.30 ± 0.94 l	5.602 ± 1.13	1.140 ± 0.72ˆa	<99%	1.45
Nickel (Ni)	4.425 ± 0.21 l	0.302 ± 0.31	0.250 ± 0.20ˆa	<99%	0.02
Lead (Pb)	BDL	BDL	BDL	BDL	BDL

All the values are Mean ±SE. (n=3); Unit of all parameters is in (mgL^{-1}) except pH, color (Co-Pt. Unit) and EC ($\mu mhoscm^{-1}$); Students *t* test (two tailed as compared to pre-treated sludge); [a]Highly significant at $p<0.001$; [b]Significant at $p<0.01$; [c]Less significant at $p<0.05$; [NS]Non-significant at $p>0.05$; BDL: Below detection Limit.

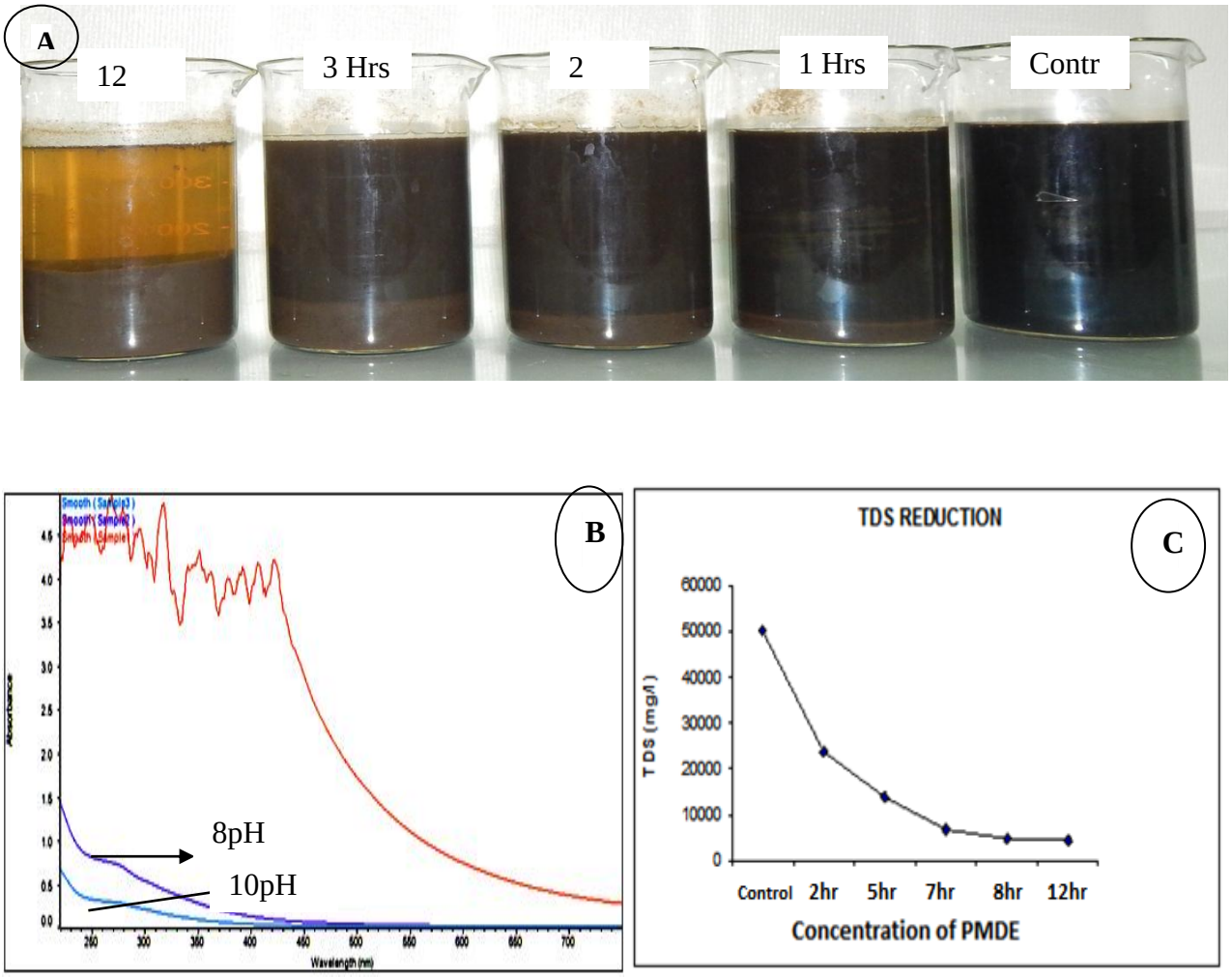

Fig: 9.4 A: TDS Reduction of PMDE along with decolorization of Effluent after different time incubation. **B: -** Comparative UV-Vis absorption spectrum of PMDE after TDS reduction and bacterial degradation. **C: -** TDS reduction pattern at various PMDE concentration.

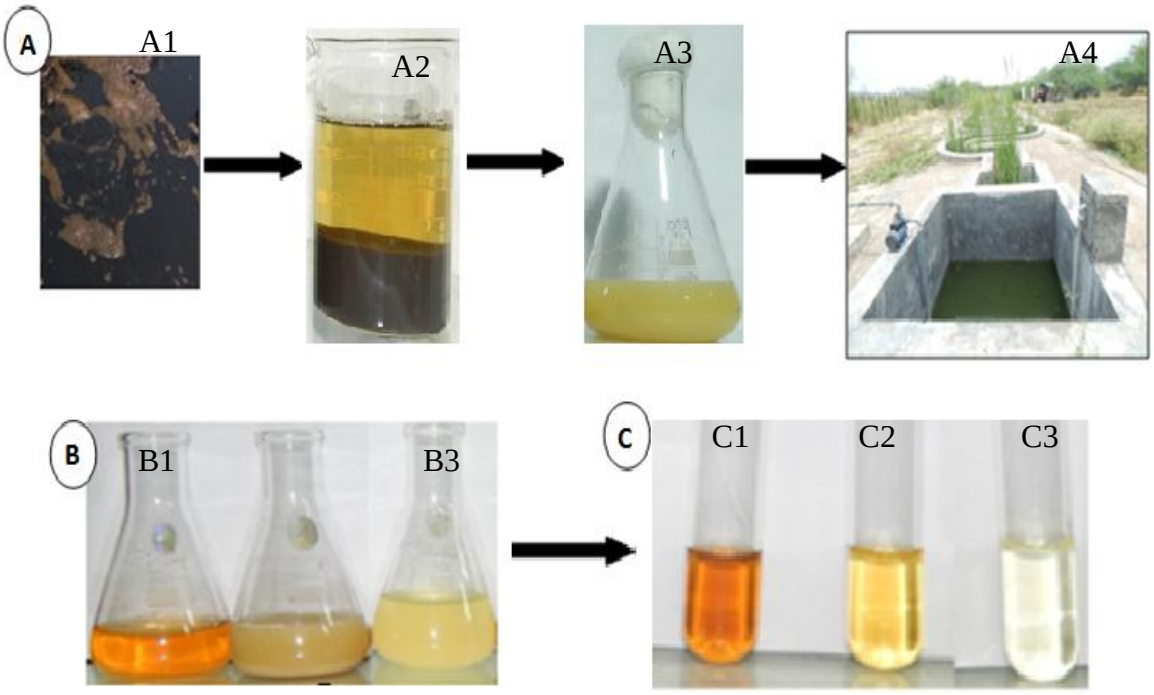

Fig: 9.5: (A-C) View of colour reduction at various stages of treatment

(A). Comparative view of PMDE colour after decolorization at pilot scale**. (B)** Biostimulation after TDS Reduction.*B1*- after TDS removal, *B2*- Bacterial growth after biostimulation process at 48hrs incubation, *B3*- Bacterial growth after biostimulation process at 96hrs incubation. **(C).** Colour reduction after biomass separation at various incubation time**.** *C1*- TDS removal, *C2*- 48hrs incubation, *C3*- 96hrs incubation.

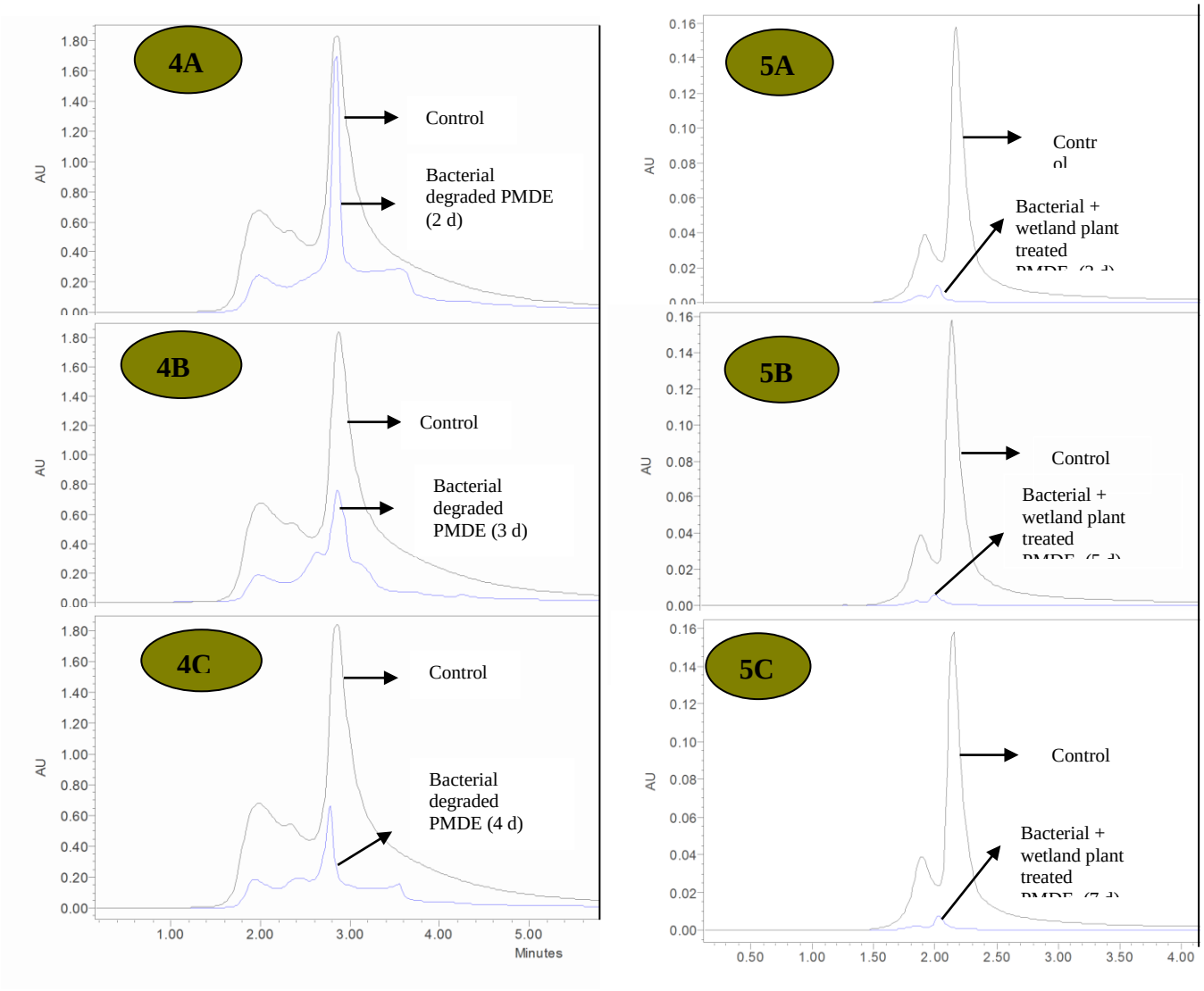

Fig: 9.6: Colour Reduction pattern in bacterial degradation at various day's incubation. 4**A.** Two days**. 4B.** 3 days. 4**C.** 4 days.

Fig: 9.7: A comparative decolorization pattern of PMDE during the wetland plant treatment after bacterial degradation at various days. 5**A.** 3 days. 5**B.** 5 days. 5**C.** 7days.

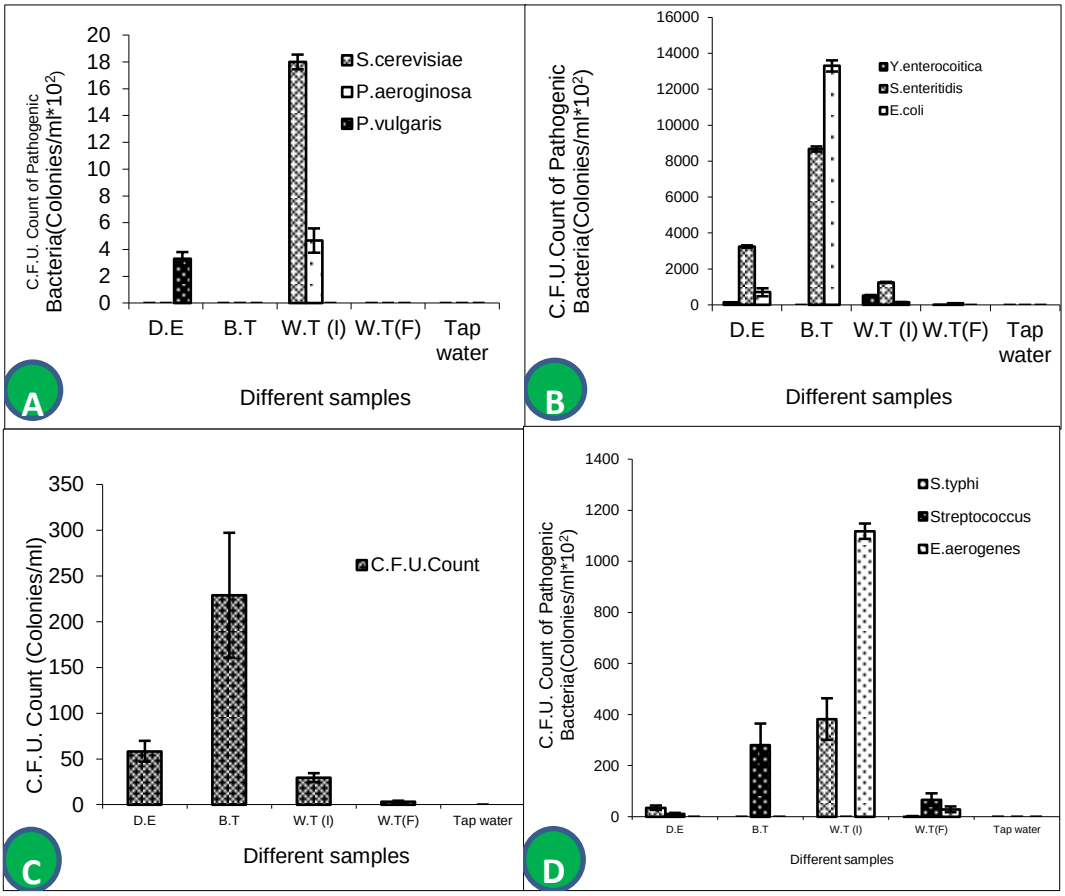

Fig: 9.8: (A-D):- Comparative reduction of bacterial count at various stages treatment of PMDE

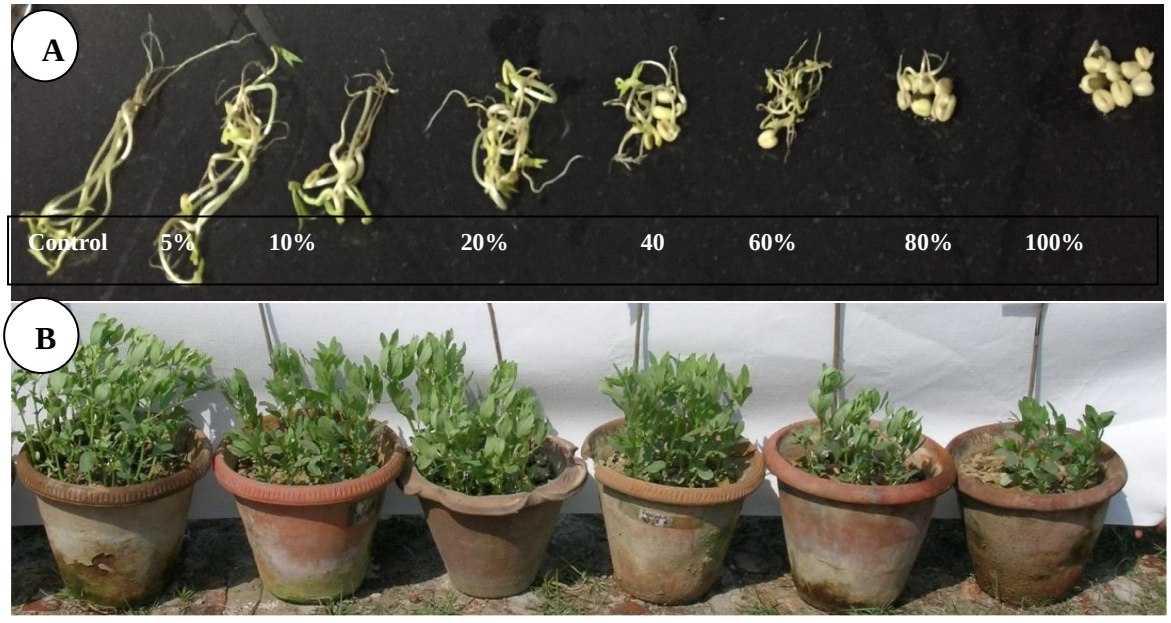

Fig: 9.9: Seed germination & plant growth assessment after PMDE decolorization.

9.5 Advantages and propects of this technology:

1. This is new process of post methanated distillery effluent decolorization with integration of bacterial treatment followed by constructed wetland for pollution prevention and environmental safety.
2. There is reduction of color (up to 98%) along with COD (96%), BOD (95%), TS (95%), phenol (93%), sulphate (90%), nitrogen (82%) and heavy metals at neutral pH in 30-50% post methanated distillery effluent (PMDE).
3. The channelized path of effluent flow may give more opportunity for degradation of recalcitrant compounds with longer interaction. In addition, the aquatic plant with adventitious root which multiply very rapidly would absorb nutrient and other compounds faster. The wetland plant species like *Phragmites cummunis, Typha angustata and Cyperus aloecupiroides* etc., makes unique bacterial association around their rhizospheres which acts as safety guard for the plant with shock load of pollutant and mineralize the toxicants
4. The decolorization of distillery effluent and reduction of pollution parameters is achieved upto environmental safe limit. This can be used for ferti-irrigation in India.
5. Hazardous compound like cyanide, arsenate and polychlorinated bi-phenyl can also be degraded.
6. Maintenance and supervision requirement are also low.
7. No additional energy source like electricity is required than those of mechanical unit.
8. Capital cost is normally cheaper than of its mechanical counterpart.
9. This is Simple bioremediation technique.

Conclusion: -

The result of this study has concluded that TDS of distillery effluent as one very important regulatory factors for degradation and detoxification process. Because, high TDS due to complexation of organic compounds inhibit the microbial growth. Bacterial growth after TDS reduction at optimized condition was found effective in Bio-stimulation & Bioaugumentation process. Further integration of treatment technology with constructed treatment process was found as promising technology where floating of effluent through root zone was based on rhizofiltration technology. Constructed wetland plant treatment after bacterial treatment was as bi-phasic treatment process. It was found cost effective & recycling of treated distillery wastewater.

Chapter-Ten
Summary

Chapter-10

Study the Degradation Mechanism of Distillery waste by Potential Bacterial Consortium for its safe Disposal and re-cycling in Environment

Summary

In India a wide network of distilleries has been established one of the most polluting agro - based industries producing enormous amounts of complex distillery effluent. The distillery effluent contains organic and inorganic pollutants which have been reported to be beneficial and have a beneficial effect at low concentration on crop yields. Melanoidin is known as a major distillery wastewater colourant. Chemically melanoidins are complex due to polymerization of amino-corbonyl complex due to polymerization of aminoacid and carbohydrate at elevated temperature. It has also been stated that melanoidins have a net negative charge; thus to form large organo-metallic complex molecules. Consequently, different heavy metals strongly bind to melanoidins in environment. Biomethanation is a common convention anaerobic method that produces methane to meet out a part of the power requirement in distilleries among the various processes available for the treatment of spent wash. Owing to the complexity of organic and inorganic pollutants present in waste, the effluent becomes more pungent and black during anaerobic treatment called methanogenesis produces PMDE (Post methanated Distillery Effluent). After anaerobic treatment, the discharged effluent causes environmental issues in marine and surface water that eventually impact aquatic flora and fauna. Therefore, prior to its discharge into the atmosphere, proper care of the distillery effluent is warranted. Hence, before and after anaerobic treatment, the precise chemical properties of different contaminants present in the distillery effluent are still to be investigated.

In the first chapter of the study, the above information was systematically revealed as an introduction. Subsequently, the objectives of the study were stated in the second chapter. Furthermore the literature review of the subject was expanded in Chapter Three, while the investigation of physicochemical properties and characterization of the different persistent organic compounds present in distillery effluent has been addressed in Chapter Four. Anaerobically treated molasses-based distilleries spent wash which is also known as post methanated distillery effluent (PMDE) 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 (λmax) 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-butyl1H, 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 *Heteropneustes fossil* fish, indicating the generation of oxidative free radicals. Similarly, inhibition of α-amylase activities in germinating *Zea mays L*. (Maize) seeds were also noted at 10% PMDE. This study confirmed the environmental toxicity of effluent with freshwater fish and *Zea mays* seeds. The chapter five of thesis has mentioned the Isolation & characterization of potential bacterial community responsible for bio-stimulation and bio-augmentation process during detoxification of Distillery waste. The analysis showed that sixteen morphologically different bacterial strains (ACS1-ACS16) were isolated in Biostimulation process and twenty morphologically different aerobic bacterial strains (IITRCS01-IITRCS20) were isolated by nutrient enrichment technique from distillery sludge by streak plate method. The tolerance behavior of MnP, laccase, and Lip was also tested on these bacterial strains. Total out of 36 overall bacterial strains, two aerobic bacterial strains (AS1and AS7) in biostimulation and (IITRCS10 - IITRCS12) were showed maximum MnP and laccase producing activity on phenol red amended GPM medium and guiacol amended B&K agar medium plated. Further, these bacterial strains were also showed higher melanoidins (3500 mg L^{-1}) tolerance activity. The potential bacterial consortium showed 40% decolourisation in biostimulation sample and up to 70% in bioaugumented consortium in presence of glucose (1%), peptone (0.1%) at optimum pH (8.1), temperature (37 °C) and shaking

speed (180 rpm) within 192 h of incubation. The reduction of colour of Maillard product correlated with shifting of absorption peaks in UV–Vis spectrophotometry analysis. UV-Vis spectrophotometric analysis of distillery effluent showed many absorption peaks between 200 and 450 nm and their absorption maximum peak was noted at 250 nm in spectrophotometric detection. Further, the changing of functional group in FT-IR data showed appearance of new peaks and GC–MS analysis of degraded sample revealed the depolymerisation of complex organometallic compounds. The toxicity evaluation using seed of *P. mungo* L. showed reduction of toxicity of effluent after bacterial treatment in Bioaugumentation and biostimulation process. Thus, this consortium might be useful for decolourisation of industrial wastewater containing high concentration of melanoidins.

In the Chapter six, the created bacterial consortium was employed for the assessment of TDS reduced wastewater, based on potential isolated bacterial strains. The study was conducted for optimal decolourisation and degradation of wastewater after TDS reduction under optimised environmental and nutritional conditions which constitute as chapter six of my thesis. 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 TDS of PMDE offers an excellent system for removal of color from PMDE and further reduction of BOD, COD for safe disposal wherein the increased microbial population has been indicated for the degradation of organic chemical contaminated soil. In this objective the role of rhizosphere bacteria for a degradation of industrial wastewater is reported wetland plants growing at polluted site. The Optimization of TDS and potential growth of many microphytes has been observed in distillery effluent contaminated site. The absorption spectrum scanning by UV-Visible spectrophotometer between 200-700 nm revealed reductions of absorption spectrum of organic compounds present in bacterial degraded sample after TDS reduction in range of 200-450 nm compared to control. The degradation and decolourisation of TDS reduced PMDE by bacterial consortium was noted by induction of manganese peroxidase and laccase activities in sample supernatant. Further, the TLC and HPLC analysis of bacterial decolourised melanoidins also showed degradation and reduction of absorption peak at (295nm), respectively. Furthermore, FT-IR and GC-MS analysis also showed the change of functional group and disappearance of ion peaks. This indicated the degradation and depolymerisation of melanoidins and cleavage of C═C,

C=O and C≡N conjugated bonds which resulted in reduction of colour. The metabolic analysis also showed the disappearance of some organic compounds and generation of new metabolites. Further the seed germination test using *P. mungo* L. showed toxicity reduction in decolorized effluent. Thus, the result revealed that the developed bacterial consortium could be used to scale up the decolourisation, degradation and detoxification process of TDS reduced PMDE for industrial application.

In Chapter 7, this is the first report of the microbial community present in sugarcane molasses-based distillery sludge and their relationships with the organo-metallic pollutants present. Samples were collected from the discharge point, 1 and 2 km meters downstream (D1, D2, and D3, respectively) and their physico-chemical properties characterised. The complex physico-chemical characteristics of distillery sludge have significant impact on the diversity and relative abundance of the bacterial community. Using QIIME, taxonomic assignment for the V3 and V4 hypervariable regions of 16S rRNA was performed. The phyla *Fermicutes, Proteobacteria, Bacteriodetes, Actinobacteria, Euryechaeota, Tenericutes and Patescibacteria* were the predominant bacteria in samples collected from all three sites. *Spirochaetes, Sinergistetes* and *Cloacimonetes* were only detected in samples from site D1.Shannon,Simpson, Chao1, and Observed-species indices indicated that site D1 (10.18, 0.0013, 36706.55 and 45653.84, respectively) has higher bacterial diversity and richness than D2 (6.66, 0.0001, 25987.71 and 49655.89, respectively) and D3 (8.31, 0.002, 30345.53 and 30654.88, respectively), suggesting the organo-metallic pollutants provided the stressor to favour the survival of microbial community that can biodegradation and detoxification them in the distillery sludge. This finding provides important information for toxicity assessment on bacterial communities in distillery industry and selection of bioremediation candidates. These bacteria are able to tolerate extreme condition and survive in the hostile environment of the distillery sludge. When the environmental pressure was reduced through mixing and dilution of the receiving water, the competitive advantage was removed and was reflected in reduced bacterial diversity. Many of the compounds identified in the sludge samples are hazardous to the environment and human health, the current treatment process is not sufficiently effective to reduce the level of organic and metal pollutants to an acceptable level, additional steps are required to improve the treatment of the distillery discharge. Bacteria detected in the most pollution site, D1, are potential candidates to remediate the organic and metal pollutants present in distillery sludge

and should be further explored as part of the solution to improve the chemical quality of the discharge. Since, the phytoremediation of complex industrial waste by native plants is an emerging green technology for eco-restoration of polluted site. Hence, before construction of wetland plant treatment system some potential native plants have been assessed for phytoextraction of heavy metals from stabilised post methanated distillery sludge. Therefore, in chapter eight of thesis has showed in detail result of phytoextraction of heavy metals.The study investigated the heavy metal accumulation by herbaceous traditional medicinal and food plants (*Achyranthus aspera, Amaranthus viridis, Basella alba, Sesbania bispinosa, Pedalium murex, and Momordica doica*) grown on complex distillery waste containing a mixture of organometallic compounds. The results revealed bioaccumulation of Mn, Cd, Fe, Cr, Cu, As, Se, Mo, and Co in their root, shoot and leaves in levels higher than the surrounding sludge. *A. aspera* was noted as root accumulator for Mn (16.95±0.1 mg/kg-1), Zn (30.12±0.3 mg/kg--1), Fe (240.4±0.1 mg/kg- -1), Co (3.19± 0.2 mg/kg--1), while Se (4.07±0.3 mg/kg--1), Mo (4.36± 0.2 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. In another study the phytoremediation potential of *Ricinus communis L*. was evaluated for heavy metals remediation via rhizospheric bacteria for distillery waste detoxification and management. Results showed that the wastewater containing high metals and other Physico-chemical parameters, which cause environmental pollution and aquatic toxicity. The identified bacterial strain produced siderophores, indole acetic acid, and ligninolytic enzymes resulted in provides nutrients and help in the mineralization of metals in plants during stress conditions as plant growth promoting rhizobacteria (PGPR). The translocation factor and Bioconcentration factor of all the metals were< 1, which reveals that these metals are stabilized in the root portion of *Ricinus communis L.* The ability of *Ricinus communis L.* to grow well in metals containing distillery waste suggests that it can be used for the removal of heavy metals. The use of *Ricinus communis* L. is an eco-friendly tool to deal with heavy metal pollution and improvement in productivity of agricultural systems.

Further, as per objective of study, the bacterial degraded PMDE was integrated with designed horizontal subsurface flow-constructed wetland (HSSF-CW) plant treatment system. The total dissolved solid (TDS) was optimized with using

various coagulants such as aluminum sulfate, aluminum chloride, sodium aluminate, ferric chloride, ferric sulfate, ferrous sulphate, lime etc, at variable concentration (10%, 20%, 40%,50% & 100%) of PMDE. But the ferric chloride showed optimum decolorization in presence of 0.34% within 6-8hrs. The (80%) decolorization period was also influence with 97% TDS reduction with mixing of ferric chloride in PMDE. Fast and prolong mixing enhanced the decolorization process. Further the efficiency of decolorization and TDS reduction was evaluated with rapid mixing variable pH and the coagulant dosage which determined the hydrolysis species for effective treatment of PMDE. The decolorized supernatant of PMDE was separated from optimized dose of coagulant. The optimum TDS reduction resulted 80% supernatant and 20% colloidal precipitated material. The PMDE supernatant was 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%) to evaluate the bacterial growth in biostimulation for reduction of colour from supernatant of PMDE. The optimum decolorization of PMDE was noted 96% in biostimulation process while the reduction of BOD & COD was noted 98% & 97% respectively. Further, the biomass separated supernatant was (96%).

The bacterial consortium of four identified bacteria were inoculated in 30% PMDE collected after extended aeration in presence of 1% glucose as additional carbon source and 0.5% peptone as nitrogen in 800 L capacity bioreactor with working capacity of 500 L at pH 8.0±0.5, 120±2 rpm and 37±2oC temp. This was incubated in tank upto 96 hrs. This showed 98% bacterial decolorization along with reduction of various pollution parameters (BOD, COD, TS, TDS, sulphate and heavy metals) upto 70-99%. 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 *Phragmites cummunis* & *Typha angustata*. 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. The reduction of BOD and COD was observed upto 97 and 98%. While the color was reduced upto 98-99%, sulphate and heavy metals was also reduced upto 95%.the color

reduction was also validated with HPLC analysis which showed the comparative reduction of coloring peak. The Physico-chemical analysis of decolorized PMDE after biofilters with constructed wetland treatment was found environmentally safe with seed germination test using Phaseolus mungo and ferti-irrigation to Vicia fabae (L.) as terrestrial test model approved by OECD,1995; USEPA, 1980.

Therefore, novel technique for biological decolorization and detoxification of post methanated distillery effluent (PMDE) in combined bacterial and constructed wetland plant treatment system for environmental safety is novel because of following reasons;

1. This is new process of post methanated distillery effluent decolorization with integration of bacterial treatment followed by constructed wetland for pollution prevention and environmental safety.
2. There is reduction of color (up to 98%) along with COD (97%), BOD (95%), TS (95%), phenol (93%), sulphate (90%), nitrogen (82%) and heavy metals at alkaline pH in 70-80% post methanated distillery effluent (PMDE).
3. The decolorization of distillery effluent and reduction of pollution parameters is achieved upto environmental safe limit. This can be used for ferti-irrigation in India.
4. There is a production of useful biomass of wetland plants during the wastewater treatment process which can be used for various small scale industries.
5. This is cost effective technique which will be applicable at industrial scale.

As per objective for development of feasible technology for sustainable developmet a patent has been granted as innovative technology entitled **"Decolorization and Detoxification of Distillery Wastewater with Combined Use of Bacteria and Constructed Wetland Treatment (2021101010)".**

Conclusion

This study concluded that Distillery Effluent after secondary treatment contains various residual organic pollutants which showed chromosomal aberration, genotoxicity, cytotoxicity and oxidative damage of DNA in A. cepa and P. mungo plant. After Biostimulation and Bioaugumentation process most dominant and persistant organic pollutants i.e. Nonacosane, Heptacosane, Hexadecanoic acid, Stearic acid, 3-(octadecyloxy) propyl ester (CAS), Octadecane, 3-ethyl-5-(2-ethylbutyl)-(CAS), benzoic acids and Octadecanoic has been detected by GC-MS analysis and degraded various treatment process. Majority of these compounds has been reported as bacteriostatic and bactericidal effects on the microbial community. In addition, some plant steroids and other detected residual organic pollutants are reported as EDCs compounds. The native plants growing at contaminated site showed high potentiality for Phytoextraction of heavy metals from organometallic complex along with phytoremediation for the Eco restoration of polluted site. A diversity of microbial community from rhizosphere has been also detected as evidence for bacteria assisted phytoremediation. The presence of mutagenic and androgenic compounds as persistent pollutants even after secondary treatment of distillery effluent makes the aquatic ecosystem and environment more attentive to tertiary treatment for pollution prevention. The proposed technology is very helpful for our nation/country and Environment for sustainable development.

Based on work, fifteen (23) original research papers have been published in high impact journal and three research papers is under consideration. While seven conference papers presented in national and international conferences. Two book chapters are published in firm of reputed publisher. Two awards and two Patent related to my PhD Topic are also published.

Chapter-Eleven

Bibliography

A**.S. Deval, H.A. Parikh, A. Kadier, K. Chandrasekhar, A.M. Bhagwat, A.K. Dik shit.** Sequential microbial activities mediated bioelectricity production from distillery wastewater using bio-electrochemical system with simultaneous waste remediation. **Int. J. Hydrogen Energy, 42 (2017), pp. 1130-1141**

Ahmed, V., Verma, M.K., Gupta, S., Mandhan, V., Chauhan, N.S., 2018. Metagenomic profiling of soil microbes to mine salt stress tolerance genes. Front. Microbiol. 9, 159.

Ahsan N, Lee SH, Lee DG, Lee H, Lee SW, Bahk JD, Lee BH (2007) Physiological and protein profiles alternation of germinating rice seedlings exposed to acute cadmium toxicity. Comptes. Rendus. Biologies. 330: 735–746.

Akunna JC, Clark M (2000) Performance of a granular-bed anaerobic baffled reactor (GRABBR) treating whisky distillery wastewater. Biores. Technol. 74: 257–261.

Ale R, Jha PK, Belbase N (2008) Effect of distillery effluent on some agricultural crops, a case of environmental injustice to local farmers in Khajura VDC, Banke. Scientific World 6(6): 68-75.

Alkhatib R, Bsoul E, Blom DA, Ghoshroy K, Creamer R, Choshroy S (2013) Microscopic analysis of lead accumulation in tobacco (*Nicotiana tabacum* var. Turkish) roots and leaves. J. Microsco. Ultrastr. 1: 57-62.

All India Distillers Association [AIDA] (2004) All India Distillers Association and Sugar Technologists of India. New Delhi: All India Distillers Association.

All India Distillers' Associations (AIDA) (2016) Annual report. NewDelhi. http://www.aidai ndia.org/annual-report.html

Allen SE (1989) Analysis of ecological Materials. 2nd ed. Oxford (England): Blackwell Scientific Publications.

A**lonso A, Sanchez P, Martinez JL (2000)** *Stenotrophomonas maltophilia* D457R contains a cluster of genes from Gram-positive bacteria involved in antibiotic and heavy metal resistance. Antimicrob. Agents. Chemo. 44: 1778–1782.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSIBLAST: A new generation of protein database search programs. Nucl. Acids Res. 25: 3389-3402.

Ameen, F., AlYahya, S., Govarthanan, M., ALjahdali, N., Al-Enazi, N., Alsamhary, K., ... & Alharbi, S. A. (2020). Soil bacteria Cupriavidus sp. mediates the extracellular synthesis of antibacterial silver nanoparticles. *Journal of Molecular Structure*, *1202*, 127233.

Angayarkanni J, Palaniswamy M, Swaminathan K (2003) Biotreatment of distillery effluent using *Aspergillus niveus*. Bull. Environ. Contam. Toxicol. 70: 268–277.

AOAC (2002) Official methods of analysis of AOAC International, 17th edn. International, AOAC.

Aoshima I, Tozawa Y, Ohmomo S, Ueda K (1985) Production of decolorizing activity for molasses pigment by *Coriolus versicolor* Ps4a. Agric. Boil Chem. 49: 2041-2045.

APHA (American Public Health Association) (2012) Standard method for examination of water and wastewater. 22nd ed. APHA, AWWA and WEF, Washigton, DC

Aravinthan, A., Govarthanan, M., Selvam, K., Praburaman, L., Selvankumar, T., Balamurugan, R., ... & Kim, J. H. (2015). Sunroot mediated synthesis and characterization of silver nanoparticles and evaluation of its antibacterial and rat splenocyte cytotoxic effects. *International journal of nanomedicine*, *10*, 1977.

Arimi MM, Zhang Y, Goetz G, Kiriamiti K, Geissen SU (2014) Antimicrobial colorants in molasses distillery wastewater and their removal technologies. Int. Biodeterior. Biodegr. 87: 34-43.

Arimi MM, Zhang Y, Götz G, Geisen S-U (2015) Treatment of melanoidin wastewater by anaerobic digestion and coagulation. Environ. Technol. 36(19): 2410–2418.

Arora DS, Chander M, Gill PK (2002) Involvement of lignin peroxidase, manganese peroxidase and laccase in degradation and selective ligninolysis of wheat straw. Int. Biodeterior. Biodegrad. 50: 115–120.

Asaithambi P, Susree M, Saravanathamizhan R, Matheswaran M (2012) Ozone assisted electrocoagulation for the treatment of distillery effluent. Desalination 297: 1–7.

Assih EA, Ouattara AS, Thierry S, Cayol JL, Labat M, Macarie H (2002) *Stenotrophomonas acidaminiphila* sp. nov., a strictly aerobic bacterium isolated from an upflow anaerobic sludge blanket (UASB) reactor. Int. J. Syst. Evolu. Microbiol. 52(2): 559-568.

Badejo AA1, Sridhar MK, Coker AO, Ndambuki JM, Kupolati WK (2015) Phytoremediation of Water Using Phragmites karka and Veteveria nigritana in Constructed Wetland. Int. J. Phytoremed. 17(9): 847-852.

Baker AJM (1981) Accumulators and excluders strategies in the response of plants to heavy metals. J Plant Nutr 3: 643–654.

Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry. Biorecovery 1: 81–126.

Barakat MA (2011) New trends in removing heavy metals from industrial wastewater. Arab. J. Chemis. 4: 361–377.

Bardiya MC, Hashia R, Chandna S (1995) Performance of hybrid reactor for anaerobic digestion of distillery effluent. J. Indian Assoc. Environ. Manag. 22 (3): 237–239.

Barrow GI, Feltham RKA (2003) Cowan and steels, manual for the identification of medical bacteria (3rd ed). Cambridge University Press, Cambridge.

Beckett PHT, Davis RD (1977) Upper critical levels of toxic elements in plants. New Phytol. 79: 95-106.

Beltrain de Heredia J, Domingues J R, Partido E (2005) Physico-chemical treatment for depuration of wine distillery wastewater (vinasses). Water Sci. Technol. 51(1): 159–166.

Beltran FJ, Alvarez PM, Rodriguez EM, Garcia-Araya JF, Rivas J (2001) Treatment of high strength distillery wastewater (cherry stillage) by integrated aerobic biological oxidation and ozonation. Biotechnol. Prog. 17: 462–467.

Beltrán FJ, García-Araya JF, Álvarez PM (1999) Wine distillery wastewater degradation. 1. Oxidative treatment using ozone and its effect on the wastewater biodegradability. J. Agric. Food Chem. 47(9): 3911–3918.

Benitez FJ, Real FJ, Acero JL, Garcia J, Sanchez M (2003) Kinetics of the ozonation and aerobic biodegradation of wine vinasses in discontinuous and continuous processes. J. Hazard. Mater. B. 101: 203–218.

Berthelet M, Whyte LG, Greer CW (1996) Rapid, direct extraction of DNA from soils for PCR analysis using polyvinylpolypyrrolidone spin columns. FEMS Microbiol. Lett. 138: 17-22.

Bharagava R, 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. J. Hazard. Mater. 180: 730-734

Bharagava RN, Chandra R (2009) Isolation and characterisation of phenolic compounds by ^{1}H NMR and mass spectrometric analysis from sugarcane molasses post methanated distillery effluent. Ind. J. Environ. Prot. 29: 873-888.

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: 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. Biores. Technol. 99: 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., Chandra, R., 2009. "Isolation and Characterization of Phenolic Compounds by 1H NMR and Mass Spectrometric Analysis from Sugarcane Molasses Post Methanated Distillery Effluent." Indian Journal of Environmental Protection 29: 873–888.

Billaud C, Maraschin C, Nicolas J (2004) Inhibition of polyphenoloxidase from apple by Maillard reaction products prepared from glucose or fructose with L-cysteine under various conditions of pH and temperature. LWT 37: 69-78.

Billore SK, Singh N, Ram HK, Sharma JK, Singh VP, Nelson RM, Dass P (2001) Treatment of a molasses based distillery effluent in a constructed wetland in central India. Water Sci. Technol. 44: 441–448.

Billore SK, Singh N, Sharma JK, Dass P, Nelson RM (1999). Horizontal subsurface flow gravel bed constructed wetland with *Phragmites Karka* in central India. Water Sci. Technol. 40(3): 163-171.

Bini C, Wahsha M, Fontana S, Maleci L (2012) Effects of heavy metals on morphological characteristics of *Taraxacum officinale* Web growing on mine soils in NE Italy. J. Geochem. Explor. 123: 101–108

Blonskaja V, Menert A, Vilu R (2003) Use of two-stage anaerobic treatment for distillery waste. Adv. Environ. Res. 7 (3): 671–678.

Blonskaja V, Zub S (2009) Possible ways for post-treatment of biologically treated wastewater from yeast factory. J. Environ. Eng. Lansc. Manag. 17(4): 189–197.

BMC Bioinformatics 12:385.

Boer CG, Obici L, De Souza CGM, Peralta RM (2006) Purification and some properties of Mn peroxidase *from Lentinula edodes*. Process Biochem. 41: 1203–1207.

Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J. I., Knight, R., Mills,D.A., Caporaso, J.G., 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat. Methods 10:57–59.

Bonugli-Santos RC, Durrant LR, Sette LD (2012) The production of ligninolytic enzymes by marine-derived basidiomycetes and their biotechnological potential in the biodegradation of recalcitrant pollutants and the treatment of textile effluents. Water, Air Soil Pollut. 223(5): 2333–2345.

Boopathy R, Tilche A (1991) Anaerobic digestion of high strength molasses wastewater using hybrid anaerobic baffled reactor. Water Res. 25(7): 785–790.

Borja R, Alba J, Banks CJ (1997) Impact of the main phenolic compounds of olive mill wastewater (OMW) on the kinetics of acetoclastic methanogenesis. Proc. Biochem. 32: 121-133.

Borja R, Martin A, Maestro R, Luque M, Durán J (1993) Enhancement of the anaerobic digestion of wine distillery wastewater by the removal of phenolic inhibitors. Bioresour. Technol. 45: 99-104.

Borkert CM, Cox FR, Tucker MR (1998) Zinc and copper toxicity in peanut, soybean, rice and corn in soil mixtures. Commun. Soil Sci. Plant Analy. 29(19-20): 2991-3005.

Boutard M, Cerisy T, Nogue P-Y, Alberti A, Weissenbach J, Salanoubat M, et al. (2014) Functional Diversity of Carbohydrate-Active Enzymes Enabling a Bacterium to Ferment Plant Biomass. PLoS Genet 10(11): e1004773. https://doi.org/10.1371/journal.pgen.1004773

Brenner DJ, Krieg NR, Staley JT, Garrity GM (2005) Bergey's manual of systematic bacteriology. New York: Springer.

Bustamante MA, Paredes C, Moral R, Moreno-Casalles J, Pérez-Espinosa A, Pérez-Murcia, MD (2005) Uses of winery and distillery effluents in

agriculture: characterization of nutrient and hazardous components. Water Sci. Technol. 51(1): 145-151.

Caizán-Juanarena, L., terHeijne, A., Weijma, J., Yntema, D., Suárez-Zuluaga, D. A., &Buisman, C. J. (2020). Screening for electrical conductivity in anaerobic granular sludge from full-scale wastewater treatment reactors. Biochemical Engineering Journal, 159, 107575.

Calheiros CSC, Teixeira A, Pires C et al. (2010) Bacterial community dynamics in horizontal flow constructed wetlands with different plants for high salinity industrial wastewater polishing. Water Res. 44: 5032–5038

Cämmerer B, Chodakowski K, Gienapp C, Wohak L, Hartwig A, Kroh L (2012) Pro-oxidative effects of melanoidin-copper complexes on isolated and cellular DNA. Eur. Food Res. Technol. 234: 663-670.

Cammerer B, Jaluschkov V, Kroh LW (2002) Carbohydrates structures as part of the melanoidins skeleton. Int. Cong. Ser.1245-269

Campanale, C., Massarelli, C., Savino, I., Locaputo, V., &Uricchio, V. F. (2020). A detailed review study on potential effects of microplastics and additives of concern on human health. International journal of environmental research and public health, 17(4), 1212.

Canle, M., Pérez, M. I. F., &Santaballa, J. A. (2017). Photocatalyzed degradation/abatement of endocrine disruptors. Current opinion in green and sustainable chemistry, 6, 101-138.

Caporaso JG, Bittinger K, Bushman FD, De-Santis TZ, Andersen GL, Knight R (2010a) PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26: 266–267.

Caporaso JG, Kuczynski J, Stombaugh et al. (2010b) QIIME allows analysis of high throughput community sequencing data. Nat. Methods 7:335–336.

Carita R, Marin-Morales MA (2008) Induction of chromosome aberrations in the *Allium cepa* test system caused by the exposure of seed to industrial effluent contaminated with azo dye. Chemosphere 72: 722-725

Carranza-Alvarez C, Alonso-Castro AJ, Alfaro-De laTorre MC, GarcIa- De La Cruz RF (2008) Accumulation and distribution of heavy metals in *Scirpus americanus* and *Typha latifolia* from an artificial lagoon in San Luis Potosı, Mexico. Water Air Soil Poll. 188: 297–309.

Cetin D, Donme G (2006) Decolorization of reactive dyes by mixed cultures isolated from textile effluent under anaerobic conditions. Enzy. Microb. Technol. 38: 926-930.

Chandra R and Pandey PK (2000) Decolourization of anaerobically-treated distillery effluent by activated charcoal adsorption method. Indian J. Environ. Prot. 21: 134-137

Chandra R, Abhishek A, Sankhwar M (2011) Bacterial decolorization and detoxification of black liquor from rayon grade pulp manufacturing paper industry and detection of their metabolic products. Bioresour. Technol. 102(11): 6429-36.

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: 78-86.

Chandra R, Bharagava RN, Rai V (2008b) Melanoidins as major colourant in sugarcane molasses based distillery effluent and its degradation. Bioresour. Technol. 99: 4648–4660.

Chandra R, Bharagava RN, Rai V, Singh SK (2009) Characterization of sucrose–glutamic acid Maillard products (SGMPs) degrading bacteria and their metabolites. Bioresour. Technol. 100: 6665–6668.

Chandra R, Kumar K, Singh J (2004) Impact of anaerobically treated and untreated (raw) distillery effluent irrigation on soil microflora, growth, total chlorophyll and protein contents of *Phaseolus aureus* L. J. Environ. Biol. 25(4): 381-385.

Chandra R, Singh H (1999) Chemical decolourisation of anaerobically treated distillery effluent. Indian J. Environ. Prot. 19(11): 833-837.

Chandra R, Singh R (2012) Decolourisation and detoxification of rayon grade pulp paper mill effluent by mixed bacterial culture isolated from pulp paper mill effluent polluted site. Biochem. Enginee. J. 61: 49–58.

Chandra R, Yadav S (2010) Potential of *Typha angustifolia* for phytoremediation of heavy metals from aqueous solution of phenol and melanoidin. Ecol. Engin. 36: 1277–1284.

Chandra R, Yadav S, (2011) Phytoremediation of Cd, Cr, Cu, Mn, Fe,Ni, Pb and Zn from aqueous solution using *Phragmites cummunis*, *Typha angustifolia* and *Cyperus esculentus*. Int. J. Phytoremed. 13(6): 580–591.

Chandra R, Yadav S, Bharagava RN, Murthy RC (2008c) Bacterial pretreatment enhances removal of heavy metals during treatment of post-methanated distillery effluent by *Typha angustata* L. J. Environ. Manag. 88:1016–1024.

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

Chandra S, Chauhan LK, Murthy RC, Saxena PN, Pande PN, Gupta SK (2005) Comparative biomonitoring of leachates from hazardous solid waste of two industries using the *Allium* test. Sci. Total. Environ. 347: 46-52.

Chandra, R., & Kumar, V. (2017). Detection of Androgenic-Mutagenic Compounds and Potential Autochthonous Bacterial Communities during *In Situ* Bioremediation of Post-methanated Distillery Sludge. *Frontiers in microbiology*, *8*, 887. https://doi.org/10.3389/fmicb.2017.00887

Chandra, R., Kumar, V., & Tripathi, S. (2018b). 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. (2018c). 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.

Chang IS, Choo KH, Lee CH, Pek UH, Koh UC, Kim SW, Koh JH (1994) Application of ceramic membrane as a pre-treatment in anaerobic digestion of alcohol-distillery wastes. J. Memb. Sci. 90 (1–2): 131–139.

Chatterjee S, Chetia M, Singh L, Chattopadhyay B, Datta S, Mukhopadhyay SK (2011) A study on the phytoaccumulation of waste elements in wetland plants of a Ramsar site in India. Environ. Moni. Assess. 178(1): 361-371.

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. Ecol. Eng. 27: 202–207.

Chaudhari PK, Mishra IM, Chand S (2005) Catalytic thermal treatment (catalytic thermolysis) of a biodigester effluent of an alcohol distillery plant. Ind. Eng. Chem. Res. 44(15): 5518–5525.

Chaudhari PK, Mishra IM, Chand S (2007) Decolourization and removal of chemical oxygen demand (COD) with energy recovery: treatment of biodigester effluent of a molasses-based alcohol distillery using inorganic coagulants. Colloids Surf. A Physicochem. Eng. Asp 296(1–3): 238–247.

Chaudhari PK, Mishra IM, Chand S (2008) Effluent treatment of alcohol distillery: catalytic thermal pretreatment (Catalytic thermolysis) with energy recovery. Chem. Eng. J. 136: 14–24.

Chen Y, Wen Y, ZhiruTang, et al. (2015) Effects of plant biomass on bacterial community structure in constructed wetlands used for tertiary wastewater treatment. *Ecol. Engin.* 84, 38–45.

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.

Cibis E, Kent CA, Krzywonos M, Garncarek Z, Garncarek B, Miskiewicz T (2002) Biodegradation of potato slops from a rural distillery by thermophilic aerobic bacteria. Biores. Technol. 85: 57– 61.

Coenye T, Vanlaere E, LiPuma JJ, Vandamme P (2004) Identification of genomic groups in the genus *Stenotrophomonas* using gyrB RFLP analysis. FEMS Immunol. Med. Microbiol. 40(3): 181-185.

Cole JR, Wang Q, Cardenas E, et al. (2009) The ribosomal database project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res. 37: D141–145.

Collins G, Foy C, McHugh S, Maho T, O'Flaherty V (2005) Anaerobic biological treatment of phenolic wastewater at 15-18ºC. Water Res. 39: 1614-1620.

Costa, O. Y., Souto, B. M., Tupinambá, D. D., Bergmann, J. C., Kyaw, C. M., Kruger, R. H., Barreto, C. C., &Quirino, B. F. (2015). Microbial diversity in sugarcane ethanol production in a Brazilian distillery using a culture-independent method. Journal of industrial microbiology & biotechnology, 42(1), 73–84. https://doi.org/10.1007/s10295-014-1533-1

D'Souza DT, Tiwari R, Sah AK, Raghukumar C (2006) Enhanced production of laccase by a marine fungus during treatment of colored effluents and synthetic dyes. Enzy. Microb. Technol. 38: 504–511.

Daga NS, Prasad CVS, Joshi JB (1986) Kinetics of hydrolysis and wet air oxidation of alcohol distillery waste. Indian Chem. Eng. 28:22–31.

Dahiya J, Singh D, Nigam P (2001a) Decolorization of synthetic and spentwash melanoidins using the white-rot fungus *Phanerochaete chrysosporium* JAG-40. Bioresour. Technol. 78 (1): 95–98.

Dahiya J, Singh D, Nigam P (2001b) Decolourisation of molasses wastewater by cells of *Pseudomonas fluorescens* immobilized on porous cellulose carrier. Biores. Technol. 78: 111–114.

Daud MK, Ali S, Variath MT, Zhu SJ (2013) Differential physiological, ultramorphological and metabolic responses of cotton cultivars under cadmium stress. Chemosphere 93(10): 2593-602.

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: 138.

Davídek T, Devaud S, Robert F, Blank I (2006) Sugar fragmentation in the maillard reaction cascade: isotope labeling studies on the formation of acetic acid by a hydrolytic β-dicarbonyl cleavage mechanism. J. Agri. Food Chem. 54(18): 6667-6676.

de Oliveira Silva J, Filho GR, da Silva Meireles et al (2012) Thermal analysis and FTIR studies of sewage sludge produced in treatment plant. The case of sludge in the city of Uberlandia-MG. Brazil. *Thermochimica Acta* 528: 72-75.

de Sousa Andrade LN, De Lima TM, Curi R, de Lauro Castrucci AM (2005) Toxicity of fatty acids on murine and human melanoma cell lines. Toxicol. In Vitro 19: 553–560.

de Wilde FGN (1987) Demineralization of a molasses distillery wastewater. Desalination 67: 481–493.

Defew, L.H., Mair, J.M., Guzman, H.M., 2005. An assessment of metal contamination in mangrove sediments and leaves from Punta Mala Bay, Pacific Panama. Marine Pollution Bulletin 50(5), 547–552

Dehorter B, Bloodeau R (1993) Isolation of an extracellular Mn-dependent enzyme mineralizing melanoidins from the white rot fungus *Trametes versicolor*. FEMS Microbial. Lett., 109: 117-122

Deng H, Ye ZH, Wong MH (2004) Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal contaminated sites in China. Environ. Poll. 132(1): 29–40.

Dhale AD, Mahajani VV (2000) Treatment of distillery waste water after bio-gas generation: Wet Oxidation, Indian J. Chem. Technol. 7(1): 11–18.

Doran PM (2009) Application of plant tissue cultures in phytoremediation research: incentives and limitations. Biotechnol. Bioeng. 103: 60–76.

Driessen W, Yspeert P (1999) Anaerobic treatment of low, medium and high strength effluent in agro-industry. Water Sci. Technol. 40: 221– 228.

Duncan, J., Bokhary, A., Fatehi, P., Kong, F., Lin, H., & Liao, B. (2017). Thermophilic membrane bioreactors: a review. Bioresource technology, 243, 1180-1193.

Dungan RS, Yates SR, Frankenberger WT (2003) Transformations of selenate and selenite by *Stenotrophomonas maltophilia* isolated from a seleniferous agricultural drainage pond sediment. Environ. Microbiol. 5: 287–295.

Dwyer J, Lant P (2008) Biodegradability of DOC and DON for UV/H_2O_2 pre-treated melanoidin based wastewater. Biochem. Eng. J. 42: 47–54.

Echavarría AP, Pagán J, Ibarz A (2013a) Antioxidant activity of the melanoidins fraction formed from D-glucose and D-fructose with L-asparagine in the Maillard reaction. Scient. Agrope. 4: 45-54.

Echavarría AP, Pagán J, Ibarz A (2013b) Optimization of Maillard reaction products isolated from sugar-amino acid model system and their antioxidant activity. Afinidad LXX 562: Abril-Junio

Echavarría AP, Pagán J, Ibarz A (2014) Kinetics of color development of melanoidins formed from fructose/amino acid model systems. Food Sci. Technol. Int. 20(2): 119-126.

Eggleston G, Vercellotti JR (2000) Degradation of sucrose, glucose and fructose in concentrated aqueous solution under constant pH conditions at elevated temperature. J. Carbohy. Chem. 19(9): 1305-1218.

Elsgaard L, Petersen SO, Debosz K (2001) Effects and risk assessment of linear alkylbenzene sulfonates in agricultural soil. 2. Short-term effects on soil microbiology. Environ. Toxicol. Chem. 20(8): 1656–1663.

España-Gamboa EI, Mijangos-Cortés JO, Hernández-Zárate G, Maldonado JAD, Alzate-Gaviria LM (2012) Methane production by treating vinasses from hydrous ethanol using a modified UASB reactor. Biotechnol. Biofue. 5: 82.

Eusébio A, Petruccioli M, Lageiro M, Federici F, Duarte JC (2004) Microbial characterization of activated sludge in jet-loop bioreactors treating winery wastewaters. J. Ind. Microbiol. Biotechnol. 31(1): 29-34.

Fernandes TCC, Mazzeo DEC, Marin-Morales MA (2007) Mechanism of micronuclei formation in polyploidizated cells of *Allium cepa* exposed to trifluralin. Pesti. Biochem. Physiolo. 88: 252-259.

Filip Z (2002) International approach to assessing soil quality by ecologically related biological parameters. Agriculture, Ecosyst Environ 88(2): 169–174.

Fiskesjo G (1985) The *Allium* as a standard en environmental monitoring. Hereditas 102: 99-102.

Fiskesjo G (1993) The *Allium* test-A potential standard protocol of assessment of environmental toxicity. In: Gorusch JW, Dwyner FJ, Ingorsoll CD et al. (Eds) Environmental Toxicology and risk assessment. American Society for Testing and Materials, Philadelphya, USA

Fiskesjo G (1997) *Allium* test for screening chemicals: evaluation of cytological parameters. In: Wang W, Gorsuch JW, Hughes JS (Eds), Plants for environmental studies. CRC Lewis Publishers, Boca Raton, New York

Fitz WJ, Wenzel WW (2002) Arsenic transformation in the soil–rhizosphere– plant system, fundamentals and potential application of phytoremediation. J. Biotechnol. 99: 259–78.

Fitzgibbon, F J, Nigam, P, Singh, D and Marchant, R (2007) Biological treatment of distillery waste for pollution-remediation. J. Basic Microbiol. 35(5): 293–301.

Fuhrman JA (2009) Microbial community structure and its functional implications. Nature 459: 193-199.

Gabbrielli R, Pandolfini T (1984) Effect of Mg and Ca on the response to nickel toxicity in a serpentine endemic and nickel-accumulating species. Physiol. Plant. 62: 540–544.

Galfati I, Bilal E, Sassi AB, Abdallah H, Zaïer A (2011) Accumulation of heavy metals in native plants growing near the phosphate treatment industry, Tunisia. Carpathian J. Earth Environ. Sci. 6(2): 85–100.

Ghosh M, Ganguli A, Tripathi AK (2002) Treatment of anaerobically digested distillery spent wash by a two stage bioreactor using *Pseudomonas putida* and *Aeromonas* sp. Proc. Biochem. 37(8): 857-862.

Ghosh M, Verma SC, Mengoni A, Tripathi AK (2004) Enrichment and identification of bacteria capable of reducing chemical oxygen demand of anaerobically treated molasses spent wash. J. Appl. Microbiol. 96: 1278-1286.

Glenn TC (2011) Field guide to next-generation DNA sequencers. Mol. Ecol. Resour. 11: 759–769.

González T, Terrón MC, Yagüe S, Zapico E, Galletti GC, González AE (2000) Pyrolysis/gas chromatography/mass spectrometry monitoring of fungal-biotreated distillery wastewater using *Trametes* sp. I-62 (CECT 20197). Rap. Commun. Mass Spect. 14: 1417-1424.

González T, Terrón MC, Yagüe S, Junca H et al. (2008) Melanoidin-containing wastewaters induce selective laccase gene expression in the white-rot fungus *Trametes* sp. I-62. Res. Microbiol. 159(2): 103-109.

Goodwin JAS, Finlayson JM, Low EW (2001) A further study of the anaerobic biotreatment of malt whisky distillery pot ale using an UASB system. Bioresour. Technol. 78: 155-160.

Goodwin JAS, Stuart JB (1994) Anaerobic digestion of malt whisky distillery pot ale using upflow anaerobic sludge blanket reactors. Bioresour. Technol. 49: 75-81.

Govarthanan, M., Mythili, R., Selvankumar, T., Kamala-Kannan, S., & Kim, H. (2018). Myco-phytoremediation of arsenic-and lead-contaminated soils by Helianthus annuus and wood rot fungi, Trichoderma sp. isolated from decayed wood. Ecotoxicology and environmental safety, 151, 279-284.

Govarthanan, M., Selvankumar, T., Manoharan, K., Rathika, R., Shanthi, K., Lee, K. J., ... & Oh, B. T. (2014). Biosynthesis and characterization of silver nanoparticles using panchakavya, an Indian traditional farming formulating agent. International journal of nanomedicine, 9, 1593.

Grisey E Laffray X, Contoz O Cavalli E, Mudry J, Aleya L (2012) The bioaccumulation performance of reeds and cattails in a constructed treatment wetland for removal of heavy metals in landfill leachate treatment (Etueffont, France). Water Air Soil Pollut. 223(4): 1723–1741.

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

Guo L, Cutright TJ (2014) Remediation of acid mine drainage (AMD)-contaminated soil by *Phragmites australis* and rhizosphere bacteria. Environ. Sci. Pollut. Res. 21(12): 7350–7360.

Gupta AK, Sinha S (2006) Chemical fractionation and heavy metals accumulation in the plants of *Sesamum indicum* (L.) var. T55 grown on soil amended with tannery sludge: selection of single extractants. Chemosphere 64: 161–173.

Gupta AK, Sinha S (2007) Phytoextraction capacity of the plants growing on tannery sludge dumping sites. Bioresour. Technol. 98: 1788–1794.

Gupta RS (2000) The phylogeny of proteobacteria: relationships to other eubacterial phyla and eukaryotes. FEMS Microbiol Revi. 24(4): 367–402.

Haarhoff J, Cleasby JL (1988) Comparing aluminum and iron coagulants for in-line filtration of cold waters. J. Am. Wks Ass. 80: 168-175.

Hallberg KB, Johnsonb D (2005) Microbiology of a wetland ecosystem constructed to remediate mine drainage from a heavy metal mine, Sci. Total. Environ. 338(1/2): 53.

Han, Y., Liu, J., Gao, X., Lin, L. 2012. Micro-environment characteristics and microbial communities in activated sludge flocs of different particle size, Bioresource Technology, Volume 124, 2012, Pages 252-258, ISSN 0960-8524, https://doi.org/10.1016/j.biortech.2012.08.008.

Harada H, Uemura S, Chen AC, Jayadevan J (1996) Anaerobic treatment of a recalcitrant distillery wastewater by a thermophilic UASB reactor. Bioresour. Technol. 55: 215-221.

Hatano K, Kanazawa K, Tomura H, Yamatsu T, Tsunoda K, Kubota K (2016) Molasses melanoidin promotes copper uptake for radish sprouts: the potential for an accelerator of phytoextraction. Environ. Sci. Pollut. Res. 23: 17656-17663.

Hatano K, Kikuchi S, Miyakawa T, Tanokura M, Kubota K (2008) Separation and characterization of the colored material from sugarcane molasses. Chemosphere 71: 1730-1737.

Hatano K, Yamatsu T (2018) Molasses melanoidin-like products enhance phytoextraction of lead through three Brassica species. Int. J. Phytoreme. DOI 10.1080/15226514.2017.1393397.

Hayase F (2000) Recent development of 3-deoxyosone related maillard reaction products. Food Sci. Technol. Res. 6(2): 79-86.

Hayase F, Kim SB, Kato H (1984) Decolorization and Degradation Products of the Melanoidins by Hydrogen Peroxide. Agric. Biol. Chem. 48 (11): 2711-2717.

Hemavanthi KL, Kumar NKH, Jagannath S (2015) Gentotoxic effect of distillery effluent on root tip cells of *Allium sativum* L. J. Appl. Biol. Biotechnol. 3(03): 038-041.

Hoagland DR, Arnon DI (1938) The water culture method for growing plants without soil. Circ-347. University of California, College of Agriculture, Agric. Exp. Stn. Berkley.

Hu, Y., Duan, C., D., Fu, D., Wu, X., W., Yan, K., Fernando, E., Karunarathna, S.C., Promputtha, I., Mortimer, P.E., Xu, J. 2020. Structure of Bacterial Communities in Phosphorus- Enriched Rhizosphere Soils.Appl. Sci. 10, no. 18: 6387. https://doi.org/10.3390/app10186387.

Hwang C, Jiang Y, Sheu S, Hsieh P, Guo J (2011) Purification and characterization of a novel glucose oxidase-like melanoidin decolorizing enzyme from *Geotrichum* sp. No. 56. Afr. J. Microbiol. Res. 5(20): 3256-3266.

Ibekwe AM, Lyon SR, Leddy M, Jacobson-Meyers M (2007) Impact of plant density and microbial composition on water quality from a free water surface constructed wetland. J. Appl. Microbiol. 102: 921–936.

Igiri, B.E., Okoduwa, S.I., Idoko, G.O., Akabuogu, E.P., Adeyi, A.O., Ejiogu, I.K., 2018. Toxicity and. bioremediation of heavy metals contaminated ecosystem from tannery wastewater: a review. J. Toxicol.2568038

Incedayi B, Tamer CE, Copur UC (2010) A research on the composition of Pomegranate molasses. J. Agri. Fac. 24: 37-47.

Jadhav HD, Savant NK (1975) Influence of added spent wash (distillery waste) on chemical and physical properties of soil. Indian J. Agric. Chem. 8: 73–84.

Jagdale HN, Sawant NK (1979) Influence of added spent wash (Distillery waste) on growth and chemical composition of immature sugarcane (*Saccharum officinarum* L.) cultivar CO-740. Indian Sugar 29(7): 433–440.

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

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

Jenkins R, Wilson EM, Angus RA, Howell WM, Kirk M (2003) Androstenedione and progesterone in the sediment of a river receiving paper mill effluent. Toxicol. Sci. 73: 53–59.

Jiang J, Zhang X, Zhu X, Li Y (2017) Removal of intermediate aromatic halogenated DBPs by activated carbon adsorption: a new approach to controlling halogenated DBPs in chlorinated drinking water. Bull. Environ. Sci. Technol. 81(5):449-454.

Jimnez AM, Borja R, Martin A (2003) Aerobic-anaerobic biodegradation o beet molasses alcoholic fermentation wastewater. Proc. Biochem. 38: 1275–1284.

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. J. Hazard. Mater. 156(1-3): 387-97.

Juhasz AL, Stanley GA, Britz ML (2000) Microbial degradation and detoxification of high molecular weight polycyclic aromatic hydrocarbons by *Stenotrophomonas maltophilia* strain VUN 10,003. Lett. Appl Microbiol. 30: 396–401.

Juwarkar A, Dutta SA (1989) Impact of distillery effluent application to land on soil microflora. Environ. Mon. Assess. 15: 201-210.

Kabata-Pendias A (2011) Trace elements in soils and plants, third Ed. CRC Press, Boca Raton, p. 365.

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: 1009–1020.

Kadlec R, Wallace S (2009) Treatment Wetlands, 2nd, CRC Press.

Kahraman S, Yesilada O (2003) Decolorization and bioremediation of molasses wastewater by white rot fungi in a semi solid state condition. Folia Microbiol. 48: 525–528.

Kalavathi DF, Uma L, Subramanian G (2001) Degradation and metabolization of the pigment—melanoidin in distillery effluent by the marine cyanobacterium *Oscillatoria boryana* BDU 92181. Enzy. Microb. Technol. 29: 246-251.

Kalra YP, Maynard DG (1991) Methods manual for forest soil and plant analysis, Information Report NOR-X-319 Forestry Canada, Northwest region, Northern Forest Centre, Edmonton, Alberta.

Kamaya Y, Kurogi Y, Suzuki K (2003) Acute toxicity of fatty acids to the freshwater green alga *Selenastrum capricornutum.* Environ. Toxicol. 18: 289–294.

Kambe TN, Shimomura M, Nomura N, Chanpornpong T, Nakahara T (1999) Decolourization of molasses wastewater by *Bacillus* sp. under thermophilic and anaerobic conditions. J. Biosci. Bioeng. 87: 119–121.

Kansal A, Rajeshwari KV, Balakrishnan M, Lata K, Kishore VVN (1998) Anaerobic digestion technology for energy recovery from industrial wastewater: a study in Indian context, TERI inform. Monitor. Environ. Sci. 3: 67–75.

Kapley A, Lampel K, Purohit HJ (2001) Rapid detection of *Salmonella* in water samples by multiplex PCR. Wat. Environ. Res. 73: 461–465.

Kaushik G, Gopal M, Thakur IS (2010) Evaluation of performance and community dynamics of microorganisms during treatment of distillery spent wash in a three stage bioreactor. Bioresour. Technol. 101: 4296-4305.

Kaushik G, Gopal M, Thakur IS (2010) Evaluation of performance and community dynamics of microorganisms during treatment of distillery spent wash in a three stage bioreactor. Bioresour. Technol. 101: 4296-4305.

Kaushik G, Thakur IS (2009) Isolation of fungi and optimization of process parameters for decolorization of distillery mill effluent. World J. Microbiol. Biotechnol. 25: 955.

Kaushik G, Thakur IS (2013) Adsorption of colored pollutants from distillery spent wash by native and treated fungus: *Neurospora intermedia*. Environ. Sci. Pollu. Res. 20(2): 1070–1078.

Kersters K, Devos P, Gillis M, Swings J, Vandamme P, Stackebrandt E (2006) Introduction to the Proteobacteria. In The Prokaryotes: A handbook on the biology of bacteria, pp. 3–37. Dworkin M, Falkow S, Rosenberg E, Schleifer KH & Stackebrandt E (Eds). New York: Springer.

Keyser M, Witthuhn RC, Ronquest LC, Britz TJ (2003) Treatment of winery effluent with upflow anaerobic sludge blanket (UASB)–granular sludges enriched with *Enterobacter sakazakii*. Biotechnol. Letters 25(22): 1893-1898.

Kim SB, Hayase F, Kato H (1985) Decolorization and degradation products of melanoidins on ozonolysis. Agric. Biol. Chem. 49(3): 785-792.

Kim SB, Hayase F, Kato H (1985) Decolourisation and degradation products of melanoidin on ozonolysis. Agric. Biol. Chem. 49: 785-792.

Kim SJ, Shoda M (1999) Batch decolorization of molasses by suspended and immobilized fungus of *Geotrichum candidum*. J. Biosci Bioeng 88(5): 586–589.

Kirk TK, Schultz E, Connors WJ, Lorenz LF, Zeikus JG (1978) Influence of culture parameters on lignin metabolism by *Phanerochaete chrysosporium*. J. Arch. Microbiol. 117: 177-185

Kobya M, Gengec E (2012) Decolourization of melanoidins by a electrocoagulation process using aluminium electrodes. Environ. Technol. 33(21): 2429-2438.

Koster IW, Cramert A (1987) Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids. Appl. Environ. Microbiol. 403-409.

Kramer U, Pickering IJ, Prince RC, Raskin I, Salt DE (2000) Subcellular localization and speculation of nickel in hyperaccumulator and non-accumulator *Thlaspi* species. Plant Physiol. 122: 1343–53

Krishna Prasad R (2009) Color removal from distillery spent wash through coagulation using *Moringa oleifera* seeds: Use of optimum response surface methodology. J. Hazard. Mater. 165(1–3): 804-811.

Krishnamoorthy S, Premalatha M, Vijayasekaran M (2017) Characterization of distillery wastewater – An approach to retrofit existing effluent treatment plant operation with phycoremediation. J. Clea. Produc. 148:735-750.

Krzywonos M, Seruga P (2012) Decolourisation of sugar beet molasses-vinasse,a high strength distillery wastewater, by Lactic acid bacteria. Pol. J. Environmental. Stud. 21(4): 943-948.

Kubik P, Leja K, Cleinska E, Spychala M, Szymanowska-Powalowska D, Czaczyk K, Grajek, W (2012) Physiological predisposition of various *Clostridium* species to synthesize 1,3-propanediol from glycerol. Process Biochem. 48: 1308-1319.

Kumar M, Infant F, Ponselvan A, Malviya JR, Srivastava VC, Mall ID (2009) Treatment of bio-digester effluent by electrocoagulation using iron electrodes, J. Hazard. Mater. 165: 345–352.

Kumar N, Bauddh K, Kumar S, Dwivedi N, Singh DP, Barman SC (2013) Accumulation of metals in weed species grown on the soil contaminated with industrial waste and their phytoremediation potential. Ecol. Engin. 61: 491-495.

Kumar P, Chandra R (2004) Detoxification of distillery effluent through *Bacillus thuringiensis* (MTCC 4714) enhanced phytoremediation potential of *Spirodela polyrrhiza* (L.) Schliden. Bull. Environ. Contam. Toxicol. 73: 903–910.

Kumar P, Chandra R (2006) Decolourisation and detoxification of synthetic molasses melanoidins by individual and mixed cultures of *Bacillus* spp.. Bioresour. Technol. 97: 2096-2102.

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

Kumar V, Wati L, Nigam P, Banat IM, Yadav BS, Singh D, Marchant R (1998) Decolourization and biodegradation of anaerobically digested sugarcane molasses spent wash effluent from biomethanation plants by white-rot fungi. Proc. Biochem. 33: 83–88.

Kumar V, Wati L, Fitz Gibbon F, Nigan P, Banat I, M, Singh D, Marchant R (1997) Bioremediation and decolorization of anaerobically digested distillery spent wash. Biotechnol. Lett. 19: 311–313.

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), 316. https://doi.org/10.1007/s13205-020-02310-5

Kumari V, Yadav A, Haq I, Kumar S, Bharagava RN, Singh SK, Raj A (2016) Genotoxicity evaluation of tannery effluent treated with newly isolated hexavalent chromium reducing *Bacillus cereus.* J. Environ. Manag. 183: 204-211.

Kupper H, Lombi E, Zhao FJ, McGrath SP (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. Planta 212: 75–84.

Laghlimi M, Baghdad B, El-Hadi H, Bouabdli A (2015) Phytoremediation mechanisms of heavy metal contaminated soils: A Rev. Open J. Ecol. 5: 375-388.

Lalov IG, Guerginov II, Krysteva A, Fartsov K (2000) Treatment of wastewater from distilleries with chitosan, Water Res. 34(5): 1503–1506.

Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt F, Good fellow M (Eds.), Nucleic acid techniques in bacterial systematics. John Wiley and Sons, New York, pp. 115–175.

Larkin MA, Blackshields G, Brown NP et al. (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23(21): 2947-2948.

Lau J, Tran C, licari P, Galazzo J (2004) Development of a high cell-density fed-bath bioprocess for the heterologous production of 6-deoxyerythronolide B in *Escherichia coli*. J. Biotechnol. 110: 95-103.

Lee, K. J., Park, S. H., Govarthanan, M., Hwang, P. H., Seo, Y. S., Cho, M., ... & Oh, B. T. (2013). Synthesis of silver nanoparticles using cow milk and their antifungal activity against phytopathogens. Materials Letters, 105, 128-131.

Lele SS, Rajadhyaksha PJ, Joshi JB (1989) Effluent treatment for alcohol distillery: thermal pretreatment with energy recovery. Environ. Prog. 8: 245–252.

Letterman RD, Driscoll CT (1988) Survey of residual aluminum in filtered water. J. Am. Wat. Wks Ass. 80: 154-158

Levy DB, Redente EF, Uphoff GD (1999) Evaluating the phytotoxicity of Pb-An tailing to big bluesteam (*Andropogon gerardii* vitamin) and switch grass (*Panicum virgatum* L.). Soil Sci. 164: 363-375.

Liakos TI, Lazaridis NK (2014) Melanoidins removal from simulated and real wastewaters by coagulation and electro-flotation. Chem. Eng. J. 242: 269-277.

Liang Y, Wong MH (2003) Spatial and temporal organic and heavy metal pollution at Mai Po Marshes Nature Reserve, Hong Kong. Chemosphere 52: 1647-1658.

Liang Z, Wang Y, Zhou Y, Liu H (2009b) Coagulation removal of melanoidins from biologically treated molasses wastewater using ferric chloride. Chem. Eng. J. 152(1): 88–94.

Liang Z, Wang Y, Zhou Y, Liu H, Wu Z (2009a) Variables affecting melanoidins removal from molasses wastewater by coagulation/flocculation. Sep. Puri. Technol. 68: 382–389.

Ligi T, Oopkaup K, Truu M et al. (2014) Characterization of bacterial communities in soil and sediment of a created riverine wetland complex using high throughput 16S rRNA amplicon sequencing. Ecol. Eng. 72: 56–66.

Liu J, Dong Y, Xu H, Wang D, Xu J (2007) Accumulation of Cd, Pb and Zn by 19 wetland plant species in constructed wetland. J. Hazard. Mater. 147(3): 947–953.

Liu J, Zhang X (2014) Comparative toxicity of new halophenolic DBPs in chlorinated saline wastewater effluents against a marine alga: halophenolic DBPs are generally more toxic than haloaliphatic ones. Water Res. 65: 64-72.

Liu L, Li Y, Li S, Hu N, He Y, Pong R, Lin D, Lu L, M Law (2012) Comparison of next-generation sequencing systems. J. Biomed. Biotechnol. 1–11.

Liu M, Zhu H, Dong B, Zheng Y, Yu S, Gao C (2013) Submerged nanofiltration of biologically treated molasses fermentation wastewater for the removal of melanoidins. Chem. Eng. J. 223: 388-394.

Loviso CL, Lozada M, Guibert LM, Musumeci MA, Sarango Cardenas S, Kuin RV, Marcos M. S, Dionisi HM (2015) Metagenomics reveals the high polycyclic aromatic hydrocarbon-degradation potential of abundant uncultured bacteria from chronically polluted subantarctic and temperate coastal marine environments. Appl. Microbiol. 119: 411-424.

M Krzywonos (2012) Decolorization of sugar beet distillery effluent using mixed cultures of bacteria of the genus *Bacillus*. Afr. J. Biotechnol. 11(14): 3464-3475.

Ma Y, Prasad MNV, Rajkumar M, Freitas H (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol. Advan. 29: 248–258.

MacFarlane GR, Koller CE, Blomberg SP (2007) Accumulation and partitioning of heavy metals in mangroves: a synthesis of fieldbased studies. Chemosphere 69(9): 1454–146.

Magoc T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*. 27: 2957–2963.

Mahgoub S, Tsioptsias C, Samaras P (2016) Biodegradation and decolorization of melanoidin solutions by manganese peroxidase yeasts. Water Sci. Technol. 73(10): 2436-45.

Mane JD, Modi S, Nagawade S, Phadnis SP, Bhandari VM (2009) Treatment of Spentwash using chemically modified bagasse and color removal studies. Bioresour. Technol. 97: 1752--1755.

Mane JD, Modi S, Nagawade S, Phadnis SP, Bhandari VM (2009). Treatment of Spentwash using chemically modified bagasse and color removal studies. Bioresour. Technol. 97: 1752–1755.

Mansur M, Suarez T, Fernandez-Larrea J, Brizuela MA, Gonzalez AE (1997) Identification of a Laccase gene family in the new lignin degrading basidiomycete CECT20197. Appl. Environ. Microbiol. 63: 2637–2646.

Martins S, Van Boekel M (2003) Melanoidins extinction coefficient in the glucose/glycine Maillard reaction. Food Chem. 83: 135-142.

Maruthi Sridhar BB, Diehl, SV, Han FX, Monts, DL, Yi, S (2005) Anatomical changes due to uptake and accumulation of Zn and Cd in Indian mustard (*Brassica juncea*). Environ. Exp. Bot. 54(2): 131–141.

Matkar LS, Gangotri MS (2003) Acute toxicity tests of sugar industrial effluents on the freshwater crab. *Barytelphusa guerini* (H. Milne Edwards) (Decapoda, Potamidea), Poll. Res. 22: 269–276.

Mazumdar K, Das S (2015) Phytoremediation of Pb, Zn, Fe, andMg with 25 wetland plant species froma papermill contaminated site in north east India. Environ. Sci. Poll. Res. Int. 22: 701–710.

McCartney DM, Oleszkiewicz JA (1991) Competition between methanogens and Sulfate reducers: effect of COD: sulfate ratio and acclimation. Water Environ. Res. 65(5): 655–664.

McGrath SP, Lombi E, Gray CW, Caille N, Dunham SJ, Zhao FJ (2006) Field evaluation of Cd and Zn phytoextraction potential by the hyperaccumulators

Thlaspi caerulescens and *Arabidopsis halleri.* Environ Poll. 141(1): 115–125.

McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from contaminated soils. Curr. Opin. Biotechnol. 14:1–6.

McGrath SP, Zhao FJ, Lombi E (2001) Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. Plant Soil 232: 207–214.

Memon AR, Aktoprakligul D, Zdemur A, Verti A (2001) Heavy metal accumulation and detoxification mechanisms in plants. Turkish J. Bot. 25: 111–121.

Meng Y, Wang Y, Han Q, Xue N, Sun Y, Gao B, Li Q (2016) Trihalomethane (THM) formation from synergic disinfection of biologically treated municipal wastewater: effect of ultraviolet (UV) irradiation and titanium dioxide photocatalysis on dissolve organic matter fractions. Chem. Eng. J. 303: 252-260.

Metcalf and Eddy (2003) Wastewater engineering: treatment and reuse, 4th edn. McGraw Hill Higher Education, New York

Migo VP, Del Rosario EJ, Matsumura M (1997) Flocculation of melanoidins induced by inorganic ions. J. Ferment. Bioeng. 83: 287–291.

Migo VP, Matsumura M, Delrosario, EJ, Kataoka H (1993) Decolorization of Molasses Wastewater Using an Inorganic Flocculent. J. Ferment. Bioeng. 75-438.

Miller RG, Kopfer FC, Kelty KC, Stober JA, Ulmer NS (1984) The occurrence of aluminum in drinking water. J. Wat. Wks Ass. 76: 84-91

Mineo H, Ohdate T, Fukumura K, Katayama T, Onaga T, Kato S, Yanaihara N (1995) Effects of benzoic acid and its analogues on insulin and glucagon secretion in sheep. European J. Pharmacol 280(2): 149-154.

Minuti L, Pellegrino RM, Tesei I (2006) Simple extraction method and gas chromatography-mass spectrometry in the selective ion monitoring mode for the determination of phenols in wine. J. Chromatogr. 1114: 263–268.

Miranda PM, Benito GG, Cristobal NS, Nieto CH (1996) Colour elimination from molasses wastewater by Aspergillus niger. Biores. Technol. 57: 229–235.

Miyata N, Iwahori K, Fujita M (1998) Manganese-independent and-dependent decolorization of melanoidin by extracellular hydrogen peroxide and peroxidases from *Coriolus hirsutus* pellets. J. Fermen. Bioenginee. 85(5): 550-553.

Miyata N, Mori T., Iwahori K, Fujita M (2000) Microbial decolourization of melanoidin-containing wastewaters: Combined use of activated sludge and the fungus *Coriolus hirsutus*. J. Biosci. Bioeng. 89: 145-150.

Mohammad P, Azarmidokht H, Fatollah M, Mahboubeh B (2006) Application of response surface methodology for optimization of important parameters in decolorizing treated distillery wastewater using Aspergillus fumigatus UB2 60. Int. Biodeteri. Biodeg. 57(4): 195–199.

Mohana S, Acharya BK, Madamwar D (2009) Distillery spent wash: treatment technologies and potential applications. J. Hazard. Mater. 163: 12-25.

Mohana S, Desai C, Madamwar D (2007) Biodegradation and decolorization of anae robically treated distillery spent wash by a novel bacterial consortium. Bioresour. Technol. 98(2): 333-339.

Moreau MM, Eades SC, Reinemeyer CR, Fugaro MN, Onishi JC (2014) Illumina sequencing of the V4 hypervariable region 16S rRNA gene reveals extensive changes in bacterial communities in the cecum following carbohydrate oral infusion and development of early-stage acute laminitis in the horse. Vet. Microbiol. 168: 436–441.

Morris JK, Knocke WR (1984) Temperature effects on the use of metal - ion coagulants for water treatment. J. Am. Wat. Wks Ass. 66: 74-79.

Morrison DA (1977) Transformation in *Escherichia coli*. J. Bacteriol. 132: 349-351.

Moyer CL, Dobbs FC, Karl, DM (1994) Estimation of diversity and community structure through restriction fragment length polymorphism distribution analysis of bacterial 16s rRNA genes from a microbial mat at an active, Hydrothermal Vent System, Loihi Seamount, Hawaii. Appl. Environ. Microbiol. 60(3): 871-879.

Muhammad F, Monteiro-Riviere NA, Riviere JE (2005) Comparative in vivo toxicity of topical JP-8 jet fuel and its individual hydrocarbon components: identification of tridecane and tetradecane as key constituents responsible for dermal irritation. Toxicol. Pathol. 33: 258-266.

Mukherjee, A., Chettri, B., Langpoklakpam, J.S., Basak, P., Prasad, A., Mukherjee, A.K., Bhattacharyya, M., Singh, A.K., Chattopadhyay, D., 2017. Bioinformatic approaches including predictive metagenomic profiling reveal characteristics of bacterial response to petroleum hydrocarbon contamination in diverse environments. Sci. Rep. 7 (1), 1–22.

Muller AK, Westergaard K, Christensen S, Sorensen SJ (2001) The effect of long-term mercury pollution on the soil microbial community. FEMS Microbiol Ecol. 36: 11–19.

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. Hazard. Mater. 186(1): 565-574. 18.

Nakhla G, Lugowski A, Patel J, Rivest V (2006) Combined biological and membrane treatment method processing wastewater to achieve dry-ditch criteria:pilot and full scale performance. Bioresour. Technol. 99: 1-14.

Narain K, Yazdani T, Bhat MM, Yunus M (2012) Effect on physico-chemical and structural properties of soil amended with distillery effluent and ameliorated by cropping two cereal plant spp. Environ. Earth Sci. 66: 977-987.

Natarajan SK, Hosamani KM, Aminabhavi TM (2006) Distillery wastewater treatment by the membrane based nanofiltration and reverse osmosis. Water Res. 40: 2349-2356.

Nazir R, Khan M, Masab M et al (2015) Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physico-

chemical parameters of soil and water collected from Tanda Dam Kohat. Pharma. Sci. Res. 7(3): 89–97

Ngo, H.H., Bui, X.T., Nghiem, L.D., Guo, W., 2020.Green technologies for sustainable water. Bioresour. Technol. 317, 123978.

Nieminen MT, Hernandez M, Novak-Frazer L et al. (2014) DL-2-hydroxyisocaproic acid attenuates inflammatory responses in a murine *Candida albicans* biofilm model. Clinical Vacc. Immunol. 21(9): 1240-5.

Nouri J, Khorasani N, Lorestani B, Karami M, Hassani AH, Yousefi N (2009) Accumulation of heavy metals in soil and uptake by plant species with phytoremediation potential. Environ. Earth Sci. 59: 315–323.

Odeigah PGC, Nurudeen O, Amund OO (1997) Genotoxicity of oil field wastewater in Nigeria. Hereditas 126: 161-167

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

Ohmomo S, Aoshima I, Tozawa Y, Sakurada N, Ueda K (1985) Purification and some properties of melanoidin decolourizing enzymes, P-3 and P-4, from mycelia of *Coriolus vericolour* Ps4a. Agric. Biol. Chem. 49: 2047–2053.

Ojijo VO, Onyango MS, Ochieng A, Otieno FAO (2010) Decolourization of melanoidin containing wastewater using south african coal fly ash. Int. J. Civil Eng. 2(1): 17-23

Ojuederie, O.B., Babalola, O.O., 2017. Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. Int. J. Environ. Res. Public Health 14 (12), 1504.

Okino LK, Machado KMG, Fabris C, Bononi VLR (2000) Ligninolytic activity of tropical rainforest basidiomycetes. World J. Microbiol. Biotechnol. 16(8-9): 889-893.

Oller I, Malato S, Sánchez-Pérez JA (2011) Combination of advanced oxidation processes and biological treatments for wastewater decontamination– a review. Sci. Total. Environ. 409(20): 4141–4166

Ondov BD, Bergman NH, Phillippy AM (2011) Interactive metagenomic visualization in a Web browser.

Onyango M, Kittinya J, Hadebe N, Ojijo V, Ochieng A (2011) Sorption of melanoidin onto surfactant modified zeolite. Chem. Ind. Chem. Eng. Q. 17(4): 385–395.

Outridge PM, Noller BN (1991) Accumulation of toxic trace elements by freshwater vascular plants. Rev. Environ. Contam. Toxicol. 121: 1e63.

Pandey SK, Tyagi P, Gupta AK (2007) Physico-chemical analysis and effect of distillery effluent on seed germination of wheat (*Triticum aestivum*), pea (*Pisum sativum*) and lady's finger (*Abelmoschus esculentus*). J. Agricul. Biol. Sci. 2(6): 35-40.

Pandey SK, Tyagi P, Gupta AK (2008) Physico-chemical analysis of treated distillery effluent irrigation response on crop plant pea (*Pisum sativum*) and wheat (*Triticum aestivum*). Life Sci. J. 84-89.

Pang, S., Zhang, S., Lv, X.Y., Han, B., Liu, K., Qiu, C., Wang, C., Wang, P., Toland, H., He, Z., 2016. Characterization of bacterial community in biofilm and sediments of wetlands dominated by aquatic macrophytes. Ecol. Eng. 97: 242–250.

Pangallo D, Simonovicova A, Chovanova K, Ferianc P (2007) Wooden art objects and the museum environment: identification and biodegradative characteristics of isolated microflora, Lett. Appl. Microbiol. 45: 87–94.

Pant D, Adholeya A (2007a) Identification, ligninolytic enzyme activity and decolorization potential of two fungi isolated from a distillery effluent contaminated site. Water Air Soil Pollu. 183: 165–176

Pant D, Adholeya A (2007b) Biological approaches for treatment of distillery wastewater: A review. Bioresour. Technol. 98(12): 2321-34

Pant D, Adholeya A (2009a) Nitrogen removal from biomethanated spent wash using hydroponic treatment followed by fungal decolorization. Environ. Eng. Sci. 26: 559–565.

Pant D, Adholeya A (2009b) Concentration of fungal ligninolytic enzymes by ultrafiltration and their use in distillery effluent decolorization. World J. Microbiol. Biotechnol. 25: 1793–800.

Park SJ, Yoon JC, Shin K et al. (2007) Dominance of endospore-forming bacteria on a Rotating Activated Bacillus Contactor biofilm for advanced wastewater treatment. J. Microbiol. 45: 113-121

Patel A, Pawar P, Mishra S, Tewari A (2001) Exploitation of marine cyanobacteria for removal of colour from distillery effluent. Ind. J. Environ. Prot. 21: 1118–1121.

Payet B, Shum Cheong Sing A, Smadja J (2006) Comparison of the concentrations of phenolic constituents in cane sugar manufacturing products with their antioxidant activities. J. Agric. Food Chem. 54: 7270-7276.

Payet B, Shum Cheong Sing A, Smadja, J (2005) Assessment of antioxidant activity of cane brown sugars by ABTS and DPPH radical scavenging assays: determination of their polyphenolic and volatile constituents. J. Agric. Food Chem. 53: 10074-10079.

Pena M, Coca M, Gonzalez G, Rioja R, Garcia MT (2003) Chemical oxidation of wastewater from molasses fermentation with ozone. Chemosphere 51: 893-900

Pennanen T, Frostegard A, Fritze H, Baath E (1996) Phospholipid fatty acid composition and heavy metal tolerance of soil microbial communities along two heavy metal-polluted gradients in coniferous forests. Appl. Environ. Microbiol. 62: 420–428.

Perkiomaki J, Tom-Petersen A, Nybroe O, Fritze H (2003) Boreal forest microbial community after long-term field exposure to acid and metal pollution and its potential remediation by using wood ash. Soil Biol. Biochem. 35: 1517–1526.

Petruccioli M, Duarte JC, Fedrerici F (2000) High rate aerobic treatment of winery wastewater using bioreactors with free and immobilized activated sludge. J. Biosci. Bioeng. 90: 381–386

Pikaev AK, Ponomarev AV, Bludenko AV, Minin VN, Elizar'eva LM (2001) Combined electronic-beam and coagulation purification of molasses distillery slops. Features of the method, technical and economic evaluation of large scale facility. Rad. Phy. Chem.61 (1): 81–87.

Płaza GA, Zjawiony I, Banat IM (2006) Use of different methods for detection of thermophilic biosurfactant-producing bacteria from hydrocarbon-contaminated and bioremediated soils. J. Petroleum Sci. Enginee. 50(1): 71-77.

Portillo MC, Villahermosa D Corzo A, Gonzalez JM (2011) Microbial community fingerprinting by differential display-denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. *77*(1): 351-354.

Prajapati A, Chaudhari P (2014) Electrochemical treatment of rice grain based distillery effluent: chemical oxygen demand and color removal, Environ. Technol. 35: 242–248.

Prajapati AK, Chaudhari PK (2015) Physicochemical treatment of distillery wastewater-a review. Chem. Eng. Commun. 202: 1098e1117.

Prasad RK, Srivastava SN (2009) Electrochemical degradation of distillery spent wash using catalytic anode: Factorial design of cexperiments. Chem. Eng. J. 146: 22–29.

Priest FG (1993) Systematics and ecology of Bacillus, In Sonenshein AL, Hoch JA, Losick R (eds.), *Bacillus subtilis* and other gram positive bacteria. American Society for Microbiology, Washinton D.C., USA p. 3-16.

Purohit HJ, Raje DV, Kapley A, Padmanabhan P, Singh RN (2003) genomics tools in environmental impact assessment. Environ. Sci. Tech. 37: 356A-363A

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Gloeckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41(D1):D590–596.

Quinn BP, Bernier UR, Geden CJ, Hogsette JA, Carlson DA (2007) Analysis of extracted and volatile components in blackstrap molasses feed as candidate house fly attractants. J. Chromatogr. 1139: 279–284

Qureshi MI, D'Amici GM, Fagioni M, Rinalducci S, Zolla L (2010) Iron stabilizes thylakoid protein–pigment complexes in Indian mustard during Cd-phytoremediation as revealed by BN-SDSPAGE and ESI-MS/MS. J. Plant Physiol. 167: 761–770.

Qureshi N, Malmberg RG (1985) Reducing aluminum residuals in finished water. J. Am. Wat. Wks Ass. 77:101-108.

R Rosselli, O Romoli, N Vitulo (2016) Direct 16S rRNA-seq from bacterial communities: a PCR-independent approach to simultaneously assess microbial diversity and functional activity potential of each taxon. Scientific Rep. 6: 32165.

Raghukumar C Mohandas C, Shailaja MS, Kamat S (2004) Simultaneous detoxification and decolorization of molasses spent wash by the immobilized

white-rot fungus *Flavodon flavus* isolated from a marine habitat. Enzyme Microb. Technol. 35: 197-202.

Raghukumar C, Rivonkar G (2001) Decolourization of molasses spent wash by the white-rot fungus *Flavodon flavus*, isolated from a marine habitat. Appl. Microbiol. Biotechnol. 55: 510–514.

Rai UK, Muthukrishnan M, Guha BK (2008) Tertiary treatment of distillery wastewater by nanofiltration, Desalination 230: 70–78.

Rajkumar M, Sandhya S, Prasad MNV, Freitas H (2012) Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol. Adva. 30(6): 1562–1574.

Ramakritinan CM, Kumaraguru AK, Balasubramanian MP (2005) Impact of distillery effluent on carbohydrate metabolism of freshwater fish, *Cyprinus carpio*. Ecotoxicol 14(7): 693-707.

Ramana S, Biswas AK, Kundu S, Saha JK, Yadava RBR (2002) Relative efficacy of different distillery effluents on growth, nitrogen fixation and yield of groundnut. Bioresour. Technol. 81: 117-121.

Ramendra Awasthi M (1992) Anaerobic and aerobic fermentation - a proven biotechnology for distillery effluent treatment. Ind. J. Environ. Prot. 12: 835–838.

Ramezani GN Darzi, Mohammadi M (2011) Removal of melanoidin from molasses spent wash using fly ash–clay adsorbents. Kor. J. Chem. Eng. 28: 1035–1041.

Ramteke DS, Wate SR, Moghe CA (1989) Comparative adsorption studies of distillery waste on activated carbon. Ind. J. Environ. Heal. 31(1): 17–24.

Rao KVM, Sresty TVS (2000) Anti oxidative parameters in the seedlings of pigeon pea (*Cajanus cajan* (L.) Mulluspaugh) in response to Zn and Ni stresses. Plant Sci. 157: 113–128.

Rashmi, I., Roy, T., Kartika, K. S., Pal, R., Coumar, V., Kala, S., &Shinoji, K. C. (2020). Organic and inorganic fertilizer contaminants in agriculture: Impact on soil and water resources. In Contaminants in Agriculture (pp. 3-41). Springer, Cham.

Ravikumar R, Vasanthi NS, Saravanan K (2011) Single factorial experimental design for decolorizing anaerobically treated distillery spent wash using *Cladosporium cladosporioides*. J Environ. Sci. Technol. 8: 97-106

Ravikumar R (2015) Effect of transport phenomena of *Cladosporium cladosporioides* on decolorization and chemical oxygen demand of distillery spent wash. Int. J. Environ. Sci. Technol. 12: 1581–1590.

Ravikumar R, Saravanan R, Vasanthi N et al. (2007) Biodegradation and decolorization of biomethanated distillery spent wash. Ind. J. Sci. Technol. 1(2): 1–6

Renella G, Mench M, Landi L, Nannipieri P (2005) Microbial activity and hydrolase synthesis in long-term Cd-contaminated soils. Soil Biol. Biochm. 37: 133–139.

Robertson AI (1985) The poisoning of roots of Zea mays by nickel ions and the protection afforded by magnesium and calcium. New Phytol. 100: 173–189.

Rodriguez E, Pickard M, Vazquez-Duhalt R (1999) Industrial dye decolourisation by laccase from ligninolytic fungi. Curr. Microbiol. 38: 27-31.

Rosselli W, Keller C, Boschi K (2003) Phytoextraction capacity of trees growing on metal contaminated soil. Plant Soil 256: 265–272.

Russell JB, Forsberg N (1986) Production of tricarballylic acid by rumen microorganisms and its potential toxicity in ruminant tissue metabolism. British J. Nutr. 56: 153-162.

Ryan RP, Monchy S, Cardinale M, Taghavi S, Crossman L, Avison MB, Berg B, van der Lelie D, Dow JM (2009) The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. Nat. Rev. Microbiol. 7: 514-525.

Saliha B. (2005) Distillery spent wash for sustainable soil helath. Kisan World *32*(18): 50-51.

Sambrook J, Russell DW (2001) Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sangave PC, Gogate PR, Pandit AB (2007) Ultrasound and ozone assisted biological degradation of thermally pretreated and anaerobically pretreated distillery wastewater. Chemosphere 68: 42–50.

Sangave PC, Pandit AB (2006a) Enhancement in biodegradability of distillery wastewater using enzymatic pre-treatment. J. Environ. Manag. 77(1): 77–85.

Sangave PC,Pandit AB (2006b) Ultrasound and enzyme assisted biodegradation of distillery wastewater. J. Environ. Manag. 80 (1):36–46.

Sanita di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41: 105–130.

Sankaran K, Premalatha M, Vijayasekaranb M, Somasundaram VT (2014) DEPHY project: Distillery wastewater treatment through anaerobic digestion and phycoremediation—A green industrial approach. Renew. Sust. Ene. Rev. 37: 634-643

Santal AR, Sing NP, Saharan BS (2016) A novel application of *Paracoccus pantotrophus* for the decolorization of melanoidins from distillery effluent under static conditions. J. Environ. Manag. 169: 78-93.

Santal AR, Singhb NP, Saharan BS (2011) Biodegradation and detoxification of melanoidin from distillery effluent using an aerobic bacterial strain SAG_5 of *Alcaligenes faecalis*. J. Hazard. Mater. 193: 319– 324.

Satyawali Y, Balakrishnan M (2007) Removal of color from biomethanated distillery spentwash by treatment with activated carbons. Bioresour. Technol. 98(14): 2629-2653.

Satyawali Y, Balakrishnan M (2008a) Wastewater treatment in molasses-based alcohol distilleries for COD and color removal: A review. J. Environ. Manage 86(3): 481−497

Satyawali Y, Balakrishnan M (2009) Performance enhancement with powdered activated carbon (PAC) addition in a membrane bioreactor (MBR) treating distillery effluent, J. Hazard. Mater. 170: 457–465.

Satyawalia Y, Balakrishnana M (2008b) Treatment of distillery effluent in a membrane bioreactor (MBR) equipped with mesh filter. Sep. Pur. Technol. 63(2): 278–286.

Saxena KK, Chauhan RRS (2003) Oxygen consumption in fish, *Labeo rohita* (Ham.) caused by distillery effluent, Ecol. Environ. Conserv. 9: 357–360.

Seghezzo L, Zeeman G, Van Lier JB, Hamelers HVM, Lettinga G (1998) A review: The anaerobic treatment of sewage in UASB and SGSB reactors. Bioresour. Technol. 65:175-190

Selvam, K., Sudhakar, C., Govarthanan, M., Thiyagarajan, P., Sengottaiyan, A., Senthilkumar, B., & Selvankumar, T. (2017). Eco-friendly biosynthesis and characterization of silver nanoparticles using Tinospora cordifolia (Thunb.) Miers and evaluate its antibacterial, antioxidant potential. Journal of Radiation Research and Applied Sciences, 10(1), 6-12.

Selvi, A., Rajasekar, A., Theerthagiri, J., Ananthaselvam, A., Sathishkumar, K., Madhavan, J., & Rahman, P. K. (2019). Integrated remediation processes toward heavy metal removal/recovery from various environments-a review. Frontiers in Environmental Science, 7, 66.

Sengar RS, Gupta S, Gautam M, Sharma A, Sengar K (2008) Occurrence, uptake, accumulation and physiological response of nickel in plants and its effect on environment. Res. J. Phtytochem. 2(2): 44–60.

Sessitsch A, Kuffner M, Kidd P, Jaco V, Wenzel WW, Fallmann K, Puschenreiterd 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.

Sharma J, Singh R (2001) Effect of nutrient supplementation on anaerobic sludge development and activity for treating distillery effluent. Biores. Technol. 79: 203–206.

Sharma V, Sharma R, Sharma KD (2002) Distillery effluent on seed germination, early seedling and pigment content of sugarbeet (*Beta vulgaris* Linn. Var. Mezzanau-Poly). J. Environ. Biol. 23: 77–80.

Sharmin F, Wakelin S, Huygens F, Hargreaves M (2013) *Firmicutes* dominate the bacterial taxa within sugar-cane processing plants. Scientific Rep 3:3107.

Sharmin F, Wakelin S, Huygens F, Hargreaves M. Firmicutes dominate the bacterial taxa within sugar-cane processing plants. Sci Rep. 2013 doi: 10.1038/srep03107

Shayegan J, Pazouki M, Afshari A (2005) Continuous decolourization of anaerobically digested distillery wastewater. Proc. Biochem. 40: 1323–1329.

Shi-Yan Y, Zhou XY, Du X et al. (2007) Three novel missense germline mutations in different exons of MSH6 gene in Chinese hereditary non-polyposis colorectal cancer families. World J. Gastroenterol. 13(37): 5021-5024.

Shu WS, Ye ZH, Lan CY, Zhang ZQ, Wong MH (2002) Lead, zinc and copper accumulation and tolerance in populations of *Paspalum distichum* and *Cynodon dactylon*. Environ Poll. 120: 445–453.

Singh NK, Pandey GC, Rai UN, Tripathi RD, Singh HB, Gupta DK (2005) Metal accumulation and ecophysiological effects of distillery effluent on *Potamogeton pectinatus* L. Bull. Environ. Contam. Toxicol. 74: 857–863.

Singh VK, Kavita K, Prabhakaran R, Jha B (2013) Cis-9-octadecenoic acid from the rhizospheric bacterium *Stenotrophomonas maltophilia* BJ01 shows quorum quenching and anti-biofilm activities. Biofouling 29: 855–867

Sirianuntapiboon S, Phothilangka PO (2004) Decolourization of molasses wastewater by a strain no. BP103 of acetogenic bacteria. J. Biores. Technol. 92: 31-39

Sirianuntapiboon S, Zohsalam P, Ohmomo S (2004) Decolorization of molasses wastewater by Citeromyces sp. WR-43-6. Process Biochem. 39: 917-924.

Sirivedhim T, Gray KA (2006) Factors affecting denitrification rates in experimental wetlands: field and laboratory studies. Ecol. Eng. 26(2): 167-181.

Smith KE, Schwab AP, Banks MK (2007) Phytoremediation of polychlorinatedbiphenyls (PCB) contaminated sediment: a greenhouse feasibility study. J. Environ. Qual. 36: 239–244

Sochacki A, Guy B, Faure O, Surmacz-Gorska J (2015) Accumulation of Metals and Boron in *Phragmites australis* planted in constructed wetlands polishing real electroplating wastewater. Int. J. Phytoreme. 17(11). doi.org/10.1080/15226514.2015.1021956

Solhi M, Shareatmadari H, Hajabbasi MA (2005) Lead and zinc extraction potential of two common crop plants, *Helianthus annuus* and *Brassica napus*. Water Air Soil Poll. 167: 59–71.

Solovchenko A, Pogosyan S, Chivkunova O et al. (2014) Phycoremediation of alcohol distillery wastewater with a novel *Chlorella sorokiniana* strain cultivated in a photobioreactor monitored on-line via chlorophyll fluorescence. Algal Res. Part B: 234-241.

Sowmeyan R, Swaminathan G (2008) Effluent treatment process in molasses-based distillery industries: A review, J. Hazard. Mat. 152(2): 453-462.

Spain, A.M., Krumholz, L.R., Elshahed, M.S., 2009. Abundance, composition, diversity and novelty of soil Proteobacteria. ISME J. Aug; 3(8):992-1000.

Sreethawong T, and Chavadej S (2008) Color removal of distillery wastewater by ozonation in the absence and presence of immobilized iron oxide catalyst, J. Hazard. Mater. 155: 486–493.

Srivastava, R. K., Shetti, N. P., Reddy, K. R., &Aminabhavi, T. M. (2020). Sustainable energy from waste organic matters via efficient microbial processes. Science of the Total Environment, 722, 137927.

Steyn PL, Segers P, Vancanneyt M, Sandra P, Kersters K, Joubert JJ (1998) Classification of heparinolytic bacteria into a new genus, *Pedobacter*, comprising four species: *Pedobacter heparinus* comb. nov., *Pedobacter piscium* comb. nov., *Pedobacter africanus* sp. nov. and *Pedobacter saltans* sp. nov. Proposal of the family *Sphingobacteriaceae* fam. nov. Int. J. Syst. Bacteriol.. 48: 165–177.

Stottmeister U, Wiessner A, Kuschk P, Kappelmeyer MK, Bederski RA, Müller H, Moormann H (2003) Effects of plants and microorganisms in constructed wetlands for 460 wastewater treatment. Biotechnol. Adva. 22: 93-117.

Subramani A, Sundaramoorthy P, Lakshmanchari AS (1995) Effect of distillery effluent on growth, yield and productivity of Vigna radiata, Poll. Res. 14: 477–482.

Swamy MM, Mall ID, Prasad B, Mishra IM (1997) Removal of phenol by adsorption on coal fly ash and activated carbon. Poll. Res. 16: 170–175.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30(12): 2725-2729.

Taylor CD, Smith OS, Gagosian R (1981) Use of microbial enrichments for the study of anaerobic degradation of cholesterol. Geochim Coscochim Acta 45:2161–2168.

Tewari PK, Batra VS, Balakrishnan M (2007) Water management initiatives in sugarcane molasses based distilleries in India, Resour. Conserv. Recycl. 52, 351–367.

Thakkar AP, Dhamankar VS, Kapdnis BP (2006) Biocatalytic decolorisation of molasses by *Phanerochaete chrysosporium*. Bioresour. Technol. 97(12): 1377–1381.

Thakur C, Srivastava VC, Mall ID (2009) Electrochemical treatment of a distillery wastewater: Parametric and residue disposal study. Chem. Eng. J. 148: 496–505.

Thomas F, Hehemann J, Rebuffet E, Czjzek M, Michel G (2011) Environmental and Gut Bacteroidetes: The Food Connection. Front Microbiol. 2: 93.

Thompi J (2000) Studies in effluent treatment. PhD (Tech.). Thesis submitted to Mumbai University.

Tiwari S, Gaur R, Singh R (2012) Decolorization of a recalcitrant organic compound (Melanoidin) by anovel thermotolerant yeast, *Candida tropicalis* RG-9. BMC Biotechnol. 12: 30.

Tiwari S, Gaur R, Singh A (2014) Distillery spentwash decolourisation by a noval consortium of *Pedicoccus acidilactici* and *Candida tropica* under static condition. Paki. J. Biolo. Sci. 17(6): 780-791.

Tiwari S, Rai P, Yadav SK, Gaur R (2013) A novel thermotolerant *Pediococcus acidilactici* B-25 strain for color, COD, and BOD reduction of distillery effluent for end use applications. Environ. Sci. Pollut. Res. Int. 20(6): 4046-4058.

Tondee T, Sirianutapiboon S (2006) Screening of melanoidin decolorization activity in yeast strain. KLIN KMUTT Library Network.

Tondee T, Sirianutapiboon S (2008) Decolorization of molasses wastewater by *Lactobacillus plantarum* No. PV71-1861. Bioresour. Technol. 99(14): 6258-6265.

Tong YP, Kneer R, Zhu YG (2004) Vacuolar compartmentalization: a second-generation approach to engineering plants for phytoremediation. Trends Plant Sci. 9: 7–9.

Tripathi, S., Sharma, P., & Chandra, R. (2021c). Distillery wastewater detoxification and management through phytoremediation employing Ricinus communis L. Bioresource Technology, 125192.

Tripathi, S., Sharma, P., & Chandra, R. (2021d). Degradation of organometallic pollutants of distillery wastewater by bacterial community in biostimulation and bioaugmentation process. Bioresource Technology, 125518.

Tripathi, S., Sharma, P., Purchase, D., Tiwari, M., Chakrabarty, D., & Chandra, R. (2021e). Biodegradation of organo-metallic pollutants in distillery wastewater employing bioaugmentation process. Environmental Technology & Innovation, 101774.

Tripathi, S., Sharma, P., Singh, K., Purchase, D., & Chandra, R. (2021b). 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., Singh, A., Mishra, A., & Chandra, R. (2021a). Organo-metallic pollutants of distillery effluent and their toxicity on freshwater fish and germinating Zea mays seeds. International Journal of Environmental Science and Technology, 1-14.

Trivedy RK, Nakate SS (2000) Treatment of diluted distillery waste by constructed wetlands. Indian J. Environ. Prot. 20: 749–753.

Tsioptsias C, Petridis D, Athanasakis N, Lemonidis I, Deligiannis A, Samaras P (2015) Post-treatment of molasses wastewater by electrocoagulation and process optimization through response surface analysis. J. Environ. Manage. 164: 104e113.

US EPA (1996) Test methods for evaluating solid waste. SW-846 Method 3050-B. pp. 1–12.

US EPA (2000) Phytoremediation of contaminated soil and ground water at hazardous waste sites. Ground water forum issue paper EPA 540/S-01/500. U.S. EPA Office of Research and Development, Cincinnati, OH.

US EPA (2002) The environment protection rules, 3A, Schedule- II, III. U.S. Environmental Protection Agency, Office of research and development, Cincinnati.

USEPA (2012) U.S. Environmental protection agency endocrine disruptor screening program universe of chemicals. Washington, DC: United States Environmental Protection Agency.

Uzal N, Gokacay CF, Demirer GN (2003) Sequential anaerobic/ aerobic) biological treatment of malt whisky wastewater. Process Biochem. 39: 279–286

Valderrama LT, Del Campo CM, Rodriguez CM, Bashan LE, Bashan Y (2002) Treatment of recalcitrant wastewater from ethanol and citric acid using the microalga Chlorella vulgaris and the macrophyte Lemna minuscula. Water Res. 36: 185–4192

Valentino, F., Moretto, G., Lorini, L., Bolzonella, D., Pavan, P., &Majone, M. (2019). Pilot-scale polyhydroxyalkanoate production from combined treatment of organic fraction of municipal solid waste and sewage sludge. Industrial & Engineering Chemistry Research, 58(27), 12149-12158.

Varjani, S., &Sudha, M. C. (2020). Occurrence and human health risk of micro-pollutants—A special focus on endocrine disruptor chemicals. In Current Developments in Biotechnology and Bioengineering (pp. 23-39). Elsevier.

Verma SR, Delea RC, Tyagi MP, Gupta SP (1974) Studies of characteristics and disposal problem of industrial effluent with reference to ISI standard; part I. Indian J. Environ. Health 16: 289-299.

Vlissidis A, Zouboulis AI (1993) Thermophilic anaerobic digestion of alcohol distillery wastewaters. Biores. Technol 43 (2): 131–140.

Vymazal J (2011) Constructed Wetlands for Wastewater Treatment: Five Decades of Experience. *Environ. Sci. Technol.* 45: 61–69

Vymazal J (2014) Constructed wetlands for treatment of industrial wastewaters: A review. Ecol. Eng. 73: 724–751.

Vymazal J, Kröpfelová L, Švehla J, Chrastný V, Štíchová J. 2009. Trace elements in *Phragmites australis* growing in constructed wetlands for treatment of municipal wastewater. Ecol. Eng. 35: 303–309

Walford SN (1996) Composition of cane juice. Proc. S. Afr Sug. Technol. Ass. 70: 265-266.

Wang, Q, Xie H, Hao Ngo H (2016) Microbial abundance and community in subsurface flow constructed wetland microcosms: role of plant presence. Environ Sci Pollut Res 1–10.

Wenzel WW, Unterbrunner R, Sommer P, Sacco P (2003) Chelate assisted phytoextraction using canola (Brassica napus L.) in outdoors pot and lysimeter experiments. Plant Soil 249: 83–96.

Wheeler DM, Power IL, Edmeades DC (1993) Effect of various metal ions on growth of two wheat lines known to differ in aluminium tolerance. Plant Soil 156: 489–492

Whitman WBEd (2009) Bergey's manual of Systematic Bacteriology Vol 3, 2nd Edition. Springer, New York

Woese CR, Weisburg WG, Hahn CM et al. (1985) The phylogeny of purple bacteria: the gamma subdivision. Syst. Appl. Microbiol. 6: 25–33.

Wolmarans B, De Villiers G (2002) Start up of a UASB effluent treatment plant on distillery wastewater. Water SA 28(1): 63-68.

Wong DW (2009) Structure and action mechanism of ligninolytic enzymes. Appl. Biochem. Biotechnol. 157(2): 174-209

Wu L, Wang J, Huang W, Wu H, Chen J, Yang Y, Zhang Z, Lin W (2015) Plant-microbe rhizosphere interactions mediated by *Rehmannia glutinosa* root exudates under consecutive monoculture. Sci. Rep. 5: 15871

Wu X, Monchy S, Taghavi S, Zhu W, Ramos J, van der Lelie D (2011) Comparative genomics and functional analysis of niche-specific adaptation in *Pseudomonas putida*. FEMS Microbial. Rev. 35: 299–323.

Yadav S, Chandra R (2012) Biodegrdation of organic compounds of molasses melanoidins (MM) from biomethanated distillery distillery spent wash (BMDS) during the decolourisation by potential bacterial consortium. Biodegradtion 23: 609–620.

Yadav S, Chandra R (2013) Detection of persistent organic compounds from biomethanated distillery spent wash (BMDS) and their degradation by manganese peroxidase and laccase producing bacterial strains. J. Environ. Biol. 34(4): 755-764.

Yadav S, Chandra R, Rai V (2011) Characterization of potential MnP producing bacteria and its metabolic products during decolourisation of synthetic melanoidins due to biostimulatory effect of d-xylose at stationary phase. Proc. Biochem. 46: 1774–1784.

Yang BY, Montgomery R (1996) The alkaline degradation of glucose: eVect of initial concentration of reactants. Carbohyd. Res. 280: 27–45.

Yang X, Feng Y, He Z, Stoffell PJ (2005) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J. Trace Elem. Med. Biol. 18: 339–353.

Yeoh BG (1997) Two-phase anaerobic treatment of cane-molasses alcohol stillage. Water Sci. Tech. 36: 441–448.

Yoon J, Cao X, Zhou Q, Ma LQ (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci. Total Environ. 368(2): 456-464.

Yu Z, Mohn WW (2001) Bacterial diversity and community structure in an aerated lagoon revealed by ribosomal intergenic spacer analyses and 16S ribosomal DNA sequencing. Appl. Environ. Microbiol. 67(4): 1565-1574.

Zhang D, Jinadasa KBSN, Gersberg RM, Liu Y, Tan SK, Ng WJ (2015a) Application of constructed wetlands for wastewater treatment in tropical and subtropical regions (2000–2013). J. Environ. Sci. (China) 30: 30–46

Zhang J, Sun QL, Zeng ZG, Chen S, Sun L (2015b) Microbial diversity in the deep-sea sediments of Iheya North and Iheya Ridge, Okinawa Trough. Microbiol. Res. 177: 43–52.

Zhang JF, Zheng YG, Liu ZQ, Shen YC (2007) Preparation of 3-ketovalidoxylamine A C-N lyase substrate: N-p-nitrophenyl-3-ketovalidamine by *Stenotrophomonas maltrophilia* CCTCC M 204024. Appl. Microbiol. Biotechnol. 73: 1275–1281.

Zhou J, Bruns MA, Tiedje JM (1996) DNA recovery from soils of diverse composition. Appl. Environ. Microbiol. 62(2): 316-322.

Chapter Twelve

Reprint

Chapter 12

Scientific Publications and Achievements:

(A) Research Papers published high impact Journals of International Repute: (20)

1. Tripathi, S., Sharma, P., & Chandra, R. (2021). Degradation of organo-mettalic pollutants of distillery wastewater by bacterial community in biostimulation and bioaugmentation process. ***Bioresource Technology*, 125518**. ,**.(IF-9.64)**

2. Sonam Tripathi, Sharma, P., & Chandra, R. (2021). Microbial community dynamics and their correlation with organo-mettalic pollutants of distillery wastewater sludge. (**Accepted in Environmental Pollution IF- 8.01)**

3. Sonam Tripathi., Sharma, P., & Chandra, R. (2021). Distillery wastewater detoxification and management through phytoremediation employing Ricinus communis L. ***Bioresource Technology*,** 125192,.**(IF-9.64)**

4. Tripathi, S., Sharma, P., Purchase, D., Tiwari, M., Chakrabarty, D., & Chandra, R. (2021). Biodegradation of organo-metallic pollutants in distillery wastewater employing bioaugmentation process. ***Environmental Technology & Innovation*, 101774.** ,**.(IF-5.21)**

5. Sonam Tripathi, Singh, K., Singh, A., Mishra, A., & 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*,** 1-14.(**IF- 3.1**)

6. Sonam Tripathi, Sharma, P., Singh, K., Purchase, D., & Chandra, R. (2021). Translocation of heavy metals in medicinally important herbal plants growing on complex organo-mettalic sludge of sugarcane molasses-based distillery waste. ***Environmental Technology & Innovation***, *22*, 101434.**IF – 5.21)**

7. Sonam Tripathi., Pandey, M. K., Malik, Y. S., Bilal, M., Dhama, K., Chaicumpa, W., & Chandra, R. (2020). Novel Coronavirus (Sars-cov-2): Molecular biology, pathogenesis, pathobiology and advances in treatment of covid-19 patients–an update. ***Journal of Experimental Biology and Agricultural Sciences***, *8*(6).

8. Sharma, P., **Tripathi, S.,** & Chandra, R. (2021). Metagenomic analysis for profiling of microbial communities and tolerance in metal-polluted pulp and paper industry wastewater. ***Bioresource Technology***, *324*, 124681. .**(IF-9.64)**

9. Pooja Sharma, **Sonam Tripathi**, Ram Chandra (2020), Highly efficient phytoremediation of metal and metalloids from the pulp paper industry waste employing *Eclipta alba* and Alternanthera philoxeroide, 2021, ***Bioresource Technology,*** 319,1-8 **(IF-9.64)**

10. Chandra, Ram, Vineet Kumar, and **Sonam Tripathi** (2018). "Evaluation of molasses-melanoidin decolourisation by potential bacterial consortium discharged in distillery effluent." ***3 Biotech*** 8.4: 187.**(IF-2.45)**

11. Sharma, P., **Tripathi, S.,** Chaturvedi, P., Chaurasia, D., & Chandra, R. (2021). Newly isolated Bacillus sp. PS-6 assisted phytoremediation of heavy metals using Phragmites communis: Potential application in wastewater treatment. ***Bioresource Technology,*** *320,* 124353. **(IF-9.64)**

12. Pooja Sharma, **Sonam Tripathi**, Ram Chandra. (2020). Phytoremediation Potential of Heavy Metal Accumulator Plants for Waste Management from Pulp Paper Industry. ***Heliyon,*** 7, 1- 10. (**IF-1.6**).

13. Pooja Sharma, **Sonam Tripathi**, Ram Chandra (2020). Characterization of Autochthonous Bacteria Capable for Degradation of Residual Organic Pollutants of Pulp Paper Mill Effluent by Biostimulation Process. ***Journal of Pure and Applied Microbiology,*** 14, 1181-1194 **(IF-0.9)**

14. Ram Chandra, Vineet Kumar, **Sonam Tripathi**, Pooja Sharma (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 phytoextraction, ***Ecological Engineering***, 111,143-156 **(IF-4.03)**

15. Pooja Sharma, **Sonam Tripathi**, Ram Chandra (2020). SARS-CoV-2 / COVID-19 and its Transmission, Prevention, Treatment and Control – An Update. ***Journal of Pure and Applied Microbiology,*** 14, 1-12, **(IF-0.1)**

16. Pooja Sharma**, Sonam Tripathi**, Ram Chandra (2020). Assessment of pollutants in pulp paper industry effluent and their toxic effect on Fenugreek (Trigonella foenum-graecum L). ***Annals of Plant and Soil Research*** 22, 3 **(UGC care Listed)**

17. Pooja Sharma, **Sonam Tripathi**, Ram Chandra (2020). Environmental Impacts of Pulp Paper Mill Effluent: Potential Source of Chromosomal Aberration and Phytotoxicity. ***International Journal of Applied Environmental Sciences***. 15, 77-92.

18. Sharma, P., **Tripathi, S.,** Vadakedath, N. and Chandra, R., 2020. In-situ toxicity assessment of pulp and paper industry wastewater on Trigonella foenum-graecum L: Potential source of cytotoxicity and chromosomal damage. ***Environmental Technology & Innovation***, p.101251.**IF – 5.26)**

19. Sharma, P., **Tripathi, S.,** Purchase, D., & Chandra, R. (2021). Integrating phytoremediation into treatment of pulp and paper industry wastewater: field observations of native plants for the detoxification of metals and their potential as part of a multidisciplinary strategy. ***Journal of Environmental Chemical Engineering,*** 105547. **IF – 5.90)**

20. Sharma, P., **Tripathi, S.,** Sirohi, R., Kim, S. H., Ngo, H. H., & Pandey, A. (2021). Uptake and mobilization of heavy metals through phytoremediation process from native plants species growing on complex pollutants: Antioxidant enzymes and photosynthetic pigments response. ***Environmental Technology & Innovation***, 101629. **IF – 5.21)**

(B) **Book Chapters:**

(1) Ram Chandra, Vineet Kumar**, Sonam Tripathi,** Pooja Sharma **(2018).** Phytoremediation of industrial pollutants and life cycle assessment. In: Phytoremediation of Environmental Pollutants, Ram Chandra, N.K. Dubey, Vineet Kumar (Eds), CRC Press, USA, pp-441-469.

(2) Sonam Tripathi, Kshitij Singh, Ram Chandra (2021) Adaptation of bacterial communities and plant strategies in amelioration and eco-restoration of organo-metallic industrial waste polluted site. Pp. 598., **ISBN:** 9780128244487.

(C) Popular Scientific Magazine Article in Hindi: Two

(1) **सोनम त्रिपाठी** एवं राम चन्द्रा (2020), कोरोना वायरस महामारी (कोविद-१९) का संक्रमण, उपचार एवं रोकथाम के उपाय, कहार- जन विज्ञानं की बहुभाषी पत्रिका त्रैमासिक, संयुक्तांक 7 (3-4) (जुलाई- दिसंबर).

(2) **राम** चन्द्रा, विनीत कुमार, **सोनम त्रिपाठी**, पूजा शर्मा (2016) कागज कारखानों से उत्सर्जित विषैले अपशिष्टों का पर्यावरण पर दुष्प्रभाव तथा सुरक्षात्मक निस्तारण की चुनौतियाँ, विषविज्ञान संदेश,आई0एस0एस0न0 0972-1746, 19-33.

(D) Paper in Conference Proceedings (one)

(1) Ram Chandra, Vineet Kumar**, Sonam Tripathi (2016)** Environmental Health Hazards of Post Methanated Distillery Waste and its Detoxification. In: Proceeding of 74th Annual Convention of STAI, ISBN-81-85871-83-3, New Delhi, pp-684-699.

(E) Awards

- **Best Poster Award,** Bioremediation of Endocrine Disrupting Chemicals (EDC) and refractory organic pollutants of pulp paper mill effluent after secondary treatment in Biostimulation process for environmental safety", Sonam Tripathi & R Chandra, 58thAnnual Conference of Association of Microbiologists of India (AMI) & International Symposium on "Microbes for Sustainable Development: Scope and Application", Babasaheb Bhimrao Ambedkar University, Lucknow, India; 16-19 Nov. 2017.

- **Best Oral Presentation Award**,"Biostimulation and bioaugumentation approach for degradation of organ metallic pollutants: Role enzymes and bacterial community" Sonam Tripathi, Pooja Sharma & Ram Chandra, INTERNATIONAL CONFERENCE ON BIOENGINEERING SOLUTIONS FOR HEALTHCARE, FOOD, ENERGY, AND ENVIRONMENT (BSHFE organized by INDIAN INSTITUTE OF TECHNOLOGY, JODHPUR and THE BIOTECH RESEARCH SOCIETY, INDIA at Jodhpur, India during April 09-10, 2021. E-2021).

(F) Memberships of Scientific Societies

(1) Life Member of **Association of Microbiologists of India (AMI)**

(2) Life Member of **The Biotech Research Society, India (BRSI)**

(3) Life Member of **Indian Science Congress Association (ISCA)**

(G) Research Paper Presented in National /International symposium and conferences

1. Sonam Tripathi, and, Ram Chandra (2016) "Bioremediation and Detoxification of Endocrine Disrupting Chemicals (EDC) and Residual Organic Pollutants of pulp paper mill effluent after secondary treatment in Biostimulation process for environmental safety". 57thAnnual Conference of Association of Microbiologists of India; Guhawati-Assam (23-26 November) (poster presentation).

2. Sonam Tripathi, Vineet Kumar, and Ram Chandra (2016) "Heavy metal phytoextraction potential of common Indian aquatic weeds and grasses from pulp & paper mill effluent after secondary treatment and their accumulation pattern in different parts" International Conference on Current Trends in Biotechnology ICCB-2016, organized by the School of Bio Sciences and technology [SBST], VIT University, Vallore, in association with The Biotech Research Society, India [BRSI] (8-10 Dec 2016). (Poster Presentation)

3. Sonam Tripathi and Ram Chandra (2017) "Bioremediation of Endocrine Disrupting Chemicals (EDC) and refractory organic pollutants of pulp paper mill effluent after secondary treatment in Biostimulation process for environmental safety". 58th Annual Conference of Association of Microbiologists of India (AMI) & International Symposium on "Microbes for Sustainable Development: Scope and Application", Babasaheb Bhimrao Ambedkar University, Lucknow, UP, INDIA (16-19 Nov. 2017). (Poster Presentation-**BEST Poster Award).**

4. Sonam Tripathi and Ram Chandra (2017) "Detection of Residual Organic Pollutants of Pulp and Paper Mill Waste and Its Detoxification by Potential Bacterium Consortium". 4th Lucknow Science Congress [LUSCON-2017] on Science Technology and Innovation for Sustainable Development, Organized by Babasaheb Bhimrao Ambedkar University, Lucknow, UP, INDIA 3-4 March, 2017. (Poster Presentation).

(H) Workshops & Training

1. Summer Training:-

Institute: CSIR-Indian Institute of Toxicology Research (CSIR-IITR), Lucknow, INDIA.
Topic: Isolation, Purification and Screening of Potential Lignolytic Enzyme Producing Bacteria from Distillery Waste
Duration: Six months (May-Oct 2015)
Supervisor: Dr. Ram Chandra (Senior Principal Scientist).

2. Certificate Course on computer concepts (CCC) conducted by National Institute of Electronics and Information Technology [NIELIT], 2018

(I) Patent Granted related to PHD Topic

1. **Australian Patent number: <u>2021101010</u> - : Decolorization and Detoxification of Distillery Wastewater with Combined Use of Bacteria and Constructed Wetland Treatment. (2021).**

2. **Australian Patent number: - <u>2021103846</u>:- Solar Photo decolourisation of Bacterial Degraded Distillery Wastewater for its Reuses in Agriculture as green technology. (2021)**

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

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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.

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Degradation of organometallic pollutants of distillery wastewater by autochthonous bacterial community in biostimulation and bioaugmentation process

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HIGHLIGHTS

- Distillery wastewater are heavily polluted with organometallic pollutants.
- Bacterial consortium and bioaugumentation process was highly effective.
- This techniques can be used at industrial scale for detoxification of environmental pollution.

ARTICLE INFO

Keywords:
Bioremediation strategy
Organic pollutants
Ligninolytic enzymes
Industrial pollution
Environmental safety

ABSTRACT

This study aimed to detoxify and degrade the organometallic pollutants from distillery wastewater by using an autochthonous microbial community *via* biostimulation and bioaugmentation process. Results revealed that the wastewater contained high concentrations of the metals i.e. Fe-2403; Zn-210.15; Cr- 22.825; Cu-73.62; Mg-27.30; Ni-14.425; and Pb-17.33 (mg L^{-1}). The biostimulation and bioaugmentation process resulted from a substantial reduction (50–70%) in the pollution load. Scanning electron microscopy analysis showed bacterial community and their relationship with complex organometallic pollutants during the chemical reactions. The major identified organic pollutants in the control (untreated) samples were acetic acid, Oxo-,trimethylsilyl ester [CAS], Hydrocinnamic acid, p-[Trimethylsiloxy]-trimethylsilyl ester and tetradecanoic acid, trimethylsilyl ester [CAS] while some new metabolic products were generated as a by-product in bioaugmentation process. Therefore, the study showed that biostimulation and bioaugmentation were successful bioremediation strategies for the detoxification of distillery wastewater and restoration of organometallic polluted sites.

1. Introduction

Industries have become important players in many developed countries' national economies; but unfortunately, they are still the main sources of environmental pollution. Industrial wastewater discharged from different industries is referred to as the primary source of environmental pollution with various threats to the researchers (Goutam et al., 2018; Jiang et al., 2019; Sharma et al., 2021a). There are several organic and inorganic pollutants in treated waste which can cause severe groundwater pollution and health risks (Tripathi et al., 2021a; Arora et al., 2018). Recently, the toxicity of organic and inorganic pollutants has been reported on human and plant life by various researchers (Sharma et al., 2021b, 2021c). As a result of their high toxicity, many of them have been classified as priority pollutants by the US Environmental Protection Agency (USEPA), World Health Organization (WHO), and Agency for Toxic Substances and Disease Registry (ATSDR). Distillery wastewater is a significant source of economic activity, but it is also one of the most significant sources of pollution due to the discharge of large volumes of black wastewater. Such dark-colored wastewater contains very high total dissolved solids (TDS), phosphate, sulfate, phenolics, biological oxygen demand (BOD), chemical oxygen demand (COD), and heavy metals (Fe, Ni, Cu, Cr, Pb, Cd, Zn), respectively (Sharma et al., 2020d). Melanoidins has been reported to consist of color contributing compounds which is known as Maillard product of amino-carbonyl complex compounds at elevated temperature and it remains abundantly present along with other organic compounds such as di-n-octyl

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Distillery wastewater detoxification and management through phytoremediation employing *Ricinus communis* L.

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HIGHLIGHTS

- Organometallic compounds cause immense ecological effects.
- *Ricinus communis* L. shows phytoremediation potential of metals.
- Reduction of BOD, COD, and metals is>70% using *Ricinus communis* L.

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Keywords:
Distillery wastewater
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ABSTRACT

This study aimed to assess the phytoremediation potential of *Ricinus communis* L. for heavy metals remediation via rhizospheric bacterial activities for distillery wastewater detoxification and management. Results revealed that distillery wastewater contained high levels of metals and other physico-chemical pollution parameters that could cause environmental pollution and aquatic toxicity. The identified bacterium produced several plant growth-promoting compounds including siderophores, ligninolytic enzymes, and indole acetic acid that resulted in nutrient enhancement and improved mineralization of metals in the plants during stress conditions. The bioconcentration factor (BCF) of all the metals examined were > 1, which showed that these metals are accumulating in the root, shoot, and leaves of *Ricinus communis* L. Most of the metals are stablised in the roots but Pb, Cd and Zn were translocated more to the shoorts (TC>1). The ability of *Ricinus communis* L. to grow in metals-containing distillery wastewater and reduce heavy metals and organic contaminants suggests that it can be used to provide an effective treatment of distillery wastewater. The use of *Ricinus communis* L. is an eco-friendly tool for the reduction of organometallic contamination and protecting agricultural land.

1. Introduction

The development of the industry is a major reason for high levels of environmental pollution (Esmaeili and Beni, 2015; Sharma and Rath, 2020). Substantial amount of metals are released and discharged directly into receving water and soil as a result of anthropogenic activities, causing problems in human health and the environment (Jiang et al., 2019; Sharma et al., 2021a). There are nearly 320 distilleries currently in operation in India, discharging approximately 1500 tons of wastewater and sludge into the aquatic environment daily. Distilleries wastewater (DWw) contains very high organic and suspended solids levels, and due to its dark color, reduces the photosynthetic activity and dissolved oxygen content in water. Melanoidins are the most recalcitrant color pollutants in DWw and known to cause environmental and health concerns (Arimi et al., 2015; Chowdhary et al., 2017). The presence of harmful carcinogenic and androgenic pollutants such as endocrine-disrupting chemicals, recalcitrant toxic compounds, phenolics, melanoidins, organic acids, heavy metals and other persistant toxic compounds made sugarcane molasses-based distilleries a major source of environmental pollution (Tripathi et al., 2021a). Reuse of distillery wastewater for crop irrigation could result in some of these metals being taken up via contaminated food and stored in the lungs and other organs (Benson et al., 2018; Huang et al., 2018; Ngo et al., 2020).

Many bacterial species, such as *Micrococcus* sp. *Flavobacterium, Pseudomonas, Bacillus* and *Enterobacter have been investigated for heavy metals bioremediation.* Bacteria have excellent biosorbent ability due to

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Biodegradation of organo-metallic pollutants in distillery wastewater employing a bioaugmentation process

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Distillery wastewater
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ABSTRACT

The objective of this work was to study the potential of a constructed bacterial consortium (comprised three strains: *Stenotrophomonas maltophilia*, *Bacillus cereus*, and *Bacillus anthracis*) to treat distillery wastewater via the bioaugmentation process. The discharged wastewater showed elevated total ammonium nitrogen (195.0 ± 1.24 mg L^{-1}), total dissolved solids (25980.6 ± 8.09 mg L^{-1}), chemical oxygen demand (20534.5 ± 3.12 mg L^{-1}), and biological oxygen demand (20534.5 ± 3.12 mg L^{-1}). High concentration of heavy metals, phenolic and organo-metallic compounds were also detected. Results showed that growing the bacterial consortium in the distillery wastewater at 37 °C supplemented with 1% glucose achieved the best color reduction (up to 90%) in 144 h. The physico-chemical quality of the treated wastewater also improved by 50%–70%. Furthermore, many of the major organic pollutants present in the distillery wastewater were degraded by the constructed consortium to below detection limit via active biotransformation and biodegradation. Heavy metals were biosorbed by the bacterial consortium, and the ligninolytic enzymes such as Lip and MnP played an important role in the degradation of the organo-metallic pollutants. The constructed bacterial consortium therefore offered a sustainable and effective solution to treat distillery wastewater.

1. Introduction

Industrialization has been the major driver in the economic development of the world. Industrial processes improved productivity and allowed for mass production, which has increased standards of living. However, many industries are also the main sources of environmental pollution. In particular, industrial wastewaters have been identified as the primary sources of environmental pollution that threaten the environment and human health (Goutam et al., 2018; Jiang et al., 2019; Sharma et al., 2021a). For example, distillery wastewater is a significant source of economic activity, but it is also a significant source of pollution due to the discharge of large volumes of black wastewater.

There are 319 distilleries in India, producing approximately 3.25 × 10^9 liters of alcohol and 40.4 × 10^{10} liters of wastewater annually (Tripathi et al., 2021b; Chandra et al., 2018a). The dark-colored wastewater contains high levels

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Translocation of heavy metals in medicinally important herbal plants growing on complex organometallic sludge of sugarcane molasses-based distillery waste

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Medicinal plants
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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

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ORIGINAL PAPER

Organo-metallic pollutants of distillery effluent and their toxicity on freshwater fish and germinating *Zea mays* seeds

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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

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NOVEL CORONAVIRUS (SARS-COV-2): MOLECULAR BIOLOGY, PATHOGENESIS, PATHOBIOLOGY AND ADVANCES IN TREATMENT OF COVID-19 PATIENTS– AN UPDATE

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ABSTRACT

The novel coronavirus (CoV), earlier named 2019-nCoV, and later as severe acute respiratory syndrome coronavirus - 2 (SARS-CoV-2) has now created havoc and panic across the globe by its severe ongoing pandemic. This virus has to date as of 23rd November 2020, killed nearly 1.4 million persons out of more than 59 million confirmed positive cases, while spreading rapidly in more than 215 countries and territories. Taxonomically, SARS-CoV-2 has been characterized in genus *Betacoronavirus,* which contains non-segmented positive-sense, single-stranded (ss) RNA genome of ~30 kb. The first two open reading frames (ORFs), ORF1a and ORF1b, of SARS-CoV-2, encode 16 non-structural proteins (nsp1-nsp16), whereas other ORFs encodes four main structural proteins (sp) [spike (s) by ORF2, envelope (E) by ORF4, membrane (M) by ORF5, nucleoprotein (N) by ORF9], and accessory proteins essential for the virus fitness, pathogenesis and host immunity evasion. Sequence alignments of SARS-CoV-2 with genomes of various coronaviruses showed 58% identity in the non-structural protein (nsp)-coding region, 43% with the structural protein (sp)-coding region and 54% with the whole genome. The full-length genome sequence of the 2019-nCoV sample showed only up to 79.60% similarity with SARS

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Metagenomic analysis for profiling of microbial communities and tolerance in metal-polluted pulp and paper industry wastewater

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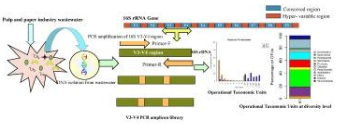
HIGHLIGHTS

- Environmental pollutants from industries cause enormous ecological impacts.
- Bacterial community has strong prospects for removing metal pollutants.
- The best approach to microbes profiling is use of metagenomic strategies.

GRAPHICAL ABSTRACT



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Keywords:
Microbial community
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ABSTRACT

This work aimed to study the profiling and efficiency of microbial communities and their abundance in the pulp and paper industry wastewater, which contained toxic metals, high biological oxygen demands, chemical oxygen demand, and ions contents. Sequence alignment of the 16S rRNA V3-V4 variable region zone with the Illumina MiSeq framework revealed 25356 operating taxonomical units (OTUs) derived from the wastewater sample. The major phyla identified in wastewater were *Proteobacteria, Bacteroidetes, Firmicutes, Chloroflexi, Actinobacteria, Spirochetes, Patesibacteria, Acidobacteria,* and others including unknown microbes. The study showed the function of microbial communities essential for the oxidation and detoxifying of complex contaminants and design of effective remediation techniques for the re-use of polluted wastewater. Findings demonstrated that the ability of different classes of microbes to adapt and survive in metal-polluted wastewater irrespective of their relative distribution, as well as further attention can be provided to its use in the bioremediation process.

1. Introduction

The metagenomic analysis is an excellent technique for quantitative characteristics of microbial communities such as existing inside the industrial wastewater treatment. Pulp and paper industry wastewater facilitates the complex microbial communities to break down organic material and facilitate the removal of emerging pollutants. The pulp and paper industry have been known one of the world's largest energy and water using industry. In India, approximately 100 million kg of hazardous pollutants are produced annually from pulp paper industries (Dey et al., 2013). The 5th largest energy consumption procedures are used by this industry; about 4% of overall energy has been used globally. In India, there are estimates of more than 850 pulp paper units, including small paper industries (Annual report of CPPRI, 2016-17). The pulp and paper manufacturing process generates a high quantity of waste. This has been reported that in 2020, 500 million tonnes of paper will be produced annually (Boguniewicz-Zabłocka and Kłosok-Bazan, 2020). The objective of this research is profiling the total microbial community growing in this contaminated wastewater and their efficiency for ex-situ bioremediation of pollutants.

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ORIGINAL ARTICLE

Evaluation of molasses-melanoidin decolourisation by potential bacterial consortium discharged in distillery effluent

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Abstract
The extracted sugarcane molasses-melanoidins showed the presence of Mn (8.20), Cr (2.97), Zn (16.61), Cu (2.55), Fe (373.95), Pb (2.59), and Ni (4.18 mg L^{-1}) along with mixture of other organic compounds which have endocrine-disrupting chemicals (EDCs) properties. A consortium of aerobic bacteria comprising *Klebsiella pneumoniae* (KU321273), *Salmonella enteric* (KU726954), *Enterobacter aerogenes* (KU726955), and *Enterobacter cloacae* (KU726957) showed the optimum decolourisation of molasses-melanoidins up to 81% through co-metabolism in the presence of glucose (1.0%) and peptone (0.2%) as a carbon and nitrogen source, respectively. The absorption spectrum scanning by UV–visible spectrophotometer between 200 and 700 nm revealed reductions of absorption spectrum of organic compounds present in bacterial degraded sample of melanoidins in range of 200–450 nm compared to control. The degradation and decolourisation of melanoidins by bacterial consortium was noted by induction of manganese peroxidase and laccase activities in sample supernatant. Furthermore, the TLC and HPLC analysis of bacterial decolourised melanoidins also showed degradation and reduction of absorption peak at (295 nm), respectively. Furthermore, FT-IR and GC–MS analysis also showed the change of functional group and disappearance of ion peaks. This indicated the degradation and depolymerisation of melanoidins and cleavage of C=C, C=O and C≡N conjugated bonds which resulted in reduction of colour. The metabolic analysis also showed the disappearance of some organic compounds and generation of new metabolites. Furthermore, the seed germination test using *Phaseolus mungo* L. showed toxicity reduction in decolourized effluent.

Keywords Melanoidins · Metabolites · PMDE decolourisation · Endocrine-disrupting chemicals · Toxicity reduction

Introduction

Melanoidins are negatively charged, complex, heterogenous non-linear polymers produced through non-enzymatic Maillard reaction (MR) between amino compounds and carbohydrates, and are highly resistant to biological and chemical degradation (Wedzicha and Kaputo 1992; Wang et al. 2011). It is a major colouring constituents of dark-brown effluent released from sugarcane molasses-based ethanol producing distillery industries. During the distillation process, an average of 12–15 L of effluent is generated per litre of ethanol production (Chandra and Kumar 2017a). Dark coloured effluent from the distilleries is one of the most obvious indicators of water pollution. Apart from the colour, the discharged distillery effluent also contains high amount of total dissolved solids (TDS) and recalcitrant pollutants in form of complex polymer containing heterocyclic nitrogenous compounds of aldehyde-amines, various heavy metals, phenolic compounds and plant derived resins and fatty acids which behave as endocrine-disrupting chemicals (EDCs) (Chandra and Kumar 2017a). The major metallic constituents in distillery effluent is reported as iron (Fe), copper (Cu), manganese (Mn), lead (Pb), zinc (Zn), cadmium (Cd), and nickel (Ni). Furthermore, it has also been reported that melanoidins have net negative charges; therefore, various heavy metals strongly bind with melanoidins to form large organo-metallic complex molecules (Migo et al. 1997). Therefore, discharged effluent after anaerobic

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Highly efficient phytoremediation potential of metal and metalloids from the pulp paper industry waste employing *Eclipta alba* (L) and *Alternanthera philoxeroide* (L): Biosorption and pollution reduction

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HIGHLIGHTS

- Discharged pulp and paper waste contains metal and metalloids.
- *Eclipta alba* (L.) and *Alternanthera philoxeroide* (L.) have phytoremediation potential of pulp and paper mill.
- Physico-chemical pollution parameter reduced more than 60%, respectively.
- Selective plant showed >1.0 Bioconcentration factor.
- This study may be effective technology for ecorestoration of polluted site.

ARTICLE INFO

Keywords:
Metal accumulation
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Bioaccumulation factor

ABSTRACT

The aims of the study was the evaluation of phytoremediation potential by *Eclipta alba* (L) and *Alternanthera philoxeroide* (L) of pulp and paper mill waste after secondary treatment which a source of aquatic and soil pollution due to huge discharge of organometallic compounds per tone of paper production. The result revealed 50% reduction of pollution parameters after in-situ phytoremediation. The comparative analysis of metal and metalloids showed the highest accumulation of Fe (2251.24 ± 64.74) in both plants. The antioxidant activity, chlorophyll and carotenoid content were increased in *E. alba* (L.) and *A. philoxeroide* (L.) respectively. From the results, it was concluded that *E. alba* (L.) and *A. philoxeroide* (L.) could be effectively used for the removal of metals and metalloids from effluent and sludge of pulp and paper mill waste that may help to reduce adverse health effects of metal accumulation in humans and animals via their food chain.

1. Introduction

There is the discharge of a huge amount of various heavy metals in the environment due to anthropogenic & industrial activities, which is a threat to human health due to these metals through the food chain and other sources (Nagarajan et al., 2020). Heavy metals are also added to an aquatic ecosystem from a variety of industries, such as metal molding, water treatment, batteries, mineral mining, nuclear power generation, metal grinding, paint, and agro-industries (Kamarudzaman et al., 2015). According to the annual report of Central Pulp and Paper Research Institute 2016–17, there are 850 pulp and paper industries in India (CPPRI, 2016). The pulp paper industry ranks 6th among the world's most polluters and produces large-scale environmental pollutants during the pulping and bleaching process (Ugurlu et al., 2007; Sharma et al., 2020). Effluents and sludge of pulp and paper mill industries after secondary treatment contain more than 250 identified toxic chemicals and harmful components like sterols, metals, fatty acids, and resin acids (Ratia et al., 2013). The dark color and high turbidity of effluent and sludge after secondary treatment due to dissolved suspended solids from the pulp and paper manufacturing process of the industry are increases the environmental and aquatic pollution and deteriorate the drinking water quality near the industry. The effluent contaminated water absorbs more light and inhibits the oxygen supply in water resources. Consequently, impart an adverse effect on aquatic life due to the abundance of resin and tannin acids. The earlier studies have observed discharge of pollutants from the paper and pulp industry after secondary

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Newly isolated *Bacillus* sp. PS-6 assisted phytoremediation of heavy metals using *Phragmites communis*: Potential application in wastewater treatment

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Highlights

- Treated wastewater contains high amount of TDS, TSS, BOD, COD, and metals.
- Biosorption of eight toxic metals by *Bacillus sp.* PS-6 were assisted phytoremediation.
- Use of *Phragmites communis* to removal of metals and EDCs pollutants.
- Strategy and opportunity to detoxification of pulp and paper industry wastewater via phytoremediation.

Abstract

This work aimed to study *Bacillus* sp. PS-6 assisted phytoremediation of metals from pulp and paper industry wastewater as a novel green technique for the removal of metals of wastewater. Results revealed that heavy metal (mg L^{-1}) contents in wastewater were reduced after in-situ phytoremediation for Fe, Cu, Zn, Cd, Mn, Ni, Pb, and As. *Phragmites communis* showed higher potential for the enrichment of Fe, Cu, Zn, Cd, Mn, Ni, Pb, and As in its rhizomes, roots, and shoots compared to leaves. The strain produced indole acetic acid, siderophores, and hydrolytic and ligninolytic enzymes, and resulted in nutrients solubilization. Results offer

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Research article

Phytoremediation potential of heavy metal accumulator plants for waste management in the pulp and paper industry

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Bioconcentration factor
Environmental analysis
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Environmental toxicology
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Toxicology

ABSTRACT

The present manuscript has focused on the heavy metal; accumulation potential by common native plants i.e. *Chenopodium album* L., *Ricinus communis*, *Ranunculus sceleratus*, and *Rumex dentatus* growing on the disposed of pulp and paper mill effluent sludge. The sludge showed the abundance of benzene propanoic acid tert- butyldimethylsilyl ester, Octadecanoic acid, TMS, Hexadecanoic acid, TMS, cinnamic acid-α-phenyl-TMS ester, β-sitosterol TMS, 4-mercaptobenzoic acid as residual complex organic compounds along with heavy metals Fe (98.30 mg/L^{-1}), Zn (51.00 mg/L^{-1}), Cu (3.21 mg/L^{-1}), Cd (9.11 mg/L^{-1}), Mn (18.27 mg/L^{-1}), Ni (5.21 mg/L^{-1}), (Hg 0.014 mg/L^{-1}) which were above the prescribed limit of environmental standard. The complexation of organic compounds with heavy metal restricts the bioavailability of metals to plants. But the metal analysis in various parts of the plant showed a significant amount of metal accumulation. Further, histological observations of root tissue through TEM showed apparent deposition of metal granules near the cell wall and vacuole as adoption features of plants. But the variable concentration of metal accumulation in different parts by various plants indicated the variable potential of tested plants with various metals. This also indicated their metal bioavailability and movement to plant tissue. Further, their bioconcentration factor (BCF) and translocation factor (TF) > 1.0 indicated the hyperaccumulation tendency of plants Mn was accumulated maximum in leaves *C. album* ($69.38\ mg/kg^{-1}$) followed by Cu ($25.75\ mg/kg^{-1}$), As ($23.20\ mg/kg^{-1}$), Fe ($20.90\ mg/kg^{-1}$) and Pb was maximum accumulated ($22.41\ mg/kg^{-1}$) in *R. cummunis* leaves. The result revealed that arsenic has been accumulated in higher amount root, shoot and leaves of all tested plants. The metal accumulator plants showed phytoremediation potential also by reducing various pollution parameters after growth on sludge. These potential plants may be used as biotechnological tools for the eco-restoration of polluted sites.

1. Introduction

Currently Industrial waste is one major source of heavy metals pollution in the environment worldwide. The agro-based industries i.e. distillery, Tannery and pulp and paper industries and thermal plants are contributing a significant amount of heavy metal contamination in soil and aquatic ecosystem along with their discharged waste (Chandra et al., 2011; Sivakumar et al., 2014; Satyawali and Balakrishnan, 2008; Sushil and Batra, 2006). The heavy metals concentration in the discharge of the above industry has been detected beyond the permissible level of environmental safety regulation (CPCB, 2007; EPA, 2003). The heavy metals pollution in the environment is not the only risk to microorganisms, zooplankton, phytoplankton, and wildlife, but also affects to human health due to its recycling through the food chain (Yang et al., 2005; Zhou et al., 2008). The paper industries manufacturing writing paper, Kraft paper, and hardboard discharge various metal mainly Fe, Mn, Zn, Cu, Cr, Cd, Ni, and Pb along with their waste (Singh and Chandra, 2019; Chandra and Singh, 2012). These heavy metals have a strong binding tendency with lignocellulosic waste due to their cations and result in the formation of the organometallic complex. Due to the complex nature of pollutants, these compounds behave as persistent organic pollutants. Therefore these heavy metals and persistent organic pollutants (POPs) are a threat to the environment for its persistence nature and harmful due to continuous bio-concentration to plant and animal tissue which adversely affects to food chain due to its tendency to accumulate in the food chain (Singh et al., 2015). In general, there is a discharge of 190–200m^3 of wastewater per ton of paper production from paper industries containing a high amount of suspended particles and dissolved

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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

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ABSTRACT

Sugarcane-molasses based distillery waste is a threat to environment for its safe disposal due to complexation of endocrine-disrupting chemicals (EDCs) containing mixture of organic pollutants. This study revealed that distillery sludge contains not only mixture of complex organic pollutants but also retains high quantity of Fe (5264.49), Zn (43.47), Cu (847.46), Mn (238.47), Ni (15.60), and Pb (31.22 mg kg^{-1}) which enhances the toxicity of sludge to the environment. The major identified organic compounds were benzene, 1-ethyl-2-methyl, benzene, 1-ethyl-4-methyl benzoic acid, 3,4,5-tris(TMS oxy), TMS ester; hexanedioic acid, dioctyl ester; stigmasterol TMS ether; 5α-cholestane,4-methylene; campesterol TMS; β-sitosterol and lanosterol. These compounds are listed under the EDCs also as per U.S. Environmental Protection Agency. However, the phytoextraction potential of growing native weeds and grasses i.e. *Argemone mexicana*, *Saccharum munja*, *Cynodon dactylon*, *Pennisetum purpureum*, *Chenopodium album*, *Rumex dentatus*, *Tinospora cordifolia*, *Calotropis procera* and *Basella alba* revealed the high accumulation of Fe, Zn, Cu, Mn, Ni, and Pb in their root and leaves compared to shoot. This indicated high accumulation and translocation capabilities of these plants. Further, the bioaccumulation coefficient factor (BCF) and translocation factor (TF) was found > 1 for majority of plants for various metals. Thus, this given strong evidence for hyperaccumulation tendency of these native weeds and grasses from complex polluted sites. Furthermore, the ultrastructural observations of root tissues also revealed the deposition of heavy metals at various cellular components without any apparent toxic effects. This indicated the variable adaptive characteristics of these plants growing at a hazardous waste polluted site. Thus, the study given a strong evidence for application of these weeds and grasses as tools for in-situ phytoremediation and eco-restoration of polluted sites.

1. Introduction

Sugarcane-molasses based distillery waste is well known as source of complex environmental pollutants due to various heavy metals containing complex organic pollutants (Chandra et al., 2008; Chandra and Kumar, 2017a, 2017b). 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). This reflects the magnitude of the environmental pollution caused by the waste generated from distillery sector all over India. The dotriacontane, lanosta-8, 24-dien-3-one, 1-methylene-3-methyl butanol, and 1-phenyl-1-propanol as androgenic and mutagenic compounds (Chandra and Kumar, 2017a), which are listed under the endocrine-disrupting chemicals (EDCs) list of USEPA (2012). The study has revealed that these organic pollutants makes organo-metallic complex with various heavy metals which are mainly iron (Fe), zinc (Zn), copper (Cu), chromium (Cr), cadmium (Cd), manganese (Mn), nickel (Ni), and lead (Pb) present in high quantity i.e. (Fe: 2403.64), (Zn: 210.624), (Cu: 73.63), (Cr: 21.84), (Cd: 1.446), (Mn: 126.292), (Ni: 13.425), (Pb: 16.332 mg kg^{-1}) (Chandra and Kumar, 2017c). The concentrations of these metals are far above than the prescribed limit in

जलतन्त्र और बाढ़

कोरोना वायरस महामारी (कोविद–19) का संक्रमण, उपचार एवं रोकथाम के उपाय

❑ प्रो0 रामचंद्रा एवं सोनम त्रिपाठी

Covid-19 has impacted the entire world order as one of the unprecedented pandemic of the century. However, it reflects a new threat of spread of animal viruses, unknown viruses or synthetic viruses, This article describes the science and management of Covid-19 with certain technical details of the threat.

वायरस जिन्हें हम विषाणु कहते हैं, यह प्रोटीन एवं न्यूक्लिक एसिड के बने हुए अति सूक्ष्म कण होते हैं, जिसकी साइज प्रायः १२० से १६० नैनोमीटर तक होती है। इनकी कोशिकाओं की वृद्धि या विभाजन अपने आप नहीं हो सकती है। इनकी वृद्धि किसी जंतु कोशिका में संक्रमण के उपरांत ही संभव होती है, विषाणु में पाए जाने वाली नुक्लिक अम्ल या तो डी–ऑक्सी राइबोनुक्लिक अम्ल होती है या तो राइबो नुक्लिक अम्ल होती है। परन्तु कोरोना वायरस में पाई जाने वाली नुक्लिक अम्ल, राइबोनुक्लिक अम्ल ही होती है इसके अलावा इसकी संरचना विशेष फूल की तरह गोल दिखती है। इसका नामकरण कोरोना सूर्य की संरचना में दिखने वाली कोरोना के आधार पर की गयी है। दिसंबर माह 2019 के प्रथम सप्ताह के समय, चीन में जिस वायरस का पता लगा था उस वायरस के लक्षण पूर्व वर्ष 2002–2003 में पाए गए वायरस, जिनकी वजह से घातक साँस की बीमारी के रूप में देखा गया था, इसको सीवियर एक्यूट रेस्पिरेटरी सिंड्रोम (सार्स) के नाम से प्रचलित हुआ था। इस महामारी के कारण चीन के अलावा अन्य देशों में कुल 8438 मौते हुई थी तथा बीमारी का प्रकोप चीन देश तक ही सीमित रहा। तदोपरांत वर्ष 2012 में इसी प्रकार के वायरस के द्वारा एक अन्य बीमारी भी अरब के देशों में रहने वाले लोगो को प्रभावित किया था जिसका नाम था मिडिल ईस्ट रेस्पिरेटरी सिंड्रोम (मर्स)। इस बीमारी के कारण 600–800 लोगों की जान चली गयी थी। यह बीमारी बहुत ही घातक रूप में देखी गयी थी क्योकि इन दोनों बिमारियों के कारण मृत्यु दर क्रमशः 9–10% एवं 35–36% पाई गयी थी, जिसके कारण इस वायरस की पुष्टि होने के कारण पुरे विश्व में एक दहशत का वातावरण फ़ैल गया, यूरोप एवं अमेरिका तथा चीन से प्राप्त आंकड़ों के आधार पर कोविद–19 के कारण मृत्युदर 4–5% के आस–पास देखी गयी, परन्तु भारत में इस महामारी के कारण मृत्युदर 1.5–2.0 प्रतिशत ही पाई गयी है जो बाद में घट करके स्वास्थ्य विभाग के आंकड़ों के आधार पर और भी कम हो गयी जो कि 0. 5–1.0 प्रतिशत तक ही देखी गयी है। जिसके दो प्रमुख कारण हो सकते है, पहला यह कि या तो बाहर के देशों में संक्रमित करने वाला वायरस के जीनोम में कुछ बदलाव आया है और या दूसरा कारण यह भी हो सकता है कि भारतीय मूल के लोगों में शारीरिक प्रतिरोधक क्षमता बहुत अच्छी है, इसका कारण रहन सहन के साथ खाना, मसाले एवं जलवायु हो सकती है। लेकिन जो वायरस दिसंबर 2019, चीन में बीमारी का कारण पाया गया उसके लक्षण पूर्व में देखे गए वायरस से भिन्न था। इसलिए दिसंबर 2019 में मिले वायरस को शुरुआत में न्यू कोरोना वायरस (n-CoV) नाम दिया गया जिसको 2019–न–कोव, 2019–n–Cov) या सार्स–कोव–2 (SARS-Cov-2) नाम दिया गया। जो वर्तमान में भयावह संक्रमण से एक महामारी का रूप ले लिया है। इन्हें विश्व स्वास्थ्य संगठन के द्वारा जनवरी 12, 2020 को इस महमारी का नाम कोरोनावायरस बीमारी – 2019 (कोविद–19) नाम दिया गया है। इस बीमारी की शुरुआत चीन के वुहान शहर के हुबई प्रान्त में दिसंबर 2019 के प्रथम सप्ताह में पाया गया, जिसमे शुरुआत में यह अनुमान लगाया गया कि इस बीमारी का संक्रमण वुहान शहर के सी–फूड मार्किट में बिकने वाले चमगादड़ों के माध्यम से हुआ है। इस बीमारी के लक्षण निमोनिया से मिलते जुलते थे तथा इसमें तेज बुखार के साथ श्वसन तंत्र पूरी तरह बंद हो जाता है। शुरुआती लक्षण में तेज बुखार के साथ पाचन तंत्र भी खराब होने के लच्छण भी मरीजों में पाए गए । इस वायरस के लिए 15 से 20 सेंटीग्रेड तापमान उत्तम पाया गया है। पूर्व वर्गीकरण के अनुसार कोरोना वायरस के पहले से ही दो मुख्य प्रजातियां ज्ञात थी उनमें एक प्रजाति जो आजकल बहुत ही चर्चा में है, उसे कोरोनावायरस कहते हैं, उसी परिवार की दूसरी प्रजाति है जिसको हम टोरो वायरस के नाम से जानते हैं । कोरोना वायरस

पर्यावरणीय सूक्ष्मजैविकि विभाग, बाबासाहेब भीमराव आंबेडकर विश्वविद्यालय (केंद्रीय विश्वविद्यालय) विद्या विहार, रायबरेली रोड, लखनऊ– 226025, यू० पी0
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कागज कारखानों से उत्सर्जित विषैले अपशिष्टों का पर्यावरण पर दुष्प्रभाव तथा सुरक्षात्मक निस्तारण की चुनौतियाँ

राम चन्द्रा, विनीत कुमार, सोनम त्रिपाठी एवं पूजा शर्मा

पर्यावरणीय सूक्ष्म जैविकी प्रभाग, पर्यावरण विषविज्ञान समूह

सी.एस.आई.आर.– भारतीय विषविज्ञान अनुसंधान संस्थान

विषविज्ञान भवन, 31, महात्मा गाँधी मार्ग लखनऊ–226001, उत्तर प्रदेश भारत

भारत को कागज के उत्पादन में विश्व के 10 वें सबसे बड़े देश के रूप में जाना जाता है। वर्तमान में हमारे देश में लगभग 618 कागज के कारखाने हैं जिसमें 565 कारखाने स्थाई रूप से तथा 58 कारखाने अस्थाई रूप से कार्यरत हैं। प्रायः 01 टन सफेद कागज बनाने हेतु 100 से 200 घन मीटर ताजे जल की आवश्यकता होती है, जिसके कारण इससे निकलने वाला बहिस्राव बहुत भारी मात्रा में निकलता रहता है। प्रायः लकड़ी को कागज बनाने में कच्चे माल के रूप में प्रयोग किया जाता है उसके मूल संरचना के करीब 40–45 प्रतिशत ही लुग्दी के रूप में सेल्यूलोज के रेशे बच पाते हैं जबकि बाकी लकड़ी के मूल

कोई आँकड़े उपलब्ध हैं।

कागज उत्पादन हेतु मुख्यतः विभिन्न पेड़ों की लकड़ियाँ जैसे–यूकेलिप्टस, पापलस, बाँस तथा चीड़ के अलावा पुनः चक्रित (रिसाईकिल्ड पेपर) पुराने कागज, गन्ने की खोई, या अन्य पौधों के रेशे को कच्चे माल के रूप में उपयोग में लाया जाता है। कागज बनाने की प्रक्रिया प्रायः दो से तीन चरणों में पूरी होती है। पहले चरण में लट्ठे को छोटे–छोटे टुकड़ों में काटकर उसे लुग्दी बनाने हेतु बड़े–बड़े डाइजेशन टैंक में कास्टिक सोडा और सोडियम सल्फाइड के मिश्रण के साथ उबाला जाता है। जिसके फलस्वरूप लकड़ी के विभिन्न अवयव घुल जाते हैं। इन अवयवो

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Phytoremediation of Industrial Pollutants and Life Cycle Assessment

Ram Chandra, Vineet Kumar, Sonam Tripathi, and Pooja Sharma

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Microbes in Land Use Change Management
2021, Pages 45-90

2 - Adaptation of bacterial communities and plant strategies for amelioration and eco-restoration of an organometallic industrial waste polluted site

Sonam Tripathi, Kshitij Singh, Ram Chandra

https://doi.org/10.1016/B978-0-12-824448-7.00005-X

Abstract

The adaptation of any microorganism is morphological or biochemical changes required to survive in their ecological niches. This indicates the evolutionary process of bacterial communities, where the individual organism becomes better suited to live in its habitat. The bacterial communities have a broad range of adaptations due to their unique cell membrane properties and versatile nutritional behavior. It may be thermophiles, psychrophiles, acidophiles, halophiles, alkalophiles, and barophiles. To maintain the variable environmental conditions bacterial cell membrane protein modification plays a crucial role in controlling their function. The genome sequence of various psychrophiles revealed the function of the cell membrane with an increased proportion of polyunsaturated and branched fatty acids to increase the fluidity at low temperature and the presence of cold shock proteins are also believed to increase translational efficiency by destabilizing secondary structure of mRNA. The plant strategies for growth and

Environmental health hazards of post methanated distillery waste...
Proceedings of 74th Annual Convention of STAI: 684 – 699 2016

ENVIRONMENTAL HEALTH HAZARDS OF POST METHANATED DISTILLERY WASTE AND ITS DETOXIFICATION

Ram Chandra*, Vineet Kumar & Sonam Tripathi

ABSTRACT

The sugarcane molasses based distillery waste water is major source of environmental pollutant due to its huge generation and complex nature. The post Methanated distillery effluent (PMDE) is being used indiscriminately by farmers also in country as irrigating water without knowing its adverse effect. The study has revealed that 1-10% PMDE showed stimulatory effect on Phaseolus mungo L. growth parameter, while above 15-20% distillery effluent showed toxic effect on plant growth parameter. Similarly, PMDE sludge having concentration ≤ 10% (w/w) act as a fertilizer. However, above than 10% of PMDE sludge in soil (w/w) both caused adverse effect on plant growth parameters and soil fertility. The research revealed that accumulation of heavy metal at ≥ 40% (w/w) concentration was highest in the root except iron and manganese. Further, the biodegradation of distillery wastewater (DWW) by using aerobic bacterial consortium comprising Bacillus licheniformis (DQ79010), Bacillus sp. (DQ779011), and Alcaligenes sp. (DQ779012) were showed that the bacterial consortium was efficient for 70% color removal in presence of glucose (1.0%) and peptone (0.1%) at pH 7.0 and temperature 37 0C. The metabolic products were also characterized by LC-MS/MS analysis. This confirmed that bacterial consortium was capable for biodegradation. Further, the seed germination test for environmental safety showed that the PMDE after bacterial treatment showed growth promoting effect.

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Babasaheb Bhimrao Ambedkar University, Lucknow

(A Central University)

Certificate

Certificate of Merit is awarded to Mr./Ms. SONAM TRIPATHI

Department of Environmental Microbiology in recognition of significant contributions in the academic activities of the University 2017-2018.

Registrar

Vice Chancellor

INTERNATIONAL CONFERENCE ON BIOENGINEERING SOLUTIONS FOR HEALTHCARE, FOOD, ENERGY, AND ENVIRONMENT (BSHFEE-2021)

CERTIFICATE

THIS IS TO CERTIFY THAT

Sonam Tripathi

Babasaheb Bhimrao Ambedkar Central University, Lucknow

HAS WON

Best Oral Presentation Award

(Bioengineering Solutions for Environment)

for the paper entitled Biostimulation and bioaugmentation approach for degradation of organometallic pollutants: Role enzymes and bacterial community authored by Sonam Tripathi, Pooja Sharma & Ram Chandra in the International Conference on Bioengineering Solutions for Healthcare, Food, Energy, and Environment, jointly organized by INDIAN INSTITUTE OF TECHNOLOGY, JODHPUR and THE BIOTECH RESEARCH SOCIETY, INDIA at Jodhpur, India during April 09-10, 2021.

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