UTILIZATION OF MULTISPECIES MICROALGAL BIOSYSTEM FOR WASTEWATER REMEDIATION AND BIOFUEL PRODUCTION

A Thesis Submitted to the
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IN

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CANDIDATE'S DECLARATION

I, **Dig Vijay Singh**, solemnly declare that the research work embodied in this thesis entitled **'Utilization of Multispecies Microalgal Biosystem for Wastewater Remediation and Biofuel Production'** carried out by me under the guidance and supervision of **Prof. Rana Pratap Singh,** Department of Energy & Environment, School of Environmental Sciences, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, India is original and is also approved by Departmental Research Committee (DRC).

I further declare that to the best of my knowledge, this thesis does not contain part of any work submitted for the award of any degree either in this University or any other University around the globe. It is further undertaken that the thesis is essentially free from all kinds of plagiarism.

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CERTIFICATE

This is to certify that the thesis titled **'Utilization of Multispecies Microalgal Biosystem for Wastewater Remediation and Biofuel Production'** submitted by **Mr. Dig Vijay Singh** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

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

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PREFACE

Water pollution and energy scarcity are the serious global issues amplified by the unprecetendent population growth, intensive urbanization and use of fossil fuels. The increasing water demand driven by escalating population, economic growth, higher living standards, and scarcity of water is becoming the main threat towards sustainable development. Wastewater management and its regular treatment continues to be one of the biggest challenges for India as industrial and municipal wastewater are getting disposed off in the rivers as yet. Wastewater discharged into water bodies poses harmful impact on the different ecosystems due to occurrence of enormous quantity of inorganic nutrients, toxic heavy metals and other hazardous pollutants. Wastewater discharged without the adequate treatment causes eutrophication, acidification and accumulation of toxic pollutants in the freshwater bodies resulting in severe water toxicity. Several treatment facilities have been recommended by the regulatory authorities but due to high cost and energy, such methods are hardly followed for the management of wastewater. Therefore, it is important to employ innovative, reliable, and sustainable approaches to tackle the increasing menace of water pollution. Management of wastewater using microalgae can offer myriad of benefits to the society over other methods of treatment. Microalgae are very promising alternative for nutrient reutilization, metal detoxification and biomass production. Inspite of multiple advantages, the wastewater treatment through microalgae is still having many technological bottlenecks as the daily as well as the seasonal fluctuation in temperature and light intensity affects the growth and biomass production potential of the microalgae. The rapidly changing environmental factors such as temperature and light affect the efficiency of microalgae by altering photosynthesis and other metabolic pathways responsible for the lipid synthesis and activity of anti-oxidative enzymes. Furthermore, employing single organism for wastewater treatment has to compete with the existing wastewater diversity which can affect the remediation potential and biomass production of the microalgae. Thus, using consortia of different compatible microalgae in a community form can be more suitable alternative to enhance the rate of wastewater remediation and to mitigate the damage posed by multiple stresses with an enhanced biomass yield for biofuel production. Furthermore, the influence of temperature and light intensities on the photosynthetic performance, lipid accumulation and defense mechanisms could be analysed to elucidate the different cellular modifications adopted by the algal consortia to manage temperature and light stresses. Therefore, the comprehensive assessment of these algal consortia treated with municipal wastewater under different light intensity and temperature conditions can make algal consortia a resilient bio-system for the wastewater remediation under the natural environmental conditions.

Thus, the present study entitled "Utilization of Multispecies Microalgal Biosystem for Wastewater Remediation and Biofuel Production" was carried out to give scientific knowledge about the ability of algal consortia to remediate wastewater and improve lipid production under the different environmental conditions. The alteration in growth kinetics, photosynthetic performance, biochemical modification, antioxidants activity and biofuel production potential were measured by using UV-Visible spectrometry, fourier transform infrared spectroscopy (FTIR), Chlorophyll fluorescence induction kinetics (OJIP) curve, and GC-MS (Gas chromatography and mass spectrophotometry) analysis.

In this thesis, the research outcomes presented was an attempt to improve the flexibility of the bio-based treatment system by screening the algal consortia tolerant to variable environmental condition. The present study encompasses multiple aspects from resource recovery, stabilization of wastewater attributes to improvement in lipid and biofuel production using wastewater as the sole nutrient media under the different light intensity and temperature regimes. The present thesis is divided into following chapters:

Chapters

Chapter I: 'Introduction' presents the outline of the work and attempts to provide the rationale of the research work along with the list of objectives to be accomplished in this thesis.

Chapter II: 'Review of Literature' provides an overview of the work done by several investigators in the field of microalgae, algal consortia, wastewater treatment, environmental stress conditions, antioxidant defense mechanism and biofuel production.

Chapter III: 'Materials and Methods' is exclusively assigned to the general methodology used during the present investigation.

Chapter IV: Microalgal competence in urban wastewater management: phycoremediation and lipid production is assigned to identify the potential of single microalgae in terms of growth, pigment content, stress biomarkers and defense mechanism to adapt and tolerate different stress conditions posed under natural environment.

Chapter V: Implication of municipal wastewater on growth kinetics, biochemical profile, and defense system of *Chlorella vulgaris* and *Scenedesmus vacuolatus* is assigned to assess the adaptability potential of monoalgal species to tolerate different wastewater concentrations. The study was conducted in plastic tubs under natural environment to screen the algal species that show prolific growth, promising remediation efficiency, lipid synthesis and potent antioxidant defense mechanism against adverse conditions.

Chapter VI: Algal consortia based metal detoxification of municipal wastewater: Implication on photosynthetic performance, lipid production, and defense responses is assigned to examine the role of selected algal consortia in treatment of municipal wastewater under laboratory conditions. The study was conducted under controlled temperature and light intensity conditions in order to observe the effect of wastewater on growth, biomass production, photosynthetic performance, biochemical profile, lipid accumulation and defense mechanisms of algal consortia.

Chapter VII: Algal consortia as the flexible biosystem for wastewater treatment: effect of different light intensities on photosynthetic performance, anti-oxidative system and biodiesel production is assigned to analyze the combined effect of light intensities (20 W/m^2 and 40 W/m^2) and wastewater concentrations (25-100%) on nutrient reutilization, metal detoxification and biochemical profile of consortia 1 and 2. The present chapter was not only aimed to screen the algal consortia tolerant to different light intensities but also to examine the effect of different light intensities on remediation efficiency, growth kinetics, lipid bio-synthesis, oxidative damage, stress mitigation mechanisms and biofuel production.

Chapter VIII: Effect of different temperature regimes on removal efficiency, photosynthetic parameters and fatty acid profile of algal consortia sheds light on the role of different temperature regimes in algal growth and biomass production. The study also focuses on different photosynthetic parameters, lipid accumulation, oxidative stress biomarkers and antioxidant responses of selected algal consortia. Apart from analyzing the effect of temperature on algal consortia, the study also aims to screen the adaptable and tolerant algal consortia keeping in consideration the growing wastewater complexity and rapidly changing temperature conditions.

Chapter IX: Competence of algal consortia under natural environment: remediation efficiency, photosynthetic performance and antioxidant activity. This chapter throws light on the resilience of algal consortia 1 and 2 to tolerate natural environmental conditions and grow vigorously with enhancement in remediation efficiency, lipid accumulation and biodiesel production. The study also examined the cellular modification such as biochemical constituent, pigment content and modification in photosynthetic parameters adopted by algal consortia against unfavorable conditions. The present chapter also aimed to examine the potential of algal consortia for large scale biomass and lipid production under outdoor environment.

Chapter X: Summary and Conclusions summarizes the major outcome of the present research work

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ABBREVIATION

µg	Microgram
µm	Micromolar
µl	Microliter
mL	Mililiter
mg	Miligram
%	Percent
cm	Centimeter
W	Watt
M	Meter
nm	Nanometer
N	Normal
M	Molar
L	Liter
mg	Milligram
mL	Millilitre
mM	Millimolar
v/v	Volume/volume
PPM	Parts per million
Fw	Fresh weight
G	Gram
UV	Ultra-Violet radiations
sp.	Species
g/L	Gram/liter
UV-VIS	Ultraviolet-visible
pH	Potential of hydrogen
EC	Electrical conductivity
TS	Total solid
TSS	Total suspended solid
TDS	Total dissolved solid
BOD	Biological oxygen demand
COD	Chemical oxygen demand

DO	Dissolved oxygen
NO_3^{-1}-N	Nitrate-nitrogen
PO_4^{-3}-P	Phosphate
Fv/Fo	Active photosystem II reaction center
Fv/Fm	Maximum quantum yield
Pi_{abs}	Performance index
Mo	Net closing rate of the reaction center
TRo/RC	Trapping flux
ABS/RC	Effective antenna size per reaction center
ET_O/RC	Electron transport per reaction center
FAME	Fatty acid methyl ester
ROS	Reactive oxygen species
FTIR	Fourier Transform Infrared spectroscopy
PS-I	Photosystem-I
Fm	Maximum fluorescence
Fo	Minimum fluorescence
US	United states
$OH^{\cdot}$	Hydroxyl radical
$^1O_2$	Singlet molecular oxygen
$O_2^{\cdot -}$	Superoxide
H_2O_2	Hydrogen peroxide
PCs	Phytochelatins
CH_2	Methylene
ATP	Adenosine triphosphate
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
RNA	Ribonucleic Acid
DNA	Deoxyribonucleic Acid
KBr	Potassium bromide
ETC	Electron transport chain
RuBisCO	ribulose-1,5-bisphosphate-carboxylase/oxygenase
FTIR	Fourier transform infrared spectroscopy
OJIP	Chlorophyll fluorescence induction kinetics
OD	Optical density
PAR	Photosynthetically active radiation

PBS	Phosphate Buffer Saline
Psi	Pounds per square inch
PS I	Photosystem I
PS II	Photosystem II
SFA	Saturated fatty acid
PUFA	Poly-unsaturated fatty acid
MUFA	Mono-unsaturated fatty acid
RC	Reaction centre
Rpm	Rotation per minute
UNESCO	United Nations Educational, Scientific, and Cultural Organization
CPCB	Central pollution control board
SGR	Specific growth rate
TAG	Triacylglycerol
MTs	Metallothioneins
CO_2	Carbon dioxide
Pb	Lead
Cd	Cadmium
Cr	Chromium
Cu	Copper
Zn	Zinc
Ni	Nickel
Fe	Iron
As	Arsenic
SOD	Superoxide dismutase
CAT	catalase
GR	Glutathione reductase
ETC	Electron transport chain

Chapter 1

Introduction

Chapter-1

Introduction

1.0 Introduction

Water is imperative and necessary resource for the survival of organisms and underpins the functioning of different ecosystem (Mushtaq et al., 2020). Inspite of being essential component of the life, water is one of the most poorly managed natural resource today. The most pervasive problem that affects the global population is inadequate access to clean water. Shrinking lakes, rapidly declining ground water table, deteriorating water quality and inadequate treatment methods has increased the scarcity of freshwater (Hoekstra, 2014). Water crises and insufficient clean water has become the world's most persistent problem now (Hanikel et al., 2020). The scarcity of clean water will more worsen in near future even in the regions currently containing adequate quantity of water for human use, if not managed properly. At world economic forum, Ban Ki-moon urged leaders from the all over the world to keep water crisis as the top agenda to prevent emerging conflicts over the freshwater. The inadequate freshwater availability and supply will hamper economic growth and accelerate the regional disputes for the water (Hightower and Pierce, 2008). Schewe et al., (2014) have reported that climate driven changes can leads to increment of 40% people living in regions with water availability of less than 500 m^3 yr^{-1}. By 2050, the population will reach to 9.5 billion globally and approximately 66% of total population will shift from rural to urban area (UN, World Urbanization Prospects: The 2014 Revision). Such population growth and migration towards urban areas will not only increase water scarcity but also leads to vast generation of wastewater (Kookana et al., 2020). Wastewater generation is increasing at alarming rate as most (70-80%) of the water supplied for domestic use is discharged without any proper treatment (Abinandan and Shanthakumar, 2015). This practice of wastewater discharge is more in developing countries like India whose wastewater management has become a key problem as the number of wastewater treatment plants is not keeping pace with rapid wastewater generation (Kaur et al., 2012). As per the reports of World Health Organization (WHO, 2000) and Central Pollution Control Board, (CPCB, 2009), 65-69% of the generated wastewater is disposed without any appropriate treatment into the environment in Asia including most of the cities of India. In India, only 51% of the wastewater generated from metropolitan cities is subjected to treatment and 49% of remaining untreated wastewater is discharged into the environment. On the other hand, the rapid industrial growth and urbanization has increased the pressure on the available resource and the major obstruction behind water quality deterioration is inappropriate discharge and mismanagement of wastewater (WHO and UNICEF, 2010).

The incessant pollution has restricted the availability of water in major parts of the world (Damania et al., 2019). This intensive global water crisis has forced world leaders to devise policies regarding judicious use, proper management and reuse of wastewater (Hightower and Pierce, 2008). The conventional methods (Fig. 1.1) for wastewater treatment disinfect, desalinise and decontaminate water however, due to energy and chemical intensive nature, such methods require huge financial investment, infrastructural facilities and technological modifications from time to time which ultimately prelude their use for continuous wastewater treatment in regular practice (Shannon et al., 2010). Even in industrialized nation, drawbacks associated with such treatment methods make it arduous to resolve several problems linked with wastewater management and its cost effectiveness (Shannon et al., 2010). Additionally, it is very difficult for the single conventional technology to simultaneously stabilize the different physico-chemical attributes of various kinds of wastewater (Ma et al., 2017).

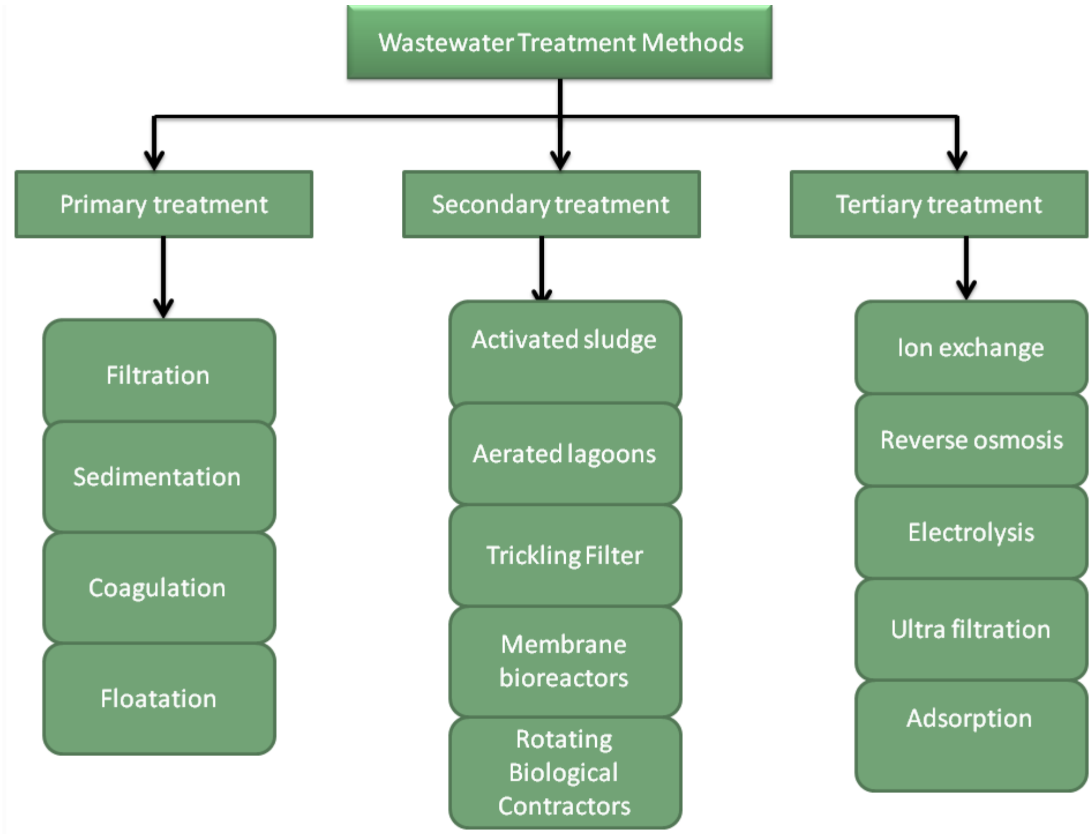


Fig. 1.1 Conventional methods for the treatment of wastewater

1.1 Types of wastewater

1.1.1 Municipal wastewater

The wastewater produced from domestic, residential, businesses and institutes is categorized as municipal wastewater. Wastewater from domestic area is usually grey coloured with bad odour (Khan and Kaafil, 2020) and contains 70% of organic compounds and about 30% of inorganic contaminants (Von Sperling, 2007).

The composition of municipal wastewater varies with source of wastewater even daily and seasonally throughout the year. The nutrients such as nitrogen and phosphorus concentration ranges from 15–90 and 5–20 mg L^{-1} in municipal wastewater (Kligerman and Bouwer, 2015). Municipal wastewater is also enriched with dissolved ions, organic matter, oil, fats, detergents and metals and when discharged into the environment, these chemicals can have detrimental impacts on the aquatic and terrestrial ecosystem (Margot et al., 2015).

1.1.2 Agro-industrial wastewater

Agro-industries and food industries generate solid, liquid and gaseous waste during the processing operations as well as treatment and disposal of waste (Sadh et al., 2018). Agro-industrial waste contains huge quantity of organic material, suspended solid along with high COD and BOD level (Leiva-Candia et al., 2014). Agriculture is considered as one of the largest consumer of water. The runoff from the agricultural fields also contains persistent pollutants in the form of the heavy metals and pesticides (Anju et al., 2010). Agro industries such as diary, food, slaughterhouse, and distillery contains huge amount of nutrients responsible for pollution of water bodies (Shen et al., 2019). Apart from agro-industries, livestock and poultry farms also contribute the significant amount of nutrients in the wastewater. Kligerman and Bouwer, (2015) reported that abundant amount of nitrogen (185 - 3213 mg L^{-1}) and phosphorus (30- 987 mg L^{-1}) is discharged as dairy and swine wastewater. Achieving zero water footprints in agriculture sector is very difficult but the water consumption pattern as well as pollution level can be decreased substantially (Brauman et al., 2013).

1.1.3 Industrial wastewater

Rapid industrial development from the past century along with improved living standards of the people together brings several environmental issues (Bulgariu and Bulgariu, 2020). Industrial wastewater is complex mixture of different toxic pollutants which even in small amount can persist and deteriorates the health of environment. Different industries such as textile (Qadir et al., 2015), electroplating (Elystia et al., 2020), paint (Nair et al., 2021), dye (Shindhal et al., 2021), paper and pulp (Usha et al., 2016), tannery industry (da Fontoura et al., 2017) and pharmaceutical industry (Hena et al., 2021) continuously releases organic matter, nutrients (N and P), dyes, heavy metals and other recalcitrant pollutants in the wastewater (Gupta et al., 2016).

Currently, energy scarcity and water pollution are the major issues at global level which need to be addressed immediately for smooth functioning of the lifeform on the planet mother Earth (de Amorim et al., 2018). The indecent planning has lead to depletion of energy reserves (Owusu and Asumadu-Sarkodie, 2016) and deterioration of freshwater resources at very alarming rate (Mushtaq et al., 2020). Energy is considered as the engine for economic and social growth of the country. This energy demand is currently being fulfilled by fossil fuel reserves (Martins et al., 2019). The developing countries undergoing modernization and industrialization requires sufficient energy to sustain the unprecedented demand of the population (Zhu, 2015). The spontaneous exploitation of fossil reserves along with population explosion, improving living standards and urbanization are the main causes of energy scarcity and climate change (Perera, 2018). Several inexhaustible options of energy like solar, wind, hydropower, and plant based biomass are being used in various countries to not only enhance the production but reduce the energy crisis in environmentally sound manner (Mata et al., 2010). The biomass is believed to fullfil almost 1/4th of the energy demands globally and also serves as the viable substrate to elevate the production of valuable bio-compounds (Briens et al., 2008). Food crops also have good potential of biofuel production; its competition along with the need of food security will be major concerns to hinder their use for energy production (Stamenković et al., 2020). Approximately, 50% of the agricultural land is required to sustain the fuel demand for transportation sector in US. The diversion of agricultural land for fuel production will creates huge imbalance in the food production and may even leads to food crisis. Exploiting energy crops for fuel production will compete with agricultural crops for land, water and nutrient resource (Alalwan et al., 2019). Thus, it is important to relay on the technique that must assist concurrently in attaining the energy targets, reduce the emission of green house gases and also enhances the availability of clean water with less consumption of the valuable natural resources (Hwang et al., 2016).

Bioremediation is gaining huge attention as the focus is shifting towards sustainable wastewater treatment, resource recovery and biomass production (Rosli et al., 2020). The term bioremediation encompasses utilization of plants, bacteria, fungi and algae for the restoration of contaminated ecosystem (Kumar et al., 2018). Among different types of bioremediation approaches, microalgae based treatment system seems very flexible and sustainable approach for the wastewater treatment (Wang et al., 2022). Exploiting microalgae to treat wastewater of diverse origin is called phycoremediation (Shackira et al., 2022). Phycoremediation is preferred for the wastewater treatment as this approach improves the water quality by facilitating extraction of nutrients and toxic pollutants from contaminated environment (Priyadharshini et al., 2021) (Fig. 1.2).

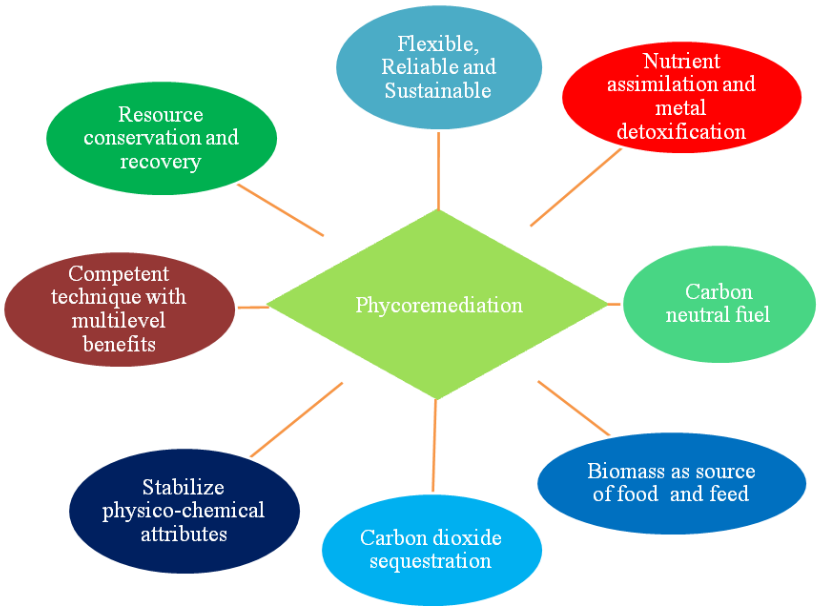


Fig. 1.2 Role of algae based wastewater remediation in environmental sustainability

1.2 Role of Microalgae

Microalgae are categorized as prokaryotic or eukaryotic (Mata et al., 2010) unicellular (Katiyar et al., 2017) group of photosynthetic organisms (Thanigaivel et al., 2022) that play essential role in sustaining the balance of aquatic ecosystem. Microalgae are ecologically most important primary producers (Katiyar et al., 2017) responsible for fixation of more than 50% of carbon dioxide of the earth Swarnalatha, et al., 2019). Both plants and microalgae have similar photosynthesis process but the sunlight conversion efficiency of microalgae is higher than terrestrial plants (Sørensen et al., 2022). Microalgae can reduce the intensity of global warming as for the production of 1 g of glucose, almost 1.57 g of the CO_2 is required by the microalgae (Yin et al., 2020). Due to high flexibility, tolerance, photosynthetic efficiency, carbon dioxide sequestration, and potential to accumulate carbohydrate/lipids, the microalgae based wastewater treatment methods are gaining global attention (Goswami et al., 2019). Microalgae based remediation is in itself the complete treatment system with capacity to resolve the different apprehensions from treatment of contaminated environment to energy production in cheap and sustainable manner (Tibbetts et al., 2015). Zuka et al. (2012) have estimated that half of industrial effluents if utilized as nutrient resource for microalgae can leads to the generation of 247 and 37 million tons of biomass and lipid respectively. Further, sorting water pollution and energy issues through the microalgae based treatment techniques may enhance the production of vital metabolites (Hannon et al., 2010) having multifarious role in food, cosmetic, pharmaceutical and neutraceutical industry (Mandotra et al., 2020) (Fig. 1.3). Further, the cost involved in wastewater treatment and concomitant algal biomass production can also be recuperated in the form of carbon credits for the several positive effects of microalgae based treatment system on the environment (Delrue et al., 2016).

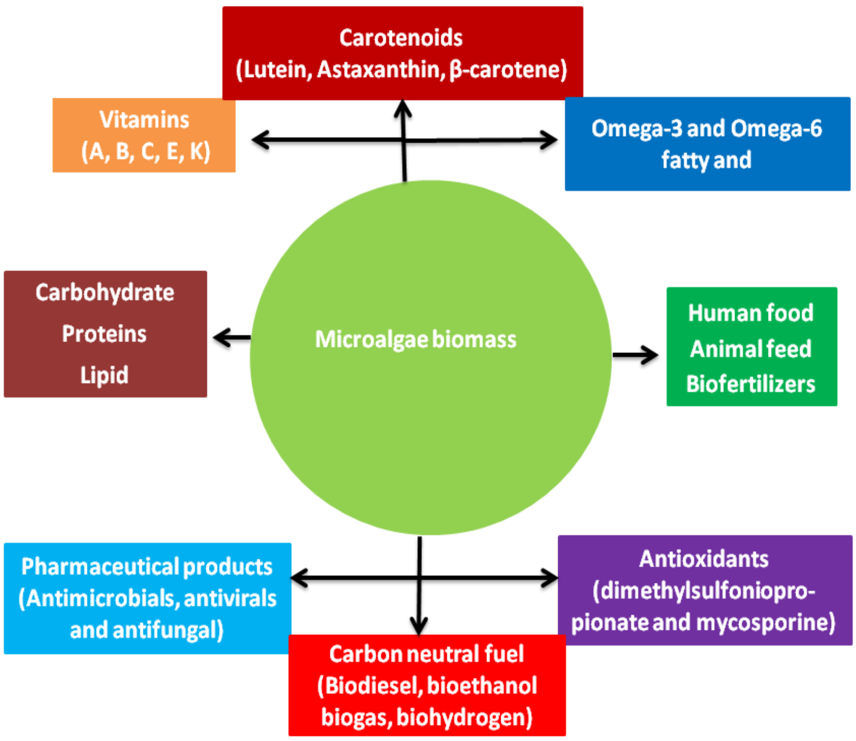


Fig. 1.3 Microalgae as the promising substrate for bioactive compounds

1.3 Phycoremediation of nutrients and heavy metals from wastewater

Wastewater has plentiful of necessary nutrients (Gupta et al., 2019) and can acts as the reasonable replacement of synthetic media (Dudek et al., 2017). Nitrogen constitutes about 1-20% of dry matter and is important for the growth, protein, lipid and carbohydrate synthesis in the algae (Zarrinmehr et al., 2019). Microalgae convert inorganic nitrogen such as nitrite, nitrate and ammonia into the organic forms (Salbitani and Carfagna, 2021). Nitrate is one of the widely used nitrogen form due to its stability and less probability of pH shift of growth media (Yaakob et al., 2021). However, nitrate assimilation is possible only after its reduction by nitrate and nitrite reductase into ammonium (Singh et al., 2021). Phosphorus is indispensable for prolific growth of microalgae (Grobbelaar, 2004) and is vital constituents of DNA, RNA, ATP and cell membrane (Geider and La Roche, 2002; Yaakob et al., 2021). The orthophosphate is most readily assimilated through phosphorylation for the phospholipids, nucleic acids, and protein synthesis by microalgae (Gupta et al., 2019). Phosphate deficiency in the growth media hampers cell division, protein synthesis (Åkerström et al., 2014), and functioning of photosynthetic machinery (Bucciarelli and Sunda, 2003). Excess phosphorus is stored as polyphosphate granules and algae use this surplus phosphorus under phosphorus deficient conditions (Singh and Pandey, 2018). Several researchers have reported that the microalgae can remove 80-100% of the nutrients from diverse sources of wastewater (Shahid et al., 2020). Utilizing microalgae for nitrogen and phosphorus removal increases the dissolved oxygen level of water and production of biomass, fertilizers and feed (Gonçalves et al., 2017).

Heavy metals are important constituent of the material used in the production of several industrial products obtained through the different industrial processes

(Ghasemi et al. 2014). Copper, chromium, cadmium, lead and arsenic are frequently detected heavy metals in the different types of wastewater discharged from various industries (Shrestha et al., 2021). Consequently, their continuous discharge and accumulation in different trophic level have enlarged the risk of exposure to diverse organisms (Ali et al., 2019). Heavy metals even in very low concentration can persist for years and have carcinogenic and mutagenic effects (Cesur et al., 2021). Elimination of metals from the environment is still a major challenge in term of the availability of operational technologies and effectiveness. Ion exchange, chemical precipitation, coagulation etc. are some of the frequently used methods for metals removal from the wastewater (Shrestha et al., 2021). These conventional techniques however, involve huge operational and maintenance cost which restrict their use for the wastewater treatment constantly (Fu and Wang, 2011). Most of the physico-chemical techniques are also ineffective at very high concentration of heavy metals (100 mg/L) and are not considered promising for wastewater remediation (Jaafari and Yaghmaeian, 2019). It becomes very cumbersome for the most of the developing countries to rely and exploit such techniques for the treatment and management of wastewater. Thus, other appropriate treatment technologies and plans are required to facilitate environmental cleanup and ecosystem functioning required for existence of life on the earth (Khattiyavong and Lee, 2019).

Microalgae remove heavy metals through multiple processes such as bioadsorption (cell adsorption), biodegradation (enzyme mediated degradation of toxicants), photo degradation (light and oxygen induced degradation), biotransformation (conversion of toxic substances into non toxic compounds) and bioaccumulation (high accumulation in cell organelles) (Sutherland and Ralph, 2019). Recently, bio-adsorption has gained tremendous significance but other strategies such as chelation and complextion are also adopted by viable algal cells for the metal removal from a contaminated environment (García-García et al., 2016). Additionally, under the metal stress environment, microalgae synthesize extracellular polymeric substances further enhancing the remediation efficiency of the wastewater treatment system (Naveed et al., 2019). Hamed et al., (2017) observed that *Scenedesmus acuminatus* can mitigate the heavy metal stress by enhancing the activity as well as concentration of different antioxidants such as proline, glutathione levels, GR and SOD. Additionally, the potential of microalgae to eliminate heavy metals from the contaminated environment depends upon the functional groups, types of metal ions, accessibility of binding groups as well as intracellular mechanisms.

1.4 Microalgae and wastewater

During phycoremediation, microalgae encounter various stressor such as nutrients, temperature, light, metals, UV radiation that affects the growth and metabolic activity of algae (Upadhyay and Mandotra, 2021). The stress conditions triggers oxidative damage in microalgae through enhanced production of ROS in the cell (Ugya et al., 2020). Reactive oxygen species ($OH^{\bullet}$, $^1O_2$, H_2O_2), the pro-oxidants accumulate in the cell both naturally and imposition of biotic as well as abiotic stress (Gauthier et al., 2020). The severity of damage depends upon the reactivity of ROS ($OH^{\cdot} > {}^1O_2 > H_2O_2 > O_2^{\cdot -}$) as $OH^{\cdot}$ being very reactive can degrade the cellular organelles and biomolecules more than H_2O_2 (Asada, 2006). The reactive oxygen species are generated at normal rate in favourable condition but the rate of ROS generation generally gets enhanced under the unfavourable environment (Sachdev et al., 2021). Increased accumulation of ROS disturbs the equilibrium resulting in oxidative damage to the cell or the cell is in "oxidative stress". The threat posed by enhanced production of ROS is in the form of lipid peroxidation, inhibition of enzymes activity and damage to protein, nucleic acid and DNA (Xie et al., 2019). The severe damage even leads to the activation of pathways related to the programmed cell death (Mishra et al., 2011). To offset the increased generation of ROS in the cell, microalgae induces the synthesis of antioxidants which apart from neutralizing of ROS maintains the osmotic balance and cell viability (Upadhyay et al., 2016). In microalgae, antioxidants modify processes responsible for prolific growth and encourage survival by maintaining redox status and modulation of gene expression (Shao et al., 2008). The antioxidants operate at different levels involving prevention of over production of radicals, scavenging of ROS and repair of the damaged biomolecules (Li et al., 2021). The action of the antioxidants to overcome the stress induced damage involves first line (SOD, CAT), second line (tocopherol and ubiquinol), third line (polymerases, glycosylases, proteases) and fourth line of antioxidant defense system (Ighodaro and Akinloye, 2018). Superoxide dismutase (SOD) and catalase acts as first line of defense and are important antioxidants of the entire defense system especially in neutralizing of the superoxide anion (Ighodaro and Akinloye, 2018). The other antioxidants like proline and ascorbic acid also help to overcome the toxicity in the cell (Ugya et al., 2020). Proline assist in stabilization of protein, enzymes, osmotic balance, maintains integrity of the membrane (Szabados and Savouré, 2010; Shafi et al., 2019), scavenges heavy metals, ROS and protects lipids from degradation (Shafi et al., 2019). Cysteine, a significant component of phytochelatins (Balzano et al., 2020) acts as precursor for glutathione (Wirtz et al., 2010) which assist in protecting the unstable macromolecules, sequester free radicals maintain photosynthesis and also play key role in neutralizing toxic derivatives produced through oxidation (Li et al., 2021).

1.5 Strategies to elevate lipid yield for biofuel production

Biomass from algae as the substrate for biofuel production is one of the important alternative to minimize the dependence on non renewable fuels (Shanmugam et al., 2021). The high lipid accumulating microalgae serve as the substrate for biodiesel production (Chen et al., 2022) while the high carbohydrate accumulating microalgae for bioethanol (Kumar et al., 2021) and biogas production (Nagappan et al., 2019). Various authors have reported that tailoring environmental conditions served as an efficient strategy to enhance the accumulation of energy rich compounds in the microalgae (Chu et al., 2020). Abiotic stress like nutrient starvation (Griffiths et al. 2014), UV radiation (Singh et al., 2019), salinity (Singh et al., 2018), light intensity and temperature are believed to elevate the accumulation of lipids in algae (Roleda et al. 2013). Stress conditions triggers modification in metabolic processes and genetic integrity leading to the accumulation of diverse metabolites and storage compounds in algae (Chu et al., 2020). Oxidative stress shifts metabolic pathways towards accumulation of carbohydrate and lipids as storage molecules in microalgae (Chokshi et al., 2015). Under stressful environment, the competition between carbohydrate and lipid synthesizing pathways occurs and the types of energy molecule accumulated depend upon the species of microalgae (Rismani-Yazdi et al., 2011). There are some factors which enhance the production of lipid include:

1.5.1 Nutrient limited growth conditions

Utilizing wastewater as the growth media depends upon the nutrient content and environmental factors (Arkronrat et al., 2016). Microalgae are promising source of lipids for biofuel production (Fal et al., 2022) but still are facing hurdles in terms of mass cultivation to sustain the fuel demand for growing population (Hannon et al. 2010). Nutrient starvation is the most feasible approach to increase the accumulation of lipid and improve composition of fatty acid in microalgae (Roy et al., 2021). The availability of nutrients in wastewater has substantial impacts on the cell growth, multiplication, lipid production as well as fatty acid composition of algal biomass (Gupta et al., 2016). Microalgae diverts carbon skeleton towards carbohydrate and lipid synthesis after utilizing proteins and pigments as nutrient source under nutrient deprived growth conditions (Pancha et al., 2014). Nutrient limited condition in culture media stimulates starch and triacylglycerol accumulation as the energy reserve to escape stressful environment (Lawton et al. 2015). Under nutrient stress conditions, the inhibited cell growth and membrane synthesis also leads to the diversion of fatty acids into triglycerides (Roleda et al., 2013).

1.5.2 Salinity

Salinity is the common abiotic stress which influence productivity of algae (Sun et al., 2018) and limit the contaminants as well as competing microorganisms (Bartley et al., 2013). Salinity is considered as the cheapest stress for large scale lipid production and eventually biofuel production (Shi et al., 2020). Sea water and saline groundwater are preferred water source for cultivation of microalgae for biofuel production (Borowitzka and Moheimani, 2010). High salinity leads to generation of stress due to extracellular osmotic pressure in microalgae (Sajjadi et al., 2018). Pancha et al., (2015) observed that salinity stress enhanced lipid accumulation after 9 days treatment to *Scenedesmus* sp. CCNM 1077 followed by 6 days and 3 days. Salinity stress strongly influences the photosynthetic process and diverts the photoassimilates towards lipid synthesis (Singh et al., 2018) especially neutral lipids (Yilancioglu et al., 2014). The increment in neutral lipid accumulation provides rigidity to cell membrane and help to regulate the mineral ion in microalgae (Lu et al., 2012). In order to overcome the damage posed by salinity stress, microalgae also stimulate the metabolic pathways responsible for restoration of turgor pressure, solute accumulation and synthesis of stress proteins (Sajjadi et al., 2018).

1.5.3 UV-radiation

UV irradiance is mainly focused to enhance the lipid biosynthesis and large scale lipid production from microalgae (Singh et al., 2019). The shorter and stronger UV radiation exposure considerably affects the composition as well as production of lipid in microalgae (Seo et al., 2019). Sijil et al., (2019) reported that microalgae treated with UV radiation for 60 minutes showed an increase of 1.60 folds in lipid content. Similarly, lipid content was increased by 33.7%, in *Ettlia* sp. after treatment with UV-A and lipid content was higher under UV-A and UV–C as compared to UV-B after treatment for 18 h (Seo et al., 2019). Mengelt and Prézelin (2005) also observed that UV-A radiation enhanced the carbon fixation potential as well as synthesis of neutral lipids in the microalgae (Balaji et al., 2014) while UV-B inhibited the synthesis of lipid in *C. vulgaris* (Ganapathy et al., 2017). Further, shorter exposure of UV radiation stimulates lipid synthesis without damaging the essential cellular components in microalgae (Seo et al., 2019).

1.5.4 Cultivation pattern

The microalgae cultivation carried under two stages is beneficial as compared to single stage cultivation (Nagappan et al., 2019). Although stress condition enhance the lipid yields but at the same time decrease the biomass productivity of the microalgae (Sun et al. 2018). Two stage cultivation has been suggested to overcome the challenges posed by nutrient limited growth conditions (Singh et al., 2016) and enhance lipid production without compromising with the biomass yield from microalgae (Xia et al., 2013). Mujtaba et al. (2012) also demonstrated that two stage cultivation improved lipid productivity as well as biomass production of *Chlorella vulgaris* and creates conditions that limits contamination under outdoor cultivation (Wensel et al., 2014). In two stage cultivation system, the microalgae are grown in the adequate nutrient conditions to obtain maximum biomass and subsequently the cells are shifted to nitrogen stressed condition to improve the lipid production (Chokshi et al., 2017). Thus, the depleted growth of microalgae under nutrient limited conditions may be balanced by multi-stage cultivation system **(**Nagappan et al., 2019)**.**

1.5.5 Stress amalgamation

Recently, simultaneous nutrients and abiotic stress has shown potential to improve lipid productivity of algae (Singh et al., 2016). The knowledge about the synergistic effect of combined stress in terms of lipid productivity is important to maximize the yield of microalgae (Sun et al., 2014). Breuer et al. (2012) studied the effect of abiotic stress on TAG accumulation in microalgae and observed that TAG accumulation increased by 40% at neutral pH and temperature condition of 27.6 °C. High lipid accumulation was also observed under multi stress of metal, salinity and nutrient in *Chlorella minutissima* (Cao et al., 2014). The combined stress of high temperature and metals initiate the shift in absorption spectra, elevates ROS generation as well as metabolic modification by altering the level of proteins, lipids and carbohydrates in the cell. The major advantage of exploiting the combined strategy is that one stress factor may compensate the negative effect of other stress factor. Moreover, under natural environment, microalgae is exposed to different stress condition and employing combined stress condition can assist in screening the highly adaptable and tolerant microalgae for lipid and biomass production (Singh et al., 2016).

1.5.6 Light and temperature

In natural environment, sunlight is the source of energy for algae (Mehan et al., 2018), but continuous seasonal variation, weather conditions and day/night cycle greatly changes the intensity of light (Castrillo et al., 2018). González-Camejo et al. (2019) suggested that the performance of microalgae is influenced by the net photon flux but not by light regime and time of the day the energy is received by microalgae. Light intensity controls the main cellular processes such as carbon fixation, physiology as well as the photosynthesis kinetics (Yang et al., 2018) and is necessary to produce the energy molecules in the form of ATP and NADPH for proper functioning of algae (Singh and Singh, 2015). The supply of proper light and its utilization by algal cells in efficient manner has been the major challenges in the wide scale cultivation of microalgae (Li et al., 2012). Light intensity has insightful affect on growth kinetics, biomass production, lipid accumulation, oil production and nutrient reutilization potential of microalgae (Gao et al., 2022). Wu et al., (2017) observed that slight change in light intensity causes considerable variation in metabolic activities and cell morphology. Microalgae respond to the changing light conditions by regulating photosynthetic machinery to absorb and utilize incident light in excellent manner (Sforza et al., 2012). Generally, the sufficient supply of light energy during the growth period increases the accumulation of neutral lipids as energy reserve in microalgae. Cheirsilp and Torpee, (2012) observed that increased light intensity during cultivation of *Chlorella* sp. and *Nannochloropsis* sp. enhanced the lipid production (2 folds) and the composition of lipid was appropriate for biofuel production. Light greatly affects the fatty acid composition of algal biomass and few studies have focused to improve the fatty acid composition of microalgae under different light intensities (He et al., 2015).

Microalgae also experience seasonal and diurnal changes in temperature along with light irradiance under natural environmental conditions (Borowitzka and Vonshak, 2017). The unprecetendent rise in temperature due to human interferences has profound impacts on the earth ecosystem (García et al., 2018). Temperature influences the cellular composition, carbon dioxide fixation, nutrient uptake and growth rate of microalgae (Singh and Singh, 2015). Fanesi et al. (2016) reported that microalgae are able to adapt under different temperature conditions by competent energy partition between non-photochemical quenching, alternative electron transport pathways as well as growth. An increase in temperature below optimum level has positive effects on cell metabolism and enzymes associated with calvin cycle (Ras et al., 2013). Cultivation of microalgae under low temperature conditions affects the metabolic pathways, lag phase and reduce the chlorophyll and protein content of microalgae (Yadav and Sen, 2017). The slower growth under adverse conditions enhances the accumulation of energy molecule as well as carotenoid to maintain the essential cellular activities of the microalgae (Christov et al., 2001). Sajjadi et al., (2018) observed that higher temperature is more harmful as compared to low temperature. Cultivation of microalgae under high temperature conditions (38° C) may cause abrupt interruption of metabolism leading to the cell death. The changes in the algal cells under high temperature are easily visible as color changes from green to brown indicating cell discoloration (Converti et al., 2009). Extreme exposure to high temperature conditions may promote photo-inhibition and affects the growth profile of algae (Gupta et al., 2019). To counter the imbalance caused by high temperature between the anabolic and catabolic processes of the cell, reduced cell volume (Atkinson et al., 2003), photosynthetic and respiration rate are considered as the acclimatization strategy adopted by microalgae *Microcystis aeruginosa*, *Scenedesmus acutus* and *Dunaliella* species (García et al., 2007). Furthermore, exposure of microalgae to varying temperature conditions play key role in accumulation of lipid and carbohydrates (Jaiswal et al., 2020). Temperature effects the lipid yield (Roleda et al., 2013) and physiological processes of the microalgae by regulating the rate of biochemical reactions in the cell (Van Wagenen et al., 2012). The enzymes responsible for carbohydrate synthesis are known to be influenced by varying temperature condition during cultivation of the microalgae (González-Fernández and Ballesteros, 2012). Temperature strongly influences the TAG profile in algal biomass by increasing the proportion of saturated fatty acid under high temperature conditions (Wu et al., 2013). On the contrary, Srirangan et al., (2015) reported that activities of enzymes that stimulate the percentage of fatty acid in microalgae were down regulated under high temperature conditions.

1.6 Monoculture vs Consortia

Microalgae are suitable bioresource for wastewater remediation due to its adaptability under saline water, as well as nutrient and metal enriched environment (Li et al., 2022). Each microalgae species has its unique characteristics in terms of growth, biomass, carbohydrates, lipid productivity and tolerance towards stress conditions (Patel et al., 2017). Employing single organism for wastewater treatment has to compete with the existing wastewater diversity and complexity (Renuka et al., 2013; Renuka et al., 2021). Furthermore multiple stresses imposed by wastewater (organic matter, nutrients variation and metal pollution) as well as culture conditions could affect the growth, biomass production and remediation potential of microalgae. Unfortunately, the single microalgae species cannot resolve multiple issues and relying on monoalgal species for wastewater treatment is not viable option for sustainable wastewater management. Furthermore, the significant development in the field of algology has taken place however; the work on algal consortia is still in its infancy stage. Algal consortia can acts as the feasible strategy to address the diverse issues from wastewater remediation to simultaneous biomass and bioenergy production (Sharma et al., 2020; Renuka et al., 2021). Consortia may leads to the development of a reluctant biosystem that facilitates the elimination of various pollutants from wastewater concomitantly (Renuka et al., 2013; Choudhary et al., 2016). Consortia offer the perfect alternative to introduce the different algal species on the basis of promising remediation potential, tolerance and lipid productivity into the wastewater treatment system (Johnson and Admassu, 2013). Therefore, employing algal consortia for wastewater treatment can serves as the advantageous system as microalgae with different metabolic rate and adaptability can certainly improve the removal of multiple pollutants and overall efficiency of the wastewater treatment system (Johnson and Admassu, 2013; Renuka et al., 2021).

Since, most of the studies related to wastewater treatment through microalgae focussed mainly on the nutrient and metal removal however, the research on photosynthetic efficiency, biochemical modifications, stress biomarkers and antioxidants defense mechanism adopted by algal consortia treated with municipal wastewater has not been thoroughly studied. The present work was based on the hypothesis that consortia may enhance the stability of the wastewater treatment system with increased biomass production, lipid yield and improve underlying defense mechanism against different wastewater concentrations. Further, the study was carried out as the holistic approach for screening of tolerant algal consortia keeping into consideration the different environment factors such as varying light intensity and temperature conditions under natural environment. On the basis of question formulated, hypothesis developed and analysis of the work reported in the pertinent literature, the following specific objectives were formulated:

1.7 Objectives

- Quality parameter analysis of wastewater collected from different locations of Lucknow
- Remediation efficiency and tolerance of the monoalgal species for natural condition of wastewater and heavy metal contamination
- Estimation of growth, biomass production, and photosynthetic performance of algal consortia treated with municipal wastewater under laboratory conditions
- Evaluation of biochemical characteristics and biofuel production efficiency of algal consortia under different light intensity and temperature conditions
- Assessment of adaptability and tolerance potential of algal consortia treated with municipal wastewater in natural environment

Chapter 2
Review of Literature

Chapter-2

Review of Literature

2.0 Review of Literature

Water is indispensable resource for the sustenance of life and balance between different ecosystem (Mushtaq et al., 2020). Since last few decades, the demand for freshwater has increased by approximately 8 times (Wada et al., 2016). The extraordinary population expansion is the forefront cause of freshwater scarcity, water pollution and energy depletion besides urbanization, industrial development, enormous wastewater generation, environmental pollution and ultimately global warming also contributes significantly (Singh et al., 2021). Much attention is being paid towards sanitation but still the problem of water pollution is worsening at rapid rate (Herrera, 2019). The unplanned establishment of cities, inferior sewage disposal system, lack of appropriate technologies and financial resource has created huge gap between wastewater generation and treatment in various developing countries especially India. As per the statistical report of UNESCO, highest quantity of water is used by agriculture sector (70%) followed by industries (22%) and domestic sector (8%) (UNESCO, 2003). As per the CPCB, India, 40 billion litres of wastewater is produced from urban areas daily and 70-80% of wastewater is discharged directly into the environment (CPCB, 2011). Amerasinghe et al., (2013) reported that wastewater generation in India may exceed to 600 million tonnes by 2030 due to massive shifting of large number of population in urban area. The substantial quantity of water used for different sectors often ends up as the wastewater. One of the prominent sources contributing to an increase in the load of contaminants is disposal of municipal wastewater. Water is used to accomplish day to day activities and discharged water is designated as municipal wastewater. This includes the water from bathrooms, kitchens and toilets (Razzak et al., 2013). The small industries in urban areas often discharge enormous quantity of wastewater to the municipal wastewater collection system (Razzak et al., 2013). Thus, municipal wastewater is the mixture of domestic, agricultural and industrial wastewater (Guldhe et al., 2017) and is abundant in nutrients, heavy metals, recalcitrant pollutants, emerging contaminants and disease causing organism (Sarkar et al., 2019) (Fig. 2.1). Certain pollutants are present in wastewater in very meagre amount but due to the lack of proficient remediation technologies, such pollutants has become the serious cause of environmental deterioration (Shahid et al., 2020). Discharging of contaminant rich wastewater are the forefront cause of serious environmental concerns (Leiva-Candia et al., 2014) such as eutrophication, hypoxia, turbidity, and loss of biota in the aquatic ecosystem (Jaiswal and Pandey, 2019).

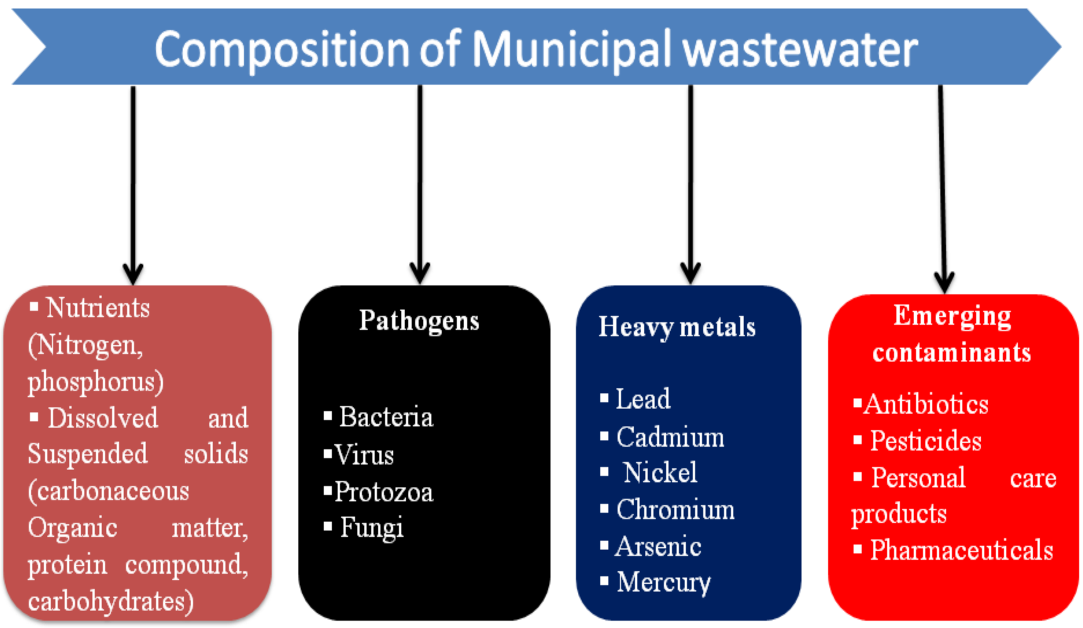


Fig. 2.1 Composition of municipal wastewater

Table 2.1. Physico-chemical attributes of wastewater

pH	7.49	8.39	7.35	7.2	7.3	8.40	6.5-11.5
EC	1165.5 (µS/cm)	2.84 (dS m^{-1})	2.15 (mS/cm)	663 mS/cm	3368 mS/cm	0.1148 S/m	-
TDS	930.34	-	1109	489	-	316	200–440
TSS	18.36	1824.42	1293.7	166	-	2.489	320–450
BOD	11.67	620.27	-	341.5	270	-	900–1100
Nitrate nitrogen	2.72	84.99	1.85	62	6.2	55.5	-
Phosphorus	3.502	124.42	2.64	7.2	2.98	25.63	-
Refrences	Mohanakavitha et al., (2019)	Kumar and Chopra, (2012)	Moondra et al., (2020)	Fito and Alemu, (2018)	Ali et al., (2021)	Pandey et al., (2019)	Mandal et al., (2010)

All values are in mg/L, otherwise mentioned

Due to the complexity of wastewater (Table 2.1), it is important to rely on the techniques which may fulfill diverse aspects such as nutrient removal, metal detoxification, energy production and meet discharging standards in eco-friendly manner. In developing countries, wastewater treatment facilities are not well structured, organized and equipped to remove diverse types of pollutants. Several physico-chemical treatments such as electro-coagulation, chemical precipitation, reverse osmosis, and ultra-filtration are utilized for wastewater tretament (Bouchelkia et al., 2016) but being expensive and energy intensive has restricted their use on wide scale (Table 2.2). It is important to mention that the wastewater after treatment through conventional techniques still contains enough quantity of contaminants

Table 2.2 Advantages and disadvantages of different wastewater treatment methods

Treatment Method	Advantages	Disadvantages	References
Advanced Oxidation process	User friendly, less maintenance	High use of chemicals and manpower,	Gautam et al., (2019)
Electro - coagulation	Less maintenance, easy to operate, no secondary pollution, less energy requirement, treat diverse wastewater	High maintenance, regular replacement of electrodes,	Syam Babu et al., (2020); Rajoria et al., (2021)
Membrane separation	High compactness, Effectively remove pollutants	Membrane fouling, high operational cost,	Hube et al., (2020)
Activated sludge	Avoid excess biomass accumulation, Low installation cost, sustainable	High operating cost, need skilled supervision	Hao et al., (2018)
Phytoremediation	Cost effective, eco-friendly restore contaminated soil, maintain soil health,	Relocate toxic metals, slow process, low uptake, time consuming, low biomass production	Farraji et al., (2016)
Mycoremediation	Promising to remediate contaminated environment, ecofriendly and efficient, tolerant to toxic pollutants	Less efficient at low pollution level, Require suitable conditions for growth and development	Kumar et al., (2021)
Phycoremediation	Quick growth, short life span, high photosynthetic efficiency, remediate wastewater of different origin, green synthesis of different biomolecules, high carbon sequestration efficiency, excellent resource reutilization potential and biofuel production	Extreme exposure limits growth and biomass synthesis, high nutrient and water requirement	John et al., (2020); Singh et al., (2021); Upadhyay et al., (2021)

(Cai et al. 2013) which can alter the pH, biological oxygen demand, dissolved oxygen, salinity and concentrations of heavy metals in receiving water bodies (Atoku et al., 2021). The complexity of wastewater, prevailing environmental condition and rapid economic development has necessities the implementation of flexible and modified technologies to meet the effluent discharge standards in different countries. Implementation of such techniques in water deficient regions is of major importance as inappropriate management of wastewater might leads to stern social and environmental conflicts (Qu et al., 2019).

2.1 Fossil fuel scarcity and alternative fuel

Dwindling fuel reserve followed by toxic pollutant released on fuel combustion is further posing risk to the environment (Islam and Mostafa, 2021). Approximately, 85% of crude oil is used for the production of transportation fuel and 10% is being utilized for various products in different industries (Tandon and Jin, 2017). The total dependence on fossil fuels is mootable issue as climate change and decline in oil reserves are continuously accelerating at rapid pace (Martin-Roberts et al., 2021). The fossil reserves are predicted to get exhausted in the near future and the world will face one of the largest challenges of the acute energy shortage (Chisti, 2007; Kamran et al., 2022). The serious impact related to the combustion of fossil fuels provide stimulus for exploitation of renewable and sustainable sources. The constant increase in the energy demand and depleting oil reserves has increased focus on biobased energy production to fulfil the energy demands of the future generations (Yana et al., 2022). Currently, 10% of the world energy demand are fulfilled by biomass energy and have huge potential to contribute more towards fulfilling the rising energy demands. In order to alleviate the ecological impacts of fossil fuels, it is imperative to enhance biofuel production on wide scale keeping sustainability of fuel on priority. The paradigm is shifting towards technologies that can address the problem of wastewater remediation and bioenergy production simultaneously (Zhang and Liu, 2022).

2.2 Role of microalgae in wastewater remediation and simultaneous biofuel production

Microalgae are gaining immense popularity as one of the neotheric and sustainable approach towards wastewater remediation and energy production (Singh et al., 2021). Microalgae are microscopic, prokaryotic or eukaryotic, chlorophyll containing organism living in both fresh as well as marine water having mechanism of photosynthesis similar to that of land plants (Hachicha et al., 2022) (Fig. 2.2) and the biomass produced has multifarious role in industrial sector.

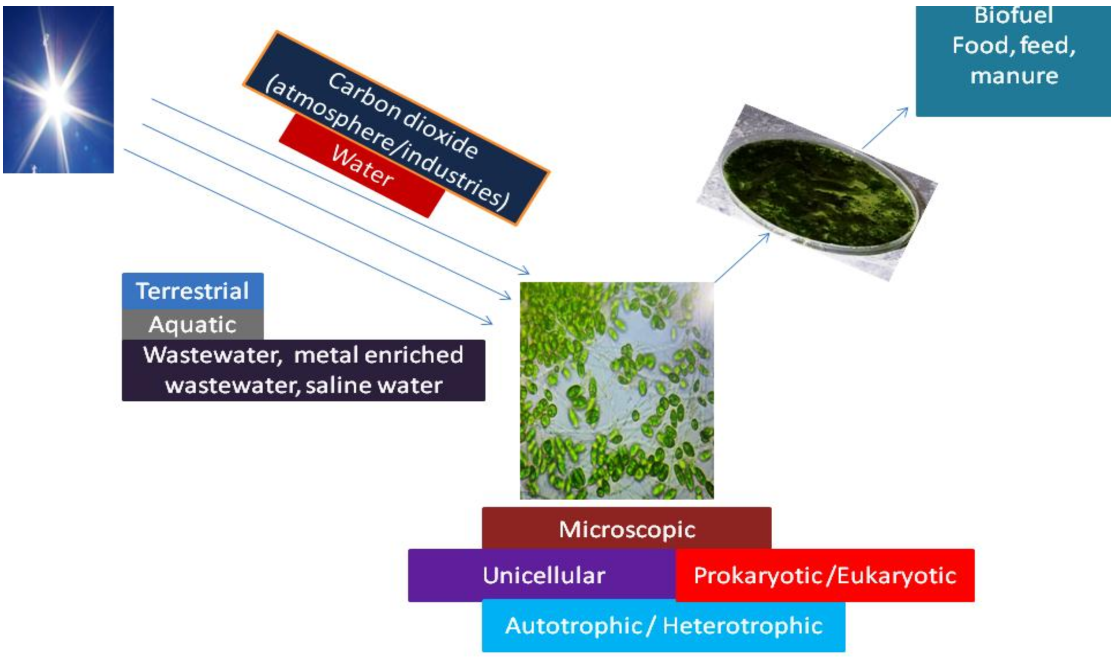


Fig. 2.2 Chracterisitics of microalgae

Microalgae synthesize their own food and play key role in maintaining the balance of different ecosystem. Microalgae, ubiquitous in almost every ecosystem are responsible for almost half of the photosynthesis on the earth (Pikula et al., 2019) and their ability to adapt in adverse conditions makes them suitable candidate for the remediation of contaminants prevalent in the contaminated environment (Lutzu et al., 2020**).** The microalgae outperform other energy feedstock due to high sunlight conversion efficiency, CO_2 fixation (Schenk et al., 2008) and abudance of carbohydrates (60%), protein (65%) and lipid (70%) (Afzal et al., 2017) make it alternate substrate for biofuel production (Khan et al., 2018). Additionally, their ability to adapt, tolerate and quick growth makes microalgae perfect organism than terrestrial plants for energy production (Costa and de Morais, 2014) and wastewater treatment (Singh et al., 2020). The nutrient and water requirement can be minimized by exploiting wastewater as the nutrient enriched growth media for microalgae (Fernández et al., 2019).

Microalgae such as *Chlorella* sp., (Asadi et al., 2019), *Scenedesmus* sp., (Morillas-España et al., 2021), *Phormidium* sp., (Das et al., 2018) *Botryococcus* sp. (Arif et al., 2020), *Spirulina* sp. (Cardoso et al., 2021), *Chlamydomonas sp.* (Qu et al., 2020) have shown tremendous potential to remove nitrate, phosphate, total dissolve solids, total solids and metals from cotaminated environment. Algal species has shown promising potential in artificial wastewater (Zhou et al., 2021), textile wastewater (Fazal et a., 2021), food processing (Gani et al., 2022), tannery wastewater (Rajalakshmi et al., 2021), aquaculture wastewater (Fan et al., 2021), diary wastewater (Kusmayadi et al., 2022), brewery wastewater (Ashraf et al., 2021), municipal wastewater (Fito and Alemu, 2019), meat processing wastewater (Hu et al., 2021) and urban wastewater (La Bella et al., 2022). The algal cultivation in diverse wastewater requires the adjustment in pH (Huo et al., 2012) as well as toxicity by dilution (Kothari et al., 2012) and mixing wastewater of different origin (Lu et al., 2016) to make it appropriate for microalgae.

The wastewater treatment through microalgae is based on the following approaches:

- Wastewater can acts as the suitable alternative in places of synthetic nutrient media.
- Microalgae based treatment system can simultaneously reutilize nutrients, degrade organic matter and detoxify toxic pollutants.
- The algal biomass can be utilized as feedstock for multiple products especially biofuel which can further enhance the economical feasibility of algal based treatment systems.

Wastewater remediation concomitantly with biofuel production is potential alternative to improve monetary viability of microalgae based treatment system (Vasistha et al., 2021). Further, microalgal biomass as source of diverse products can improve human development index by providing green energy, employment, income and other health benefits (Katiyar et al., 2017).

2.3 Microalgae based nutrient resource recovery from wastewater

Nutrients are abundantly available in the environment (Nazari-Sharabian et al., 2018) but atmospheric deposition (Richon, et al., 2018), sewage (Zhang et al., 2021), manures and fertilizers are exeggerating the problem of nutrient pollution (Sommer and Knudsen, 2021). Nutrients are essential for proper functioning and growth of live forms but overdischarge can have adverse health as well as ecological effects (Nieder et al., 2018). The nitrogen and phosphorus discharged in domestic wastewater after human metabolism is an important component of global nitrogen and phosphorus cycle (Reinhard et al., 2017). Nutrient mainly nitrogen from human excreta is responsible for approximately 15-20% of anthropogenic reactive nitrogen per year (Larsen et al., 2016). The treatment facilities exploited for the removal of nutrients are incapable to remove different nitrogen forms from the wastewater (Abeysiriwardana-Arachchige et al., 2020)**.** The conventional techniques for nutrients removal from wastewater are neither economical nor facilitate the nutrient resource recovery and reuse (Shi et al., 2014). The excessive nutrient discharge overstimulate the growth of aquatic flora causing severe consequences such as algal bloom formation, depletion of dissolved oxygen and loss of aquatic biota (Cravo et al., 2022).

Unlike plants, microalgae lacks root system for the uptake of nutrients and relies totally on small size and large surface area for fixation of carbon dioxide, uptake of water and nutrients (Udaiyappan et al., 2017). The nitrogen and phosphorus remediation efficiency of algae from wastewater is dependent upon their availability as well as interaction between pH, light intensity, temperature and other micro-organisms (Delgadillo-Mirquez et al., 2016). Nitrogen is essential component of cell and is involved in the synthesis of protein, chlorophyll (Wang et al., 2013) and genetic material (Silva et al., 2015). The nitrogen content in algae ranges from 1->10% and is strongly dependent upon the type, concentration as well as availability of nitrogen source in the growth media (Richmond, 2004). Microalgae *Tetradesmus obliquus* grown in municipal wastewater recovered nitrogen (76.99–100%) and phosphorus (97.64–100%) and nutrient level decreased considerably with exposure time (Kong et al., 2021). Microalgae *Chlorella pyrenoidosa* cultivated in riboflavin manufacturing industry effluent for 8 days leads to decline of nitrogen (64.5%) and phosphorus (82.2%) with good biomass productivity (1.25 g/L) (Sun et al. 2013). Caporgno et al. (2015) also observed that microalgae *Chlorella kessleri* after 11 days of cultivation in urban wastewater removed nitrogen (96%) and phosphorus (99%) with biomass productivity of 2.70 g/L. After nitrogen, phosphorus is another essential macronutrient vital for several biomolecules (RNA, DNA, ATP) in the cell (Juneja et al., 2013). Phosphorus is key nutrient which play significant role in growth and energy metabolism in microalgae. The content of the phosphorus in the biomass ranges from 0.05%-3.3% (Grobbelaar, 2004). Phosphorus is non renewable resource and it is believed to get exhausted in the coming years (Elser, 2012). Orthophosphate is the preferred phosphorus form assimilated by algae as compared to other inorganic and organic forms (Lavrinovičs et al. 2020). Phosphorus in the form of orthophosphate is taken up by active mechanism whereas passive mechanism is responsible for influx of tiny proportion of inorganic phosphorus (Cembella et al., 1982). However, phosphorus present in abundant amount is assimilated by microalgae at elevated rate as luxury uptake and is stored in the vacuole as polyphosphate granules. These granules are utilized as the source of phosphorus under phosphorus limited condition by microalgae (Gupta et al. 2019). Ahmad et al. (2019) observed that *Tetradesmus obliquus* efficiently removed 94% of phosphorus through absorption, cellular assimilation, volatization as well as precipitation from municipal wastewater.

2.4 Nutrients limited growth conditions as the cheap factor for enhanced lipid accumulation

Modulation of the nitrogen and phosphorus level in the growth media not only influences the composition but also the lipid accumulation in algae (Zhang et al. 2019). Under nutrient limited conditions, microalgae alter the composition of biomass by stimulating the accumulation of either carbohydrate or lipid (Chen et al., 2015) or by altering the protein or pigment content of microalgae (Pancha et al., 2014). Nitrogen deprived culture conditions is one of the efficient strategy (Shi et al., 2017) for lipid and carotenoid synthesis as well as accumulation of triglycerides (Juneja et al., 2013). Adams et al. (2013) reported that during the early stage of nitrogen starvation, the carbon fixation exceed the carbon demand and the excess carbon is directed towards biosynthesis of storage compounds like carbohydrate and lipids by microalgae. Anand and Arumugam, (2015) observed that lipid content was increased by 2.27 times in *Scenedesmus quadricauda* under nitrogen limited culture condition. Algal species such as *Botryococcus braunii, Chlamydomonas, Scenedesmus* and *Chlorella* have been recoganized as the efficient assimilators of nutrients along with lipids accumulation (Abdel-Raouf et al., 2012). Similarly, phosphorus deprived conditions affects energy requiring processes and enhance accumulation of TAGs in microalgae (Muhlroth et al., 2017). The phosphorus deficient growth conditions not only influence the biosynthesis of chlorophyll but also metabolic activities of microalgae (Liang et al., 2013). Li et al. (2014) observed that phosphorus limited wastewater conditions increased lipid accumulation by 32% in *C. protothecoides* while *Chlorella* species showed lipid accumulation of 53% and lipid productivity of 23.45 mg L^{-1} (Wong et al., 2017). The reasons responsible for lipid accumulation in algal cells under nutrient limited conditions could be attributed to diverting of photosynthetically fixed carbon towards lipid synthesis (Roy et al., 2021). Although, the nitrogen starved growth conditions stimulated the lipid accumulation but, the biomass yield declined by more than 80% which ultimately affects the economic feasibility of algae for biofuel production (Suparmaniam et al., 2022). The decrease in biomass productivity under nitrogen limited condition can be overcomed by using nitrogen source which enhances the lipid accumulation without compromising with biomass production, cell metabolism and physiology (Olofsson et al., 2014). Tang et al. (2011) reported that biomass yield of *T. obliquus* was increased by 2-3 folds using nitrate as the nitrogen source than ammonia and urea. Suparmaniam et al., (2022) observed that drawbacks associated with nitrogen deficiency can be compensated by the supplementation of optimum concentration of phosphorus as an efficient strategy to enhance the biomass yield. The increment in biomass yield could be attributed to sufficient supply of phosphorus that was used by the algal cells to sustain the activity of different enzymes responsible for synthesis of lipid, energy molecules and genetic material (Chu et al. 2013). The excess supply of phosphorus (≤ 45 mg/L) under nitrogen deficient conditions not only stimulates growth but also increase the biomass production (10.2%) and lipid productivity (39.3%) in *Chlorella vulgaris* (Suparmaniam et al., 2022). Furthermore, in order to improve the lipid and biomass productivity simultaneously, the two stage cultivation system has been considered as the feasible approach to overcome the limitation posed by the nutrient starved growth conditions (Roy et al., 2021).

2.5 Heavy metals: source and remediation by conventional techniques

Heavy metal is the most commonly used term globally and defined as metal having specific density greater than 5 g/cm^3 (Mohan et al., 2021). The classification of heavy metals is based on atomic number, mass and density but still no collectively accepted definition has been proposed (Duffus, 2002). The term heavy metals has been associated with metals and metalloids which even in small quantity can have detrimental impacts on the living organisms (Duffus, 2002; Pujari and Kapoor, 2021). Both essential and non essential heavy metals are released through natural phenomena as well as athropogenic interferences (Meena et al., 2018).

Heavy metals are persistent, nondegradable, carcogenic, metagenic and tertagoneic (Tchounwou et al., 2012). Due to toxicity, bioaccumulation and non-biodegradable nature, heavy metals accumualtion has emerged as the severe environmental problem (Rehman et al., 2021). Metal accumulation is major concern for organisms particularly humans get contaminated through the process of biomagnifications (Chojnacka, 2010). Industries such as paper, dyeing, plastic, agrochemicals, textile, paint, cement and steel are continuously discharging metal enriched wastewater (Kobielska et al. 2018; Cui et al., 2021; Elgarahy et al., 2021; Özdemir et al., 2021). Fuel combustion, military operations, and agrochemicals also releases significant quantity of metals in the nearby ecosystem (Zhen-Guo *et al.*, 2002). The other major industries such as lead acid batteries, mining, tannery, dye and waste dumpsites also contributes towards environmental pollution (Pure Earth, 2016). As per WHO recommendation for drinking water, the acceptable limits for Pb and Cd should not go beyond 0.001 and 0.003 mg/L, respectively (WHO, 1998/2003). As per EPA, 2009, the permissible limits of Pb, Cr, Cd, Zn, and Ni in drinking water should not be above 0.015, 0.1, 1.3, 0.005, 5, and 0.04 mg/L, respectively (EPA, 2009).

Heavy metals are the major component of the wastewater discharged from the different sectors of the society. Heavy metals laden wastewater can contaminate soil, groundwater and surface water ecosystems (Sadeghi et al., 2022). Different techniques such as chemical precipitation (Chen et al., 2018), ion exchange (Dong et al., 2018), reverse osmosis (Thaçi and Gashi, 2019), electrocoagulation (Fakorede and Adewumi, 2020) and adsorption (Khulbe and Matsuura, 2018) has been exploited for wastewater treatment but such techniques failed to accomplish the targets of economical and sustainable management of wastewater. Since, heavy metals are resistant to degradation, the only possible way to reduce their concentration is to remove such metals from cycling process followed by recovery and reuse. In order to reduce metal concentration and their impacts on human health, several types of plant based biomass has been examined by scientists for metal removal from contaminated ecosystem. Environmental Protection Agency (EPA) has also encouraged the development of economical and novel techniques for protection of environment from different types of pollutants.

2.6 Role of microalgae in heavy metals removal from contaminated environment

Microalgae are considered as the promising organisms that can remove diverse metals from wastewater (Goswami et al., 2020) (Table 2.3). The remediation potential is dependent upon the type of heavy metal, concentration of metals, biological organism and prevailing environmental condition during the process of bioremediation (Pratush et al., 2018). In order to grow profusely and detoxify metals, microalgae have evolved certain mechanisms responsible for imparting tolerance against metal contaminated environment (Kumar et al., 2015). Mehta and Gaur, (2001a) reported that Cu and Ni removal by *Chlorella vulgaris* was in the range of 37%-42% with initial concentration of 2.5 and 10 ppm respectively. Venkatesan and Sathiavelu, (2022) reported that *Scenedesmus* sp. removed 72.80% of Cr from tannery wastewater while, *T. obliquus* eliminated 50-65% of Cu and Ni within 15-30 minutes from wastewater (Rugnini et al., 2019). Several constitutive mechanisms are exhibited by microalgae to remove metals from diverse ecosystem and serve as potential candidate for treatment of metal enriched wastewater (Tripathi and Poluri, 2021). In aquatic ecosystem, microalgae sequester large amount of heavy metals and their ability to absorb and further metabolize metals is mainly due to large surface/volume ratio (Anjana et al., 2007), metal binding groups (Leong and Chang, 2020) and efficient metal uptake rate, transport and storage system (Inthorn et al., 2002).

Table 2.3 Remediation efficiency of microalgae treated with wastewater under different operating conditions

Wastewater	Metal removal	Microalgae	Operating conditions	Refrences
Tannery wastewater	Cr-81.2–96%, Pb-75–98%	*Scenedesmus* sp.	Temperature: 27° C, Light intensity and duration: 4000 lux, 16 (light) : 8 (dark)	Ajayan et al., (2015)
Industrial wastewater effluent	Cr-97.57%, Pb: 94% Fe: 97.94% , Cu: 91.77% , As: 66%	*Anabaena variabilis*	Light intensity: 3000 Lux, Temperature: 25˚ C	Elsadany, (2018)
Municipal wastewater	Cd, Ni, Pb- 100%	*Scenedesmus* sp.	Duration: 14 days	Tripathi et al., (2019)
Textile mill wastewater	Fe-74.47%, Cd-17%	*Chlorococcum humicola*	Duration: 6 days, Algal inoculums: 1.52–1.81 × 10^7 cells/mL, Temperature: 30.4˚C, Light intensity: 145 µmol/m^2/s	Borah et al., (2020)
Wet market wastewater	Fe: 59.33%, Zn: 79.65%, Cu: 100% Fe: 65.76%, Zn: 84.14%	Scenedesmus sp.	Duration: 8 days	Jais et al., (2017); Wan Mohamad Apandi, and Matias Peralta, (2015)
Domestic wastewater	Zn: 71.5%, Fe: 51.2%, Cd: 83.5%, Mn: 97.2%	*Botryococcus* sp.	Duration: 18 days	Gani et al., (2017)
Mining wastewater	Fe: 99%, Pb: 95%, Zn: 52%, Cu: 94%	*Arthrospira platensis*	pH>7.1	Randrianarisona et al., (2021)

Adsorption, complexation, precipitation and bioaccumulation are the mechanisms adopted by microalgae to eliminate the toxic metals (Fernandez et al., 2018) (Fig. 2.3). Microlgae can eliminate metals and protect the biota from the detrimental impacts of higher metal concentrations (Randrianarison and Ashraf, 2017). Living algal biomass primarily shows two-phase kinetic sorption. In first phase, algae remove heavy metals through passive process and in second phase, by the active transport of metals utilizing metabolic energy (Monteiro et al., 2012). The first line of defence that the microalgal cells evolved in the contaminated environments is the reduced uptake of pollutants by binding to negatively charged functional groups of the cell wall (Pradhan et al., 2019). Adsorption to algal cell surface occurs by ionic exchange; covalent bond formation and also binding with exopolysaccharides that reduce metal concentration and protect microalgae from metal damage (Leong and Chang, 2020). The intracellular metal transport occurs when the extracellular concentration is more as the binding groups may become saturated and enable the metal transport into cytoplasm (Monteiro et al., 2012). Bioaccumulation of heavy metals inside the cell is very slow process and metals are transported inside the cytoplasm using metabolic energy followed by binding with certain sulphur containing compounds such as cysteine (Pradhan et al., 2019). Like cysteine, histidine, glutamate and proline play key role in the chelation and metal detoxification (Kumar et al., 2015). Heavy metals through binding with multivalent carriers and chelating proteins may enter into the cell by the process of endocytosis. Sharma et al., (2016) observed that vacuolar sequestration is an important mechanism of pollutants detoxification driven by transporters and vacuolar pump. The biosynthesis of peptides such as phytochelatins and metallothioneins under metal stress is one of the favored mechanism of metal detoxification. Balzano et al. (2020) reported that phytochelatis (PCs) and metallothioneins (MTs) has been observed in microalgae exposed to metal contaminated environment. Several researchers reported that the metal like cadmium, lead, copper, nickel, zinc and mercury stimulates the PCs production in microalgae (Tripathi and Poluri, 2021). The length of PCs is key for the formation of stable complexes with metals as stability increases with the length of the PCs. Additionally, microalgae also adapt self protection mechanism under heavy metal stress by stimulating chelate formation, metal exclusion, enhance antioxidants activity and regulate gene expression related to tolerance towards heavy metals (Gómez-Jacinto et al., 2015).

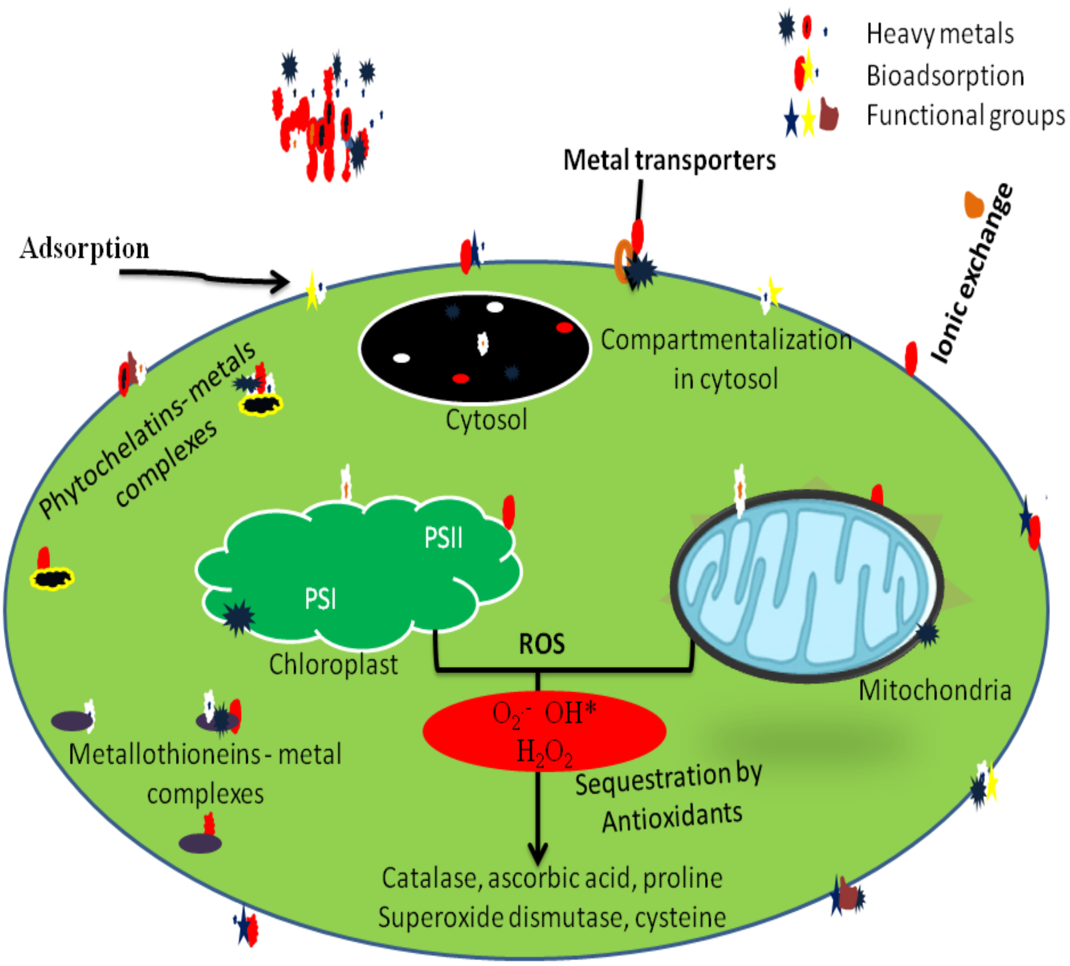


Fig. 2.3 Mechanisms adopted by microalgae for metal removal from contaminated ecosystem

2.7 Effect of heavy metals on microalgae

The photosystem II (PS-II) is very sensitive to metal damage (Moustaka et al., 2018). Heavy metals directly affects the light as well dark reaction and also alter the functioning of the stomata (Appenroth, 2010). Kupper et al., (2002) reported that heavy metals perturbs the light harvesting complex II by displacing magnesium with copper and zinc and influence the activity of PS II in microalgae (Fig. 2.4). Metals directly react with multiple sites in electron transport chain (ETC) (Rai et al., 1991) and inactivate the reaction centres of PS II (Prasad et al., 1991). Heavy metals distort the structure of the chloroplast membrane, suppress the activity of photosystems, obstruct the electron transfer in photosystems, reduce pigment formation, inhibit the process of photosynthesis (Suresh Kumar et al., 2014) and also perturbs the activity of light harvesting as well as oxygen evolving complex (Pontevedra-Pombal et al., 2012). Rezayian et al., (2019) observed that ROS generation occurs in PSII when the excitation energy to photosystems is limited. The amplified generation of ROS damages the photosystem II reaction centre and eventually results in loss of cell vitality (Dutta et al., 2018). Metals target light harvesting complex of photosystems, inhibit activity of nitrate reductase, glutamate synthetase and carbonic anhydrase (Ackova, 2018) as well as biosynthesis of chlorophyll (Zamani-Ahmadmahmoodi et al., 2020). Heavy metals also affect enzyme activity through displacement of essential metal ion (Hossain et al., 2012) as some heavy metals replace magnesium from RUBISCO leading to the inhibition in their activity (Van Assche and Clijsters, 1986). At higher concentration, heavy metals affect carbon dioxide assimilation by binding with RUBISCO and inhibits the activity of RUBP carboxylase which lead to cellular changes by decreasing photosynthetic rate, pigment content and quantum yield of PS-II (Song et al., 2019). Metals stimulates the morphological, metabolic and physiological alterations leading to degradation of polyunsaturated fatty acids (PUFA) (Xiong et al., 2017), inhibit growth and normal functioning of the cell (Dutta et al., 2018). Sun et al., (2018) also observed that PUFA are essential structural component

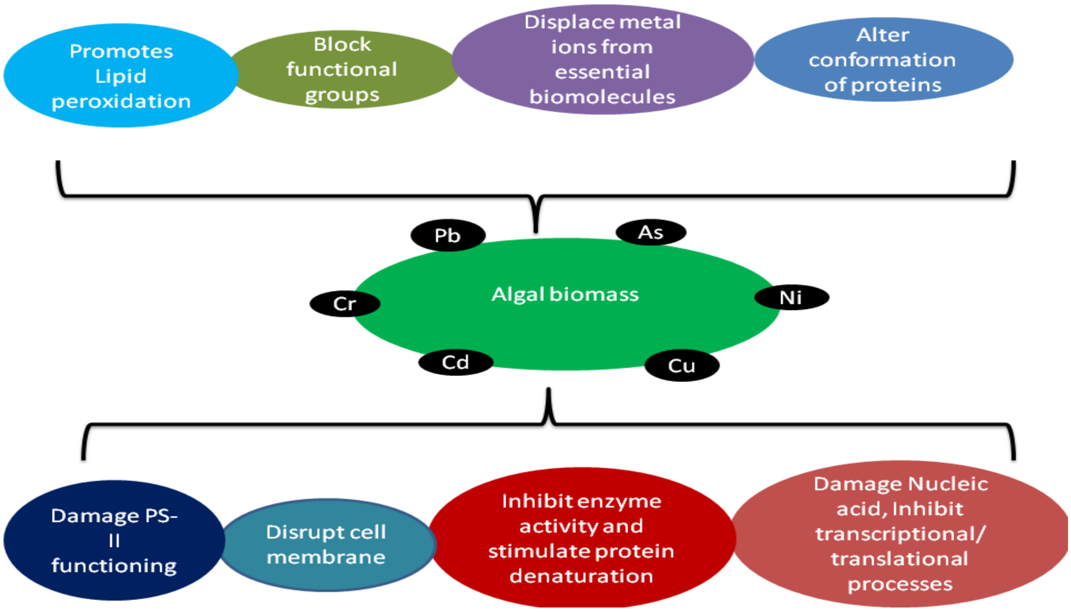


Fig. 2.4 Effect of metals on the cellular components of algae

but their peroxidation affect membrane fluidity leading to interruption of physiological and functional activities of microalgae.

2.8 Effect of light intensity and temperature regimes on microalgae

Light is main abiotic factor responsible for proper growth, photosynthesis and biomass production in microalgae and plants (Ho et al., 2012). Light intensity to a great extent affects the cellular composition, metabolic pathways as well as economic efficiency (He et al., 2015) and maintaining proper light intensity has been the major bottleneck in the commercial cultivation of microalgae. The growth kinetics increases with increase in light intensity but enhancement above the saturation level have lethal effects on growth and survival of microalgae (Straka and Rittmann, 2018). Under increasing light intensity condition, the microalgal growth kinetics is divided into four phases comprising lag (no change in growth), light limitation (increased growth with intensity of light), light saturation (growth is not dependent on light intensity) and light inhibition phase (decreased growth of algae with increasing light intensity) (Ogbonna and Tanaka, 2000). Patel et al., (2019) observed that beyond saturation point, the cellular biomolecules synthesis decreased with light intensity. In *Chlorella vulgaris*, light limitation was observed between 0-9 W/m^2, light saturation phase occurred between 9-18 W/m^2 while light inhibition was completely observed above 18 W/m^2 (Yeh et al. 2010). Several species of microalgae showed an increase in biomass with a light intensity which could be attributed to enhanced reproduction up to saturation point and beyond that decrease in biomass production could be due to photo-inhibition (Barbara et al., 2013). It is well recognized that the photo-damage is related to light intensity (Simionato et al., 2013). Under high light intensity condition, excess energy promotes photodamage to the cell by reducing the acceptors and reaction centres of PS-II (Straka, and Rittmann, 2018; Fettah et al., 2021). The photoinhibition could also be due to the disruption of chloroplast lamellae and enzymetic activity responsible for carbon dioxide fixation (Juneja et al., 2013). Photo-inhibition leads to decrease in biomass as excess light supply may not be absorbed by the photosynthetic apparatus (Dall'Osto et al., 2019). Gururani et al., (2015) reported through physiological and biochemical studies that microalgae prevent the damage by down-regulating the activity of PS-II under light stress conditions. Znad et al., (2012) observed that supply of higher light intensity damages the PS-II activity and eventually carbon supply for the photosynthesis while low light leads to photolimitaion which ultimately affects the growth and biomolecules synthesis in microalgae. Destruction of the photosynthetic machinery under high light irradiance occurs due to variety of oxidative radicals produced during the process of photosynthesis (Sharma et al., 2020). Microalgae *Selenastrum capricornutum* and *C. vulgaris* exposed to low light/darkness and copper stress showed that ROS generation increased under the light condition indicating the light induced disturbance to the photosynthetic machinery (Knauert and Knauer, 2008). Microalgae *C. reinhardtii* after treatment with heavy metals under high light conditions showed increased metal accumulation and efficient tolerance expressed in the form of prolific growth as compared to low light intensity (Cheloni et al. 2014). Photosynthetic organisms also possess potent defense mechanism against higher light intensity (Shi et al., 2017). Cheloni et al. (2014) suggested that high light conditions upregulates gene expression responsible for the increased activity of enzymatic antioxidants (Nowicka, 2022). However, microalgae also possess array of antioxidants such as carotenoids (Guedes et al., 2011), superoxide dismutase (Chen, et al., 2020), proline, ascorbic acid, cysteine, glutathione reductase and catalase which may compensate the adverse affects posed by high light stress conditions (Shi et al., 2017).

2.8.1 Light and temperature induced lipid synthesis in microalage

Consistent variation in light intensity for longer time period triggers changes in cellular structures, compositions and functioning of the microalgae (Orefice et al., 2016). Light irradiance influences carbon sequestration as well as its allocation by modifying the cellular metabolism (Rismani-Yazdi et al., 2012; He et al., 2015)) and plays pivotal role in lipids and carbohydrate accumulation in microalgae (Iasimone et al. 2018). Wahidin et al., (2013) reported that supply of light exceeding the optimum level trigger the enhanced synthesis of lipid in *Scenedesmus abundance* and *Nanochloropsis sp*. Oxidative stress is responsible for changing carbon metabolic flux from glycolysis to the oxidative pentose phosphate pathway which leads to the accumulation of reduction equivalents in the form of NADPH. In order to protect the cell from excessive reduction equivalents, the lipids are accumulated as it require more reduction equivalents in place of proteins and carbohydrates (Shi et al., 2017). Microalgae not only adjust the carbon allocation favouring lipid accumulation but also amend their lipid metabolism towards accumulation of neutral lipid (TAG) under unfavourable conditions (He et al., 2018).

The fatty acid composition of biomass is important to determine the quality of the biofuel as it influence the combustion efficiency as well as heating power of engine (Talebi et al., 2013). In microalgae, the light dependent changes in fatty acids may be an adaptive mechanism to different light conditions (Yun et al., 2020). Gouveia and Oliveira (2009) observed that oil extracted from the biomass of *Chlorella vulgaris, Nannochloropsis oleabundans,* and *Scenedesmus obliquus* contains 50-65% of unsaturated fatty acids and 17-40% of palmitic acid. Saturated and monounsaturated fatty acids are main component of neutral lipid and their content increased by exposure of microalgae to higher light intensity (Hu et al., 2008). Microalgae like *Scenedesmus obliquus* exposed to light conditions of 180 µmol $m^{-2}s^{-1}$ showed the notable increase in percentage of different fatty acids as well as carbohydrate in algal biomass (Ho et al., 2012). Similarly, Sun et al., (2014) demonstrated that percentage of palmitic, stearic, and oleic acid was increased to a significant level after exposure to 300 µmol $m^{-2}s^{-1}$. Thus, effective utilization of light is imperative factor in algal technology and play importants role in determining growth, biomass and production of energy rich compounds (Nzayisenga et al., 2020). Furthermore, deeper understanding upto molecular level on light use efficiency and cellular mechanisms under high irradiance can assist to optimize algal cultivation along with biofuel production (Orefice et al., 2016).

Temperature is the other predominant factors which affects the algal growth (Chaisutyakorn et al., 2018). The global temperature is increasing at swift rate and several organisms are unable to adjust with the escalating temperature. Seasonal fluctuations, day to day variation and abrupt changes in temperature manipulate the growth profile and biomass production efficiency of algae (Sajjadi et al., 2018). Most of the species of microalgae are able to carry out their cell divisions and photosynthetic process under wide temperature range (15-30˚ C) and the optimum temperature differs with the species of microalgae (Sajjadi et al., 2018). Cultivating microalgae under optimal conditions enhance growth rate exponentially and supplementing extreme low or high temperature either slows or cease the growth, cell functioning, metabolism, and biomass production (Bechet et al., 2017). Roleda et al. (2013) observed that under low temperature conditions (10 °C), growth declined significantly and microalgae sustained the metabolic activities without undergoing cell division. In photosynthetic system, the PS-II is most sensitive for thermal stress. Exposure to temperature stress affect different processes such as membrane stacking, integrity and ion conductivity (Zhang and Sharkey, 2009). High temperature affects the cell growth but photosynthesis is more often inhibited before other cellular function (Haldimann and Feller, 2004). The most common effect of high temperature is inhibition of charge separation and oxygen evolving capability of photosystem II that eventually leads to the production of free radicals (Ras et al. 2013). The biological processes responsible for adaptation under unfavourable conditions are very complex. Under high temprature exposure, different mechanisms such as alteration in membrane permeability, unsaturated fatty acid composition (Chaisutyakorn et al., 2018), protective antioxidants (Snider et al., 2010) and increased flow of electron are adopted to mitigate the temperature stress (Zhang and

Sharkey, 2009). Some species of microalgae are able to grow upto temperature condition of 40 °C while some are very sensitive and cannot even tolerate the temperature below 25 °C (Converti et al. 2009). It has been reported that *Scenedesmus obliquus* showed declined growth rate as well as biomass productivity above 30 ºC (Martínez et al. 2000) while Li et al. (2011) observed that *Scenedesmus* sp. showed maximum growth at 25º C with good adaptation under temperature range of 10-30 ºC. Several authors have obtained optimum temperature conditions of 20 °C, 30°C and 35 °C for *Nannochloropsis oculata*, *Chlorella vulgaris* (Converti et al. (2009) and *A. Dimorphus* respectively (Chokshi et al., 2015). Guedes et al. (2011a) reported that *T. obliquus* showed higher biomass productivity (3 times) at 30° C as compared to 20 °C. The microalgae *A. dimorphus* showed an increase of 68% in biomass productivity at 35 °C while declined biomass productivity at 38 °C (Chokshi et al., 2015). The two-step method utilized to investigate the effect of two temperatures on microalgae revealed that temperature conditions of 30 °C increased the lipid production as compared to ambient temperature (Venkata Subhash et al., 2014). Previously, it has been also observed that decreasing temperature from 30 °C to 25 °C in *Chlorella vulgaris* showed considerable increase of lipid content (2.5 folds) without interfering in the growth of microalgae (Converti et al., 2009). Whereas, decreasing temperature from 25 °C to 20 °C showed increment in lipid content by 1.7 folds with 8% loss of growth rate in *Scenedesmus* sp. (Xin et al. 2011). The divergence from ambient temperature causes modification in fatty acids composition of microalgae (Pachiappan et al., 2015). The C16-C18 is the common fatty acid in microalgae after treatment under high temperature conditions. The higher content of C16-C18 fatty acids increases the suitability of microalgae for biofuel production (Knothe, 2009). James et al. (2013) observed that the accumulation of

TAG was increased with temperature in *C. reinhardtii*, while in *Desmodesmus* sp. lipid content remained stable under wide temperature range of 25° C to 40° C suggesting that enzymes responsible for lipid synthesis were not sensitive to varying temperature conditions. The enhancement in fatty acid saturation in microalgae under high temperature maximize hydrophobic interactions and counterbalance the increased cell membrane fluidity (Srirangan et al., 2015) while the increase in the degree of unsaturation influence the cetane number and oxidative stability of biofuel (Adu-Mensah et al., 2019).

2.9 Stress induced ROS generation in microalgae

In aerobic organisms, reactive oxygen species (ROS) like**,** hydroxyl radical (OH°), singlet oxygen (O_2), hydrogen peroxide (H_2O_2), and superoxide anion (O_2^-) occurs frequently under normal as well as stressful conditions (Ighodaro and Akinloye, 2018; Gauthier et al., 2020). Reactive oxygen species generation is slow under favourable conditions but the process accelerates after exposure of microalgae to adverse conditions (Ugya et al., 2020) (Fig. 2.5). At lower concentration, ROS play essential role by acting as signalling molecule that alter gene expression as well as defense mechanisms in the cell (Sachdev et al., 2021). The generation of ROS is accelerated when the gap between production of reduction equivalents does not match the consumption leading to leakage of electrons from electron transport chain (Gechev et al., 2006). The condition that results in the ROS generation has been categorized as oxidative stress. Chloroplast, mitochondria or peroxisomes are major sites responsible for generation of ROS in the cell (Gill and Tuteja, 2010) (Fig. 4).ROS are produced regularly in organelles (chloroplast, mitochondria) responsible

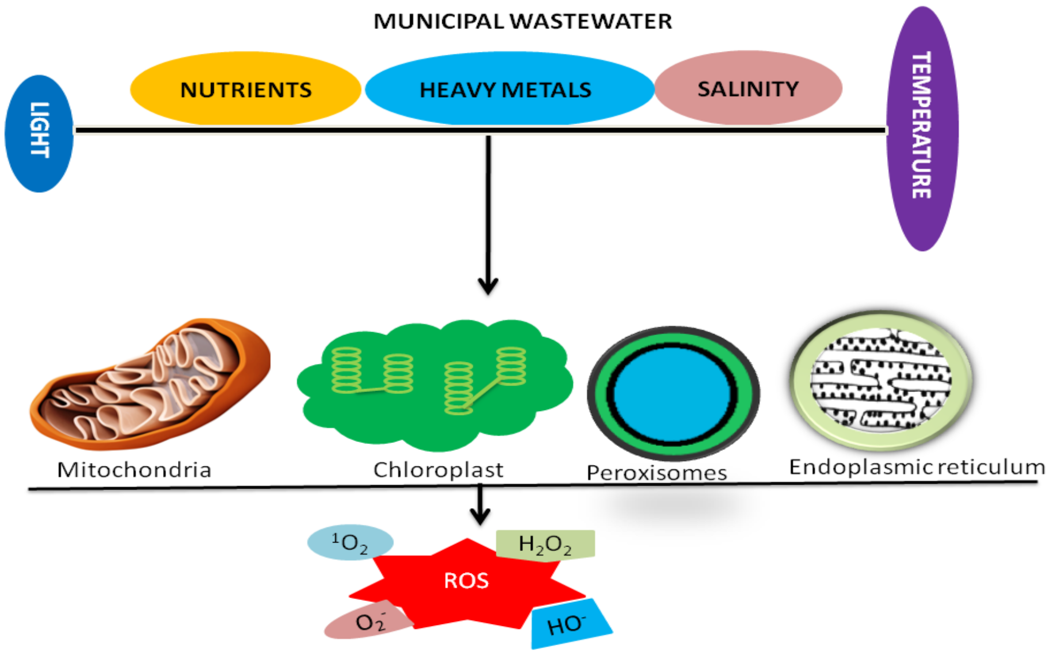


Fig. 2.5 Factors responsible for enhanced generation of ROS in microalgae

for oxygen metabolism in microalgae (Apel and Hirt, 2004). Out of the total oxygen consumption, 1-2% is involved in the formation of the ROS (Bhattacharjee, 2005). Oxygen is also involved in several kinds of reaction that leads to the formation of different ROS in the cell having detrimental impacts on the cellular biomolecules (Juan et al., 2021). The oxygen produced by the process of photosynthesis accepts electron through ETC and leads to the formation of superoxide radical (O_2^-) (Juan et al., 2021). Like plants, the whole process of photosynthesis occurs in chloroplasts as it contains a structured thylakoid membrane and light harvesting system that assists in accomplishing the photosynthesis in algae (Kumar et al., 2021).

The role of ROS totally depends upon their concentration in the cell (Chokshi et al., 2020). At low concentration, it serve as the signalling molecules, influence gene expression as well as important regulator of the growth, metabolism and response to different biotic and abiotic stress conditions (Talaat, 2019). In some cases, oxidative stress acts as store house of signals that organism use to make adjustment in the structure as well as gene expression to several environmental perturbations (Shcolnick and Keren, 2006). On the contrary, unfavourable growth conditions accelerate ROS formation which upon reaction degrade biomolecules, impairs the functioning of cell organelles, alter cell structure and also causes mutagenesis (Rezayian et al., 2019). The steady electron flow and lipids in cell membrane increases the possibility of the aerobic organism to oxidative damage (Von Moos and Slaveykova, 2014). Defense system involves the secretion of proteins, elevated storage of energy molecules, enhanced activities of enzymatic and non enzymatic system protects from oxidative damage and ensure to withstand the stress conditions (Shao et al., 2008). If the genomic responses to the stress are not sufficient, the vital functioning of the cell is hindered and the responses liable for the cell death are triggered (Shao et al., 2008).

2.10 Defense responses against oxidative stress

Antioxidants are essential compounds responsible for scavenging of ROS, provide protection to vital biomolecules and repair the damage caused by the oxidative stress (Halliwell, 2007). The presence of toxic pollutants evokes the oxidative damage through rapid generation of ROS (Li et al., 2016a) which causes damage to DNA, membrane stability, disturbs nutrient status and functioning of vital proteins and enzymes in the cell (Tamás et al., 2014). In order to ensure survival in contaminated environment, the microalgae has evolved a extensive mechanism to detoxify and promote tolerance against the toxic pollutants. The upregulation of the antioxidants activity has been observed in microalgae exposed to different stress conditions (Chokshi et al., 2017). The redox homeostasis of the cell cultivated under stressful environment is sustained by both enzymatic and non-enzymatic antioxidants

(Upadhyay et al., 2014). Nowicka et al. (2020) observed that enhanced activity of antioxidants occurs more predominantly in microalgae exposed to redox active than inactive metals. Non enzymatic antioxidants interfere in the process that leads to the production of ROS while enzymatic antioxidants repair the damage induced by ROS and remove the oxidised macromolecules from the cell (Pikula et al., 2019).

2.10.1 Superoxide dismutase (SOD)

SOD is ubiquitous, indispensible and endogenous antioxidants of plants defence system (Ighodaro and Akinloye, 2018) and is found widely in aerobic organisms/cell organelles susceptible to oxidative damage (Gill and Tuteja, 2010). SOD serve as the indicators of stress condition and extent of cell repair in microalgae (Li et al., 2020). In microalgae, SOD is one of the key enzymes responsible for annulment of ROS generated through oxidative stress. SOD is foremost antioxidant against superoxides and the enhanced activity is related to increased generation of superoxide (Raychaudhuri and Deng, 2008). SOD prevent damage induced by ROS through conversion of superoxide radical at very fast rate to H_2O_2 (Ma et al., 2021) and also protects the cell by preventing the formation of hydroxyl radical through Haber Weiss reaction (Gill and Tuteja, 2010). SOD production is also believed to be increased with metal concentration indicating protective role of SOD under heavy metals stress (Leong and Cheng et al., 2021). The higher SOD in microalgae signifies efficient defence activity against superoxide produced by abundant heavy metal concentration in wastewater (Danouche et al., 2020).

2.10.2 Catalase (CAT)

CAT is heme containing tetrameric protein (Rezayian, et al., 2019) present in all aerobic organisms (Zhang et al., 2018). CAT has huge potential than other antioxidants as single molecule can neutralize almost 6 million hydrogen peroxide (H_2O_2) in one minute (Ścibior et al., 2006). H_2O_2 is the most stable ROS produced after neutralization of superoxides and their concentration in the cell is reduced by the higher activity of catalase (De Zwart et al. 1999**).** Catalase is responsible for conversion of H_2O_2 to simple forms having no impacts on the cell (Kato et al., 2021) (Fig. 2.6). Catalase not only scavenges H_2O_2 but also protect the cell from the attack of other lipid derived oxides (Ali and Alqurainy, 2006). Catalase ability to lower the H_2O_2 concentration assist in maintaining essential physiological responses in the cell (Góth et al., 2004). The collective efforts of both SOD and CAT is termed as first line defense which is responsible for providing protection to the cell from oxidative damage (Ighodaro and Akinloye, 2018). Antioxidants like CAT and SOD activity is influenced by heavy metals as their proper structure and activity is dependent upon the several essential metal like iron or manganese in the cell (Janku et al., 2019).

2.10.3 Ascorbic acid

Ascorbic acid is ubiquitous in microalgae and is found in higher level in photosynthetic cells. Ascorbic acid is also one of the strong antioxidants responsible for maintaining of cellular integrity and protection from the oxidative stress (Khan and Ashraf, 2008). The increased ascorbic acid accumulation is mainly due to their role in photosynthesis and photoprotection (Smirnoff, 2018). Due to regenerative nature, ascorbic acid is powerful antioxidants that in combination with vitamin E sequesters reactive oxygen species either directly or through enzymatic catalysis (Akram et al., 2017). Shao et al., (2008) stated that ascorbic acid maintain the level of ROS within the tolerance level in the cell. Yabuta et al., (2007) observed that the expression of gene responsible for synthesis of ascorbic acid is light dependent and continuous light intensity upregulates the synthesis of ascorbic acid.

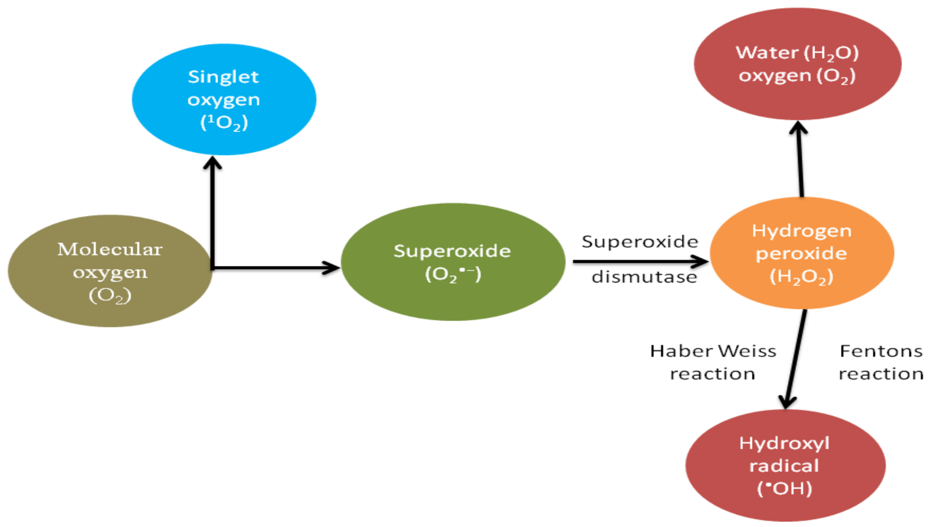


Fig. 2.6 Mechanisms of ROS generation and sequestration in microalgae

Ascorbic acid also assists to modulate the fundamental function under both favorable and unfavorable conditions (Akram et al., 2017). Additionally, ascorbate-glutathione pair regulates developmental process through exploitation of oxidative metabolism (Pignocchi and Foyer, 2003).

2.10.4 Proline

Antioxidants mainly found in an all the compartments of the cell are necessary for the survival of organism under stress conditions (Chen and Dickman, 2005). Proline is essential antioxidant play vital role in responding effectively under diverse stress conditions (Ghosh et al., 2022). Salinity, metals, light and other biotic stress has been observed to elevate the proline synthesis and accumulation in plants (Szabados and Savouré, 2010; Zouari et al., 2019). Proline is considered as osmolyte that protect cellular structures form oxidative radicals (Shafi et al., 2019), modulate functions of mitochondria, manipulate cell proliferation and trigger genes responsible for recovery from stress conditions (Szabados and Savouré, 2010). Proline acts as signalling molecules and is also responsible for initiation of several signalling processes (Ghosh et al., 2022), stabilization of protein structure; prevent membrane disruption (Singh et al., 2015), protect lipids (Okuma et al., 2004) and enzymes from degradation (Islam et al., 2009) and also assists in regulating the pH of cytosol (Koca et al., 2007). Under metal stress, proline improves tolerance by forming chelates with metals, alleviates the oxidative stress by ameliorating the activity of antioxidants (Zouari et al., 2019) and also reduces the accumulation of ROS in the cell (Shafi et al., 2019). Proline alleviates the damage and removes singlet oxygen by means of physical quenching and hydroxyl radicals by the assistance of chemical reactions (Alia et al., 2001). Like ascorbic acid, proline is a non enzymatic antioxidant which has potential to evade programmed cell death (Forlani et al., 2019). Proline under stress conditions functions as the molecular chaperone that apart from improving the activity of different enzymes (Szabados and Savouré, 2010), sustain the protein integrity, prevent protein aggregation under high temperature conditions (Rajendrakumar, 1994), and protect the activity of nitrate reducates under metal stress (Sharma and Dubey, 2005).

2.10.5 Cysteine

Cysteine serves as precursor of sulphur containing peptides, proteins and several other compounds directly or indirectly involved in the metal detoxification (Leong and Chang, 2020). Tripathi and Poluri, (2021) reported that phytochelatins could be synthesised as a reponse to metal concentration in the cell by microalgae. Metal chelators like metallothioneins (MTs) and phytochelatins form complexes with metals and depletes their availability in the cell (Tripathi and Poluri, 2021). Phytochelatins are very small peptides consisting of 5-11 amino acids while metallothioneins are long chain peptides containing large number of amino acids (Pinto et al., 2003). The synthesis of phytochelatins occurs in cytosol and transported to vacuole through ATP-binding cassette (ABC) transporters (Manara, 2012). Cysteine is essential constituent of MtIII (Cobbett and Goldsbrough 2002) and play key role in production and activation of phytochelatins (Perales-Vela et al., 2006). Howe and Merchant, (1992) reported that MtIII are vital for detoxification of metals from contaminated environment and sequester considerable amount of cytosolic cadmium from the cell (Tripathi and Poluri, 2021). The tolerance of microalgae to metal pollutants is not only due to the production of MtIII but also the length of MtIII thiol peptides (Perez-Rama et al., 2001). Metallothioneins are responsible for the transport of non essential heavy metals into different compartment of the cell and supply of essential metals to the cell (Capdevila and Atrian, 2011). MtIII have significant roles in metal detoxification (Chatterjee et al., 2020), sulphur metabolism, regulation of metal homeostasis (Garza-Lombó et al., 2018) and protection from oxidative damage (Tripathi and Poluri, 2021). Torricelli et al. (2004) observed that the concentration of cysteine was higher in microalgal species having effective tolerance towards metal pollution. Besides phytochelatin, cysteine enhance glutathione production that further accelerate the sequestration of toxic pollutants (Netto et al., 2007), stabilize unstable macromolecules, maintain photosynthesis and also play vital role in neutralizing toxic derivatives produced through lipid peroxidation (Li et al., 2020).

2.11 Role of algal consortia in wastewater treatment

In algal biotechnology, the single microalgae are used mainly for commercial application and experimental purposes as each species of microalgae have certain desired characteristics. In reality, the axenic culture of single algae is very difficult to be maintained due to presence of several micro-organisms in wastewater (Carney et al., 2016). Furthermore, the wastewater complexity in the form of organic matter, high nutrient load and toxic heavy metals affects the remediation efficiency, algal growth and biomass production. To adapt under multiple stress conditions, single algal species may not be able to address multiple aspects from wastewater remediation to biofuel production. In order to overcome the challenges, the most promising strategy is to mix different microalgae species having synergistic effect on growth and biomass production. The appraisal of the ability of the consortia for remediation of wastewater concomitantly with biomass production can prove very promising for environmental protection. González-Fernandez et al., (2011) reported that consortia has proven very productive and effective as compared to monoculture. Huy et al., (2018) observed that algal consortia consisting *Chlorella* and *Scenedesmus* removed 31 and 481 mgL^{-1} of phosphorus and nitrogen from wastewater. Similarly, Chinnasamy et al., (2010) reported that consortia of native microalgae recovered more than 96% of nutrient with lipid accumulation of 6.82% and biomass productivity of 9.2–17.8 tonnes ha^{-1} $year^{-1}$. Das et al. (2018) also suggested that consortia can prove very effective both in terms of wastewater remediation and biomass production.

Exploiting algal consortia can be the practicable approach to increase treatment efficiency and bioenergy production as each microalgal species has different nutrient requirement, detoxification mechanisms and environmental adaptability. Consortia boost nutrient uptake, enhance tolerance to biological contaminants and toxic pollutants widespread in wastewater (Renuka et al., 2013). Algal consortia can substantially enhance the nutrient reutilization provided adequate amount of nutrients are supplied in the growth media **(**Dayana Priyadharshini et al., 2021). Consortia can also increase the flexibility of the algae based wastewater treatment system by increasing the resistance towards salinity, nutrient deficiency and metal pollutants (Stockenreiter et al., 2016). Algal cosortia for wastewater remediation is advantageous as lose of one species due to toxicity of pollutants in wastewater or antagonistic interaction is overcomed by other species (Shahid et al., 2020). Co-culturing different microalgae also helps in autofloccution and may assist in decreasing the harvesting cost of biomass (Dayana Priyadharshini et al., 2021).

Due to varying wastewater complexity, the selection of indigenous and tolerant algal species for the formation of consortia may efficiently mitigate the damage and amplify the algal productivity in terms of lipid and biomass. Consortia developed for the remediation of wastewater along with biofuel production must contain species having high tolerance to toxic pollutants while some should be able to enhance the lipid productivity under nutrient limited condition or toxic pollutants condition. Consortia consisting of microalgal species having unique capabilities to remove several toxic pollutants and capacity to vigorously grow under harsh conditions should be incoporated for treatment of wastewater. The major concern in consortia is the interaction between different microalgae species that can play fundamental role in determining the success of the remediation process. Using algal consortia that exhibits symbiotic relationships can serve as the efficient strategy not only in terms of wastewater remediation but also the biomass production. Therefore, exploiting algal consortia could improve the competence of algae based system for biofuel production and simultaneously stabilize different attributes of the municipal wastewater in cost-effective manner. Additionally, the novel approach of relying on algal consortia based systems is economical and sustainable bio-filtration system for wastewater remediation, lipid yield, biomass and biofuel production.

Chapter 3

Material and Methods

Chapter-3

Material and Methods

3.0 Material and methods

Due to cost and energy intensive technological advancement along with inability to remove multiple pollutants from wastewater, the sustainable process for the treatment of wastewater is gaining huge momentum. The integrated approach involving wastewater treatment and energy production is of utmost importance to resolve the water management issues. Algae based treatment is economical and reliable in several manners from wastewater remediation, carbon dioxide sequestration to biofuel production. Thus, the holistic approach from screening of tolerant species to flexible biosystem for treatment of wastewater and biofuel production was carried out.

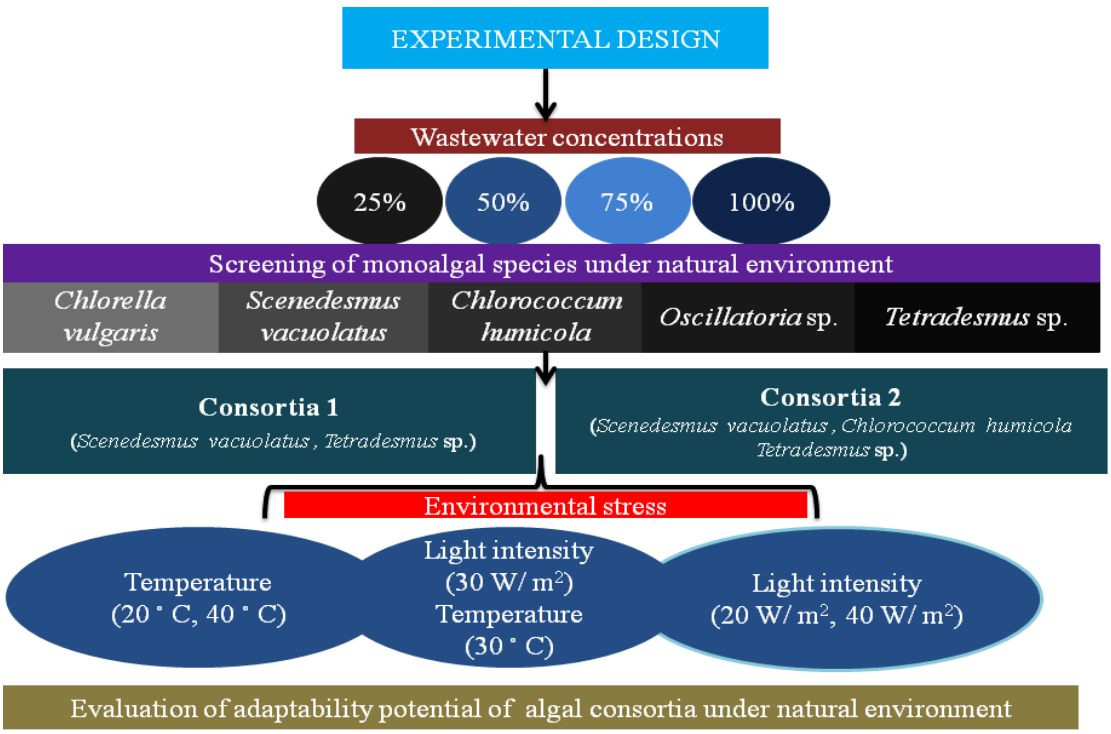


Fig 3.1 Experimental design of the present study

In the study, the following methodology was used for assessment of wastewater treatment, biochemical profile, lipid accumulation, defense mechanisms and biofuel production.

3.1 Collection, isolation and characterization of microalgae

The pure culture of *Chlorococcum humicola*, *Scenedesmus vacuolatus*, *Chlorella vulgaris* were obtained from the Department of Environmental Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow whereas *Oscillatoria* sp. and *Tetradesmus* sp. were isolated near wastewater collection sites of Charbagh.

Microalgae sample collected near wastewater site was cultured in the BG-11 media. The growth media- BG-11 was prepared as mentioned by the Stainer et al. (1971).

Table 3.1 Composition of BG-11 growth media for microalgae

Chemical	**Concentration (g/L)**	**Chemical**	**Concentration (g/L)**
Sodium Nitrate	1.5	Boric acid	2.86
Potassium hydrogen phosphate	0.04	Manganese chloride tetrahydrate	1.81
Magnesium sulphate heptahydrate	0.075	Zinc sulphate heptahydrate	0.22
Calcium chloride	0.036	Sodium molybdate	0.39
Citric acid	0.006	Copper sulphate	0.079
Ferric ammonium citrate	0.006	Cobalt nitrate hexahydrate	0.049
Ethylenediamine tetraacetic acid	0.001	Trace metal mix A5	1 mL
Sodium bicarbonate	0.02	Distilled water	983 mL

The algal sample after microscopic examination was found to be the mixture of three microalgae. The single species was isolated after following regular scientific procedures. To isolate the species, it is important to grow algae as the pure culture. The following methods (agar plate method, capillary method, successive dilution) were adopted to purify the algal strain from natural environment.

3.2 Material required

Agar Agar, growth media, plastic petri dish, micropipette, inoculation loop, para film, laminar flow chamber

3.2.1 Agar plate technique for isolation of pure microalgae

For isolation of pure strains, agar plate techniques are widely used in the laboratory. The prepared agar plate was used for the inoculation of mixed algal strains. The process was performed in laminar flow chamber to avoid any contamination from outside environment.

3.2.2 Preparation of agar plate and inoculation

- The laminar was sterilized with ethanol and UV light was kept on for 30 minutes for complete sterilization.
- In this method, the nutrient media (15 g) along with agar (1.5%) and distilled water (1 L) added to conical flask was sterilized by autoclaving (121°C and 15 psi).
- After autoclaving, the flask was allowed to cool for 30 minutes and media (20 mL) was poured into the sterilized petri plates.
- To avoid the contamination, the process of transferring of media to petri plates was carried out near the spirit lamp and the plates were allowed to solidify.
- After solidification, the desired amount of algal inoculums was transferred to plate directly with the help of micropipette or inoculation loop. The lid of the

plate was opened just enough to streak the plate in order to minimize the exposure of plate to external environment.

- The algal cells transferred with the help of micropipette onto the petri plate were spread in all direction for the homogenous distribution of cells on the entire plate. In another method, the metal loop was sterilized by keeping the wire loop near the blue zone of the burner till it become red hot. The sterilized loop was used for the streaking of algal cells onto the plates.
- The plates were kept in culture room under controlled conditions for proper growth of the isolated microalgae. The algal cells grow and form colonies after 7-10 days depending upon the size of inoculums.
- Once the colonies were formed in the petri plate, the algal cells were taken for identification under microscope. Lastly, the plates were wrapped in polythene bag and kept in refrigerator until further needed.

3.3 Successive dilutions

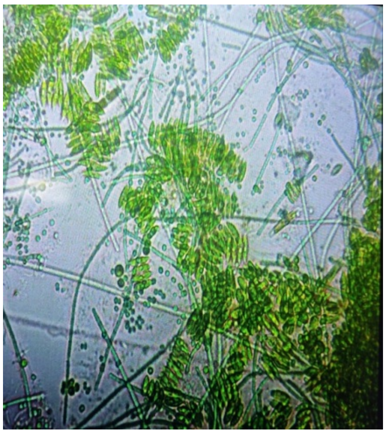
3.3.1 Material required

Test tubes, laminar, burner, sterilized tips

3.3.2 Procedure

The serial dilution was carried out in bio-safety cabinet to avoid any contamination. The 10 test tubes after proper numbering was filled with sterilized BG-11 nutrient media (9 mL). The 1 mL algal inoculum taken with micropipette was transferred to the first test tubes. Thereafter, the 1 mL from first test tubes was transferred to the second test tubes followed by thorough mixing. The same procedure was repeated for the remaining test tubes.

After the cell start growing in the test tube, the microscopic examination of the cells was carried out. The contaminated test tubes were rejected. The more diluted test tubes were used to obtain the pure culture of microalgae. The pure cultures after agar plating and serial dilution were utilized for the characterization of microalgae.



Single microalgae

3.4 Wastewater collection

Wastewater was collected from different sites of Lucknow such as Charbagh (26°49' 57"N, 80° 56' 08" E), Pakapul (26° 52' 21" N 80° 54' 58" E), Kukrail (26° 52' 48" N 80° 57' 57" E) and Jiamau (26° 50' 50" N 80° 57' 37" E) (Fig. 3.2).

Fig. 3.2 Wastewater collection sites

Different concentration of wastewater (25%, 50%, 75%, and 100%) was formed by carrying out dilution through double distilled water (Table 3.2). The 600 mL of different wastewater concentrations were inoculated with algal consortia in a 1 L flask under laboratory conditions.

Table 3.2 Different concentrations of municipal wastewater utilized for experimental work

Wastewater concentrations	Wastewater	Distilled water
Control (BG-11 media)	-	600 mL
25% WW	150 mL	450 mL
50% WW	300 mL	300 mL
75% WW	450 mL	150 mL
100% WW	600 mL	-

3.5 Experimental design

Initially, the single species were tested for their tolerance to different wastewater concentration under natural environment (Fig. 3.3).

Fig 3.3 Cultivation of microalgae in plastic tubs under natural environment

The experiment was conducted in 3 L plastic tubs (Length-36 cm, depth-12 cm) using municipal wastewater as the nutrient media. The algal species with excellent remediation potential were utilized for formation of consortia.

3.5.1 Preparation of mass culture

The glassware utilized for the present study was routinely autoclaved at 15 lb/inch^2 and temperature of 121° C. The flasks were tightly closed with the help of cotton plugs and wrapped with aluminum foil before placing in autoclave for sterilization. The axenic cultures of selected microalgae were grown in 1 L Erlenmeyer flask containing 300 mL autoclaved BG-11 media. Microalgal cells were grown for 15 days under controlled laboratory conditions (10 W/m^2 for 16 hours at 24± 2^0 C) and the mass culture of each algal species was maintained in BG-11 media.

3.5.2 Preparation of algal consortia

The algal biomass was centrifuged (5000 ×g) and mixed to form different consortia using BG-11 as the growth media (Fig. 3.4). Different consortia formed were allowed to acclimatize and growth rate was taken at regular intervals. Consortia showing excellent growth were taken as indicators of positive interaction between different algal species.

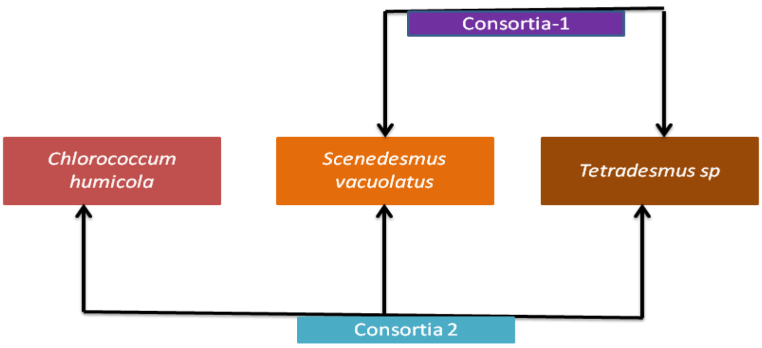


Fig. 3.4 Formation of consortia from different monoalgal species

The algal consortia formed were employed for wastewater treatment under different light intensities (20 W/m^2, 40 W/m^2) and temperature (20° C, 40° C) conditions (Fig. 3.5). The experiments were carried out in triplicates. Constant shaking of flasks was done at regular intervals to encourage proper mixing of nutrients and avoid settling down of the cells. The experiments lasted for 15-20 days and per day growth were observed by taking absorbance at 650 nm**.** The algal biomass harvested after 15-20 days was used for the analysis of different biochemical parameters.

Fig. 3.5 Cultivation of different algal consortia under laboratory condition

3.6 Analysis of physico-chemical attributes of wastewater

The wastewater analysis was carried out by APHA, (2005). The physico-chemical of wastewater like pH, electrical conductivity (EC), total dissolved solids (TDS) were analyzed with the help of Systronics water analyser- 371. The total solid (TS), total suspended solids (TSS) of wastewater was analyzed by gravitation method; biological oxygen demand (BOD) by modified Winkler method, nitrate-nitrogen (NO_3^{-1}-N) by brucine-sulphanilic and phosphate (PO_4^{-3}-P) by ammonium molybdate method.

3.6.1 Heavy metal estimation

For heavy metal analysis, the wastewater sample of 10 ml before and after treatment were digested using Di-acid HCl : $HClO_4$ (3:1) at 90 ^{0}C on hot-plate. The samples were digested until the white precipitates were formed at the bottom of the 50 ml flask. Finally, the samples were diluted upto 50 mL by distilled water and filtered through Whatman filter paper No. 42. The heavy metal analysis was carried out through Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent 7500 cx).

Percent decrease in metal concentration = Final value – Initial value / Final value * 100

3.7 Biochemical analysis of microalgae consortia:

3.7.1 Growth profile, specific growth rate (μ), **biomass production and productivity**

Different microalgae and algal consortia were grown in 1 L Erlenmeyer flask containing 600 mL of BG-11 media. The per day growth of selected microalgae were monitored at 650 nm using spectrophotometer (Shimadzu, Japan 1601).

The specific growth rate of microalgae (μ) was calculated as per the formula suggested by Yu et al. (2017):

$$\mu\ (d^{-1}) = \ln (X_2 - X_1) / (t_2 - t_1)$$

Where X_1= Initial density at t_1 time;

X_2= Final density at t_2 time

Biomass productivity of microalgae was calculated by the following formula

$$\text{Biomass production (mg L}^{-1}) = (B_2 - B_1) * 1000$$

$$\text{Biomass productivity (mg L}^{-1}\text{d}^{-1}) = (B_2 - B_1) / (t_2 - t_1)$$

Where B_1=Biomass production at t_1: B_2=Biomass production at t_2

3.7.2 Pigment content

The pigment content in microalgae was estimated through the methods of Arnon (1949). 100 mg of the fresh microalgae biomass was crushed with the help of mortar and pestle in 3 mL chilled 80% acetone (v/v) under controlled conditions. The homogenized cell was centrifuged at 8,000 x g for 10 minutes. After centrifugation, the supernatant was used for the estimation of pigment at the wavelength of 663, 645, 480 and 510 nm using spectrophotometer (UV – 1601, Shimadzu, Japan). The carotenoid content was determined using the formula given by Duxbury and Yentsuch, (1956).

3.7.3 Chlorophyll a fluorescence

Chlorophyll fluorescence of treated and untreated algae under different environmental conditions was analyzed by Pulsed amplitude-modulated fluorimeter (PAM) (Aqua Pen-C, Czech Republic). The PAM was set to a pulse intensity of 3000 μmol photon m^{-2} s^{-1} for 10s and light/dark induction adopted curves for Chl irradiance levels ranging from 0 to 480 μ mol photon m^{-2} s^{-1}. The fresh sample of consortia was dark-adapted for 15 minutes at fixed excitation (450 nm) and emission wavelengths of 450 nm and 650 nm for the measurement of the OJIP curve. Microalgae cultivated under different experimental conditions were examined for different physiological parameters such as active photosystem II reaction center (Fv/Fo), maximum quantum yield (Fv/Fm), performance index (Pi_{abs}), Net closing rate of the reaction center (Mo), Trapping flux (TRo/RC), effective antenna size per reaction center (ABS/RC) and electron transport per reaction center (ET_O/RC) (Singh et al., 2019).

$$\text{Quantum yield (Fv/Fm)} = \frac{\text{Maximum fluorescence (Fm) - Minimum fluorescence (Fo)}}{\text{Maximum fluorescence (Fm)}}$$

Effective antenna size per reaction center = (Mo/Vj)/[1-(Fo/Fm]

Trapping flux = (TRo/ABS) * (ABS/RC)

Electron transport per reaction center = (TRo/RC)* (1-Vj)

3.7.4 Protein content

The protein concentration in the algal consortia were estimated by following the method of Lowry et al., (1951) modified by Herbert et al., (1971). For measurement of protein, 100 mg of fresh algal biomass was digested using 0.1 N sodium hydroxide at 100^0 C for 10 minutes. The reagent A was prepared by mixing 50 mL sodium hydroxide (1 N) and 5 mL sodium carbonate (0.2%) and followed by the addition of 1 mL copper sulphate (0.5%) and 1 mL sodium potassium tartarate (1.0%). Reagent A (2.5 mL) added to the reaction mixture was incubated at 25° C for 15 min. Finally, 0.5 ml Folin-Ciocalteu's was added and the mixture was quickly centrifuged. The intensity of the resulting blue colour was determined spectrophotometrically at 650 nm. The protein content in the sample was estimated using bovine serum albumin for the preparation of standard curve.

3.7.5 Carbohydrate content

Carbohydrate estimation was carried out through the method of Hedge and Hofreiter, (1962). Anthrone reagent was prepared by adding 0.029 gm of anthrone in 100 mL sulphuric acid. For carbohydrate estimation, fresh algal biomass was mixed with 3 ml of anthrone reagent followed by boiling at 100^0 C for 10 minutes in water bath. The samples after cooling at room temperature were taken for absorbance at 600 nm using spectrophotometer.

3.7.6 Lipid content

Lipid estimation was carried out by the method of Knight et al. (1972). The phospho-vanillin reagent was prepared by mixing 0.06 gm vanillin in 1 mL ethanol, 40 mL of ortho-phosphoric acid and 9 mL of double distilled water. For lipid estimation, 100 mg algal biomass was mixed with 2 ml of 98% sulphuric acid, followed by boiling for 10 minutes. After boiling, 5 ml phospho-vanillin reagent was added and the mixture was incubated at 37 ^{0} C for 20 minutes. The mixture was further used for lipid estimation by taking absorbance at 530 nm.

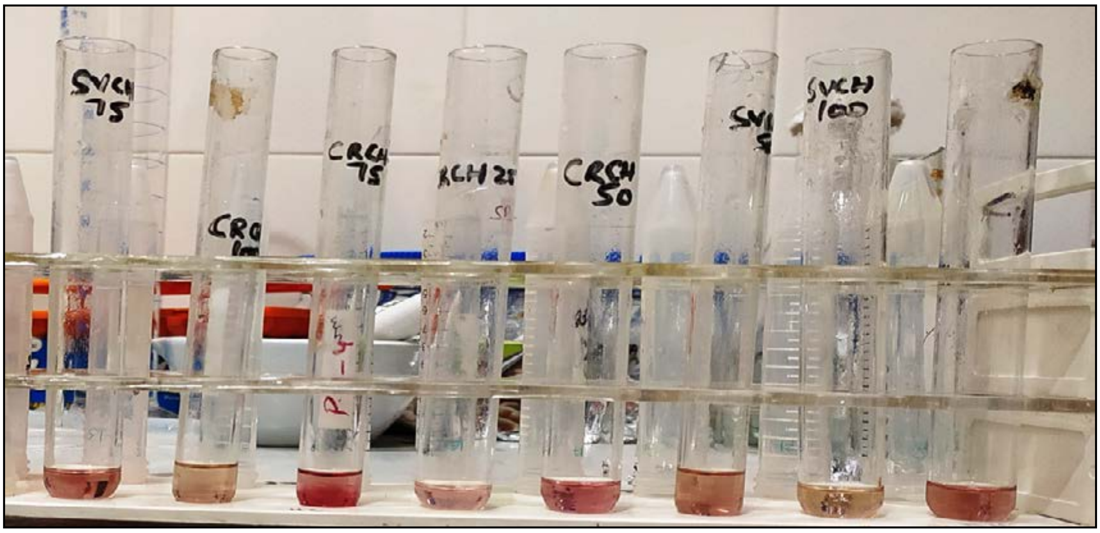


Fig. 3.6 Extraction of lipid from algal consortia

3.7.7 Lipid extraction

The lipid extraction from algal biomass was carried out using method of Bligh and Dyer, (1959). Algal biomass harvested after 20 days was used for the extraction of lipid. In this method, the 500 mL of algal suspension was centrifuged (5000 rpm) and the pellet collected was washed thrice with distilled water. The algal cell pellet was dried for overnight at 70˚ C in oven and the biomass was grounded with the help of mortar and pestle. After weighing, the powdered biomass was washed with neutral phosphate buffer saline. To the algal biomass, 0.5 mL PBS and glass beads were added followed by vortexing of the suspension for 10 minutes. Further, the phosphoric acid and sulfuric acid (0.4% v/v) in the ratio of 2:0.75 added to the mixture was incubated for 5 minutes (50˚ C). The mixture was again centrifuged at 2000 rpm for 5 minutes. The chloroform: methanol (2:1) was added to the biomass andafter vortexing for 5 minutes; the mixture was left at 25˚ C for 24 hours. The distilled water (1 mL) and 2 mL chloroform: methanol solution added to the mixture was again vortexed and finally centrifuged at 4000 rpm. The distinct two phases are formed, the top layer was aspired and the bottom layer was transferred to another vial followed by washing with NaCl solution (5%) to remove impurities. The lipid content in algal biomass was calculated by the following formula:

$$\text{Lipid content (\%)} = A/B \times 100$$

A= weight of lipid; B= weight of algal biomass

The lipid extracted was further utilized for the fatty acid methyl ester (FAME) profiling through gas chromatography-mass spectrometry.

3.8 Estimation of Oxidative stress markers

3.8.1 Hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) concentration in algal biomass was estimated by the method of Velikova et al. (2000). The fresh algal biomass soon after harvesting was mixed with trichloroacetic acid (0.1%) followed by centrifugation at 8000 ×g. 1 ml of filtrate was mixed with 0.5 ml phosphate buffer (10 mM) and 1 ml of potassium iodide (1M). Finally, the absorbance was recorded at 390 nm using a spectrophotometer and the concentration of H_2O_2 in algal sample was calculated from the standard curve of known H_2O_2 concentrations.

3.8.2 Thiobarbituric acid reactive species (TBARS)

Thiobarbituric acid reactive species (TBARS) in algal biomass were estimated by the method of Heath and Packer, (1968). Lipid peroxidation in terms of TBARS was determined in microalgae after treatment with wastewater under different light and temperature conditions. The reagent A was prepared by mixing 0.5 % thiobarbituric acid in 20% trichloroacetic acid. Through this method, algal biomass (100 mg) was mixed with 2 ml of trichloroacetic acid (5%) and centrifuged (10000×g) for 10 minutes. The 0.5 mL of filtrate was mixed with reagent A and resultant mixture was heated at 95^0 C for 60 minutes followed by immediate cooling. The mixture was again centrifuged and the absorbance was finally recorded at 532 nm and 600 nm. The optical density obtained at 600 nm was deducted from non-specific turbidity. The TBARS concentration in the samples was calculated by extinction coefficient 155 mM^{-1} cm^{-1}.

3.9 Estimation of different antioxidants in algal consortia

3.9.1 Proline estimation

Proline content in the microalgae samples was estimated by using the method of Bates et al, (1973). For proline estimation, acid ninhydrin was prepared by mixing ninhydrin ninhydrin (1.25 g), glacial acetic acid (30 mL) and phosphoric acid (20 mL). Fresh samples of the microalgae (250 mg) were crushed in sulfosalicyclic acid (3%) and the mixture was centrifuged at 10,000 × g. The mixture was further mixed with 1 mL GAA and acid ninhydrin. The reaction mixture was heated (95° C) for 60 minutes followed by cooling for 5 minutes. Finally, the 4 ml toluene was added and extraction was carried out by vortexing the mixture for 15 seconds. The upper layer was taken out and the absorbance of lower layer was recorded at 520 nm.

3.9.2 Cysteine estimation

Cysteine content in algal consortia treated with wastewater was estimated by the method of Gaitonde (1967). Reagent A was prepared by dissolving ninhydrin (250 mg) in glacial acetic acid (6 mL) and hydrochloric acid (4 mL). In this method, the fresh 500 mg microalgae sample crushed in 2 mL chilled perchloric acid (5%) was centrifuged at 10,000 ×g for 10 minutes. After centrifugation, 1 mL of the supernatant, glacial acetic acid and reagent A was mixed properly and heated for 30 minutes at 100^0 C. Finally, the absorbance of the samples was recorded at 560 nm using double beam UV- spectrophotometer.

3.9.3 Ascorbic acid estimation

Ascorbic acid was estimated through the method of Kampfenkel et al. (1995). 2, 6-dichlorophenolindophenol sodium salt dehydrates (DCPIP) was prepared by dissolving 100 mg DCPIP in 1 L of distilled water. Before use, the DCPIP solution was diluted with distilled water (10/25). The fresh algal samples (100 mg) were homogenized in 2 mL extracting solution containing 3 gm trichloroacetic acid and 0.075 gm ethylenediamine tetraacetic acid. The mixture was centrifuged at 3000 × g for 5 minutes. After centrifugation, 1 mL supernatant was mixed with 5 mL of DCPIP and absorbance was taken at 525 nm.

3.10 Estimation of different enzymes in microalgae consortia

3.10.1 Preparation of enzyme extract

For enzymatic antioxidants estimation, 100 mg of fresh biomass of consortia was mixed with 100 mM phosphate buffer (pH-7), ethylenediamine tetraacetic acid (1mM), and a pinch of polyvinyl polypyrrolidone. The mixture was centrifuged at low temperature (4^0 C) for 10 minutes at 12000 × g. The extract was utilized for the estimation of following enzymes:

3.10.2 Superoxide dismutase (SOD)

SOD activity in algal consortia was analyzed by following the method of Nishikimi and Rao, (1972). The reaction mixture was prepared by adding sodium pyrophosphate buffer, phenazine methanosulphate, nitroblue tetrazolium and enzyme extract, respectively. After adding different chemicals, the volume was raised to 2.8 mL and the reaction was started using 0.2 mL of NADH (0.78 mM). Finally, the reaction mixture was incubated at 30^0 C and 1 mL of glacial acetic acid was used to stop the reaction. The absorbance was recorded at 560 nm on spectrophotometer.

3.10.3 Catalase activity

The catalase (CAT) activity in algal samples was examined by the method of Aebi (1984). The fresh algal biomass (100 mg) mixed with phosphate buffer (50 mM) and ethylenediamine tetraacetic acid (1 mM) was centrifuged at 6,000 g. The reaction mixture was prepared by mixing 0.5 ml hydrogen peroxide (40 mM), 1.3 mL phosphate buffer and crude extract (0.2 mL). The absorbance was taken at 240 nm for 90 seconds. The H_2O_2 consumption was calculated by 39.4 mM^{-1} cm^{-1}as the extinction coefficient.

3.10.4 Glutathione reductase (GR)

The GR activity in microalgae was examined following the method of Smith et al., (1988). The reaction mixture (1 mL) was prepared by adding enzyme extract along with 0.2 mL phosphate buffer (50 mM), ethylene diamine tetraacetic acid (0.2 mM), freshly prepared 5, 5’-dithiobis nitro benzoic acid (2 mM) solution and 0.1 mL GSSG. The reaction was started by adding 0.1 mL nicotinamide adenine dinucleotide phosphate (2 mM) to the mixture. The mixture was properly shaken and the absorbance of yellow colour was recorded at 412 nm at the gap of 15 second for 3 minutes.

3.11 Fourier transforms infrared spectroscopy (FTIR) of algal biomass

FTIR spectroscopy was used to distinguish the modification in the macromolecular composition of algal consortia after treatment with municipal wastewater under different light and temperature conditions. The algal consortia after reaching saturation phase were dried and grounded into very fine particles using mortar and pistle. The powdered biomass after proper mixing with potassium bromide (1:10) was used for formation of transculent disk-shaped pellet at 150 lbs using hydraulic press. The IR absorbance was measured (4000-500 cm^{-1}) in the FTIR machine for analysis (Thermo- Scientific Nicole 6700, USA). The background correction of absorbance was carried out by using omics software. The spectra obtained were used to analyze of composition of treated samples as compared to control under different environmental conditions. The biochemical constituents in algal biomass were assessed after comparing the absorption spectra with characteristic absorbance of different macromolecules (Duygu et al., 2012).

3.12 Statistical analysis

The statistical analysis of different parameters of wastewater and microalgae were performed by using the Statistical Package for Social Sciences software. One way analysis of variance was used to assess the comparison between different wastewater treatments. Duncan multiple range test was used to differentiate the effect of different wastewater concentrations on consortia. Similar alphabets were used to show the non-significant changes in different parameters treated with wastewater. Standard deviation were represented in the form of bar in different figures.

Chapter 4

Microalgal competence in urban wastewater management: Phycoremediation and lipid production

Chapter-4

Microalgal Competence in Urban Wastewater Management: Phycoremediation and Lipid Production

4.0 Introduction

Water resource is mainly contaminated by direct disposal of wastewater from different sources which disturbs the balance of aquatic ecosystem (Singh et al., 2020; Upadhyay et al., 2021). Therefore, appropriate approach is required for management of wastewater at point source as well as non point source. Urban (municipal) wastewater is abundant in different essential nutrients, organic matter and heavy metals (Hammer, 2020). Conventional techniques are not able to efficiently resolve the different pollutants from urban wastewater (Singh et al., 2020). In comparison to existing technologies for wastewater treatments (Li et al., 2020), the microalgae seems to be a promising tool for the treatment of nutrient enriched municipal wastewater (Singh et al., 2020). Microalgae has shown tremendous potential to adapt in diverse kinds of wastewater from dairy, leather, textile and food processing industries (Daneshvar et al., 2019**;** Goswami *et al.,* 2019).

Apart from water pollution, the major challenge is to reduce the dependence on the fossil based fuel as source of energy (Abas et al., 2015). Exploiting renewable energy source like wind, solar and biomass can play key role in attaining the future demands of energy (Owusu and Asumadu-Sarkodie, 2016). The algal biomass could serve as the alternative energy source with capacity to address numerous environmental issues simultaneously (Singh et al., 2020). The problem of wastewater pollution can be resolved using microalgae as the suitable bioresource for the treatment of wastewater (Hannon et al., 2010: Upadhyay et al., 2021). Besides mitigation of wastewater related pollution, the algal biomass generated can be used as the substrate for the green energy (Singh et al., 2020). Luo et al., (2016) observed that microalgae *Coelastrella* sp. effectively removed nutrients (90%) from swine wastewater. Microalgae *Scenedesmus bijuga* and *Oscillatoria quadripunctulata* have been observed to remove the copper and lead from contaminated environment (Ajayan et al., 2011). Furthermore, *Oscillatoria*, *Chlamydomonas*, *Scenedesmus* and *Chlorella* have shown exceptional potential to remove and tolerate different concentration of metal pollutants (Abdel-Raouf et al., 2012**).** Therefore, utilizing microalgae could be a sustainable approach for the treatment of wastewater (Pacheco et al., 2015). Extensive studies has been carried on microalgae for nutrient and metal removal but a very little effort has been made to examine the cellular modification and defense activities. Microalgae *Chlorococcum humicola*, *Tetradesmus* sp. and *Oscillatoria* sp. were used for municipal wastewater treatment and simultaneous lipid production. The present investigation includes study of growth response of these algal strains, nutrient removal efficiency and cellular defense mechanism against the toxicants of wastewater and modifications in cell constituents including lipid molecules in selected microalgae.

4.1 Results and discussion

4.1.1 Physico-chemical characteristics of wastewater

The physico-chemical attributes of municipal wastewater collected from Lucknow was analysed and the results are mentioned in table 4.1. The municipal wastewater showed the presence of different heavy metals such as Fe (1809.33±14.10

Table 4.1 Changes in physicochemical attributes of wastewater treated with *C. humicola, Oscillatoria* sp. and *Tetradesmus* sp. All values are in mg/L otherwise mentioned. All values are in means ±SD (n=3). ANOVA post hoc DMRT (p≤0.05) was done to check the significance difference between the variables. Identical letter indicates no significant difference with in the variables.

Parameters	Initial concentration	Algal species	Control	25% WW	50% WW	75% WW	100% WW
pH	8.4±0.25	*CH*	7.54±0.049[a]	9.02±0.048[d]	8.86±0.051[cd]	8.95±0.044[ab]	9.00±0.053[d]
		*O*s	7.35±0.040[a]	9.16±0.068[d]	9.29±0.032[e]	9.08±0.068[cd]	9.09±0.058[d]
		TS	8.12±0.093[b]	8.52±0.181[bc]	8.66±0.183[c]	9.07±0.101[d]	8.54±0.153[c]
EC (µS cm^{-1})	856±4.58	*CH*	4.91±0.200[a]	122±5.56[b]	210±6.00[c]	316±4.58[d]	385±6.00[d]
		*O*s	7.27±0.152[a]	134±3.00[b]	222±6.00[c]	357±7.54[d]	405±5.56[e]
		TS	4.8±0.457[a]	110.33±5.95[b]	200.33±5.75[c]	305±7.15[d]	335.66±7.44[d]
TDS	305±4.04	*CH*	5.52±0.213[a]	77.44±0.216[b]	102.28±2.030[c]	122.10±1.360[d]	149.80±1.380[e]
		*O*s	3.91±0.218[a]	88.63±1.896[b]	106.80±1.747[c]	128.77±1.036[d]	140.46±2.297[e]
		TS	2.41±0.018[a]	64±4.470b	92±13.39[b]	115±21.03[cd]	135±18.5[de]
TSS	122.6±2.27	*CH*	3.83±0.65[a]	24.33±1.305[b]	31.33±0.849[c]	52.33±0.935[d]	60.33±1.652[e]
		*O*s	5.20±0.91[a]	26.33±1.443[b]	39.66±1.200[c]	55.66±0.648[d]	65.33±1.073[e]
		TS	1.82±0.76[a]	23.33±3.05[b]	30±4.580[c]	61.66±6.42[e]	70.33±4.04[e]

TS	420.17±5.03	*CH*	9.07±0.040[a]	103.77±1.162[b]	135.66±1.598[c]	173.76±1.466[d]	214.50±1.733[e]
		*O*s	9.43±0.045[a]	113.50±2.601[b]	150.88±2.495[c]	185.91±1.203[d]	202.86±1.185[e]
		TS	4.72±0.652[a]	90±0.892[b]	122.33±17.48[c]	178.45±25.64[d]	200.98±15.51[e]
BOD	45±2.08	*CH*	0.21±0.005[a]	3.33±0.160[c]	8.36±0.235[e]	15.66±0.896[g]	20.36±1.098[i]
		*O*s	0.48±0.008[a]	4.60±0.700[c]	9.46±0.653[f]	17.73±1.160[h]	23.40±1.113[j]
		TS	0.42±0.022[a]	1.33±0.136[b]	7.62±0.974[d]	16.41±1.60[gh]	20.03±2.12[i]
DO	ND	*CH*	6.34±0.402[h]	3.01±0.366[f]	2.03±0.045[e]	0.64±0.0112[bc]	0.183±0.060[a]
		*O*s	6.20±0.490[h]	2.77±0.081[ef]	1.02±0.059[d]	0.53±0.0172[b]	0.140±0.045[a]
		TS	7.40±0.178[i]	5.33±0.136[g]	2.45±0.236[e]	0.843±0.178[c]	0.360±0.136[b]
NO_3-N	39.44±1.90	*CH*	2.06±0.170[b]	5.56±0.368[d]	8.46±0.414[f]	13.83±0.343[i]	16.23±0.436[k]
		*O*s	2.36±0.470[b]	7.26±0.202[e]	10.31±0.408[h]	14.22±0.458[j]	19.50±0.372[l]
		TS	1.17±0.309[a]	4.82±0.284[c]	9.68±0.27[g]	12.8±0.44[i]	14.30±0.686[j]
PO_4^{-3}	16.72±0.57	*CH*	0.167±0.010[a]	0.426±0.013[b]	0.751±0.014[c]	1.33±0.031[e]	1.80±0.036[f]
		*O*s	0.164±0.010[a]	0.422±0.011[b]	0.773±0.013[c]	1.43±0.022[e]	2.02±0.084[g]
		TS	0.136±0.042[a]	0.363±0.062[b]	0.813±0.103[d]	1.30±0.139[e]	1.96±0.126[f]

All values are in mg/L, otherwise mentioned, ND-Not detected, CH: *Chlorococcum humicola*, Os: *Oscillatoria* sp., Ts: *Tetradesmus* sp.

µg/L), Pb (8.14±0.090 µg/L), Cd (2.072±0.050 µg/L), Cr (3.02±1.08 µg/L), Cu (2.05±0.051 µg/L), Zn (12.22±0.765 µg/L), Ni (13.45±0.576 µg/L) and As (0.25±0.005 µg/l), respectively.

4.1.2 Variation in wastewater characteristics with microalgae

The characteristics of wastewater were analyzed after treatment with selected microalgae (Table 4.1). The physico-chemical parameter; pH shifts towards alkalinity throughout the range of wastewater concentration after algal treatment as compared to their respective untreated wastewater. The result also showed that dissolved oxygen (DO) was increased in different wastewater concentrations after algal treatment. The results on DO level of wastewater after treatment by *Tetradesmus* sp., *C. humicola* and *Oscillatoria* sp. showed an increase of 5.33, 3.01 and 2.77 folds respectively, at 25% concentration of wastewater and subsequent decline in DO level was observed with wastewater concentration gradient (Table 4.1). The results further exhibited that highest reduction in EC, TDS, TSS, TS and BOD were found in selected microalgae grown in BG-11 media than wastewater treated microalgae. At 25% wastewater concentration, the reduction in EC, TDS, TSS, TS and BOD was found to be 87.11%, 79.01%, 80.72%, 78.57% and 97.04% with *Tetradesmus sp.* while the said parameters were reduced by 85.74%, 80.15%, 74.60%, 77.14 % , 92.6%, and 84.34%, 70.94%, 78.52%, 72.98% 89.77%, by *C. humiocla* and *Oscillatoria* sp. respectively. The results further revealed that *Tetradesmus* sp. showed the highest reduction of 76.39%, 69.83% and 64.36%, 62.29% in EC and TDS at 50% and 75% concentration of wastewater, respectively. At 50% concentration of wastewater, *Tetradesmus* sp. showed highest removal efficiency of 70.88%, 75.53%, 83.06% while with *C. humicola*, the removal efficiency of 58.64%, 57.31%, 65.20% in TS, TSS and BOD was observed at 75% concentration of wastewater. At 100% wastewater concentration, the results revealed that the highest reduction in EC (60.86%), TDS (55.73%), TS (52.16%) and BOD (55.48%) was observed with *Tetradesmus sp.* followed by *C. humiocla* and *Oscillatoria* sp. respectively. The reduction in total suspended and dissolved solid content of wastewater could be due to flocculation and assimilation of the nutrients by microalgae (Kumar et al., 2013). The decline in the BOD level may be attributed to microbial load in wastewater that utilizes the oxygen released by microalgae for degradation of organic matter (Su et al., 2012). The TDS and BOD removal by selected microalgae was found to be higher than that reported by El-Sheekh et al. (2016). The results also revealed that the nitrogen removal efficiency of 97.02%, 94.77% and 94.01% was found highest in untreated *Tetradesmus* sp., *C. humicola* and *Oscillatoria* sp. as compared to wastewater treated one. At 25% concentration of municipal wastewater, the nitrogen removal efficiency was found to be 87.77% and 85.90% in *Tetradesmus* sp. and *C. humicola* which was the highest as compared to *Oscillatoria* sp. (81.59%). At 75% and 100% concentration of wastewater, the results showed the highest decline in nitrate nitrogen level of wastewater was 67.54% and 63.74% with *Tetradesmus* sp., while lowest was observed with *C. humicola* (64.93%, 58.84%) and *Oscillatoria* sp. (63.94%, 50.55%). The phosphorus removal efficiency of 97.97%, 97.51% and 97.55% was also found highest in untreated *Tetradesmus* sp. *Oscillatoria* sp. and *C. humicola* than wastewater treated microalgae (Table 4.1). Further, the results on phosphorus removal by *Tetradesmus* sp., *C. humicola* and *Oscillatoria* sp. exhibited removal efficiency of about 94.59%, 93.66% and 93.72, at 25% concentration of wastewater, respectively. The phosphorus removal from wastewater was found to be the highest (> 93%) at 25% wastewater and ~ 89% at 50% wastewater in the treated algae. At 100%

wastewater (undiluted), the phosphorus (73.20%) removal by *C. humicola* was higher than *Tetradesmus* sp (70.88%) and *Oscillatoria* sp. (69.94%). Both nutrients (nitrogen and phosphorus) are important for microalgae and higher uptake from the wastewater is responsible for excellent growth and cellular functioning (Singh et al., 2020; Upadhyay et al., 2021). Thus, decreased nutrient level in treated wastewater could be ascribed to their assimilation and sequestration by microalgae (Rajasulochana and Preethy, 2016). The substantial reduction of nutrients from wastewater by *Chlamydomonas polypyrenoideum* has been reported by Kothari et al. (2013). Xu et al. (2015) reported that *Scenedesmus obliquus* removed 70-88% and 58-74% of total phosphorus and nitrogen from the wastewater, respectively. The BOD, TDS, nitrogen and phosphorus removal by selected microalgae from the municipal wastewater was higher than that reported by earlier workers (El-Sheekh et al., 2016; Tripathi et al., 2019).

4.1.3 Removal of heavy metals from wastewater through microalgae

The different municipal wastewater concentration treated with microalgae revealed a significant reduction in the metal concentrations after 16 days. The heavy metal removal efficiency of *C. humicola* at 25% concentration of wastewater was found to be the highest for Ni (88.1%), Cd (85.47%), As (84.40%) while *Tetradesmus* sp. for Zn (82.40%), Cu (87.67%), Fe (86.33%) and *Oscillatoria* sp. showed efficient removal for Pb (85.87%) and Cr (78.03). The treatment of 50% concentration of wastewater, *C. humicola* exhibited metal removal efficiency in the order of Ni>Zn>Pb>As >Fe>Cd>Cu>Cr, whereas metal removal after treatment with same dilution (50%, v/v) of wastewater was in the order of Cu>Fe>Pb>Zn>Ni>As>Cd>Cr and Zn>Ni>As>Cd>Cu>Fe>Pb>Cr with *Tetradesmus sp.* and *Oscillatoria* sp.

respectively (Fig. 4.1). The reduction in metal contents at 100% wastewater was in the order of Fe (61.56%), Cu (61.19%), Zn (61.04%), As (56.19%), Ni (55.29%), Pb (51.78%), Cd (49.26%) and Cr (43.65%) after treatment with *Tetradesmus* sp. while lowest was observed with *Oscillatoria* sp. The results suggested that *Tetradesmus sp.* and *C. humicola* was more effective in removing different heavy metals from 100% wastewater than the *Oscillatoria* sp. Earlier reports showed that heavy metals removal efficiency of *C. vulgaris* and *C. salina* was in the range of 13.61–100 % (El-Sheekh et al., 2016), while our study showed metal removal efficiency of 43.65-87.83%, 31.87-88.10%, 24.17-87.76% with *Tetradesmus sp., C. humicola* and *Oscillatoria* sp., respectively (Fig. 4.1). However, the metal removal efficiency of selected microalgae in present study was comparable to that reported by Ajayan et al. (2015). Perhaps, this difference in the metal removal efficiency of algal strain might be attributed to nature of the wastewater and metal tolerance characteristics of individual algal species. Earlier workers have demonstrated that the reduction of metals from wastewater depends upon the chelation, compartmentalization and accumulation of metabolites inside the algal cells (Emamverdian et al., 2015; Singh et al., 2020). It has been suggested that microalgae experience different stresses in the presence of wastewater and start synthesizing phytochelatin or exopolymers thereby, protect microalgae from damage (Cassier-Chauvat and Chauvat, 2015). The other adaptive mechanisms might involve alterations in the metabolic pathways associated with protective cellular response of microalgae, which ensures their growth in the presence of wastewater stress (Piotrowska-Niczyporuk et al., 2015). Overall, *Tetradesmus* sp., and *C. humicola* were more effective in removal of organic matter, nitrogen and heavy metals from different wastewater concentrations as compared to *Oscillatoria* sp.

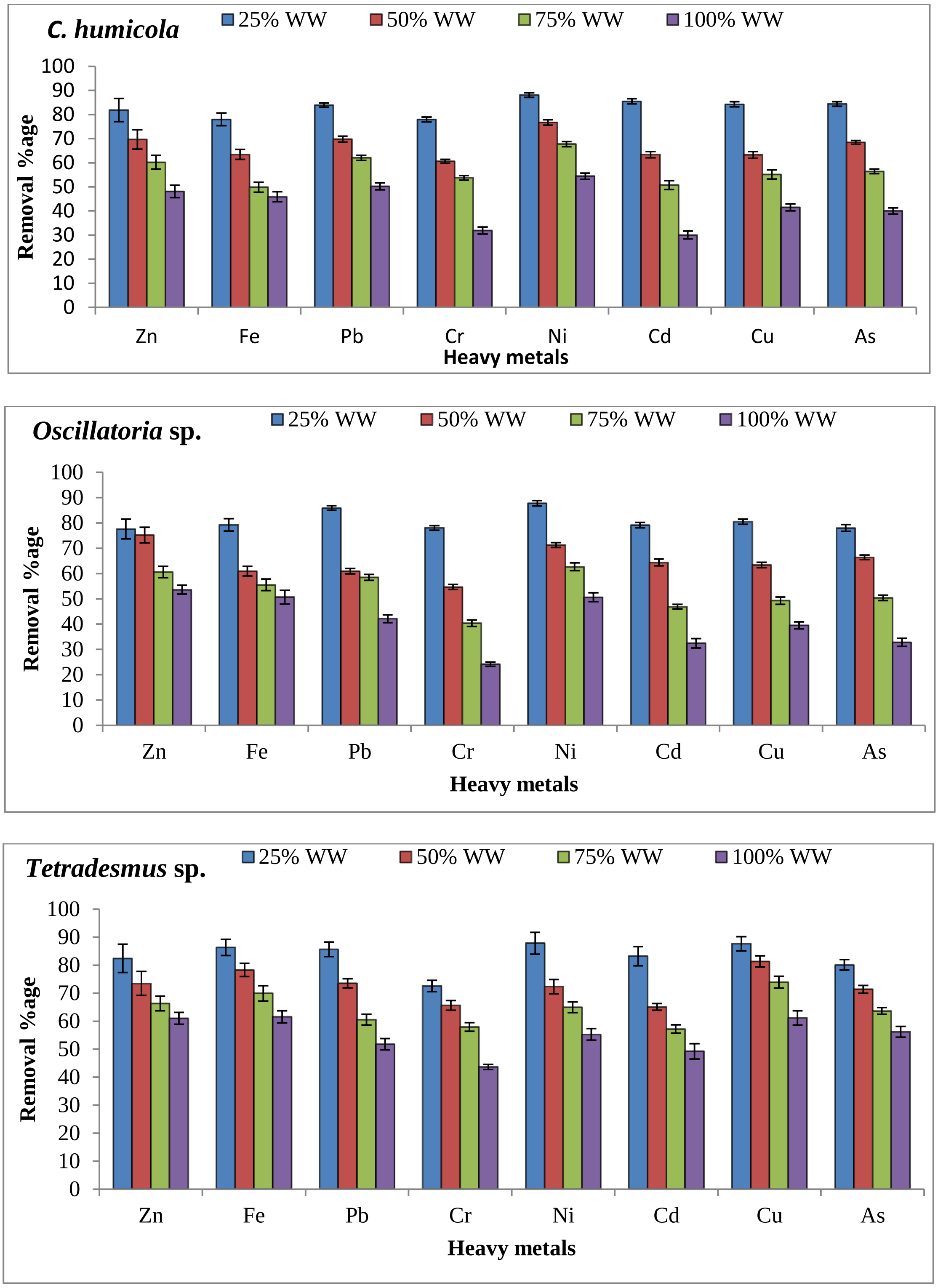


Fig 4.1 Heavy metals removal efficiency of *C. humicola, Oscillatoria* sp. and *Tetradesmus* sp. treated with different concentrations of municipal wastewater

4.2 Growth response of microalgae to wastewater

Growth characteristics of selected microalgae treated with different municipal wastewater concentrations (25%, 50%, 75% and 100 %, v/v) were analyzed by measuring absorbance at 650 nm on per day basis (Fig. 4.2). The results showed slow growth upto 3rd day in selected microalgae treated with wastewater than control. The initial slow growth could be due to channelization of energy for maintaining homeostasis by microalgae under municipal wastewater. The results also showed that *C. humicola, Tetradesmus* sp. and *Oscillatoria* sp. treated with 75% and 100% wastewater concentrations showed reduction in the exponential phase (8 days), which could be attributed to wastewater induced toxicity to microalgae (Nabizadeh et al., 2006). The decreased growth could also be due to metal pollutants mediated interruption in the essential metabolic processes such as photosynthesis and cell division (Arunakumara and Zhang, 2008). It was observed that *Tetradesmus* sp. showed highest growth rate at different concentration of wastewater as compared to *C. humicola,* and *Oscillatoria* sp. Further, the results exhibited maximum growth rate at 50% of municipal wastewater in selected microalgae (Fig. 4.2). The growth curves reflected that that 25% and 50% wastewater concentration enhanced the growth of microalgae with respect to control.

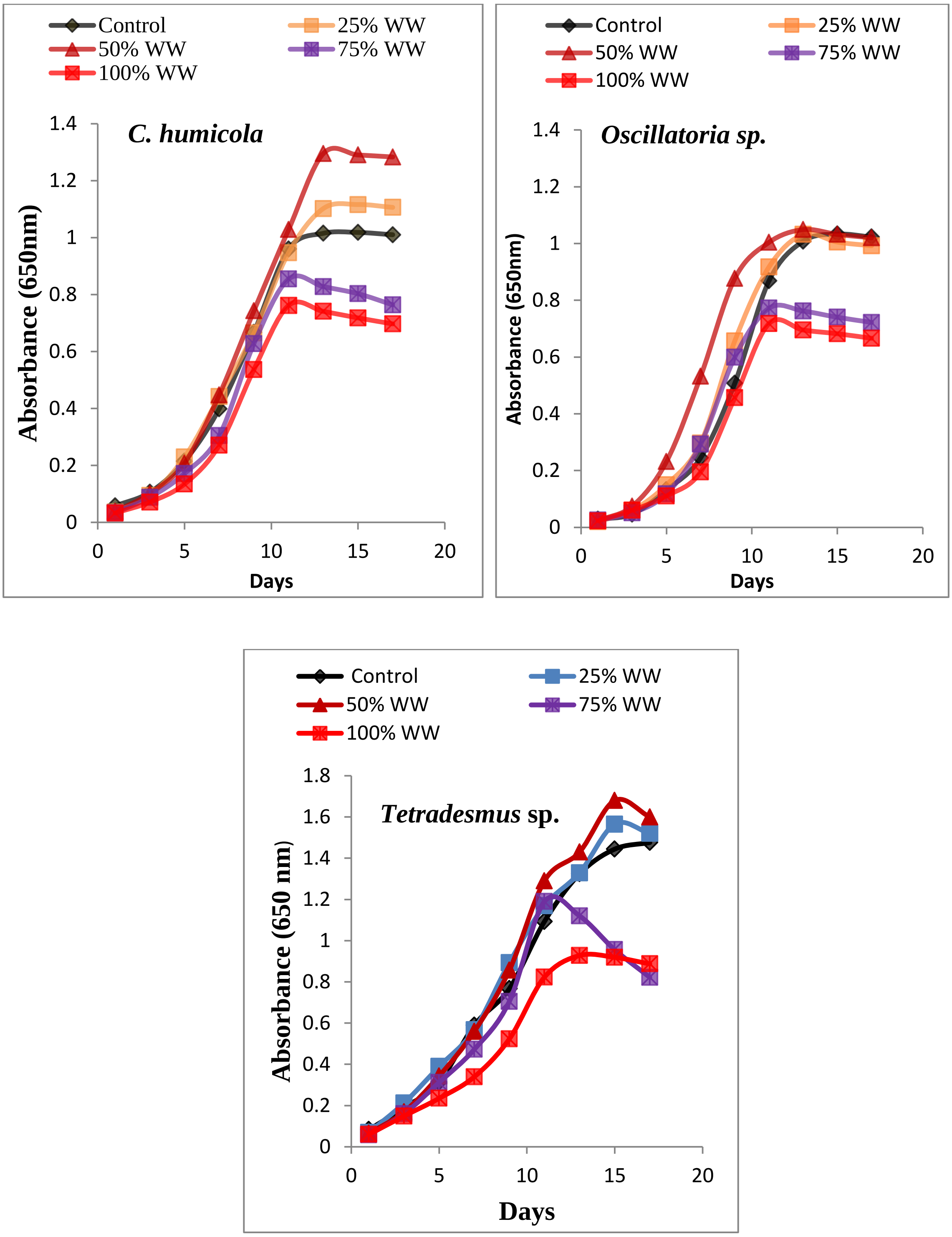


Fig.4.2 Growth pattern of *C. humicola* , *Oscillatoria* sp. and *Tetradesmus* sp. under different wastewater concentrations

4.3 Effect of municipal wastewater on pigment content of microalgae

The pigments (total chlorophyll and carotenoids) content is important indicator of adaptation potential of microalgae grown under different environmental conditions. It was observed that chlorophyll content increased by 78.97%, 26.54%, and 17.85% in *C. humicola, Oscillatoria* sp. and *Tetradesmus* sp. respectively treated with 25% municipal wastewater. The results showed the maximum increase of total chlorophyll content in *C. humicola, Tetradesmus* sp. and *Oscillatoria* sp. at 50% concentration of wastewater were about 54%, 57% and 52% higher than the control (Table 4.2). At 75% and 100% concentration of wastewater, the chlorophyll content was increased by 82% and 50% in *Tetradesmus* sp. whereas 2.70% and 19.51% in case of *C. humicola* while percent decline of 3.08% and 17.28% in case of *Oscillatoria* sp. The decrease in chlorophyll content may be due to toxic metal pollutants (Maleva et al., 2012), which affects the photosynthetic process by altering the activity of enzymes involved in the Calvin cycle (Aggarwal et al., 2012). Kumar and Shin, (2017) further suggested that impairment in the chlorophyll biosynthesis affects the growth of microalgae. The carotenoid pigment showed an increasing trend with wastewater concentration gradient when compared with their respective control. The carotenoid content was increased by 1.54, 2.07, 1.79 and 1.48 folds in *Tetradesmus* sp., after treatment with 25%, 50%, 75% and 100% wastewater concentration. In case of *C. humicola*, an increase of 1.25 and 1.39 folds while 1.11 and 1.50 folds in carotenoid content in *Oscillatoria* sp. was observed at 50% and 75% concentration of wastewater, respectively. Further, the maximum increase in the carotenoids content of *C. humicola* (1.71 fold) and *Oscillatoria* sp. (1.90 fold) was observed at 100% concentration, as compared to control (Table 4.2). The increased carotenoid synthesis with wastewater concentration gradient could be due to their role as antioxidants which maintain stability, maintain ROS balance, prevent membrane degradation and lipid peroxidation (Aboul-Enein et al., 2003; Sun et al., 2014; Singh et al., 2019). The results also revealed that carotenoids was less sensitive towards different concentrations of wastewater and increased carotenoids accumulation offered protection against reactive oxygen species (Upadhyay et al., 2021).

4.4 Protein, carbohydrate and lipids content

Microalgae *C. humicola*, *Tetradesmus* sp. and *Oscillatoria* sp. grown in different concentration of wastewater showed highest increase in the protein content of *C. humicola* (27.07%) at 25% wastewater, whereas in case of *Tetradesmus* sp. (37.50%) and *Oscillatoria* sp. (33.33%), it was observed at 50% concentration of wastewater (Table 4.2).

At 75% and 100% concentrations of wastewater, a decline in the protein content of *C. humicola* (0.43% and 4.80%) and *Oscillatoria* sp. (39.62%, and 30.07%) were observed in comparison to control. In case of *Tetradesmus* sp., an increase of 20.83% in protein content was observed at 75% wastewater concentration while declined by 16.66% at 100% concentration of wastewater. The declined protein synthesis in selected microalgae at 100% wastewater concentration could be due to inhibited enzyme activity responsible for protein synthesis (Carfagna et al., 2013). Patra et al. (2004) also reported that decline in the protein content might be due to binding of metals with sulphydryl groups of proteins which leads to protein deformation. The results of present study reflected that the protein content in *Oscillatoria* sp. was lower as reported by Sundaramoorthy et al. (2016).

Table 4.2 Effect on total chlorophyll (mg g^{-1} fw), carotenoids (mg g^{-1} fw), carbohydrate (mg L^{-1} fw), protein (mg g^{-1} fw) lipid (mg L^{-1}), TBARS (µmol g^{-1}fw) and H_2O_2 (µmol g^{-1}fw) content in microalgae *C. humicola, Oscillatoria* sp. and *Tetradesmus* sp. cultivated under different wastewater concentrations. All the values are means ±S.D. ANOVA post hoc DMRT ($p \leq 0.05$) was done to check the significance difference between the variables. Identical letter indicates no significant difference with in the variables.

Parameters	Microalgae	Control	Wastewater concentration			
			25% WW	**50% WW**	**75% WW**	**100% WW**
TC	*CH*	0.033±0.0014^{d}	0.051±0.0032^{f}	0.059±0.0009^{g}	0.032±0.0012^{d}	0.026±0.0019^{c}
	*O*s	0.016±0.0016^{a}	0.020±0.0009^{b}	0.024±0.0029^{bc}	0.015±0.0013^{a}	0.013±0.0007^{a}
	Ts	0.028±0.008^{c}	0.033±0.001^{d}	0.044±0.002^{e}	0.051±0.003^{f}	0.041±0.001^{e}
Carotenoids	*CH*	0.158±0.006^{c}	0.192±0.056^{e}	0.199±0.007^{e}	0.220±0.004^{f}	0.271±0.010^{g}
	*O*s	0.080±0.009^{a}	0.077±0.003^{a}	0.089±0.007^{a}	0.120±0.008^{b}	0.152±0.007^{c}
	Ts	0.082±0.003^{a}	0.127±0.004^{b}	0.170±0.006^{d}	0.147±0.007^{c}	0.122±0.005^{b}
Carbohydrate	*CH*	20.39±0.113^{d}	31.62±0.631^{f}	26.47±0.383^{e}	19.86±0.214^{c}	17.59±0.266^{bc}
	*O*s	24.69±0.256^{e}	26.80±0.201^{e}	27.75±0.571^{e}	20.03±0.209^{bc}	20.50±0.155^{c}
	Ts	15.31±1.37^{ab}	25.4±1.07^{e}	34.16±1.91^{g}	16.24±1.90^{ab}	12.78±0.830^{a}

Protein	*CH*	0.022±0.0013[g]	0.029±0.0010[h]	0.028±0.0012[h]	0.022±0.0018[g]	0.021±0.0022[f]
	*O*s	0.013±0.0009[d]	0.010±0.0014[c]	0.015±0.0016[e]	0.008±0.0027[a]	0.009±0.0011[b]
	Ts	0.024±0.002[gh]	0.030±0.001[hi]	0.033±0.001[j]	0.029±0.003[h]	0.025±0.005[gh]
Lipid	*CH*	0.117±0.009[a]	0.155±0.011[bc]	0.189±0.013[d]	0.164±0.019[bcd]	0.157±0.022[bc]
	*O*s	0.136±0.016[ab]	0.158±0.011[bc]	0.172±0.017[cd]	0.156±0.018[bc]	0.145±0.014[abc]
	Ts	0.143±0.013[ab]	0.174±0.006[cd]	0.198±0.006[e]	0.177±0.011[cd]	0.137±0.007[ab]
TBARS	*CH*	3.76±0.666[c]	4.94±0.440[d]	5.05±0.166[e]	5.59±0.333[ef]	6.02±0.881[f]
	*O*s	2.25±0.763[b]	4.62±0.333[d]	7.52±0.927[g]	10.21±0.600[h]	12.25±0.499[i]
	Ts	1.75±0.166[a]	2.15±0.345[b]	3.65±0.331[c]	4.73±0.632[d]	5.48±0.577[ef]
H_2O_2	*CH*	11.75±2.595[a]	50.23±9.856[d]	102.03±5.969[g]	112.18±3.230[g]	195.42±7.030[j]
	*O*s	22.85±3.218[b]	102.14±4.700[g]	157.39±5.390[h]	178.43±2.250[i]	210.80±6.540[k]
	Ts	8.10±0.654[a]	31.53±1.05[c]	80.98±2.23[e]	93.44±4.12[f]	144.12±7.67[h]

TC: Total chlorophyll, TBARS: Thiobarbituric acid reactive subastances, H_2O_2: Hydrogen peroxide, CH: *C. humicola*, *O*s: *Oscillatoria* sp., Ts: *Tetradesmus* sp.

The results exhibited that lipid content was increased in *C. humicola* (32.12%), *Tetradesmus* sp. (21.67%) and *Oscillatoria* sp. (15.59%) at 25% concentration of wastewater. At 50% concentration of wastewater, the lipid content in *C. humicola*, *Tetradesmus* sp. and *Oscillatoria* sp. was increased by 61.00%, 27.77% and 20.98%, respectively. The results also showed that highest increase in the lipid content was observed in case of *C. humicola* treated with 75% and 100% wastewater as compared to *Tetradesmus* sp. and *Oscillatoria* sp. The enhanced lipid accumulation may be attributed to wastewater induced modification in microalgae (Li et al., 2018). The increased lipid content may be also due to modification in PS-II centres and enhanced synthesis of glycerol, sugar and proteins (Sun et al., 2014). Furthermore, carbon redistribution and conversion of starch under stress condition could also leads to lipid accumulation in microalgae (Tan and Lee, 2016). Similarly, an increase of 65.90%, 123.12%, and 6.07% in carbohydrate content was observed at 25% 50% and 75%, while subsequent decline of 16.52% was observed in *Tetradesmus* sp. at 100% concentration of wastewater as compared to control. In *C. humicola,* carbohydrate content was found to be the highest at 25% concentration of wastewater, followed by 50% concentration of wastewater. Increase in the carbohydrate content of *C. humicola* (55% and 29.81%) than the *Oscillatoria* sp., (8.54% and 12.39%) was observed at 25% and 50% concentration of wastewater, respectively. The increased carbohydrate content at 25% and 50% wastewater concentration as compared to control might be due to low toxicity as well as efficient utilization of nutrient resources by microalgae. At 75% and 100% wastewater concentration, the decline in carbohydrate content was observed in *C. humicola* (2.57%, 13.73%) and *Oscillatoria* sp. (18.85%, 16.96%). The decreased carbohydrate content at 75% and 100% municipal wastewater concentration could be ascribed to decline in the chlorophyll content, interference in the cell division and inhibition of photosynthesis in microalgae.

4.5 Lipid peroxidation and hydrogen peroxide (H_2O_2)

Cultivating microalgae in contaminated environment leads to lipid peroxidation which is measured in terms of TBARS content (Upadhyay et al., 2016). The results showed the highest TBARS content in *Oscillatoria* sp. as compared to *Tetradesmus* sp. and *C. humicola*. At 100% concentration of wastewater, the TBARS content in *C. humicola* and *Tetradesmus* sp. was increased by 1.60 and 3.12 folds respectively while, it was enhanced by 5.38 fold in *Oscillatoria* sp. when compared with their respective control (Table 4.2). In case of *Tetradesmus* sp., TBARS content was observed to increase by 1.22, 2.08 and 2.69 folds while 1.31, 1.34 and 1.48 folds in *C. humicola,* 2.04, 3.28 and 4.47 folds in *Oscillatoria* sp. at 25%, 50% and 75% concentration of wastewater. TBARS is a by-product of lipid peroxidation and key indicator of toxicity (Upadhyay et al., 2020). Similarly, the level of H_2O_2 in *Tetradesmus* sp., *C. humicola*, and *Oscillatoria* sp. was increased by 9.15**,** 9.22 and 16.62 folds respectively, in comparison to control at 100% concentration of wastewater. At 50% and 75% concentration of wastewater, the highest increase of 8.68 and 9.54 folds in H_2O_2 was observed in *Oscillatoria* sp. The results also showed that H_2O_2 in *Tetradesmus* sp. (6.62, 7.64 folds) and *C. humicola* (6.88, 7.80 folds) was also increased after treatment with 50% and 75% concentration of wastewater. As compared to untreated microalgal cells, accumulation of H_2O_2 in selected microalgae was increased with successive increase in the concentration of wastewater. Higher intracellular concentration of H_2O_2 elevate the oxidative damage through oxidation of thiol rich compounds and interrupt the activity of enzymes of calvin cycle (Upadhyay et al., 2016). It also stimulates the production of hydroxyl radical (Phaniendra et al.,

2015) and initiates cell death in case of extreme exposure of microalgae to stress conditions (Dat et al., 2000).

4.6 Effect of different wastewater concentration on antioxidant activity of microalgae

4.6.1 Cysteine, proline and ascorbic acid

Exposure to stress conditions elevates the accumulation of ROS in microalgae which is subjected to intracellular scavenging either by enzymatic scavengers (SOD, CAT, GR) or by antioxidative compounds (ascorbic acid, proline, cysteine). Proline content in *Tetradesmus* sp., *C. humicola* and *Oscillatoria* sp. was increased by 4.63, 4.04 and 3.12 fold, respectively, at 100% concentration of wastewater as compared to control. The results further revealed that proline content was increased by 1.46, 3.17 and 4.29 folds in *Tetradesmus* sp while 1.74, 2.42 and 3.33 folds in *C. humicola*, 1.26, 2.08 and 2.91 folds in *Oscillatoria* sp. at 25%, 50% and 75% concentration of wastewater. Proline protects the algal cells (Upadhyay et al., 2016) by stabilizing proteins, maintaining intracellular pH and cell homeostasis (Szabados and Savoure, 2010). An increase in the accumulation of proline with wastewater concentration gradient in microalgae could be their involvement in cell signalling and cell recovery (Hayat et al., 2012). The enhanced accumulation of proline may be due to their role in the removal of metal pollutants from the wastewater (Tripathi and Gaur, 2004). The cysteine content in *C. humicola*, *Tetradesmus* sp. and *Oscillatoria* sp. at 25% concentration of wastewater exhibited an increase of 2.64, 1.89 and 1.44 folds, respectively, as compared to control. However, the highest enhancement in cysteine level was observed in *C. humicola* (4.92 fold) and *Tetradesmus* sp. (3.75 folds) while lowest in *Oscillatoria* sp. (3.40 fold) at 100% concentration of wastewater. The results further exhibited that cysteine content was increased by 3.53 and 4.07 folds in

C. humicola , 2.70 and 3.32 folds in *Tetradesmus* sp. while 2.35, and 2.98 folds in *Oscillatoria* sp. after treatment with 50% and 75% concentration of wastewater. The lowest cysteine accumulation at 25% wastewater could be attributed to decrease in the load of metals with increase in the dilution of wastewater. The enhancement in cysteine accumulation may improve the metals sequestration by acting as precursor of metal chelating compounds such as glutathione, phytochelatins and metallothioneins (Balzano et al., 2020). Ascorbic acid is another important anti-oxidative molecule of plant defense system with its vital role in the cell growth and metabolism (Pehlivan, 2017). The results revealed that highest increase in the ascorbic acid was observed in *C. humicola* (4.97, 5.38, 5.93, 9.25 folds) followed by *Tetradesmus* sp. (3.15, 3.42. 2.27, 3.48 folds) and *Oscillatoria* sp. (1.25, 1.01, 1.26, 1.64 folds) at different municipal wastewater concentrations (Fig. 4.3). The increased ascorbic acid content may regulates photosynthesis, stimulate activity of hormones, sustain redox balance and alleviate damage by sequestration of oxidative radicals (Gallie et al., 2013). Thus, the increased generation of reactive species may serves as the trigger for accumulation of ascorbic acid in selected microalgae.

4.6.2 Superoxide dismutase, catalase and glutathione reductase

The superoxide dismutase (SOD) activity in selected microalgae was observed to increase with municipal wastewater concentration gradient. The highest SOD activity was observed in *Tetradesmus* sp. (1.54 folds), *C. humicola* (1.43 folds) and *Oscillatoria* sp. (1.26 fold) at 100% wastewater concentration than control. In *Tetradesmus sp.*, the activity of SOD was also increased at 25% (1.06 folds), 50% (1.15 folds), and 75% (1.76 folds) concentration of wastewater.

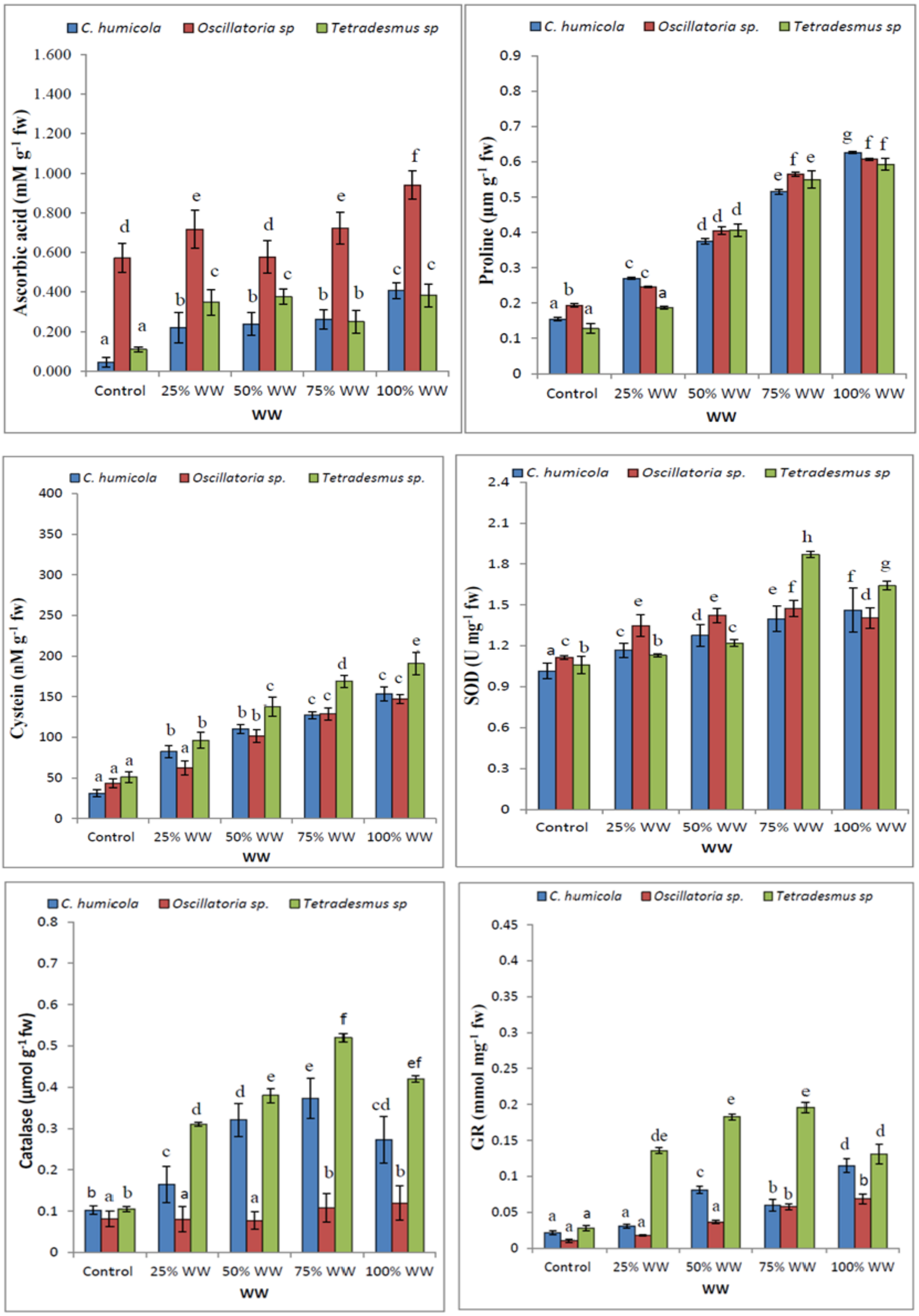
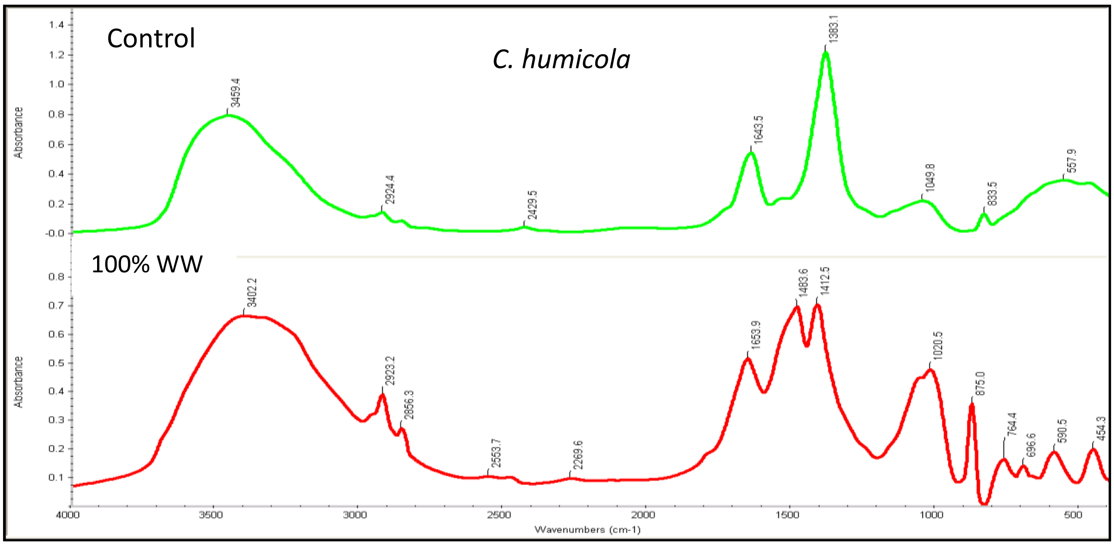


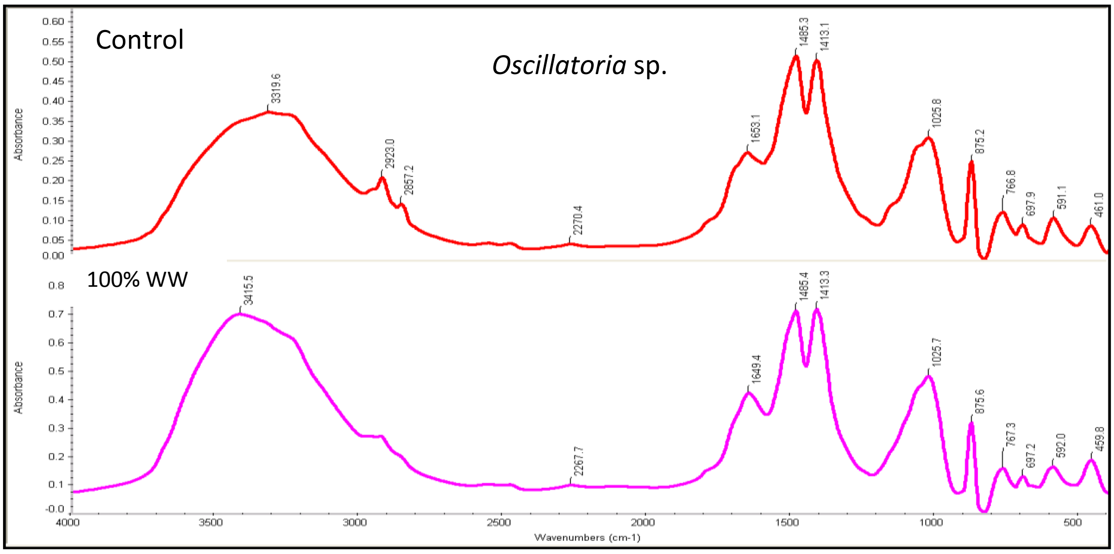
Fig.4.3 Effect on antioxidants (Proline, cysteine and ascorbic acid) and enzymatic activities (SOD, catalase and GR) in microalgae *C. humicola*, *Oscillatoria* sp. and *Tetradesmus* sp. treated with different wastewater concentration.

It was further observed that SOD activity was increased in *C. humicola* (1.15-1.37 folds) and *Oscillatoria* sp. (1.20-1.32 folds) at 25%-75% municipal wastewater (Fig. 4.3). Thus, the increased SOD activity may be due to ROS and microalgae respond to offset the cell damage by stimulating the activity of SOD. SOD protect the aerobic organism from oxidative damages by sequestration of superoxide and also prevent the formation of hydroxyl radical (Ighodaro and Akinloye, 2018; Singh et al., 2018). Besides, the activity of catalase in *Tetradesmus* sp. (4.95 folds) and *C. humicola* (3.63 folds) was maximum at 75% municipal wastewater concentration while in *Oscillatoria* sp., an increase of 1.48 folds was found at 100% concentration of wastewater in comparison to control. Further, an increase of 2.95 and 3.61 folds in catalase activity was observed in *Tetradesmus* sp. at 25% and 50% wastewater while 1.60 and 3.12 folds in case of *C. humicola*. The catalase is highly specific towards H_2O_2 sequestration and neutralizes it without consuming extra energy (Sharma et al., 2012; Shoaib and Sibi, 2018). The GR activity in *C. humicola*, *Tetradesmus* sp. and *Oscillatoria* sp. was enhanced by 5.30, 4.67 and 6.67 folds at 100% wastewater as compared to control (Fig. 4.3). The maximum GR activity of 7 folds was found to be in case of *Tetradesmus* sp. treated with 75% wastewater. At 25% and 50% municipal wastewater, the highest increase in GR activity was observed in *Tetradesmus* sp (4.95, 6.53 folds) followed by *C. humicola* (1.43, 3.75 folds) and *Oscillatoria* sp. (1.74, 3.54 folds). GR is an important antioxidant which play key role under stress condition by neutralizing ROS and sustain the balance of reduced glutathione in the cell (Ding et al., 2016; Rezayian et al., 2019). Therefore, the enhancement in the activity of different enzymatic activity with wastewater concentration gradient could be the response towards wastewater imposed stress conditions.

4.7 FTIR spectroscopy of algal biomass

The FTIR spectroscopy is the technique used to examine the cellular composition of the algal biomass. The spectra of untreated and municipal wastewater treated microalgae between 3410-2850 cm^{-1} showed the asymmetry of hydroxyl group in algal biomass (Fig. 4.4). The broad peaks in *C. humicola* (3415.5 cm^{-1}), *Oscillatoria* sp. (3402 cm^{-1}) and *Tetradesmus sp.* (3388.9 cm^{-1}) at 100% municipal wastewater reflected the presence of OH group containing compounds in algal biomass which may be responsible metal removal from wastewater (Ajayan et al., 2015).





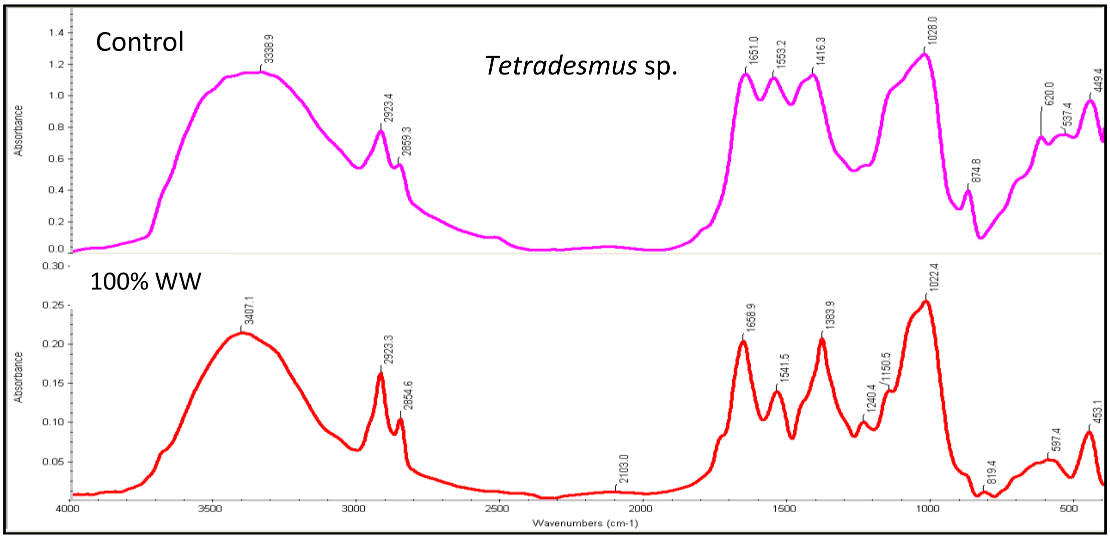


Fig.4.4 FTIR analysis of microalgae *C. humicola, Oscillatoria* sp. and *Tetradesmus* sp. treated with different concentration of wastewater

The IR absorption between 2900-3000 cm^{-1} is associated with the methyl and methylene groups (Laurens and Wolfrum, 2011) and the higher peaks in treated *C. humicola* and *Tetradesmus sp.* indicates lipid accumulation. Further, the absorption peaks between 1500-1700 cm^{-1} reflected the stretching of amide I (C=O) and amide II (N-H) (Giordano et al., 2001), which also indicated the variation of protein content in microalgae. The region between 900-1200 cm^{-1} is linked to carbohydrates (Murdock and Wetzel, 2009) and the complex overlapping bands reflects the larger variation of carbohydrate in microalgae after treatment with 100% municipal wastewater. Overall, the results of FTIR spectroscopy showed higher absorption peaks in *Tetradesmus* sp. and *C. humicola* which reflected that different functional groups responsible for metal removal were abundant in algal biomass.

Chapter 5

Implication of municipal wastewater on growth kinetics, biochemical profile, and defense system of Chlorella vulgaris and Scenedesmus vacuolatus

CHAPTER-5

Implication of Municipal Wastewater on Growth Kinetics, Biochemical Profile and Defense System Of *Chlorella vulgaris* and *Scendesmus vacuolatus*

5.0 Introduction

Water scarcity along with energy depletion and environmental deterioration are the leading global problems that need to be addressed for smooth functioning of different ecosystem (Singh et al., 2021). Wastewater management is one of the biggest problem in India as the technological as well as economic constraints are hampering the proper treatment of wastewater (Upadhyay et al., 2021). Wastewater not only affects the different qualities of water but also disturb the balance of the recipient ecosystem (Rajasulochana and Preethy, 2016). Thus, appropriate treatment of the municipal wastewater is required prior to their discharge into the environment (Singh et al., 2022).

The research on suitable treatment technologies is increasing in order to improve the quality parameters as well as water reuse (Ahmed et al., 2021). The conventional technologies like electro-coagulation, chemical precipitation, trickling filter and activated sludge used to treat wastewater are costly, energy-intensive and incompetent to deal with complexity of wastewater (Singh et al., 2020). Therefore, the sustainable treatment methods are required that improve the water quality as per the discharge standards (Upadhyay et al., 2021). Due to simple growth requirement, microalgae are gaining substantial attention for the treatment of wastewater (Peter et al., 2021). Additionally, utilizing microalgae for the wastewater treatment can certainly reduce the reliance on the water, energy and nutrients which ultimately can have multiple benefits for the society (Singh et al., 2020).

Algae are autotrophic, oxygen-evolving and faster growing organisms than terrestrial plants (Randrianarison and Ashraf, 2017). Wastewater of different origin has been exploited as the nutrient media for growth of microalgae (Chokshi et al., 2016). Earlier, it has been reported that that *Chlorella vulgaris* showed promising potential to remove nitrogen (98%) from wastewater (Liu, et al., 2017). Microalgae have also shown potential to remove different heavy metals from wastewater (Ajayan et al., 2011). Recently, microalgae *Chlorella pyrenoidosa* (45.45%) and *Scenedesmus acutus* (57.14%) have shown excellent potential to remove cadmium at neutral pH (Chandrashekharaiah et al., 2021). Similarly, *Chlorella* sp. and *Scenedesmus* sp. removed 8.07% and 5.13% cadmium from growth media containing 1 ppm of cadmium. Though, microalgae have shown tremendous remediation efficiency (Zhou et al., 2014) but exposure to heavy metals could lead to cellular toxicity which impairs growth, biomass production (Jais et al., 2017), pigment synthesis and photosynthetic activity of algae (Singh et al., 2021). At the same time, microalgae also stimulate the antioxidants activity in order to reduce the damage under stressed conditions (Danouche et al., 2020; Singh et al., 2022). Thus, the treatment efficiency can be improved by selecting the stress tolerant algal species that can counterbalance the damage and maintain the overall growth and productivity of microalgae. Therefore, two widely known species of microalgae that is *Chlorella vulgaris* and *Scenedesmus vacuolatus* were exploited to treat different concentrations of municipal wastewater under natural condition.

5.1 Results and discussion

5.1.1 Variation in wastewater characteristics treated with *Chlorella vulgaris* and *Scenedesmu vacuolatus*

The quality parameters of wastewater before and after treatment with microalgae are mentioned in table 5.1. The initial pH of 8.1 was observed in wastewater and after treatment with *C. vulgaris* and *S. vacuolatus*, the increment in pH was observed which may be attributed to the use of CO_2 from wastewater. The EC was reduced by 86.00% and 77.04% with *C. vulgaris* while 86.38% and 75.15% with *S. vacuolatus* at 25% and 50% municipal wastewater, respectively in comparison to initial EC concentration. The results also depicted that *S. vacuolatus* (61.41%) and *C. vulgaris* (60.32%) showed excellent reduction in EC at 100% municipal wastewater concentration. It was also observed that TDS, TS and TSS was reduced by 91.31%, 90.75%, and 89.17% in case of wastewater (25%) treated with *C. vulgaris* while the reduction efficiency of 90.59%, 90.55%, and 89.69% was observed with *S. vacuolatus*. Further, the results exhibited that *S. vacuolatus* showed highest TDS and TS reduction efficiency at 50% and 75% concentration of wastewater. Similarly, the highest TDS (65.97%) and TS (70.43%) reduction was observed with *S. vacuolatus* treated with 100% of municipal wastewater. The significant decline in TDS level of different wastewater concentration may be ascribed to utilization of nutrient resource by microalgae (Vinodhini and Soundhari, 2019; Singh et al., 2021). The result showed that BOD level of treated wastewater was reduced significantly after treatment with *C. vulgaris* and *S. vacuolatus* (Table 5.1). It was observed that *S. vacuolatus* (93.40%) and *C. vulgaris* (91.87%) showed highest reduction efficiency of BOD at 25% of wastewater concentration. The results further showed the highest BOD reduction with *S. vacuolatus* (83.17% and 50.12%) at 50% and

100% municipal wastewater concentration. At 75% wastewater, the highest reduction efficiency of 67.36% in BOD level was observed with *C. vulgaris*. The considerable decline in BOD level of municipal wastewater could be due to reduction of organic matter of wastewater after treatment with microalgae (Singh et al., 2020, Singh et al., 2021). The initial DO level of municipal wastewater was nil which indicated the highly polluted wastewater. The results showed an increase of 2.74 folds in DO level after treatment of 25% wastewater with *S. vacuolatus* while significant decline was observed with further increment in wastewater concentration. The decline in DO level after 25% wastewater concentration might be attributed to different metal pollutants in wastewater which eventually influence the photosynthesis and metabolic pathways of algae (Singh et al., 2021). The results related to different water quality parameters were in agreement with Upadhyay et al., (2021).

5.1.2 Nutrient resource recovery

Nutrient like nitrate-nitrogen and phosphorus are important for the proper functioning of different cellular organelles (Singh et al., 2021). At 25% municipal wastewater, the nitrogen and phosphorus removal efficiency of 87.74-88.90% and 91.34-92.28% was observed with *S. vacuolatus* and *C. vulgaris*, respectively, (Table 5.1). It was also observed that *C. vulgaris* removed 79.06% and 63.35% of nitrate-nitrogen at 50% and 75% municipal wastewater while the removal efficiency of 78.25% and 66.14% was observed in case of *S. vacuolatus*. Further, the removal efficiency of 53.86% and 59.11% for nitrate nitrogen was observed in the case of *C. vulgaris* and *S. vacuolatus,* respectively. The results also depicted the highest phosphorus removal at 25% (93.28%), 50% (89.13%), 75% (87.40%) and 100% (83.59%) wastewater treatment to *S. vacuolatus* while, 91.34%, 88.22%, 85.12% and

Table 5.1 Alteration in physico-chemical characteristics of different municipal wastewater concentrations after treatment with *Chlorella vulgaris* and *Scenedesmus vacuolatus*

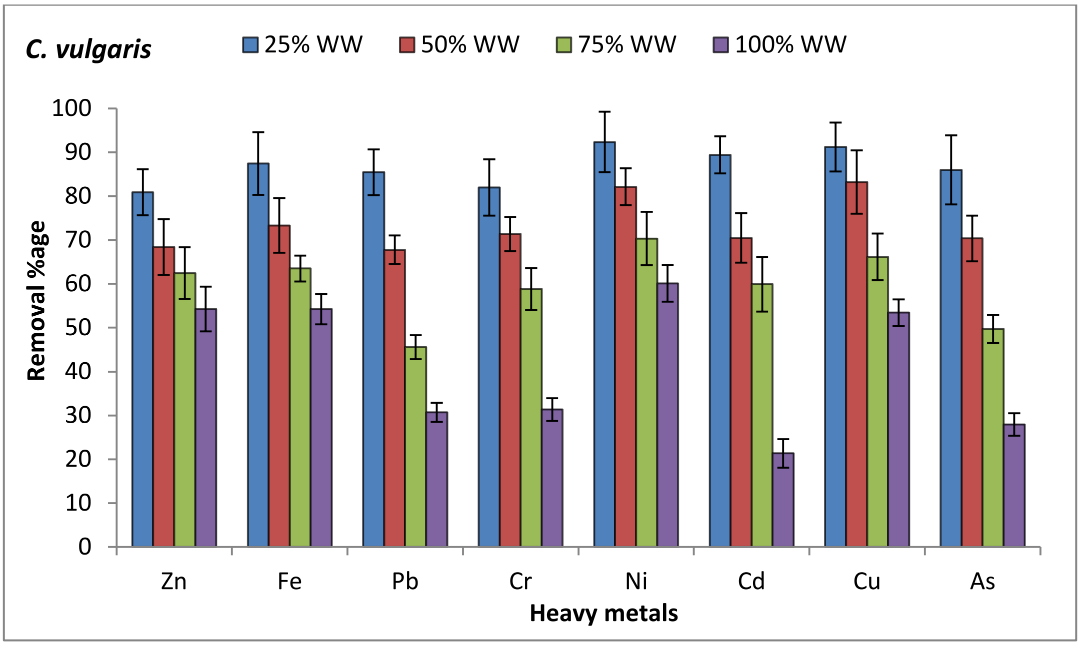
Parameters	Initial concentration	Microalgae	BG-11 treated microalgae	Final concentration			
				25%	50%	75%	100%
pH	8.2±0.054	CV	8.85±0.036^{b}	8.92±0.022^{b}	8.95±0.037^{b}	8.90±0.053^{b}	9.06±0.063^{c}
		SV	9.15±0.047^{d}	8.75±0.036^{a}	9.32±0.067^{e}	9.23±0.037^{e}	9.14±0.036^{cd}
EC (µS cm^{-1})	793±9.06	CV	2.81±0.142^{a}	111±2.518^{b}	182±5.516^{c}	251±3.053^{e}	314±6.414^{f}
		SV	2.44±0.237^{a}	108±4.514^{b}	197±2.510^{d}	261±4.046^{e}	306±3.512^{f}
TDS	194±8.79	CV	2.41±0.018^{a}	21±4.032^{b}	37±3.222^{c}	53±2.737^{d}	73±6.502^{e}
		SV	2.23±0.035^{a}	20±3.227^{b}	28±3.726^{c}	50±4.509^{d}	66±6.285^{e}
TSS	276±11.04	CV	3.83±0.650^{a}	24.3±2.510^{b}	35.66±5.033^{c}	50.00±7.548^{d}	75.66±5.032^{e}
		SV	5.20±0.916^{a}	26.3±1.522^{b}	41.00±5.567^{c}	54.66±7.631^{d}	84.00±6.243^{e}
TS	487±15.76	CV	6.24±0.677^{a}	45±3.733^{b}	73±6.557^{c}	103±5.772^{d}	149±12.660^{e}
		SV	7.43±0.822^{a}	46±5.681^{b}	68±8.389^{c}	96±7.236^{d}	144±13.421^{e}
BOD	41±2.434	CV	0.42±0.022^{a}	3.33±0.136^{b}	8.43±0.372^{d}	13.38±0.290^{e}	22.50±0.599^{g}
		SV	0.34±0.037^{a}	2.70±0.392^{b}	6.90±0.410^{c}	14.13±0.928^{e}	20.45±0.502^{f}
DO	-	CV	5.22±0.189^{a}	2.33±0.136^{b}	1.10±0.236^{b}	0.59±0.178^{c}	0.26±0.036^{d}
		SV	5.50±0.199^{a}	2.74±0.089^{b}	1.40±0.136^{b}	0.71±0.268^{c}	0.30±0.078^{d}
NO_3-N	32.8±5.45	CV	0.266±0.010^{a}	4.01±0.039^{b}	6.86±0.039^{c}	12.01±0.256^{e}	15.13±0.426^{g}
		SV	0.312±0.040^{a}	3.63±0.053^{b}	7.13±0.214^{c}	11.10±0.246^{d}	12.40±0.373^{e}
PO_4^{-3}	12.6±3.89	CV	0.420±0.008^{a}	1.09±0.036^{c}	1.48±0.013e	1.87±0.049^{g}	2.15±0.036^{i}
		SV	0.366±0.010^{a}	0.84±0.049^{b}	1.34±0.018^{d}	1.58±0.017^{f}	2.06±0.080^{h}

CV; *Chlorella vulgaris*, SV; *Scenedesmus vacuolatus*.

82.93% was observed in case of *C. vulgaris*. The noteworthy phosphorus removal efficiency may be due to nitrogen sufficient conditions which stimulated the accumulation of phosphorus in microalgae (Beuckels et al., 2015). The considerable nutrient reduction from different wastewater concentrations may be their utilization for growth as well as biomass synthesis (Rosli et al., 2020). Furthermore, the removal of nutrients from wastewater depends upon algal species as well as the pH of media used for the growth of microalgae. The increase in pH after treatment of wastewater with selected microalgae may promotes nutrients precipitation which also may be responsible for removal of nutrients from wastewater (Vandamme et al., 2012; Singh et al., 2021). The excellent nutrient removal from wastewater has been also reported by Rasoul-Amini et al., (2014).

5.1.3 Heavy metal removal

Heavy metals toxicity is the most common stress encountered by different organisms (Ghori et al., 2019). Heavy metals are considered highly toxic and even small quantity can severely disturb different ecosystem. The results exhibited that heavy metal like Fe (1776.34 µg/L) was maximum in municipal wastewater followed by Ni (13.04 µg/L), Zn 10.78 (µg/L), Pb (9.27 µg/L), Cr (5.2 µg/L), Cd (3.12 µg/L), Cu (3.78µg/L), and As (0.25 µg/L). At 25% municipal wastewater, the highest removal efficiency for Cd (92.08%), Pb (87.85%), As (87.26%), Cr (86.93%) and Zn (82.51%) was observed with *S. vacuolatus* while *C. vulgaris* showed highest removal efficiency for Fe (92.34%), Cu (91.21%), and Ni (87.42%). The results also exhibited that *C. vulgaris* showed highest removal efficiency for Cu (83.23%), Ni (82.15%), Fe (73.29%) and Cd (70.47%) while removal efficiency of 75.14% (As), 73.97% (Cr), 73.60 (Zn) and 70.10% (Pb) was observed in case of *S. vacuolatus* treated with 50% of municipal wastewater.



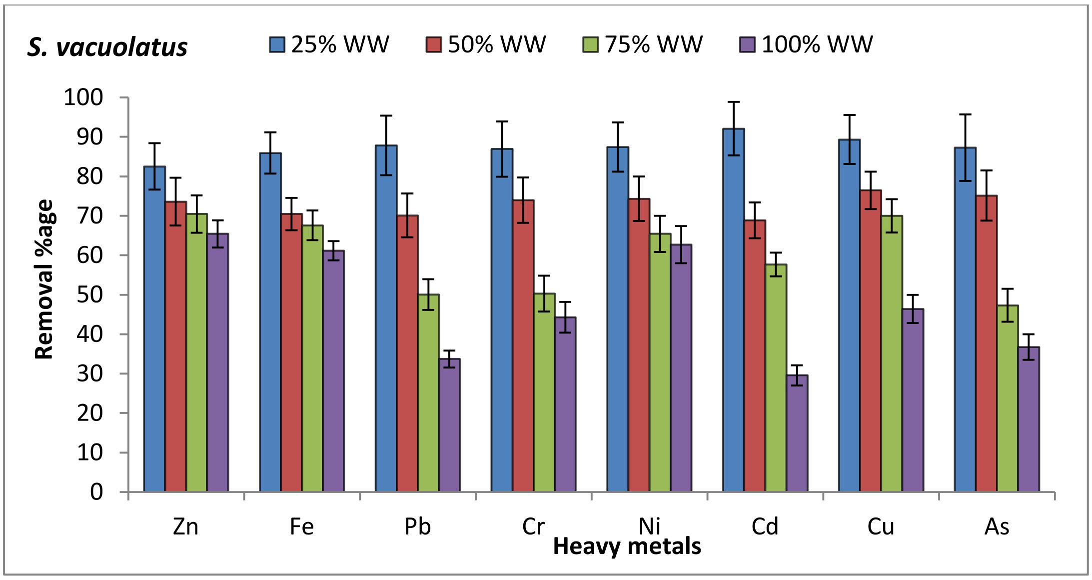


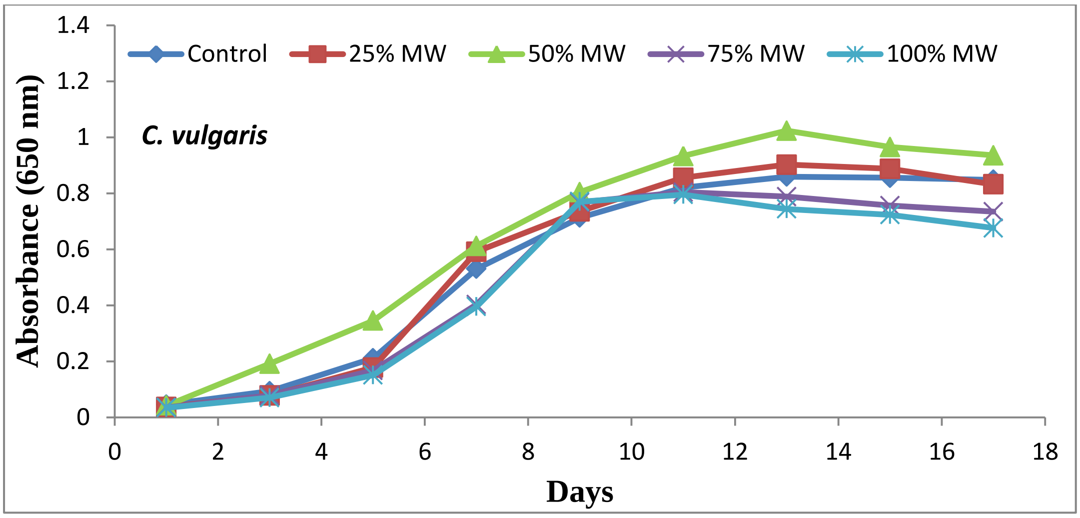
Fig. 5.1 Heavy metal removal efficiency (% age) of microalgae treated with different concentration of municipal wastewater collected from Lucknow.

At 75% municipal wastewater, *C. vulgaris* showed highest removal for Ni (70.33%), Cd (59.92%), Cr (58.81%) and As (49.70%) whereas *S. vacuolatus* efficiently removed Zn (70.45%), Cu (69.98%), Fe (67.58%) and Pb (50.07%). Furthermore, the removal efficiency of 65.45%, 62.69%, 61.12%, 44.29%, 36.75%, 33.73% and 29.58% was observed for Zn, Ni, Fe, Cr, As, Pb and Cd with *S. vacuolatus* treated with 100% concentration of municipal wastewater (Fig. 5.1). The remarkable metal removal by selected microalgae at lower wastewater concentrations (25-50%) may be ascribed to lower toxicity and sufficient functional groups on cell surface. The tremendous metal removal potential of microalgae may be due to hydroxyl, amides, carbonyl and carboxyl groups on algal cell surface that bind metal and ensure their removal from wastewater (Kumar et al., 2020). Furthermore, the phytochelatins could also assist microalgae in metal sequestration (Balzano et al., 2020). Overall, the results reflected that *S. vacuolatus* was more effective in metal removal from wastewater than *C. vulgaris.*

5.2 Growth pattern under wastewater environment

The growth pattern of selected microalgae was recorded for 16 days after treatment with different municipal wastewater concentrations (25%-100%). The results showed restricted growth for two days which may be due to acclimatization of both microalgae to different wastewater and environmental conditions. The enhancement in the growth was observed after 2^{nd} day in both microalgae treated with different municipal wastewater concentrations. The results exhibited that the growth profile was highest at 25% and 50% wastewater concentration as compared to 75% and 100%. The specific growth of 0.0708 day^{-1} was observed in case of *S. vacuolatus* and 0.0558 day^{-1} in case of *C. vulgaris treated* with 50% wastewater (Fig. 5.2). The results also showed that specific growth rate was decreased at higher wastewater concentration in both microalgae. The highest growth as well as specific growth upto

50% municipal wastewater concentration may be due to less toxicity to microalgae after dilution of wastewater. Metals hamper the growth of microalgae by interfering in cell division and also lead to cell death under extreme exposure to metal pollutants (Naorbe et al., 2018). The metal induced toxicity and growth inhibition has been also reported by Upadhyay et al., (2016). Therefore, higher growth rate indicated superior adaptability of *S. vacuolatus* under different concentrations of municipal wastewater.



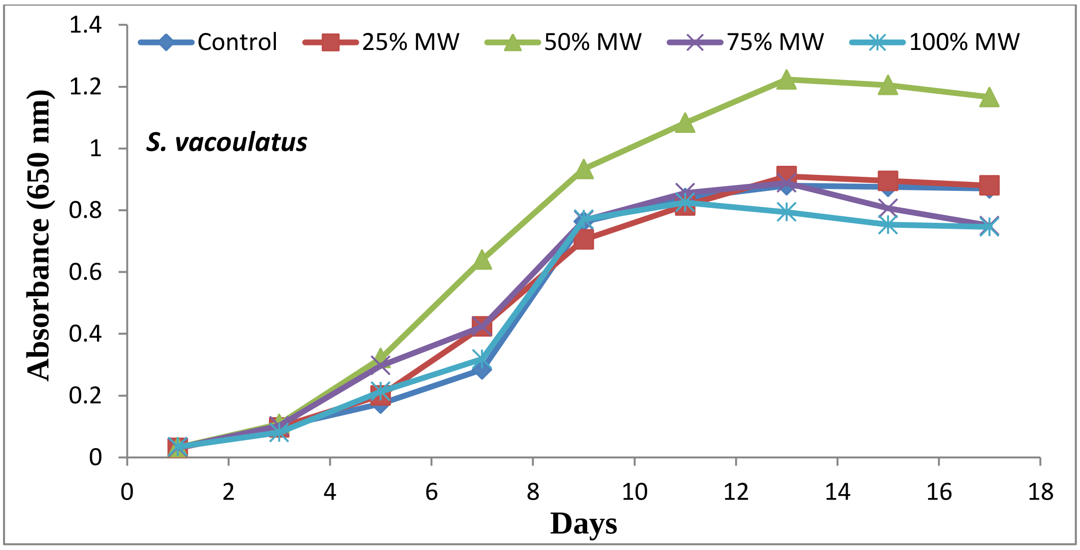


Fig. 5.2 Alteration in growth pattern of *Chlorella vulgaris* and *Scenedesmus vacoulatus* under different concentration of municipal wastewater

5.3 Biochemical profiling

5.3.1 Total chlorophyll and carotenoid content

In this study, the pigment content in microalgae *C. vulgaris* and *S. vacuolatus* showed significant variation after treatment with municipal wastewater. The results depicted that chlorophyll content was increased in *C. vulgaris* (1.75 folds) and *S. vacuolatus* (1.23 folds) after treatment with 25% concentration of municipal wastewater. The results also showed that the highest increase of 2.13 folds in chlorophyll content was observed in case of *C. vulgaris* while, 2.75 folds in *S. vacuolatus* after treatment with 50% municipal wastewater. At 75% and 100% wastewater concentration, an increase of 1.08 and 1.02 folds in chlorophyll content was observed in case of *C. vulgaris* and in *S. vacuolatus*, it was increased by 1.09 and 1.00 folds. The increased chlorophyll content might be attributed to the cellular mechanisms responsible for alleviation of the damage posed by wastewater. The results further depicted that total chlorophyll content in *C. vulgaris* and *S. vacuolatus* was decreased significantly at 75% and 100% as compared to 25% and 50% municipal wastewater. The decreased total chlorophyll content may be ascribed to metal induced damage to photosystems as well as inhibited activity of enzymes (Farooqi et al., 2021). Metals may also disturb the ETC chain or replace the essential ions from the chlorophyll molecule which eventually leads to chlorophyll degradation (Yang et al., 2015). It was observed that carotenoid content was increased by 1.30 and 1.05 folds in *C. vulgaris* and *S. vacuolatus* treated with 25% wastewater (Table 5.2). Similarly, an increase of 1.49 and 1.39 folds was also observed in *C. vulgaris* and *S. vacuolatus* after treatment with 50% wastewater. Further, *C. vulgaris* and *S. vacuolatus* also showed an increase of 1.12 and 1.31 folds in carotenoid content at

75% while, 1.06 and 1.10 folds at 100% municipal wastewater concentration than control. The increased carotenoid accumulation with wastewater may be due to its role as antioxidants in microalgae under adverse operating and environmental conditions. Slama et al., (2017) observed that apart from serving as the accessory pigment, carotenoids also protect the algal cell from to stress conditions. Earlier, the increased carotenoid accumulation after exposure of microalgae to stress conditions may be ascribed to scavenging of singlet oxygen by carotenoids (Hu et al., 2018). Furthermore, carotenoids prevent lipid peroxidation, maintain cell growth and stability of cell membrane (Upadhyay et al., 2021). Thus, enhanced carotenoid accumulation may be the defensive mechanism to sustain the cellular activity under different wastewater concentrations.

5.3.2 Protein and carbohydrate content

The results revealed an increase of 56.08% and 12.06% in *C. vulgaris* and *S. vacuolatus* at 25% wastewater concentration while, it was increased by 77.82% and 65.86% at 50% concentration of municipal wastewater. Furthermore, *C. vulgaris* showed an increase of 22.17% and 15.21% after treatment with 75% and 100% wastewater while, in case of *S. vacuolatus*, an increase of 6.20% in protein content was observed at 75% wastewater concentration. The decline in protein content at higher wastewater concentration as compared to lower wastewater concentration may be attributed to inhibited activity of enzymes and protein synthesizing system (Hossain et al., 2012). It was also observed that *C. vulgaris* treated with 25% and 50% wastewater showed an increase of 45% and 67% in carbohydrate content while, an increase of 4.29% and 56.53% was observed in case of *S. vacuolatus* (Table 5.2). The results further depicted that carbohydrate content was decreased at 75% and 100% as

Table 5.2 Effect of different wastewater concentration on total chlorophyll (mg g^{-1} fw), carotenoids, (mg g^{-1} fw), carbohydrate (mg L^{-1} fw), protein (mg g^{-1} fw), lipid content (mg L^{-1}), TBARS (µmol g^{-1}fw) and H_2O_2 (µmol g^{-1}fw) in microalgae *Chlorella vulgaris* and *Scenedesmus vacuolatus*

Wastewater concentration	Total Chlorophyll	Carotenoids	Protein	Carbohydrate	Lipid	TBARS	H_2O_2
Chlorella vulgaris							
Control	0.036±0.0006[a]	0.202±0.002[a]	0.023±0.0013[a]	13.40±0.091[b]	0.251±0.023[a]	2.58±0.288[a]	8.69±3.05[a]
25% MW	0.064±0.0012[e]	0.263±0.001[c]	0.035±0.0018[e]	22.44±0.823[d]	0.431±0.033[b]	4.73±0.166[b]	83.29±3.92[c]
50% MW	0.077±0.0029[f]	0.302±0.031[d]	0.040±0.0011[f]	19.56±0.381c	0.633±0.067[c]	5.26±0.331[c]	173.6±8.70[e]
75% MW	0.039±0.0029[ab]	0.227±0.007[b]	0.028±0.0017[bc]	15.07±0.430[ab]	0.669±0.037[c]	6.23±0.326[d]	268.5±9.88[g]
100% MW	0.037±0.0019[a]	0.215±0.003[b]	0.026±0.0010[b]	14.13±0.459[a]	0.450±0.021[b]	7.41±0.577[e]	365.24±4.93[h]
Scenedesmus vacuolatus							
Control	0.042±0.0023[bc]	0.226±0.003[b]	0.029±0.0015[bc]	15.37±0.531[ab]	0.274±0.019[a]	2.90±0.288[a]	11.64±1.78[a]
25% MW	0.053±0.0021[d]	0.238±0.006[bc]	0.032±0.0011[d]	16.03±0.418[b]	0.662±0.024[c]	4.19±0.288[b]	67.55±6.57[b]
50% MW	0.118±0.0023[g]	0.315±0.006[e]	0.048±0.0024[g]	24.06±1.022[e]	0.827±0.020[d]	5.37±0.166[c]	141.88±3.08[d]
75% MW	0.047±0.0019[c]	0.298±0.003[d]	0.030±0.0013[cd]	19.48±0.770c	0.872±0.057[d]	6.02±0.166[d]	143.33±4.83[d]
100% MW	0.043±0.0024[bc]	0.250±0.007[c]	0.028±0.0016[bc]	15.12±1.110[ab]	0.705±0.055[c]	7.09±0.294[e]	227.16±5.39[f]

compared to 25% and 50% wastewater concentration. The increment in carbohydrate content at lower wastewater concentration may be attributed to prolific growth of microalgae. The decline in carbohydrate synthesis could be ascribed to chlorophyll degradation as well as decreased photosynthetic rate of microalgae. Furthermore, damage to chloroplast may affect the photosynthetic performance which eventually affects the carbohydrate synthesis in microalgae (Sun et al., 2014).

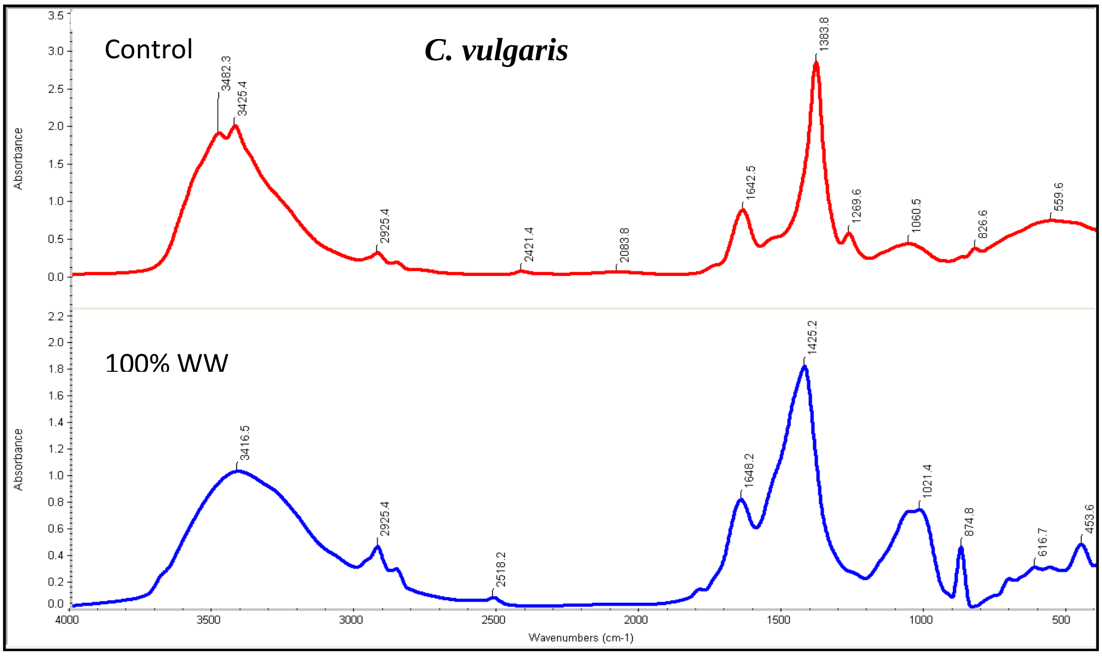
5.3.3 Lipid content

The results showed that the lipid content in selected microalgae was increased with wastewater concentration as compared to control. It was observed that *C. vulgaris* showed an increase in lipid accumulation at 25% (1.71 folds), 50% (2.52 folds), 75% (2.66 folds) and 100% (1.79 folds) wastewater concentration while, an increase of 2.41, 3.01, 3.18 and 2.57 folds was observed in case of *S. vacuolatus*. The increased lipid accumulation in wastewater treated microalgae may be ascribed to residual organic carbon (Lin and Wu, 2015). The increment in lipid accumulation may be also due to the cellular responses in order to sustain the energy for accomplishment of the metabolic processes (Zhu et al., 2017). Further, abundance of trace elements may elevate the accumulation of lipid in microalgae (Leong et al., 2020). Stress conditions like salinity and nutrient imbalance could be also responsible for enhancement of lipid accumulation in microalgae. The increased lipid synthesis in microalgae treated with municipal wastewater was in agreement with Upadhyay et al., (2021).

5.4 FTIR spectroscopy

FTIR spectroscopy is the simple technique to determine the cellular composition of microalgae. The results showed that the absorption spectra between

1700-1000 cm^{-1} revealed the presence of amides and carboxylic groups in the algal biomass (Upadhyay et al., 2021). Further, the spectral peaks in the region between ~ 1280–1200 cm^{-1} and ~1500–1400 cm^{-1} reflected the presence of hydroxyl and carboxyl groups (Fig. 5.3). The presence of different functional groups in algal biomass may elevate the removal efficiency for heavy metals (Michalak et al., 2018). Moreover, it was also observed that absorption peak at 2925 cm-^{1} were more pronounced in wastewater treated microalgae than control. The absorption peak between 3000-2800 cm^{-1} could be ascribed to methylene group in the microalgae (Singh et al., 2021). However, the sharp absorption peak (2925 cm^{-1}) reflected the lipid accumulation potential of *S. vacuolatus* was higher as compared to *C. vulgaris*. Overall, the different absorption peak showed by FTIR spectroscopy reflected that *S. vacuolatus* could be the promising organism for the metal removal and lipid accumulation.



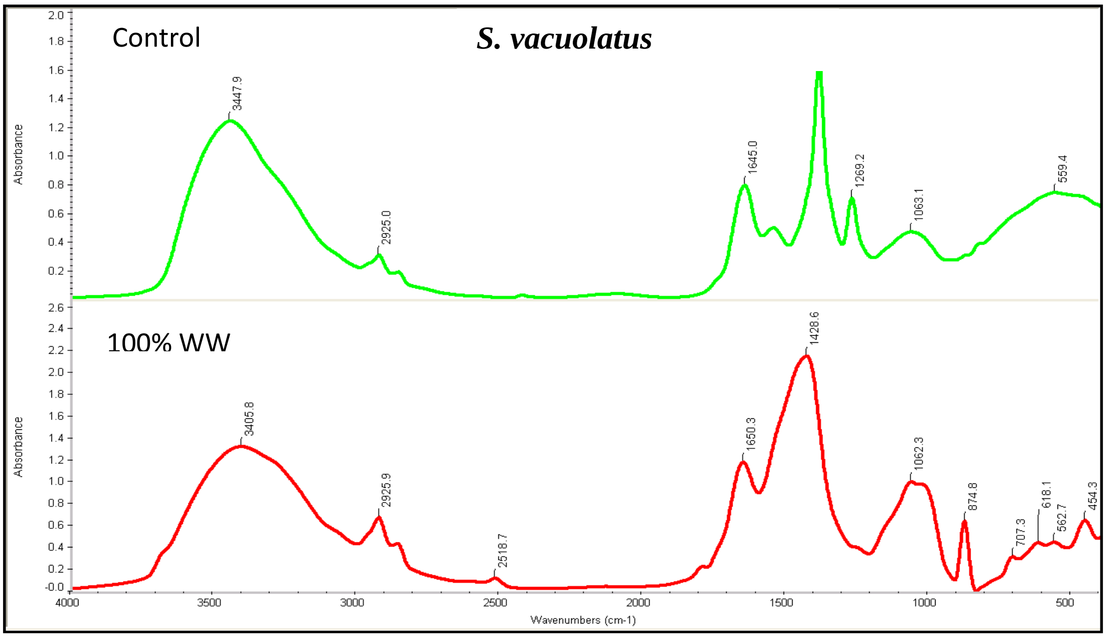


Fig. 5.3 Comparison of FTIR spectra of *Chlorella vulgaris* and *Scenedesmus vacuolatus* treated with BG-11 media (Control) and municipal wastewater (100%)

5.5 Lipid peroxidation and H_2O_2

TBARS are the direct measure of lipid peroxidation and cellular toxicity (Belda et al., 2021). It was observed that TBARS content was increased in microalgae *C. vulgaris* (2.86 folds) and *S. vacuolatus* (2.44 folds) treated with 100% municipal wastewater. The results further depicted an increase of 1.82, 2.03 and 3.40 folds in *C. vulgaris* while 1.44, 1.85 and 2.07 folds in *S. vacuolatus* after treatment with 25%, 50%, and 75% concentration of municipal wastewater. The increased TBARS content in treated microalgae as compared to control might be due to wastewater induced damage to microalgae. Upadhyay et al., (2016) also observed the increased TBARS content in microalgae after wastewater treatment could be ascribed to lipid peroxidation and damage to cellular membrane. It was observed that *C. vulgaris* and *S. vacuolatus* showed an increase of 9.58 and 5.80 folds in the H_2O_2 content at 25% wastewater whereas, an increase of 42.02 and 19.51 folds was observed at 100%

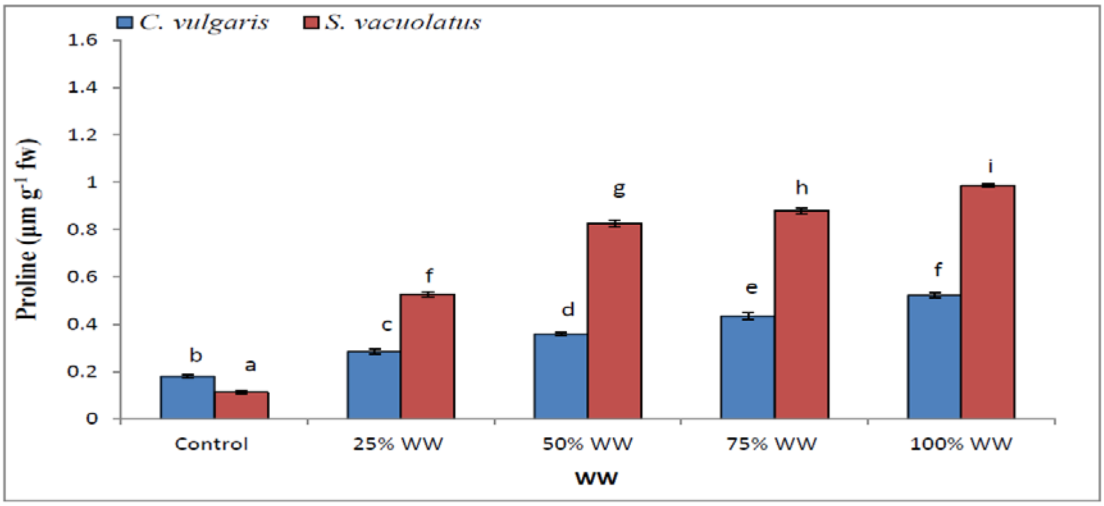
concentration of municipal wastewater (Table 5.2). At 50% and 75% wastewater, *C. vulgaris* showed an increase of 19.97% and 30.89% in H_2O_2 content while, 12.18% and 12.31% increase in case of *S. vacuolatus* respectively, than control. The low oxidative stress markers at 25% and 50% wastewater concentration may be attributed to less toxicity and tolerance of microalgae to stress conditions (Kerchev et al., 2019). However, the increased H_2O_2 accumulation in wastewater treated (75%, 100%) microalgae may inhibit cell functioning by inducing oxidative damage. The enhancement in H_2O_2 content with metal stress has been also reported by Tahira et al., (2019). Additionally, increased oxidative stress markers at 100% municipal wastewater concentration may be due to damage to lipid and thiol-rich compounds in selected microalgae. Higher oxidative stress markers in *C. vulgaris* treated with wastewater indicates inferior adaptability and tolerance mechanisms. Overall, the results exhibited that *S. vacuolatus* efficiently adapted under different wastewater concentration as evident from the less content of oxidative stress markers.

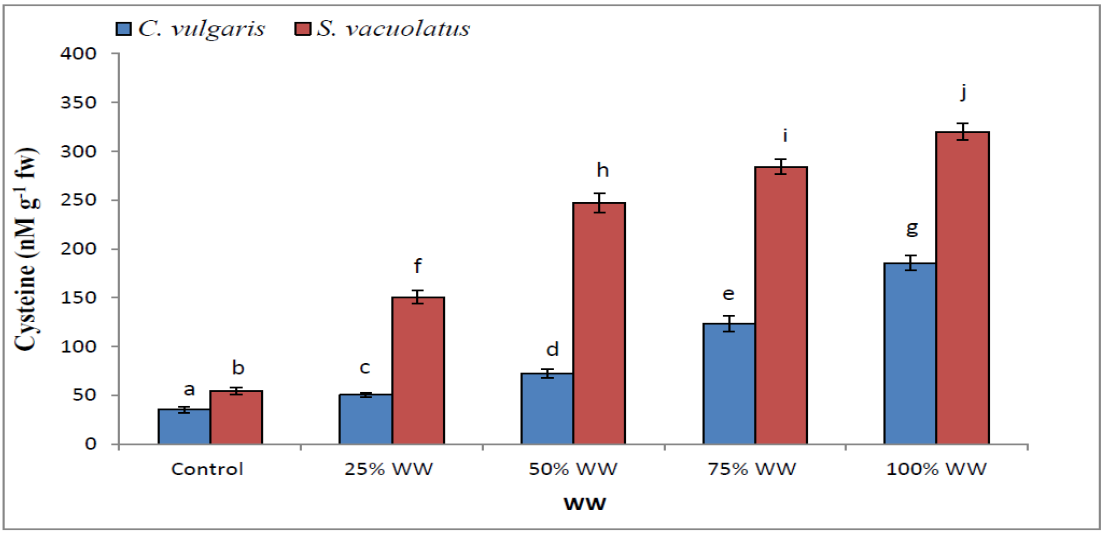
5.6 Antioxidants induced tolerance in microalgae treated with municipal wastewater

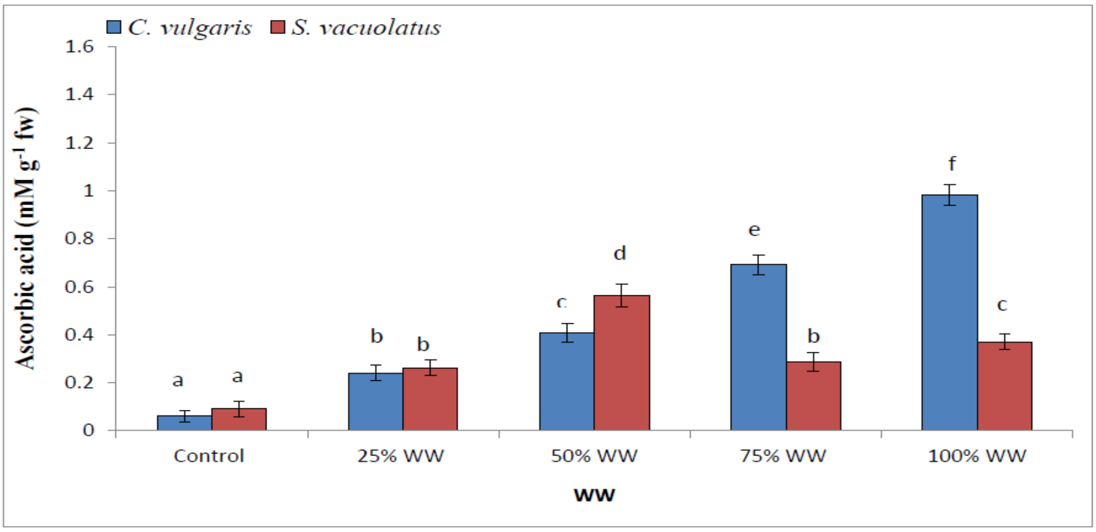
5.6.1 Non-enzymatic antioxidants (Cysteine, proline, and ascorbic acid)

Antioxidants alleviate the damage posed by oxidative stress and maintain the intracellular processes responsible for the prolific growth of microalgae (Singh et al., 2021). The results depicted that proline content was increased by 1.57 and 4.71 folds in *C. vulgaris* whereas an increase of 2.90 and 8.80 folds was observed in case of *S. vacuolatus* after treatment with 25% and 100% wastewater concentration. Further, proline content was also increased in *C. vulgaris* (1.99 and 7.37) and *S. vacuolatus* (2.41 and 7.84 folds) treated with 75% and 100% of wastewater concentration (Fig. 5.4). Proline, an osmolyte play essential role in protecting the microalgae under adverse conditions (Mutale-joan et al., 2021). The increased proline accumulation with wastewater concentration gradient indicates that it might assist in maintaining osmotic balance and confers protection against oxidative damage. The results were in agreement with Singh et al. (2021). It was also observed that wastewater concentration of 100% enhanced the cysteine content by 5.27 and 5.88 folds in *C. vulgaris* and *S. vacuolatus,* respectively in comparison to their respective control. The results further showed that cysteine content was increased in *C. vulgaris* (2.04 and 3.50 folds) and *S. vacuolatus* (4.54 and 5.22 folds) after treatment with 50% and 75% wastewater concentration respectively, than control (Fig. 5.4). The enhancement in cysteine content with municipal wastewater may be attributed to their active involvement in sequestration and detoxification of heavy metal. Cysteine also stimulates the production of glutathione which has important role in metal detoxification and scavenging of toxic derivatives triggered by stressful environment (Singh et al., 2022). Thus, increased cysteine accumulation may be responsible for sustaining growth and the metabolic activities by alleviating the heavy metal concentration and oxidative damage posed by different municipal wastewater concentrations. It was observed that ascorbic acid increased by 3.88 and 15.85 folds in microalgae *C. vulgaris* treated with 25% and 100% wastewater concentration, respectively. The results further depicted that ascorbic acid content in *S. vacuolatus* was increased at 50% (6.14 folds), 75% (3.11 folds) and 100% (4.03 folds) wastewater concentration. Ascorbic acid is water soluble non-enzymatic antioxidants which play protective role against stress conditions (Del Mondo et al., 2020) and also protect algae from oxidative damage (Akram et al., 2017). The increased ascorbic acid may maintain the cellular balance by scavenging of ROS and also sustain the activity of different metal containing enzymes in the cell (Roy et al., 2021).

Therefore, the increment in ascorbic acid might protects the cellular organelles from oxidative damage thereby confers the tolerance towards stress conditions.







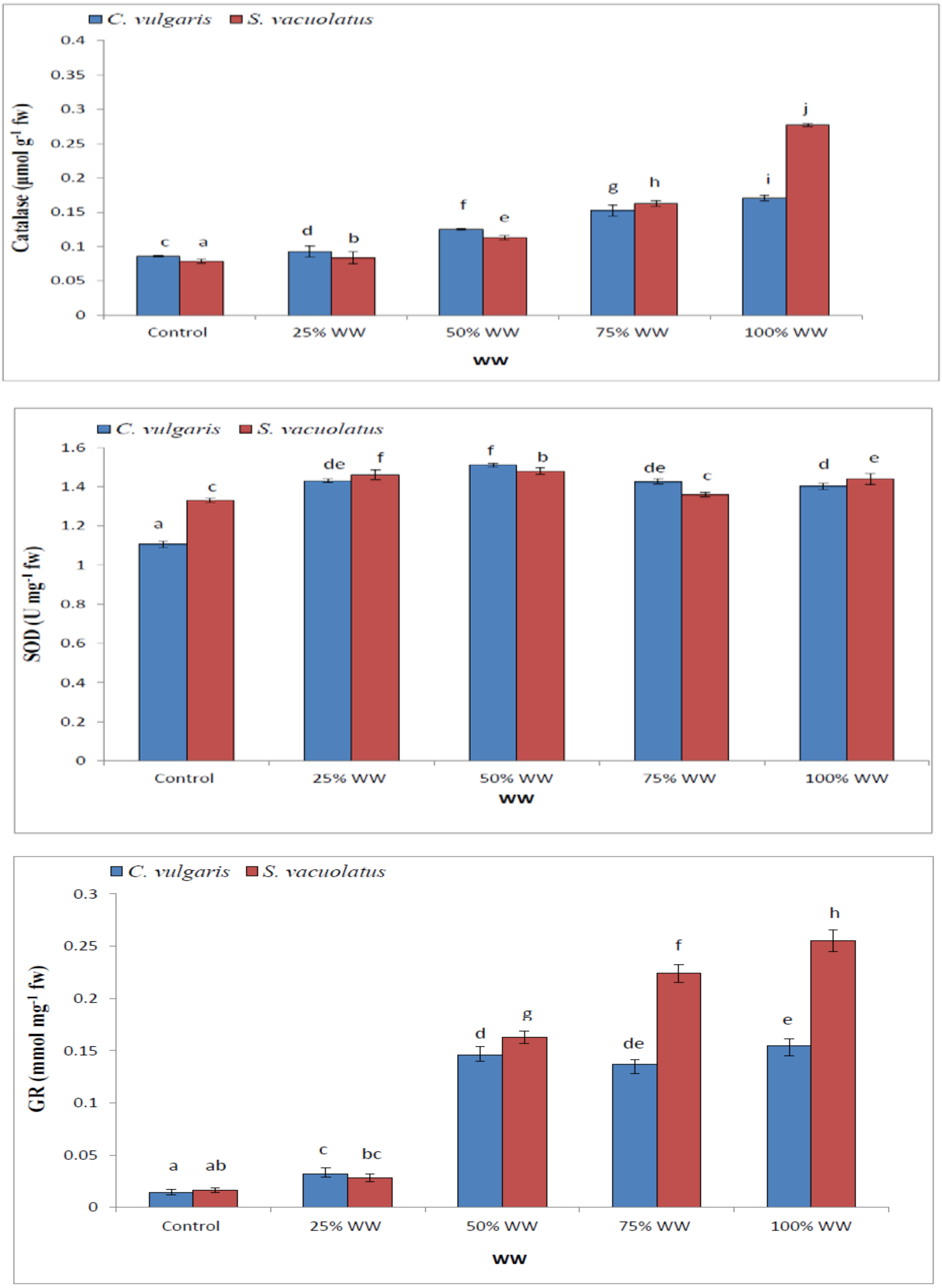


Fig. 5.4 Modification in non-enzymatic and enzymatic antioxidants activity of microalgae cultivated under different municipal wastewater conditions. All the values are means ±S.D. ANOVA post hoc DMRT ($p \leq 0.05$) was done to check the significance difference between the variables. Identical letter indicates no significant difference with in the variables.

5.6.2 Enzymatic antioxidants

The results revealed that enzymatic antioxidants like SOD, CAT and GR in selected microalgae showed increasing trend with municipal wastewater concentration. It was observed that SOD activity in *C. vulgaris* and *S. vacuolatus* was increased with wastewater concentration as compared to control. The results exhibited that SOD activity showed an increase of 1.29, 1.36, 1.29 and 1.09 folds in *C. vulgaris.* while 1.26, 1.11, 1.02 and 1.08 folds in *S. vacuolatus* after treatment with 25%, 50%, 75% and 100% municipal wastewater concentration (Fig. 5.4). The increment in SOD activity after treatment with wastewater may be the protective mechanism to scavenge the superoxide radical under wastewater stress (Singh et al., 2022). Therefore, the increased SOD activity after wastewater treatment may be due to their direct involvement in the neutralization of superoxide radicals. Furthermore, the results exhibited an increase of 1.06 and 3.53 folds in catalase activity in *S. vacuolatus* treated with 25% and 100% municipal wastewater. At 50% and 75% wastewater, an increase of 1.46 and 1.78 folds was found to be in *C. vulgaris* while 1.44 and 2.07 folds in *S. vacuolatus*. The enhancement in catalase activity in selected microalgae after wastewater treatment may be due to increased production of H_2O_2. Ugya et al. (2021) also observed that increment in the catalase activity was responsible for H_2O_2 degradation and protects the cell from oxidative stress. Therefore, the increased activity of catalase in selected microalgae after municipal wastewater treatment may alleviate the generation of H_2O_2 in microalgae exposed to stress conditions. It was further observed that microalgae *S. vacuolatus* (15.63 folds) and *C. vulgaris* (11.07 folds) showed increment in the GR activity after treatment with 100% wastewater concentration. The results also exhibited an increase in GR activity at 25% (2.30

folds), 50% (10.40 folds) and 75% (9.76 folds) wastewater concentration in *C. vulgaris* while, an increase of 1.73, 9.97 and 13.71 folds was observed in case of *S. vacuolatus*. The increased GR activity with municipal wastewater concentration gradient in both selected microalgae could be attributed to different metal pollutants in wastewater. Earlier, Danouche et al. (2020) also reported increased GR activity in heavy metal treated *Scenedesmus obliqus*. GR protects microalgae from damage by maintaining the glutathione level which alleviates the ROS accumulation and sustain redox balance of the cell (Couto et al., 2016; Singh et al., 2021). Thus, the enhanced GR activity in *S. vacuolatus* and *C. vulgaris* could be attributed to the wastewater induced ROS generation which was also evident from the increased TBARS and H_2O_2 level in microalgae. In order to show tolerance to stress conditions, microalgae regulate the activity of different antioxidants to alleviate the cellular damage (Pradhan et al., 2020). Therefore, the increased activity of enzymatic and non-enzymatic antioxidants in *S. vacuolatus* and *C. vulgaris* could be a tolerance response that alleviate ROS accumulation, enhance metal detoxification and mitigate wastewater induced oxidative damage. Overall, utilizing tolerant algal species for wastewater remediation not only enhance the metal removal but also elevate the flexibility of algal based system for the treatment of municipal wastewater.

Chapter 6

Algal consortia based metal detoxification of municipal wastewater : Implication on photosynthetic performance, lipid production, and defense responses

Chapter-6

Algal consortia based metal detoxification of municipal wastewater : Implication on photosynthetic performance, lipid production and defense response

6.0 Introduction

Globally, the scarcity of freshwater is increasing and the demand for water is also intensifying at swift rate. Wastewater generation and treatment continuous to be biggest challenges for India (Schellenberg et al., 2020). In India, the 63% of the untreated wastewater is discharged from class 1 and class 2 cities (CPCB, 2013) and out of total wastewater treatment capacity (18.6%), only 13.5% is treated in efficient manner (CPCB, 2017c). Besides, the major part (>50%) of the country are facing extreme water shortage and the future prediction indicates even grimmer availability of water (WBSCD, 2019). Lucknow, the capital city of Uttar Pradesh generates 363.82 MLD of wastewater with a treatment capacity of only 12% for total generated wastewater (Baral et al., 2020). Almost 88% of the sewage water in Lucknow is discharged without any proper treatment, which can be the forefront cause of the deteriorating health of waterbodies.

Municipal wastewater varies in pH, nutrients, salinity, as well as metal load, and existing technologies fail to work efficiently under the varying wastewater composition. Thus, it became very important to exploit sustainable approaches for the management of wastewater (Leong and Cheng, 2020, Singh et al., 2020). Microalgae-based wastewater treatment systems are environmentally secure which makes it a progressively alluring approach for resource recuperation in developing countries

(Upadhyay et al., 2021). Besides nitrogen and phosphorus, wastewater is abundant in different heavy metals which are difficult to be removed by monoalgal species. Metals enriched wastewater can inhibit growth, biomass production and amplify oxidative damage by interrupting the functioning of photosystems (Tripathi and Poluri, 2021). Therefore, utilizing monolagal species cannot be suitable approach to address different issues related to wastewater pollution. Although, extensive studies has been carried on microalgae but exploiting algal consortia for municipal wastewater treatment and lipid production is still in its infancy stage. Algal consortia may also enhances the flexibility of the treatment system as the decresed treatment efficiency due to inefficiency of one species may be balanced by other algal species (Renuka et al., 2013). Furthermore, algal species differes in adaptability potential, nutrient requirement and biochemical profile thus, each algal species can itself can serve as a dynamic biosystem for wastewater treatment and biomass production. Earlier, Ji et al., (2019) reported that consortia of *Chlorella vulgaris* and *Bacillus licheniformis* removed total nitrogen (88.82%) and orthophosphate (84.87%) from wastewater. Sharma et al., (2020) also reported that algal consortia grown in municipal wastewater removed different metals with a simultaneous increase in lipid production (31.3%). Under metal exposure, microalgae also elevates the activity superoxide dismutase, catalase, and glutathione reductase to offset the damage caused by oxidative stress (Danouche et al., 2020). Therefore, the integrated approach considering multiple aspects like photosynthetic performance, stress markers, and antioxidative responses requires extensive reasearch to screen competent consortia for municipal wastewater treatment and biofuel production.

6.1. Results and discussion

6.1.1 Changes in physico-chemical attributes of wastewater after treatment with algal consortia

6.1.1.1 pH and electrical conductivity (EC)

The varying composition of wastewtaer can act as the natural stress for microalgae. The pH of 7.6±0.233 was observed for raw wastewater (Table 6.1) and afer treatment with consortia 1 and 2, the highest increase of 28.15% and 22.76% in pH was observed at 75% and 25% concentration of municipal wastewater. The results also showed an increase of 14.34% and 8.02% in pH treated with consortia 1 and 2, respectively at 100% wastewater concentration. The results further exhibited that the pH of wastewater was significantly increased after treatment with algal consortia 1 and 2. The enhancement in pH could be ascribed to basic substances released after the degradation of organic compounds (Su et al., 2011). It was observed that EC decresed by 85.28% and 88.27% with consortia 1 and 2 treated with 25% concentration of municipal wastewater. The results further depicted the maximum decrese of 63.39%, 79.79% and 73.08% in EC was observed with algal consortia 1 treated at 50%, 75% and 100% wastewater concentration. The noticable decline in EC may be due to assimilation of nutrients and dissolved salts by microalgae from wastewater (Singh et al., 2020).

6.1.1.2 Total solid (TS), total suspended solids (TSS), total dissolved solids (TDS)

The results depicted that the highest decrease in solids was observed with consortia 1 and 2 grown in BG-11 media. It was observed that TSS and TS content was decreased by 81.76% and 81.74% with consortia 1 while 77.55% and 85.25%

with consortia 2 at 25% of wastewater concentration. Algal consortia 1 and 2 also showed the decline of 33.66% and 25.66% in TSS while the TS was declined by 45.08% and 36.09% at 100% wastewater concentration (Table 6.1). In case of TDS, the algal consortia 1 showed highest reduction of 76.70%, 72.10%, and 59.53% after treatment with 50%, 75% and 100% municipal wastewater, respectively. The declined TDS of municipal wastewater may be attributed to utilization of macro and the micronutrients by algal consortia (Upadhyay et al., 2021).

6.1.1.3 Biological oxygen demand (BOD) and Dissolved oxygen (DO)

BOD and DO are important quality parameters which directly indicates about the contamination level of wastewater. The results depicted that initial DO level was nil and the level of BOD was highest at 100% concentration of municipal wastewater. The results further exhibited that the highest DO level was observed with consortia 1 (5.30 mg/L) and consortia 2 (5.04 mg/L) at 25% municipal wastewater. At 75% and 100% wastewater, the highest DO level was observed after treatment with consortia 1 than consortia 2. The increased DO level of wastewater may be ascribed to improved algal growth as well as pigment biosynthesis of microalage after wastewater treatment. Contrary, the BOD level was decreased after treatment of different wastewater concentrations with consortia 1 and 2. The results further showed the significant decline in the BOD level (47.32 - 90.18%) of different wastewater concentration treated with algal consortia 2. The decline in BOD level of wastewater after treatment with consortia may be ascribed to organic matter degradation, nutrient removal and metal detoxification. Ummalyma and Sukumaran (2014) reported that BOD level was decreased after growing microalage in dairy effluent for 15 days.

Table 6.1 Percentage change in physico-chemical attributes of wastewater treated with consortia 1 and 2

Wastewater Parameters	Initial characteristics	Wastewater concentrations	Consortia-1	% change	Consortia-2	% change
pH	7.6±0.233	Control	8.66±0.220[ab]	13.94	9.70±0.015[de]	27.63
		25%	9.66±0.132[d]	27.10	9.33±0.152[c]	22.76
		50%	9.35±0.089[c]	23.02	9.18±0.026[c]	20.78
		75%	9.74±0.109[de]	28.15	8.73±0.075[b]	14.86
		100%	8.69±0.022[ab]	14.34	8.21±0.177[a]	8.02
EC (µS cm^{-1})	836±11.29	Control	2.90±0.024[a]	99.65	2.45±0.040[a]	99.70
		25%	123±5.507[c]	85.28	98.0±7.505[b]	88.27
		50%	169±8.504[d]	79.79	201±9.022[e]	75.95
		75%	225±5.567[f]	73.08	240±7.498[f]	71.29
		100%	306±10.06[g]	63.39	330±10.69[h]	60.52
TDS	588±9.12	Control	1.19±0.013[a]	99.79	2.13±0.015[a]	99.63
		25%	101±3.145[c]	82.82	75.0±2.000[b]	87.24
		50%	137±3.141[d]	76.70	152±5.516[d]	74.14
		75%	164±8.532[d]	72.10	188±5.507[e]	68.02
		100%	239±4.472[f]	59.35	247±3.055[f]	57.99
TSS	142.6±5.89	Control	4.13±0.826[a]	97.10	4.00±0.270[a]	97.19
		25%	26.0±3.000[b]	81.76	32±4.162[b]	77.55
		50%	50.6±3.055[c]	64.51	63±5.508[d]	55.82
		75%	70.6±5.859[d]	50.47	90±4.509[e]	36.88
		100%	94.6±4.163[e]	33.66	106±4.041[f]	25.66

TS	712±5.07	Control	5.025±1.08[a]	99.29	4.95±0.270[a]	99.30
		25%	130±4.670[c]	81.74	105±4.160[b]	85.25
		50%	182±7.310[e]	74.43	165±7.370[d]	76.82
		75%	346±2.610[f]	51.40	326±5.130[f]	54.21
		100%	391±2.08[g]	45.08	455±4.160[h]	36.09
BOD	43±0.489	Control	0.68±0.008[a]	98.41	0.43±0.065[a]	99.00
		25%	4.60±0.178[b]	89.30	4.22±0.265[b]	90.18
		50%	9.46±0.273[c]	78.00	10.24±0.350[d]	76.18
		75%	17.7±0.136[e]	58.83	16.89±0.368[f]	60.79
		100%	23.4±0.263[g]	45.58	22.65±1.453[g]	47.32
DO	-	Control	6.40±0.217[e]	-	6.80±0.244[f]	-
		25%	5.30±0.277[d]	-	5.04±0.378[d]	-
		50%	3.73±0.345[c]	-	3.94±0.161[c]	-
		75%	1.36±0.130[b]	-	0.933±0.155[b]	-
		100%	0.54±0.073[a]	-	0.326±0.095[a]	-
NO_3^-	38.80±2.56	Control	0.741±0.034[a]	98.09	0.910±0.537[a]	97.65
		25%	2.161±0.348[b]	94.43	3.152±0.358[c]	91.87
		50%	4.676±0.429[d]	87.94	5.257±0.614[e]	86.45
		75%	7.163±0.195[f]	81.53	7.504±0.217[g]	80.65
		100%	8.152±0.217[h]	78.98	8.247±0.379[h]	78.74
PO_4^{-3}	16.47±2.04	Control	0.396±0.095[a]	97.59	0.514±0.059[a]	96.87
		25%	0.763±0.090[b]	95.36	0.786±0.744[b]	95.22
		50%	1.140±0.143[c]	93.07	1.230±0.135c	92.53
		75%	2.010±0.126[e]	87.79	1.696±0.116[d]	89.70
		100%	2.606±0.202[f]	84.17	2.646±0.202[f]	83.93

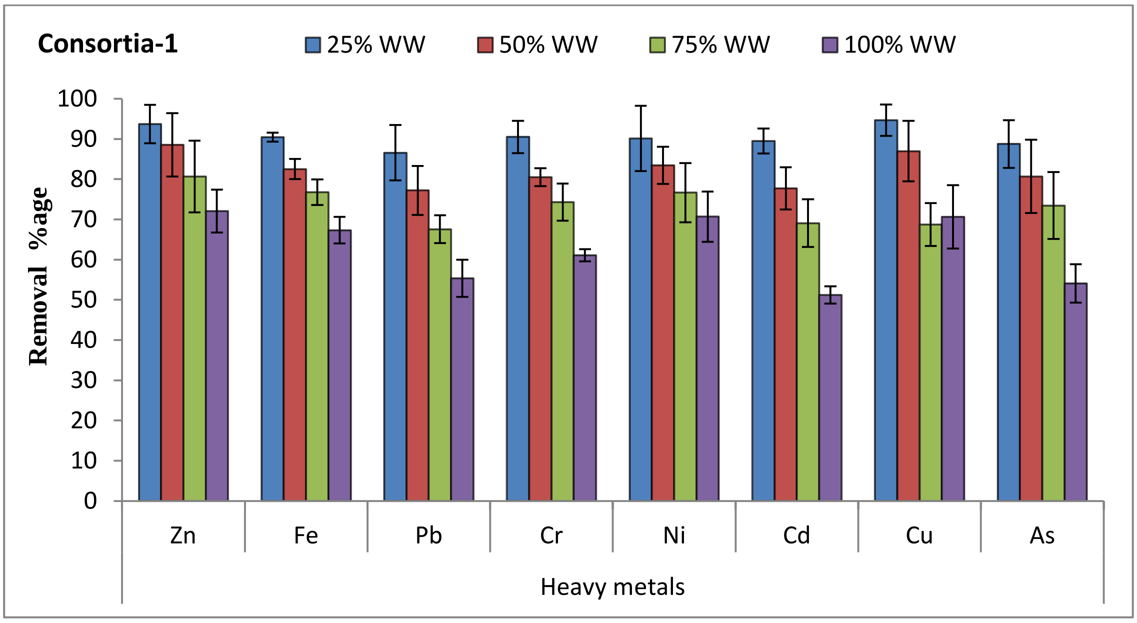
Therefore, decreased BOD level of different wastewater concentration can be linked with the improvement in water quality parameters after growing algal consortia 1 and 2 in municipal wastewater.

6.1.1.4 Nitrogen and phosphorus

Nutrients plays vital role in proper cell functioning, metabolic processes, and cell cycle (Upadhyay et al. 2021). The results depicted that the highest decrease of 94.43%, 87.94%, 81.53%, and 78.98% in nitrogen content was observed with consortia 1 treated with 25%, 50%, 75% and 100% concentration of wastewater, respectively. In case of consortia 2, the nitrate nitrogen was decreased by 91.87%, 86.45%, 80.65% and 78.74% after treatment with different concentration of municipal wastewater. Earlier, Choudhary et al. (2016) reported microlage grown in rural wastewater progressively decreased the nitrate level (70%) below the discharge standards. Thus, the tremendrous nitrogen removal efficiency observed in case of consortia 1 could be due to its significant role in growth promotion, biomolecule synthesis and eventually biomass production. It was also observed that phosphorus content was decreased with consortia 1 (95.36%) and consortia 2 (95.22%) at 25% wastewater concentration. At 100% wastewater concentration, the decline of 84.17% and 83.93% in phosphorus content was found to be with consortia 1 and 2, respectively. Furthermore, the results also showed that the highest phosphorus removal of 93.07% and 89.70% in case of consortia 1 and 2 treated with 75% wastewater while 93.07% and 92.53% at 50% of municipal wastewater concentration. Sharma et al., (2020) observed that consortia of microalage recovered >80% of nutrients after treatment with different wastewater concentrations. The excellent removal of nutrients from municipal wastewater could be ascribed to their involvement in growth, photosynthetic process, energy conversion, lipid and biomass synthesis (Singh et al., 2020; Singh et al., 2021). Furthermore, the incresed pH after treatment of different wastewater concentration with algal consortia may enhance removal efficiency by precipitation of phosphorus.

6.1.1.5 Heavy metals

Discharge of heavy metals can perturb the quality of environment which can have severe affects on humans (Singh et al., 2020). Essential metals (Fe, Zn, Cu, Ni) have an important role in cell metabolism while non essential metals (Pb, As, Cd, Cr) are toxic with no role inside the cell. The concentration of heavy metals in wastewater was in the order of Fe (1415.35 µg/L), Ni (12.47 µg/L), Zn (9.72 µg/L), Pb (8.72 µg/L), Cu (6.28 µg/L), Cr (4.32 µg/L), Cd (4.30 µg/L), and As (0.65 µg/L) respectively. The results showed that the removal efficiency of >85% was observed for Fe, Pb, Cr, Cd, Cu, and As while >50% for Fe, Pb, Cd, Cu, Ni, and Zn with selected consortia at 25% and 100% of wastewater concentration, respectively. The results showed the maximum reduction efficiency with consortia 1 for Fe (93.05%), Cr (91.88%), Pb (86.69%), and As (88.91%) at 25% wastewater concentration while the lowest was observed in case of consortia 2 for Cd (51.25%), As (54.08%), Pb (55.35%), Cr (61.10%), and Ni (70.71%) at 100% wastewater concentration. Consortia 1 also showed the highest removal efficiency of 87.98%, 84.19%, 83.79%, and 78.99% for Cr, Fe, Ni and Cd at 50% concentration of municipal wastewater. At 75% wastewater concentration, the maximum removal efficiency of 82.59%, 82.37%, 82.36%, 77.97%, 74.91% and 71.85% was found for Zn, Cu, Cr, Ni, Pb and Cd with consortia 1 while 76.79% and 73.46% for Fe and As with algal consortia 2 (Fig. 6.1).



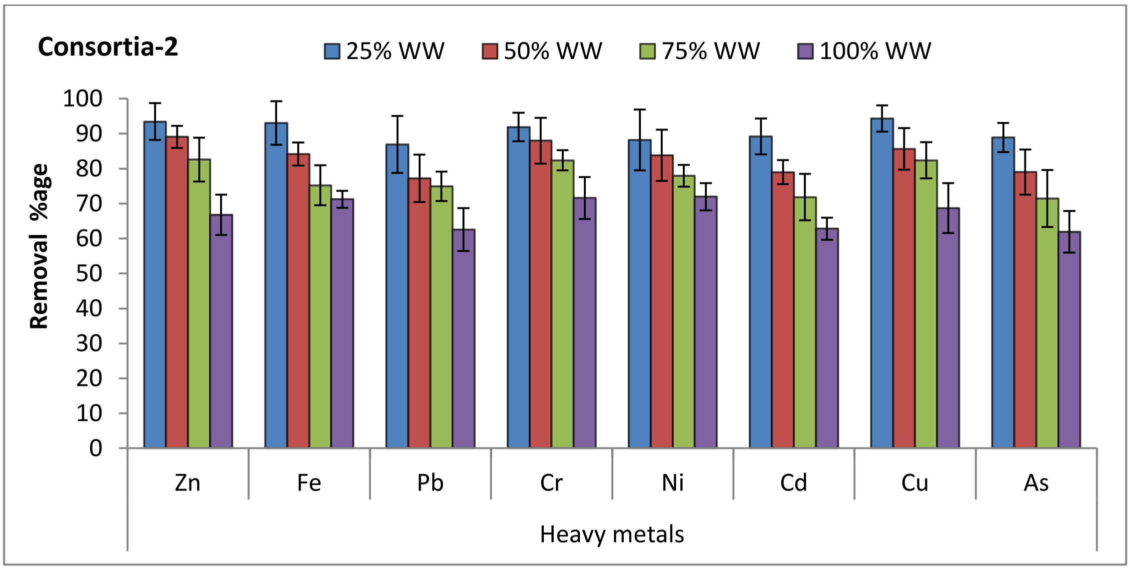


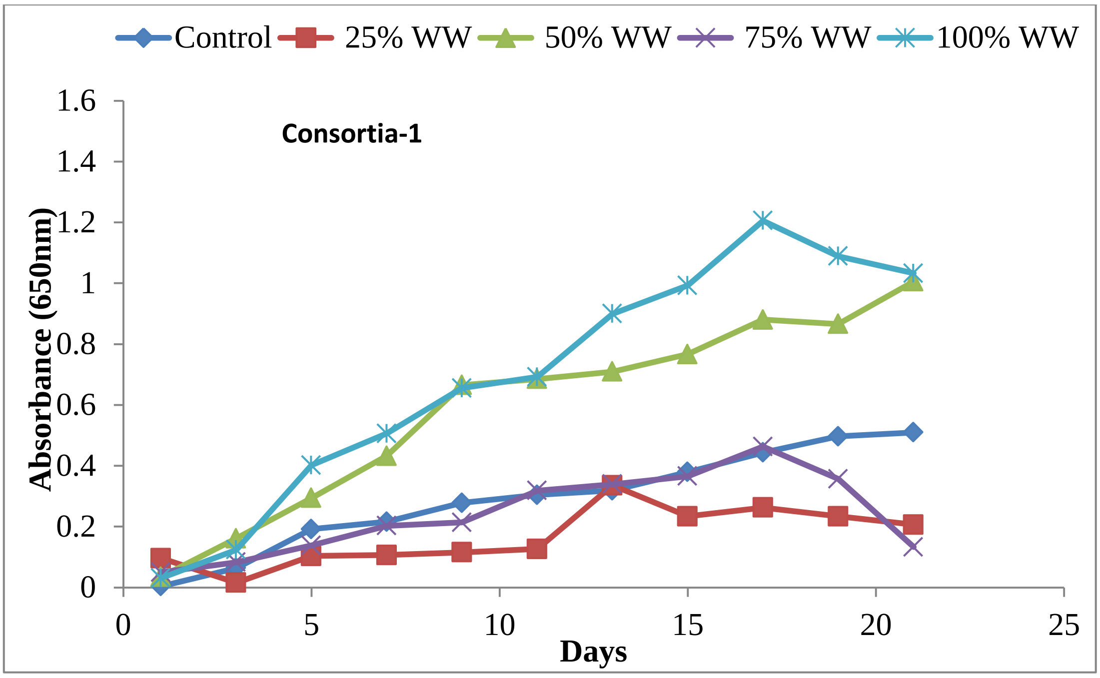
Fig. 6.1 Heavy metal removal efficiency of microalgae consortia treated with different concentrations of municipal wastewater

The maximum removal of heavy metals after treatment with municipal wastewater could be due to bio-adsorption and bio-accumulation which are mainly adopted by microalgae for remediation of contaminated wastewater (Sharma et al., 2020; Singh et al., 2020). Moreover, the excellent removal efficiency of algal consortia may be due to presence of different functional groups like monomeric alcohols, hydroxyl and carboxyl groups in algal biomass. Furthermore, immobilization, chelate formation, stimulation of antioxidant system, and gene expression could also be mechanisms adopted by microalage against heavy metal (Leong and Chang, 2020; Singh et al., 2021). Therefore, the wastewater after treatment though microalgae can certainly overcome eutrophication, algal bloom formation and also protect the recipient ecosystem from further deterioration.

6.2. Effect of different wastewater concentrations on algal consortia

6.2.1 Growth and biomass productivity

Growth is an important indicator of toxicity and informs about the functioning of microalgae. The growth of algal consortia 1 and 2 was recorded and the continuous increase in growth rate was observed upto 20 days in both consortia grown in BG-11 media. The results depicted that algal consortia 1 and 2 treated with 25% municipal wastewater started fading after 12^{th}-14^{th} days. The discoloration and subsequent decline in the growth could be attributed to deficiency of nutrients after treatment of consortia 1 and 2 with 25% wastewater concentration. The results further showed the superior growth of consortia 1 and 2 treated with wastewater concentration of 100% than control (Fig. 6.2). Similarly, the specific growth rate of both algal consortia was also observed highest at 100% wastewater and the lowest at 25% concentration of municipal wastewater (Table 6.2). The sufficient supply of essential nutrients is neccesay for the proper growth of microalgae (Prajapati et al., 2013). Upadhyay et al., (2021) also observed the increment in the specific growth rate of algal consortia after treatment with municipal wastewater. The highest growth rate of consortia 1 and 2 could be attributed to either nutrient sufficiency or increased tolerance by increment in activity of antioxidative compounds in algal consortia after treatment with 100% wastewater (Upadhyay et al., 2021; Singh et al., 2021).



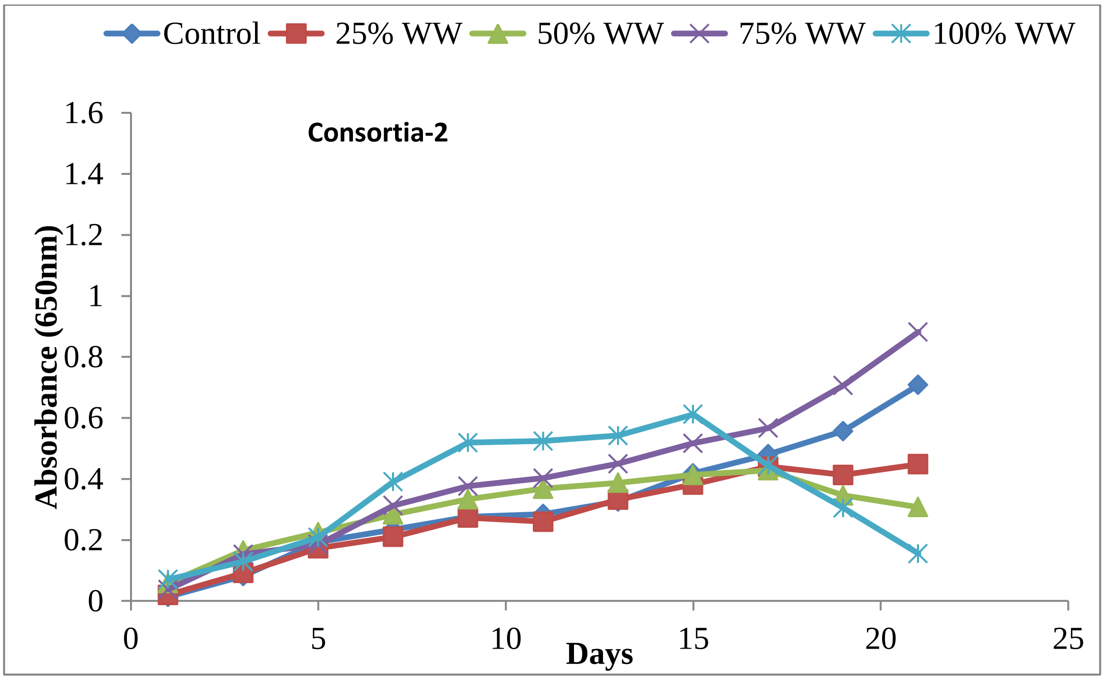


Fig. 6.2 Growth pattern of selected consortia 1 and 2 treated with different concentrations of wastewater

The results also showed that biomass production was highest in selected consortia 1 and 2 at 100% as compared to 25%, 50% and 75% of wastewater concentration. Similarly, highest biomass productivity was found in wastewater (100%) treated algal consortia than control. It was also observed that biomass productivity of 374 mg/L/d was observed in case of consortia 1 than consortia 2 (134 mg/L/d) (Table 6.2). The increased biomass productivity in wastewater treated algal consortia may be attributed to sufficient nutrient load and enhancement in the synthesis of intracellular biomolecules. Upadhyay et al. (2021) reported that the increment in biomass synthesis could be due to enhancement in cell proliferation of microalgae after wastewater treatment. Furthermore, the maximum biomass production and productivity in treated consortia reflected that undiluted municipal wastewater could be exploited to enhance the biomass production of microalgae.

Table 6.2 Variation in growth rate and biomass production of selected consortia treated with different wastewater concentrations collected from Lucknow

Wastewater concentration	Specific growth rate (day^{-1})	Biomass Production (mg/L)	Biomass Productivity (mg/L/ day)
Consortia 1			
Control	0.0384 ± 0.004^{c}	2610 ± 18.22^{g}	261 ± 5.05^{g}
25% WW	0.0174 ± 0.001^{b}	830 ± 9.45^{b}	83 ± 1.89^{b}
50% WW	0.039 ± 0.005^{c}	1110 ± 9.12^{c}	111 ± 1.79^{c}
75% WW	0.0485 ± 0.007^{e}	1240 ± 11.12^{d}	124 ± 1.95^{d}
100% WW	0.0405 ± 0.005^{d}	3740 ± 25.45^{h}	374 ± 5.42^{h}
Consortia 2			
Control	0.0347 ± 0.002^{c}	1610 ± 12.14^{e}	161 ± 2.56^{e}
25% WW	0.0161 ± 0.002^{ab}	780 ± 8.34^{a}	78 ± 1.72^{a}
50% WW	0.0124 ± 0.003^{a}	1050 ± 7.56^{c}	105 ± 1.79^{c}
75% WW	0.0421 ± 0.006^{d}	2090 ± 14.32^{f}	209 ± 2.85^{f}
100% WW	0.0420 ± 0.008^{d}	1340 ± 15.15^{d}	134 ± 2.09^{d}

6.2.2 Chlorophyll content

The results exhibited that consortia 1 and 2 showed maximum increase of 2.16 and 1.40 folds in total chlorophyll content at 100% concentration of wastewater. It was also observed that chlorophyll content was increased by 1.16, 1.33 and 1.38 folds in consortia 1 at 25%, 50% and 75% wastewater concentration, respectively. In case of consortia 2, an increase of 1.05 and 1.00 folds in chlorophyll content was observed at 50% and 75% wastewater concentration, respectively (Table 6.3). Earlier, Sharma et al. (2020) reported that 75% wastewater concentration was optimum to elevate the chlorophyll content in algal consortia. However, in this study, the increment in chlorophyll content in consortia 1 and 2 was also observed after treatment with 100% wastewater in comparison to their respective control. The enhancement in total chlorophyll content with municipal wastewater could be ascribed to the utilization of wastewater components as raw material for photosynthesis (Sutherland et al., 2015). Further, the results depicted an increase of 1.46 and 2.22 folds in carotenoids content in consortia 1 while, consortia 2 showed an increase of 2.11 and 2.22 folds at 75% and 100% municipal wastewater concentration, respectively. The results further showed the highest increment of 1.88 and 2.66 folds in carotenoid content in case of consortia 2 while, it was increased by 1.26 and 1.41 folds in consortia 1 treated with 25% and 50% of wastewater concentration, respectively (Table 6.3). The increment in the carotenoid content after treatment of algal consortia with wastewater may asssit in light harvesting as well as seuqestration of ROS in the cell (Singh et al., 2019; Singh et al., 2021). Therefore, increased carotenoids synthesis may mitigate the oxidative damage caused by stress condition like varying pH, salinity, nutrients, and metals after cultivation of microalgae in wastewater.

Table 6.3 Effect of wastewater concentrations on biochemical parameters and oxidative stress markers of microalgae consortia 1 and 2

WW Concentrations	Total chlorophyll (mg g^{-1} fw)	Carotenoids (mg g^{-1} fw)	Protein (mg g^{-1} fw)	Carbohydrate (mg L^{-1})	Lipid (mg L^{-1})	TBARS (mmol g^{-1} fw)	H_2O_2 (µmol g^{-1} fw)
Consortia 1							
Control	0.018±0.002^{a}	0.015±0.006^{b}	0.019±0.0001^{ab}	10.22±0.195^{a}	0.045±0.002^{a}	2.04±0.166^{ab}	1.29±0.251^{c}
25%	0.021±0.004^{ab}	0.019±0.002^{bc}	0.013±0.0003^{a}	15.63±0.317^{b}	0.204±0.059^{c}	2.47±0.179^{b}	3.85±0.107^{e}
50%	0.024±0.004^{ab}	0.021±0.003^{c}	0.043±0.0006^{c}	24.93±0.247^{c}	0.471±0.050^{e}	2.25±0.333^{ab}	0.736±0.172^{ab}
75%	0.025±0.003^{ab}	0.022±0.004^{c}	0.069±0.0004^{d}	17.00±0.448^{b}	0.366±0.062^{d}	2.36±0.445^{abc}	0.241±0.029^{a}
100%	0.039±0.008^{d}	0.033±0.008^{e}	0.088±0.0008^{e}	26.93±0.331^{e}	0.216±0.001^{c}	2.79±0.166^{bc}	2.53±0.047^{d}
Consortia 2							
Control	0.020±0.002^{ab}	0.009±0.001^{a}	0.020±0.0002^{b}	29.54±0.092^{f}	0.113±0.003^{b}	1.72±0.116^{a}	0.225±0.025^{a}
25%	0.018±0.001^{a}	0.017±0.002^{b}	0.012±0.0001^{a}	16.96±0.130^{b}	0.137±0.002^{b}	3.44±0.166^{cd}	1.09±0.063^{c}
50%	0.021±0.004^{ab}	0.024±0.002^{cd}	0.023±0.0001^{b}	21.26±0.302^{d}	0.081±0.006^{a}	3.01±0.881^{c}	0.30±0.106^{a}
75%	0.020±0.006^{ab}	0.019±0.004^{bc}	0.048±0.0007^{c}	46.75±0.130^{g}	0.279±0.084^{c}	4.08±0.726^{e}	0.130±0.050^{a}
100%	0.028±0.005^{bc}	0.020±0.006^{c}	0.041±0.0004^{c}	18.79±0.458^{b}	0.037±0.001^{a}	5.05±0.999^{f}	4.07±0.045^{f}

6.2.3 Chlorophyll fluorescence

Chlorophyll fluorescence is a fast method to examine the effect of municipal wastewater on photosystems. The OJIP curve was used to examine the photosynthetic performance and electron transport in consortia 1 and 2 treated with different concentrations of municipal wastewater. It was observed that Fv/Fm and Fv/Fo ratio in consortia 1 and 2 was increased by 1.13-1.57 folds at 100% concentration of wastewater in comparison to respective control. The results also exhibited that Fv/Fo was increased at 25% (1.40 folds) 50% (1.50 folds) and 75% (1.30 folds) wastewater treatment to consortia 1 while, in case of consortia 2, an increase of 1.70, 1.21 and 1.30 folds was observed at 25%, 75% and 100% of wastewater concentration respectively, than control. The results further exhibited that Fv/Fm ratio showed maximum increase in consortia 1 (1.20 folds) and consortia 2 (1.26 folds) after treatment with 100% concentration of municipal wastewater. Further, consortia 1 also showed an increase 1.16, 1.19, 1.01 and 1.20 folds in Fv/Fm after treatment with different concentration of municipal wastewater. It was also observed that Fv/Fm was increased in consortia 2 treated with 75% (1.10 folds) and 100% (1.13 folds) concentration of wastewater (Table 6.4). The increased Fv/Fo and Fv/Fm ratio reflected improvement in photosystem-II activity in wastewater treated algal consortia 1 and 2 than control. Mo value reflects the slope of the induction curve and the results revealed that Mo value increased with wastewater concentration which represents the increase in the reduced Q_A as compared to oxidized Q_A. The results revealed that Mo value was increased in consortia 1 (1.45 folds) and consortia 2 (1.44 folds) treated with 50% municipal wastewater. Further, an increase of 1.39 and 1.26 folds was observed in case of consortia 1 and 2 treated with 75% municipal wastewater. The increased Mo in consortia 1 and 2 could be attributed to interference in the functioning of PS-II. The results showed that Mo value was decreased significantly in consortia 1 and 2 treated with 25% and 100% municipal wastewater. Furthermore, the lowest Mo value at 100% wastewater than control represents low toxicity which might be due to efficient utilization of nutrient and strong defense responses against wastewater (Subhadra, 2011). The results depicted that Pi_{abs} value was increased in consortia 1 (2.35 folds) and consortia 2 (1.14 folds) at 25% wastewater concentration while, an increase of 1.48 and 1.19 folds was observed in case of consortia 1 and 2 treated with 100% and 75% municipal wastewater, respectively. It was also observed that Pi_{abs} value was increased in algal consortia 1 (1.01 folds) and consortia 2 (1.75 folds) treated with 50% municipal wastewater. The increased Pi_{abs} value in algal consortia 1 and 2 reflected increased cell vitality and photosynthetic efficiency (Singh et al., 2019) which eventually indicated that municipal wastewater could be utilized as the growth media with minimum impact on cellular processes. The results also showed that ABS/RC, TRo/RC, and ETo/RC ratio was decreased with municipal wastewater in consortia 1 and 2 as compared to control. The decreased ratio may be attributed to increased number of active reaction centres (Strasser et al., 2004) reflecting proper excitation of electron and photophosphorylation which might be responsible for increased growth and biomass production of algal consortia. The increased reaction centres with wastewater concentration promote the transport of electron, Q_A reduction and also indicates the high photosynthetic capacity of consortia (Markou et al., 2017). Furthermore, the increased value of Fv/Fm, Fv/Fo and Pi_{Abs} substantiate that municipal wastewater improved the photosynthetic performance of algal consortia 1 and 2.

Table 6.4 Effect of different wastewater concentrations on chlorophyll fluorescence of microalgae consortia 1 and 2

Wastewater concentration	Fv/F0	Fv/Fm	Mo	Pi_{abs}	ABS/RC	TRo/RC	ETo/RC
Consortia-1							
Control	$1.037±0.038^{bc}$	$0.509±0.012^{b}$	$0.204±0.013^{b}$	$2.033±0.039^{c}$	$1.869±0.063^{g}$	$0.952±0.025^{d}$	$0.748±0.025^{c}$
25%	$1.462±0.050^{cd}$	$0.594±0.020^{b}$	$0.142±0.009^{a}$	$4.797±0.071^{gh}$	$1.099±0.042^{a}$	$0.652±0.012^{a}$	$0.832±0.029^{d}$
50%	$1.563±0.073^{e}$	$0.610±0.022^{c}$	$0.297±0.014^{c}$	$2.064±0.062^{c}$	$1.364±0.047^{c}$	$0.832±0.018^{b}$	$0.535±0.015^{b}$
75%	$1.080±0.042^{bc}$	$0.519±0.013^{b}$	$0.284±0.019^{c}$	$1.336±0.059^{b}$	$1.691±0.053^{ef}$	$0.878±0.020^{bc}$	$0.594±0.013^{bc}$
100%	$1.569±0.055^{e}$	$0.611±0.025^{c}$	$0.221±0.016^{bc}$	$3.023±0.089^{de}$	$1.196±0.041^{abc}$	$0.731±0.016^{ab}$	$0.510±0.009^{b}$
Consortia-2							
Control	$0.958±0.025^{b}$	$0.489±0.018^{a}$	$0.249±0.015^{bc}$	$1.371±0.48^{b}$	$1.858±0.039^{g}$	$0.909±0.031^{bc}$	$0.661±0.012^{bc}$
25%	$1.634±0.036^{ef}$	$0.620±0.029^{c}$	$0.255±0.016^{bc}$	$2.943±0.061^{d}$	$1.575±0.032^{cd}$	$0.977±0.037^{d}$	$0.722±0.016^{c}$
50%	$0.699±0.021^{a}$	$0.412±0.015^{a}$	$0.361±0.025^{e}$	$0.506±0.015^{a}$	$2.403±0.074^{h}$	$0.989±0.038^{d}$	$0.628±0.023^{bc}$
75%	$1.167±0.043^{bcd}$	$0.539±0.024^{b}$	$0.316±0.023^{d}$	$1.273±0.040^{b}$	$1.637±0.033^{cd}$	$0.882±0.027^{b}$	$0.565±0.020^{b}$
100%	$1.254±0.040^{bcd}$	$0.556±0.017^{b}$	$0.195±0.013^{b}$	$2.475±0.046^{cd}$	$1.138±0.036^{ab}$	$0.633±0.017^{a}$	$0.438±0.017^{b}$

Overall, the results depicted that different concentrations of municipal wastewater sustained the proper electron transport and PS- II activity thus can be used as a nutrient-enriched resource for biomass production.

6.3 Effect of municipal wastewater on different biochemical parameters of consortia

6.3.1 Protein content

Protein, carbohydrate, and lipid are important cell constituents and their content in the cell is a very important determinant of growth conditions (Singh et al., 2019). It was observed that protein content declined significantly after treatment with 25% wastewater concentration in consortia 1 and 2 as compared to control. The decline in protein content may be due to decreased availability of essential nutrient in growth media after exponential phase of cell cycle. The results also depicted that protein content was increased in consortia 1 (2.26 folds) and consortia 2 (1.15 folds) treated with 50% concentration of municipal wastewater. Further, the results depicted that protein content was highest in consortia 1 (0.088±0.0008 mg g^{-1} fw) and consortia 2 (0.048±0.0007 mg g^{-1} fw) after treatment with 100% and 75% wastewater concentration (Table 6.3). Sharma et al., (2020) also reported the increment in protein content in different consortia upto 75% wastewater while declining trend was observed at 100% wastewater concentration. The increased protein content after wastewater treatment (100%) may be ascribed to synthesis of stress protein which enhance tolerance towards stress conditions (Choudhary et al., 2007). Moreover, the sufficient nutrients level in the municipal wastewater may elevate the synthesis of proteins in algal consortia 1 and 2.

6.3.2 Carbohydrate content

The results depicted that carbohydrate content in algal consortia 1 was increased by 1.52, 2.43 and 2.63 folds at 25%, 50% and 100% municipal wastewater concentration than control. In case of consortia 2, the results showed the increase in carbohydrate content at 75% wastewater concentration while the significant decline was observed at 25%, 50%, and 100% wastewater in comparison to control. The decline in carbohydrate synthesis may be ascribed to the use of carbohydrate and chlorophyll as nutrient sources under nutrient-scarce growth conditions. However, the increment in carbohydrate synthesis in wastewater treated consortia 1 could be attributed to modifications in photosynthetic machinery, enhancement in cell division, and synthesis of proteins and osmoprotectants (Sun et al., 2014). Overall, the results showed that carbohydrate content was also found highest in consortia 1 treated with 100% wastewater which indicates that municipal wastewater can be used as source of nutrients for algal consortia without supplementation of additional nutrients in the growth media. Additionally, carbohydrates and protein are vital indicators of algal growth (Singh et al., 2018) and also play an instrumental role in maintaining osmotic balance, carbon partitioning, and photosynthesis in microalgae (Rosa et al., 2009).

6.3.3 Lipid content

Microalgae stimulate lipid accumulation by storing the photo-assimilates in the form of lipid which ensure their survival under stress conditions. It was observed that lipid content in algal consortia 1 was increased by 4.52 and 10.46 folds after treatment with 25% and 50% municipal wastewater in comparison to control. The enhancement in the lipid accumulation could be attributed to nutrient limited condition created by dilution of wastewater which shifted the metabolic pathways towards lipid accumulation. The increased lipid accumulation in nutrient-deficient conditions has also been reported by Rai et al., (2017). It was also observed that lipid accumulation in algal consortia 1 was increased by 8.13 and 4.80 folds at 75% and 100% municipal wastewater, respectively. In case of the algal consortia 2, the maximum increase of 1.21 and 2.46 folds in lipid content was found to be at 25% and 75% wastewater concentration, respectively. Further, the results exhibited that lipid content was found to be highest in wastewater treated consortia 1 than consortia 2. The higher accumulation of lipid in algal consortia treated with different concentrations of municipal wastewater may be attributed to nutrient imbalance, nitrogen and phosphorus deficiency, salinity, and heavy metals. Such stress conditions may modify metabolic pathways and divert the photosynthetically fixed carbon into storage molecules which leads to accumulation of lipids in algal consortia. Overall, the results depicted that algal consortia 1 and 2 showed considerable variation in the accumulation of lipid after treatment with different concentrations of municipal wastewater. Furthermore, lipid accumulation potential varies with algal species as different mechanisms are responsible for directing carbon from carbohydrate synthesis towards lipid accumulation (Hounslow et al., 2021). Therefore, the enhancement in lipid accumulation in algal consortia treated with different concentrations of municipal wastewater may be attributed to the cumulative effect of different stress factors imposed by wastewater and operating conditions.

6.4 FTIR spectroscopy

FTIR spectroscopy was used to examine the effect of municipal wastewater on the composition of algal consortia. The results revealed that the wastewater treated algal consortia showed higher peak height between 3600-3300 cm^{-1}, 3000-2800 cm^{-1}, 1750-1400 cm^{-1}, and 1200-900 cm^{-1} as compared to the control (Fig. 6.3). The absorption peak between 3600-3300 cm^{-1} could be due to hydroxyl groups in treated consortia 1 and 2. The presence of hydroxyl groups might be responsible for the sorption of metals to the cell surface (Ajayan et al., 2015). The absorption peaks in the region between 1200-900 cm^{-1} exhibited the presence of carbohydrates (Singh et al., 2021; Upadhyay et al., 2021), and the sharp peaks at 100% municipal wastewater reflected enhancement in carbohydrate accumulation in the treated consortia 1 and 2. It was observed that the spectral peaks of treated consortia showed major variation between 1700-1000 cm^{-1} which depicts stretching of C=O, bending vibration of C-H, and rocking vibration of CH_2 bonds of different functional groups (Singh et al., 2021). The spectral region between 3000-2800 cm^{-1} represents stretching of C-H groups of CH_2 while a sharp peak in the region between 1700-1500 cm^{-1} exhibited stretching of C=O groups which indicate the presence of carbonyl functional group in the biomass (Ajayan et al., 2015). Furthermore, two regions of FTIR spectra are commonly used for the assessment of lipid content (Laurens and Wolfrum, 2011) i.e the methyl and methylene groups (2800– 3000 cm^{-1}) and the vibrational stretching of ester bonds (1740 cm^{-1}). FTIR spectroscopy depicted that only consortia 1 showed a peak between 1750-1740 cm^{-1} which is a direct indicator of the neutral lipid accumulation. Moreover, the appearance of higher spectral peaks between 3000-2800 cm^{-1} in consortia treated with 100% wastewater as compared to control may be responsible for symmetrical stretching of CH_3 groups of lipids (Forfang et al., 2017; Singh et al., 2021). Furthermore, the FTIR spectra revealed that the lipid accumulation potential of treated consortia 1 and 2 was higher as compared to that of their respective control. Therefore, the increment in neutral lipid accumulation at 100% of municipal wastewater concentration may eventually increase the biofuel production potential of consortia 1. The enhancement in lipid accumulation suggested that utilizing municipal wastewater can be the feasible approach to improve the production of biofuel in eco-friendly manner. Overall, FTIR spectra reflected that lipid, proteins and carbohydrates were in abundant amounts in wastewater treated consortia 1 and 2 than control. Thus, the dominance of macromolecules in consortia further reflected the presence of several functional groups in algal biomass which may help in adsorption of different heavy metals from municipal wastewater. The results were in agreement with Laurens and Wolfrum, (2011); Singh et al., (2021).

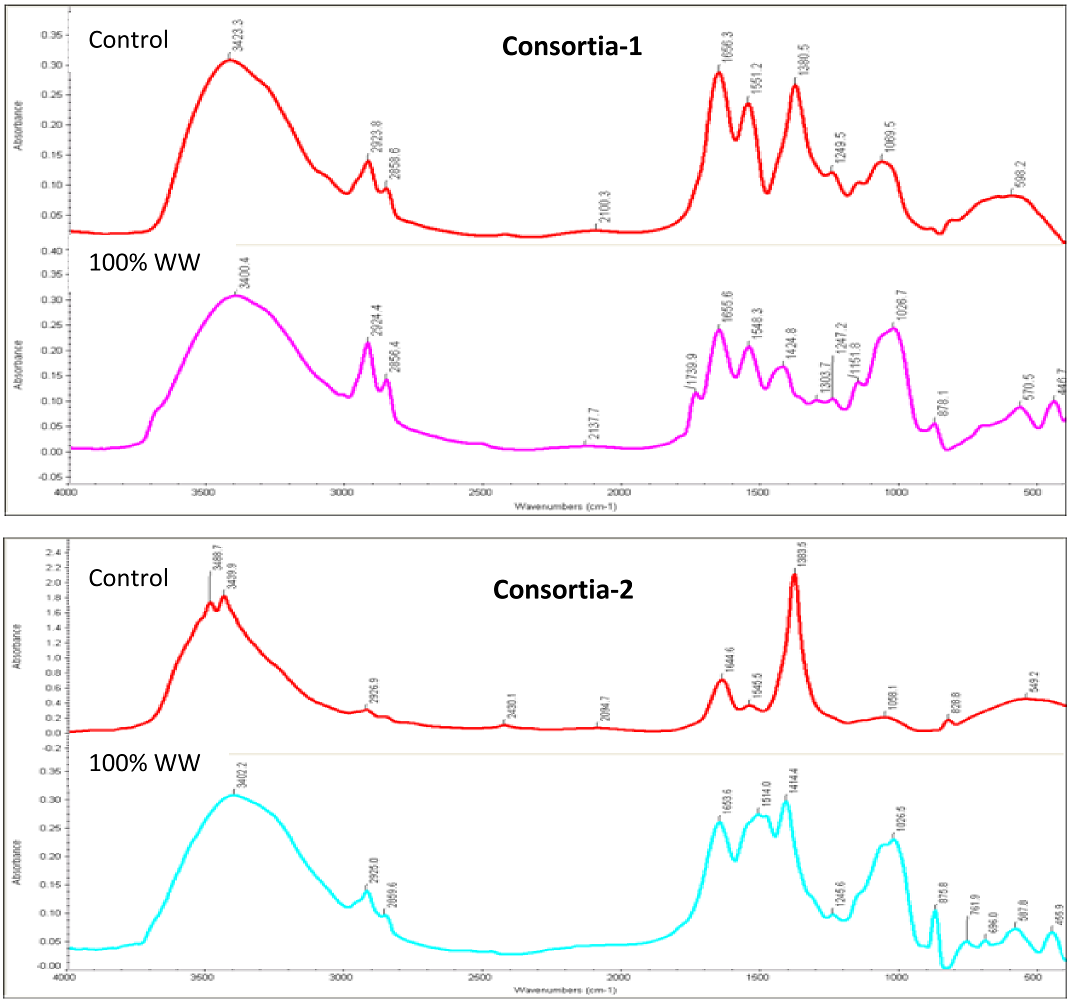


Fig.6.3 Comparison of FTIR spectra of consortia 1 and 2 treated with BG-11 and municipal wastewater (100%)

6.5 Oxidative stress markers

TBARS are the indicators of cellular toxicity induced by temperature, light, salinity, and toxic pollutants. The results revelaed that that TBARS content in consortia 1 was increased by 1.21, 1.10, 1.15 and 1.36 folds while in case of consortia 2, it was increased by 2.00, 1.75, 2.37 and 2.93 folds after treatment with 25%, 50%, 75% and 100% of municipal wastewater in comparison to their respective control (Table 6.3). The lowest TBARS content in algal consortia 1 treated with municipal wastewater might be attributed to efficient tolerance, cellular modifications and enhanced antioxidants activity that mitigated the oxidative damage posed by ROS. It was also observed that H_2O_2 content was increased by 2.98 and 1.96 folds in consortia 1 while, an increase of 4.84 and 18.08 folds was observed in case of consortia 2 after treatment with 25% and 100% wastewater concentration. The enhancement in oxidative stress markers like TBARS and H_2O_2 could be attributed to stress imposed by different wastewater concentrations. The highest increase of TBARS content in consortia 2 could be ascribed to structural and functional alteration caused by metal pollutants with wastewater concentration gradient. The increased TBARS content at higher wastewater concentration indicates the presence of stress conditions which initiated lipid peroxidation in selected algal consortia. Similarly, the H_2O_2 content was also amplified with the level of stress and vice versa. Algal consortia 1 and 2 treated with municipal wastewater showed significant increment in H_2O_2 content in comparison to control. The increased H_2O_2 content in consortia 2 treated with municipal wastewater may be attributed to enhancement in ROS generation due to binding of metals and thiol groups which eventually damage cell memebrane and disturbs the redox status of the cell (Leong and Chang, 2020; Singh et al., 2021).

Sharma et al. (2020) also reported the increment in oxidative stress markers in *Scenedesmus obliquus* after treatment with heavy metals. Therefore, less accumulation of oxidative stress markers in algal consortia 1 reflected remarkable tolerance potential to adapt under different wastewater concentrations.

6.6 Wastewater induced non-enzymatic and enzymatic antioxidants activity in consortia

Antioxidants ensure survival, promote growth and maintain homeostasis under different stresful environment. It was observed that consortia 1 showed an increase of 1.00 and 1.02 folds in ascorbic acid content at 75% and 100% wastewater concentration. The results further revealed that ascorbic acid content in algal consortia 2 was declined significantly at different concentration of municipal wastewater as compared to control (Fig. 6.4). Ascorbic acid is highly abundant and a water-soluble antioxidant, which besides positively influencing various aspects in the cell also acts as an enigmatic component of defense armory (Anjum et al., 2014; Singh et al., 2021). Under adverse conditions, ascorbic acid also maintains the balance of ROS and ensures protection by regulating the activity of the metal-containing enzymes in the cell (Leong and Chang, 2020). Therefore, the increased ascorbic acid content could be the mechanism for scavenging of ROS thereby protects cellular components from oxidative damage. The results further depicted that proline content was increased by 1.33 and 1.06 folds in consortia 1 and 2, respectively at 100% wastewater concentration (Fig. 6.4). It was also observed that the proline content was increased by 1.06 and 1.11 folds in consortia 1 treated with 25% and 75% of wastewater concentration.

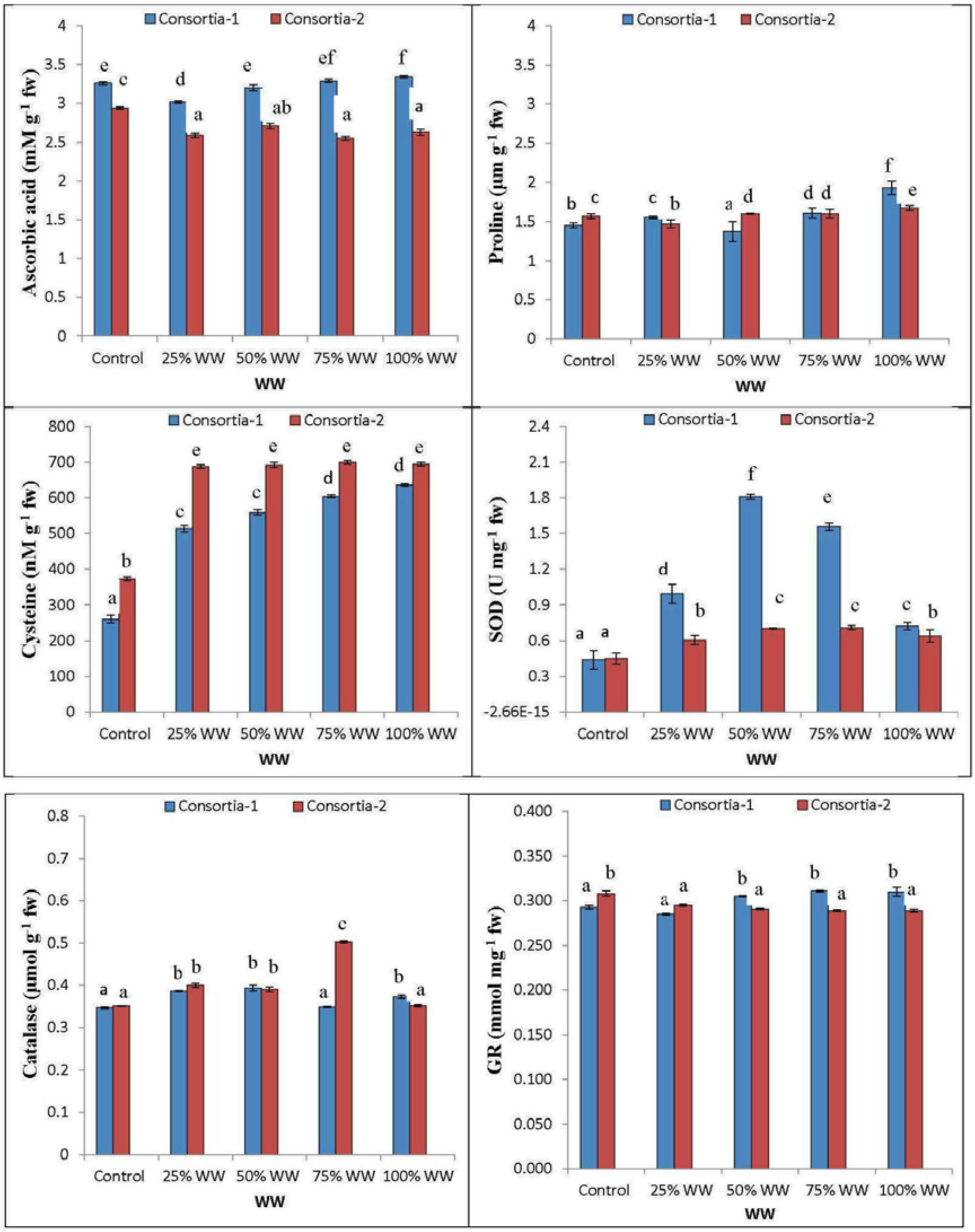


Fig. 6.4 Effect of different municipal wastewater concentrations on non-enzymatic (ascorbic acid, proline, cysteine) and enzymatic antioxidant (superoxide dismutase, catalase and glutathione reductase) system of microalgae consortia 1 and 2

In case of consortia 2, an increase of 1.01 and 1.01 folds was observed at 50% and 75% wastewater concentration respectively. The increment of proline content may be ascribed to the presence of different heavy metals which may leads to accumulation of proline in algal consortia 1 and 2 after wastewater treatment (100%). Proline protects microalgae from damage by forming complexes with metals; scavenges free radicals, helps in osmoregulation, and enzyme protection (Gupta et al., 2017). Thus, increased proline accumulation may maintain cell homeostasis and protect cellular components from oxidative damage under different wastewater concentrations. Further, the results revealed that cysteine content was increased by 1.97, 2.15, 2.32 and 2.44 folds in consortia 1 treated with municipal wastewater while, in case of consortia 2, an increase of 1.84, 1.55, 1.87 and 1.86 folds was observed at 25%, 50%, 75% and 100% concentration of municipal wastewater respectively, as compared to control. The increased cysteine content in algal consortia 1 and 2 treated with different concentration of municipal wastewater may be attributed to their active involvement in metal sequestration and stress mitigation. Besides, cysteine also serves as the precursor of metal chelating proteins that can further assist in the reduction of ROS (Leong and Chang, 2020; Singh et al., 2021). Metal-binding proteins like metallothioneins has been observed as the celluar mechanism in microalage *Scenedesmus vacuolatus* and *Tetraselmis suecica* for detoxification of metals (Le Faucheur et al., 2005). Furthermore, phytochelatins could be also synthesized by microalgae as a response to alleviate cellular metal concentration (Leong and Chang, 2020). Overall, the increased activity of non-enzymatic antioxidants may be a tolerance mechanism adopted by algal consortia 1 and 2 to sequester metals and decrease their concentration from the cell.

Superoxide dismutase (SOD), ubiquitous in aerobic organisms is very prone to oxidative stress. The results showed an increase of 1.64 and 1.42 folds in SOD activity in consortia 1 and 2 at 100% wastewater concentration. while at 50%, it was increased by 4.14 and 1.56 folds in consortia 1 and 2 respectively, as compared to respective control. It was also observed that SOD activity increased by 2.27, 4.14 and 3.56 folds in consortia 1 while 1.34, 1.56 and 1.57 folds in consortia 2 treated with 25%, 50% and 75% concentration of municipal wastewater. The results also depicted an increase in the catalase activity in consortia 1 (1.07 folds) and consortia 2 (1.00 folds) treated with 100% municipal wastewater. Further, it was observed that catalase activity increased by 1.11, 1.13 and 1.00 folds in consortia 1 while, an increase of 1.13, 1.11, and 1.43 folds was observed in case of consortia 2 after treatment with 25%, 50% and 75% concentration of municipal wastewater, respectively in comparison to their respective control. Both SOD and CAT are considered as the first and second line of defense, respectively (Leong and Chang, 2020). Increased SOD activity with wastewater concentration might be ascribed to heavy metals-induced oxidative stress which leads to the generation of superoxides in microalgae (Danouche et al., 2020; Singh et al., 2021). SOD neutralizes superoxide and consequently protects the cellular structure from further oxidative damage. Danouche et al., (2020) reported that the activity of SOD in microalgae increased with the dose of heavy metals in the growth media. Thus, the significant increase in the activity of SOD in consortia 1 may be attributed to increased heavy metal exposure with the wastewater concentration gradient. Among different ROS, H_2O_2 can cause severe toxicity to macromolecules. CAT sequesters H_2O_2 and ensures the survival of microalgae under a stressful environment (Leong and Chang, 2020; Singh et al., 2021). Thus, enhanced production of SOD and catalase may be the main reason for decreased TBARS and H_2O_2 in consortia 1 as compared to consortia 2. Therefore, elevated level of SOD and catalase, particularly in consortia 1 along a wastewater concentration gradient indicates the protective role of these enzymatic antioxidants under stress condition. The results further showed that the glutathione reductase (GR) activity enhanced by 1.04, 1.06, 1.05 folds in consortia 1 at 50%, 75% and 100% while a significant decline was observed in consortia 2 at different wastewater concentrations as compared to control. The declined GR activity at 100% might be due to interference in ROS generation and sequestration. The enhancement in GR activity with wastewater concentration in consortia 1 can be due to their active involvement in synthesis of metal-chelating proteins thus, GR may augment the metal removal from different wastewater concentrations. Previously, increased GR activity has also been observed in *Chlorella vulgaris* exposed to heavy metals such as Pb (Danouche et al., 2020). Consequently, GR prevents damage to cellular constituents by sustaining the balance of reduced glutathione as well as the sulfur-containing compound in the cell (Yousuf et al., 2012; Singh et al., 2021). Furthermore, GR performs multiple functions by increasing the growth, development, and metabolize ROS and its products from the cell (Aoyama and Nakaki, 2015). Thus, the increased activities of enzymatic and non-enzymatic antioxidants may enhance metal detoxification potential, improve tolerance, adaptability and ensure survival by repairing the damage caused by different metals in wastewater. Therefore, in order to amplify the sustainable treatment of wastewater, the microalgae possessing competent defense responses should be exploited for remediation of different wastewater.

Chapter 7

Algal consortia as the flexible bio-system for wastewater treatment: effect of different light intensities on photosynthetic performance, anti-oxidative system and biodiesel production

CHAPTER-7

Algal consortia as the flexible bio-system for wastewater treatment: effect of different light intensities on photosynthetic performance, anti-oxidative system and biodiesel production

7.0 INTRODUCTION

The start of 21^{ist} century has shifted the world towards speedy industrialization and brisk urbanization that leads to crisis of freshwater, energy and clean environment (Khan et al., 2019). Rapid water quality deterioration and the dearth of freshwater (for human use owing to improper management of water in current scenario is the major apprehension for the global community (Abinandan and Shanthakumar, 2015). In India, majority of the treatment facilities for wastewater treatment is deployed in urban areas representing 31% of total population and still enormous wastewater is discharged without necessary treatment into the aquatic environment (Choudhary et al., 2016). Wastewater discharged prior to requisite treatment causes eutrophication, acidification and rapid accumulation of toxic pollutants in the freshwater reservoir (Singh et al., 2020). Several treatment facilities like coagulation, photodegradation, reverse osmosis and ozonation have been employed but due to energy and cost intensive nature, such techniques cannot be universally employed for the treatment of wastewater (Bolisetty et al., 2019). Bioremediation using living organisms is gaining huge impetus (Han et al., 2022) as such techniques can resolve multiple problems from nutrient recuperation to pollutants detoxification in economical and eco-friendly manner (Singh et al., 2021). Because of high photosynthetic rate and unique capability to withstand environmental perturbation, microalgae seem suitable organism for the wastewater treatment (Singh et al., 2021). In order to enhance the remediation potential and lipid production, several techniques in the form of changing light intensity, temperature, and nutrients have been adopted but the studies are still lacking in response to microalgae consortia. Wide scale wastewater treatment and biomass production has been limited by light intensity however, understanding the light dependent activity of microalgae is of profound importance in designing the treatment system, performance prediction and suitable conditions for cultivation of microalgae.

Light is an important environmental factor necessary for growth, biomolecules synthesis, metabolic pathways and photosynthesis in microalgae (He et al., 2015). The supply of sufficient light during cultivation of microalgae in wastewater not only influences the remediation efficiency (Iasimone et al., 2018) but also the growth and biomass production (Mata et al., 2012). Previously, it has been observed that *Scenedesmus* sp. showed increase in biomass productivity (255 to 452 mg L^{-1} d^{-1}) after increasing intensity of light from 50-250 μmol m^{-2} s^{-1} (Liu et al., 2012). Cheirsilp et al., (2012) further reported that increasing light intensity during cultivation of *Chlorella* sp. and *Nannochloropsis* sp. enhanced the lipid production by two times and composition of lipid was suitable for biofuel production. Light also changes the fatty acid composition that could improve biofuel quality extracted from microalgae (Nzayisenga et al., 2020). Therefore, altering light intensity can acts as the major factors which may be responsible for modifying the metabolic pathways for enhanced accumulation of key metabolites in the cell. Since, studies related to utilization of microalgae consortia for wastewater treatment under different light intensities are very limited; thus, this study was conducted to examine the effect of light intensities on remediation efficiency, chlorophyll fluorescence, lipid production and defense mechanisms of algal consortia. Furthermore, examining remediation potential, chlorophyll fluorescence, biochemical composition and anti-oxidative responses under different light conditions can be helpful in identification of the microalgae consortia well adaptable to the changing environmental conditions that eventually augment the success of the outdoor cultivation of microalgae. Therefore, two different algal consortia were employed to resolve the growing crisis of wastewater treatment and energy scarcity.

7.1 Results and discussion

7.1.1 Effect of light intensity (20 W/m^2 and 40 W/m^2) on remediation efficiency of algal consortia

The physico-chemical characteristics are the vital determinant of water quality and results exhibited that both algal consortia significantly improved electrical conductivity (EC), total solid (TS), biological oxygen demand (BOD), and dissolved oxygen (DO) of wastewater. At 40 W/m^2, the highest percent increase of 21.01% and 19.62% in pH was found to be at 75% wastewater with consortia 1 and 2 while, 19.36% and 14.43% was observed in case of consortia 1 and 2 at 100% of wastewater concentration. Similarly, the increase in pH was observed at 25% (5.82%) and 50% (8.35%) concentration of municipal wastewater treated with consortia 1 and 2, respectively. At 20 W/m^2, consortia 1 showed highest percentage change of 15.50% and 23.79% in pH at 75% and 100% wastewater concentration while 16.45% and 21.39% with consortia 2 at 25% and 50% wastewater, respectively. The results further depicted that wastewater after treatment with algal consortia, pH of wastewater shifted towards alkalinity which could be due to the use of inorganic carbon in the form of bicarbonate from wastewater. The results also depicted that wastewater after treatment through consortia 1 and 2 showed highest increment in dissolved oxygen level at 25% while lowest increment at 100% wastewater concentration. At 20 W/m^2, the results revealed that consortia 2 treated wastewater showed highest increase in DO level at 25% (4.43 mg/L), 50% (3.94 mg/L), 75% (1.18 mg/L) and 100% (0.453 mg/L) concentration of wastewater. At 40 W/m^2, the DO level of 4.62 mg/L (25%), 3.67 mg/L (50%), 2.11 mg/L (75%) and 0.436 mg/L (100%) was also observed in case of consortia 2. The results further showed that pH and DO level was increased significantly at both light intensities with selected algal consortia. The increment in pH and DO level could be attributed to fast cell proliferation that increased the assimilation of inorganic carbon and photosynthetic rate of algal consortia. At 40 W/m^2, highest remediation efficiency of 84.98%, 84.30%, 85.63%, 85.61% and 93.35% was observed for EC, TSS, TDS, TS and BOD at 25% wastewater while lowest reduction of 42.15% (TSS), 48.36%% (TDS), 48.63% (TS) was also observed in case of consortia 1 treated with 100% concentration of wastewater, respectively. Furthermore, consortia 1 showed highest removal efficiency for EC (76.99%), TSS (71.61%), TDS (75.63%), TS (75.50%) and BOD (80.82%) at 50% wastewater concentration. At 75%, the highest reduction of 65.55%, 55.05%, 65.27%, 63.56% and 68.75% in EC, TSS, TDS, TS and BOD was observed with consortia 1 as compared to control. At 20 W/m^2, the removal efficiency of 81.64%, 80.99%, 84.18%, 87.71% for EC, TSS, TDS and BOD was shown by consortia 1 treated with 25% wastewater concentration. Furthermore, consortia 1 also showed the maximum reduction efficiency of 65.43%, 67.32%, 69.71%, 77.08% and 49.25% for EC, TSS, TDS, TS, and BOD at 50% wastewater concentration while 49.26%, 53.55%, 66.71% in EC, TSS, and BOD at 75% concentration of wastewater, (Table 7.1). At 100%, the results further depicted the maximum decline of EC (38.64%), TSS (37.42%) and BOD (51.36%) with consortia 1 while, TDS (47.09%) and TS (44.73%) with consortia 2. The present study revealed that highest reduction percentage in different quality parameters were observed at 25% of the wastewater concentration with algal consortia 1 under different light intensities. The considerable variation in EC, TS, TDS, TSS and BOD removal at different wastewater concentration may be attributed to dilution which decreases the amount of different pollutants and eventually increases the removal efficacy of consortia. Moreover, the oxygen released by microalgae is utilized by the microbial population to degrade the organic material that results in decreased BOD level of wastewater (Fito and Alemu, 2019).

7.1.2 Nitrogen and phosphorus remediation

Nitrogen is vital constituent of various molecules of the cell (Sajjadi et al., 2018). At 40 W/m^2, the results depicted that consortia 1 showed highest nitrate-nitrogen removal efficiency at 25% (93.37%), 75% (78.71%) and 100% (74.25%) of wastewater concentration while, consortia 2 (85.28%) at 50% concentration of wastewater. Similarly, at 20 W/m^2, the maximum removal efficiency of 92.00%, 82.11%, 76.48%, and 75.20% was observed with consortia 1 while 90.77%, 83.05%, 75.60% and 70.14% with consortia 2 at 25%, 50%, 75% and 100% of wastewater concentration. The results also exhibited that maximum decrease in level of phosphorus was observed with consortia 1 at 25% (93.42%), 50% (89.75%) and 100% (77.56%) concentration of wastewater while with consortia 2 (83.24%) at 75% concentration of wastewater.

Table 7.1 Variation in different physico-chemical attributes of municipal wastewater treated with consortia 1 and 2 under different light intensities

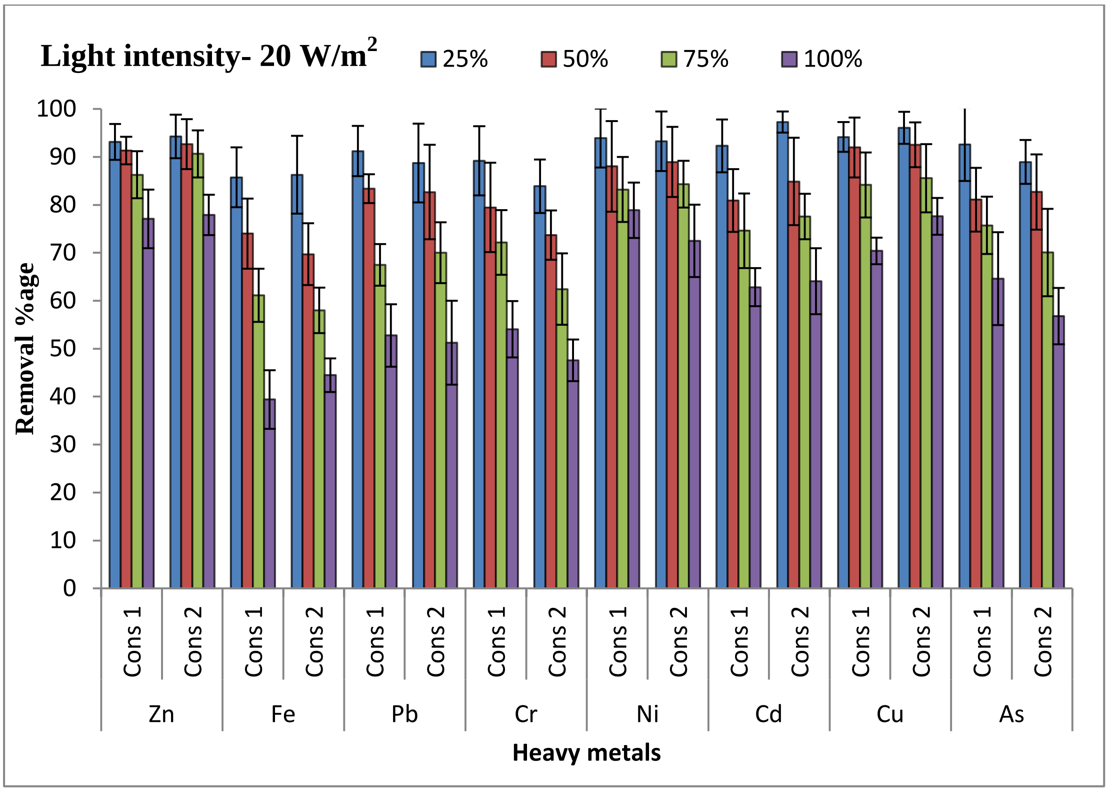
Parameters	Initial concentration	WW	Consortia-1		Consortia-2	
			20 W/m^2	40 W/m^2	20 W/m^2	40 W/m^2
pH	7.9	Control	8.79±0.200[b]	8.83±0.225[b]	9.56±0.150[f]	8.85±0.145[b]
		25%	9.15±0.160[c]	8.36±0.225[a]	9.20±0.110[c]	8.12±0.155[a]
		50%	9.65±0.290	9.28±0.076[c]	9.59±0.110[f]	9.35±0.195[d]
		75%	9.35±0.240[d]	9.56±0.077[de]	9.10±0.210[b]	9.45±0.116[e]
		100%	9.78±0.110[e]	9.43±0.073[d]	8.95±0.280[b]	9.04±0.243[b]
EC (µS/cm)	710	Control	2.31±0.26[a]	2.09±0.21[a]	2.23±0.015[a]	1.89±0.14[a]
		25%	148±7.17[b]	121±3.10[b]	170±8.02[b]	151±3.32[b]
		50%	280±8.59[d]	186±5.04[c]	382±13.01[d]	276±4.23[c]
		75%	411±8.04[f]	279±4.15[d]	419±10.14[e]	365±7.13[d]
		100%	497±7.32[g]	367±6.26[e]	527±11.67[f]	442±6.34[e]
TDS	550	Control	1.81±0.071[a]	3.30±0.212[a]	1.72±0.070[a]	4.10±0.110[a]
		25%	87±6.94[b]	79±1.34[b]	108±2.000[b]	96.67±1.66[b]
		50%	169±12.07[c]	134±1.88[c]	174±5.516[c]	159.67±1.23[c]
		75%	252±18.95[d]	191±1.45[c]	224±5.507[d]	230±2.45[d]
		100%	319±10.58[e]	284±3.02[d]	291±3.055[d]	260.67±3.32[d]
TSS	155	Control	3.83±0.650[a]	2.79±0.304[a]	4.00±0.22[a]	4.00±0.43[a]
		25%	29.46±0.36[b]	24.33±0.514[b]	33±0.63[b]	28±0.88[b]
		50%	50.66±0.750[c]	44±0.982[c]	61±0.79[c]	50±0.69[c]
		75%	72.0±1.65[d]	69.67±0.900[c]	86±0.52[d]	73±0.96[d]
		100%	97.0±1.43[e]	89.67±1.05[d]	113±1.23[e]	86±1.19[d]

TS	705	Control	6.37±0.74[a]	5.32±0.49[a]	6.66±0.86[a]	5.80±0.37[a]
		25%	119.67±1.01[b]	101±1.14[b]	151±1.24[b]	124±2.06[b]
		50%	212.66±1.84[d]	172±1.62[c]	220±1.79[c]	190±2.55[b]
		75%	317±2.06[e]	255.79±2.29[d]	302±2.76[d]	260±2.98[c]
		100%	401±3.33[f]	360.65±2.82[e]	388±3.65[e]	332±2.66[d]
BOD	45	Control	0.386±0.068[a]	0.516±0.051[a]	0.306±0.056[a]	0.43±0.065[a]
		25%	5.53±0.261[b]	2.99±0.179[b]	7.04±0.805[b]	4.02±0.0454[b]
		50%	10.31±0.909[c]	8.63±0.225[c]	13.43±1.60[c]	9.37±1.55[b]
		75%	14.98±1.30[d]	14.06±1.48[d]	18.63±1.61[d]	15.22±1.91[d]
		100%	21.89±2.11[f]	18.6±1.84[e]	22.17±1.45[e]	23.97±1.46[e]
DO		Control	5.53±0.036[e]	6.1±0.025[f]	5.13±0.205[f]	5.56±0.039[f]
		25%	4.41±0.276[d]	4.56±0.186[d]	4.43±0.182[e]	4.62±0.275[e]
		50%	2.87±0.202[c]	2.66±0.186[c]	3.94±0.161[d]	3.67±0.402[d]
		75%	0.978±0.115[b]	1.06±0.125[b]	1.18±0.187[b]	2.11±0.225[c]
		100%	0.381±0.044[a]	0.414±0.028[a]	0.453±0.110[a]	0.436±0.087[a]
NO_3-N	35	Control	0.429±0.021[a]	0.362±0.051[a]	0.400±0.030[a]	0.304±0.037[a]
		25%	2.80±0.250[b]	2.32±0.110[b]	3.23±0.458[b]	2.78±0.301[b]
		50%	6.26±0.807[c]	5.50±0.302[c]	5.93±0.700[d]	5.15±0.209[c]
		75%	8.23±0.741[e]	7.45±0.816[d]	8.54±0.460[e]	8.90±0.483[e]
		100%	8.68±0.550[e]	9.01±0.666[f]	10.45±0.812[f]	10.09±0.851[f]
PO_4^{3-}	15.82	Control	0.36±0.021[a]	0.274±0.062[a]	0.546±0.025[a]	0.66±0.021[a]
		25%	1.04±0.169[c]	0.654±0.089[b]	1.20±0.019[c]	0.986±0.057[b]
		50%	1.62±0.254[d]	1.47±0.156[d]	1.89±0.421[f]	1.42±0.212[d]
		75%	2.96±0.476[f]	1.68±0.121[d]	2.65±0.410[h]	2.14±0.378[g]
		100%	3.55±0.612[g]	2.27±0.139[g]	3.92±0.723[i]	2.24±0.310[g]

At 40 W/m^2, highest reduction in phosphorus level was at 25% (95.86%) and 75% (89.38%) with consortia 1 while, consortia 2 showed maximum reduction efficiency at 50% (91.02%) and 100% (85.85%) wastewater concentration (Table 7.1). In present investigation, discrepancy in nutrient removal efficiency between consortia 1 and 2 could be attributed to microalgal species, metabolic rate and their adaptability to thrive under different wastewater and light intensity conditions. Choudhary et al., (2016) observed that mixture of different microalgae efficiently reutilized 80-100% of inorganic nutrients from wastewater. The results also depicted that nitrogen and phosphorus removal potential enhanced with light intensity which could be attributed to increased metabolic activities of consortia assimilating additional nutrients for synthesis of cellular bio-molecules and biomass production. The differential nutrients removal efficiency of consortia 1 and 2 might be attributed to protein rich microalgal species that resulted in enhanced assimilation of nitrogen from wastewater (Fan et al., 2020). The elevated nutrient removal could also be due to their important role in the synthesis of essential cellular components such as chlorophyll, genetic material and energy molecules (Emparan et al., 2019). Moreover, light directly influence the growth rate thereby alters the nutrient removal efficiency of microalgae from growth media. In present study, the maximum nutrients removal efficiency of algal consortia 1 and 2 after exposure to higher light intensity might be attributed to light induced elevated carbon and nutrients removal from wastewater, which was in accordance with Liu et al., (2018). The present study provided the adequate evidences that consortia 1 and 2 has promising potential to recover nutrients from wastewater treated under different light intensities.

7.1.3 Heavy metals detoxification

The heavy metal removal from contaminated environment is very difficult and challenging but using microalgae can provide new avenues of metal detoxification in sustainable manner. The different heavy metals in wastewater were analyzed and the results revealed that Fe (1514.48±9.45 µg/L), Ni (15.30±0.706 µg/L), Zn (13.04±0.415 µg/L) Pb (8.72±0.125 µg/L), Cd (3.98±0.067 µg/L), Cr (3.70±0.088 µg/L), Cu (12.20±0.559 µg/L) and As (0.62±0.025 µg/L) were present in municipal wastewater. At 40 W/m^2, the highest removal efficiency for Pb (91.21%), Cr (89.18%), Ni (93.96%), As (92.63%) was observed with consortia 1 while consortia 2 showed maximum removal efficiency for Zn (94.27%), Fe (86.26%), Cd (97.28%), Cu (96.08%) at 25% concentration of wastewater. At 50% wastewater concentration, consortia 2 exhibited maximum remediation efficiency for Zn (92.66%), Cd (84.96%), Ni (88.95%), Cu (92.53%) and As (82.68) while consortia 1 effectively removed Pb (83.39%), Fe (74.01%) and Cr (79.45%). The results also showed the maximum removal of Fe (61.25%) and As (75.71%) was observed with consortia 1 while, consortia 2 efficiently removed Zn (90.64%), Pb (70%), Ni (84.31%), Cd (77.57%), and Cu (85.57%) after treatment with 75% concentration of municipal wastewater. Furthermore, at 100% of wastewater concentration, consortia 1 showed highest remediation efficiency for Pb (52.76%), Cr (54.05%), Ni (78.88%) and As (64.62%) while consortia 2 for Zn (77.91%), Fe (44.48%), Cd (64.07%) and Cu (77.62%). At 20 W/m^2, consortia 1 also showed maximum removal efficiency for Zn (89.11-97.73%), Pb (77.23-86.25%) and Cr (72.43-83.16%) at 25% and 50% concentration of wastewater.



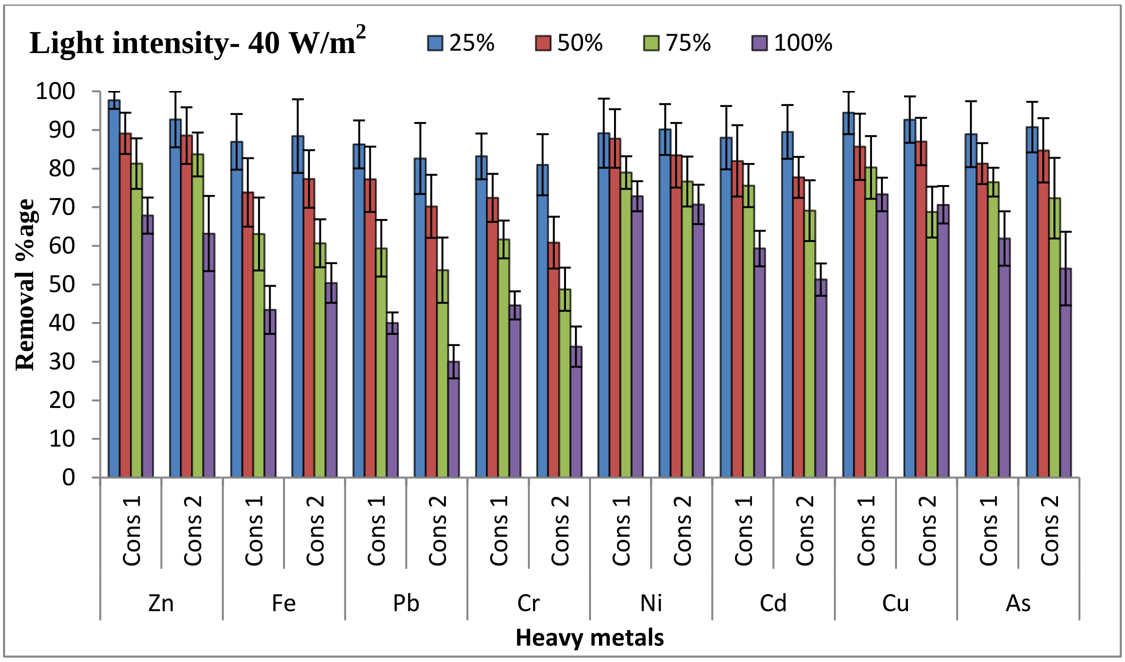


Fig. 7.1 Heavy metal remediation potential of selected consortia treated with wastewater under different light intensities (20 W/m^2 and 40 W/m^2)

Similarly, highest reduction in Fe (77.32-88.43%) and As (84.71-90.77%) at 25% and 50% concentration of wastewater was observed in case of consortia 2. The results further depicted that consortia 1 efficiently removed Pb (40-59.37%), Cr (44.59-61.62%), Ni (72.87-79.01%), Cd (59.29-75.59%), Cu (73.32-80.32%) and As (61.90-76.46%) at 75% and 100% concentration of wastewater than consortia 2 (Fig. 7.1). In present study, the maximum heavy metal removal efficiency at 40 W/m^2 as compared to 20 W/m^2 after treatment with different wastewater concentration might be attributed to the abundance of cellular macromolecules exhibiting different functional groups for binding of metals (Hwang et al., 2016). Salama et al., (2019) reported the removal of heavy metals from contaminated environment may be ascribed to bioadsorption and biosorption. The improved metal removal with light intensity could also be ascribed to growth rate that may augment the availability of active binding sites. Furthermore, different mechanisms like immobilization, chelation, exclusion, and gene expression may also elevate the metal removal efficiency of microalgae from wastewater (Leong and Chang, 2020). In this study, the heavy metal removal from wastewater under different light intensity using algal consortia was also reflected by Fourier transform infrared spectroscopy (FTIR).

FTIR is promising technique to examine the presence of diverse functional groups and macromolecular content in microalgae (Fig. 7.2). In present study, the higher spectral peaks in the region between 3500-900 cm^{-1} in selected algal consortia at 100% wastewater concentration treated under different light intensities expressed the presence of multiple functional groups. Carbohydrate (1200-900 cm^{-1}) reflects aldehyde and ketones functional groups, lipids (3000-2800 cm^{-}1) mostly contain ester and alcohol groups while proteins (1700-1500 cm^{-1}) contain thiols, alcohols,

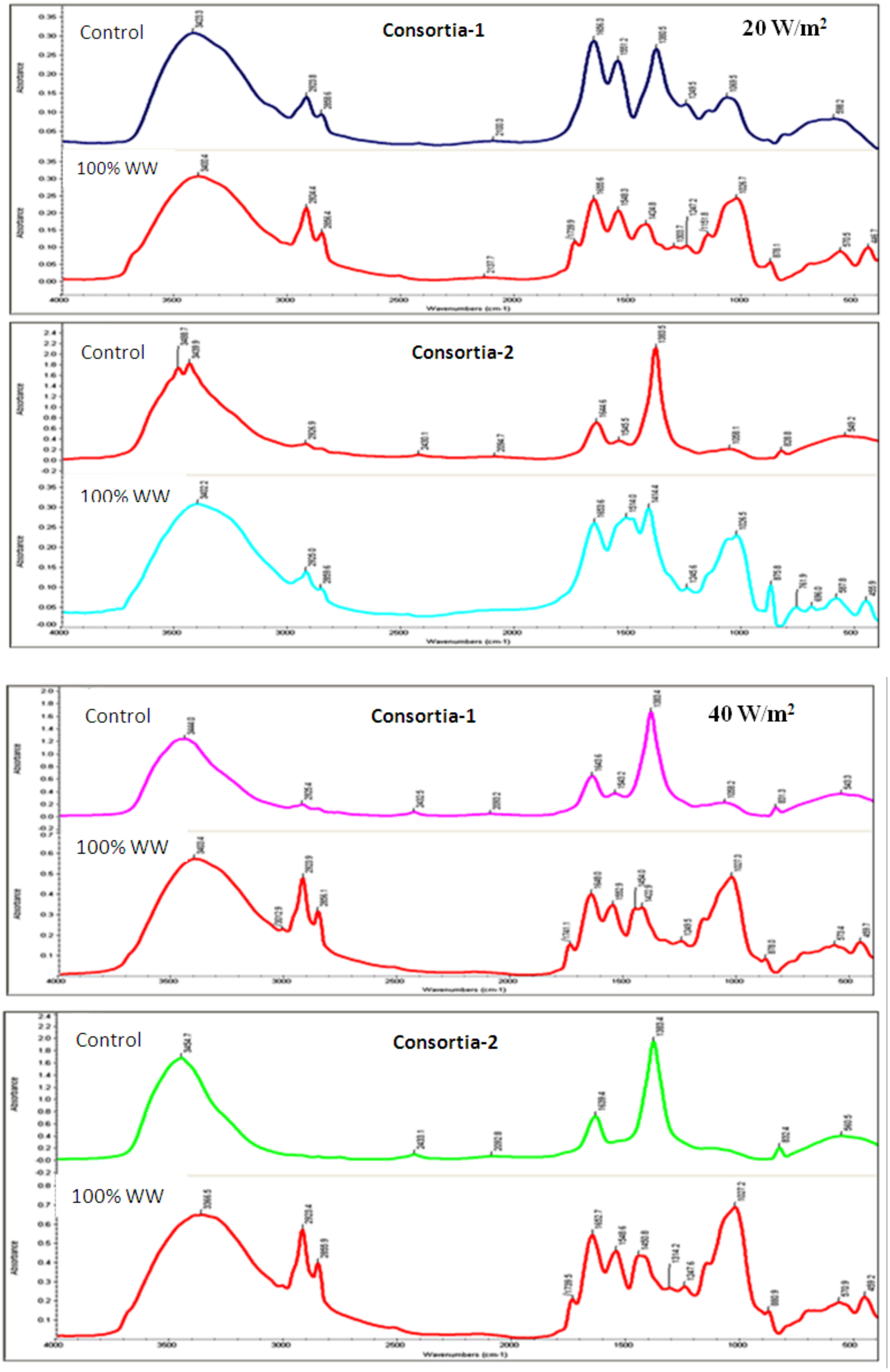


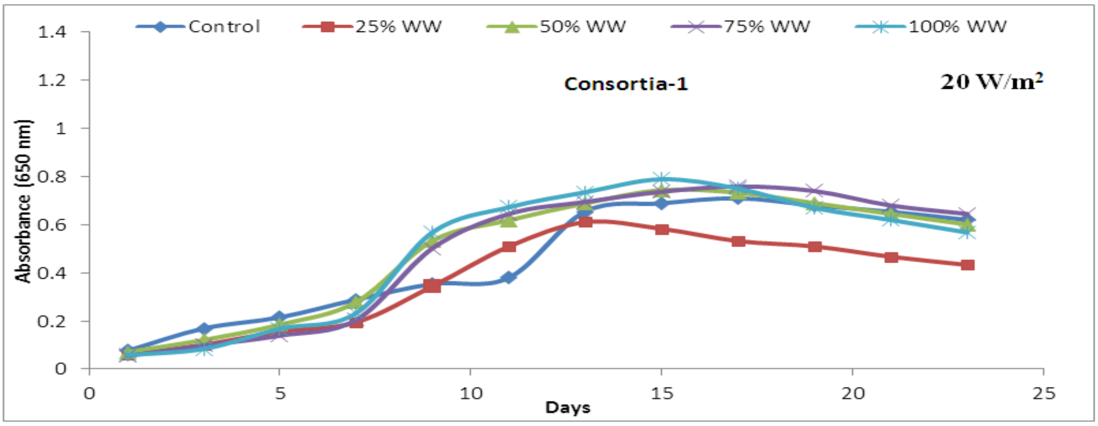
Fig. 7.2 FTIR spectroscopy of consortia 1 and 2 treated with 100% concentration of wastewater under light intensity of 20 W/m^2 and 40 W/m^2

thioethers and carboxylic acids groups (Sultana et al., 2020). Further, broad stretching of peak in consortia 1 and 2 at 3550-3400 cm^{-1} indicated that hydroxyl (OH) groups may adsorb metals from contaminated environment (Ajayan et al., 2015). Leong and Cheng (et al., 2020) also observed that algal biomass contains diverse active functional groups which provide enough number of binding sites for bio-adsorption of heavy metals thus, reduce their concentration from the wastewater. Therefore, the enhancement of different macromolecule may improve the metal removal efficiency of consortia 1. Furthermore, the decline in level of metal from wastewater after exposure to different light intensities and wastewater concentrations clearly indicated that higher light intensity could improve the metal remediation by enhancing the synthesis of different bio-molecules in consortia 1.

7.2 Growth responses and biomass production

The growth profile of selected consortia under different wastewater concentrations was recorded on alternate days. Microalgae consortia treated with 100% wastewater concentration exhibited maximum growth at 20 W/m^2 and 40 W/m^2 as compared to their respective control. The selected algal consortia treated with 25% wastewater faded after 10^{th} day (40 W/m^2) and 13^{th} day (20 W/m^2). The discolouration at 25% wastewater concentration could be ascribed to either light induced damage or accelerated nutrient assimilation at exponential phase leading to nutrient deficiency at later stages. At 50-100% wastewater, the continuous growth of consortia was observed upto 16 days in selected consortia under light intensities of 20 W/m^2 and 40 W/m^2 (Fig. 7.3). The results also exhibited that growth rate of 0.027 $day^{-1}$and 0.043 day^{-1} was observed with consortia 2 grown in BG-11 media under both light intensities. It was also observed that highest growth rate of 0.033 day^{-1} and 0.063 day^{-}

[1] was observed with consortia 1 and 2 at 20 W/m^2 and 40 W/m^2, respectively after treatment with 100% concentration of wastewater. Comparing both light intensities, it was observed that specific growth rate



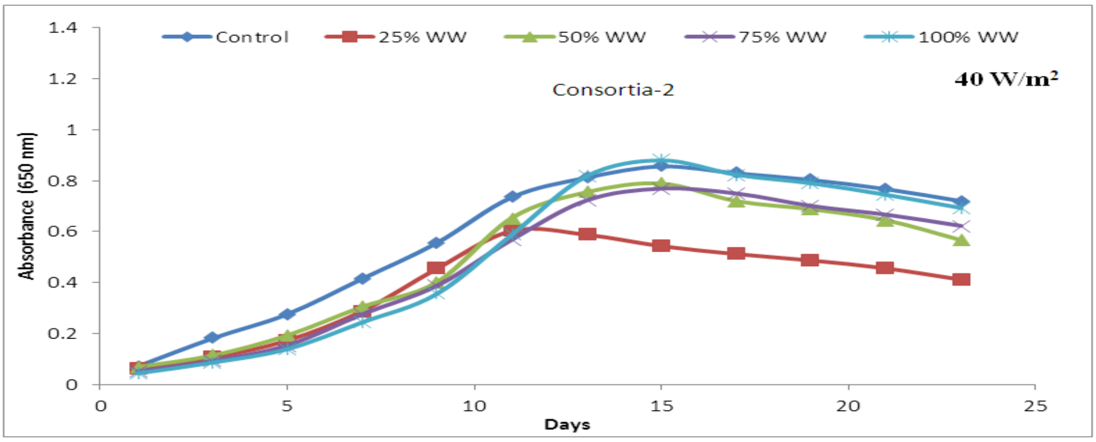


Fig. 7.3 Growth profile of consortia 1 and 2 treated with wastewater under different light intensities (20 W/m^2 and 40 W/m^2)

was highest at 40 W/m^2. The results also showed the maximum biomass production was observed with consortia 2 (2350 mg/L) at 40 W/m^2 while, under 20 W/m^2, the biomass production of 1010 mg/L was observed with consortia 1 grown in BG-11 media. At 20 W/m^2, the highest biomass production of 1340 mg/L and 1550 mg/L was observed at 25% and 50% in case of consortia 1 while 1450 mg/L and 1780 mg/L at 75% and 100% concentration of wastewater with consortia 2.

Table 7.2 Effect of light intensity on specific growth rate and biomass production of consortia treated with different wastewater concentration

Wastewater (WW) concentration	Specific growth rate (day^{-1})		Biomass Production (mg/L)	
	20 W/m^2	40 W/m^2	20 W/m^2	40 W/m^2
Consortia 1				
Control	0.0243±0.004[c]	0.0292±0.005[d]	1010±14.51[a]	1990±14.34[d]
25% WW	0.0029±0.004[a]	0.0324±0.007[e]	1340±16.34[b]	1700±16.23[c]
50% WW	0.0291±0.006[d]	0.0348±0.007[e]	1550±12.13[bc]	1860±23.12[cd]
75% WW	0.0159±0.009[b]	0.0431±0.009[f]	1420±12.78[b]	2250±14.13[e]
100% WW	0.0331±0.007[e]	0.0604±0.011[g]	1630±14.34[c]	3470±18.19[f]
Consortia 2				
Control	0.0277±0.007[c]	0.0437±0.005[d]	910±15.31[a]	2350±14.25[f]
25% WW	0.0006±0.0001[a]	0.0344±0.005[cd]	1110±18.19[b]	1510±15.78[c]
50% WW	0.0091±0.0004[b]	0.0447±0.008[d]	1280±11.34[b]	2080±17.45[e]
75% WW	0.0202±0.005[c]	0.0546±0.011[e]	1450±14.12[c]	2650±12.47[g]
100% WW	0.0306±0.008[c]	0.0639±0.013[f]	1780±16.23[d]	3020±12.98[h]

At 40 W/m^2, maximum biomass production of 1700 mg/L and 3470 mg/L was observed in case of consortia 1 at 25% and 100% wastewater concentration. However, consortia 2 showed highest biomass production at 50% (2080 mg/L) and 75% (2650 mg/L) of wastewater concentration (Table 7.2). Interestingly, the results revealed that the consortia 1 and 2 treated with municipal wastewater under two light intensities showed that 40 W/m^2 stimulated the growth rate and biomass yield of consortia 1 and 2 as compared to 20 W/m^2. The higher biomass production at 40 W/m^2 might be attributed to utilization of light energy by algal consortia for cell division and lipid/carbohydrates accumulation. The increase in biomass production with light intensity might be also due to absorption of excess light energy by the photosynthetic apparatus of microalgae (Metsoviti et al., 2019). Overall, the results concluded that the growth rate as well as biomass production of microalgae increased with light intensity and the same has been reported by George et al., (2014).

7.3 Effect of light intensity and wastewater on pigments and photosynthetic performance of consortia

The photosynthetic pigment in microalgae is essential parameter to examine the adaptation capacity, cellular viability as well as photosynthetic capacity under different light intensities. At 40 W/m^2, consortia 2 showed highest increase in total chlorophyll content at 25% (1.13 folds), 50% (2.00 folds), 75% (2.18 folds) while, 1.56 folds in consortia 1 treated with 100% concentration of wastewater. Similarly, at 20 W/m^2, the maximum increase of 1.75 and 2.50 folds was observed with consortia 2 at 25% and 50% while 3.71 and 4.00 folds in total chlorophyll content with consortia 1 at 75% and 100% concentration of wastewater (Table 7.3). The results also depicted that pigment content in consortia 1 and 2 increased considerably with wastewater concentrations and light intensities in comparison to control. The increased chlorophyll content indicated that wastewater and light intensity instead of interfering in PS-II functioning stimulated the photosynthetic rate and chlorophyll synthesis in consortia 1 and 2. The increased growth rate with light intensity and wastewater concentration may also be responsible for improved chlorophyll content of consortia. Gayathri and Rajasree, (2017) observed that changing light intensity bring considerable changes in the biochemical composition and chlorophyll content of microalgae. Previously, Bhatnagar et al. (2011) also reported that chlorophyll synthesis increased after growing microalgae in industrial and municipal wastewater. The other reason could be that the darker colour of wastewater encouraged chlorophyll synthesis as a strategy to improve light absorption (da Silva Ferreira and Sant' Anna, 2017). In addition, the increment in the pigment biosynthesis with

Table 7.3 Modification in biochemical parameters and oxidative stress markers in consortia 1 and 2 treated at light intensities of 20 W/m^2 and 40 W/m^2

WW Concent-rations	Algal consorti	Light intensity	Total chlorophyll (mg g^{-1} fw)	Carotenoids (mg g^{-1} fw)	Protein (mg g^{-1} fw)	Carbohydrate (mg L^{-1} fw)	Lipid (mg L^{-1} fw)	TBARS (µmol g^{-1}fw)	H_2O_2 (µmol g^{-1}fw)
Control	Consortia-1	20 W/m^2	0.007±0.0006^{a}	0.011±0.0011^{b}	0.0051±0.0001^{b}	9.94±0.581^{a}	0.114±0.0010^{b}	1.26±0.087^{a}	1.59±0.257^{a}
25%			0.011±0.0004^{ab}	0.0099±0.0002^{a}	0.0042±0.0004^{b}	19.02±1.74^{c}	0.075±0.0027^{a}	3.31±0.444^{c}	7.24±0.876^{e}
50%			0.015±0.0006^{c}	0.0183±0.0002^{bc}	0.0066±0.0002^{d}	21.69±2.89^{d}	0.113±0.0043^{b}	1.78±0.054^{ab}	2.93±0.378^{b}
75%			0.026±0.0007^{d}	0.0121±0.0003^{b}	0.0058±0.0005^{c}	32.76±4.03^{f}	0.134±0.0032^{bc}	1.33±0.077^{a}	4.84±0.144^{c}
100%			0.028±0.0009^{d}	0.0157±0.0007^{b}	0.0102±0.0007^{e}	21.42±2.12^{d}	0.139±0.0025^{bc}	2.98±0.093^{b}	6.79±0.343^{d}
Control	Consortia-2		0.008±0.0005^{a}	0.0094±0.0001^{a}	0.0032±0.0002^{a}	15.56±0.434^{b}	0.115±0.018^{b}	1.65±0.065^{ab}	1.72±0.234^{a}
25%			0.014±0.0007^{ab}	0.0096±0.0004^{a}	0.0025±0.0001^{a}	22.62±1.88^{d}	0.085±0.007^{a}	4.62±0.676^{d}	6.65±0.414^{d}
50%			0.020±0.0005^{c}	0.0096±0.0004^{a}	0.0047±0.0004^{b}	26.62±2.34^{e}	0.143±0.009^{d}	5.16±0.342^{e}	7.04±0.323^{e}
75%			0.017±0.0009^{c}	0.0106±0.0017^{b}	0.0050±0.0003^{b}	32.49±3.03^{f}	0.122±0.004^{b}	5.26±0.456^{e}	7.89±0.305^{ef}
100%			0.019±0.0004^{c}	0.0092±0.0008^{a}	0.0059±0.0006^{c}	31.82±2.56^{f}	0.131±0.019^{bc}	7.09±0.892^{f}	9.88±0.568^{g}
Control	Consortia-1	40 W/m^2	0.025±0.0002^{bc}	0.016±0.0027^{a}	0.0023±0.001^{a}	11.42±0.59^{a}	0.170±0.0010^{b}	1.72±0.273^{a}	2.94±0.151^{a}
25%			0.016±0.0001^{a}	0.010±0.0032^{a}	0.0032±0.003^{b}	28.76±5.62^{e}	0.161±0.0027^{b}	4.91±0.343^{b}	5.68±0.219^{c}
50%			0.031±0.0002^{d}	0.037±0.0012^{c}	0.0040±0.002^{c}	19.34±3.43^{bc}	0.290±0.0043^{e}	4.94±0.515^{b}	4.46±0.392^{b}
75%			0.039±0.0002^{de}	0.018±0.0021^{a}	0.0102±0.002^{f}	35.82±8.38^{f}	0.251±0.0032^{d}	6.01±0.727^{cd}	6.59±0.274^{d}
100%			0.050±0.0003^{e}	0.042±0.0028^{d}	0.0062±0.001	18.94±2.07^{bc}	0.203±0.0025^{c}	9.49±0.901^{e}	11.35±0.617^{g}
Control	Consortia-2		0.022±0.0002^{ab}	0.018±0.0010^{a}	0.0040±0.0001^{c}	14.2±2.41^{b}	0.153±0.0014^{a}	1.93±0.072^{a}	2.65±0.027^{a}
25%			0.025±0.0001^{bc}	0.025±0.0068^{b}	0.0047±0.0005^{c}	23.42±5.21^{d}	0.142±0.0015^{a}	4.82±0.126^{b}	9.53±1.03^{f}
50%			0.044±0.0001^{e}	0.042±0.0020^{d}	0.0071±0.0006^{e}	28.49±2.51^{e}	0.236±0.0029^{d}	5.12±0.345^{c}	7.56±0.413^{e}
75%			0.032±0.0008^{d}	0.036±0.0010^{c}	0.0059±0.0003^{d}	19.69±2.32^{bc}	0.316±0.0025^{f}	5.80±0.765^{c}	9.67±0.632^{f}
100%			0.048±0.0001^{e}	0.017±.0015^{a}	0.0048±0.0002^{c}	11.85±1.66^{a}	0.329±0.0055^{f}	11.34±0.555^{f}	18.05±0.885^{h}

wastewater concentration under low light intensity (20 W/m^2) conditions increases the light capturing potential which may be the response adopted by microalgae to compensate the photo-limitation. Therefore, the increased chlorophyll content seems a compensatory mechanism adopted by microalgae under light limited conditions to absorb and utilize light in efficient manner (Ferreira et al., 2016).

In order to gain more insight related to effect of different light intensities and wastewater concentration on photosynthetic health of consortia, different photosynthetic parameters such as Fv/Fo, Fv/Fm, Pi_{abs}, Mo, TRo/RC, ABS/RC and ET_O/RC were estimated. The photosynthetic performance of consortia 1 and 2 treated with 25-100% wastewater concentration was analyzed under different light intensities. At 40 W/m^2, the results revealed that Fv/Fo was increased by 20.45%, 21.06%, 13.04% and 19.21% with consortia 1 while 29.42%, 33.43%, 29.91% and 36.21% with consortia 2 at 25%, 50%, 75% and 100% concentration of wastewater, respectively. Similarly, F_v/F_m ratio showed an increase of 4.41%, 4.54%, 2.94, 4.27% and 6.43%, 7.26%, 6.57%, 7.67% with consortia 1 and 2 at different concentrations (25-100%) of wastewater, respectively. At 20 W/m^2, the results showed that ratio of Fv/Fo was increased with wastewater concentration gradient in the order of 53.31% (25%), 69.58% (50%), 61.45% (75%), 79.75% (100%) with consortia 1. In the case of consortia 2, it was found to be increased by 4.79% and 4.22% at 50% and 75% concentration of wastewater (Table 7.4). It was also observed that F_v/F_m ratio was in the order of 19.39%, 23.72%, 21.65% and 26.17% with consortia 1 at 25%, 50%, 75% and 100% concentration of wastewater while, an increase of 1.67% and 1.52% in F_v/F_m was observed with consortia 2 at 50% and 75% concentration of wastewater. F_v/F_m represents the quantum efficiency and increased value indicates that the activity of the PS II was improved at higher light intensity (40 W/m^2) as well as wastewater concentration gradient. Fv/Fo represents number of oxygen evolving reaction centres thus estimates the photosynthetic capacity of microalgae exposed to different light intensity (Vadiveloo et al., 2013). The increased Fv/Fo ratio with light intensity indicates negligible interference in electron transport chain and photosynthetic capacity of microalgae (Pereira et al., 2000). The results of photosynthetic fluorescence revealed that both Fv/Fm and Fv/Fo were higher in selected consortia exposed to 40 W/m^2 as compared to 20 W/m^2 signifying that higher light intensity (40 W/m^2) enhanced the photosynthetic performance of consortia 1. Further, the results exhibited that Mo was increased by 10.08% and 88.72% in consortia 1 and 2 at 25% concentration of wastewater under light intensity of 20 W/m^2. However, Mo value was decreased at 25% wastewater concentration with consortia 1. In the case of consortia 2, a significant increase of 43.60%, 26.31% and 39.09% was observed above 25% concentration of wastewater (Table 7.4). At 40 W/m^2, the results exhibited that Mo value was highest in control sample thereafter, the decline in Mo value was observed at different wastewater concentration. Mo represents closing rate of reaction centre and decreased value along wastewater concentration gradient confirms the higher photosynthetic efficiency of selected consortia under 40 W/m^2. Further, the Pi_{abs} value was increased along wastewater concentration gradient in consortia 1 by 57.39%, 118.46%, 150% and 195.8% while declining trend was observed with consortia 2 as compared to control at 20 W/m^2.

Table 7.4 Effect of light intensities (20 W/m^2 and 40 W/m^2) on the active photosystem II reaction centre (Fv/Fo), the quantum yield of photosystem II (Fv/Fm), net closing rate of the reaction center (Mo), photosynthetic performance index (Pi$_{abs}$), apparent antenna size (ABS/RC), trapping flux per reaction centre (TRo/RC) and electron transport per reaction centre (ET$_O$/RC) of consortia 1 and 2

Wastewater concentration	Algal consortia	Fv/Fo	Fv/Fm	Mo	Pi $_{abs}$	ABS/RC	TRo/RC	ETo/RC
Light intensity-20 W/m^2								
Control	**Consortia-1**	1.131^{a}	0.531^{a}	0.228^{de}	2.009^{a}	1.815^{e}	0.963^{d}	0.735^{c}
25%		1.734^{b}	0.634^{b}	0.251^{e}	3.162^{b}	1.417^{cd}	0.898^{c}	0.648^{b}
50%		1.918^{d}	0.657^{b}	0.218^{d}	4.389^{c}	1.371^{c}	0.901^{c}	0.683^{b}
75%		1.826^{c}	0.646^{b}	0.183^{c}	5.040^{d}	1.298^{bc}	0.839^{b}	0.655^{b}
100%		2.033^{e}	0.670^{bc}	0.177^{b}	5.944^{e}	1.159^{a}	0.777^{a}	0.600^{a}
Control	**Consortia-2**	1.919^{d}	0.657^{b}	0.133^{a}	7.990^{g}	1.313^{bc}	0.863^{b}	0.730^{c}
25%		1.817^{c}	0.645^{b}	0.251^{e}	3.386^{b}	1.425^{cd}	0.919^{c}	0.668^{b}
50%		2.011^{e}	0.668^{b}	0.191^{c}	5.469^{e}	1.274^{ab}	0.851^{b}	0.660^{b}
75%		2.000^{e}	0.667^{b}	0.168^{b}	6.390^{f}	1.313^{bc}	0.876^{b}	0.707^{c}
100%		1.877^{d}	0.652^{b}	0.185^{c}	5.085^{d}	1.226^{ab}	0.800^{ab}	0.615^{a}
Light intensity-40 W/m^2								
Control	**Consortia-1**	2.967^{ab}	0.748^{b}	0.097^{d}	19.998^{a}	1.006^{e}	0.753^{e}	0.656^{cd}
25%		3.574^{d}	0.781^{cd}	0.072^{b}	34.031^{b}	0.784^{a}	0.613^{a}	0.541^{a}
50%		3.592^{d}	0.782^{cd}	0.072^{b}	35.289^{b}	0.925^{cd}	0.723^{e}	0.651^{cd}
75%		3.354^{c}	0.770^{c}	0.060^{a}	38.761^{c}	0.817^{b}	0.629^{b}	0.569^{a}
100%		3.537^{d}	0.780^{cd}	0.064^{a}	38.785^{c}	0.856^{b}	0.667^{bc}	0.603^{b}
Control	**Consortia-2**	2.698^{a}	0.730^{a}	0.086^{c}	20.121^{a}	0.952^{d}	0.694^{d}	0.609^{b}
25%		3.492^{cd}	0.777^{cd}	0.068^{a}	35.647^{b}	0.857^{b}	0.666^{bc}	0.598^{b}
50%		3.600^{e}	0.783^{cde}	0.063^{a}	40.879^{d}	0.893^{bc}	0.699^{d}	0.636^{c}
75%		3.505^{cd}	0.778^{cd}	0.066^{a}	37.167^{c}	0.907^{c}	0.706^{d}	0.639^{c}
100%		3.675^{e}	0.786^{cde}	0.062^{a}	41.459^{d}	0.783^{a}	0.615^{a}	0.553^{a}

At 40 W/m^2, an increase of 70.17%, 76.46%, 93.82% and 93.94% in Pi_{abs} value was observed with consortia 1 while with consortia 2, 77.16%, 103.16%, 84.71% and 106.04% at 25%, 50%, 75% and 100% concentration of wastewater (Table 7.4). Pi_{abs} is measure of cell vitality (Gururani et al., 2018) and the increased Pi_{abs} value with light intensity to that of control in the present study reflects healthy algal cells. The increased Pi_{abs} also signifies high photosynthetic performance (Negi et al., 2016) and efficient tolerance against metal pollutants. Thus, in present study, the increased Pi_{abs} value with wastewater concentrations and light intensity is prime indicators of better cell vitality, electron transport as well as photosynthetic efficiency of consortia 1 than consortia 2. In case of consortia 1, the ABS/RC, TRo/RC and ETo/RC ratio was observed to decrease significantly with different wastewater concentrations under 20 W/m^2 and 40 W/m^2. In consortia 2, it was observed that the ratio of TRo/RC increased at 25% (1.06 folds) and 75% (1.01 folds) concentration of wastewater after treatment at 20 W/m^2. At 40 W/m^2, the results also showed that ETo/RC was decreased significantly in consortia 1 while with consortia 2, an increase of 1.04 and 1.04 folds was observed at 50% and 75% concentration of wastewater. The decreased ABS/RC reflects the activation of reaction centre (Strasser et al., 2004) which stimulates photophosphorylation and eventually leads to enhanced cell growth and biomass production. The ABS/RC represents the antenna size and it was found to decrease in both consortia at different wastewater concentration than control. Previously, it has been reported that stress condition increases the antenna size (Dao et al., 2016) but present study showed decreased antenna size which might be attributed to competent adaptability of selected consortia under different light and wastewater conditions. In present study, the decreased TRo/RC value in consortia 1 treated with different wastewater concentration and light intensity may be due to presence of large number of reaction centres that promotes the transport of electron and Q_A reduction (Markou et al., 2017). Similarly, the ratio of ETo/RC was also decreased with wastewater which may be due to abundance of active RC and excellent photosynthetic efficiency of selected algal consortia (Zhao et al., 2017).

As per the different photosynthetic parameters, the results revealed that consortia 1 was more adaptable to low as well as high light intensity while consortia 2 showed good adaptability at higher light intensity. Thus, overall results suggested that consortia 1 could be exploited for mass cultivation under different light intensities.

7.3.1 Carotenoid content

The carotenoid content in selected consortia was increased with wastewater concentration and light intensity as compared to control. At 40 W/m^2, consortia 1 showed highest increase of 2.62 folds in carotenoid content at 100% wastewater while, in consortia 2, the maximum increase (2.31 folds) was found at 50% concentration of wastewater. The carotenoid content was also increased in consortia 1 (1.12 folds) and consortia 2 (2.00 folds) at 75% concentration of wastewater. At 20 W/m^2, consortia 1 showed an increase of 1.66, 1.10 and 1.42 folds in carotenoid content at 50%, 75% and 100% while, an increase of 1.02, 1.02 and 1.12 folds in consortia 2 treated with 25%, 50% and 100% of wastewater concentration (Table 7.3)**.** The enhancement in carotenoid accumulation with wastewater and light intensity might be accredited to their involvement in protection of photosynthetic machinery from oxidative damage. Earlier, it has been reported that under stress condition, carotenoid prevent oxidative damage by acting as precursor of signaling molecule, scavenge free radicals and hamper formation of triplet chlorophyll (Sharma et al., 2012; Coulombier et al., 2021). Apart from anti-oxidative role, carotenoid contributes in trapping of excess light energy under high light intensity conditions (Singh et al., 2010). Increased carotenoid content may also be attributed to their role in protection of PS II against photo-inhibition (Solovchenko et al., 2008). The increased accumulation of carotenoids may regulate factors responsible for proper growth, cell cycle, as well as cell signaling (Fiedor and Burda, 2014). Thus, enhancement of carotenoid under wastewater concentration and light intensity in this study could be a palliative measure adopted by algal consortia to mitigate the light and wastewater induced oxidative damage.

7.4 Effect of municipal wastewater concentrations and light intensities on protein, lipid and carbohydrate content of algal consortia

The intensity of light strongly influences the cell growth, pigment biosynthesis and composition of the algal biomass. At 40 W/m^2, the protein content was increased in consortia 1 (1.39 folds) and consortia 2 (1.17 folds) at 25% of wastewater concentration. The highest increase of 1.77 folds in consortia 1 was found to be at 50% wastewater concentration while, 4.43 folds in case of consortia 2 at 75% wastewater concentration (Table 7.3). The protein content was also increased in consortia 1 (2.69 folds) and consortia 2 (1.20 folds) at 100% concentration of wastewater than respective control. At 20 W/m^2, the protein content in consortia 1 (2.00 folds) and consortia 2 (1.84 folds) was increased at 100% of wastewater concentration. The results also showed an increase of 1.29 and 1.13 folds in protein content in consortia 1 while 1.46 and 1.56 folds in consortia 2 at 50% and 75% of wastewater concentration. Furthermore, protein content was decreased in both consortia at 25% in comparison to control (Table 7.3). However, the increased protein content observed in selected consortia above 25% wastewater concentration may be either ascribed to stimulation of protein synthesizing machinery or synthesis of stress/chelating protein to avoid damage to cellular components. Ghosh and Xu, (2014) also reported that enhanced protein synthesis under the stress condition not only improves the tolerance towards the abiotic stress but also sustain metabolic processes in the cell. Therefore, in the present study, the enhancement in protein synthesis may be the stress mitigation strategy adopted by algal consortia under different light intensities and wastewater concentrations.

At 40 W/m^2, consortia 1 showed that the maximum increase of 3.13 folds in carbohydrate content at 75% concentration of wastewater. Moreover, consortia 1 also showed an increase of 2.51 and 1.69 folds while, in consortia 2, it was increased by 1.64 and 2.00 folds at 25% and 50% wastewater concentration, respectively. The results also depicted an increase of 1.65 folds in carbohydrate content after treatment of 100% wastewater concentration to consortia 1 as compared to control. At 20 W/m^2, the carbohydrate content in case of consortia 1 was increased by 1.91, 2.18, 3.29 and 2.15 folds at 25%, 50%, 75% and 100% wastewater concentration. The results also showed that carbohydrate content in consortia 2 was increased at 25% (1.45 folds), 50% (1.71 folds), 75% (2.08 folds) and 100% (2.04 folds) of wastewater concentration. The increased carbohydrate accumulation could be attributed to elevated cell proliferation, photosynthetic efficiency as well as the osmotic balance of the cell (Sami et al., 2016). Therefore, the increased biomass production, carbohydrate, chlorophyll and protein content may be ascribed to efficient utilization of light and wastewater for prolific growth and macromolecules synthesis by selected consortia.

Lipids perform metabolic activities inside the cell by maintaining the structure and functions of cell membrane. At light intensity of 40 W/m^2, consortia 1 and 2 showed an increase of 2.15 and 1.19 folds in lipid content at 100% concentration of wastewater. At 50% and 75% wastewater concentration, an increase of 1.54 and 2.06 folds was observed in consortia 1 while 1.70 and 1.47 folds in case of consortia 2. At 20 W/m^2, consortia 1 showed an increase of 1.17 and 1.06 folds while 1.21 and 1.13 folds in consortia 2 at 75% and 100% wastewater concentration. At 50% wastewater, the maximum enhancement of 1.24 folds was observed in consortia 2 (Table 7.3). The results also exhibited selected algal consortia 1 and 2 treated with 25% wastewater concentration showed decline in lipid accumulation at both light intensities in comparison to untreated consortia**.** Overall, the selected consortia after treatment with 50%-100% wastewater concentration under 20 W/m^2 and 40 W/m^2 showed elevated lipid accumulation than untreated consortia. The improvement could be attributed to nutrient exhausted conditions favoring the accumulation of lipid in microalgae (Shen et al., 2015). In present study, elevated lipid accumulation under higher light intensity as compared to low light intensity could be the mechanism adopted by microalgae to protect the cellular components from degradation. He et al., (2015) also observed that higher light intensity may affect the photosynthetic rate, anabolic reactions and diverts the photosynthetically fixed carbon and energy towards lipid biosynthesis. Under high light intensity, the increased lipid accumulation and fatty acid synthesis may also be attributed to increased activity of enzymes such as acetyl-CoA carboxylase, desaturase, ATP citrate lyase, and glucose permease (Arroyo et al., 2011; Gim et al., 2014). Furthermore, the elevated synthesis of lipid in consortia 1 and 2 was also shown by FTIR spectroscopy. The FTIR absorption spectra depicted that consortia 1 and 2 treated with 100% wastewater concentration at 20 W/m^2 and 40 W/m^2 showed higher lipid peak compared to respective control as evident by the occurrence of absorption peak between 3000-2800 cm^{-1} and 1750 cm^{-1} (Upadhyay et al., 2021). The results exhibited that consortia 1 treated with 100% wastewater concentration under light intensity of 20 W/m^2 and 40 W/m^2 showed spectral peak between 1741-1739 cm^{-1} which could be directly linked with accumulation of neutral lipid. The FTIR spectroscopy also showed the spectral peaks at 2924.2 cm^{-1} and 2856.4 cm^{-1} in consortia 1 while 2925.0 cm^{-1} and 2859.6 cm^{-1} in case of consortia 2, respectively at 20 W/m^2 (Fig. 7.2). Similarly, at 40 W/m^2, consortia 1 and 2 showed the intense peaks between 3000-2800 cm^{-1} after treatment with 100% wastewater concentration. Earlier, reports revealed that absorption peaks between 3000-2800 cm^{-1} represent "lipid band spectra" which may arise due to C-H group of methylene (Sudhakar and Premalatha, 2015). Furthermore, the absorption peaks between 1700-1500 cm^{-1} represents the bending of C-N, C=O, N-H bond of proteins and the peaks were more prominent in wastewater treated consortia. It was observed that the spectral peak between 1200-900 cm^{-1} were higher in treated consortia 1 and 2 which reflected that both light intensities induced carbohydrate accumulation. The results also showed that 40 W/m^2 induced higher carbohydrate accumulation in wastewater treated consortia 1 and 2 as compared to 20 W/m^2. Overall, the results reflected that both light intensities (20 W/m^2 and 40 W/m^2) enhanced the lipid accumulation in consortia 1 after treatment with 100% of wastewater concentration. Therefore, the enhancement in the synthesis of different bio-molecules may be ascribed to cellular modifications adopted by algal consortia to adjust under different light intensities and wastewater concentrations.

7.5 FAME analysis

The composition and percentage of fatty acid in algal biomass is the key factor that affects the properties of the biofuel (He et al., 2015). The compositions of fatty acid were analyzed to determine the biodiesel production potential of consortia 1 and 2 treated with municipal wastewater (100%) under different light intensities (20 W/m^2 and 40 W/m^2). In this study, consortia 1 treated with wastewater (100%) showed the highest content of C16:0 (26.89%) and C18:2 (12.78%) at 40 W/m^2 while 18:0 (7.24%), C18:1 (16.04%), and C18:3 (12.80%) at 20 W/m^2. In case of consortia 2 treated with 100% of wastewater concentration, C16:0 (26.72%) and C18:0 (6.86%) were observed in maximum amount at 20 W/m^2 while C18:1 (14.45%), C18:2 (12.89%) and C18:3 (14.79%) were found to be highest at 40 W/m^2 (Fig. 7.4). The fatty acids containing C-16 and C-18 carbon atoms reflect the good quality biodiesel (Knothe, 2009). Kumar et al., (2019) observed that considerable increase in the fatty acid such as C16:0 and C18:1 indicated that higher light intensity improved the quality of biofuel. In the present study, both light intensities (20 W/m^2 and 40 W/m^2) and wastewater (100%) improved the percentage of important fatty acid in terms of biofuel production. Singh et al., (2015) further suggested that the C18:3 content should not be above the specified limits (12%). However, in the present study, the C18:3 (10.52%) was below the specified value in consortia 2 treated with 100% wastewater concentration at 20 W/m^2 thus, can serve as the suitable feedstock for biodiesel production. The results also showed that saturated fatty acid content was highest (42.30%) in consortia 1 whereas lowest was observed in case of consortia 2 (37.36%) treated under light intensity of 40 W/m^2. Furthermore, in case of consortia 1, the maximum monounsaturated (MUFA-26.60%) and polyunsaturated fatty acid (PUFA-26.93%) content was observed at 20 W/m^2 whereas with consortia 2, the highest MUFA (25.98%) and PUFA content (29.45%) was at 40 W/m^2.

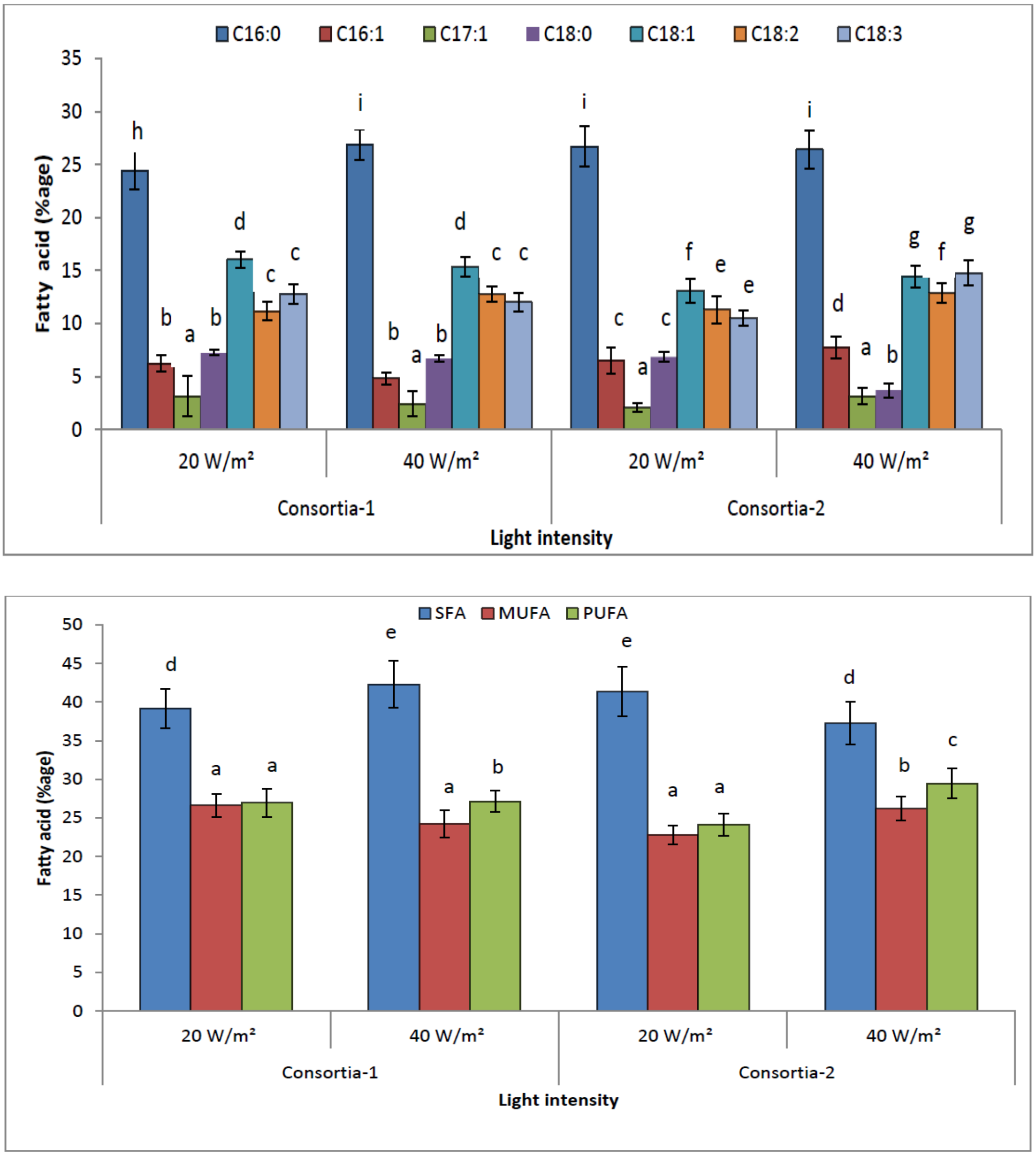


Fig. 7.4 Fatty acid profiling of post harvested biomass of consortia 1 and 2 treated under light intensity of 20 W/m^2 and 40 W/m^2

The considerable percentage of MUFA is advantageous as it increases the oxidative stability, cold flow and combustion efficiency of biodiesel (He et al., 2015). Overall, the results revealed that light intensity of 40 W/m^2 and wastewater concentration (100%) enhanced the synthesis of important fatty acid in consortia 1 while 20 W/m^2 in case of consortia 2. Thus, it can be concluded that consortia 1 grown under different light intensities and wastewater concentration (100%) can certainly improves the qualities of biofuel.

7.6 Oxidative stress markers: thiobarbituric acid reactive substances (TBARS) and hydrogen peroxide (H_2O_2)

Microalgae trigger certain physiological processes to adapt under the fluctuating environmental conditions. At 40 W/m^2, highest increase of 5.51 and 6.39 folds in TBARS content was found to be with consortia 1 and 2, at 100% wastewater concentration. At 25%, 50% and 75% concentration of wastewater, consortia 1 showed an increase of 2.85, 2.87 and 3.49 folds in TBARS content while an increase of 2.49, 2.65, and 3.00 folds was found in case of consortia 2 than control. At 20 W/m^2, consortia 1 and 2 showed an increase of 2.62 and 2.80 folds in TBARS content at 25% while, 1.41 and 3.12 folds at 50% wastewater concentration. The results showed an increase of 1.05 and 3.18 folds in TBARS content in consortia 1 and 2 at 75% concentration of wastewater. Similarly, TBARS content in algal consortia 1 and 2 was increased by 2.36 and 4.29 folds at 100% wastewater concentration. Furthermore, this study clearly reflected that 40 W/m^2 along with wastewater enhanced the TBARS content in consortia 2 than consortia 1.

At 40 W/m^2, hydrogen peroxide (H_2O_2) content was increased by 3.86 and 5.73 folds in consortia 1 and 2 treated with 100% of wastewater concentration. The results also depicted that consortia 2 showed highest increase in H_2O_2 content at 25% (3.02 folds), 50% (2.40 folds) and 75% (3.06 folds) concentration of wastewater. In case of consortia 1, the maximum increase was observed at 25% (1.93 folds), 50% (1.51 folds) and 75% (2.24 folds) of municipal wastewater concentration (Table 7.3). Similarly, at 20 W/m^2, H_2O_2 content was found to increase by 3.86, 4.09, 4.58 and 5.74 folds in consortia 2 while, in consortia 1, it was increased by 4.55, 1.84, 3.04, and 4.27 folds with wastewater concentration ranging from 25-100%, respectively. Further, the results concluded that both TBARS and H_2O_2 content exhibited maximum increase at 100% wastewater and light intensity of 40 W/m^2 which reflects the lipid peroxidation and elevated ROS generation in consortia 2. Wastewater enriched with different heavy metals poses oxidative damage by inhibiting photosynthesis, increase acidity of cytoplasm and disturb cellular homeostasis (Gauthier et al., 2020). Chaula et al., (2019) also reported that increased TBARS accumulation is widely used as the index of lipid peroxidation and cellular toxicity. Light intensity may elevate the ROS generation by affecting the reaction centres of PSII resulting in alteration of growth rate, cell physiology and biochemical activity of microalgae (Zhang et al., 2017). The selected consortia 1 and 2 cultivated under higher light intensity absorb excess energy that is either transferred to electron acceptors or dissipated in the form of heat which may lead to ROS generation in the cell. Further, excess ROS accumulation may disintegrate and destabilizes cell membrane by reacting with polyunsaturated fatty acids and induce chain reaction in the presence of hydroperoxides as well as intermediate products of lipid peroxidation

(Melegari et al., 2012). Overall, the results showed that the consortia 2 were more sensitive to oxidative damage as compared to consortia 1. Thus, due to the less TBARS and H_2O_2 content after treatment with wastewater and light intensity, consortia 1 could be employed to remediate wastewater under different environmental conditions.

7.7 Effect of light intensity and wastewater on antioxidant activity of algal consortia

7.7.1 Non-enzymatic antioxidants

Under stressful environment, microalgae produce array of antioxidants to offset the oxidative damage induced by different reactive oxygen species (ROS) to the microalgae. At 40 W/m^2, the highest increase in ascorbic acid was found in consortia 1 (1.30 folds) and consortia 2 (1.14 folds) at 50% and 75% wastewater concentration, respectively. In case of consortia 2, it was enhanced by 1.04 folds while decline in ascorbic acid was found at 25% concentration of wastewater in case of consortia 1. Moreover, consortia 1 also showed the highest increase of 1.15 folds in ascorbic acid at 100% wastewater concentration. The results showed the maximum increase of cysteine content in consortia 1 at 25% (1.68 folds), 50% (2.00 folds), 75% (2.80 folds) and 100% (2.11 folds) concentration of wastewater. In case of consortia 2, an increase of 1.09, 1.30, 1.67 and 2.09 folds in cysteine content was observed at different concentration of municipal wastewater. The results also depicted an increase of 1.04, 1.27, 1.20 and 1.32 folds in proline content in consortia 1 while 1.26, 1.47, 1.59 and 1.64 folds in consortia 2 at 25%, 50%, 75% and 100% concentration of wastewater (Fig 7.5).

At 20 W/m^2, consortia 2 showed maximum increment of ascorbic acid at 50% (1.00 folds), 75% (1.13 folds) and 100% (1.23 folds) of wastewater concentration. It was also observed that ascorbic acid content was increased by 1.03 and 1.16 folds in algal consortia 1 at 75% and 100% of municipal wastewater concentration. Furthermore, cysteine content in consortia 1 was increased by 1.30-1.44 folds while 1.55-2.38 folds in consortia 2 at 25-100% concentration of municipal wastewater. The results also exhibited an increase of 1.19 and 1.19 folds in proline content in algal consortia 1 and 2 treated at 100% wastewater concentration. Comparing different wastewater concentrations, the results exhibited that proline content decreased significantly upto 75% concentration of wastewater than control. Overall, consortia exposed to 20 W/m^2 and 40 W/m^2 showed differential responses towards different concentrations of wastewater (25-100%).

In the present investigation, the increased ascorbic acid accumulation may be responsible for growth, biosynthesis of hormones, photosynthetic process and acclimatization to unfavorable environmental conditions (Bartoli et al., 2017). Ascorbic acid may also assist in photo-protection by directly serving as antioxidants, sequester ROS, or as catalyst for enzymes of xanthophyll cycle which dissipate the excess light energy to prevent photo-inhibition (Bartoli et al., 2017). Therefore, accumulation of ascorbic acid under different light intensity may play indispensible role in photo-protection and against photo-oxidative damage. Similarly, proline protects enzymes, maintain osmo-regulation, stabilize synthesis of proteins and scavenge ROS, thereby encourage microalgae to adjust the intracellular mechanism under stressful environment (Liang et al., 2013).

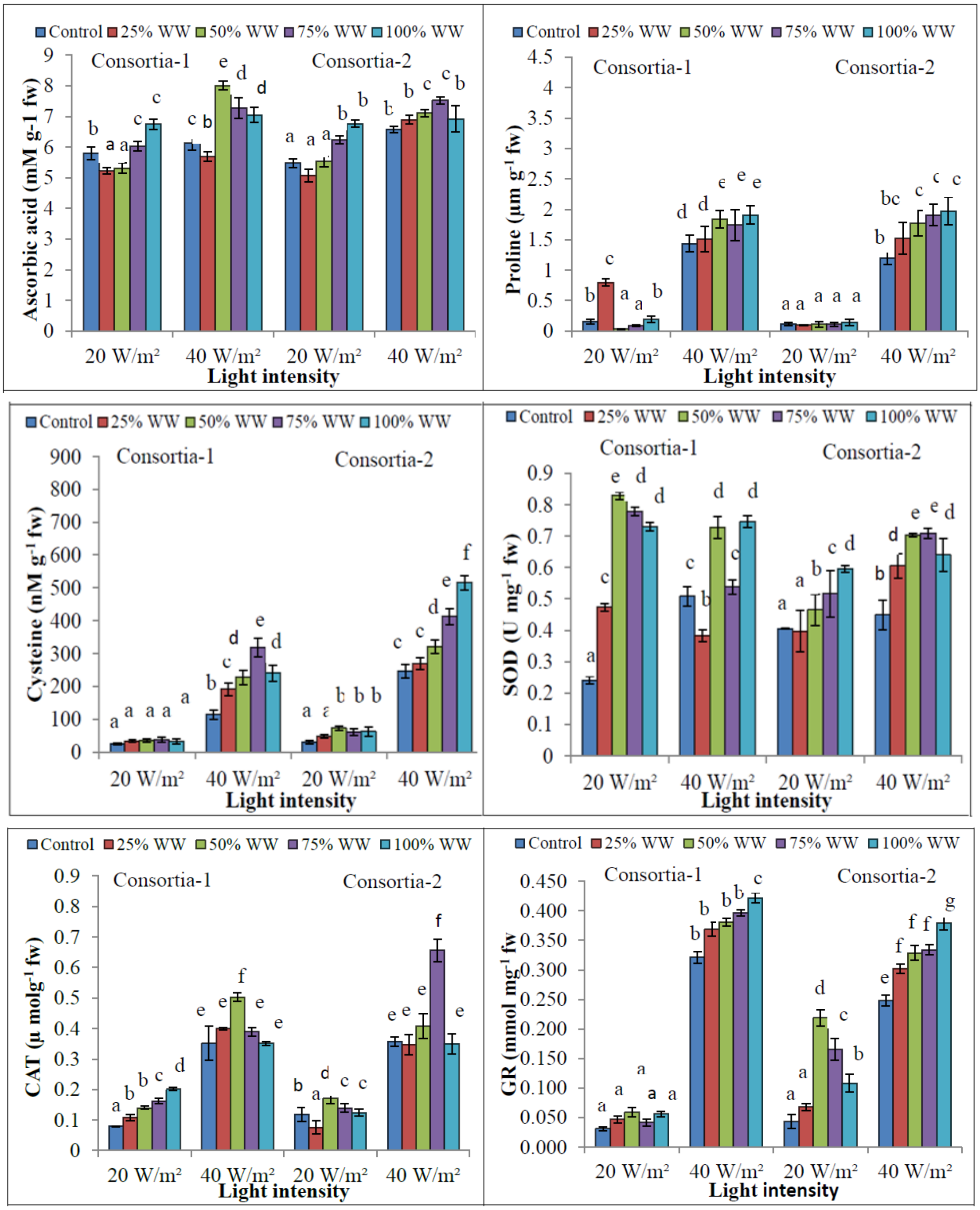


Fig. 7.5 Alteration in non-enzymatic and enzymatic antioxidants in consortia 1 and 2 treated with different wastewater concentrations (25-100%) and light intensities (20 W/m^2, 40 W/m^2)

The increased production of proline maintains homeostasis; regulate osmotic as well as redox balance and mitigate the oxidative damage posed by different environmental conditions (Ghosh et al., 2022). Moreover, the increased proline accumulation with wastewater and light intensity may be attributed to their active role in metal detoxification as well as antioxidant and signaling molecule inside the cell (Ghosh *et al.,* 2022). Cysteine is also essential component of several proteins and acts as the precursor of antioxidants and defense compounds that ensure survival under unfavorable environmental conditions (Álvarez et al., 2012). The increased cysteine content with wastewater and light intensity may be important for proper functioning of chloroplast and play vital role in survival under long term exposure of light (Speiser et al., 2015). Furthermore, superior antioxidant potential in response to different light intensity and wastewater conditions suggested that both can be the effective external factor to modulate the defense responses in selected consortia.

7.7.2 Enzymatic antioxidants

In order to understand the ability of microalgae to resist the oxidative stress under light stress, the activity of the ROS scavenging antioxidants such as SOD, CAT and GR were examined in algal consortia 1 and 2. At 40 W/m^2, consortia 2 showed an increase of 1.34 folds in SOD activity at 25% concentration of wastewater. The results further depicted that SOD activity increased by 1.43, 1.05 and 1.46 folds in consortia 1 while 1.56, 1.57, and 1.42 folds in consortia 2 treated with 50%, 75% and 100% concentration of wastewater, respectively. It was also observed that catalase activity was increased by 1.13, 1.43, 1.11 and 1.00 folds in consortia 1 at 25-100% wastewater concentration. In case of consortia 2, maximum increase of 1.14 and 1.83 in catalase activity was found to be at 50% and 75% of wastewater concentration. The results also exhibited that consortia 2 showed decline in catalase activity at 25% and 100% concentration of wastewater. Further, consortia 1 showed increased GR activity at 100% (1.31 folds), followed by 75% (1.21), 50% (1.18 folds) and 25% (1.14 folds) wastewater concentration. Similarly, consortia 2 showed an increase of 1.23, 1.32, 1.34, and 1.52 folds at 25%, 50%, 75% and 100% concentration of wastewater (Fig. 7.5). At 20 W/m^2, the results depicted an increase of 1.96, 3.44, 3.23 and 3.02 folds in SOD activity in consortia 1 at different concentrations of municipal wastewater (25-100%). Similarly, the consortia 2 showed an increase of 1.14, 1.47 and 1.27 folds in SOD activity at 50%, 75% and 100% concentration of wastewater. The results also showed an increase of 1.35, 1.75, 2.02 and 2.52 folds in catalase activity with consortia 1 at 25%, 50%, 75%, and 100% wastewater concentration while, with consortia 2, an increase of 1.43, 1.17 and 1.04 folds was observed at 50%, 75% and 100% wastewater concentration. The results also depicted the an increase of 1.51, 1.90, 1.35 and 1.80 folds in GR activity in consortia 1 while 1.58, 5.09, 3.83 and 2.51 folds in case of consortia 2 at 25%, 50% 75% and 100% of municipal wastewater, respectively.

Consortia exposed to varying light intensity may accelerate ROS generation through production of excessive electrons in electron transport chain (He et al., 2015). Milne et al., (2009) reported that exposure to higher light intensity elevates the superoxide and peroxide generation and the appropriate activity of SOD is necessary to reduce toxicity in microalgae (Deng et al., 2017). He et al., (2015) observed the 3-5 folds increase in the activity of SOD under high light than low light indicating their involvement in the scavenging of light induced oxidative radicals. Therefore, in present study, the increased activity of SOD after treatment with wastewater under different light intensity in microalgae consortia ensure protection by scavenging of the superoxide radical and alleviate oxidative damage. Catalase has high hydrogen peroxide specificity and degrades hydrogen peroxide in energy efficient manner as compared to other antioxidants. Catalase eliminates hydrogen peroxide through photo-respiratory oxidation, β-oxidation of fatty acids, and also with the assistance of other enzyme systems (Corpas et al., 2008). He et al., (2015) reported the increased catalase activity by 2.43- 2.53 folds in microalgae grown under high light than low light. Similarly, in present study, the significant increase in catalase activity observed under low as well as high light intensity could assist the selected algal consortia to neutralize the hydrogen peroxide concentration from the cell. Thus, enhancement in the activity of enzymatic antioxidants such as SOD and catalase at both light intensities may maintain the integrity of cellular structures and prevents lipid peroxidation by neutralizing the superoxide, hydro-peroxide and H_2O_2 level from the cell. Further, in present study, the increased activity of glutathione reductase (GR) with wastewater concentration and light intensity may ensure cell survival and increase tolerance towards photoxidative damage by modulation of redox potential of glutathione (Lin et al., 2018). GR imparts tolerance against stress conditions by maintaining the level of glutathione and sulfhydryl group containing compounds in the cell (Yousuf et al., 2012). Finally, the present investigation suggested that defense mechanisms in the form of different antioxidants play key role in sustaining growth of algal consortia by scavenging the excessive ROS and alleviate photo-inhibition under light stress (Zhang et al., 2017). Therefore, under different light intensity and wastewater concentrations, modification in the activity of antioxidants leads to great plasticity which makes algal consortia one of the superior and tolerant organisms for bio-removal of toxic metals with concurrent increment in biomass and lipid production.

Chapter 8

Effect of different temperature regimes on removal efficiency, photosynthetic parameters and fatty acid profile of algal consortia

Chapter-8

Effect of different temperature regimes on removal efficiency, photosynthetic parameters and fatty acid profile of algal consortia

8.0 Introduction

Inadequate sanitation deteriorates environmental health and restricts the economic growth of the country (WHO & UNICEF, 2010). The clean environment is necessary for living organisms and healthy environment requires 50-80% reduction of carbon dioxide from the atmosphere and 50% enhancement in the water and energy resource availability (Ng et al., 2017). The 2030 Agenda has identified improvement in water quality as important goal for sustainable development of different member countries (Lutzu et al., 2020). As per the statistical report of 2014, 3700 billion m^3 of the freshwater was consumed globally for different human activities and majority of water is disposed as wastewater (Diniz et al., 2017). Wastewater frequently contains inorganic and organic compounds which can directly pose serious problems to aquatic organisms and indirectly deteriorate human health by interacting with food chain. Wastewater enriched with diverse contaminants adversely influence the water quality parameters and makes it unfit for different organisms (Lutzu et al., 2020). Several wastewater treatment techniques have been adopted but drawbacks associated with such system have restricted their use for wastewater remediation.

Due to environmental-friendliness, bioremediation through bacteria, plants, microalgae, and fungi seems viable alternative over conventional treatment methods (Leong and Chang, 2020). Microalgae have exceptional characteristics such as small size, large surface area, high photosynthetic efficiency and ability to grow under variety of stressful conditions as compared to plants, bacteria and fungi (Cameron et al., 2018). Different environmental factors such as light intensity, light duration and temperature during affects the remediation efficiency. Additionally, such external factors greatly influence the growth profile, lipid and biomass yield of microalgae (Saranya and Shanthakumar, 2020).

Among different stimuli, temperature is an important external variable that influence the algal growth by affecting the rate of chemical reactions, diffusion through cell membrane, electron transport chain as well as activity of enzymes (Nogueira et al., 2015). Temperature strongly influences the metabolic activities, macromolecular composition, photosynthetic process as well as absorption and consumption of energy (Ferro et al., 2018). Microalgae have ability to adapt under wide range of temperature however; the response to stress conditions depends upon their origin (de Boer et al., 2005). An optimum temperature of 15–25° C for prolific growth has been observed in different species of microalgae (Singh and Singh, 2015). Ma et al., (2017) observed that lipid productivity of microalgae was maximum at room temperature (25° C) as compared to lower temperature (4° C, 10° C, and 15° C).

The continuous rise in temperature and the future prediction of further enhancement in global temperature, it become critically important to understand the responses adopted by different organisms against heat stress (Légeret et al. 2016). Algae may possess several adaptive and acclimation mechanism to survive under the high temperature stress. The tolerance mechanism involves different ion transporters, compatible solutes, heat shock proteins, antioxidants and several other factors responsible for signaling cascades and control of transcription are stimulated to mitigate the heat stress induced biochemical as well as physiological alterations (Hasanuzzaman et al., 2013). Fluctuation in temperature occurs more frequently under natural environment and mixing different microalgae species may address the problem by acting as the rigid system under different environmental conditions. The flexibility of algal consortia to adapt under different temperature conditions can represent the long term solution for future large scale outdoor cultivation. Thus, screening algal consortia that withstand wide temperature ranges can play key role in the large scale outdoor cultivation. The present investigation relied on the hypothesis that increased temperature may accelerate the remediation efficiency along with lipid and biomass synthesis. The study was expected to provide insight related to biochemical alteration and defense responses of algal consortia in response to climate change (Xing et al., 2022). Therefore, the effect of different temperature regimes on algal physiology, biomass composition, lipid production, antioxidants defense system and biofuel production was analyzed along with growth and chlorophyll fluorescence as the prime indicators of the tolerant and productive microalgae. Thus, the fundamental studies on removal efficiency, biomass production, chlorophyll fluorescence, defense responses and biofuel production will be realistic approach for screening of the algal species adaptable to different temperature regimes.

8.1 Results and discussion

8.1.1 Physico-chemical attributes of treated wastewater

At 20˚ C, it was observed that pH of wastewater increased by 15.64% and 20.00% with consortia 1 and 2 at 25% concentration of wastewater. At 50% and 75% wastewater concentration, an increase of 18.62% and 19.32% in pH was found to be with consortia 1 while, 28.55% and 24.34% with consortia 2 respectively. The pH value was also increased with consortia 1 (24.86%) and consortia 2 (23.02%) at 100% concentration of municipal wastewater. At 40˚ C, the results showed the highest increase of 17.76% and 24.73% in pH with consortia 1 and 2 at 50% concentration of wastewater. The result also showed the lowest increase in pH with consortia 1 (5.26%) and consortia 2 (7.89%) at 100% concentration of municipal wastewater (Table 8.1). The increment in pH value could be attributed to intensive algal growth resulting in recovering of dissolved inorganic carbon from wastewater (Delgadillo-Mirquez et al., 2016). Furthermore, the alkaline pH value of wastewater after treatment may be ascribed to the utilization carbon dioxide, carbonate and bicarbonates from wastewater by algal consortia (Iasimone et al., 2018).

At 20˚ C, highest decrease in EC (87.38%), TSS (86.33%), TDS (90.55%) and TS (88.57%) was shown by consortia 2 at 25% of municipal wastewater. The results also depicted that consortia 1 (64.06-79.13%) showed the maximum removal efficiency for EC, TDS, TSS and TS at 50% and 75% wastewater concentration as compared to consortia 2 (58.52-78.07%). At 100%, consortia 1 showed the removal efficiency of 61.21%, 53.09%, 74.17% and 57.30% whereas consortia 2 of 53.50%, 46.36%, 71.71% and 50.21% for EC, TDS, TSS and TS, respectively. Similarly, the level of BOD with algal consortia 1 was observed to decrease by 85.23%, 73.38%, 70.71% and 64.75% while 84.22%, 72.85%, 67.76%, and 60.00% with algal consortia 2 at different municipal wastewater concentrations (25%-100%). At 40˚ C, the highest decline of 87.73% and 89.07% for EC and TSS was observed with consortia 1 while 91.18% and 89.20% for TDS and TS with consortia 2 treated with 25% concentration of wastewater. Further, consortia 1 showed maximum removal efficiency of 78.62%, 82.20%, 83.95% and 83.33% for EC, TDS, TSS and TS at 50% wastewater concentration while 57.12%, 67.96% and 63.46% for EC, TDS and TS at 75% concentration of wastewater, respectively. At 100% wastewater concentration, the highest reduction of 50.11%, 48.98% and 49.33% for EC, TDS, and TS was also observed with consortia 1. In case of BOD, the decline of 88.92%, 78.42%, 67.02%

and 52.22% while 86.99%, 78.80%, 60.98% and 47.17% was observed with consortia 1 and 2 at different wastewater concentrations (Table 8.1). In this study, the removal of nutrients by selected consortia from wastewater could be responsible for decreased EC, TDS and BOD level of wastewater. Balaji et al., (2016a) also observed that due to the presence of several reactive groups on cell surface, microalgae form complexes with nutrients and pollutants leading to flocculation which eventually results in the decline of dissolved solids and suspended solid from wastewater. At 20˚ C and 40˚ C, the selected consortia showed highest DO level at 25% with subsequent decline at higher wastewater concentrations. At 40˚ C, the highest increase in DO was observed upto 50% concentration of wastewater with selected algal consortia and thereafter the significant decline in DO level was at 40˚ C as compared to 20˚ C. The increased DO level at different concentration of wastewater may be attributed to enhanced activity of microalgae in the form of cell growth, cell number, chlorophyll synthesis and photosynthetic rate. Thus, higher removal efficiency and increased DO content at different wastewater concentration under 20˚ C and 40˚ C could be attributed to less metal pollutants, lower cellular toxicity as well as proper cell growth of consortia 1 and 2.

8.1.2 Nitrogen and phosphorus removal from municipal wastewater

At 20˚ C, consortia 1 showed the highest nitrate nitrogen removal at 25% (89.96%), 50% (75.55%), 75% (78.65%) and 100% (72.59%) concentration of wastewater. In case of consortia 2, the removal efficiency of 88.94%, 78.19%, 76.59% and 68.70% was observed at different wastewater concentration (25-100%). Similarly, the consortia 2 showed the highest decline of 93.13% and 90.91% in phosphorus content at 25% and 50% of municipal wastewater whereas, 88.70% and 85.90% with consortia 1 at 75% and 100% wastewater concentration. At 40˚ C, the maximum nitrate-nitrogen removal in case of consortia 2 (97.57%) was found to be at 25% of municipal wastewater concentration while, with consortia 1 at 50% (90.21%), 75% (73.84%) and 100% (66.62%) wastewater concentration. The results also revealed that consortia 2 showed highest phosphorus removal efficiency at 25% (96.61%) and 50% (93.90%) concentration of wastewater. In case of consortia 1, the removal efficiency of 81.72% and 80.16% was found to be at 75% and 100% concentration of wastewater (Table 8.1). The results of the study also depicted that remediation efficiency was excellent in wastewater treated by consortia 1 at 20˚ C and 40˚ C as compared to consortia 2. Moreover, both algal consortia showed maximum remediation efficiency upto 50% wastewater concentration at 40˚ C as compared to 20˚ C. In the present study, different temperature regimes affect the metabolic rate, bio-adsorption process and growth profile of algae which ultimately leads to differential nutrients removal from wastewater. Delgadillo-Mirquez et al., (2016) also reported that nutrient removal and algal growth is considerably effected by temperature. The higher nutrients remediation efficiency by selected consortia at 40˚ C could be attributed to increased cell proliferation leading to the elevated uptake of nutrient resource from wastewater. Furthermore, the excellent nutrient removal efficiency could be attributed to biosorption and the nutrients recovered are utilized to accomplish metabolic activities and surplus energy is stored as lipids/carbohydrate in algae (Sharma et al., 2020). The subsequent decline in nutrient level of wastewater could be attributed to utilization of organic and inorganic compounds by algal consortia for its prolific growth (Mohsenpour et al., 2021). Overall, the excellent removal efficiency could be ascribed to increased metabolic activity triggered by higher temperature conditions (40˚ C).

Table 8.1 Percentage change in physico-chemical attributes of wastewater treated with consortia 1 and 2 at temperature regimes of 20˚ C and 40˚ C

Parameters	Initial concentration	WW	Consortia-1				Consortia-2			
			20˚ C	% change	40˚ C	% change	20˚ C	% change	40˚ C	% change
pH	7.6	Control	8.82±0.200	13.83	8.43±0.156	10.92	8.95±0.225	17.76	8.62±0.145	13.42
		25%	9.01±0.163	15.65	8.23±0.119	8.29	9.12±0.225	20.00	8.63±0.155	13.55
		50%	9.34±0.298	18.63	8.95±0.114	17.76	9.77±0.276	28.55	9.48±0.195	24.74
		75%	9.42±0.240	19.32	8.52±0.218	12.11	9.45±0.312	24.34	8.85±0.116	16.45
		100%	9.49±0.116	24.87	8.00±0.283	5.26	9.35±0.244	23.03	8.20±0.125	7.89
EC (µS/cm)	856	Control	3.68±0.071	99.57	3.12±0.078	99.64	3.23±1.12	99.62	2.86±1.09	99.67
		25%	125±7.174	85.40	105±8.029	87.73	108±10.74	87.38	128±8.54	85.05
		50%	254±8.590	70.33	183±13.01	78.62	228±8.59	73.36	210±9.88	75.47
		75%	224±8.044	73.83	367±10.14	57.13	398±9.83	58.53	398±9.72	53.50
		100%	332±7.325	61.21	427±11.67	50.12	355±8.34	53.50	470±10.96	45.09
TDS	550	Control	1.86±0.260	99.68	1.48±0.212	99.75	1.62±0.195	99.73	1.20±0.123	99.80
		25%	75±6.94	87.29	64±6.12	89.15	55.73±3.21	90.55	52.0±1.98	91.19
		50%	135±9.07	77.12	105±4.10	82.20	152±5.01	74.24	111±2.34	81.19
		75%	212±7.95	64.07	189±7.34	67.97	239±6.12	59.49	235±4.56	60.17
		100%	258±9.58	56.27	301±9.12	48.98	295±8.23	50.00	359±8.89	39.15
TSS	167	Control	1.45±0.050	99.21	1.12±0.073	99.39	1.68±0.049	99.08	1.18±0.088	99.36
		25%	26.33±2.06	85.61	20±1.34	89.07	25±1.22	86.34	27±1.45	85.25
		50%	43±2.87	76.50	29.37±1.76	83.95	47.34±1.89	74.13	34.23±1.32	81.30
		75%	38.19±3.12	79.13	78±3.67	57.38	40.12±1.45	78.08	67±2.54	63.39
		100%	43.12±2.14	76.44	93±3.22	49.18	47.23±1.09	74.19	81±2.89	55.74

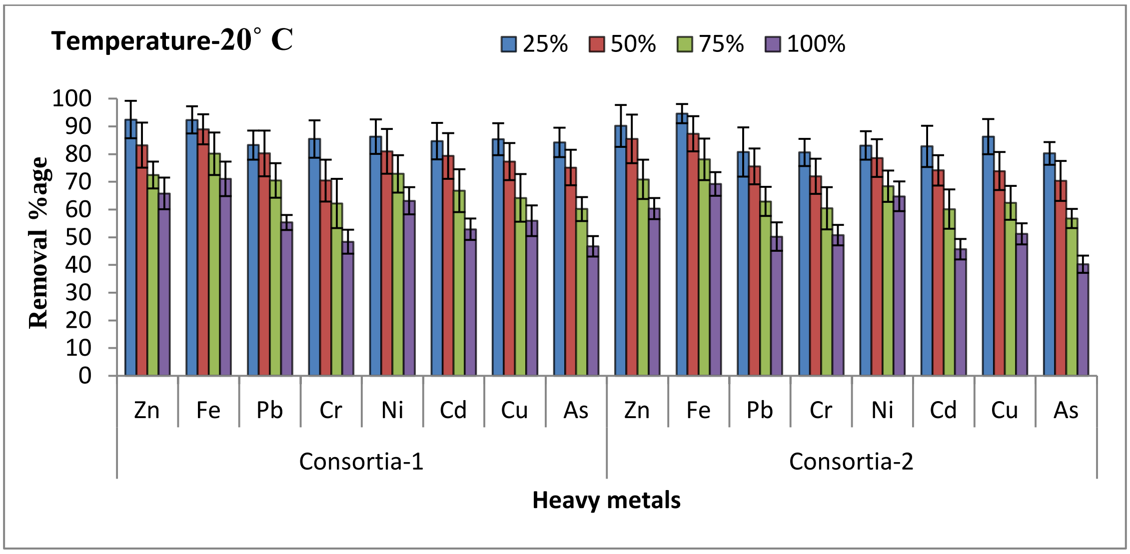
		Control	4.12±0.94	99.41	3.32±0.45	99.52	3.40±0.63	99.51	2.76±0.37	99.60
		25%	105±3.04	86.00	93±2.45	87.60	85.67±1.95	88.58	81±1.33	89.20
TS	705	50%	185±3.92	75.33	125±3.76	83.33	192±3.21	74.40	160.23±2.68	78.64
		75%	250±5.12	66.67	274±4.34	63.47	290±3.82	61.33	284±3.54	62.13
		100%	301±5.95	59.87	380±5.34	49.33	351±4.59	53.20	435±5.56	42.00
		Control	0.716±0.068	98.75	0.586±0.051	98.98	0.843±0.056	98.53	0.506±0.065	99.12
		25%	8.45±0.261	85.24	6.34±0.179	88.92	9.03±0.290	84.22	7.44±0.454	87.00
BOD	57.23	50%	15.23±0.909	73.39	12.35±0.225	78.42	15.69±1.668	72.58	12.13±1.223	78.80
		75%	16.76±1.611	70.71	18.87±1.793	67.03	18.45±1.304	67.76	22.33±1.481	60.98
		100%	20.17±2.56	64.76	27.34±3.013	52.23	22.89±2.113	60.00	30.23±1.847	47.18
		Control	6.87±0.676	-	7.12±0.598	-	7.01±0.501	-	7.27±0.432	-
		25%	4.37±0.276	-	5.01±0.195	-	4.93±0.233	-	5.34±0.378	-
DO	-	50%	2.56±0.202	-	2.66±0.278	-	3.01±0.412	-	3.67±0.156	-
		75%	1.23±0.115	-	0.823±0.056	-	1.02±0.078	-	0.783±0.032	-
		100%	0.567±0.040	-	0.344±0.029	-	0.454±0.033	-	0.297±0.020	-
		Control	0.834±0.054	98.07	0.462±0.014	98.93	0.912±0.038	97.89	0.374±0.029	99.14
		25%	4.34±0.705	89.96	1.42±0.578	96.72	4.78±0.514	88.95	1.05±0.567	97.57
NO_3-N	43.24	50%	10.57±1.45	75.56	4.23±0.613	90.22	9.43±1.18	78.19	5.38±0.756	87.56
		75%	9.23±0.756	78.65	11.31±1.78	73.84	10.12±0.856	76.60	1.02±0.093	64.94
		100%	11.85±0.934	72.59	14.43±1.24	66.63	13.53±1.49	68.71	17.34±1.81	59.90
		Control	0.523±0.044	96.88	0.212±0.016	98.73	0.402±0.043	97.60	0.301±0.052	98.20
		25%	1.23±0.077	92.65	0.878±0.054	94.76	1.15±0.098	93.13	0.567±0.071	96.61
PO_4^{3-}	16.74	50%	1.89±0.101	88.71	1.54±0.132	90.80	2.67±0.213	84.05		93.91
		75%	2.23±0.149	86.68	3.06±0.241	81.72	1.52±0.123	90.92	3.33±0.273	80.11
		100%	2.36±0.124	85.90	3.32±0.190	80.17	3.02±0.278	81.96	4.35±0.512	74.01

WW: Wastewater, All values are in mg/L, otherwise mentioned

8.1.3 Heavy metals removal from wastewater

Temperature affects the toxico-kinetics of different heavy metals which eventually have significant effect on removal efficiency and bioaccumulation potential of microalgae. The heavy metals (µg/L) such as Fe (1555.74±11.26 µg/L), Cu (7.20±0.074), Pb (6.86±1.12), Ni (13.43±0.953), Zn (12.76±0.968) Cr (6.46±0.454), Cd (5.19±0.345), As (0.42±0.065) were observed in municipal wastewater collected from Lucknow.

At 20˚ C, highest removal of 92.44% (Zn), 86.28% (Ni), 85.45% (Cr), 84.25% (As), 83.26% (Pb) was observed in case of consortia 1 while 94.56% (Fe), 86.26% (Cu), 82.78% (Cd) in case of consortia 2 at 25% concentration of municipal wastewater. At 50% municipal wastewater concentration, consortia 1 showed highest removal efficiency of 88.92% (Fe), 80.98% (Ni), 80.26% (Pb), 77.34 (Cu), 75.15% (As) and 85.45% (Zn), 74.15% (Cd), 72.02% (Cr) in case of consortia 2. The results also revealed that consortia 1 treated with 75% concentration of wastewater showed maximum removal efficiency of 80.17% (Fe), 72.89% (Ni), 72.45% (Zn), 70.45% (Pb), 66.82% (Cd), 64.19% (Cu), 62.19% (Cr) and 60.20% (As). At 100% concentration of wastewater, the remediation efficiency of 65.78% (Zn), 55.98% (Cu), 55.34% (Pb), 52.89% (Cd), 46.71% (As) and 73.41% (Fe), 64.76% (Ni), 50.76% (Cr) was observed with consortia 1 and 2, respectively. At 40˚ C, the removal efficiency of 98.34%, 90.21%, 90.13%, 89.56% and 86.43% for Fe, Ni, Cu, As and Pb was observed with consortia 1 whereas, consortia 2 removed 97.23%, 91.34% and 84.76% of Zn, Cd, and Cr at 25% concentration of municipal wastewater. At 50% concentration of wastewater, consortia 1 showed maximum remediation efficiency for Pb (83.26%), Cd (82.12%), Cu (80.34%), Cr (75.45%) and As (78.23%). It was found



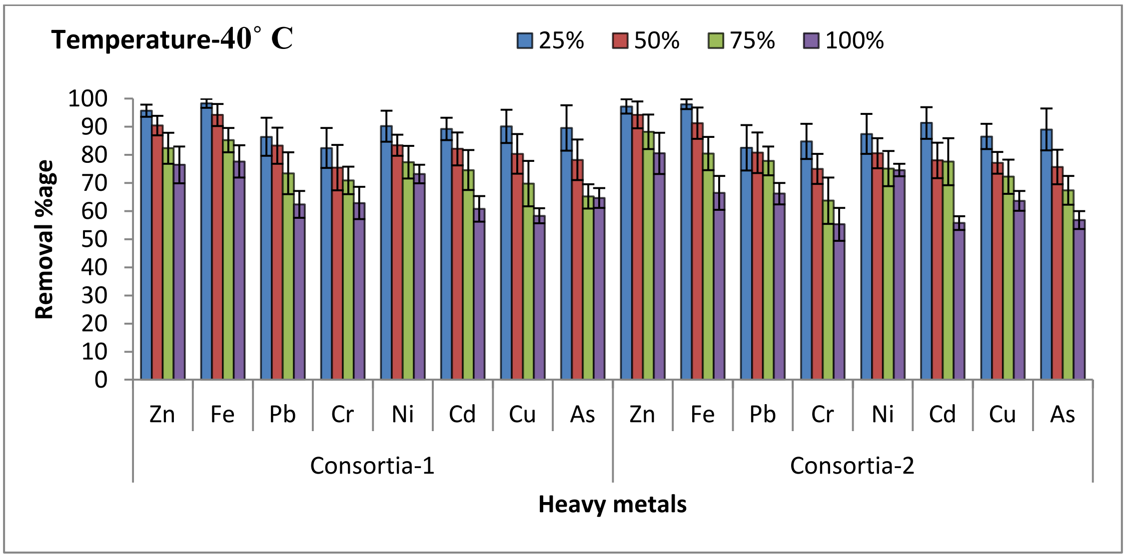
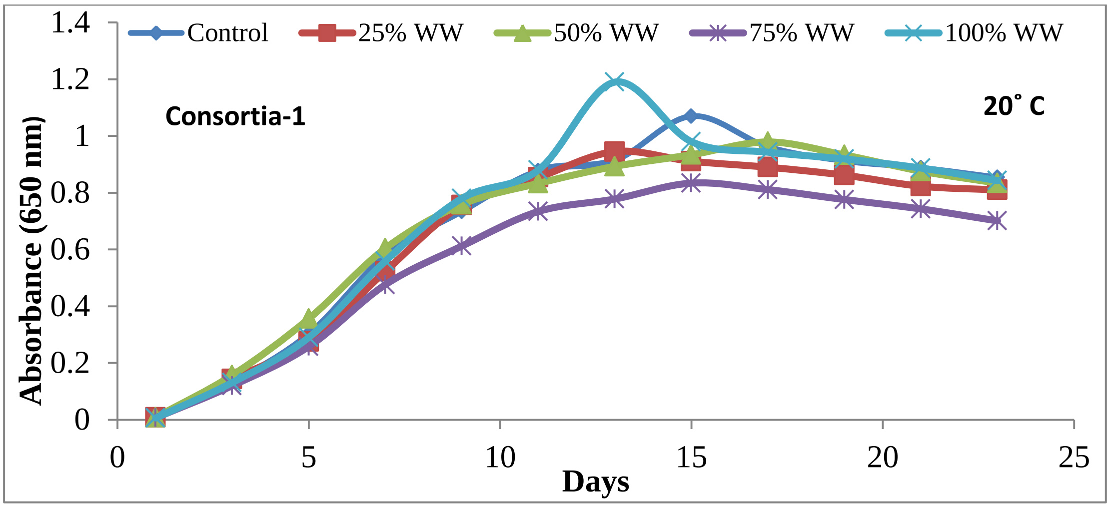


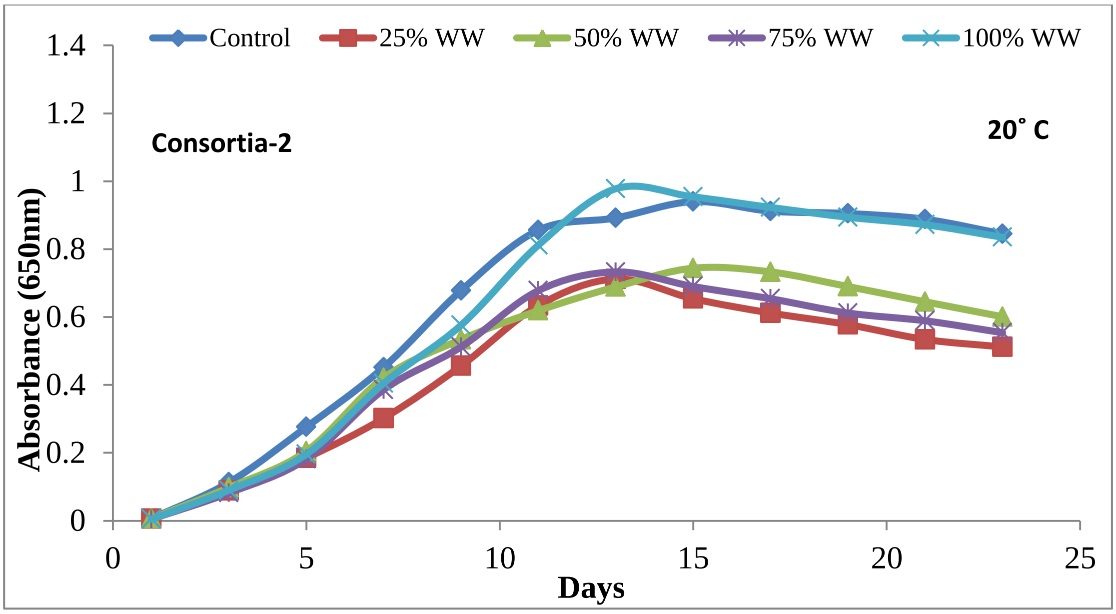
Fig. 8.1 Effect of different temperature regimes on the metal remediation efficiency of algal consortia treated with different wastewater concentrations

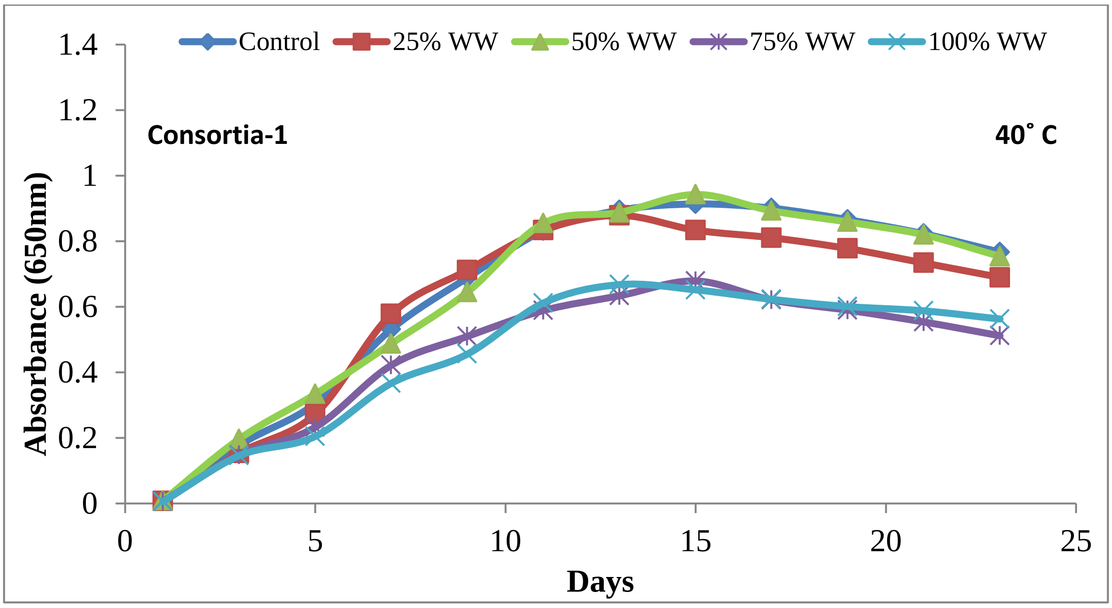
that consortia 2 removed 88.19%, 77.57%, 72.23% and 67.34% of Zn, Cd, Cu and As while consortia 1 removed 85.23%, 77.37%, 73.45% and 70.89% of Fe, Ni, Pb and Cr from 75% of wastewater concentration. The results further exhibited that consortia 1 showed highest removal efficiency for Fe (77.67%), As (64.62%), Cr (62.88%), Cd (60.78%), while consortia 2 for Zn (80.56%), Ni (74.59%), Cu (63.62%) and Pb (66.23%) at 100% wastewater concentration (Fig. 8.1). The results further revealed that selected consortia 1 and 2 showed efficient removal efficacy for different heavy metals. The excellent removal efficiency could be attributed to adsorption of positively charged metals to negative charged functional groups. Pradhan et al., (2019) also observed that adsorption of heavy metals to cell surface of microalgae can occur due to ion exchange, binding with uronic acid and covalent bond formation between cell surface and heavy metals. The results further exhibited that remediation efficiency of selected consortia was highest at 40˚ C as compared to 20˚ C which could be attributed to abundance of binding sites on cell surface or enhanced synthesis of metal cheating protein by algal consortia. Meena et al., (2005) reported that temperature elevates the bio-adsorption process by either enhancing the binding sites or suppress the thickness of cell wall which makes metals approachable to different binding sites on the cell surface. Furthermore, the increase in pH after wastewater treatment with algal consortia may augment the net negative charge and facilitates the removal of metal from wastewater.

8.2 Effect of temperature regimes and wastewater concentration on growth and biomass production of algal consortia

The growth and biomass production is greatly affected by different factors such as media composition, light intensity, light duration and temperature. Temperature during the cultivation of microalgae is the major factor that determines the rate of chemical reactions occurring in the cell. It was observed that selected consortia grown under 40˚ C showed accelerated growth up to 7th day thereafter a decline in growth was observed as compared to 20˚ C**.** At 20˚ C, the maximum growth as well as specific growth rate was found to be at 100% of wastewater concentration, whereas under 40˚ C, the selected consortia showed maximum growth potential at 50% wastewater concentration (Fig. 8.2).


Control
25% WW
50% WW
75% WW
100% WW
Consortia-1
20˚ C
Absorbance (650 nm)
Days
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
5
10
15
20
25


Control
25% WW
50% WW
75% WW
100% WW
Consortia-2
20˚ C
Absorbance (650nm)
Days
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
5
10
15
20
25


Control
25% WW
50% WW
75% WW
100% WW
Consortia-1
40˚ C
Absorbance (650nm)
Days
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
5
10
15
20
25

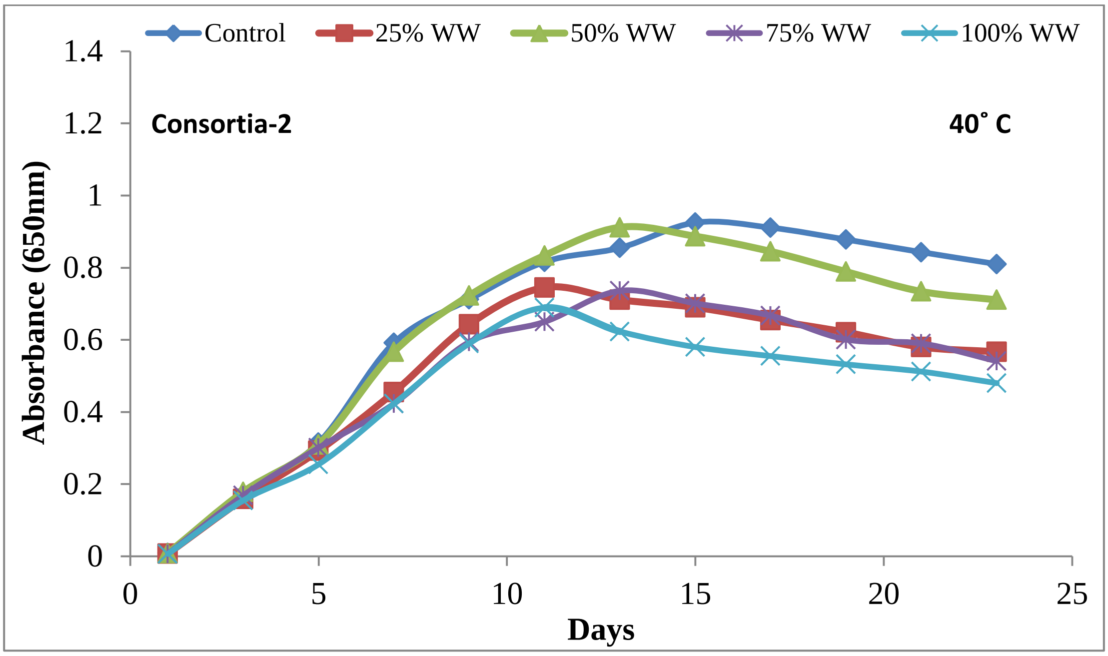


Fig 8.2 Growth profile of consortia 1 and 2 treated with wastewater concentrations (25-100%) under different temperature regimes (20˚ C, 40˚ C)

The subsequent increase in the growth at 20˚ C may be credited to favourable temperature condition during the cultivation of algal consortia 1 and 2. The results also depicted that 75% and 100% concentration of wastewater inhibited the growth of consortia 1 and 2 after treatment at 40˚ C. The inhibited growth may be ascribed to interruption of metabolic processes and cessation of cell proliferation by irreversible inactivation of enzyme (Brindhadevi et al., 2021). It was observed that biomass production enhanced with concentration gradient in consortia 1 and 2 at 20˚ C. The results depicted the highest biomass production with consortia 1 (890 mg/L) grown in BG-11 media at 20˚ C while, consortia 2 (1270 mg/L) at 40˚ C. At 20˚ C, the biomass production of 950 mg/L, 1380 mg/L, and 1720 mg/L was observed with consortia 1 at 25%, 50%, and 100% while, 1490 mg/L with consortia 2 at 75% wastewater concentration, respectively. Similarly at 40˚ C, the maximum biomass production of 1280 mg/L, 2010 mg/L, 1550 mg/L and 1830 mg/L was observed with consortia 2 at

25%, 50%, 75% and 100% municipal wastewater concentration (Table 8.2). Interestingly, biomass production of selected consortia treated with 50% wastewater was increased significantly which indicated that consortia 1 and 2 have potential to tolerate the higher temperature conditions. Earlier reports revealed that microalgae cultivated at higher temperature (35˚ C) showed decline in biomass production by 30% which could be attributed to temperature induced damage to microalgae (Subhash et al., 2014). However, in the present investigation, enhancement in biomass production with temperature was observed with consortia 1 and 2 upto 50% while decreased biomass production at 75% and 100% wastewater concentration. The decrease in the biomass production with temperature may be attributed to low solubility of carbon dioxide (Raeesossadati et al., 2014) or toxicity imposed by higher temperature and wastewater concentrations.

Table 8.2 Effect of different temperature and municipal wastewater concentrations on specific growth rate and biomass production of consortia 1 and 2

Wastewater concentration	Specific growth rate		Biomass Production mg/L	
	20˚ C	40˚ C	20˚ C	40˚ C
Consortia-1				
Control	0.024±0.004[a]	0.029±0.003[ab]	890±5.12[a]	1180±8.23[b]
25% WW	0.033±0.005[bc]	0.043±0.005[d]	950±5.78[a]	1250±10.23[b]
50% WW	0.029±0.002[ab]	0.060±0.006[f]	1380±8.17[c]	1755±15.12[e]
75% WW	0.032±0.006[bc]	0.038±0.005[c]	1160±9.23[b]	1270±12.67[b]
100% WW	0.040±0.007[d]	0.047±0.009[d]	1720±12.12[d]	1520±14.23[c]
Consortia-2				
Control	0.027±0.006[a]	0.034±0.008[bc]	830±6.23[a]	1270±12.34[b]
25% WW	0.030±0.008[b]	0.051±0.011[e]	945±7.12[a]	1280±8.45[b]
50% WW	0.035±0.006[bc]	0.064±0.012[f]	1300±7.45[c]	2010±14.23[f]
75% WW	0.020±0.002[a]	0.043±0.006[d]	1490±10.23[cd]	1550±12.19[c]
100% WW	0.038±0.007[d]	0.046±0.010[d]	1630±11.56[d]	1830±17.43[e]

8.3 Effect on pigment composition and photosynthetic performance

Temperature alters several physiological processes by either increasing or decreasing the biochemical reactions occurring in different cellular compartments (Van Wagenen et al., 2012). At 20˚ C, an increase of 1.35, 1.54, 2.00 and 2.70 folds in total chlorophyll content was observed with consortia 1 while 1.25, 1.64, 2.46 and 5.35 folds with consortia 2 at 25%, 50%, 75% and 100% concentration of wastewater (Table 8.3). At 40˚ C, consortia 1 showed highest increase of 1.78 and 2.29 folds in total chlorophyll content at 25% and 50% concentration of wastewater. In case of consortia 2, the highest increase in total chlorophyll content was at 75% (1.17 folds) while decreased chlorophyll synthesis was found to be in selected consortia at 100% concentration of municipal wastewater. This study also depicted that total chlorophyll declined significantly at higher temperature (40˚ C) and wastewater concentration (100%) in both algal consortia. The decreased total chlorophyll content at higher wastewater concentration in heat stressed cells at 40˚ C could be attributed to interference in electron transport of photosynthetic system, damage to chlorophyll biosynthesis machinery and photosynthetic efficiency of consortia (Chokshi et al., 2020). Ras et al., (2013) observed that the declined growth and photosynthesis may be ascribed to imbalance between energy demand and production as well as inactivation of enzymes involved in the process of photosynthesis. The results also revealed that total chlorophyll content increased at different concentration of wastewater in selected consortia at 20˚ C while at 40˚ C, the enhancement was observed upto 50% and 75% wastewater concentration in consortia 1 and 2, respectively. The increased chlorophyll content could also be attributed to suitable quantity of essential trace elements in wastewater which play indispensible role in different physiological as well as

Table 8.3 Effect of different temperature regimes (20° C, 40° C) and wastewater concentrations (25%-100%) on total chlorophyll (mg g^{-1} fw), carotenoids (mg g^{-1} fw), protein (mg g^{-1} fw), carbohydrate (mg L^{-1} fw), lipid (mg L^{-1} fw), TBARS (µmol g^{-1} fw) and H_2O_2 (µmol g^{-1} fw) in consortia 1 and 2

WW Concent-rations	Algal consortia	Total chlorophyll	Carotenoids	Protein	Carbohydrate	Lipid	TBARS	H_2O_2
Control	Consortia -1	0.0031±0.0003	0.012±0.001^{b}	0.0020±0.001^{b}	13.45±2.32^{a}	0.192±0.011^{b}	1.79±1.20^{a}	3.44±0.066^{ab}
25%		0.0042±0.0001	0.017±0.008^{a}	0.0031±0.002^{b}	22.89±1.61^{c}	0.220±0.023^{a}	4.88±0.865^{c}	5.16±0.360^{c}
50%		0.0048±0.0002	0.020±0.001^{bc}	0.0037±0.003^{d}	26.09±2.51^{d}	0.226±0.008^{b}	7.59±1.05^{e}	5.59±0.640^{c}
75%		0.0062±0.0003	0.019±0.001^{b}	0.0051±0.001^{c}	24.89±1.69^{f}	0.249±0.017^{bc}	6.02±1.34^{d}	10.81±1.11^{e}
100%		0.0084±0.0002	0.021±0.008^{b}	0.0089±0.001^{e}	21.16±1.29^{d}	0.235±0.007^{bc}	9.78±1.15^{g}	11.60±0.92^{f}
Control	Consortia-2	0.0028±0.0001^{a}	0.019±0.001^{a}	0.0034±0.0001^{a}	11.12±0.434^{b}	0.156±0.012^{b}	2.12±0.078^{ab}	2.87±0.520^{a}
25%		0.0035±0.0007^{ab}	0.064±0.004^{a}	0.0039±0.0003^{a}	18.49±0.744^{d}	0.161±0.007^{a}	4.05±0.093^{c}	6.12±0.580^{d}
50%		0.0046±0.0003^{c}	0.053±0.003^{a}	0.0044±0.0005^{b}	28.72±2.04^{e}	0.187±0.004^{d}	7.62±0.42^{e}	5.16±0.377^{c}
75%		0.0069±0.0009^{c}	0.048±0.005^{b}	0.0054±0.0003^{b}	23.69±1.92^{f}	0.230±0.011^{b}	8.12±0.76^{f}	11.73±0.905^{f}
100%		0.015±0.0008^{c}	0.038±0.008^{a}	0.0072±0.0007^{c}	17.69±0.823^{f}	0.204±0.008^{bc}	11.06±0.84^{h}	17.09±1.48^{g}

Control	**Consortia-1**	0.0037±0.0002[a]	0.018±0.007[a]	0.0043±0.0005[a]	26.28±1.13[a]	0.264±0.014[b]	3.76±1.04[a]	6.60±0.470[a]
25%		0.0066±0.0006[ab]	0.019±0.009[a]	0.0060±0.0003[b]	29.88±1.24[e]	0.313±0.014[b]	7.69±1.19[b]	11.49±0.630[b]
50%		0.0085±0.0003[c]	0.020±0.008[c]	0.0074±0.0003[c]	30.83±1.57[bc]	0.326±0.020[e]	9.33±1.32[c]	17.74±0.659[d]
75%		0.0017±0.0002[d]	0.024±0.003[a]	0.0066±0.0002[f]	27.31±1.14[f]	0.405±0.012[d]	15.56±1.40[d]	20.32±1.24[e]
100%		0.0012±0.0003[d]	0.026±0.008[d]	0.0052±0.0002	22.51±1.09[bc]	0.390±0.013[c]	22.15±1.28[e]	27.74±1.76[f]
Control	**Consortia-2**	0.0041±0.0001[ab]	0.015±0.001[a]	0.0035±0.0003[c]	29.65±1.07[b]	0.197±0.018[a]	3.47±0.280[a]	5.87±0.450[a]
25%		0.0064±0.0001[bc]	0.024±0.001[b]	0.0048±0.0004[c]	30.12±1.29[d]	0.210±0.020[a]	6.29±0.312[b]	10.09±0.620[b]
50%		0.0092±0.0005[e]	0.030±0.002[d]	0.0087±0.0003[e]	33.16±1.40[e]	0.236±0.022[d]	10.34±0.789[c]	14.34±0.913[c]
75%		0.0048±0.0002[d]	0.032±0.003[c]	0.0051±0.0004[d]	27.96±1.31[bc]	0.264±0.029[f]	18.14±0.567[d]	25.69±2.63[f]
100%		0.0038±0.0005[e]	0.024±0.004[a]	0.0032±0.0002[c]	24.89±1.51[a]	0.260±0.032[f]	25.41±0.723[ef]	31.29±3.85[g]

biochemical processes such as growth, photosynthetic efficiency, electron transport chain, chlorophyll and metabolite biosynthesis (Jalmi et al., 2018). In present study, the increased total chlorophyll content may be credited to excellent photosynthetic rate, electron transport and less oxidative damage posed by low temperature as compared to high temperature conditions. Pigments perform multiple functions in the cell such as harvesting of light, scavenging of ROS, energy dissipations as well as stabilization of cellular structures (Mulders et al., 2014). At 20˚ C, consortia 2 showed the highest increase of 3.36, 2.78, 2.52 and 2.00 folds in carotenoid content at 25-100% of municipal wastewater concentration. At 40˚ C, consortia 2 further showed the maximum increase of 1.60, 2.00, 2.13 and 1.60 folds in carotenoids at municipal wastewater concentration ranging from 25-100% (Table 8.3). Markou and Nerantzis, (2013) reported that carotenoid synthesis is affected by temperature and their accumulation in the cell is elevated by ROS generation. The present study depicted that temperature along with different wastewater concentrations increased carotenoid synthesis in consortia 1 and 2. The increased accumulation of carotenoid under different operational condition might protect the microalgae from oxidative stress. Das and Roychoudhury, (2014) also observed that carotenoids prevent oxidative damage to photosynthetic apparatus by reducing the formation of $^1O_2$ and also helps in dissipating surplus excitation energy in the form of heat. Therefore, the carotenoid accumulation in consortia 1 and 2 might be attributed to defensive responses adopted by microalgae to prevent cellular damage instigated by different wastewater concentrations and temperature regimes.

The adequate assessment of metabolic processes is important to examine the behaviour of microalgae exposed to different stress conditions. The chlorophyll fluorescence assists to estimate the physiological status of photosynthetic apparatus in algal consortia. The changes in photosynthetic fluorescence is directly or indirectly related to ATP synthesis, photolysis of water, transport of electron in electron transport chain and pH gradient across thylakoid membrane. At 20˚ C, the results showed that active photosystem II reaction centre (Fv/Fo) increased by 3.57, 2.42, 1.97 and 1.30 folds while quantum yield (Fv/Fm) was increased by 2.29, 1.94, 1.78 and 1.21 folds in consortia 1 at 25%, 50%, 75% and 100% concentration of wastewater. In case of consortia 2, an increase of 1.11 and 1.10 folds in Fv/Fo while 1.08 and 1.01 folds in Fv/Fm was found at 25% and 75% wastewater concentration, respectively (Table 8.4). The Mo values was observed to decrease significantly with wastewater concentration in consortia 1 while in consortia 2, an increase was observed at 50% (1.52 folds), 75% (1.40 folds) and 100% (1.25 folds) of wastewater concentration. It was also observed that performance index (Pi_{abs}) value increased by 35.51, 27.31, 6.68 and 3.05 folds in consortia 1 at different wastewater concentrations whereas, consortia 2 showed an increase of 1.67 folds at 25% wastewater concentration. The ABS/RC and TRo/RC ratio in consortia 1 showed an increase of 1.32 and 1.61 folds at 100% wastewater and significantly declined at 25-75% concentration of municipal wastewater. The ETo/RC in consortia 1 was also increased by 1.23, 1.32, 1.34 and 1.36 folds at 25%, 50%, 75% and 100% concentration of municipal wastewater. Similarly, in consortia 2, the significant increase in ABS/RC (1.09-1.38 folds), TR_O/RC (1.07-1.30 folds), and ETo/RC (1.15-1.23 folds) was found to be at 25-100% of municipal wastewater (Table 8.4).

At 40˚ C, the ratio of Fv/Fo was enhanced by 1.38, 1.43 and 1.06 folds while, Fv/Fm ratio by 1.19, 1.20, and 1.03 folds in consortia 1 treated with 25%, 50% and 100% of wastewater concentration in comparison to untreated consortia. In consortia 2, an increase of 1.51 (Fv/Fo) and 1.23 folds (Fv/Fm) was observed at 25%, thereafter, the decline in Fv/Fo as well as Fv/Fm was observed with increasing concentration gradient. The results further depicted that Mo value increased after treatment of 75% (1.19 folds) and 100% (1.07 folds) wastewater concentration to consortia 1 whereas, consortia 2 showed increment at 50% (1.15 folds), 75% (1.14 folds) and 100% (3.63 folds) of wastewater concentration. It was also found that Pi_{abs} value was increased by 2.23 folds in consortia 2 at 25% while 4.45, 3.43, and 1.07 folds in consortia 1 at 25%, 50% and 100% wastewater concentration. ABS/RC ratio was increased by 1.41, 1.16, 1.55 and 6.38 folds with wastewater concentration gradient in consortia 2 while, in the case of consortia 1, an increase of 1.57 and 1.16 folds was observed at 75% and 100% of wastewater concentration. The TR_O/RC ratio was also found to increase in consortia 1 after treatment with 75% (1.11 folds) and 100% (1.21 folds) of wastewater concentration. Similarly, consortia 2 showed an increase in TR_O/RC ratio at 25% (1.75 folds) and 100% (2.31 folds) of wastewater concentration. Consortia 1 also showed an increase of 1.09, 1.08 and 1.26 folds in ETo/RC ratio at 50%, 75% and 100% wastewater concentration while in consortia 2, an increase of 2.01 and 1.86 folds was observed at 25% and 100% concentration of wastewater, respectively (Table 8.4).

Microalgae exposed to different temperature alter the quantum yield and primary electron transport that eventually affects the photosynthetic performance of microalgae. Fv/Fo represents number of oxygen evolving reaction centres thus estimates the photosynthetic capacity of microalgae (Vadiveloo et al., 2013). The highest increment in Fv/Fo at lower concentration of wastewater could be attributed to excellent photosynthetic performance of consortia. The Fv/Fm ratio serves as the important indicator of photosynthetic performance and also measure the potential of PSII to absorb light energy for photosynthesis (Qiao et al., 2015). Fv/Fm is also an

Table 8.4 Changes in photosynthetic performance of algal consortia 1 and 2 cultivated in municipal wastewater under temperature regimes of 20˚ C and 40˚ C

Wastewater (WW) concentration	Algal consortia	Fv/Fo	Fv/Fm	Mo	Pi $_{abs}$	ABS/RC	TRo/RC	ETo/RC
Temperature -20˚ C								
Control	**Consortia-1**	0.275[a]	0.216[ab]	0.780[g]	0.035[a]	6.726[de]	1.452[bc]	0.672[c]
25% WW		0.983[e]	0.496[ef]	0.290[b]	1.243[e]	2.253[a]	1.116[b]	0.827[e]
50% WW		0.667[cd]	0.421[de]	0.185[a]	0.956[d]	4.567[b]	0.982[a]	0.892[e]
75% WW		0.542[c]	0.386[d]	0.456[d]	0.234[c]	6.789[de]	1.268[bc]	0.905[ef]
100% WW		0.358[ab]	0.263[c]	0.639[f]	0.107[b]	8.912[g]	2.348[d]	0.915[ef]
Control	**Consortia-2**	0.235[a]	0.190[a]	0.418[d]	0.064a	5.515[c]	1.048[ab]	0.629[c]
25% WW		0.261[a]	0.207[ab]	0.349[c]	0.107[b]	5.434[c]	1.126[b]	0.777[cd]
50% WW		0.217[a]	0.179[a]	0.636[f]	0.033[a]	7.649[f]	1.365[bc]	0.729[cd]
75% WW		0.237[a]	0.192[a]	0.588[e]	0.039[a]	6.141[d]	1.176[b]	0.589[b]
100% WW		0.234[a]	0.190[a]	0.410[d]	0.069[a]	6.027[d]	1.143[b]	0.522[a]
Temperature- 40˚ C								
Control	**Consortia-1**	0.755[d]	0.430[c]	0.251[b]	0.921[c]	2.028[b]	0.872[ab]	0.621a
25% WW		1.048[e]	0.512[d]	0.110[a]	4.103[g]	1.325[a]	0.678[a]	0.569[a]
50% WW		1.085[e]	0.520[d]	0.147[a]	3.168[f]	1.587[a]	0.826[ab]	0.679[ab]
75% WW		0.535[c]	0.348[b]	0.300[bc]	0.430[b]	2.794[c]	0.975[ab]	0.674[ab]
100% WW		0.804[d]	0.446[c]	0.269[b]	0.993[c]	2.369[b]	1.056[ab]	0.787[c]
Control	**Consortia-2**	0.754[d]	0.432[c]	0.219[b]	1.103[cd]	2.012[b]	0.865[ab]	0.646[ab]
25% WW		1.140[ef]	0.533[d]	0.212[b]	2.463[e]	2.841[c]	1.514[c]	1.302[d]
50% WW		0.527[c]	0.345[b]	0.254[b]	0.491[b]	2.341[b]	0.808[a]	0.554[a]
75% WW		0.332[ab]	0.249[ab]	0.250[b]	0.225[ab]	3.136[d]	0.781[a]	0.532[a]
100% WW		0.185[a]	0.156[a]	0.796[d]	0.022[a]	12.838[e]	2.000[d]	1.204[c]

appropriate indicator of stress condition during the growth of photosynthetic organisms (Zobayed et al., 2005). The significant decline in Fv/Fm value in consortia 2 above 25% wastewater concentration at 40˚ C might be attributed to photoinhibition and reversal damage to important component of PS II. The results also showed that

Fv/Fm increased in consortia 1 and 2 at 20˚ C which might be ascribed to efficient functioning of photosynthetic apparatus and photosystem II as well as high energy conversion efficiency of microalgae (Chen et al., 2017). Furthermore, higher Fv/Fo and Fv/Fm reflects that the consortia 1 were efficient to mitigate the damage posed by different wastewater concentrations and temperature regimes.

Pi_{abs} value reflects about the functional activity of PSI, PSII and activity of electron transport (Sadvakasova et al., 2016). The results reflected that Pi_{abs} value exhibited increment with wastewater concentration at 20˚ C and 40˚ C in consortia 1 than consortia 2. The increased Pi_{abs} value indicates the remarkable cellular activity and quantum yield of PS II caused by increment of active reactive centres (RCs) and decreased QB-non-reducing centres (Sadvakasova et al., 2016). Pi_{abs} value also measure cell vitality (Negi et al., 2016) and increased value with wastewater concentration at 20˚ C and 40˚ C could be due to strong tolerance and adaptability of consortia 1 as compared to control. The considerable increase in Pi_{abs} value under 20˚ C and 40˚ C exhibited that consortia 1 was more tolerant to temperature and wastewater stress as compared to consortia 2. Mo represents the net closing rate of RC and the results revealed the significant increase in Mo value with temperature and wastewater concentrations in consortia 2. The increased Mo value could be attributed to faster closing rate of reaction centres and inhibited activity of PS II (Markou et al., 2017). Therefore, the enhanced Mo value could be directly linked to increased trapping flux with wastewater concentration gradient and temperature. Furthermore, the results depicted an increase in the flux ratio ABS/RC, TRo/RC, ETo/RC with temperature and wastewater concentrations. ABS/RC reflects the number of all reaction centres to the active reaction centres (Mathur et al., 2011). In this study, the increased ABS/RC value in consortia 2 reflects the elevated number of inactive reaction centres as compared to active reaction centres. The increased ABS/RC at higher temperature may be attributed to disturbance in the balance of energy supply and consumption by different redox reaction which eventually leads to inactivation of reaction centres of PS-II (Di Pippo et al., 2012). TRo/RC ratio is the measure of maximum rate of trapping of electron by reaction centres. In the present investigation, both algal consortia showed increased TRo/RC ratio after treated with wastewater under different temperature regimes. The increased TRo/RC could be attributed to closing of reaction centres and dissipation of energy in heat form render Q_A reduction and transport of electrons in photosystems (Markou et al., 2017). The ETo/RC was increased with wastewater concentration gradient and temperature in consortia 1 while in case of consortia 2, the highest increment was found to be at 25% and 100% of wastewater concentration after treatment at 40˚ C. ETo/RC reflects the re-oxidation of reduced Q_A and also the activity of active reaction centres (Mathur et al., 2011). The amplified ETo/RC value indicated that increased number of inactive reaction centre leads to poor electron transport efficiency. Overall, the results depicted that consortia 1 exhibited excellent photosynthetic performance, electron transport and minimum inhibition of active reaction centres under two different temperature regimes as compared to consortia 2.

8.4 Effect of temperature and municipal wastewater on biochemical profile of algal consortia 1 and 2

8.4.1 Protein content

Temperature changes the physiological processes of microalgae by either increasing or decreasing the biochemical reaction in the cell (Van Wagenen et al., 2012). At 20˚ C, the result revealed that protein content was increased in consortia 1 (4.45 folds) and consortia 2 (2.11 folds) treated with 100% wastewater concentration. As per the result of the present study, protein content in consortia 1 was also increased by 1.55, 1.85 and 2.55 folds whereas 1.14, 1.29 and 1.58 folds with consortia 2 at 25%, 50% and 75% concentration of wastewater. Similarly, at 40˚ C, consortia 1 showed an increase of 1.39, 1.72 and 1.53 folds whereas, 1.37, 2.48, and 1.45 folds in consortia 2 treated with 25%, 50% and 75% concentration of municipal wastewater. Furthermore, the protein content was further increased in consortia 1 (1.20 folds) treated with 100% of wastewater concentration (Table 8.3). The increased protein synthesis with wastewater concentration may be due to excellent nutrient removal potential, proficient growth or defense responses resulting in low toxicity to consortia. Sathasivam and Ki, (2019) reported that exposure to temperature conditions above optimum level may lead to heat stress and algal consortia may respond by stimulating the synthesis of proteins responsible for heat stress mitigation. Moreover, the decreased protein synthesis in consortia 2 might be due to temperature (40˚ C) and wastewater (100%) induced damage to cellular organelles leading to protein denaturation and inhibited enzyme activity. Furthermore, exposure to high temperature decreases protein content by disturbing structural conformation of protein and enzymatic activity of the cell (Renaud et al., 2002).

8.4.2 Carbohydrate and lipid content

Carbohydrate and lipid synthesis in consortia 1 and 2 was estimated after treatment with different wastewater concentrations at 20˚ C and 40˚ C. At 20˚ C, carbohydrate content was increased by 1.70, 1.93, 1.85, 1.57 folds in consortia 1 while 1.66, 2.58, 2.13, 1.59 folds in consortia 2 at 25%, 50%, 75% and 100% concentration of wastewater, respectively. At 40˚ C, an increase of 1.13, 1.70, 1.03 in carbohydrate synthesis in consortia 1 was found at 25%, 50%, 75% wastewater concentration while with consortia 2, an increase of 1.01 and 1.11 was found to be at

25% and 50% wastewater concentration (Table 8.3). The results also revealed that increased carbohydrate accumulation at 25-100% wastewater concentration may be credited to efficient carbon fixation by selected consortia at 20˚ C. The decline in carbohydrate synthesis at 75% and 100% as compared to 25% and 50% wastewater concentration under 40˚ C may be due to impairment of metabolic activities as well as oxidative damage to consortia 1 and 2. Furthermore, the decreased carbohydrate content noticed after cultivation at 40˚ C and 75-100% wastewater concentration may be ascribed to conversion of intracellular carbohydrate to lipids. At 20˚ C, the selected algal consortia 1 and 2 showed maximum increase of 1.29 and 1.47 folds in lipid content at 75% concentration of municipal wastewater. In case of consortia 1, the lipid content was also found to increase by 1.14, 1.17 and 1.22 folds whereas, 1.03, 1.19 and 1.30 folds in consortia 2 at 25%, 50% and 100% of municipal wastewater. Similarly, at 40˚ C, the lipid content was found to increase by 1.18, 1.23, 1.53 and 1.47 folds in consortia 1 while, consortia 2 showed an increase of 1.06, 1.19, 1.34, and 1.31 folds at 25% -100% concentration of municipal wastewater (Table 8.3). Overall, lipid accumulation in consortia 1 and 2 was increased with wastewater concentration and temperature conditions than untreated consortia (BG-11). In the present study, the deceased nutrient utilization potential with wastewater concentration under 20˚ C and 40˚ C may indirectly create the nutrient deficient condition in the cell favouring the pathways for lipid accumulation. Li et al., (2010) also observed the enhancement of lipid accumulation in microalgae which could be attributed to nitrogen deficiency induced at higher temperature condition (25^{o} C). Furthermore, increased lipid accumulation under high temperature conditions may be ascribed to the preferable utilization of lipid as the storage molecule by microalgae (Sayegh and Montagnes, 2011). In this study, the results suggested that both temperatures regimes (20˚ C and 40˚ C) improved cell growth and metabolic activities which may contributes towards the protein and lipid accumulation in algal consortia. Similar results of higher lipid accumulation under different temperature regimes have been observed by Xu et al., (2019).

8.5 Analysis of composition of algal biomass through FTIR spectroscopy

FTIR is the simple tool used for examining different functional groups as well as macromolecular composition of algal biomass under diverse environmental condition (Singh et al., 2019). Different functional groups in algal consortia were characterized by absorption frequency from FTIR spectra. The absorption peaks between 4000-2500 cm^{-1} might be credited to compounds having C-H, N-H, and O-H bonds while peaks between 2000-1500 cm^{-1} may be due to the presence of compounds having double bonds. In present study, the broad peaks between 3550-3300 cm^{-1} were observed in wastewater treated consortia 1 and 2 under both temperature conditions which may possibly be due to O-H, N-H group of carbohydrate and secondary amines (Ansari, et al., 2017). The broadening of peaks could also be due to interaction between the metals and functional groups (Michalak et al., 2018). The FTIR spectroscopy further showed that the spectral peaks between 1120–1030 cm^{-1} in consortia 1 and 2 treated at 20˚ C and 40˚ C reflects the stretching vibration of C-H representing the presence of enzymatic antioxidants (Ansari, et al., 2017). Similarly, the spectral peak at 1240.8 cm^{-1} and 1063.9 cm^{-1} in consortia 1 treated with 100% wastewater concentration at 40˚ C exhibited C– –O and C– –N bond of carboxylic acid pertaining to flavonoids and phenolic compounds (Elumalai and Velmurugan, 2015). Furthermore, the distinct peaks between 1700-1600 cm-1 in wastewater treated consortia 1 and 2 at 20˚ C and 40˚ C reflects the stretching and bending of amide 1

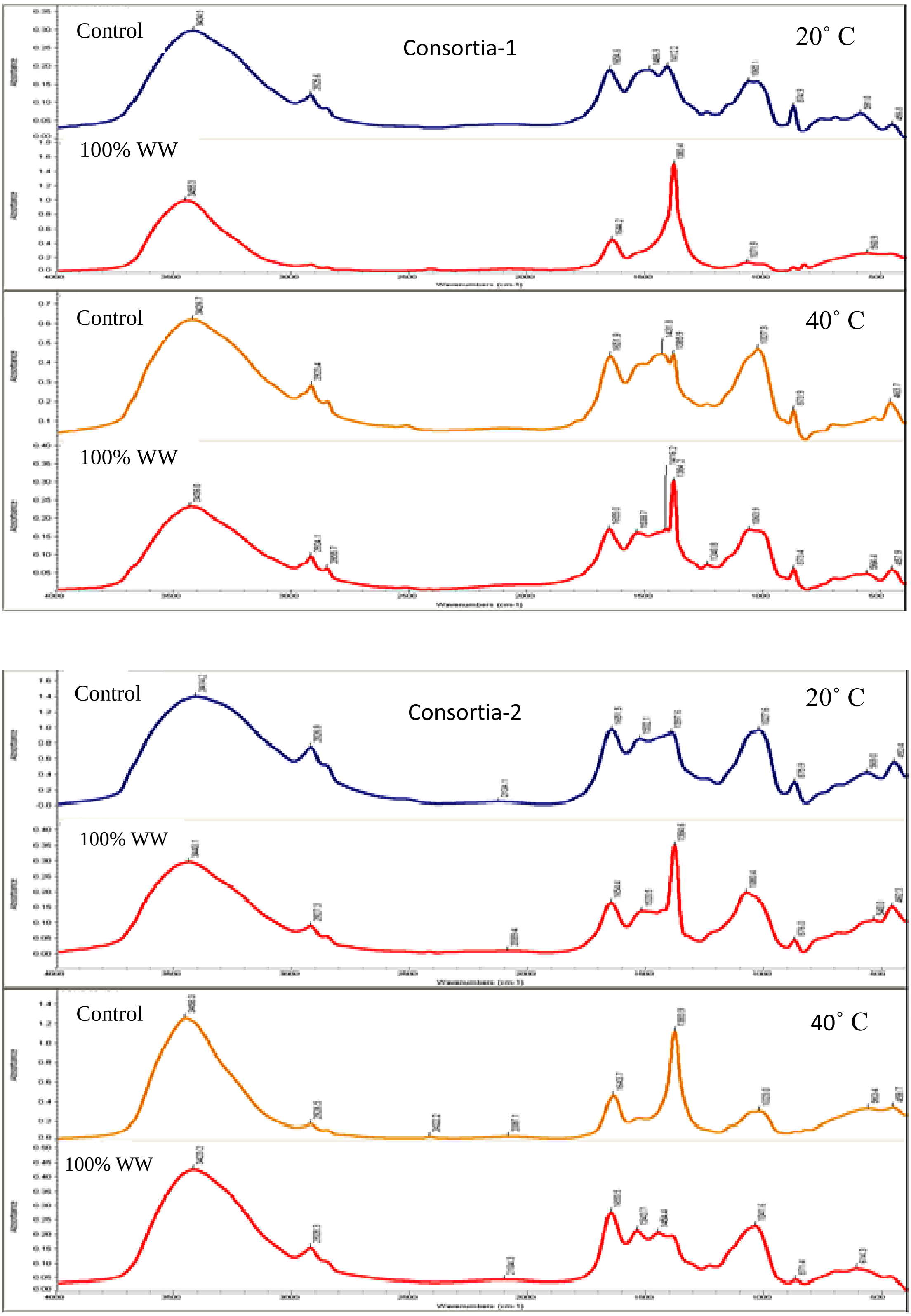


Fig. 8.3 Effect of municipal wastewater (100%) on the composition of consortia 1 and 2 grown under different temperature regimes.

group of proteins. In treated consortia, the sharp peaks between 1200-1000 cm^{-1} reflects the abundance of carbohydrate in algal biomass (Ansari, et al., 2017). The results also depicted that consortia 1 exposed to 40˚ C showed reductions in carbohydrate accumulation as compared to consortia 2. In present study, the distinct absorption peaks between 2924.1-2855.7 cm^{-1} reflects the lipid accumulation in consortia 1 and 2 (Fig 8.3). Ajayan et al., (2015) reported that peaks between 3000-2800 cm^{-1}depicts the presence of methylene and peaks between 1700-1500 cm^{-1} reflected the presence of triglycerides. The FTIR spectroscopy also depicted the higher lipid peaks in consortia 1 treated with wastewater concentration of 100% at 20˚ C and 40˚ C than consortia 2. Overall, the results depicted that apart from excellent lipid accumulation potential, FTIR spectroscopy showed that consortia 1 was enriched with different enzymatic antioxidants as well as flavonoids and phenolic compounds which may be responsible for imparting tolerance towards different wastewater and temperature regimes.

8.6 Fatty acid methyl ester (FAME) profile of algal consortia 1 and 2

The FAME analysis was carried out in algal consortia treated with wastewater (100%) under different temperature regimes. The results showed abundance of C-16 and C-18 carbon atoms in selected consortia at 20˚ C and 40˚ C. The results also reflected that palmitic acid (C16:0) was found to be in dominant amount in consortia 1 (22.34%) and consortia 2 (21.45%) after treatment with wastewater concentration of 100% at 40˚ C. It was also observed that C18:3 (14.76%), C18:1 (13.67%), C18:2 (12.43%) and C18:0 (7.50%) was found to be in maximum amount in consortia 1 while, C18:3 (14.22%), C18:1 (13.29%) and C18:2 (11.67%) in consortia 2 at 20˚ C (Fig. 8.4). Thus, due to the dominance of fatty acid (C-16 and C-18), the biodiesel extracted from consortia 1 and 2 could have excellent fuel properties (Pandey et al., 2019). On the other hand, unsaturated fatty acid (USFA) has tremendous lubricant properties and their abundance in the treated algae could further augment the qualities of biodiesel (Kumar et al., 2019).

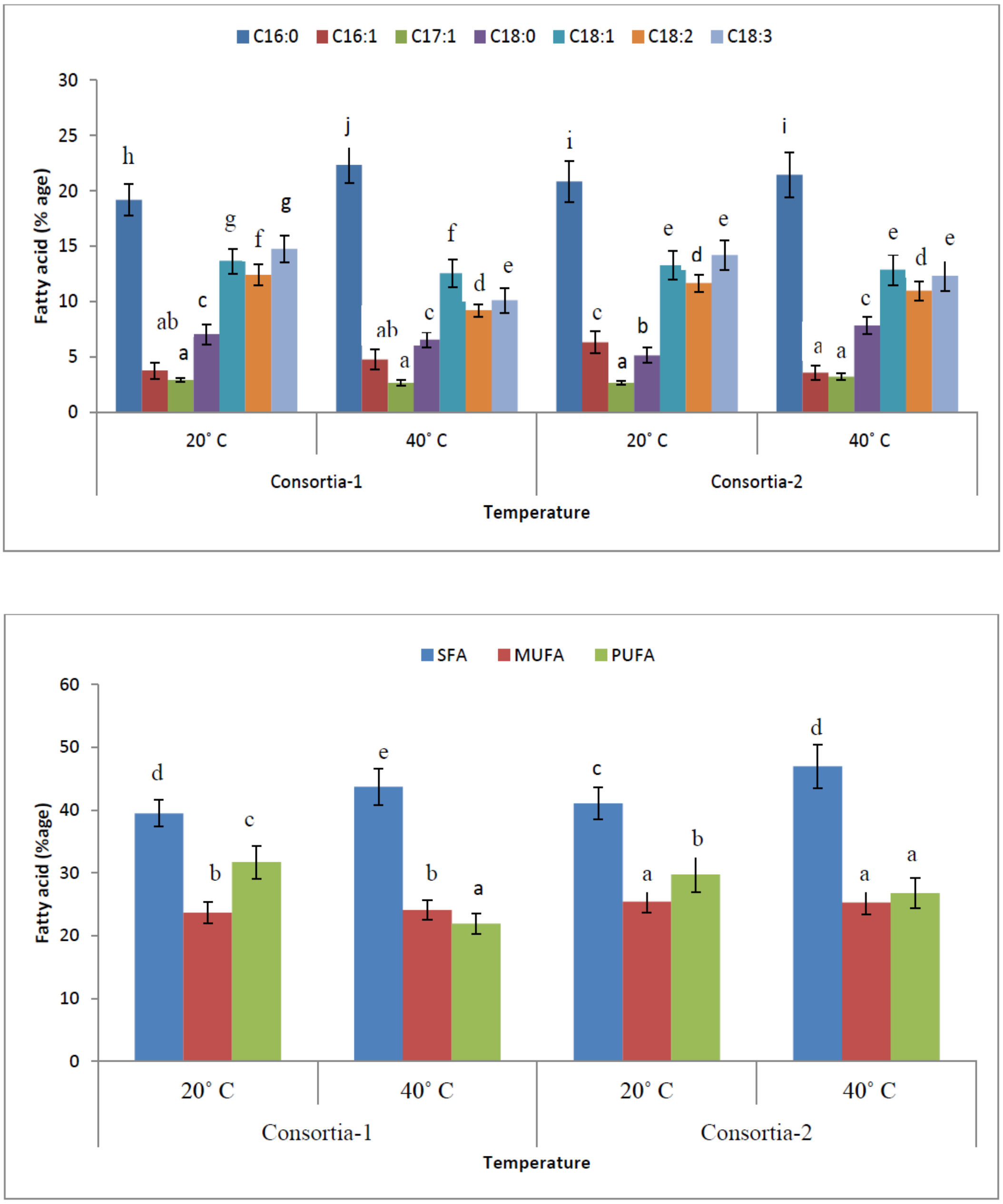


Fig. 8.4 Fatty acid profiling of algal consortia 1 and 2 treated with wastewater (100%) under different temperature regimes (20˚ C, 40˚ C)

Furthermore, the percentage of different fatty acids exhibited significant variations in the selected consortia treated with wastewater (100%) at 20˚ C and 40˚ C. In present study, the percentage of saturated fatty acid (SFA) were higher in consortia 2 (46.99%) and consortia 1 (43.70%) treated with wastewater (100%) and temperature regimes of 40˚ C. The monounsaturated (MUFA-25.42%) and polyunsaturated fatty acid (PUFA-31.71%) was also observed highest in consortia 2 and 1 treated with 100% wastewater at 20˚ C, respectively. Overall, the highest percentage of SFA depicted that consortia 1 and 2 cultivated under 40˚ C could enhance the qualities of biodiesel. Furthermore, exposure of consortia 1 and 2 to low temperature (20˚ C) decreases the SFA content with the considerable increase in the PUFA content. Gonzalez-Silvera et al., (2017) also observed that low temperature favours the accumulation of lipids with high content of unsaturated fatty acid responsible for maintaining membrane fluidity. The results of this study are in concordance with Carneiro et al., (2020).

8.7 Oxidative stress biomarkers: TBARS and H_2O_2

At 20˚ C, the highest increase in thiobarbituric acid reactive substances **(**TBARS) was found in consortia 1 (5.46 folds) and consortia 2 (5.21 folds) at 100% concentration of municipal wastewater. In consortia 1, TBARS was increased significantly at 25% (2.72 folds), 50% (4.24 folds) and 75% (3.36 folds) concentration of wastewater. Further, consortia 2 showed an increment of 1.91, 3.59 and 3.83 folds at 25%, 50% and 75% wastewater concentration. At 40˚ C, consortia 1 showed highest increment in TBARS content at 25% (2.04 folds) while, maximum increment was observed in case of consortia 2 at 50% (2.97 folds) 75% (5.22 folds) and 100% (7.32 folds) of wastewater concentration. Furthermore, consortia 2 showed the maximum increment of 5.95 and 5.33 folds in hydrogen peroxide (H_2O_2) content at 20˚ C and 40˚ C, respectively, treated with 100% wastewater concentration. At 20˚ C, H_2O_2 content was increased by 1.50, 1.62, 3.14, and 3.37 folds in consortia 1 while in consortia 2, an increase of 1.42, 1.79, 4.08, and 5.95 folds was found after treatment with 25%, 50%, 75% and 100% concentration of wastewater. At 40˚ C, H_2O_2 was found to increase by 1.74, 2.68, 3.07 and 4.20 folds in consortia 1 at 25%, 50%, 75% and 100% concentration of wastewater. In case of consortia 2, the H_2O_2 content increased at 25% (1.71 folds), 50% (2.44 folds), 75% (4.37 folds) and 100% (5.33 folds) concentration of wastewater (Table 8.3). Further, the results also showed that oxidative stress markers were more pronounced in consortia 2 at higher temperature (40˚ C) and wastewater concentration (75%-100%). TBARS is the stress biomarker which indicates about the oxidative cell damage (Chokshi et al., 2015). The results depicted that the wastewater concentration and temperature dependent increase of TBARS content in treated algal consortia in comparison to untreated consortia. The increased TBARS level with wastewater concentration and temperature regimes confirms the oxidative damage to the microalgae. Tripathi et al., (2021) observed that metal after getting attached to functional groups on the surface of microalgae may eventually gets accumulated in the cell and amplify the reactive species which poses damage to lipid biosynthesis. The strategy adopted by selected consortia to two temperature regimes may be different however the disturbance in ROS equilibrium was the common affect of high and low temperature stress (Chokshi et al., 2020). Furthermore, exposure to higher temperature may interfere in the charge separation as well as oxygen evolving capacity of PS-II and eventually results in the generation of ROS. The ROS may interrupt the cellular equilibrium by disturbing the membrane integrity and stimulate fatty acid peroxidation (Shekh et al., 2021).

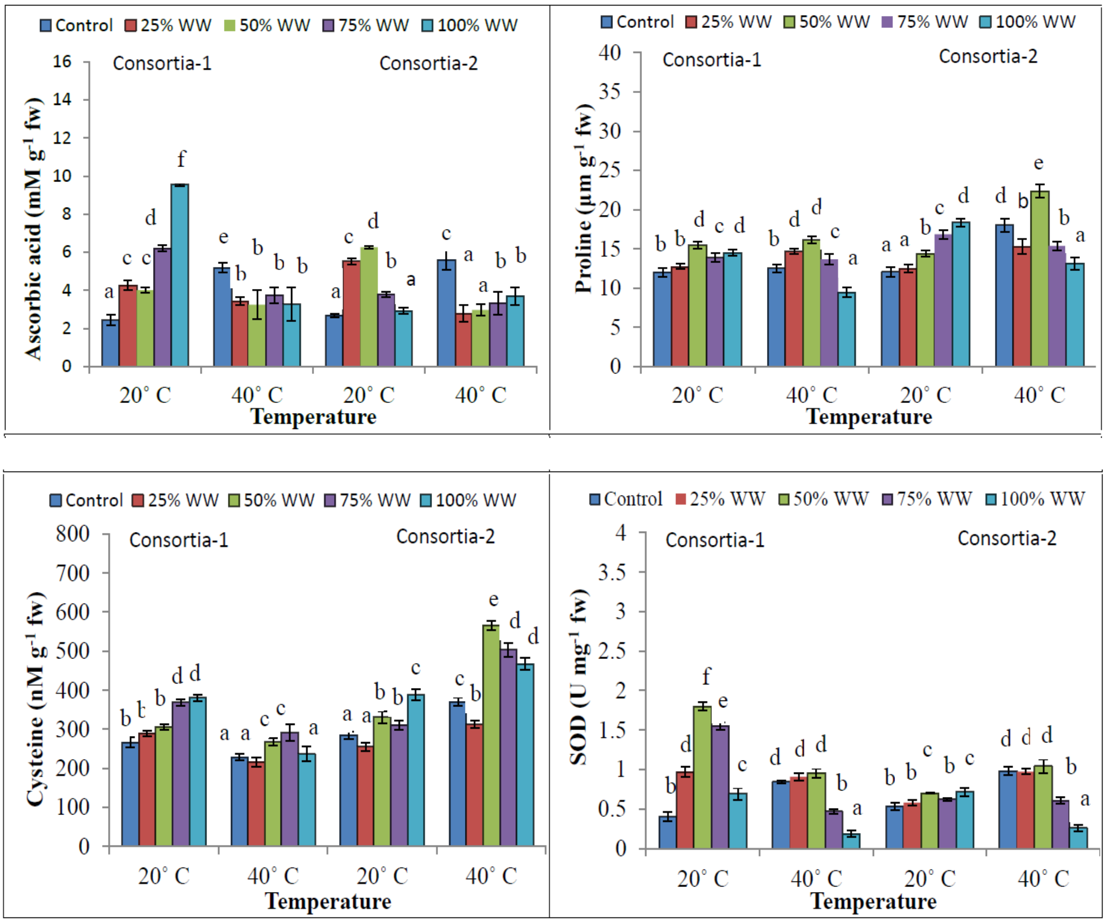
Moreover, temperature stimulates the enhanced generation of oxidative radicals, shift absorption spectra and cellular restructuring by modifying the level of proteins, fatty acids, and carbohydrates in the cell (Yong et al., 2018).

8.8 Effect of temperature regimes and wastewater concentration on antioxidants (Non-enzymatic and enzymatic antioxidants) activity of algal consortia

Fluctuating temperature induces several changes by increasing generation of ROS in microalgae (Chokshi et al., 2015). The temperature induced ROS generation is counterbalanced by different antioxidants (Gill and Tuteja, 2010). At 20˚ C, the results showed that ascorbic acid was increased at 25% (2.06 folds) and 50% (2.33 folds) wastewater concentration in consortia 2 while, 75% (2.55 folds) and 100% (3.93 folds) concentration of wastewater in case of consortia 1. The results also exhibited that proline content increased at 25% (1.06 folds) and 50% (1.29 folds) wastewater concentration in consortia 1 while, in consortia 2, an increase of 1.39 and 1.51 folds was observed at 75% and 100% of wastewater concentration. Similarly, the cysteine content in consortia 1 was increased by 1.15, 1.38 and 1.43 folds while in case of consortia 2, 1.16, 1.09 and 1.36 folds after treatment with 50%, 75% and 100% wastewater concentration, respectively (Fig. 8.5). At 40˚ C, a significant decline in ascorbic acid content was found to be in consortia 1 and 2 at different municipal wastewater concentration in comparison to control. The results also depicted that proline content in case of consortia 1 was increased by 1.17, 1.29 and 1.09 folds at 25%, 50% and 75% while an increase of 1.23 folds was found to be at 50% wastewater concentration in case of consortia 2. Moreover, consortia 1 also showed an increase of 1.17, 1.27 and 1.03 folds while, 1.52, 1.35 and 1.26 folds in cysteine content in consortia 2 at 50%, 75% and 100% wastewater concentration. The upregulation in the activity of different antioxidants under abiotic stress conditions is the response towards elevated generation of ROS (Qiao et al., 2021). Ascorbic acid acts as the enzyme cofactor, scavenge ROS, regulate photosynthesis, synthesis of hormone and regeneration of antioxidants (Pehlivan, 2017). The increased accumulation of ascorbic acid at 20˚ C in the present study may assist to sustain the proper functioning of photosynthetic system by scavenging of excessive oxidative radicals from the cell. Gallie, (2013) also reported that ascorbic acid is vital for photosynthetic function of cell as it protect the photosynthetic apparatus from the oxidative damage. The results further depicted the increment in proline accumulation in selected algal consortia treated with different wastewater concentration and temperature regimes. The increment in proline accumulation may maintain the redox balance and repair damaged cellular structures in consortia 1 and 2 cultivated under different wastewater and temperature conditions. Chokshi et al., (2015) also observed the significant increment in proline content at temperature conditions of 35° C and 38° C. Thus, in the present study, the higher proline accumulation may be due to its role as compatible solute, ROS scavenger, sustain redox balance and stabilize the cellular structure (Danouche et al., 2022). The results also showed that the cysteine accumulation enhanced at 20˚ C and 40˚ C along with different concentrations of wastewater. The increased cysteine accumulation could be attributed to their role in scavenging of different ROS under unfavourable conditions (Upadhyay et al., 2016).

At 20˚ C, consortia 1 showed the highest increase of 2.42, 4.50, 3.87 and 1.72 folds in SOD activity at 25%, 50%, 75% and 100% of municipal wastewater concentration. Catalase activity was also increased by 3.14 folds at wastewater concentration of 50% in consortia 1 while, consortia 2 showed an increase of 2.33, 3.96 and 5.00 folds at 25%, 75% and 100% concentration of municipal wastewater.

The results of present study also showed an increase of 1.01, 1.04, 1.10 folds in GR activity in consortia 1 at 25%, 75% and 100% concentration of wastewater, respectively (Fig. 8.5). At 40˚ C, an increase of 1.07 and 1.13 folds in SOD activity was shown by consortia 1 at 25% and 50% while, in case of consortia 2, an increase of 1.06 folds was at 50% concentration of wastewater. The results also depicted an increase of 1.14, 1.00 and 1.12 folds in catalase activity with consortia 1 at 25%, 50% and 75% while 1.60, and 1.12 folds with consortia 2 at 25% and 50% wastewater concentration. Moreover, wastewater concentration above 50% resulted in decreased SOD and catalase activity in consortia 2 than consortia 1. Similarly, an increase of 1.00, 1.04, 1.02, 1.01 folds in GR activity was observed with consortia 1 while 1.04, 1.08, 1.07, 1.06 folds with consortia 2 at 25-100% concentrations of municipal wastewater.



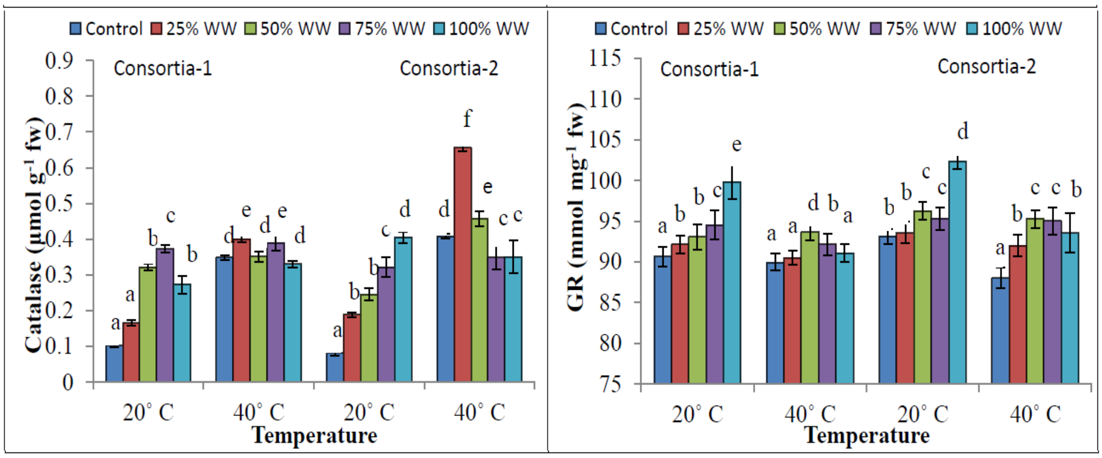


Fig. 8.5 Antioxidant activity of consortia 1 and 2 treated with municipal wastewater under different temperature regimes

SOD is an important enzymatic antioxidants widely present in aerobic organism and their activity varies with the temperature (Chokshi et al., 2020), metals (Danouche et al., 2020), salinity and UV radiation (Singh et al., 2018). The increased activity of enzymatic antioxidants in this study reflects the elevated generation of $O_2^{\bullet-}$, H_2O_2, and $OH^{\bullet}$ radicals in the cell under different wastewater concentration at 20˚ C. The increased SOD activity may acts as first defense line by mediating the conversion of O_2^{-1} into H_2O_2 (Qi et al., 2022) thus, prevents the generation and initiation of series of reaction responsible for ROS generation. Chakrabortty and Pradhan (2011) also observed that the enzymatic antioxidants like SOD and CAT showed increment in their activity below 50˚ C while the activity of glutathione reductase declined at the temperature ranging from 20˚ C to 50˚ C. In this study, the higher SOD and CAT activity at 20˚ C indicated that consortia 1 and 2 possess strong defensive mechanism to adjust under different wastewater concentrations. Moreover, the increment in catalase activity was in accordance with Choksi et al., (2015). The results further depicted the declined SOD and CAT activity in consortia 1 and 2 at higher wastewater concentration (75%, 100%) and temperature condition (40˚ C). The declined SOD and

CAT activity could be linked with the enhancement in the oxidative stress biomarkers such as TBARS and H_2O_2. The results also exhibited an increased GR activity with wastewater concentrations and temperature regimes in consortia 1 and 2. The increased GR activity confers tolerance to microalgae by regeneration of GSH, sustains the ratio of GSH/GSSG and alters the redox state of essential components of ETC **(**Singh et al., 2018)**.** Under temperature stress, GR also protects the functioning of PS-II by maintaining the transport of electron to the acceptor side of PS-II and increases the stability of PS-II complexes (Gill et al., 2013). Thus, in this study, the increased GR activity in consortia 1 and 2 may assist to sustain the ROS balance and the functioning of photosynthetic machinery. Further, the results of this study strengthen the evidence that GR play key role in protecting and maintaining the photosynthesis process under different temperature and wastewater concentration. The considerable increment in activity of antioxidants might be the adaptive strategy to mitigate the oxidative damage induced by temperature stress (Chokshi et al. 2020). Overall, the outcome of the present investigation reflected that consortia proficiently adopted different stress mitigation mechanisms such as alteration in cell physiology, enhancement in non enzymatic and enzymatic activity to avoid oxidative damage to the cell.

Chapter 9

Competence of algal consortia under natural environment: remediation efficiency, photosynthetic performance and antioxidants activity

Chapter-9

Competence of algal consortia under natural environment: remediation efficiency, photosynthetic performance and antioxidants activity

9.0 Introduction

Rapidly growing population and human interferences is posing serious challenges especially in terms of depleting energy reserves (Owusu and Asumadu-Sarkodie, 2016) and mounting water pollution problem (Liyanage and Yamada, 2017). The indecent planning has lead to depletion of energy reserves (Owusu and Asumadu-Sarkodie, 2016) and deterioration of freshwater resources at very alarming rate (Mushtaq et al., 2020). The foremost reason is rapid use of energy and water resource for industrial, agricultural and household purposes. Almost 69% of the India's population is still residing in rural areas and generates huge quantity of wastewater but are lacking the proper treatment system for efficient management of wastewater (Choudhary et al., 2016). Wastewater from different sources disposed into the water bodies poses severe threat to human and animal life by depleting the freshwater quality owing to abundance of macronutrients, micronutrients and other toxic pollutants. Wastewater is more troublesome problem in the populated countries like India and treatment before disposal is necessary to combat the damage posed by different pollutants (Shahid et al., 2020). The treatment facilities are devoid of the innovative techniques that can sustain the energy demands and remediate wastewater in environmentally sound manner. Several approaches has been employed for wastewater treatment (Wang et al., 2017a) but merely microalgae can stabilize the physico-chemical attributes of wastewater with simultaneous detoxification of multiple pollutants in very cheap and sustainable manner (Singh et al., 2020). Microalgae are also the sole organism that can efficiently remediate wastewater and concomitantly produce biomass for bio-energy production (Khan et al., 2018).

Wastewater from different source is affluent in both macro as well as micronutrients necessary for proper growth of microalgae (Khan et al., 2018). Beside nutrients, wastewater has plentiful of heavy metals and recalcitrant pollutants that can have deleterious impacts on the recipient ecosystem (Mushtaq et al., 2020). Heavy metals can also help microalgae to accomplish several functional roles as some metals are very essential for protein, enzymes and antioxidants activity (Andrade et al., 2004). At higher concentrations, heavy metals can cause deleterious effects by stimulating lipid per-oxidation in microalgae. In order to respond effectively to the oxidative stress posed by reactive oxygen species (ROS), microalgae stimulates the activity of defense system that detoxifies metals from contaminated environment in diligent manner (Shivaji and Dronamaraju, 2019).

The success of integration techniques can be made possible by selecting resilient microalgae species having vigorous growth and efficient bioremediation potential for nutrients and toxic pollutants. Qin et al. (2016) while comparing monoculture and consortia for purification of diary wastewater observed that consortia were more efficient in treating wastewater and simultaneously lipids accumulated was appropriate for biofuel production. Consortium in comparison to monoculture are more adaptable, stable and also have high pollutant removal rate along with biomass production (Chen et al., 2015). Environmental condition varies with regions so it is necessary to exploit native microalgae species and form different combination of consortia that have wider adaptability, excellent metal removal efficiency and also grow vigorously in the prevailing environmental conditions. Therefore, this study was conducted to examine the potential of the algal consortia treated with municipal wastewater under natural environment.

9.1 Results and discussion

9.1.1 Effect on physico-chemical attributes; nutrient load and metal concentration after treatment of municipal wastewater with algal consortia

Different attributes of wastewater before and after treatment were analyzed and are mentioned in table 9.1. The results showed that highest increase of 47.61% in pH was observed in case of consortia 2 treated with 100% concentration of municipal wastewater. The results also exhibited that DO level of municipal wastewater treated with algal consortia 1 and 2 was enhanced as compared to the initial concentration. It was further observed that EC, TDS, TSS, TS and BOD was decreased by 59.15%, 53.85%, 90.52%, 55.85% and 73.08% in case of consortia 1 while 63.81%, 56.06%, 87.04%, 55.64% and 69.26% in case of consortia 2. Similarly, the reduction of 77.44% and 64.37% in the nitrate nitrogen and phosphorus concentration was observed in case of consortia 1 while 82.92% and 70.47% with consortia 2 at 100% wastewater concentration (Table 9.1). Overall, the results depicted that consortia 2 showed promising potential to reutilize the nitrogen and phosphorus resource from the wastewater. In the present study, the decreased EC, TDS, BOD, nitrate-nitrogen and phosphorus content could be ascribed to the utilization of nutrients, salts and organic matter as the source of energy for cellular growth. The excellent nutrient assimilation from wastewater by algal consortia could be utilized for cell growth, bio-compound synthesis and biomass production. The decreased suspended solids and nutrients level in wastewater treated with consortia 1 and 2 could also be attributed to flocculation stimulated by the increased pH level of the treated water. Whitton et al., (2015) also observed that microalgae assimilate nutrients from wastewater and acts as the natural oxygen pump in water bodies.

Table 9.1 Effect on physico-chemical attributes of wastewater after treatment with consortia 1 and 2 under natural conditions

Parameters	**Initial concentration**	**Consortia-1**		**Consortia-2**	
		Control	**100% WW**	**Control**	**100% WW**
pH	7.56±0.028	10.67±0.145^{b}	9.68±0.576^{a}	11.16±0.610^{bc}	9.65±0.340^{a}
EC (µS/cm)	1016.67±54.55	6.12±0.080^{a}	415.23±9.51^{b}	5.46±0.105^{a}	367.9±6.64^{b}
TDS	556.66±46.72	3.39±0.210^{a}	256.89±3.67^{b}	3.14±0.095^{a}	244.56±6.88^{b}
TSS	5.34±0.354	0.474±0.029^{a}	0.506±0.012^{b}	0.536±0.060^{b}	0.692±0.082^{c}
TS	567.89±5.76	3.46±0.100^{a}	250.67±5.13^{b}	4.93±0.320^{a}	251.89±8.02^{b}
BOD	65.66±3.00	0.630±0.055^{a}	17.67±1.63^{b}	0.847±0.069^{a}	20.18±1.75^{b}
DO	-	8.37±0.266^{b}	2.56±0.33^{a}	8.44±0.345^{b}	2.41±0.193^{a}
NO_3-N	42.87±2.34	1.04±0.186^{a}	9.67±0.445^{c}	1.21±0.070^{a}	7.32±0.265^{b}
PO_4^{3-}	10.33±0.890	0.623±0.155^{a}	3.68±±0.205^{b}	0.650±0.123^{a}	3.05±0.201^{b}

All values are in mg/L, otherwise mentioned; WW- Wastewater

The initial concentration of different metals in wastewater were also analyzed and the results showed that Zn (11.14±0.673 µg/L), Fe (1719.12±15.129 µg/L), Pb (12.76±0.845 µg/L), Cd (4.87±0.176 µg/L), Cr (5.12±0.312 µg/L), Cu (5.15±0.767 µg/L), Ni (15.74±0.456 µg/L), and As (0.631±0.089 µg/L) were above the optimum limit in wastewater. The municipal wastewater after treatment was algal consortia 1 and 2 depicted that metal removal efficiency of consortia 2 was higher as compared to consortia 1. The removal efficiency of 57.23%, 73.70%, 64.23%, 53.12%, 62.49%, 48.12%, 60.34%, 31.21% and 70.29%, 62.23%, 67.69%, 60.11%, 50.56%, 55.43%, 68.46%, 45.23% for Fe, Zn, Pb, Cr, Ni, Cd, Cu, As was observed in case of consortia 1 and 2 respectively, after treatment with 100% concentration of wastewater (Fig 9.1). The significant removal of metals from the wastewater may be attributed to binding with cell surface which may facilitate their transport into the cell. The metals detoxification could be also attributed to phytochelatins that elevated the removal efficiency of selected algal

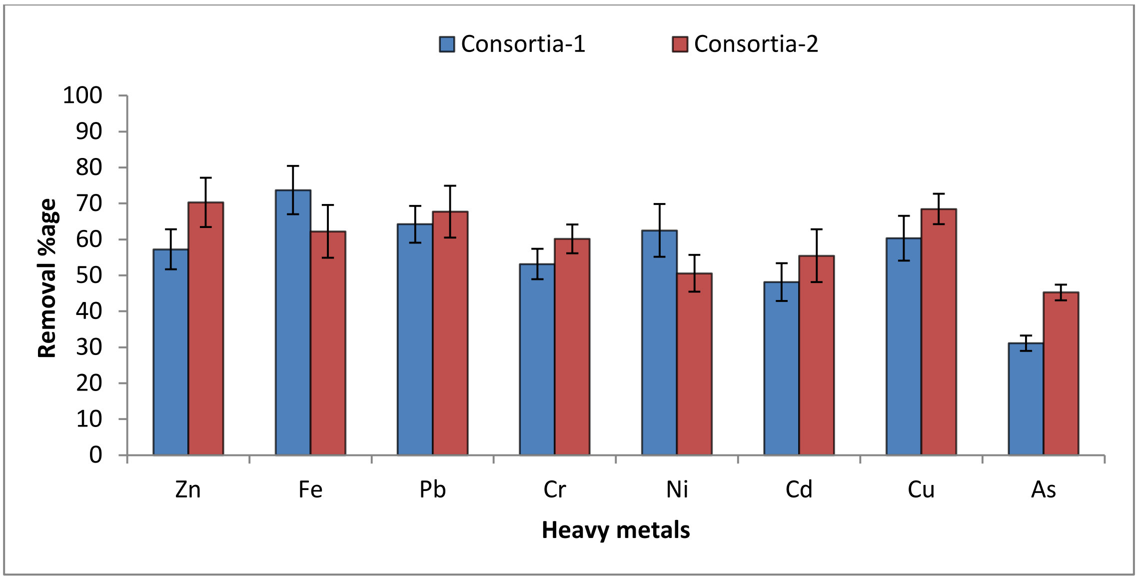


Fig. 9.1 Heavy metal detoxification efficiency of algal consortia 1 and 2 grown under natural environment

consortia. Tripathi and Poluri, (2021) reported that phytochelatins protect the microalgae from heavy metal damage by acting as signalling molecule, antioxidants and reduces the concentration by binding with heavy metals. In the present study, functional groups in algal biomass may improve the remediation efficiency was also evident from FTIR spectra.

FTIR spectroscopy is vital for determination of different functional groups in algal biomass treated with municipal wastewater. Based on composition and presence of functional group, each band in bio-molecule is assigned to specific molecular group. The relative abundance of carbohydrates, proteins, and lipids in consortia treated with BG-11 and wastewater were compared for carbohydrates (900–1100 cm^{-1}), lipids (1738 cm^{-1}; 3000-2800 cm^{-1}) and proteins (1540; 1658 cm^{-1}). The FTIR spectroscopy of the algal biomass depicted absorption spectra in the range from 800–400 cm^{-1}, 1400–1000 cm^{-1}, 1700–1500 cm^{-1} and 4000–3000 cm^{-1} which represents aliphatic, alcoholic, carboxylic and hydroxyl groups, respectively (Kumar et al., 2019). The sharp spectral peaks at 1060.0 cm-1 and 1077.9 cm-1 in wastewater treated consortia 1 and 2 could be attributed to presence of polysaccharide (Werner et al., 2007).The FTIR results showed absorption peaks at 1656.0 cm^{-1} and 1656.6 cm^{-1} in untreated consortia 1 and 2 could be attributed to amide I and II bands of protein whereas spectral peaks in region between 1300-1450 cm^{-1} in treated as well as untreated consortia indicated CH_3 and COOH groups associated with proteins and lipids (Fig. 9.2). The results showed that the spectral peaks at 1534.5 cm^{-1} disappeared in treated consortia 1 while consortia 2 showed spectral peaks at 1554.9 cm^{-1} treated with 100% concentration of wastewater. Furthermore, the absorption peak in the range between 1800-1000 cm^{-1} reflects the presence of C–O, C=O, and C=C in algal biomass (Hasan et al., 2022). The shifting and broadness of peak between 3400-3300 cm^{-1} in consortia 1 and 2 from 3323.3 to 3307.2 and 3370.9 to 3375.0 cm^{-1} respectively, may be due to interaction between metals and functional groups. The shifting of absorption peak in treated consortia could be attributed to formation of complexes between metals and OH groups (Li et al., 2017). Moreover, the spectral peaks between 3500–3200 cm^{-1} could be attributed to abundance of proteins and lipids in the algal biomass (Arun et al., 2017). Therefore, the FTIR spectroscopy depicted that prevalence of distinct functional groups may leads to the bio-adsorption of metal pollutants from the municipal wastewater.

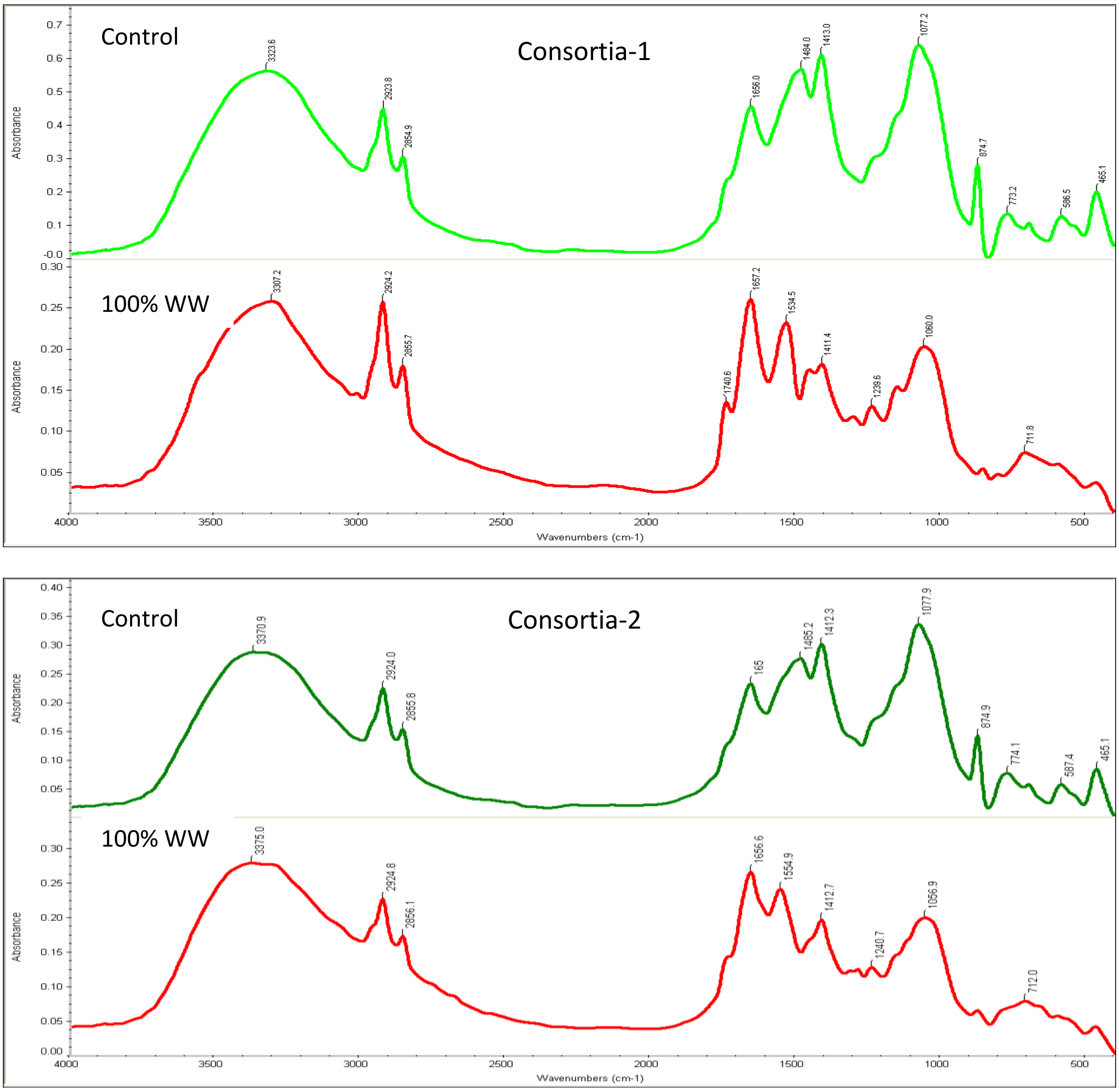


Fig. 9.2 Analysis of algal biomass through FTIR spectroscopy after treatment with municipal wastewater

9.2 Modification in biochemical profile of algal consortia 1 and 2

The results showed that growth (Fig. 9.3) and biomass production (Table 9.2) was decreased significantly in wastewater treated consortia 1 and 2. The results also showed that biomass production was highest in case of consortia 2 (4.296 mg/L/day) than consortia 1 (3.333 mg/L/day) treated municipal wastewater (100%). The inhibited growth rate and biomass production in treated consortia than their control might be due to the

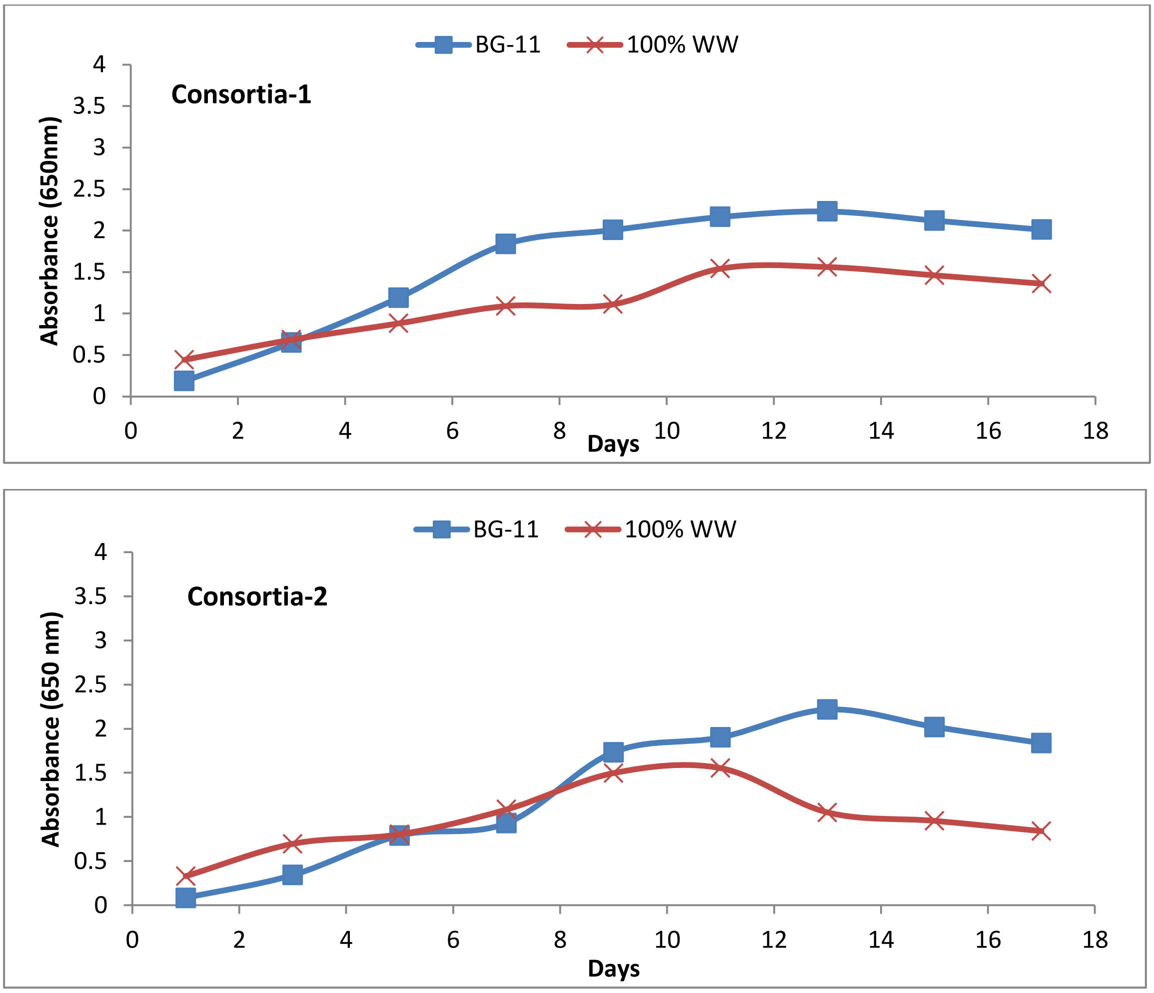


Fig. 9.3 Effect of municipal wastewater on growth profile of microalgae consortia 1 and 2 cultivated under natural conditions

wastewater contamination as depicted by initial EC, TDS, TS, BOD and nutrient level of municipal wastewater. The results were in concurrence with Gonzaslez-Camejo et al., (2017). The results further showed that total chlorophyll content was decreased by 62.09% and 10.25% in case of consortia 1 and 2 respectively, as compared to their respective control (Table 9.2). It was also observed that that carbohydrate content was decreased by 43.70% and 7.21% in consortia 1 and 2 treated with 100% concentration of municipal wastewater. The decreased total chlorophyll and carbohydrate content could be attributed to heavy metals and fluctuating environmental conditions stimulated oxidative damage to microalgae. Reduction in chlorophyll content could be ascribed to chlorosis (Piotrowska-Niczyporuk et al., 2015), leakage of electrolytes and triplet chl formation (Sharma et al., 2012) that damage photosystem and photosynthetic machinery of microalgae (Cardona et al., 2018). Pontevedra-Pombal et al., (2012) also observed that heavy metals distort the structure of the chloroplast membrane, suppress the activity of photosystems, ETC and also perturbs the activity of light harvesting as well as oxygen evolving complex. It was further observed that carotenoid content was increased by 1.57 and 2.23 folds in treated consortia 1 and 2 as compared to their respective control. The increased carotenoid content in algal consortia 1 and 2 might help to sustain the balance of ROS in the cell. Cirulis et al., (2013) also reported that carotenoids protect microalgae from ROS damage and their concentration in cell increases at low level of oxidative stress. Furthermore, carotenoids serve as antioxidant, protect from oxidative damage and assist in amplifying the tolerance capacity of microalgae (Goiris et al., 2012). The results also exhibited an increase of 1.40 folds in protein content in case of consortia 2 while, the decline of 16% was observed in case of consortia 1. The decline of chlorophyll and protein content in consortia 1 might be attributed to their use as source of nutrient under adverse conditions (Carfagna et al., 2013). Moreover, the decrease in protein content in algal consortia 1 may be ascribed to diversion of carbon skeleton from protein synthesis towards accumulation of TAG thereby protects algae from oxidative damage (Msanne et al., 2012). The decline in biochemical parameters like protein, carbohydrate and chlorophyll in microalgae exposed to stress signify that cell has revamped its composition in response to oxidative stress imposed by heavy metals (Arora et al., 2017). The results further exhibited that the lipid content was increased by 4.01 and 1.17 folds in wastewater treated consortia 1 and 2 in comparison to their respective control (Table 9.2). In the present study, the enhanced lipid accumulation in wastewater treated consortia 1 and 2 could be attributed to nutrient imbalance, salinity and toxic pollutants. Furthermore, under natural condition, the fluctuating light and temperature conditions may also serve as the stress factor which elevates the lipid accumulation in algal consortia. The increased lipid content is also predicated at the outlay of proteins and carbohydrates in microalgae (Arora et al., 2017). The results were in agreement with Saranya and Shanthakumar, (2019). It was also evident from the FTIR absorption spectra that two distinct fingerprints representing triglycerides accumulation were present in biomass of algal consortia. The absorption peak at 1740.6 cm^{-1} in wastewater treated consortia 1 may be attributed to lipid triglycerides and also represents the abundance of fatty acids in the microalgae. The results further depicted that spectral peak between 3000-2800 cm^{-1} was higher in treated consortia 1 and 2 than their respective control. The higher absorption peaks (3000-2800 cm^{-1}) in municipal wastewater treated consortia 1 and 2 is associated with asymmetric vibration of C-H bands of methyl and methylene groups (Wagner et al., 2010). Overall, the spectral peak confirms that municipal wastewater induced higher lipid accumulation in wastewater treated consortia 1 cultivated under natural conditions.

Table 9.2 Effect of municipal wastewater on biomass production, pigment content, biochemical profile, oxidative stress biomarkers and antioxidant activity of consortia 1 and 2 cultivated under natural conditions

Parameters	Consortia-1		Consortia-2	
	Control	100% WW	Control	100% WW
Biomass production (mg/L)	5.592±0.195[c]	3.330±0.191[a]	5.972±0.510[c]	4.296±0.444[b]
Total chlorophyll (mg g^{-1} fw)	0.124±0.045[c]	0.047±0.002[a]	0.117±0.014[bc]	0.105±0.006[b]
Carotenoids (mg g^{-1} fw)	1.43±0.086[c]	0.909±0.069[b]	1.401±0.115[c]	0.627±0.051[a]
Carbohydrate (mg L^{-1} fw)	8.58±1.71[b]	4.83±0.933[a]	12.19±0.758[c]	11.31±0.722[c]
Protein (mg g^{-1} fw),	1.58±0.096[a]	1.36±0.047[a]	2.34±0.339[b]	3.28±0.388[c]
Lipid (mg L^{-1})	0.224±0.033[a]	0.899±0.039[b]	1.10±0.073[c]	1.29±0.066[c]
TBARS (µmol g^{-1}fw)	8.04±0.298[b]	12.62±0.458[c]	5.42±0.201[a]	6.93±0.622[ab]
H_2O_2 (µmol g^{-1}fw)	13.16±2.35[b]	20.18±1.57[c]	10.96±0.685[a]	15.29±1.09[bc]
Ascorbic acid (mM g^{-1} fw)	0.296±0.010[a]	0.613±0.074[b]	0.357±0.059[a]	0.869±0.095[b]
Cysteine (nM g^{-1} fw)	14.14±2.72[a]	34.26±2.03[c]	22.36±1.17[b]	28.99±0.699[bc]
Proline (µm g^{-1} fw)	0.032±0.008[a]	0.052±0.005[a]	0.023±0.002[a]	0.077±0.004[b]
SOD (U mg^{-1} fw)	364.91±15.12[b]	639.69±12.08[c]	231.06±5.57[a]	313.26±7.12[ab]
Catalase (µmol g^{-1} fw)	0.458±0.046[a]	1.21±0.165[b]	0.511±0.038[a]	1.07±0.179[b]
GR (mmol mg^{-1} fw)	8.13±0.348[a]	9.74±0.477[b]	8.52±0.274[a]	10.21±0.430[c]

9.3 Photosynthetic performance of algal consortia grown under natural environment

The chlorophyll fluorescence is the accurate alternative to examine the metabolic activity and photo-protective mechanism adopted by the cell (Solovchenko et al., 2022). The results reflected that Fv/Fo ratio was increased by 41.65% folds in wastewater treated consortia 2 as compared to control. The Fv/Fo was decreased significantly by 48.62% in consortia 1 treated with 100% municipal wastewater (Fig. 9.4). Fv/Fo reflects the efficiency of oxygen evolving complex (Guo et al., 2016) and the results depicted that efficiency of wastewater treated consortia 2 was higher as compared to consortia 1. $F_v/F_{m,}$ reflects the photosynthesis efficiency and the value between 0.6-0.65 is considered as the indicator of healthy algae (Parkhill et al., 2001). The results exhibited that highest Fv/Fm was observed in treated (0.435) consortia 2 than untreated consortia (0.336). It was also observed that Fv/Fm was increased by 29.46% in consortia 2 while, the decrease of 53.22% in Fv/Fm ratio was observed in case of consortia 1. The notable decline in Fv/Fm value in consortia 1 could be attributed to either damage or down regulation of PS II activity as a response to stress conditions. Furthermore, the decreased Fv/Fm value could be ascribed to aggravated oxidative damage, reduction of proteins content as well as the photosynthetic rate which in combination posed cumulative damage to the functioning of PS-II. The Pi_{abs} is essential parameter which reflects the functioning of PS-I and PS-II. Pi_{abs} also provides quantitative information of the photosynthetic performance under unfavourable conditions (Strasser et al., 2004). The Pi_{abs} indicates overall photosynthetic performance and results showed that the photosynthetic performance of consortia 2 was

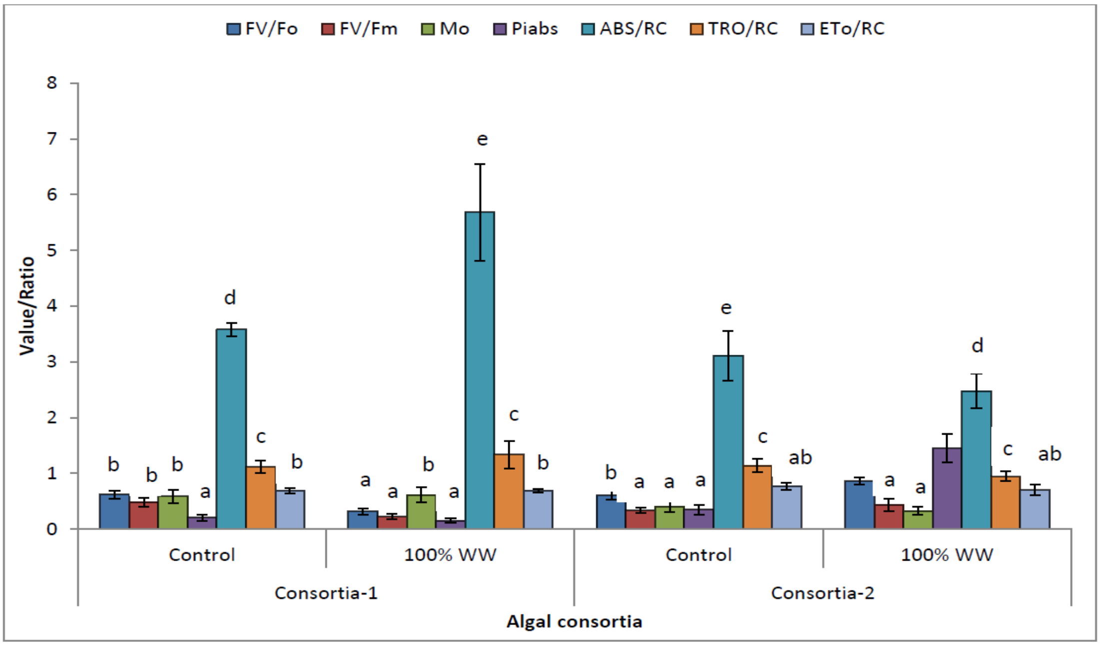


Fig. 9.4 Effect of municipal wastewater on active photosystem II reaction centre (Fv/Fo), quantum efficiency of photosystem II (Fv/Fm), net closing rate of reaction centre (Mo), photosynthetic performance index (Pi_{abs}), apparent antenna size of reaction centre (ABS/RC), trapping flux (TRo/RC) and electron transport per reaction centre (ETo/RC) in algal consortia 1 and 2 grown under natural environment

higher than consortia 1. The results showed that Pi_{abs} value was increased by 320.28% in case of consortia 2 as compared to untreated consortia. There was about 28.36% decrease in the photosynthetic performance of consortia 1 grown in the municipal wastewater. The decreased Pi_{abs} value in treated consortia 1 indicates toxicity to algal cells. It has been reported that metals affects the functioning of PSII and electron transport chain by replacing iron between QA and QB binding sites (Li et al., 2012) which eventually disturbs the equilibrium of ROS production and scavenging. The increased ROS production also impairs the defense responses of microalgae (Singh and Singh, 2022). Furthermore, the results revealed that Mo, ABS/RC and TRo/RC were increased by 4.09%, 58.93% and 19.62% in consortia 1 treated with municipal wastewater. However, the decrease of 19.01%, 20.46%, 17.36% and 8.93% in Mo, ABS/RC, TRo/RC and ETo/RC ratio was observed in the case of consortia 2 as compared to control (Fig. 9.4). The total flow of energy in the pigment antenna (ABS) and in the PSII centre (TRo) was normalized against the reaction centre (RC) (Wang et al., 2012). The treated cells showed higher value of Mo, ABS/RC, TRo/RC and ETo/RC ratio in case of consortia 1 in comparison to control. This result could be interpreted in terms of decrease in the active reaction centre (RC) in the wastewater grown cells of consortia 1 than consortia 2. Overall, the results of different photosynthetic parameters reflected that consortia 2 showed greater flexibility under natural environmental conditions than consortia 1.

9.4 Oxidative stress markers

The balance of ROS production and their scavenging is disturbed by stress condition leading to elevated production of ROS which have deleterious impacts on the cellular macromolecules (Sharma et al., 2012). In the present study, the results depicted that TBARS content was increased in consortia 1 (1.56 folds) and consortia 2 (1.27 folds) treated with 100% municipal wastewater. The increased TBARS accumulation could be attributed to wastewater triggered damage which perturbs the cellular balance of algal consortia. Elbaz et al., (2010) also observed that TBARS, a lipid peroxidation by-product enhanced with the exposure of heavy metal to the microalgae. Similarly, the H_2O_2 content was increased in consortia 1 (1.53 folds) and consortia 2 (1.39 folds) after treatment with municipal wastewater than control (Table 9.2). The increased concentration of H_2O_2 could be attributed to the oxidative damage imposed by metal pollutants, salts, nutrients and environmental conditions in selected algal consortia. Danouche et al., (2020) also observed the increased H_2O_2 accumulation may be due to the disruption of cellular balance, damage to cell membrane and essential bio-molecules after treatment with heavy metals. The enhancement in H_2O_2 content under stress indicates the damage to the lipid layer which leads to the accumulation of TBARS in the cell (Wei et al., 2011). The wastewater induced oxidative stress as evident from the increased concentration of TBARS and H_2O_2 in algal consortia 1 and 2 than control may be mitigated by the combination of biochemical alteration, enzymatic and non enzymatic pathways. Overall, the result depicted that consortia 2 showed excellent flexibility than consortia 1 under natural conditions. The increased accumulation of oxidative stress markers in algal consortia treated with wastewater is in concurrence with Sharma et al., (2020) and Singh et al., (2021).

9.5 Enzymatic and non- enzymatic antioxidants

In the present study, an increase of 2.07, 1.62 and 2.42 folds in ascorbic acid, proline and cysteine content was observed in case of consortia 1 treated with 100% municipal wastewater (Table 9.2). The results also showed that ascorbic acid, proline and cysteine was increased by 2.43, 3.34 and 1.29 folds in case of consortia 2. The increased content of ascorbic acid may maintain the cellular balance and protect the cell from ROS (Leong and Chang, 2020). The increment in proline accumulation may protect enzymes, maintain osmoregulation, and scavenge ROS, thereby encourage microalgae to adjust the intracellular mechanism under stressful environment. Szabados and Savoure, (2010) also observed that proline is involved in the stabilization of protein, enzymes, maintains integrity of the membrane and osmotic balance of the cell. Furthermore, proline ameliorates the tolerance of microalgae to metals stress by reducing the damage triggered by heavy metals (Singh et al., 2015). The increased cysteine content could serves as precursor of sulphur containing peptides, proteins and several other compounds which are directly or indirectly involved in the metal detoxification from the contaminated environment (Leong and Chang, 2020). Therefore, present study revealed that the increased non-enzymatic antioxidants may protect the essential cellular components from oxidative damage and ensure cell survival under harsh conditions.

The results further depicted an increase of 1.75, 2.64 and 1.19 folds in SOD, catalase, GR activity in consortia 1 while, an increase of 2.09 and 1.19 folds in catalase and GR was observed in consortia 2 treated with 100% wastewater (Table 9.2). The increased activity of SOD, catalase, GR in consortia 1 may assist to maintain the cellular balance by scavenging hydroxyl, superoxide and peroxide radicals instigated by wastewater and unfavorable conditions under natural conditions. Shi et al., (2017) also observed that enzymatic antioxidants such as SOD and catalase converts the reactive oxygen species into non toxic form thus, prevents the damage to macromolecules and helps in maintaining the cell vitality. Similarly, GR not only aids in removal of H_2O_2 but also in oxidized ascorbate regeneration (Gest et al., 2013). Thus, the enhanced activity of enzymatic antioxidant may assist the algal consortia to negate the oxidative damage by scavenging ROS and maintain their balance in the cell.

Chapter 10

Summary and Conclusions

Chapter-10

Summary and Conclusion

Extraordinary population expansion is the forefront cause of freshwater, land, energy scarcity along with urbanization, industrial development, enormous waste generation, environmental pollution and ultimately global warming. Water pollution is the serious environmental issues which need to be addressed by developing stringent policies, planning and sustainable approaches. In India, the majority of the treatment facilities for wastewater management are deployed in urban areas but still, huge quantity of wastewater is discharged without any necessary treatment into the environment. The leading global problems require considerable attention to ensure the smooth functioning of life. Due to the incompetent treatment systems, the disposal of wastewater interrupts the functioning of biota and ultimately perturb the stability of aquatic ecosystem. The inefficient nutrient removal through secondary treatment and huge investment as well as energy required in tertiary treatment, it become important to develop the techniques which can transform the treatment plants from energy consumer to energy producers. Additionally, the concerns related to depleting energy reserves are raising with the growing population. The swift decline in fossil fuel reserves and rising price has increased the focus on renewable fuel that has potential to offset the challenges posed by fossil fuel scarcity. Currently, no standalone techniques have been devised that can address the problem of energy and water scarcity simultaneously. Compared to different physico-chemical methods, microalgae can serve as the important biological organisms that simultaneously meet the targets of wastewater treatment and energy production in sustainable manner.

Besides improving the vital attributes of the wastewater, the biomass obtained can be the cheap feedstock for the biofuel production.

Fluctuation of temperature and light intensity within day, day to day and with season is the natural phenomenon. Furthermore, under natural conditions, maintaining axenic and optimum environmental condition is impossible. Thus, screening algal consortia that can grow under different temperature and light intensity conditions can be the appropriate solution for wastewater treatment and biomass production. It is also unclear that whether the defense mechanism stimulated in the form of enzymatic and non enzymatic antioxidants assist in regulating the physiological and metabolic pathways against temperature and light stress. The aim of this study was to screen algal consortia that have ability to grow efficiently in the wastewater with excellent removal efficiency as well as promising potential of lipid production. Therefore, the systematic studies were carried out to examine the effect of municipal wastewater, light intensity and temperature on algal growth, biomass production, lipid accumulation, photosynthetic performance, biochemical profile, stress biomarkers, antioxidants system and eventually biofuel production of algal consortia. The present study will also help to screen the algal consortia adaptable under different light intensity and temperature regimes.

The first objective was to screen the monoalgal species that can adapt under natural environmental conditions. The monoalgal species were selected and their potential to survive under natural conditions was analyzed in terms of growth, pigment content and defense responses. In this study, the algal species *Chlorococcum humicola*, *Tetradesmus* sp. and *Oscillatoria* sp. were examined for their tolerance potential against different wastewater concentrations under natural environment. The results revealed that selected microalgae reutilized nutrient resource and removed heavy metals from municipal wastewater. Microalgae *Tetradesmus* sp. and *C. humicola* efficiently removed toxic metal pollutants from wastewater than the *Oscillatoria* sp. as evident from the improvement in the water quality parameters such as EC, TDS, TS, BOD and DO level of water. The total chlorophyll (82%), protein (37.50%) and carbohydrate content (123.12%) was observed highest in *Tetradesmsus* sp. after treatment with different concentration of wastewater while the maximum increase in lipid content (39.81%) was observed with *C. humicola* at 75% wastewater concentration. Furthermore, a compositional alteration showed by FTIR spectroscopy clearly demonstrated that wastewater (100%) enhanced lipid synthesis which was maximum in *C. humicola* and *Tetradesmus* sp. than the *Oscillatoria* sp. As compared to *Oscillatoria* sp., a greater increase in the level of anti-oxidative compounds in *Tetradesmus* sp. and *C. humicola* with simultaneous decline in the oxidative stress markers exhibited tolerant behaviour against the wastewater induced toxicity. In order to counterbalance the wastewater induced toxicity, *Tetradesmus* sp. showed highest proline (4.63 folds), catalase (4.95 folds), superoxide dismutase (1.76 folds) and glutathione reductase (7.00 folds) while ascorbic acid (9.25 folds) and cysteine (4.92 folds) activity was highest in *C. humicola* at different concentrations of wastewater. Similarly, the growth pattern, cellular modification, oxidative stress markers, and defense responses of *Chlorella vulgaris* and *Scenedesmus vacuolatus* were analyzed after treatment with municipal wastewater (25-100%). The results revealed that the nutrients and heavy metal removal was highest at 25% of wastewater concentration while maximum specific growth rate (0.0708 day^{-1}), total chlorophyll (0.118 mg g^{-1} fw), and carbohydrate content (24.06 mg L^{-1} fw) was observed with *Scenedesmus vacuolatus* at 50% concentration of wastewater. Under natural environment, the algal species showed declined pigment and carbohydrate synthesis at 75% and 100%

concentration of wastewater. Furthermore, the lesser accumulation of thiobarbituric acid reactive species and hydrogen peroxide while increment in antioxidants activity may be responsible for imparting tolerance to *Scenedesmus vacuolatus* against different concentrations of municipal wastewater. Under natural conditions, 50% and 100% wastewater concentration was suitable for excellent growth and lipid accumulation in *Scenedesmus vacuolatus*, respectively. Overall, it was observed that *Scenedesmus vacuolatus, C. humicola* and *Tetradesmus* sp. were tolerant to different wastewater concentrations and exhibited higher nutrient capturing potential, growth rate and lipid production due to their proficient cellular defense system.

In the 2^{nd} objective, the effort was made to form the algal consortia on the basis of nutrient reutilization, metal detoxification potential, lipid production and antioxidants defense response of monoalgal species under natural environment. Two algal consortia (consortia 1: *Tetradesmus sp.* + *Scenedesmus vacuolatus* and consortia 2: *Chlorococcum humicola* + *Scenedesmus vacuolatus* + *Tetradesmus sp.*) were employed for municipal wastewater treatment under laboratory conditions (light intensity 30 W/m^2 and temperature: 30° C). The results of remediation efficiency, growth profile and biomass production reflected greater adaptability and tolerance of consortia 1 against different concentrations of municipal wastewater. The photosynthetic parameters such as Fv/Fo, Fv/Fm, and Pi_{abs} were increased by 1.20-2.35 folds in consortia 1 treated with municipal wastewater (25-100%). Additionally, FTIR spectroscopy showed neutral lipid accumulation (1750 cm^{-1}) in consortia 1 after treatment with wastewater (100%). The oxidative stress markers showed considerable decline in consortia 1 as compared to consortia 2. Interestingly, increased antioxidant activity (1.02-4.14 folds) reflected that oxidative damage to consortia 1 was counterbalanced by the strong defense mechanisms. Overall, remediation efficiency, photosynthetic performance and antioxidants activity suggested that the consortia 1 can be used for efficient wastewater treatment and lipid production in sustainable manner.

In the 3rd objective, suitability of municipal wastewater as growth media for consortia was analyzed by examining the phyco-remediation potential, growth profile, biomass production and photosynthetic efficiency of consortia 1 and 2 under light intensities of 20 W/m^2 and 40 W/m^2. By the end of experiments under laboratory condition, significant decline of 40-90% in different physico–chemical attributes such as EC, TDS, BOD, nitrogen, phosphorus and heavy metals was observed with consortia 1 and 2 under light intensity of 40 W/m^2. Additionally, abundance of lipid, protein, and carbohydrates showed by FTIR spectroscopy in treated consortia 1 and 2 exhibits different functional groups for bio-adsorption of metal from wastewater. Higher light intensity (40 W/m^2) induced neutral lipid accumulation in consortia 1 was reflected by the absorption peak at 1750 cm^{-1} from FTIR spectra. Furthermore, the maximum total chlorophyll content and photosynthetic performance revealed that light intensity of 20 W/m^2 and 40 W/m^2 increased the functioning of PS II in consortia 1 than consortia 2. Simultaneously, the elevated ascorbic acid, proline, cysteine, suproxide dismutase, catalase and glutathione reductase activity may offset the damage posed by wastewater and light intensity. The results also exhibited that fatty acid responsible for good quality biofuel production were in abundant percentage in consortia 1 and 2 treated with wastewater (100%) under light intensity of 20 W/m^2 and 40 W/m^2. Overall, consortia 1 were competent to adapt under different wastewater concentrations and light intensities as evident by the increment in growth, photosynthetic performance, antioxidant activity and fatty acid composition while consortia 2 showed promising potential at 40 W/m^2.

Under natural conditions, the lack of control on temperature is major limitation for large scale cultivation of microalgae. Therefore, it is very imperative to examine the effects of different temperature regimes on remediation efficiency, growth, lipids, antioxidant activity and biofuel production in algal consortia. The results exhibited that temperature regime of 40˚ C enhanced the nutrient and metal removal efficiency of selected consortia with maximum in consortia 1. Highest photosynthetic performance and lipid accumulation indicated efficient tolerance and adaptability of consortia 1 at 20˚ C and 40˚ C. Further, the fatty acid profile revealed the highest percentage of SFA in consortia 1 (43.70%) and consortia 2 (46.99%) treated with wastewater (100%) at temperature regimes of 40˚ C. Furthermore, the maximum increment in TBARS (7.32 folds) and H_2O_2 (5.95 folds) was observed in consortia 2 at 40˚ C and 20˚ C, respectively. Antioxidants like ascorbic acid (3.93 folds), SOD (4.50 folds) and GR (1.10 folds) activity was also observed to increase in consortia 1 with wastewater concentration gradient at 20˚ C than 40˚ C. Overall, the results suggested that consortia 1 showed high photosynthetic efficiency, lipid production, antioxidant activity and dominance of fatty acid at 20˚ C and 40˚ C. Thus, consortia 1 can serve as the flexible system for wastewater treatment and bio-energy production under different temperature regimes.

The operating conditions in the laboratory and natural environment are totally different therefore; the selected algal consortia were examined for tolerance and adaptability under natural conditions. The results showed that consortia 2 was efficient in recovering nitrate-nitrogen (82.92%) and phosphorus (70.47%) from municipal wastewater. Further, the results exhibited highest metal removal efficiency in case of consortia 2 treated with municipal wastewater (100%). The excellent remediation efficiency of consortia 1 and 2 was also evident from the FTIR spectra.

The results further showed that total chlorophyll, carbohydrate and protein content was decreased significantly in consortia 1 treated with municipal wastewater than control. However, an increase of 4.01 and 1.17 folds in lipid content was observed in treated algal consortia 1 and 2 in comparison to their respective control. The enhancement in lipid accumulation was also confirmed through FTIR spectroscopy. The results exhibited the improved photosynthetic performance as evident from increased Fv/Fo, Fv/Fm and Pi_{abs} value in consortia 2. The results further depicted the maximum increase in TBARS (1.56 folds) and H_2O_2 content (1.53 folds) in wastewater treated consortia 1. In case of antioxidants, the maximum increase in ascorbic acid (2.43 folds), proline (3.34 folds) and cysteine (1.29 folds) was observed in consortia 2 while highest SOD (1.75 folds), catalase (2.64 folds), and GR (1.19 folds) activity was observed in consortia 1. The increased activity of antioxidants in selected algal consortia may assist to maintain the cellular balance by scavenging hydroxyl, superoxide and peroxide radicals instigated by wastewater and unfavorable conditions under natural environment. Overall, it can be concluded that due to remarkable nutrient recovery, metal removal and photosynthetic performance, consortia 2 could be exploited for wastewater remediation under natural environment.

Overall, algal consortia 1 showed excellent growth rate, biomass production, lipid yield, photosynthetic performance and biofuel production after treatment with wastewater under laboratory conditions while consortia 2 showed proficient ability under natural conditions. Therefore, wastewater treatment system utilizing microalgae consortia can serve as the futurists approach to deal with water pollution and energy scarcity simultaneously. This work provided the experimental evidence that consortia of different microalgae can be the practical approach to treat wastewater and augment biofuel production in economical and eco-friendly manner. Thus, the synergistic association of different microalgae may be the neoteric paradigm with multilevel benefits such as resource reutilization, heavy metal detoxification, and excellent defense mechanisms along with suitable fatty acid composition for biodiesel production.

Main outcome of the study:

- Microalgae *Scenedesmus vacuolatus*, *Tetradesmus* sp. and *Chlorococcum humicola* was more competent in removal of dissolved solids, organic matter, nutrients and metal pollutants from wastewater as evident from the improvement in the water quality parameters.
- Monoalgal species and their consortia were efficient to recover the nutrient resource and detoxify metals at 25% and 50% as compared to 75% and 100% wastewater concentration.
- Wastewater concentration of 50% improved growth, total chlorophyll and carbohydrate synthesis in *Scenedesmus vacuolatus*, *Tetradesmus* sp. and *Chlorococcum humicola*.
- Synergistic interactions and the perfect utilization of nutrient resource from wastewater stimulated the prolific growth of algal consortia 1 and 2.
- Maximum nutrients and metal removal along with efficient growth and biomass production reflected greater adaptability of consortia 1 than consortia 2.
- Algal consortia 1 was promising one to withstand different light intensities and temperature regimes as compared to consortia 2.
- Different municipal wastewater concentrations, light intensities and temperature regimes improved the photosynthetic performance of consortia 1.

- Under natural conditions, excellent photosynthetic performance confirmed that consortia 2 could serve as the promising bio-system to adapt in municipal wastewater.
- Lipid accumulation potential of treated consortia 1 was higher than consortia 2 under laboratory as well natural conditions.
- Under different light intensities and temperature regimes, FTIR spectroscopy confirmed wastewater induced TAG accumulation in algal consortia 1.
- Algal consortia 1 and 2 showed excellent performance at higher light intensity (40 W/m^2) in terms of growth, biomass production, photosynthetic efficiency and antioxidants activity.
- Increment in antioxidant activity in consortia 1 at 40 W/m^2 confirmed that oxidative stress was attenuated by the amplified activity of non-enzymatic and enzymatic antioxidants.
- Antioxidants like ascorbic acid, superoxide dismutase and glutathione reductase activity amplified in consortia 1 treated with wastewater concentrations at 20˚ C.
- FTIR spectroscopy showed that consortia 1 was enriched with different enzymatic antioxidants as well as phenolic compounds that could be responsible for imparting tolerance against wastewater (100%) and temperature stress (40˚ C).
- Light intensity (40 W/m^2) and temperature (40˚ C) enhanced the percentage of SFA in consortia 1 and 2.
- Palmitic acid was the dominants fatty acid in wastewater (100%) treated consortia 1 and 2 at different light intensity and temperature regimes.
- Promising remediation potential, photosynthetic performance and antioxidants activity reflected that consortia 1 and 2 could be exploited for wastewater treatment under both laboratory as well as natural conditions.

References

References

Abas, N., Kalair, A., and Khan, N. (2015). Review of fossil fuels and future energy technologies. *Futures*, 69: 31-49.

Abdel-Raouf, N., Al-Homaidan, A.A., and Ibraheem, I.B.M. (2012). Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences*, 19: 257–275.

Abeysiriwardana-Arachchige, I.S.A., Munasinghe-Arachchige, S.P., Delanka-Pedige, H.M.K., and Nirmalakhandan, N., (2020). Removal and recovery of nutrients from municipal sewage: Algal vs. conventional approaches. *Water Research*, 175, 115709.

Abinandan, S., and Shanthakumar, S. (2015). Challenges and opportunities in application of microalgae (Chlorophyta) for wastewater treatment: a review. *Renewable and Sustainable Energy Reviews*, 52, 123-132.

Aboul-Enein, A.M., El-Baz, F.K., El-Baroty, G.S., Youssef, A.M., and Abd El-Baky, H.H. (2003). Antioxidant activity of algal extracts on lipid peroxidation. *Journal of Medical Science,* 3, 87-98.

Ackova, D.G. (2018). Heavy metals and their general toxicity on plants. *Plant Science Today* 5, 15–19.

Adams, C., Godfrey, V., Wahlen, B., Seefeldt, L., and Bugbee, B. (2013). Understanding precision nitrogen stress to optimize the growth and lipid content tradeoff in oleaginous green microalgae. *Bioresource Technology*, 131, 188-194.

Adu-Mensah, D., Mei, D., Zuo, L., Zhang, Q., and Wang, J. (2019). A review on partial hydrogenation of biodiesel and its influence on fuel properties. *Fuel*, 251, 660-668.

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, 105, 121-126.

Afzal, I., Shahid, A., Ibrahim, M., Liu, T., Nawaz, M., Mehmood, M.A. (2017). Microalgae: A promising feedstock for energy and high-value products. *Algae Based Polymers, Blends, and Composites,* 55.

Aggarwal, A., Sharma, I., Tripathi, B.N., Munjal, A.K., Baunthiyal, M., and Sharma, V. (2012). Metal toxicity and photosynthesis. *Photosynthesis: overviews on recent progress and future perspectives*, 229-236.

Ahmad, J., Naeem, S., Ahmad, M., Usman, A, R., and Al, -Wabel, M,I. (2019). A critical review on organic micropollutants contamination in wastewater and removal through carbon nanotubes. *Journal of Environmental Management*, 246, 214-228.

Ahmed, S.F., Mofijur, M., Nuzhat, S., Chowdhury, A.T., Rafa, N., Uddin, M.A., Inayat, A., Mahlia, T.M.I., Ong, H.C., Chia, W.Y., and Show, P.L. (2021). Recent developments in physical, biological, chemical, and hybrid treatment techniques for removing emerging contaminants from wastewater. *Journal of Hazardous Materials*, 125912.

Ajayan, K.V., Selvaraju, M., and Thirugnanamoorthy, K. (2011). Growth and heavy metals accumulation potential of microalgae grown in sewage wastewater and petrochemical effluents. *Pakistan Journal of Biological Sciences*, 14(16), 805

Ajayan, K.V., Selvaraju, M., Unnikannan, P., and Sruthi, P. (2015). Phycoremediation of tannery wastewater using microalgae *Scenedesmus* species. *International Journal of Phytoremediation*, 17, 907-916.

Åkerström, A.M., Mortensen, L.M., Rusten, B., and Gislerød, H.R. (2014). Biomass production and nutrient removal by *Chlorella* sp. as affected by sludge liquor concentration. *Journal of Environmental Management*, 144, 118-124.

Akram, N.A., Shafiq, F., and Ashraf, M. (2017). Ascorbic acid-a potential oxidant scavenger and its role in plant development and abiotic stress tolerance. *Frontiers in Plant Science*, 8, 613.

Alalwan, H.A., Alminshid, A.H., and Aljaafari, H.A. (2019). Promising evolution of biofuel generations. Subject review. *Renewable Energy Focus*, 28, 127-139.

Ali, A.A., and Alqurainy, F. (2006). Activities of antioxidants in plants under environmental stress. *The lutein-prevention and treatment for diseases*, 187-256.

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

Ali, M., Masood, A., and Saleem, M. (2021). Microalgae cultivation in wastewater for simultaneous nutrients removal and biomass production. *International Journal of Energy and Environmental Engineering*, 12(3), 475–485.

Alia, M.P., and Matysik, J. (2001). Effect of proline on the pro-duction of singlet oxygen. Amino. Acids, 21, 195-200.

Álvarez, C., Ángeles Bermúdez, M., Romero, L.C., Gotor, C., and García, I. (2012). Cysteine homeostasis plays an essential role in plant immunity. *New Phytologist*, 193(1), 165-177.

Amerasinghe, P., Bhardwaj, R.M., Scott, C., Jella, K., and Marshall, F. (2013). Urban wastewater and agricultural reuse challenges in India (Vol. 147). IWMI.

Anand, J., and Arumugam, M. (2015). Enhanced lipid accumulation and biomass yield of *Scenedesmus quadricauda* under nitrogen starved condition. *Bioresource Technology*, 188, 190-194.

Anderson, R.A., and Kawachi, M. (2005). Microalgae isolation techniques. *Algal Culturing Techniques*, 83.

Andrade, L.R., Farina, M., and Filho, G.M.A. (2004). Effects of copper on *Enteromorpha flexuosa* (Chlorophyta) in vitro. *Ecotoxicology and Environmental Safety*, 58, 117–125

Anjana, K., Kaushik, A., Kiran, B., and Nisha, R. (2007). Biosorption of Cr(VI) by immobilized biomass of two indigenous strains of cyanobacteria isolated from metal contaminated soil. *Journal of Hazardeous Materials*, 148, 383-386.

Anju, A., Ravi S.P., and Bechan, S. (2010). Water pollution with special reference to pesticide contamination in India. *Journal of Water Resource and Protection*.

Anjum, N.A., Gill, S.S., Gill, R., Hasanuzzaman, M., Duarte, A.C., Pereira, E., Ahmad, I., Tuteja, R., and Tuteja, N. (2014). Metal/metalloid stress tolerance in plants: role of ascorbate, its redox couple, and associated enzymes. *Protoplasma*, 251, 1265-1283.

Ansari, F.A., Singh, P., Guldhe, A., and Bux, F. (2017). Microalgal cultivation using aquaculture wastewater: integrated biomass generation and nutrient remediation. *Algal Research*, 21, 169-177.

Aoyama, K., and Nakaki, T. (2015). Glutathione in cellular redox homeostasis: association with the excitatory amino acid carrier 1 (EAAC1). Molecules 20, 8742-8758.

Apel, K., and Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology,* 55, 373-399.

APHA, (2005). Standard methods for the examination of water and wastewater. 21st Edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC.

Appenroth, K.J. (2010). Definition of “heavy metals” and their role in biological systems. In *Soil heavy metals* (pp. 19-29). Springer, Berlin, Heidelberg.

Arif, M., Bai, Y., Usman, M., Jalalah, M., Harraz, F.A., Al-Assiri, M.S., Li, X., Salama, E.S., and Zhang, C. (2020). Highest accumulated microalgal lipids (polar and non-polar) for biodiesel production with advanced wastewater treatment: role of lipidomics. *Bioresource Technology*, 298, 122299.

Arkronrat, W., Deemark, P., and Oniam, V. (2016). Growth performance and proximate composition of mixed cultures of marine microalgae (*Nannochloropsis* sp. & *Tetraselmis* sp.) with monocultures. *Songklanakarin Journal of Science and Technology*, 38(1), 1-5.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Betavulgaris*. *Plant Physiology*, 24, 1.

Arora, N., Gulati, K., Patel, A., Pruthi, P.A., Poluri, K.M., and Pruthi, V. (2017). A hybrid approach integrating arsenic detoxification with biodiesel production using oleaginous microalgae. *Algal Research*, 24, 29-39.

Arroyo, J., Hutzler, J., Bermejo, C., Ragni, E., García- Cantalejo, J., Botías, P., Piberger, H., Schott, A., Sanz, A.B., and Strahl, S. (2011). Functional and genomic analyses of blocked protein O- mannosylation in baker's yeast. *Molecular microbiology*, 79(6), 1529-1546.

Arun, J., Shreekanth, S.J., Sahana, R., Raghavi, M.S., Gopinath, K.P., and Gnanaprakash, D. (2017). Studies on influence of process parameters on hydrothermal catalytic liquefaction of microalgae (*Chlorella vulgaris*) biomass grown in wastewater. *Bioresource Technology*, 244, 963-968.

Arunakumara, K.K.I.U., and Zhang X. (2008). Heavy metal bioaccumulation and toxicity with special reference to microalgae. *Journal of Ocean University of China*, 7, 60-64.

Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology*, 141(2), 391-396.

Asadi, P., Rad, H.A., and Qaderi, F. (2019). Comparison of *Chlorella vulgaris* and *Chlorella sorokiniana* in post treatment of dairy wastewater treatment plant effluents. *Environmental Science and Pollution Research*, 26(28), 29473-29489.

Ashraf, A., Ramamurthy, R., and Rene, E.R. (2021). Wastewater treatment and resource recovery technologies in the brewery industry: Current trends and emerging practices. *Sustainable Energy Technologies and Assessments*, 47, 101432.

Atkinson, D., Ciotti, B.J., and Montagnes, D.J. (2003). Protists decrease in size linearly with temperature: ca. 2.5% C− 1. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1533), 2605-2611.

Atoku, D.I., Ojekunle, O.Z., Taiwo, A.M., and Shittu, O.B. (2021). Evaluating the efficiency of *Nostoc commune*, *Oscillatoria limosa* and *Chlorella vulgaris* in a phycoremediation of heavy metals contaminated industrial wastewater. *Scientific African*, 12, 00817.

Balaji, S., Kalaivani, T., Shalini, M., Gopalakrishnan, M., Rashith Muhammad, M.A., Balzano, S., Sardo, A., Blasio, M., Chahine, T.B., Dell'Anno, F., Sansone, C., and Brunet, C. (2020). Microalgae metallothioneins and phytochelatins and their potential use in bioremediation. *Frontiers in Microbiology*, 11, 517.

Balaji, S., Kalaivani, T., Shalini, M., Gopalakrishnan, M., Rashith Muhammad, M.A., Balan, R., and Suraishkumar, G. (2014). Simultaneous increases in specific growth rate and specific lipid content of *Chlorella vulgaris* through UV-induced reactive species. *Biotechnology Progress*, 30, 291–299.

Balaji, S., Kalaivani, T., Shalini, M., Gopalakrishnan, M., Rashith Muhammad, M.A., and Rajasekaran, C. (2016). Sorption sites of microalgae possess metal binding ability towards Cr (VI) from tannery effluents—a kinetic and characterization study. *Desalination and Water Treatment*, 57, 14518-14529.

Baral, S.S., Dionisi, D., Maarisetty, D., Gandhi, A., Kothari, A., Gupta, G. and Jain, P. (2020). Biofuel production potential from wastewater in India by integrating anaerobic membrane reactor with algal photobioreactor. *Biomass and Bioenergy*, 133, 105445.

Barbara, G., Tomas, M., Giorgio, G.M., Alberto, B., and Eleonora, S. (2013). Cultivation of *Scenedesmus obliquus* in photobioreactors: Effects of light intensities and light-dark cycles on growth, productivity, and biochemical composition. *Applied biochemistry and biotechnology*, 172(5), 2377-2389.

Bartley, M.L., Boeing, W.J., Corcoran, A.A., Holguin, F.O., and Schaub, T. (2013). Effects of salinity on growth and lipid accumulation of biofuel microalga *Nannochloropsis salina* and invading organisms. *Biomass and Bioenergy*, 54, 83-88.

Bartoli, C.G., Buet, A., Gergoff Grozeff, G., Galatro, A., and Simontacchi, M. (2017). Ascorbate-glutathione cycle and abiotic stress tolerance in plants. In *Ascorbic acid in plant growth, development and stress tolerance* (pp. 177-200). Springer, Cham.

Bates, L.S., Waldren, R.P., and Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39, 205-207.

Béchet, Q., Laviale, M., Arsapin, N., Bonnefond, H., and Bernard, O. (2017). Modeling the impact of high temperatures on microalgal viability and photosynthetic activity. *Biotechnology for Biofuels*, 10(1), 1-11.

Belda, M.T., Vargas-Guerrero, J., Martin-betancor, K., Pulido-Reyes, G., González-Pleiter, M., Leganes, F., Rosal, R., and Fernandez-Piñas, F. (2021).

Understanding nanoplastics toxicity and their interaction with engineered cationic nanopolymers in microalgae by physiological and proteomic approaches. *Environmental Science: Nano,* 8(8), 2277-2296.

Beuckels, A., Smolders, E., and Muylaert, K. (2015). Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment. *Water research*, 77, 98-106.

Bhatnagar, A., Chinnasamy, S., Singh, M., and Das, K.C. (2011). Renewable biomass production by mixotrophic algae in the presence of various carbon sources and wastewaters. *Applied Energy*, 88, 3425–3431

Bhattacharjee, S. (2005). Reactive oxygen species and oxidative burst: roles in stress, senescence and signal transducation in plants. *Current Science*, 1113-1121.

Binnal, P., and Babu, P.N. (2017). Optimization of environmental factors affecting tertiary treatment of municipal wastewater by *Chlorella protothecoides* in a lab scale photobioreactor. *Journal of Water Process Engineering*, 17, 290-298.

Bligh, E.G., and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal Biochemical Physiology*, 37, 911–917.

Bolisetty, S., Peydayesh, M., and Mezzenga, R. (2019). Sustainable technologies for water purification from heavy metals: review and analysis. *Chemical Society Reviews*, 48(2), 463-487.

Borah, D., Kennedy, B., Gopalakrishnan, S., Chithonirai, A., and Nooruddin, T. (2020). Bioremediation and biomass production with the green microalga *Chlorococcum humicola* and textile mill effluent (TE). *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 90(2), 415-423.

Borowitzka, M.A., and Moheimani, N.R. eds. (2013). *Algae for biofuels and energy* (Vol. 5, pp. 133-152). Dordrecht, The Netherlands, Springer.

Borowitzka, M.A., and Vonshak, A. (2017). Scaling up microalgal cultures to commercial scale. *European Journal of Phycology*, 52(4), 407-418.

Bouchelkia, N., Mouni, L., Belkhiri, L., Bouzaza, A., Bollinger, J.C., Madani, K., and Dahmoune, F. (2016). Removal of lead (II) from water using activated carbon developed from jujube stones, a low-cost sorbent. *Separation Science and Technology*, 51(10), 1645-1653.

Brauman, K.A., Siebert, S., and Foley, J.A. (2013). Improvements in crop water productivity increase water sustainability and food security—a global analysis. *Environmental Research Letters*, 8(2), p.024030.

Breuer, G., Lamers, P.P., Martens, D.E., Draaisma, R.B., and Wijffels, R.H. (2012). The impact of nitrogen starvation on the dynamics of triacylglycerol accumulation in nine microalgae strains. *Bioresource Technology*, 124, 217-226.

Briens, C., Piskorz, J., and Berruti, F. (2008). Biomass valorization for fuel and chemicals production--A review. *International Journal of Chemical Reactor Engineering*, *6*(1).

Brindhadevi, K., Mathimani, T., Rene, E.R., Shanmugam, S., Chi, N.T.L., and Pugazhendhi, A. (2021). Impact of cultivation conditions on the biomass and lipid in microalgae with an emphasis on biodiesel. *Fuel*, 284, p.119058.

Bucciarelli E., and Sunda W.G. (2003). Influence of CO_2, nitrate, phosphate, and silicate limitation on intracellular dimethylsulfoniopropionate in batch cultures of the coastal diatom *Thalassiosira pseudonana*. *Limnology and Oceanography*, 48, 2256–2265.

Bulgariu, L., and Bulgariu, D. (2020). Bioremediation of toxic heavy metals using marine algae biomass. In *Green Materials for Wastewater Treatment* (pp. 69-98). Springer, Cham.

Cai, T., Park, S.Y., and Li, Y. (2013). Nutrient recovery from wastewater streams by microalgae: status and prospects. *Renewable and Sustainable Energy Reviews*, 19, 360-369.

Cameron, H., Mata, M.T., and Riquelme, C. (2018). The effect of heavy metals on the viability of *Tetraselmis marina* AC16-MESO and an evaluation of the potential use of this microalga in bioremediation. *PeerJ*, 6, 5295.

Cao, J., Yuan, H., Li, B., and Yang, J. (2014). Significance evaluation of the effects of environmental factors on the lipid accumulation of *Chlorella minutissima* UTEX 2341 under low-nutrition heterotrophic condition. *Bioresource Technology*, 152, 177-184

Capdevila, M., and Atrian, S. (2011). Metallothionein protein evolution: a miniassay. *Journal of Biological Inorganic Chemistry*, 16, 977–989

Caporgno, M.P., Taleb, A., Olkiewicz, M., Font, J., Pruvost, J., Legrand, J., and Bengoa, C. (2015). Microalgae cultivation in urban wastewater: nutrient removal and biomass production for biodiesel and methane. *Algal Research*, 10, 232-239.

Cardona, T., Shao, S., and Nixon, P.J. (2018). Enhancing photosynthesis in plants: the light reactions. *Essays in Biochemistry*, 62, 85-94.

Cardoso, L.G., Duarte, J.H., Costa, J.A.V., de Jesus Assis, D., Lemos, P.V.F., Druzian, J.I., de Souza, C.O., Nunes, I.L., and Chinalia, F.A. (2021). *Spirulina* sp. as a bioremediation agent for aquaculture wastewater: production of high added value compounds and estimation of theoretical biodiesel. *BioEnergy Research*, 14(1), 254-264.

Carfagna, S., Lanza, N., Salbitani, G., Basile, A., Sorbo, S., and Vona, V. (2013). Physiological and morphological responses of Lead or Cadmium exposed *Chlorella sorokiniana* 211-8K (Chlorophyceae). *Springer Plus*, 2, 1-7.

Carneiro, M., Cicchi, B., Maia, I.B., Pereira, H., Zittelli, G.C., Varela, J., Malcata, F.X., and Torzillo, G. (2020). Effect of temperature on growth, photosynthesis and biochemical composition of *Nannochloropsis oceanica*, grown outdoors in tubular photobioreactors. *Algal Research*, 49, 101923.

Carney, L.T., Wilkenfeld, J.S., Lane, P.D., Solberg, O.D., Fuqua, Z.B., Cornelius, N.G., Gillespie, S., Williams, K.P., Samocha, T.M., and Lane, T.W. (2016). Pond crash forensics: presumptive identification of pond crash agents by next generation sequencing in replicate raceway mass cultures of *Nannochloropsis salina*. *Algal Research*, 17, 341-347.

Cassier-Chauvat, C., and Chauvat, F. (2015). Responses to oxidative and heavy metal stresses in cyanobacteria: Recent advances. *International Journal of Molecular Science*, 16, 871–886.

Castrillo, M., Díez-Montero, R., and Tejero, I. (2018). Model-based feasibility assessment of a deep solar photobioreactor for microalgae culturing. *Algal Research*, 29, 304-318.

Cembella, A.D., Antia, N.J., Harrison, P.J. (1982). The utilization of inorganic and organic phosphorous compounds as nutrients by eukaryotic microalgae: a multidisciplinary perspective: part I. *Critical Reviews in Microbiology*, 10, 317-391.

Central Pollution Control Board, (2009). Status of water supply and wastewater generation and treatment in Class-I cities and Class- II towns of India—a report by CPCB.

Cesur, A., Zeren Cetin, I., Abo Aisha, A.E.S., Alrabiti, O.B.M., Aljama, A.M.O., Jawed, A.A., Cetin, M., Sevik, H., and Ozel, H.B. (2021). The usability of *Cupressus arizonica* annual rings in monitoring the changes in heavy metal concentration in air. *Environmental Science and Pollution Research*, 28(27), 35642-35648.

Chaisutyakorn, P., Praiboon, J., and Kaewsuralikhit, C. (2018). The effect of temperature on growth and lipid and fatty acid composition on marine microalgae used for biodiesel production. *Journal of Applied Phycology*, 30(1), 37-45.

Chakraborty, U., and Pradhan, D., (2011). High temperature-induced oxidative stress in *Lens culinaris*, role of antioxidants and amelioration of stress by chemical pre-treatments. *Journal of Plant Interaction*, 6, 43–52

Chandrashekharaiah, P.S., Sanyal, D., Dasgupta, S., and Banik, A. (2021). Cadmium biosorption and biomass production by two freshwater microalgae *Scenedesmus acutus* and *Chlorella pyrenoidosa*: An integrated approach. *Chemosphere*, 269, 128755.

Chatterjee, S., Kumari, S., Rath, S., Priyadarshanee, M., and Das, S. (2020). Diversity, structure and regulation of microbial metallothionein: Metal resistance and possible applications in sequestration of toxic metals. *Metallomics*, 12(11), 1637-1655.

Chaula, D., Laswai, H., Chove, B., Dalsgaard, A., Mdegela, R., and Hyldig, G. (2019). Fatty acid profiles and lipid oxidation status of sun dried, deep fried, and smoked sardine (*Rastrineobola argentea*) from Lake Victoria, Tanzania. *Journal of Aquatic Food Product Technology*, 28, 165-176.

Cheirsilp, B., and Torpee, S. (2012). Enhanced growth and lipid production of microalgae under mixotrophic culture condition: effect of light intensity, glucose concentration and fed-batch cultivation. *Bioresource Technology*, 110, 510-516.

Cheloni, G., Cosio, C., and Slaveykova, V.I. (2014). Antagonistic and synergistic efects of light irradiation on the effects of copper on *Chlamydomonas reinhardtii*. *Aquatic Toxicology*, 155, 275–282.

Chen, C., and Dickman, M.B. (2005). Proline suppresses apoptosis in the fungal pathogen Colletotrichum trifolii. *Proceedings of the National Academy of Sciences*, 102, 3459-3464.

Chen, G., Zhao, L., and Qi, Y. (2015). Enhancing the productivity of microalgae cultivated in wastewater toward biofuel production: a critical review. *Applied Energy*, 137, 282-291.

Chen, H., Xia, A., Zhu, X., Huang, Y., Zhu, X., and Liao, Q. (2022). Hydrothermal hydrolysis of algal biomass for biofuels production: A review. *Bioresource Technology*, 344, 126213.

Chen, H., Zhou, W., Chen, W., Xie, W., Jiang, L., Liang, Q., Huang, M., Wu, Z., and Wang, Q. (2017). Simplified, rapid, and inexpensive estimation of water primary productivity based on chlorophyll fluorescence parameter Fo. *Journal of Plant Physiology*, 211, 128-135.

Chen, Q., Yao, Y., Li, X., Lu, J., Zhou, J., and Huang, Z. (2018). Comparison of heavy metal removals from aqueous solutions by chemical precipitation and characteristics of precipitates. *Journal of Water Process Engineering*, 26, 289-300.

Chen, S., Wang, L., Feng, W., Yuan, M., Li, J., Xu, H., Zheng, X., and Zhang, W. (2020). Sulfonamides-induced oxidative stress in freshwater microalga *Chlorella vulgaris*: Evaluation of growth, photosynthesis, antioxidants, ultrastructure, and nucleic acids. *Scientific Reports*, 10(1), 1-11.

Chinnasamy, S., Bhatnagar, A., Hunt, R.W., and Das, K.C. (2010). Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresource technology*, 101, 3097-3105.

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances*, 25, 294–306.

Chojnacka, K. (2010). Biosorption and bioaccumulation–the prospects for practical applications. *Environment international*, 36(3), 299-307.

Chokshi, K., Pancha, I., Ghosh, A. and Mishra, S., 2016. Microalgal biomass generation by phycoremediation of dairy industry wastewater: an integrated approach towards sustainable biofuel production. *Bioresource technology*, 221, 455-460.

Chokshi, K., Pancha, I., Ghosh, A., and Mishra, S. (2017). Nitrogen starvation-induced cellular crosstalk of ROS-scavenging antioxidants and phytohormone enhanced the biofuel potential of green microalga *Acutodesmus dimorphus*. *Biotechnology for Biofuels*, 10(1), 60.

Chokshi, K., Pancha, I., Trivedi, K., George, B., Maurya, R., Ghosh, A., and Mishra, S. (2015). Biofuel potential of the newly isolated microalgae *Acutodesmus dimorphus* under temperature induced oxidative stress conditions. *Bioresource Technology*, 180, 162-171.

Chokshi, K., Pancha, I., Trivedi, K., Maurya, R., Ghosh, A., and Mishra, S. (2020). Physiological responses of the green microalga *Acutodesmus dimorphus* to temperature induced oxidative stress conditions. *Physiologia Plantarum*, 170(4), 462-473.

Choudhary, M., Jetley, U.K., Khan, M.A., Zutshi, S., and Fatma, T. (2007). Effect of heavy metal stress on proline, malondialdehyde, and superoxide dismutase activity in the cyanobacterium *Spirulina platensis*-S5. *Ecotoxicology and Environmental Safety*, 66, 204-209.

Choudhary, P., Prajapati, S. K., and Malik, A. (2016). Screening native microalgal consortia for biomass production and nutrient removal from rural wastewaters for bioenergy applications. *Ecological Engineering*, 91, 221-230.

Christov, C., Pouneva, I., Bozhkova, M., Toncheva, T., Fournadzieva, S., and Zafirova, T. (2001). Influence of temperature and methyl jasmonate on *Scenedesmus incrassulatus*. *Biologia Plantarum*, 44(3), 367-371.

Chu, F., Cheng, J., Li, K., Wang, Y., Li, X., and Yang, W. (2020). Enhanced lipid accumulation through a regulated metabolic pathway of phosphorus luxury uptake in the microalga *Chlorella vulgaris* under nitrogen starvation and phosphorus repletion. *ACS Sustainable Chemistry & Engineering*, 8(22), 8137-8147.

Chu, F.F., Chu, P.N., Cai, P.J., Li, W.W., Lam, P.K. and Zeng, R.J. (2013). Phosphorus plays an important role in enhancing biodiesel productivity of *Chlorella vulgaris* under nitrogen deficiency. *Bioresource Technology*, 134, 3421-346.

Cobbett, C., and Goldsbrough, P. (2002). Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. *Annual Review of Plant Biology*, 53(1), 159-182.

Converti, A., Casazza, A.A., Ortiz, E.Y., Perego, P., and Del Borghi, M. (2009). Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chemical Engineering and Processing: Process Intensification*, 48(6), 1146-1151.

Corpas, F.J., Palma, J.M., Sandalio, L.M., Valderrama, R., Barroso, J.B., and Luis, A. (2008). Peroxisomal xanthine oxidoreductase: characterization of the enzyme from pea (*Pisum sativum* L.) leaves. *Journal of Plant Physiology*, 165, 1319-1330.

Costa, J.A.V., and de Morais, M.G. (2014). Chapter 1—An open pond system for microalgal cultivation. In: Soccol, A.P.-J.L.C.R. (Ed.), Biofuels From Algae. Elsevier, Amsterdam, pp. 122.

Coulombier, N., Jauffrais, T., and Lebouvier, N. (2021). Antioxidant compounds from microalgae: A review. *Marine drugs*, 19(10), 549.

Couto, N., Wood, J., and Barber, J. (2016). The role of glutathione reductase and related enzymes on cellular redox homoeostasis network. Free radical biology and medicine, 95, 27-42.

CPCB (2017c). *Status of STPs*. Available online at: https://cpcb.nic.in/status-of-stps/ (accessed July 01, 2019).

CPCB (Central Pollution Control Board), (2011). http://unstats.un.org/ unsd/environment/envpdf/pap_wasess3b6india.pdf.pp. 1–9.

CPCB, (2013). *Performance Evaluation of Treatment Plants in India under funding of NRDC*.Availableonline at: http://www.indiaenvironmentportal.org.in/files/ file/STP__REPORT.pdf (accessed December 12, 2019).

Cravo, A., Barbosa, A.B., Correia, C., Matos, A., Caetano, S., Lima, M.J., and Jacob, J. (2022). Unravelling the effects of treated wastewater discharges on the water quality in a coastal lagoon system (Ria Formosa, South Portugal): Relevance of hydrodynamic conditions. *Marine Pollution Bulletin*, 174, 113296.

Cui, D., Zhang, P., Li, H., Zhang, Z., Song, Y., and Yang, Z. (2021). The dynamic changes of arsenic biotransformation and bioaccumulation in muscle of freshwater food fish crucian carp during chronic dietborne exposure. *Journal of Environmental Sciences*, 100, 74-81.

da Fontoura, J.T., Rolim, G.S., Farenzena, M., and Gutterres, M. (2017). Influence of light intensity and tannery wastewater concentration on biomass production and nutrient removal by microalgae Scenedesmus sp. *Process Safety and Environmental Protection*, 111, 355-362.

da Silva Ferreira, V., and Sant'Anna, C. (2017). Impact of culture conditions on the chlorophyll content of microalgae for biotechnological applications. *World Journal of Microbiology and Biotechnology*, 33(1), 1-8.

Dall'Osto, L., Cazzaniga, S., Guardini, Z., Barera, S., Benedetti, M., Mannino, G., Maffei, M.E., and Bassi, R. (2019). Combined resistance to oxidative stress and reduced antenna size enhance light-to-biomass conversion efficiency in *Chlorella vulgaris* cultures. *Biotechnology for Biofuels*, 12(1), 1-17.

Damania, R., Desbureaux, S., Rodella, A.S., Russ, J., and Zaveri, E. (2019). Quality Unknown: The Invisible Water Crisis. Washington, DC: World Bank. © World Bank, https://openknowledge.worldbank.org/handle/10986/ 32245 License: CC BY 3.0 IGO. http://hdl.handle.net/10986/32245, 142.

Daneshvar, E., Zarrinmehr, M.J., Koutra, E., Kornaros, M., Farhadian, O., and Bhatnagar, A. (2019). Sequential cultivation of microalgae in raw and recycled dairy wastewater: microalgal growth, wastewater treatment and biochemical composition. *Bioresource Technology,* 273, 556-564.

Danouche, M., El Ghachtouli, N., El Baouchi, A., and El Arroussi, H. (2020). Heavy metals phycoremediation using tolerant green microalgae: Enzymatic and non-enzymatic antioxidant systems for the management of oxidative stress. *Journal of Environmental Chemical Engineering*, 8(5), 104460

Danouche, M., El Ghatchouli, N., and Arroussi, H. (2022). Overview of the management of heavy metals toxicity by microalgae. *Journal of Applied Phycology*, 1-14.

Dao, Ly H.T., and Beardall, J., (2016). Effects of lead on two green microalgae *Chlorella* and *Scenedesmus*: photosystem II activity and heterogeneity. *Algal Research*, 16, 150-159.

Das, C., Ramaiah, N., Pereira, E., and Naseera, K. (2018). Efficient bioremediation of tannery wastewater by monostrains and consortium of marine *Chlorella* sp. and *Phormidium* sp. *International Journal of Phytoremediation*, 20(3), 284-292.

Das, K., and Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Science*, 2, 53.

Dat, J., Vandenabeele, S., Vranová, E., Van Montagu, M., Inzé, D., and Van Breusegem, F. (2000). Dual action of the active oxygen species during plant stress responses. *Cellular and Molecular Life Science*, 57, 779–795.

Dayana Priyadharshini, S., Suresh Babu, P., Manikandan, S., Subbaiya, R., Govarthanan, M., and Karmegam, N. (2021). Phycoremediation of wastewater for pollutant removal: A green approach to environmental protection and long-term remediation. *Environmental Pollution*, 290, 117989.

De Amorim, W.S., Valduga, I.B., Ribeiro, J.M.P., Williamson, V.G., Krauser, G.E., Magtoto, M.K., and de Andrade, J.B.S.O. (2018). The nexus between water, energy, and food in the context of the global risks: An analysis of the interactions between food, water, and energy security. *Environmental impact assessment review*, 72, 1-11.

de Boer, M.K., Koolmees, E.M., Vrieling, E.G., Breeman, A.M. and van Rijssel, M., (2005). Temperature responses of three *Fibrocapsa japonica* strains (Raphidophyceae) from different climate regions. *Journal of Plankton Research*, 27, 47-60.

de Zwart, L.L., Meerman, J.H., Commandeur, J.N., and Vermeulen, N.P. (1999). Biomarkers of free radical damage: applications in experimental animals and in humans. *Free Radical Biology and Medicine*, 26, 202-226.

Del Mondo, A., Smerilli, A., Sané, E., Sansone, C., and Brunet, C. (2020). Challenging microalgal vitamins for human health. *Microbial Cell Factories*, 19(1), 1-23.

Delgadillo-Mirquez, L., Lopes, F., Taidi, B., and Pareau, D. (2016). Nitrogen and phosphate removal from wastewater with a mixed microalgae and bacteria culture. *Biotechnology Reports*, 11, 18-26.

Delrue, F., Álvarez-Díaz, P.D., Fon-Sing, S., Fleury, G., and Sassi, J.F. (2016). The environmental biorefinery: Using microalgae to remediate wastewater, a win-win paradigm. *Energies*, 9, 132.

Deng, X.Y., Cheng, J., Hu, X.L., Wang, L., Li, D. and Gao, K., 2017. Biological effects of TiO2 and CeO2 nanoparticles on the growth, photosynthetic activity, and cellular components of a marine diatom *Phaeodactylum tricornutum*. *Science of the Total Environment*, 575, 87-96.

Di Pippo, F., Ellwood, N.T.W., Guzzon, A., Siliato, L., Micheletti, E., De Philippis, R., and Albertano, P.B. (2012). Effect of light and temperature on biomass, photosynthesis and capsular polysaccharides in cultured phototrophic biofilms. *Journal of Applied Phycology*, 24, 211-220.

Ding, S., Jiang, R., Lu, Q., Wen, X., and Lu, C. (2016). Glutathione reductase 2 maintains the function of photosystem II in *Arabidopsis* under excess light. *Biochimica Biophysica Acta*, 1857, 665-677.

Diniz, G.S., Silva, A.F., Araújo, O.Q., and Chaloub, R.M. (2017). The potential of microalgal biomass production for biotechnological purposes using wastewater resources. *Journal of Applied Phycology*, 29(2), 821-832.

Dong, L., Hou, L.A., Wang, Z., Gu, P., Chen, G., and Jiang, R. (2018). A new function of spent activated carbon in BAC process: Removing heavy metals by ion exchange mechanism. *Journal of Hazardous Materials*, 359, 76-84.

Dudek, M., Dębowski, M., Zieliński, M., and Nowicka, A. (2017). Use of a wastewater after anaerobic pretreatment to microalgae *Platymonas subcordiformis* growth. *Ecological Engineering*, 18, 14–20.

Duffus, J.H. (2002) "Heavy metal"—a meaningless term? *Pure and Applied Chemistry*, 74, 793–807

Duque, D., Montoya, C., Botero, L.R. (2019). Cadmium (Cd) tolerance evaluation of three strains of microalgae of the genus *Ankistrodesmus*, *Chlorella* and *Scenedesmus*. Rev.fac.ing.univ 92, 60-69.

Dutta, S., Mitra, M., Agarwal, P., Mahapatra, K., De, S., Sett, U., and Roy, S. (2018). Oxidative and genotoxic damages in plants in response to heavy metal stress and maintenance of genome stability. *Plant Signaling & Behavior*, 13, 1460048.

Duxbury, A.C., and Yentsch, C.S. (1956). Plankton pigment monograph. *Journal of Marine Research*, 15, 93–101.

Duygu, D.Y., Udoh, A.U., Ozer, T.B., Akbulut, A., Erkaya, I.A., Yildiz, K., and Guler, D. (2012). Fourier transform infrared (FTIR) spectroscopy for identification of *Chlorella vulgaris* Beijerinck 1890 and *Scenedesmus obliquus* (Turpin) Kützing 1833. *African Journal of Biotechnology*, 11, 3817-3824.

Elbaz, A., Wei, Y.Y., Meng, Q., Zheng, Q., and Yang, Z.M. (2010). Mercury-induced oxidative stress and impact on antioxidant enzymes in *Chlamydomonas reinhardtii*. *Ecotoxicology and Environmental Safety*, 19, 1285-1293.

Elgarahy, A.M., Elwakeel, K.Z., Mohammad, S.H., and Elshoubaky, G.A. (2021). A critical review of biosorption of dyes, heavy metals and metalloids from wastewater as an efficient and green process. *Cleaner Engineering and Technology*, 4, 100209.

Elsadany, A. (2018). The use of microalgae in bioremediation of the textile wastewater effluent. *Natural Science*, 16, 98-104.

Elser, J.J. (2012). Phosphorus: a limiting nutrient for humanity?. *Current Opinion in Biotechnology*, 23(6), 833-838.

El-Sheekh, M.M., Farghl, A.A., Galal, H.R., and Bayoumi, H.S. (2016). Bioremediation of different types of polluted water using microalgae. *Rendiconti Lincei*, 27(2), 401-410.

Elumalai, K., and Velmurugan, S. (2015). Green synthesis, characterization and antimicrobial activities of zinc oxide nanoparticles from the leaf extract of *Azadirachta indica* (L.). *Applied Surface Science*, 345, 329–336.

Elystia, S., Edward, H.S., and Putri, A.E. (2020). Removal of chromium (VI) and chromium (III) by using *Chlorella* sp Immobilized at Electroplating Wastewater. In *IOP Conference Series: Earth and Environmental Science*, 515, 012078.

Emamverdian, A., Ding, Y., Mokhberdoran, F., and Xie, Y. (2015). Heavy metal stress and some mechanisms of plant defense response. The Scientific World Journal.

Fakorede, E.O., and Adewumi, J.R. (2020). Effectiveness of electro-coagulation treatment method on the physio-chemical parameters and heavy metals in rubber latex wastewater. *lautech Journal of Engineering and Technology*, 14, 1-9.

Fal, S., Benhima, R., El Mernissi, N., Kasmi, Y., Smouni, A., and El Arroussi, H. (2022). Microalgae as promising source for integrated wastewater treatment and biodiesel production. *International Journal of Phytoremediation*, 24(1), 34-46.

Fan, F., Xu, R., Wang, D., and Meng, F. (2020). Application of activated sludge for odor control in wastewater treatment plants: Approaches, advances and outlooks. *Water Research*, 181, 115915.

Fan, S., Ji, B., Abu Hasan, H., Fan, J., Guo, S., Wang, J., and Yuan, J. (2021). Microalgal–bacterial granular sludge process for non-aerated aquaculture wastewater treatment. *Bioprocess and Biosystems Engineering*, 44(8), 1733-1739.

Fanesi, A., Wagner, H., Becker, A., and Wilhelm, C. (2016). Temperature affects the partitioning of absorbed light energy in freshwater phytoplankton. *Freshwater Biology*, 61, 1365-1378.

Farooqi, Z.U.R. (2021). Phytoremediation of inorganic pollutants: An eco-friendly approach, its types and mechanisms. *Plant and Environment*, 1, 110-129.

Farraji, H., Zaman, N.Q., Tajuddin, R., and Faraji, H. (2016). Advantages and disadvantages of phytoremediation: A concise review. *International Journal of Environmental Science and Technology*, 2, 69-75.

Fazal, T., Rehman, M.S.U., Javed, F., Akhtar, M., Mushtaq, A., Hafeez, A., Din, A.A., Iqbal, J., Rashid, N., and Rehman, F. (2021). Integrating bioremediation of textile wastewater with biodiesel production using microalgae (*Chlorella vulgaris*). *Chemosphere*, 281, 130758.

Fernández, F.A., Sevilla, J.M.F., and Grima, E.M. (2019). Costs analysis of microalgae production. In *Biofuels from algae* (pp. 551-566). Elsevier.

Fernández, P.M., Viñarta, S.C., Bernal, A.R., Cruz, E.L., and Figueroa, L.I. (2018). Bioremediation strategies for chromium removal: current research, scale-up approach and future perspectives. *Chemosphere*, 208, 139-148.

Ferraz, M.C.M., Boaventura, R.A.R., Vilar, V.J.P., and Pires, J.C.M. (2015). Towards sustainable microalgal biomass production by phycoremediation of a synthetic wastewater: A kinetic study. *Algal Research*, 11, 350-358.

Ferreira, V.S., Pinto, R.F., and Sant'Anna, C. (2016). Low light intensity and nitrogen starvation modulate the chlorophyll content of *Scenedesmus dimorphus*. *Journal of Applied Microbiology*, 120(3), 661-670.

Ferro, L., Gorzsás, A., Gentili, F.G., and Funk, C. (2018). Subarctic microalgal strains treat wastewater and produce biomass at low temperature and short photoperiod. *Algal Research*, 35, 160-167.

Fettah, N., Derakhshandeh, M., Tezcan Un, U., and Mahmoudi, L. (2022). Effect of light on growth of green microalgae *Scenedesmus quadricauda*: influence of light intensity, light wavelength and photoperiods. *International Journal of Energy and Environmental Engineering*, 1-10.

Fiedor, J., and Burda K. (2014). Potential role of carotenoids as antioxidants in human health and disease. *Nutrients*, 6, 466–488.

Fito, J., and Alemu, K. (2019). Microalgae–bacteria consortium treatment technology for municipal wastewater management. *Nanotechnology for Environmental Engineering*, 4(1), 1-9.

Forfang, K., Zimmermann, B., Kosa, G., Kohler, A., and Shapaval, V. (2017). FTIR spectroscopy for evaluation and monitoring of lipid extraction efficiency for oleaginous fungi. *PLoS One*, 12, 170611.

Forlani, G., Trovato, M., Funck, D., and Signorelli, S. (2019). Regulation of proline accumulation and its molecular and physiological functions in stress defence. In *Osmoprotectant-mediated abiotic stress tolerance in plants* (pp. 73-97). Springer, Cham.

Fu, F., and Wang, Q. (2011). Removal of heavy metal ions from wastewaters: a review. J.G. Ellis, "Chemistry of wastewater," *In Environmental and*

Ecological Chemistry, Volume II, p. 452, Department of Civil, Construction and Environmental Engineering, Ames, Iowa, USA.

Gaitonde, M.K. (1967). A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. *Biochemical Journal*, 104, 627.

Gallie, D.R. (2013). L-ascorbic acid: a multifunctional molecule supporting plant growth and development. *Scientifica*.

Ganapathy, K., Chidambaram, K., Janarthanan, R., and Ramasamy, R. (2017). Effect of UV-B radiation on growth, photosynthetic activity and metabolic acitivities of *Chlorella vulgaris*. *Journal of Microbiology and Biotechnol*ogy, 6, 53–60.

Gani, P., Apandi, N.M., Mohamed Sunar, N., Matias-Peralta, H.M., Kean Hua, A., Mohd Dzulkifli, S.N., and Parjo, U.K. (2022). Outdoor phycoremediation and biomass harvesting optimization of microalgae *Botryococcus* sp. cultivated in food processing wastewater using an enclosed photobioreactor. *International Journal of Phytoremediation*, 1-13.

Gani, P., Sunar, N.M., Matias-Peralta, H., Parjo, U.K., and Oyekanmi, A.A. (2017). Green approach in the bio-removal of heavy metals from wastewaters. In *MATEC Web of Conferences* (Vol. 103, p. 06007). EDP Sciences.

Gao, K., Xue, C., Yang, M., Li, L., Qian, P., Gao, Z., Gao, Z., and Deng, X. (2022). Optimization of light intensity and photoperiod for growing *Chlorella sorokiniana* on cooking cocoon wastewater in a bubble-column bioreactor. *Algal Research*, 62, 102612.

García, F., Freile-Pelegrín, Y., and Robledo, D. (2007). Physiological characterization of *Dunaliella* sp. (Chlorophyta, Volvocales) from Yucatan, Mexico. *Bioresource Technology*, 98, 1359-1365.

García, F.C., Bestion, E., Warfield, R., and Yvon-Durocher, G. (2018). Changes in temperature alter the relationship between biodiversity and ecosystem functioning. *Proceedings of the National Academy of Sciences*, 115, 10989-10994.

García-García, J.D., S´anchez-Thomas, R., Moreno-S´anchez, R. (2016). Bio-recovery of non-essential heavy metals by intra- and extracellular mechanisms in free-living microorganisms. *Biotechnology Advances*, 34, 859–873.

Garza-Lombó, C., Posadas, Y., Quintanar, L., Gonsebatt, M.E., and Franco, R. (2018). Neurotoxicity linked to dysfunctional metal ion homeostasis and xenobiotic metal exposure: redox signaling and oxidative stress. *Antioxidants & Redox Signaling*, 28, 1669-1703.

Gautam, P., Kumar, S., and Lokhandwala, S. (2019). Advanced oxidation processes for treatment of leachate from hazardous waste landfill: A critical review. *Journal of Cleaner Production*, 237, 117639.

Gauthier, M.R., Senhorinho, G.N.A., and Scott, J.A. (2020). Microalgae under environmental stress as a source of antioxidants. *Algal Research*, 52, 102104.

Gayathri, S., and Rajasree, S.R.R. (2017). Influence of photoperiod, light intensity and temperature for optimizing biomass and pigment productivities of *Chlorella salina*. *Research Journal of Pharmaceutical, Biological and Chemical Science*, 8, 671-678.

Gechev, T.S., Van Breusegem, F., Stone, J.M., Denev, I., and Laloi, C. (2006). Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. *Bioessays*, 28, 1091-1101.

Geider, R.J., and La Roche, J. (2002). Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. *European Journal of Phycology*, 37, 1-17.

George, B., Pancha, I., Desai, C., Chokshi, K., Paliwal, C., Ghosh, T., and Mishra, S. (2014). Effects of different media composition, light intensity and photoperiod on morphology and physiology of freshwater microalgae *Ankistrodesmus falcatus* – a potential strain for bio-fuel production. *Bioresource Technology*, 171, 367–374.

Ghasemi, M., Naushad, M., Ghasemi, N., and Khosravi-Fard, Y. (2014). Adsorption of Pb (II) from aqueous solution using new adsorbents prepared from agricultural waste: adsorption isotherm and kinetic studies. *Journal of Industrial and Engineering Chemistry*, 20, 2193-2199.

Ghori, N.H., Ghori, T., Hayat, M.Q., Imadi, S.R., Gul, A., Altay, V., and Ozturk, M. (2019). Heavy metal stress and responses in plants. *International Journal of Environmental Science and Technology*, 16, 1807-1828.

Ghosh, D., and Xu, J. (2014). Abiotic stress responses in plant roots: a proteomics perspective. *Frontiers in Plant Science*, 5, 6.

Ghosh, U.K., Islam, M.N., Siddiqui, M.N., Cao, X., and Khan, M.A.R. (2022). Proline, a multifaceted signalling molecule in plant responses to abiotic stress: understanding the physiological mechanisms. *Plant Biology*, 24, 227-239.

Gill, S.S., and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, 48(12), 909-930.

Gill, S.S., Anjum, N.A., Hasanuzzaman, M., Gill, R., Trivedi, D.K., Ahmad, I., Pereira, E., and Tuteja, N. (2013). Glutathione and glutathione reductase: a boon in disguise for plant abiotic stress defense operations. *Plant Physiology and Biochemistry*, 70, 204-212.

Gim, G.H., Kim, J.K., Kim, H.S., Kathiravan, M.N., Yang, H., Jeong, S.H., and Kim, S.W. (2014). Comparison of biomass production and total lipid content of freshwater green microalgae cultivated under various culture conditions. *Bioprocess and Biosystem Engineering*, 37, 99

Giordano, M., Kansiz, M., Heraud, P., Beardall, J., Wood, B., and McNaughton, D. (2001). Fourier transform infrared spectroscopy as a novel tool to investigate changes in intracellular macromolecular pools in the marine microalga *Chaetoceros muellerii* (Bacillariophyceae). *Journal of Phycology*, 37(2), 271-279.

Goiris, K., Muylaert, K., Fraeye, I., Foubert, I., De Brabanter, J., and De Cooman, L., 2012. Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. *Journal of Applied Phycology*, 24, 1477-1486.

Gómez-Jacinto, V., García-Barrera, T., Gómez-Ariza, J.L., Garbayo-Nores, I., Vílchez-Gonçalves, A.L., Pires, J.C., and Simões, M. (2017). A review on the use of microalgal consortia for wastewater treatment. *Algal Research*, 24, 403-415.

Gómez-Jacinto, V., García-Barrera, T., Gómez-Ariza, J.L., Garbayo-Nores, I., and Vílchez-Lobato, C. (2015). Elucidation of the defence mechanism in microalgae *Chlorella sorokiniana* under mercury exposure. Identification of Hg–phytochelatins. *Chemico-Biological Interactions*, 238, 82-90.

Gonçalves, A.L., Pires, J.C., and Simões, M. (2017). A review on the use of microalgal consortia for wastewater treatment. *Algal Research*, 24, 403-415.

González-Camejo, J., Viruela, A., Ruano, M. V., Barat, R., Seco, A., and Ferrer, J. (2019). Effect of light intensity, light duration and photoperiods in the performance of an outdoor photobioreactor for urban wastewater treatment. *Algal Research*, 40, 101511.

González-Fernández, C., Mahdy, A., Ballesteros, I., and Ballesteros, M. (2016). Impact of temperature and photoperiod on anaerobic biodegradability of microalgae grown in urban wastewater. *International Biodeterioration & Biodegradation*, 106, 16-23.

Gonzalez- Silvera, D., Pérez, S., Korbee, N., Figueroa, F.L., Asencio, A.D., Aboal, M., and López- Jiménez, J.Á. (2017). Effects of global change factors on fatty acids and mycosporine- like amino acid production in *Chroothece richteriana* (Rhodophyta). *Journal of Phycology*, 53, 999-1009.

Gonzáslez-Camejo, J., Serna-García, R., Viruela, A., Pachés, M., Durán, F., Robles, A., Ruano, M.V., Barat, R., and Seco, A. (2017). Short and long-term experiments on the effect of sulfide on microalgae cultivation in tertiary sewage treatment. *Bioresource Technology*, 244, 15–22.

Goswami, G., Makut, B.B., and Das, D. (2019). Sustainable production of bio-crude oil via hydrothermal liquefaction of symbiotically grown biomass of microalgae-bacteria coupled with effective wastewater treatment. *Scientific Reports*, 9, 1-12.

Goswami, R.K., Agrawal, K., Mehariya, S., Molino, A., Musmarra, D., and Verma, P. (2020). Microalgae-based biorefinery for utilization of carbon dioxide for production of valuable bioproducts. In *Chemo-Biological Systems for CO_2 Utilization* ed. Kumar, A. and Sharma, S. pp. 203–228. Boca Raton: CRC Press.

Góth, L., Rass, P., and Páy, A. (2004). Catalase enzyme mutations and their association with diseases. *Molecular Diagnosis*, 8, 141-149.

Gouveia, L., and Oliveira, A.C. (2009). Microalgae as a raw material for biofuels production. *Journal of Industrial Microbiology and Biotechnology*, 36, 269-274.

Griffiths, M.J., van Hille, R.P., and Harrison, S.T. (2014). The effect of nitrogen limitation on lipid productivity and cell composition in *Chlorella vulgaris*. *Applied Microbiology and Biotechnology*, 98, 2345-2356.

Grobbelaar, J.U. (2004). *In: Richmond, A. (Ed.), Handbook of Microalgal Culture: Biotechnology and Applied Phycology*. Blackwell Publishing Ltd., Oxford, pp. 97-115.

Guedes, A.C., Amaro, H.M., Pereira, R.D., and Malcata, F.X. (2011). Effects of temperature and pH on growth and antioxidant content of the microalga *Scenedesmus obliquus*. *Biotechnology Progress*, 27, 1218–1224.

Guldhe, A., Kumari, S., Ramanna, L., Ramsundar, P., Singh, P., Rawat, I., and Bux, F. (2017). Prospects, recent advancements and challenges of different wastewater streams for microalgal cultivation. *Journal of Environmental Management*, 203, 299-315.

Guo, R., Wang, H., Suh, Y.S., Ki, J.S. (2016). Transcriptomic profiles reveal the genome-wide responses of the harmful dinoflagellate *Cochlodinium polykrikoides* when exposed to the algicide copper sulfate. BMC Genomics, 17:29

Gupta, P.L., Lee, S.M., and Choi, H.J. (2016). Integration of microalgal cultivation system for wastewater remediation and sustainable biomass production. *World Journal of Microbiology and Biotechnology*, 32(8), 1-11.

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

Gupta, S., Pawar, S.B., Pandey, R.A., Kanade, G.S., and Lokhande, S.K. (2019). Outdoor microalgae cultivation in airlift photobioreactor at high irradiance and temperature conditions: effect of batch and fed-batch strategies, photoinhibition, and temperature stress. *Bioprocess and Biosystems Engineering*, 42, 331-344.

Gupta, V., Jatav, P.K., Verma, R., Kothari, S.L., and Kachhwaha, S. (2017). Nickel accumulation and its effect on growth, physiological and biochemical parameters in millets and oats. *Environmental Science and Pollution Research*, 24, 23915-23925.

Gururani, M.A., Venkatesh, J., and Tran, L.S.P. (2015). Regulation of photosynthesis during abiotic stress-induced photoinhibition. *Molecular Plant*, 8, 1304-1320.

Gururani, M.A., Venkatesh, J., Ghosh, R., Strasser, R.J., Ponpandian, L.N., and Bae, H. (2018). Chlorophyll-a fluorescence evaluation of PEG-induced osmotic stress on PSII activity in Arabidopsis plants expressing SIP1. *Plant Biosystem, International Journal Dealing with all aspects of Plant Biology*, 152, 945–952.

Hachicha, R., Elleuch, F., Ben Hlima, H., Dubessay, P., de Baynast, H., Delattre, C., Pierre, G., Hachicha, R., Abdelkafi, S., Michaud, P., and Fendri, I. (2022). Biomolecules from microalgae and cyanobacteria: Applications and market survey. *Applied Sciences*, 12, 1924.

Haldimann, P., and Feller, U. (2004). Inhibition of photosynthesis by high temperature in oak (*Quercus pubescens* L.) leaves grown under natural conditions closely correlates with a reversible heat- dependent reduction of the activation state of ribulose- 1, 5- bisphosphate carboxylase/oxygenase. *Plant, Cell & Environment*, 27, 1169-1183

Halliwell, B. (2007). Biochemistry of oxidative stress. *Biochemical Society Transactions*, 35, 1147–1150.

Hamed, S.M., Selim, S., Klöck, G., and AbdElgawad, H. (2017). Sensitivity of two green microalgae to copper stress: growth, oxidative and antioxidants analyses. *Ecotoxicology and Environmental Safety*, 144, 19-25.

Hammer, D.A. ed., (2020). Constructed wetlands for wastewater treatment: municipal, industrial and agricultural. CRC Press.

Han, P., Teo, W.Z., and Yew, W.S. (2022). Biologically engineered microbes for bioremediation of electronic waste: Wayposts, challenges and future directions. *Engineering Biology*, 6(1), pp.23-34.

Hanikel, N., Prévot, M.S., and Yaghi, O.M. (2020). MOF water harvesters. *Nature Nanotechnology*, 15(5), 348-355.

Hannon, M., Gimpel, J., Tran, M., Rasala, B., and Mayfield, S. (2010). Biofuels from algae: challenges and potential. *Biofuels*, 1, 63-784.

Hao, X.D., Li, J., van Loosdrecht, M.C.M., and Li, T.Y. (2018). A sustainability-based evaluation of membrane bioreactors over conventional activated sludge processes. *Journal of Environmental Chemical Engineering*, 6(2), 2597–2605.

Hasan, M., Khalekuzzaman, M., Alamgir, M., Datta, P., and Kabir, S.B. (2022). A new energy-positive technological approach for wastewater treatment and bioenergy generation using a photo anoxic baffled reactor (PABR). *International Journal of Environmental Science and Technology*, 19, 995-1006.

Hasanuzzaman, M., Nahar, K., Alam, M., Roychowdhury, R., and Fujita, M. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences*, 14(5), 9643-9684.

Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., and Ahmad, A. (2012). Role of proline under changing environments: a review. *Plant Signaling & Behavior*, 7(11), 1456-1466.

He, Q., Yang, H., and Hu, C. (2018). Effects of temperature and its combination with high light intensity on lipid production of *Monoraphidium dybowskii* Y2 from semi-arid desert areas. *Bioresource Technology*, 265, 407-414.

He, Q., Yang, H., Wu, L., and Hu, C. (2015). Effect of light intensity on physiological changes, carbon allocation and neutral lipid accumulation in oleaginous microalgae. *Bioresource Technology*, 191, 219-228.

Heath, R.L., and Packer, L. (1968). Photoperoxidation in isolated chloroplasts: Kinetics and stoichiometry of fatty acid peroxidation. *Archieves of Biochemistry and Biophysics*, 125, 189-198.

Hedge, J.E., Hofreiter, B.T. (1962). *In: Carbohydrate Chemistry* 17 (Eds Whistler R L and Be Miller, J N) Academic Press New York.

Hena, S., Gutierrez, L., and Croué, J.P. (2021). Removal of pharmaceutical and personal care products (PPCPs) from wastewater using microalgae: A review. *Journal of Hazardous Materials*, 403, 124041.

Herbert, D., Phipps, P.J., and Strange, R.E., (1971). Chemical analysis of microbial cells, pp 209–344 - In: Norris, J. R. & Ribbons, D. W. (Eds.): Methods in microbiology, Vol. 5B, Academic Press, London, 695 pp.

Herrera, V. (2019). Reconciling global aspirations and local realities: challenges facing the sustainable development goals for water and sanitation. *World Development*, 118, 106-117.

Hightower, M., and Pierce, S.A. (2008). The energy challenge. *Nature*, 452, 285-286.

Ho, S.H., Chen, C.Y., and Chang, J.S. (2012). Effect of light intensity and nitrogen starvation on CO_2 fixation and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N. *Bioresource Technology*, 113, 244-252.

Hoekstra, A.Y. (2014). Water scarcity challenges to business. *Nature climate change*, 4, 318-320.

Hossain, M.A., Piyatida, P., da Silva, J.A.T., and Fujita, M. (2012). Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. *Journal of Botany*.

Hounslow, E., Evans, C.A., Pandhal, J., Sydney, T., Couto, N., Pham, T.K., Gilmour, D.J., and Wright, P.C. (2021). Quantitative proteomic comparison of salt stress in *Chlamydomonas reinhardtii* and the snow alga *Chlamydomonas nivalis* reveals mechanisms for salt-triggered fatty acid accumulation via reallocation of carbon resources. *Biotechnology for Biofuels*, 14, 1-25.

Howe, G., and Merchant, S. (1992). Heavy metal-activated synthesis of peptides in *Chlamydomonas reinhardtii*. *Plant Physiology,* 98, 127–136.

Hu, J., Nagarajan, D., Zhang, Q., Chang, J.S., and Lee, D.J. (2018). Heterotrophic cultivation of microalgae for pigment production: A review. *Biotechnology Advances*, 36, 54-67.

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., and Darzins, A. (2008). Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *The Plant Journal*, 54, 621-639.

Hu, X., Meneses, Y.E., Stratton, J., Lau, S.K., and Subbiah, J. (2021). Integration of ozone with co-immobilized microalgae-activated sludge bacterial symbiosis for efficient on-site treatment of meat processing wastewater. *Journal of Environmental Management*, 285, 112152.

Hube, S., Eskafi, M., Hrafnkelsdóttir, K.F., Bjarnadóttir, B., Bjarnadóttir, M.Á., Axelsdóttir, S., and Wu, B. (2020). Direct membrane filtration for wastewater treatment and resource recovery: A review. *Science of the Total Environment*, 710, 136375.

Huo, S., Wang, Z., Zhu, S., Zhou, W., Dong, R. and Yuan, Z., 2012. Cultivation of *Chlorella zofingiensis* in bench-scale outdoor ponds by regulation of pH using dairy wastewater in winter, South China. *Bioresource Technology*, 121, 76-82.

Huy, M., Kumar, G., Kim, H.W., and Kim, S.H. (2018). Photoautotrophic cultivation of mixed microalgae consortia using various organic waste streams towards remediation and resource recovery. *Bioresource Technology*, 247, 576-581.

Hwang, J.H., Church, J., Lee, S.J., Park, J. and Lee, W.H., 2016. Use of microalgae for advanced wastewater treatment and sustainable bioenergy generation. *Environmental Engineering Science*, 33(11), 882-897.

Iasimone, F., Panico, A., De Felice, V., Fantasma, F., Iorizzi, M., and Pirozzi, F. (2018). Effect of light intensity and nutrients supply on microalgae cultivated in urban wastewater: Biomass production, lipids accumulation and settleability characteristics. *Journal of Environmental Management*, 223, 1078-1085.

Ighodaro, O.M., and Akinloye, O.A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*, 54, 287-293.

Inthorn, D., Sidtitoon, N., Silapanuntakul, S., and Incharoensakdi, A. (2002). Sorption of mercury, cadmium and lead by microalgae. *Science Asia*, 28(3), 253-261.

Islam, M.S., and Mostafa, M.G. (2021). Environmental hazards of petroleum refinery in Bangladesh: a review. *Petroleum and Chemical Industry International*, 4, 1-4.

Jaafari, J., and Yaghmaeian, K. (2019). Optimization of heavy metal biosorption onto freshwater algae (*Chlorella* coloniales) using response surface methodology (RSM). *Chemosphere*, 217, 447-455.

Jais, N.M., Mohamed, R.M.S.R., Al-Gheethi, A.A., and Hashim, M.A. (2017). The dual roles of phycoremediation of wet market wastewater for nutrients and heavy metals removal and microalgae biomass production. *Clean Technologies and Environmental Policy*, 19, 37-52.

Jaiswal, D., and Pandey, J. (2019). Hypoxia and associated feedbacks at sediment-water interface as an early warning signal of resilience shift in an anthropogenically impacted river. *Environmental research*, 178, 108712.

Jaiswal, K.K., Banerjee, I., Singh, D., Sajwan, P., and Chhetri, V. (2020). Ecological stress stimulus to improve microalgae biofuel generation: a review. *Octa Journal of Bioscience*, 8, 48-54.

Jalmi, S.K., Bhagat, P.K., Verma, D., Noryang, S., Tayyeba, S., and Singh, K. (2018). Traversing the links between heavy metal stress and plant signaling. *Frontiers in Plant Science*, 9, 12.

James, G.O., Hocart, C.H., Hillier, W., Price, G.D., and Djordjevic, M.A. (2013). Temperature modulation of fatty acid profiles for biofuel production in nitrogen deprived *Chlamydomonas reinhardtii*. *Bioresource Technology*, 127, 441-447.

Janku, M., Luhova, L., and Petřivalsky, M. (2019). On the origin and fate of reactive oxygen species in plant cell compartments. *Antioxidants* (Basel). 8(4), 1–15.

Ji, X., Li, H., Zhang, J., Saiyin, H., Zheng, Z. (2019). The collaborative effect of *Chlorella vulgaris-Bacillus licheniformis* consortia on the treatment of municipal water. *Journal of Hazardous Materials*, 365, 483-493.

John, E.M., Sureshkumar, S., Sankar, T.V., and Divya, K.R. (2020). Phycoremediation in aquaculture; a win-win paradigm. *Environmental Technology Reviews*, 9, 67-84.

Johnson, K.R., and Admassu, W. (2013). Mixed algae cultures for low cost environmental compensation in cultures grown for lipid production and wastewater remediation. *Journal of Chemical Technology & Biotechnology*, 88, 992-998.

Juan, C.A., Pérez de la Lastra, J.M., Plou, F.J., and Pérez-Lebeña, E. (2021). The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *International Journal of Molecular Sciences*, 22, 4642.

Juneja, A., Ceballos, R.M., and Murthy, G.S. (2013). Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: a review. *Energies*, 6(9), 4607-4638.

Kampfenkel, K., Van Montagu, M., and Inze, D. (1995). Dehydroascorbate from plant tissue. *Analytical Biochemistry*, 225, 165-167.

Kamran, S.S., Haleem, A., Bahl, S., Javaid, M., Nandan, D., and Verma, A.S. (2022). Role of smart materials and digital twin (DT) for the adoption of electric vehicles in India. *Materials Today: Proceedings*, 52, 2295-2304.

Katiyar, R., Gurjar, B.R., Biswas, S., Pruthi, V., Kumar, N., and Kumar, P. (2017). Microalgae: an emerging source of energy based bio-products and a solution for environmental issues. *Renewable and Sustainable Energy Reviews*, 72, 1083-1093.

Kato, N., Nelson, G., and Lauersen, K.J. (2021). Subcellular localizations of catalase and exogenously added fatty acid in *Chlamydomonas reinhardtii*. *Cells*, 10(8), 1940.

Kaur, R., Wani, S.P., Singh, A.K., and Lal, K. (2012). Wastewater production, treatment and use in India. In *National Report presented at the 2nd regional workshop on Safe Use of Wastewater in Agriculture* (pp. 1-13).

Kerchev, P., van der Meer, T., Sujeeth, N., Verlee, A., Stevens, C.V., Van Breusegem, F., Gechev, T. (2019). Molecular priming as an approach to induce tolerance against abiotic and oxidative stresses in crop plants. *Biotechnology Advances*, 107503.

Khan, A., and Ashraf, M. (2008). Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environmental and Experimental Botany*, 63(1-3), 224-231.

Khan, A.Z., Shahid, A., Cheng, H., Mahboob, S., Al-Ghanim, K.A., Bilal, M., and Nawaz, M.Z. (2018). Omics technologies for microalgae-based fuels and chemicals: Challenges and opportunities. *Protein and Peptide Letters*, 25, 99-107.

Khan, M.K., Teng, J.Z., Khan, M.I., and Khan, M.O. (2019). Impact of globalization, economic factors and energy consumption on CO_2 emissions in Pakistan. *Science of the Total Environment*, 688, 424-436.

Khan, S.S., and Kaafil, S.F. (2020). A comparative study of grey water generated from commercial and residential buildings. *International Journal of Environment and Waste Management*, 25, 487-497.

Khattiyavong, C., and Lee, H.S. (2019). Performance simulation and assessment of an appropriate wastewater treatment technology in a densely populated growing city in a developing country: A case study in Vientiane, Laos. *Water*, 11, 1012.

Khulbe, K.C., and Matsuura, T. (2018). Removal of heavy metals and pollutants by membrane adsorption techniques. *Applied Water Science*, 8, 1-30.

Kligerman, D.C., and Bouwer, E.J. (2015). Prospects for biodiesel production from algae-based wastewater treatment in Brazil: A review. *Renewable and Sustainable Energy Reviews,* 52, 1834–1846.

Knauert, S., and Knauer, K. (2008). The role of reactive oxygen species in copper toxicity to two freshwater green algae. *Journal of Phycology,* 44, 311–319.

Knight, J.A., Anderson, S., and Rawle, J.M. (1972). Chemical basis of the sulfo-phospho-vanillin reaction for estimating total serum lipids. *Clinical chemistry*, 18, 199-202.

Knothe, G. (2009). Improving biodiesel fuel properties by modifying fatty ester composition. *Energy & Environmental Science*, 2, 759-766.

Kobielska, P.A., Howarth, A.J., Farha, O.K., and Nayak, S. (2018). Metal–organic frameworks for heavy metal removal from water. *Coordination Chemistry Reviews*, 358, 92-107.

Koca, H., Bor, M., Özdemir, F., and Türkan, İ. (2007). The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environmental and experimental Botany*, 60, 344-351.

Kong, W., Kong, J., Lyu, H., Ma, J., Yuan, P., Wang, Z., Shen, B. and Feng, S., 2021. Integrating municipal wastewater treatment with CO_2 fixation and fatty acid production by cultivating *Tetradesmus obliquus*. *Journal of Cleaner Production*, 320, 128916.

Kookana, R.S., Drechsel, P., Jamwal, P., and Vanderzalm, J. (2020). Urbanisation and emerging economies: Issues and potential solutions for water and food security. *Science of the Total Environment*, 732, 139057.

Kothari, R., Pathak, V.V., Kumar, V., and Singh, D.P. (2012). Experimental study for growth potential of unicellular alga *Chlorella pyrenoidosa* on dairy waste water: an integrated approach for treatment and biofuel production. *Bioresource Technology*, 116, 466-470.

Kothari, R., Prasad, R., Kumar, V., and Singh, D.P. (2013). Production of biodiesel from microalgae *Chlamydomonas polypyrenoideum* grown on dairy industry wastewater. *Bioresource Technology*, 144, 499-503.

Kumar, A., Yadav, A. N., Mondal, R., Kour, D., Subrahmanyam, G., Shabnam, A. A., and Malyan, S.K. (2021). Myco-remediation: A mechanistic understanding of contaminants alleviation from natural environment and future prospect. *Chemosphere,* 284, 131325.

Kumar, G., Nguyen, D.D., Huy, M., Sivagurunathan, P., Bakonyi, P., Zhen, G., and Chang, S.W. (2019). Effects of light intensity on biomass, carbohydrate and fatty acid compositions of three different mixed consortia from natural ecological water bodies. *Journal of Environmental Management*, 230, 293–300.

Kumar, K.S., Dahms, H.U., Won, E.J., Lee, J.S., and Shin, K.H. (2015). Microalgae–a promising tool for heavy metal remediation. *Ecotoxicology and Environmental Safety*, 113, 329-352.

Kumar, K.S., Shin, K.H. (2017). Effect of copper on marine microalga *Tetraselmis suecica* and its influence on intra-and extracellular iron and zinc content. *Korean Journal of Ecology and Environment*, 50, 16-28.

Kumar, M., Singh, A.K., and Sikandar, M. (2020). Biosorption of Hg (II) from aqueous solution using algal biomass: Kinetics and isotherm studies. *Heliyon*, 6, 03321.

Kumar, P.K., Krishna, S.V., Naidu, S.S., Verma, K., Bhagawan, D., and Himabindu, V. (2019). Biomass production from microalgae *Chlorella* grown in sewage, kitchen wastewater using industrial CO_2 emissions: comparative study. *Carbon Resources Conversion*, 2, 126-133.

Kumar, P.K., Krishna, S.V., Verma, K., Pooja, K., Bhagawan, D., and Himabindu, V. (2018). Phycoremediation of sewage wastewater and industrial flue gases for biomass generation from microalgae. *South African Journal of Chemical Engineering*, 25, 133-146.

Kumar, S., Isha, A., Chandra, R., Malik, A., and Vijay, V.K. (2021). Algal biomass: A promising source for future bioenergy production. In *Bioenergy Engineering* (pp. 131-152). CRC Press.

Kumar, S., Sharma, D.G., Tripathi, J.K., Ahirwar, S., and Singh, S.K. (2013). A study of the effectiveness of sewage treatment plants in Delhi region. *Applied Water Science*, 3, 57–65.

Kumar, V., and Chopra, A.K. (2012). Monitoring of physico-chemical and microbiological characteristics of municipal wastewater at treatment plant, Haridwar City (Uttarakhand) India. *Journal of Environmental Science and Technology*, 5, 109-118.

Kumar, V., Sharma, N., Jaiswal, K.K., Vlaskin, M.S., Nanda, M., Tripathi, M.K., and Kumar, S. (2021). Microalgae with a truncated light-harvesting antenna to maximize photosynthetic efficiency and biomass productivity: Recent advances and current challenges. *Process Biochemistry*, 104, 83–91.

Kupper, H., Setlik, I., Spiller, M., Kupper, F.C., and Prasil, O. (2002). Heavy metal-induced inhibition of photosynthesis: Targets of in vivo heavy metal chlorophyll formation. *Journal of Phycology*, 38, 429-441.

Kusmayadi, A., Lu, P.H., Huang, C.Y., Leong, Y.K., Yen, H.W., and Chang, J.S. (2022). Integrating anaerobic digestion and microalgae cultivation for dairy wastewater treatment and potential biochemicals production from the harvested microalgal biomass. *Chemosphere*, 291, 133057.

Larsen, T.A., Hoffmann, S., Luthi, C., Truffer, B., and Maurer, M. (2016).Emerging solutions to the water challenges of an urbanizing world. *Science*, 352, 928–933.

Laurens, L.M., and Wolfrum, E.J. (2011). Feasibility of spectroscopic characterization of algal lipids: chemometric correlation of NIR and FTIR spectra with exogenous lipids in algal biomass. *Bioenergy Research*, 4, 22-35.

Lavrinovičs, A., Mežule, L., and Juhna, T. (2020). Microalgae starvation for enhanced phosphorus uptake from municipal wastewater. *Algal Research*, 52, 102090.

Lawton, R.J., de Nys, R., Magnusson, M.E., and Paul, N.A. (2015). The effect of salinity on the biomass productivity, protein and lipid composition of a freshwater macroalga. *Algal Research*, 12, 213-220.

Le Faucheur, S., Behra, R., and Sigg, L. (2005). Phytochelatin induction, cadmium accumulation, and algal sensitivity to free cadmium ion in *Scenedesmus vacuolatus. Environmental Toxicology and Chemistry: An International Journal*, 24, 1731-1737.

Légeret, B., Schulz-Raffelt, M., Nguyen, H.M., Auroy, P., Beisson, F., Peltier, G., Blanc, G., and Li-Beisson, Y. (2016). Lipidomic and transcriptomic analyses of *Chlamydomonas reinhardtii* under heat stress unveil a direct route for the conversion of membrane lipids into storage lipids. *Plant Cell and Environment*, 39, 834–847

Leiva-Candia, D.E., Pinzi, S., Redel-Macías, M.D., Koutinas, A., Webb, C., and Dorado, M.P. (2014). The potential for agro-industrial waste utilization using oleaginous yeast for the production of biodiesel. *Fuel*, 123, 33–42.

Leong, Y.K., and Chang, J.S. (2020). Bioremediation of heavy metals using microalgae: recent advances and mechanisms. *Bioresource Technology*, 303, 122886.

Li X, Yang C, Zeng G, Wu S, Lin Y, Zhou Q, Lou W, Du C, Nie L, Zhong Y. 2020. Nutrient removal from swine wastewater with growing microalgae at various zinc concentrations. *Algal Research*, 46:101804.

Li, C., Zheng, C., Fu, H., Zhai, S., Hu, F., Naveed, S., Zhang, C., and Ge, Y. (2021). Contrasting detoxification mechanisms of *Chlamydomonas reinhardtii* under Cd and Pb stress. *Chemosphere*, 274, 129771.

Li, F., Srivatsa, S.C., Batchelor, W., and Bhattacharya, S. (2017). A study on growth and pyrolysis characteristics of microalgae using Thermogravimetric Analysis-Infrared Spectroscopy and synchrotron Fourier Transform Infrared Spectroscopy. *Bioresource Technology*, 229, 1-10.

Li, M., Ahammed, G. J., Li, C., Bao, X., Yu, J., and Huang, C. (2016a). Brassinosteroid ameliorates zinc oxide nanoparticles-induced oxidative stress by improving antioxidant potential and redox homeostasis in tomato seedling. *Frontiers in Plant Science*, 7, 615.

Li, X., Hu, H.Y., and Zhang, Y.P. (2011) Growth and lipid accumulation properties of a freshwater microalga *Scenedesmus* sp. under different cultivation temperature. *Bioresource Technology*, 102, 3098–3102.

Li, X., Sun, X., Zhou, X., He, Z., Lin, K., Chen, X. and Jiang, G. (2022). Fabrication of immobilized algal-bacterial beads and its application in synthetic mariculture tail water treatment. *Process Safety and Environmental Protection*.

Li, X., Yang, C., Zeng, G., Wu, S., Lin, Y., Zhou, Q., Lou, W., Du, C., Nie, L., and Zhong, Y. (2020). Nutrient removal from swine wastewater with growing microalgae at various zinc concentrations. *Algal Research*, 46, 101804.

Li, X., Yang, W.L., He, H., Wu, S., Zhou, Q., Yang, C., Zeng, G., Luo, L., Lou, W. (2018). Responses of microalgae *Coelastrella* sp. to stress of cupric ions in treatment of anaerobically digested swine wastewater. *Bioresource technology*, 251, 274-279.

Li, Y., Han, D., Hu, G., Sommerfeld, M., and Hu, Q. (2010) Inhibition of starch synthesis results in overproduction of lipids in *Chlamydomonas reinhardtii*. Biotechnol Bioeng 107, 258–268

Li, Y., Han, F., Xu, H., Mu, J., Chen, D., Feng, B., and Zeng, H. (2014). Potential lipid accumulation and growth characteristic of the green alga *Chlorella* with combination cultivation mode of nitrogen (N) and phosphorus (P). *Bioresource Technology*, 174, pp.24-32.

Li, Y., Zhou, W., Hu, B., Min, M., Chen, P., and Ruan, R.R. (2012). Effect of light intensity on algal biomass accumulation and biodiesel production for mixotrophic strains *Chlorella kessleri* and *Chlorella protothecoide* cultivated in highly concentrated municipal wastewater. *Biotechnology and Bioengineering*, 109, 2222-2229.

Li, Z., Xing, F., and Xing, D. (2012). Characterization of target site of aluminum phytotoxicity in photosynthetic electron transport by fluorescence techniques in tobacco leaves. *Plant and Cell Physiology*, 53, 1295-1309.

Liang, K., Zhang, Q., Gu, M., and Cong, W. (2013). Effect of phosphorus on lipid accumulation in freshwater microalga *Chlorella* sp. *Journal of Applied Phycology*, 25(1), 311-318.

Lin, T.H., Rao, M.Y., Lu, H.W., Chiou, C.W., Lin, S.T., Chao, H.W., Zheng, Z.L., Cheng, H.C., and Lee, T.M. (2018). A role for glutathione reductase and glutathione in the tolerance of *Chlamydomonas reinhardtii* to photo- oxidative stress. *Physiologia Plantarum*, 162(1), 35-48.

Lin, T.S., and Wu, J.Y. (2015). Effect of carbon sources on growth and lipid accumulation of newly isolated microalgae cultured under mixotrophic condition. *Bioresource Technology*, 184, 100-107.

Liu, J., Yuan, C., Hu, G., and Li, F. (2012). Effects of light intensity on the growth and lipid accumulation of microalga *Scenedesmus* sp. 11-1 under nitrogen limitation. *Applied Biochemistry and Biotechnology*, 166, 2127-2137.

Liu, X., Ying, K., Chen, G., Tao, Y., Cai, Z., and Wang, J. (2018). Influence of light intensity on microalgal growth, nutrients removal and capture of carbon in the wastewater under intermittent supply of CO_2. *Journal of Chemical Technology and Biotechnology*, 93, 3582-3589.

Liu, X., Ying, K., Chen, G., Zhou, C., Zhang, W., Zhang, X., Cai, Z., Holmes, T., and Tao, Y. (2017). Growth of *Chlorella vulgaris* and nutrient removal in the wastewater in response to intermittent carbon dioxide. *Chemosphere*, 186, 977-985.

Liyanage, C.P., and Yamada, K. (2017). Impact of population growth on the water quality of natural water bodies. *Sustainability*, 9(8), 1405.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193, 265-275.

Lu, N., Wei, D., Chen, F., and Yang, S.T. (2012). Lipidomic profiling and discovery of lipid biomarkers in snow alga *Chlamydomonas nivalis* under salt stress. *European Journal of Lipid Science and Technology*, 114, 253-265.

Lu, S., Zhang, X., Wang, J., and Pei, L. (2016). Impacts of different media on constructed wetlands for rural household sewage treatment. *Journal of Cleaner Production*, 127, 325-330.

Luo, L., He, H., Yang, C., Wen, S., Zeng, G., Wu, M., Zhou, Z., Lou, W. (2016). Nutrient removal and lipid production by *Coelastrella* sp. in anaerobically and aerobically treated swine wastewater. *Bioresource Technology*, 216, 135-141.

Lutzu, G.A., Ciurli, A., Chiellini, C., Di Caprio, F., Concas, A., and Dunford, N.T. (2020). Latest developments in wastewater treatment and biopolymer production by microalgae. *Journal of Environmental Chemical Engineering*, 104926.

Ma, C., Wen, H., Xing, D., Pei, X., Zhu, J., Ren, N., and Liu, B., (2017). Molasses wastewater treatment and lipid production at low temperature conditions by a microalgal mutant *Scenedesmus* sp. Z-4. *Biotechnology for Biofuels*, 10(1), 1-13.

Ma, X., Chen, Y., Liu, F., Zhang, S., and Wei, Q. (2021). Enhanced tolerance and resistance characteristics of *Scenedesmus obliquus* FACHB-12 with K3 carrier in cadmium polluted water. *Algal Research*, 55, 102267.

Maleva, M.G., Nekrasova, G.F., Borisova, G.G, Chukina, N.V., and Ushakova, O.S. (2012). Effect of heavy metals on photosynthetic apparatus and antioxidant status of Elodea. *Russian Journal of Plant Physiology*, 59, 190-197.

Manara, A. (2012). Plant responses to heavy metal toxicity. In *Plants and heavy metals* (pp. 27-53). Springer, Dordrecht.

Mandal, T., Maity, S., Dasgupta, D., and Datta, S. (2010). Advanced oxidation process and biotreatment: Their roles in combined industrial wastewater treatment. *Desalination*, 250(1), 87-94.

Mandotra, S.K., Upadhyay, A.K., and Ahluwalia, A.S. (2020). *Algae: Multifarious Applications for a Sustainable World*. Springer Nature.

Margot, J. (2015). Micropollutant removal from municipal wastewater-From conventional treatments to advanced biological processes. *Swiss Federal Institute of Technology Lausanne: Lausanne, Switzerland*.

Markou, G., and Nerantzis, E. (2013). Microalgae for high-value compounds and biofuels production: a review with focus on cultivation under stress conditions. *Biotechnology Advances*, 31, 1532-1542.

Markou, G., Dao, L.H., Muylaert, K., and Beardall, J. (2017). Influence of different degrees of N limitation on photosystem II performance and heterogeneity of *Chlorella vulgaris*. *Algal Research*, 26, 84-92.

Martınez, M.E., Sánchez, S., Jimenez, J.M., El Yousfi, F., and Munoz, L. (2000). Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*. *Bioresource Technology*, 73, 263-272.

Martin-Roberts, E., Scott, V., Flude, S., Johnson, G., Haszeldine, R.S., and Gilfillan, S. (2021). Carbon capture and storage at the end of a lost decade. *One Earth*, 4, 1569-1584.

Martins, F., Felgueiras, C., Smitkova, M., and Caetano, N. (2019). Analysis of fossil fuel energy consumption and environmental impacts in European countries. *Energies*, 12(6), 964.

Mata, T.M., Martins, A.A., and Caetano, N.S. (2010). Microalgae for biodiesel production and other applications: A review. *Renewable and Sustinable Energy Reviews*, 14(1), 217-232.

Mata, T.M., Melo, A.C., Simoes, M., and Caetano N.S., (2012). Parametric study of a brewery effluent treatment by microalgae *Scenedesmus obliquus*. *Bioresource Technology*, 107, 151-158.

Mathur, S., Allakhverdiev, S.I., and Jajoo, A. (2011). Analysis of high temperature stress on the dynamics of antenna size and reducing side heterogeneity of Photosystem II in wheat leaves (*Triticum aestivum*). *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1807(1), 22-29.

Meena, A.K., Mishra, G.K., Rai, P.K., Rajagopal, C., and Nagar, P.N. (2005). Removal of heavy metal ions from aqueous solutions using carbon aerogel as an adsorbent. *Journal of Hazardous Materials*, 122(1-2), 161-170.

Meena, R.A.A., Sathishkumar, P., Ameen, F., Yusoff, A.R.M., and Gu, F.L. (2018). Heavy metal pollution in immobile and mobile components of lentic ecosystems—a review. *Environmental Science and Pollution Research*, 25, 4134-4148.

Mehan, L., Verma, R., Kumar, R., and Srivastava, A. (2018). Illumination wavelengths effect on *Arthrospira platensis* production and its process applications in River Yamuna water treatment. *Journal of Water Process Engineering*, 23, 91-96.

Mehta, S.K., and Gaur, J.P. (2001). Characterization and optimization of Ni and Cu sorption from aqueous solution by *Chlorella vulgaris*. *Ecological Engineering*, 18 (1), 1-13.

Melegari, S.P., Perreault, F., Moukha, S., Popovic, R., Creppy, E.E., and Matias, W.G. 2012. Induction to oxidative stress by saxitoxin investigated through lipid peroxidation in Neuro 2A cells and *Chlamydomonas reinhardtii* alga. *Chemosphere*, 89(1), 38-43.

Mengelt, C., and Prézelin, B.B. (2005). UV-A enhancement of carbon fixation and resilience to UV inhibition in the genus Pseudo-nitzschia may provide a competitive advantage in high UV surface waters. Marine Ecology Progress Series, 301, 81–93.

Metsoviti, M.N., Papapolymerou, G., Karapanagiotidis, I.T., and Katsoulas, N. (2019). Comparison of growth rate and nutrient content of five microalgae species cultivated in greenhouses. *Plants*, 8, 279.

Michalak, I., Mironiuk, M., and Marycz, K. (2018). A comprehensive analysis of biosorption of metal ions by macroalgae using ICP-OES, SEM-EDX and FTIR techniques. *PloSone*, 13, 0205590.

Milne, A., Davey, M.S., Worsfold, P.J., Achterberg, E.P., and Taylor, A.R. (2009). Real- time detection of reactive oxygen species generation by marine phytoplankton using flow injection—chemiluminescence. *Limnology and Oceanography: Methods*, 7, 706-715.

Mishra, S., Jha, A.B., and Dubey, R.S. (2011). Arsenite treatment induces oxidative stress, upregulates antioxidant system, and causes phytochelatin synthesis in rice seedlings. *Protoplasma*, 248, 565-577.

Mohan, I., Goria, K., Dhar, S., Kothari, R., Bhau, B.S., and Pathania, D. (2021). Phytoremediation of heavy metals from the biosphere perspective and solutions. *Pollutants and Water Management: Resources, Strategies and Scarcity*, 95-127.

Mohanakavitha, T., Shankar, K., Divahar, R., Meenambal, T., and Saravanan, R. (2019). Impact of industrial wastewater disposal on surface water bodies in Kalingarayan canal, Erode district, Tamil Nadu, India. *Archives of Agriculture and Environmental Science*, 4, 379-387.

Mohsenpour, S.F., Hennige, S., Willoughby, N., Adeloye, A., and Gutierrez, T. (2021). Integrating micro-algae into wastewater treatment: a review. *Science of the Total Environment*, 752, 142168

Monteiro, C.M., Castro, P.M., and Malcata, F.X. (2012). Metal uptake by microalgae: underlying mechanisms and practical applications. *Biotechnology Progress*, 28, 299-311.

Moondra, N., Jariwala, N.D., and Christian, R.A. (2020). Microalgae based wastewater treatment: a shifting paradigm for the developing nations. *International Journal of Phytoremediation*, 1–10.

Morillas-España, A., Sánchez-Zurano, A., Lafarga, T., del Mar Morales-Amaral, M., Gómez-Serrano, C., Acién-Fernández, F.G., and González-López, C.V. (2021). Improvement of wastewater treatment capacity using the microalga *Scenedesmus* sp. and membrane bioreactors. *Algal Research*, 60, 102516.

Moustaka, J., Ouzounidou, G., Sperdouli, I., and Moustakas, M. (2018). Photosystem II is more sensitive than photosystem I to Al^{3+} induced phytotoxicity. *Materials*, 11, 1772.

Msanne, J., Xu, D., Konda, A.R., Casas-Mollano, J.A., Awada, T., Cahoon, E.B., and Cerutti, H. (2012). Metabolic and gene expression changes triggered by nitrogen deprivation in the photoautotrophically grown microalgae *Chlamydomonas reinhardtii* and *Coccomyxa* sp. C-169. *Phytochemistry,* 75, 50-59.

Mujtaba, G., Choi, W., Lee, C.-G., and Lee, K. (2012). Lipid production by *Chlorella vulgaris* after a shift from nutrient-rich to nitrogen starvation conditions. *Bioresource Technology*, 123, 279-283.

Mulders, K.J., Lamers, P.P., Martens, D.E., and Wijffels, R.H. (2014). Phototrophic pigment production with microalgae: biological constraints and opportunities. *Journal of Phycology*, 50, 229-242.

Murdock, J.N., and Wetzel, D.L. (2009). FT-IR microspectroscopy enhances biological and ecological analysis of algae. *Applied Spectroscopy Reviews*, 44, 335-361.

Mushtaq, N., Singh, D.V., Bhat, R.A., Dervash, M.A., and bin Hameed, O. (2020). Freshwater contamination: sources and hazards to aquatic biota. *In Fresh Water Pollution Dynamics and Remediation* (pp. 27-50). Springer, Singapore.

Mutale-joan, C., Rachidi, F., Mohamed, H.A., Mernissi, N.E., Aasfar, A., Barakate, M., Mohammed, D., Sbabou, L., and Arroussi, H.E. (2021). Microalgae-cyanobacteria–based biostimulant effect on salinity tolerance mechanisms, nutrient uptake, and tomato plant growth under salt stress. *Journal of Applied Phycology*, 1-17.

Nabizadeh, R., Kazem, N., Reza, S. (2006). Biosorption of lead (II) and cadmium (II) from aqueous solutions by protonated *Sargassum* sp. biomass. *Biotechnology*, 5, 21-26.

Nagappan, S., Devendran, S., Tsai, P.C., Dahms, H.U., and Ponnusamy, V.K. (2019). Potential of two-stage cultivation in microalgae biofuel production. *Fuel,* 252, 339–349.

Nair, S., Manu, B., and Azhoni, A. (2021). Sustainable treatment of paint industry wastewater: current techniques and challenges. *Journal of Environmental Management*, 296, 113105.

Naorbe, M.C., and Serrano Jr, A.E. (2018). Effects of heavy metals on cell density, size, specific growth rate and chlorophyll a of *Tetraselmis tetrathele* under controlled laboratory conditions. *Aquaculture, Aquarium, Conservation & Legislation*, 11, 589-597.

Naveed, S., Li, C., Lu, X., Chen, S., Yin, B., Zhang, C., and Ge, Y. (2019). Microalgal extracellular polymeric substances and their interactions with metal (loid) s: A review. *Critical Reviews in Environmental Science and Technology*, 49, 1769-1802.

Nazari-Sharabian, M., Ahmad, S., and Karakouzian, M. (2018). Climate change and eutrophication: a short review. *Engineering, Technology and Applied Science Research*, 8, 3668.

Negi, S., Barry, A.N., Friedland, N., Sudasinghe, N., Subramanian, S., Pieris, S., Holguin, F.O., Dungan, B., Schaub, T., and Sayre, R. (2016). Impact of nitrogen limitation on biomass, photosynthesis, and lipid accumulation in *Chlorella sorokiniana*. *Journal of Applied Phycology*, 28(2), 803-812.

Netto, L.E., de Oliveira, M.A., Monteiro, G., Demasi, A.P., Cussiol, J.R., Discola, K.F., Demasi, M., Silva, G.M., Alves, S.V., Faria, V.G., and Horta, B.B. (2007). Reactive cysteine in proteins: protein folding, antioxidant defense, redox signaling and more. *Comparative Biochemistry and Physiology*, 146, 180–193.

Ng, I.S., Tan, S.I., Kao, P.H., Chang, Y.K., and Chang, J.S. (2017). Recent developments on genetic engineering of microalgae for biofuels and bio- based chemicals. *Biotechnology Journal*, 12(10), 1600644.

Nieder, R., Benbi, D.K., and Reichl, F.X. (2018). Reactive water-soluble forms of nitrogen and phosphorus and their impacts on environment and human health. In *Soil components and human health* (pp. 223-255). Springer, Dordrecht.

Nishikimi, M., and Rao, N.A. (1972). The occurrence of superoxide anion in there action of reduced phenazinemethosulphate and molecular oxygen. *Biochemical and Biophysical Research Communications,* 48, 849–854.

Nogueira, D.P.K., Silva, A.F., Araújo, O.Q., and Chaloub, R.M. (2015). Impact of temperature and light intensity on triacylglycerol accumulation in marine microalgae. *Biomass and Bioenergy*, 72, 280-287.

Nowicka, B. (2022). Heavy metal–induced stress in eukaryotic algae—mechanisms of heavy metal toxicity and tolerance with particular emphasis on oxidative stress in exposed cells and the role of antioxidant response. *Environmental Science and Pollution Research*, 1-52.

Nowicka, B., Fesenko, T., Walczak, J., and Kruk, J. (2020). The inhibitor-evoked shortage of tocopherol and plastoquinol is compensated by other antioxidant mechanisms in *Chlamydomonas reinhardtii* exposed to toxic concentrations of cadmium and chromium ions. *Ecotoxicology and Environmental Safety*, 191, 110241.

Nzayisenga, J.C., Farge, X., Groll, S.L., and Sellstedt, A. (2020). Effects of light intensity on growth and lipid production in microalgae grown in wastewater. *Biotechnology for Biofuels*, 13, 1-8.

Ogbonna, J.C., and Tanaka, H. (2000). Light requirement and photosynthetic cell cultivation—Development of processes for efficient light utilization in photobioreactors. *Journal of Applied Phycology*, 12, 207–218.

Okuma, E., Murakami, Y., Shimoishi, Y., Tada, M., and Murata, Y. (2004). Effects of exogenous application of proline and betaine on the growth of tobacco cultured cells under saline conditions. *Soil Science and Plant Nutrition*, 50, 1301-1305.

Olofsson, M., Lamela, T., Nilsson, E., Bergé, J.P., Del Pino, V., Uronen, P., and Legrand, C. (2014). Combined effects of nitrogen concentration and seasonal changes on the production of lipids in *Nannochloropsis oculata*. *Marine drugs*, 12, 1891-1910.

Orefice, I., Chandrasekaran, R., Smerilli, A., Corato, F., Caruso, T., Casillo, A., Corsaro, M.M., Dal Piaz, F., Ruban, A.V., and Brunet, C. (2016). Light-induced changes in the photosynthetic physiology and biochemistry in the diatom *Skeletonema marinoi*. *Algal Research*, 17, 1–13

Owusu, P.A., and Asumadu-Sarkodie, S. (2016). A review of renewable energy sources, sustainability issues and climate change mitigation. *Cogent Engineering*, 3, 1167990.

Özdemir, S., Yalçın, M.S., and Kılınç, E. (2021). Preconcentrations of Ni (II) and Pb (II) from water and food samples by solid-phase extraction using *Pleurotus ostreatus* immobilized iron oxide nanoparticles. *Food Chemistry*, 336, 127675.

Pacheco, M.M., Hoeltz, M., Moraes, M.S., and Schneider, R.C. (2015). Microalgae: Cultivation techniques and wastewater phycoremediation. *Journal of Environmental Science and Health; A Toxic and Hazardeous Substances and Environmental Engineering*, 50, 585-601.

Pachiappan, P., Prasath, B.B., Perumal, S., Ananth, S., Devi, A.S., Kumar, S.D., and Jeyanthi, S. (2015). Isolation and culture of microalgae. *In Advances in Marine and Brackishwater Aquaculture* (pp. 1-15). Springer, New Delhi

Paliwal, C., Mitra, M., Bhayani, K., Bharadwaj, V.S., Ghosh, T., and Dubey, S., (2017). Abiotic stresses as tools for metabolites in microalgae. *Bioresource Technology*.

Pancha, I., Chokshi, K., George, B., Ghosh, T., Paliwal, C., Maurya, R., and Mishra, S. (2014). Nitrogen stress triggered biochemical and morphological changes in the microalgae *Scenedesmus* sp. CCNM 1077. *Bioresource Technology*, 156, 146-154.

Pancha, I., Chokshi, K., Maurya, R., Trivedi, K., Patidar, S.K., Ghosh, A., and Mishra, S. (2015). Salinity induced oxidative stress enhanced biofuel production potential of microalgae *Scenedesmus* sp. CCNM 1077. *Bioresource Technology*, 189, 341–348.

Pandey, A., Srivastava, S., and Kumar, S. (2019). Isolation, screening and comprehensive characterization of candidate microalgae for biofuel feedstock production and dairy effluent treatment: a sustainable approach. *Bioresource Technology*, 121998.

Parkhill, J.P., Maillet, G., and Cullen, J.J. (2001). Fluorescence-based maximal quantum yield for PSII as a diagnostic of nutrient stress. *Journal of Phycology*, 37, 517–529.

Patel, A.K., Joun, J.M., Hong, M.E., and Sim, S.J. (2019). Effect of light conditions on mixotrophic cultivation of green microalgae. *Bioresource Technology*, 282, 245-253.

Patra, M., Bhowmik, N., Bandopadhyay, B., Sharma, A. (2004). Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. *Environmental and Experimental Botany*, 52, 199-223.

Pehlivan, F.E. (2017). Vitamin C: An antioxidant agent. Vitamin C, 23-35.

Perales-Vela, H.V., Pena-Castro, J.M., and Canizares-Villanueva, R.O. (2006). Heavy metal detoxification in eukaryotic microalgae. *Chemosphere*, 64, 1–10

Pereira, W.E., de Siqueira, D.L., Martínez, C.A., and Puiatti, M. (2000). Gas exchange and chlorophyll fluorescence in four citrus rootstocks under aluminium stress. *Journal of Plant Physiology*, 157, 513-520.

Perera, F. (2018). Pollution from fossil-fuel combustion is the leading environmental threat to global pediatric health and equity: Solutions exist. *International journal of Environmental Research and Public Health*, 15, 16.

Perez-Rama, M., Alonso, J.A., López, C.H., and Vaamonde, E.T. (2002). Cadmium removal by living cells of the marine microalga *Tetraselmis suecica*. *Bioresource Technology*, 84, 265-270.

Peter, A.P., Khoo, K.S., Chew, K.W., Ling, T.C., Ho, S.H., Chang, J.S., and Show, P.L. (2021). Microalgae for biofuels, wastewater treatment and environmental monitoring. *Environmental Chemistry Letters*, 1-14.

Phaniendra, A., Jestadi, D.B., and Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*, 30, 11-26.

Pignocchi, C., and Foyer, C.H. (2003). Apoplastic ascorbate metabolism and its role in the regulation of cell signalling. *Current Opinion in Plant Biology*, 6(4), 379-389.

Pikula, K.S., Zakharenko, A.M., Aruoja, V., Golokhvast, K.S., and Tsatsakis, A.M. (2019). Oxidative stress and its biomarkers in microalgal ecotoxicology. *Current Opinion in Toxicology*, 13, 8-15.

Piotrowska-Niczyporuk, A., Bajguz, A., Talarek, M., Bralska, M., and Zambrzycka, E. (2015). The effect of lead on the growth, content of primary metabolites, and antioxidant response of green alga *Acutodesmus obliquus* (Chlorophyceae). *Environmental Science and Pollution Research,* 22, 19112-19123.

Pontevedra-Pombal, X., Rey-Salgueiro, L., García-Falcón, M.S., Martínez-Carballo, E., Simal-Gándara, J., and Martínez-Cortizas, A. (2012). Pre-industrial accumulation of anthropogenic polycyclic aromatic hydrocarbons found in a blanket bog of the Iberian Peninsula. *Environmental Research*, 116, 36-43.

Pradhan, B., Baral, S., Patra, S., Behera, C., Nayak, R., MubarakAli, D., and Jena, M. (2020). Delineation of gamma irradiation (60Co) induced oxidative stress by decrypting antioxidants and biochemical responses of microalga, *Chlorella* sp. *Biocatalysis and Agricultural Biotechnology*, 25, 101595.

Pradhan, D., Sukla, L.B., Mishra, B.B., and Devi, N. (2019). Biosorption for removal of hexavalent chromium using microalgae *Scenedesmus* sp. *Journal of Cleaner Production*, 209, 617-629.

Prajapati, S.K., Kaushik, P., Malik, A., and Vijay, V.K. (2013). Phycoremediation coupled production of algal biomass, harvesting and anaerobic digestion: possibilities and challenges. *Biotechnology Advances*, 31, 1408-1425.

Prasad, S.M., Singh, J.B., Rai, L.C., and Kumar, H.D. (1991). Metal-induced inhibition of photosynthetic electron transport chain of the cyanobacterium *Nostoc muscorum*. *FEMS Microbiology Letters*, 82, 95-100.

Pratush, A., Kumar, A., and Hu, Z. (2018). Adverse effect of heavy metals (As, Pb, Hg, and Cr) on health and their bioremediation strategies: a review. *International Microbiology*, 21, 97-106.

Pujari, M., and Kapoor, D. (2021). Heavy metals in the ecosystem: Sources and their effects. In *Heavy Metals in the Environment* (pp. 1-7). Elsevier.

Pure Earth, 2016. World's Worst Pollution Problems. The Toxics Beneath Our Feet. https://www.worstpolluted.org/docs/WorldsWorst2016Spreads.pdf

Qadir, I., and Chhipa, R.C. (2015). Critical evaluation of some available treatment techniques for textile and paper industry effluents: a review. *American Chemical Science Journal*, 6, 77-90.

Qiao, T., Zhao, Y., Zhong, D.B., and Yu, X. (2021). Hydrogen peroxide and salinity stress act synergistically to enhance lipids production in microalga by regulating reactive oxygen species and calcium. *Algal Research*, 53, 102017.

Qiao, Y., Rong, J., Chen, H., He, C., and Wang, Q. (2015). Non-invasive rapid harvest time determination of oil-producing microalgae cultivations for biodiesel production by using chlorophyll fluorescence. *Frontiers in Energy Research*, 3, 44.

Qin, L., Wang, Z., Sun, Y., Shu, Q., Feng, P., Zhu, L., Xu, J., and Yuan, Z. (2016). Microalgae consortia cultivation in dairy wastewater to improve the potential of nutrient removal and biodiesel feedstock production. *Environmental Science and Pollution Research*, 23, 8379-8387.

Qu, J., Wang, H., Wang, K., Yu, G., Ke, B., Yu, H.Q., Ren, H., Zheng, X., Li, J., Li, W.W., and Gao, S. (2019). Municipal wastewater treatment in China: Development history and future perspectives. *Frontiers of Environmental Science & Engineering*, 13(6), 1-7.

Qu, W., Show, P.L., Hasunuma, T., and Ho, S.H. (2020). Optimizing real swine wastewater treatment efficiency and carbohydrate productivity of newly microalga *Chlamydomonas* sp. QWY37 used for cell-displayed bioethanol production. *Bioresource Technology*, 305, 123072.

Raeesossadati, M.J., Ahmadzadeh, H., McHenry, M.P., and Moheimani, N.R. (2014). CO_2 bioremediation by microalgae in photobioreactors: impacts of biomass and CO_2 concentrations, light, and temperature. *Algal Research*, 6, 78-85.

Rai, L.C., Singh, A.K. and Mallick, N. (1991). Studies on photosynthesis, the associated electron transport system and some physiological variables of *Chlorella vulgaris* under heavy metal stress. *Journal of Plant Physiology*, 137(4), 419-424.

Rai, V., Muthuraj, M., Gandhi, M.N., Das, D., and Srivastava, S. (2017). Real-time iTRAQ-based proteome profiling revealed the central metabolism involved in nitrogen starvation-induced lipid accumulation in microalgae. *Scientific Reports*, 7, 1-16.

Rajalakshmi, A.M., Silambarasan, T., and Dhandapani, R. (2021). Small scale photo bioreactor treatment of tannery wastewater, heavy metal biosorption and CO_2 sequestration using microalga *Chlorella* sp.: a biodegradation approach. *Applied Water Science*, 11(7), 1-12.

Rajasulochana, P., and Preethy, V. (2016). Comparison on efficiency of various techniques in treatment of waste and sewage water–A comprehensive review. *Resource-Efficient Technologies*, 2, 175-184.

Rajendrakumar, C.S., Reddy, B.V., and Reddy, A.R. (1994). Proline-protein interactions: protection of structural and functional integrity of M4 lactate dehydrogenase. *Biochemical and biophysical research communications*, 201, 957-963.

Rajoria, S., Vashishtha, M., and Sangal, V.K. (2021). Review on the treatment of electroplating industry wastewater by electrochemical methods. *Materials Today: Proceedings*, 47, 1472-1479.

Randrianarison, G., and Ashraf, M.A. (2017). Microalgae: a potential plant for energy production. *Geology, Ecology, and Landscapes*, 1, 104-120.

Randrianarisona, G., Ashrafa, M.A., and Zaonarivelob, J.R. (2021). The potentiality of Arthrospira platensis microalgal species for mining wastewater bioremediation by biosorption removal of heavy metals (Zn^{2+}, Cu^{2+}, Pb^{2+}, Fe^{2+}). *Desalination and Water Treatment*, 239, 1-12.

Ras, M., Steyer, J.P., and Bernard, O. (2013). Temperature effect on microalgae: a crucial factor for outdoor production. *Reviews in Environmental Science and Bio/Technology*, 12(2), 153-164.

Rasoul-Amini, S., Montazeri-Najafabady, N., Shaker, S., Safari, A., Kazemi, A., Mousavi, P., Mobasher, M.A., and Ghasemi, Y. (2014). Removal of nitrogen and phosphorus from wastewater using microalgae free cells in bath culture system. *Biocatalysis and Agricultural Biotechnology*, 3, 126-131.

Raychaudhuri, S.S., and Deng, X.W. (2000). The role of superoxide dismutases (SODs) in combating oxidative stress in higher plants. *Botanical Review*, 66, 89–98.

Razzak, S.A., Hossain, M.M., Lucky, R.A., Bassi, A.S., and de Lasa, H. (2013). Integrated CO_2 capture, wastewater treatment and biofuel production by microalgae culturing—A review. *Renewable and Sustainable Energy Reviews*, 27, 622–653.

Rehman, A.U., Nazir, S., Irshad, R., Tahir, K., ur Rehman, K., Islam, R.U., and Wahab, Z. (2021). Toxicity of heavy metals in plants and animals and their uptake by magnetic iron oxide nanoparticles. *Journal of Molecular Liquids*, 321, 114455.

Reinhard, C.T., Planavsky, N.J., Gill, B.C., Ozaki, K., Robbins, L.J., Lyons, T.W., Fischer, W.W., Wang, C., Cole, D.B., and Konhauser, K.O. (2017). Evolution of the global phosphorus cycle. *Nature*, 541, 386-389.

Renaud, S.M., Thinh, L.V., Lambrinidis, G., and Parry, D.L. (2002). Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. *Aquaculture*, 211, 195-214.

Renuka, N., Ratha, S. K., Kader, F., Rawat, I., and Bux, F. (2021). Insights into the potential impact of algae-mediated wastewater beneficiation for the circular bioeconomy: A global perspective. *Journal of Environmental Management*, 297, 113257.

Renuka, N., Sood, A., Ratha, S.K., Prasanna, R., and Ahluwalia, A.S. (2013). Evaluation of microalgal consortia for treatment of primary treated sewage effluent and biomass production. *Journal of Applied Phycology*, 25(5), 1529-1537.

Rezayian, M., Niknam, V., and Ebrahimzadeh, H. (2019). Oxidative damage and antioxidative system in algae. *Toxicology reports*, 6, 1309-1313.

Richmond, A. (2004). Biological principles of mass cultivation. In: Richmond A (ed) Handbook of microalgal mass, culture: biotechnology and applied phycology. CRC Press, Blackwell Publishing Company, Oxford, pp 566

Richon, C., Dutay, J.C., Dulac, F., Wang, R., Balkanski, Y., Nabat, P., Aumont, O., Desboeufs, K., Laurent, B., Guieu, C., and Raimbault, P. (2018). Modeling the impacts of atmospheric deposition of nitrogen and desert dust-derived phosphorus on nutrients and biological budgets of the Mediterranean Sea. *Progress in Oceanography*, 163, 21-39.

Rismani-Yazdi, H., Haznedaroglu, B.Z., Bibby, K., Peccia, J. (2011). Transcriptome sequencing and annotation of the microalgae *Dunaliella tertiolecta*: Pathway description and gene discovery for production of next-generation biofuels. *BMC Genomics*, 12, 148.

Roleda, M.Y., Slocombe, S.P., Leakey, R.J., Day, J.G., Bell, E.M., and Stanley, M.S. (2013). Effects of temperature and nutrient regimes on biomass and lipid production by six oleaginous microalgae in batch culture employing a two-phase cultivation strategy. *Bioresource Technology*, 129, 439-449.

Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J.A., Hilal, M., and Prado, F.E. (2009). Soluble sugars-metabolism, sensing and abiotic stress a complex network in the life of plants. *Plant Signaling and Behavior*, 4, 388–393.

Rosli, S.S., Kadir, W.N.A., Wong, C.Y., Han, F.Y., Lim, J.W., Lam, M.K., Yusup, S., Kiatkittipong, W., Kiatkittipong, K., and Usman, A. (2020). Insight review of attached microalgae growth focusing on support material packed in photobioreactor for sustainable biodiesel production and wastewater bioremediation. *Renewable and Sustainable Energy Reviews*, 134, 110306.

Roy, M., Bera, S., and Mohanty, K. (2021). Nutrient starvation-induced oxidative stress-mediated lipid accumulation in *Tetradesmus obliquus* KMC24. *Journal of Applied Phycology*, 33, 3617-3635.

Roy, U.K., Nielsen, B.V., and Milledge, J.J. (2021). Antioxidant production in *Dunaliella*. *Applied Sciences*, 11, 3959.

Rugnini, L., Ellwood, N.T.W., Costa, G., Falsetti, A., Congestri, R., and Bruno, L. (2019). Scaling-up of wastewater bioremediation by *Tetradesmus obliquus*, sequential bio-treatments of nutrients and metals. *Ecotoxicology and Environmental Safety*, 172, 59-64.

Sachdev, S., Ansari, S.A., Ansari, M.I., Fujita, M., and Hasanuzzaman, M. (2021). Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. *Antioxidants*, 10, 277.

Sadeghi, H., Fazlzadeh, M., Zarei, A., Mahvi, A.H., and Nazmara, S. (2022). Spatial distribution and contamination of heavy metals in surface water, groundwater and topsoil surrounding Moghan's tannery site in Ardabil, Iran. *International Journal of Environmental Analytical Chemistry*, 102, 1049-1059.

Sadh, P.K., Duhan, S., and Duhan, J.S. (2018). Agro-industrial wastes and their utilization using solid state fermentation: a review. *Bioresources and Bioprocessing*, 5, 1-15.

Sadvakasova, A.K., Akmukhanova, N.R., Zayadan, B.K., Matorin, D.N., Protopopov, F.F., Alekseev, A.A., and Bolatkhan, K. (2016). Pigment mutants of the green microalga Chlamydomonas reinhardtii: Morphological properties and photosynthetic performance. *Russian Journal of Plant Physiology*, 63(4), 443-450.

Sajjadi, B., Chen, W.Y., Raman, A.A.A., and Ibrahim, S. (2018). Microalgae lipid and biomass for biofuel production: A comprehensive review on lipid enhancement strategies and their effects on fatty acid composition. *Renewable and Sustainable Energy Reviews*, 97, 200-232.

Salama, E.S., Roh, H.S., Dev, S., Khan, M.A., Abou-Shanab, R.A.I., Chang, S.W., Jeon, B.H. (2019). Algae as a green technology for heavy metals removal from various wastewater. *World Journal of Microbiology and Biotechnology*, 35(5), 75.

Salbitani, G., and Carfagna, S. (2021). Ammonium utilization in microalgae: A sustainable method for wastewater treatment. *Sustainability*, 13(2), 956.

Sami, F., Yusuf, M., Faizan, M., Faraz, A., and Hayat, S. (2016). Role of sugars under abiotic stress. *Plant Physiology and Biochemistry*, 109, 54–61.

Saranya, D., and Shanthakumar, S. (2019). Green microalgae for combined sewage and tannery effluent treatment: Performance and lipid accumulation potential. *Journal of Environmental Management*, 241, 167–178.

Saranya, D., and Shanthakumar, S. (2020). Effect of culture conditions on biomass yield of acclimatized microalgae in ozone pre-treated tannery effluent: A simultaneous exploration of bioremediation and lipid accumulation potential. *Journal of Environmental Management*, 273, 111129.

Sarkar, B., Mandal, S., Tsang, Y.F., Vithanage, M., Biswas, J.K., Yi, H., Dou, X. and Ok, Y.S. (2019). Sustainable sludge management by removing emerging contaminants from urban wastewater using carbon nanotubes. In Industrial and Municipal Sludge (553-571). Butterworth-Heinemann.

Sathasivam, R., and Ki, J.S. (2019). Heat shock protein genes in the green alga *Tetraselmis suecica* and their role against redox and non-redox active metals. *European Journal of Protistology*, 69, 37-51.

Sayegh, F.A., and Montagnes, D.J. (2011). Temperature shifts induce intraspecific variation in microalgal production and biochemical composition. *Bioresource Technology*, 102, 3007-3013.

Schenk, P.M., Thomas-Hall, S.R., Stephens, E., Marx, U.C., Mussgnug, J.H., Posten, C., Kruse, O., and Hankamer, B. (2008). Second generation biofuels: high-efficiency microalgae for biodiesel production. *Bioenergy Research*, 1(1), 20-43.

Schewe, J., Heinke, J., Gerten, D., Haddeland, I., Arnell, N.W., Clark, D.B., Dankers, R., Eisner, S., Fekete, B.M., Colón-González, F.J., and Gosling, S.N. (2014). Multimodel assessment of water scarcity under climate change. *Proceedings of the National Academy of Sciences*, 111(9), 3245-3250.

Ścibior, D., and Czeczot, H. (2006). Katalaza – budowa, właściwości, funkcje. Postęp Hig Med Dosw. 60, 170-180.

Seo, S.H., Srivastava, A., Han, M.S., Lee, H.G., and Oh, H.M. (2019). Maximizing biomass and lipid production in *Ettlia* sp. by ultraviolet stress in a continuous culture. *Bioresource Technology*, 121472.

Sforza, E., Simionato, D., Giacometti, G.M., Bertucco, A., and Morosinotto, T. (2012). Adjusted light and dark cycles can optimize photosynthetic efficiency in algae growing in photobioreactors. *PloS one*, 7, 38975.

Shafi, A., Zahoor, I., and Mushtaq, U. (2019). Proline accumulation and oxidative stress: Diverse roles and mechanism of tolerance and adaptation under salinity stress. In *Salt Stress, Microbes, and Plant Interactions: Mechanisms and Molecular Approaches* (269-300). Springer, Singapore.

Shahid, A., Malik, S., Zhu, H., Xu, J., Nawaz, M.Z., Nawaz, S., Alam, M.A., and Mehmood, M.A. (2020). Cultivating microalgae in wastewater for biomass production, pollutant removal, and atmospheric carbon mitigation; a review. *Science of the Total Environment*, 704, 135303.

Shanmugam, S., Hari, A., Kumar, D., Rajendran, K., Mathimani, T., Atabani, A.E., Brindhadevi, K., and Pugazhendhi, A. (2021). Recent developments and strategies in genome engineering and integrated fermentation approaches for biobutanol production from microalgae. *Fuel*, 285, 119052.

Shannon, M.A., Bohn, P.W., Elimelech, M., Georgiadis, J.G., Marinas, B.J., and Mayes, A.M. (2010). Science and technology for water purification in the coming decades. *Nanoscience and technology: a collection of reviews from nature Journals*, 337-346.

Shao, H.B., Chu, L.Y., Lu, Z.H., and Kang, C.M. (2008). Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. *International Journal of Biological Sciences*, 4, 8.

Sharma, A., Kumar, V., Shahzad, B., Ramakrishnan, M., Singh Sidhu, G.P., Bali, A.S., Handa, N., Kapoor, D., Yadav, P., Khanna, K., and Bakshi, P. (2020). Photosynthetic response of plants under different abiotic stresses: a review. *Journal of Plant Growth Regulation*, 39, 509-531.

Sharma, J., Kumar, V., Kumar, S.S., Malyan, S.K., Mathimani, T., Bishnoi, N.R., and Pugazhendhi, A. (2020). Microalgal consortia for municipal wastewater treatment–Lipid augmentation and fatty acid profiling for biodiesel production. *Journal of Photochemistry and Photobiology B: Biology*, 202, 111638.

Sharma, P., and Dubey, R.S. (2005) Modulation of nitrate reductase activity in rice seedlings under aluminium toxicity and water stress: role of osmolytes as enzyme protectant. *Journal of Plant Physiology*, 162, 854– 864.

Sharma, P., Jha, A.B., Dubey, R.S., and Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*.

Sharma, S.S., Dietz, K.J., and Mimura, T. (2016). Vacuolar compartmentalization as indispensable component of heavy metal detoxification in plants. *Plant Cell and Environment*, 39, 1112–1126.

Shcolnick, S., and Keren, N. (2006). Metal homeostasis in cyanobacteria and chloroplasts. Balancing benefits and risks to the photosynthetic apparatus. *Plant Physiology*, 141, 805-810.

Shekh, A., Sharma, A., Schenk, P.M., Kumar, G., and Mudliar, S. (2021). Microalgae cultivation: photobioreactors, CO_2 utilization, and value- added products of industrial importance. *Journal of Chemical Technology & Biotechnology,* 97., 1064-1085.

Shen, Q.H., Gong, Y.P., Fang, W.Z., Bi, Z.C., Cheng, L.H., Xu, X.H., and Chen, H.L. (2015). Saline wastewater treatment by *Chlorella vulgaris* with simultaneous algal lipid accumulation triggered by nitrate deficiency. *Bioresource Technology*, 193, 68-75.

Shen, X., Zhang, M., Bhandari, B., Gao, Z. (2019). Novel technologies in utilization of byproducts of animal food processing: A review. *Critical Reviews in Food Science and Nutrition*, 59, 3420-30.

Shi, J., Podola, B., and Melkonian, M. (2014). Application of a prototype-scale Twin-Layer photobioreactor for effective N and P removal from different process stages of municipal wastewater by immobilized microalgae. *Bioresource Technology*, 154, 260-266.

Shi, K., Gao, Z., Shi, T.Q., Song, P., Ren, L.J., Huang, H., and Ji, X.J. (2017). Reactive oxygen species-mediated cellular stress response and lipid accumulation in oleaginous microorganisms: the state of the art and future perspectives. *Frontiers in Microbiology*, 8, 1–9.

Shi, T.Q., Wang, L.R., Zhang, Z.X., Sun, X.M., and Huang, H. (2020). Stresses as first-line tools for enhancing lipid and carotenoid production in microalgae. *Frontiers in Bioengineering and Biotechnology*, 8, 610.

Shindhal, T., Rakholiya, P., Varjani, S., Pandey, A., Ngo, H.H., Guo, W., Ng, H.Y., and Taherzadeh, M.J. (2021). A critical review on advances in the practices and perspectives for the treatment of dye industry wastewater. *Bioengineered*, 12, 70-87.

Shivaji, S., and Dronamaraju, S.V. (2019). *Scenedesnus rotundus* isolated from the petroleum effluent employs alternate mechanisms of tolerance to elevated levels of Cadmium and Zinc. *Scientific reports*, 9, 1-15.

Shoaib, H., and Sibi, G. (2018). Antioxidant system response in green microalga *chlorococcopsis minuta* against nutrient stress in growth media. *Asian Joural of Biological Sciences*, 11(4), 210-216.

Shrestha, R., Ban, S., Devkota, S., Sharma, S., Joshi, R., Tiwari, A.P., Kim, H.Y., and Joshi, M.K. (2021). Technological trends in heavy metals removal from industrial wastewater: A review. *Journal of Environmental Chemical Engineering*, 9, 105688.

Sijil, P.V., Sarada, R., and Chauhan, V.S. (2019). Enhanced accumulation of alpha-linolenic acid rich lipids in indigenous freshwater microalga *Desmodesmus* sp.: The effect of low-temperature on nutrient replete, UV treated and nutrient stressed cultures. *Bioresource Technology*, 273, 404-415.

Silva, N.F.P., Gonçalves, A.L., Moreira, F.C., Silva, T.F.C.V., Martins, F.G., Alvim-Ferraz, M.C.M., Boaventura, R.A.R., Vilar, V.J.P., and Pires, J.C.M. (2015). Towards sustainable microalgal biomass production by phycoremediation of a synthetic wastewater: A kinetic study. *Algal Research*, 11, 350-358.

Simionato, D., Basso, S., Giacometti, G.M., and Morosinotto, T. (2013). Optimization of light use efficiency for biofuel production in algae. *Biophysical Chemistry*, 182, 71-78.

Singh, A.K., and Pandey, A.K. (2018). Microalgae: an ecofriendly tool for the treatment of industrial wastewaters and biofuel production. In *Recent Advances in Environmental Management* (pp. 167-195). CRC Press.

Singh, D.V., and Singh, R.P. (2022). Algal consortia based metal detoxification of municipal wastewater: Implication on photosynthetic performance, lipid production, and defense responses. *Science of the Total Environment*, 814, 151928.

Singh, D.V., Bhat, R.A., Upadhyay, A.K., Singh, R., and Singh, D.P. (2021). Microalgae in aquatic environs: a sustainable approach for remediation of heavy metals and emerging contaminants. *Environmental Technology & Innovation*, 21, 101340.

Singh, D.V., Upadhyay, A.K., Singh, R., and Singh, D.P. (2021). Microalgal competence in urban wastewater management: phycoremediation and lipid production. *International Journal of Phytoremediation*, 1-11.

Singh, M., Kumar, J., Singh, S., Singh, V.P., Prasad, S.M., and Singh, M.P.V.V.B. (2015). Adaptation strategies of plants against heavy metal toxicity: a short review. *Biochemistry & Pharmacology*, 4, 2167-0501.

Singh, P., Guldhe, A., Kumari, S., Rawat, I., and Bux, F. (2015). Investigation of combined effect of nitrogen, phosphorus and iron on lipid productivity of microalgae *Ankistrodesmus falcatus* KJ671624 using response surface methodology. *Biochemical Engineering Journal*, *94*, pp.22-29.

Singh, P., Guldhe, A., Kumari, S., Rawat, I., and Bux, F. (2016). Combined metals and EDTA control: an integrated and scalable lipid enhancement strategy to alleviate biomass constraints in microalgae under nitrogen limited conditions. *Energy Conversion and Management*, 114, 100-109.

Singh, R., Upadhyay, A.K., Chandra, P., and Singh, D.P. (2018). Sodium chloride incites reactive oxygen species in green algae *Chlorococcum humicola* and *Chlorella vulgaris*: implication on lipid synthesis, mineral nutrients and antioxidant system. *Bioresource Technology*, 270, 489-497.

Singh, R., Upadhyay, A.K., Singh, D.V., Singh, J.S., and Singh, D.P. (2019). Photosynthetic performance, nutrient status and lipid yield of microalgae *Chlorella vulgaris* and *Chlorococcum humicola* under UV-B exposure. *Current Research in Biotechnology*, 1, 65-77.

Singh, S.P., and Singh, P. (2015). Effect of temperature and light on the growth of algae species: a review. *Renewable and Sustainable Energy Reviews*, 50, 431-444.

Slama, I., M'Rabet, R., Ksouri, R., Talbi, O., Debez, A., and Abdelly, C. (2017). Effects of salt treatment on growth, lipid membrane peroxidation, polyphenol content, and antioxidant activities in leaves of *Sesuvium portulacastrum* L. *Arid Land Research and Management*, 31, 404-417.

Smirnoff, N. (2018). Ascorbic acid metabolism and functions: A comparison of plants and mammals. *Free Radical Biology and Medicine*, 122, 116-129.

Smith, I.K., Vierheller, T.L., and Thorne, C.A. (1988). Assay of glutathione reductase in crude tissue homogenates using 5, 5′-dithiobis (2-nitrobenzoic acid). *Analytical Biochemistry*, 175, 408-413.

Snider, J.L., Oosterhuis, D.M., and Kawakami, E.M. (2010). Genotypic differences in thermotolerance are dependent upon prestress capacity for antioxidant protection of the photosynthetic apparatus in *Gossypium hirsutum*. *Physiologia plantarum*, 138(3), 268-277.

Solovchenko, A., Lukyanov, A., Vasilieva, S., and Lobakova, E. (2022). Chlorophyll fluorescence as a valuable multitool for microalgal biotechnology. *Biophysical Reviews*, 1-11.

Solovchenko, A.E., Khozin-Goldberg, I., Didi-Cohen, S., Cohen, Z., and Merzlyak, M.N. (2008). Effects of light intensity and nitrogen starvation on growth, total fatty acids and arachidonic acid in the green microalga *Parietochloris incisa*. *Journal of Applied Phycology*, 20(3), 245-251.

Sommer, S.G., and Knudsen, L. (2021). Impact of Danish livestock and manure management regulations on nitrogen pollution, crop production, and economy. *Frontiers in Sustainability*, 2, 20.

Song, X., Yue, X., Chen, W., Jiang, H., Han, Y., and Li, X. (2019). Detection of cadmium risk to the photosynthetic performance of Hybrid Pennisetum. *Frontiers in Plant Science*, 10, 798.

Sørensen, M., Andersen-Ranberg, J., Hankamer, B., and Møller, B.L. (2022). Circular biomanufacturing through harvesting solar energy and CO_2. *Trends in Plant Science*.

Speiser, A., Haberland, S., Watanabe, M., Wirtz, M., Dietz, K.J., Saito, K., and Hell, R. (2015). The significance of cysteine synthesis for acclimation to high light conditions. *Frontiers in Plant Science*, 5, 776.

Srirangan, S., Sauer, M.L., Howard, B., Dvora, M., Dums, J., Backman, P., and Sederoff, H. (2015). Interaction of temperature and photoperiod increases growth and oil content in the marine microalgae *Dunaliella viridis*. *PloS one*, 10(5), 0127562.

Stainer, R.Y., Kunisawa, R., Mandel, M., and Cohen-Bazire, G. (1971). Purification and properties of unicellular blue –green algae (order Chroococcales). *Bacteriological Rev*iews, 32, 171-205

Stamenković, O.S., Siliveru, K., Veljković, V.B., Banković-Ilić, I.B., Tasić, M.B., Ciampitti, I.A., and Prasad, P.V.V. (2020). Production of biofuels from sorghum. *Renewable and Sustainable Energy Reviews*, 124, 109769.

Stockenreiter, M., Haupt, F., Seppälä, J., Tamminen, T., and Spilling, K. (2016). Nutrient uptake and lipid yield in diverse microalgal communities grown in wastewater. *Algal Research*, 15, 77-82.

Strasser, R.J., Tsimilli-Michael, M., and Srivastava, A. (2004). Analysis of the chlorophyll a fluorescence transient. In *Chlorophyll a fluorescence* (pp. 321-362). Springer, Dordrecht.

Su, Y., Mennerich, A., and Urban B. (2012). Synergistic cooperation between wastewater-born algae and activated sludge for wastewater treatment:

influence of algae and sludge inoculation ratios. *Bioresource Technology*, 105, 67–73.

Su, Y., Mennerich, A., and Urban, B. (2011). Municipal wastewater treatment and biomass accumulation with a wastewater-born and settleable algal-bacterial culture. *Water Research*, 45, 3351-3358.

Subhadra, B. (2011). Algal biorefinery- based industry: an approach to address fuel and food insecurity for a carbon- smart world. *Journal of the Science of Food and Agriculture*, 91, 2-13.

Subhash, G.V., Rohit, M.V., Devi, M.P., Swamy, Y.V., and Mohan, S.V. (2014). Temperature induced stress influence on biodiesel productivity during mixotrophic microalgae cultivation with wastewater. *Bioresource Technology*, 169, 789-793.

Sudhakar, K., and Premalatha, M. (2015). Characterization of micro algal biomass through FTIR/TGA/CHN analysis: application to *Scenedesmus* sp. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, 37, 2330-2337.

Sultana, N., Hossain, S.Z., Mohammed, M.E., Irfan, M.F., Haq, B., Faruque, M.O., Razzak, S.A., and Hossain, M.M. (2020). Experimental study and parameters optimization of microalgae based heavy metals removal process using a hybrid response surface methodology-crow search algorithm. *Scientific reports*, 10, 1-15.

Sun, X., Cao, Y., Xu, H., Liu, Y., Sun, J., Qiao, D., and Cao, Y. (2014). Effect of nitrogen-starvation, light intensity and iron on triacylglyceride/carbohydrate production and fatty acid profile of *Neochloris oleoabundans* HK-129 by a two-stage process. *Bioresource Technology*, 155, 204-212.

Sun, X., Wang, C., Li, Z., Wang, W., Tong, Y., and Wei, J. (2013). Microalgal cultivation in wastewater from the fermentation effluent in Riboflavin (B2) manufacturing for biodiesel production. *Bioresource Technology*, 143, 499-504.

Sun, X., Zhong, Y., Huang, Z., and Yang, Y. (2014). Selenium accumulation in unicellular green alga *Chlorella vulgaris* and its effects on antioxidant enzymes and content of photosynthetic pigments. *PLoS One*, 9, 112270.

Sun, X.M., Geng, L.J., Ren, L.J., Ji, X.J., Hao, N., Chen, K.Q., and Huang, H. (2018). Influence of oxygen on the biosynthesis of polyunsaturated fatty acids in microalgae. *Bioresource Technology*, 250, 868-876.

Sun, X.M., Ren, L.J., Bi, Z.Q., Ji, X.J., Zhao, Q.Y., and Huang, H. (2018). Adaptive evolution of microalgae *Schizochytrium* sp. under high salinity stress to alleviate oxidative damage and improve lipid biosynthesis. *Bioresource Technology*, 267, 438–444.

Sun, X.M., Ren, L.J., Zhao, Q.Y., Ji, X.J., and Huang, H. (2018). Microalgae for the production of lipid and carotenoids: a review with focus on stress regulation and adaptation. *Biotechnology for Biofuels*, 11, 1-16.

Sundaramoorthy, B., Thiagarajan, K., Mohan, S., Mohan, S., Rao, P.R., Ramamoorthy, S., and Karan, R.K.C. (2016). Biomass characterization and phylogenetic analysis of microalgae isolated from estuaries: Role in phycoremediation of tannery effluent. *Algal Research*, 14, 92–99.

Suparmaniam, U., Lam, M.K., Lim, J.W., Yusup, S., Tan, I.S., Lau, S.Y., Kodgire, P., and Kachhwaha, S.S. (2022). Influence of environmental stress on microalgae growth and lipid profile: a systematic review. *Phytochemistry Reviews*, 1-24.

Suresh Kumar, K., Dahms, H.U., Lee, J.S., Kim, H.C., Lee, W.C., and Shin, K.H. (2014). Algal photosynthetic responses to toxic metals and herbicides assessed by chlorophyll a fluorescence. *Ecotoxicology and Environmental Safety*, 104, 51–71.

Sutherland, D.L., and Ralph, P.J. (2019). Microalgal bioremediation of emerging contaminants – Opportunities and challenges. *Water Research*, 164, 114921.

Sutherland, D.L., Montemezzani, V., Howard-Williams, C., Turnbull, M.H., Broady, P.A., and Craggs, R.J. (2015). Modifying the high rate algal pond light environment and its effects on light absorption and photosynthesis. *Water Research*, 70, 86-96.

Swarnalatha, G.V., Shekh, A., Sijil, P.V., Madhubalaji, C.K., Chauhan, V.S., and Sarada, R. (2019). Carbon dioxide sequestration by microalgae. In *Handbook of algal technologies and phytochemicals* (pp. 63-75). CRC Press.

Syam Babu, D., Anantha Singh, T.S., Nidheesh, P.V., and Suresh Kumar, M. (2020). Industrial wastewater treatment by electrocoagulation process. *Separation Science and Technology*, 55, 3195-3227.

Szabados, L., and Savoure, A. (2010). Proline: a multifunctional amino acid. *Trends in Plant Science*, 15, 89-97.

Tahira, S., Khan, S., Samrana, S., Shahi, L., Ali, I., Murad, W., Rehman, Z., and Azizullah, A. (2019). Bio-assessment and remediation of arsenic (arsenite As-III) in water by *Euglena gracilis*. *Journal of Applied Phycology*, 31(1), 423-433.

Talaat, N.B. (2019). Role of reactive oxygen species signaling in plant growth and development. *Reactive oxygen, nitrogen and sulfur species in plants: production, metabolism, signaling and defense mechanisms*, 225-266.

Talebi, A.F., Tabatabaei, M., Mohtashami, S.K., Tohidfar, M., and Moradi, F. (2013). Comparative salt stress study on intracellular ion concentration in marine and salt-adapted freshwater strains of microalgae. *Notulae Scientia Biologicae*, 5(3), 309-315.

Tamás, M.J., Sharma, S.K., Ibstedt, S., Jacobson, T., and Christen, P. (2014). Heavy metals and metalloids as a cause for protein misfolding and aggregation. *Biomolecules*, 4, 252–267.

Tan, K.W.M., and Lee, Y.K. (2016). The dilemma for lipid productivity in green microalgae: importance of substrate provision in improving oil yield without sacrificing growth. *Biotechnology for biofuels*, 9, 255.

Tandon, P., and Jin, Q. (2017). Microalgae culture enhancement through key microbial approaches. *Renewable and Sustainable Energy Reviews*, 80, 1089-1099.

Tang, D., Han, W., Li, P., Miao, X., and Zhong, J. (2011). CO_2 biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO_2 levels. *Bioresource Technology,* 102, 3071-3076.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., and Sutton, D.J. (2012). Heavy metal toxicity and the environment. In Molecular, clinical and environmental toxicology (pp. 133-164). Springer, Basel.

Thaçi, B.S., and Gashi, S.T. (2019). Reverse osmosis removal of heavy metals from wastewater effluents using biowaste materials pretreatment. *Polish Journal of Environmental Studies,* 28, 337-341.

Thanigaivel, S., Priya, A.K., Dutta, K., Rajendran, S., and Vasseghian, Y. (2022). Engineering strategies and opportunities of next generation biofuel from microalgae: A perspective review on the potential bioenergy feedstock. *Fuel*, 312, 122827.

Tibbetts, S.M., Whitney, C.G., MacPherson, M.J., Bhatti, S., Banskota, A.H., Stefanova, R., and McGinn, P.J. (2015). Biochemical characterization of microalgal biomass from freshwater species isolated in Alberta, Canada for animal feed applications. *Algal Research,* 11, 435–447.

Torricelli, E., Gorbi, G., Pawlik-Skowron´ ska, B., di Toppi, L.S., and Corradi, M.G. (2004). Cadmium tolerance, cysteine and thiol peptide levels in wild type and chromium-tolerant strains of *Scenedesmus acutus* (Chlorophyceae). *Aquatic Toxicology,* 68, 315–323.

Tripathi, B.N., and Gaur, J.P. (2004). Relationship between copper-and zinc-induced oxidative stress and proline accumulation in *Scenedesmus* sp. *Planta,* 219, 397-404.

Tripathi, R., Gupta, A., and Thakur, I.S. (2019). An integrated approach for phycoremediation of wastewater and sustainable biodiesel production by green microalgae, *Scenedesmus* sp. ISTGA1. *Renewable Energy,* 135, 617-625.

Tripathi, S., and Poluri, K.M. (2021). Adaptive and tolerance mechanism of microalgae in removal of cadmium from wastewater. In *Algae* (pp. 63-88). Springer, Singapore.

Tripathi, S., and Poluri, K.M. (2021). Heavy metal detoxification mechanisms by microalgae: Insights from transcriptomics analysis. *Environmental Pollution*, 117443.

Tripathi, S., and Poluri, K.M. (2021). Metallothionein-and phytochelatin-assisted mechanism of heavy metal detoxification in microalgae. In *Approaches to the Remediation of Inorganic Pollutants* (pp. 323-344). Springer, Singapore.

Tripathi, S., Arora, N., Pruthi, V., and Poluri, K.M. (2021). Elucidating the bioremediation mechanism of *Scenedesmus sp. IITRIND2* under cadmium stress. *Chemosphere*, 283, 131196.

Udaiyappan, A.F.M., Hasan, H.A., Takriff, M.S., and Abdullah, S.R.S. (2017). A review of the potentials, challenges and current status of microalgae biomass applications in industrial wastewater treatment. *Journal of Water Process Engineering*, 20, 8-21.

Ugya, A.Y., Ari, H.A., and Hua, X. (2021). Microalgae biofilm formation and antioxidant responses to stress induce by *Lemna minor* L., *Chlorella vulgaris*, and *Aphanizomenon flosaquae*. *Ecotoxicology and Environmental Safety*, 221, 112468.

Ugya, A.Y., Imam, T.S., Li, A., Ma, J., and Hua, X. (2020). Antioxidant response mechanism of freshwater microalgae species to reactive oxygen species production: a mini review. *Chemistry and Ecology*, 36(2), 174-193.

Ummalyma, S.B., and Sukumaran, R.K. (2014). Cultivation of microalgae in dairy effluent for oil production and removal of organic pollution load. *Bioresource Technology*, 165, 295-301.

UN. *World Urbanization Prospects: The 2014 Revision*. (United Nations, Department of Economic and Social Affairs, Population Division, New York, NY 10017, USA, 2015).

UNESCO (2003) http://www.unesco.org/new/en/natural-sciences/environment/water/wwap/wwdr/wwdr1-2003

Upadhyay, A.K., and Mandotra, S.K. (2021). Constructed wetland and microalgae: a revolutionary approach of bioremediation and sustainable energy production. In *Algae* (pp. 27-40). Springer, Singapore.

Upadhyay, A.K., Mandotra, S.K., Kumar, N., Singh, N.K., Singh, L., and Rai, U.N. (2016). Augmentation of arsenic enhances lipid yield and defense responses in alga *Nannochloropsis* sp. *Bioresource Technology*, 221, 430-437.

Upadhyay, A.K., Singh, N.K., and Rai, U.N. (2014). Comparative metal accumulation potential of Potamogeton pectinatus L. and Potamogeton crispus L.: Role of enzymatic and non-enzymatic antioxidants in tolerance and detoxification of metals. *Aquatic Botany*, 117, 27-32.

Upadhyay, A.K., Singh, R., Singh, D.V., Singh, L., Singh, D.P. (2021). Microalgal consortia technology: A novel and sustainable approach of resource reutilization, waste management and lipid production. *Environmental Technology & Innovation*, p.101600.

Usha, M.T., Chandra, T.S., Sarada, R., and Chauhan, V.S. (2016). Removal of nutrients and organic pollution load from pulp and paper mill effluent by microalgae in outdoor open pond. *Bioresource Technology*, 214, 856-860.

Vadiveloo, A., Moheimani, N.R., Alghamedi, R., Cosgrove, J.J., Alameh, K., and Parlevliet, D. (2016). Sustainable cultivation of microalgae by an insulated glazed glass plate photobioreactor. *Biotechnology Journal*, 11, 363-374.

Vadiveloo, A., Moheimani, N.R., Kosterink, N.R., Cosgrove, J.J., Parlevliet, D., Gonzalez-Garcia, C. and Lubián, L.M., 2016. Photosynthetic performance of two *Nannochloropsis* spp. under different filtered light spectra. *Algal research*, 19, 168-177.

Van Assche, F., and Clijsters, H. (1986). Inhibition of photosynthesis in *Phaseolus vulgaris* by treatment with toxic concentration of zinc: effect on ribulose-1, 5-bisphosphate carboxylase/oxygenase. *Journal of Plant Physiology*, 125, 355-360.

Van Wagenen, J., Miller, T.W., Hobbs, S., Hook, P., Crowe, B., and Huesemann, M. (2012). Effects of light and temperature on fatty acid production in *Nannochloropsis salina*. *Energies*, 5(3), 731-740.

Vandamme, D., Foubert, I., Fraeye, I., Meesschaert, B., and Muylaert, K. (2012). Flocculation of *Chlorella vulgaris* induced by high pH: Role of magnesium and calcium and practical implications. *Bioresource Technology,* 105, 114–119.

Vasistha, S., Khanra, A., Clifford, M., and Rai, M.P. (2021). Current advances in microalgae harvesting and lipid extraction processes for improved biodiesel production: A review. *Renewable and Sustainable Energy Reviews*, 137, 110498.

Velikova, V., Yordanov, I., and Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Science*, 151, 59-66.

Venkata Subhash, G., Rohit, M.V., Prathima, Devi, M., Swamy, Y.V., and Venkata Mohan, S. (2014). Temperature induced stress influence on biodiesel productivity during mixotrophic microalgae cultivation with wastewater. *Bioresource Technology,* 169, 789-793.

Venkatesan, P., and Sathiavelu, M. (2022). Effective kinetic modeling and phycoremediation of Cr (IV) ions from tannery effluent by using microalgae–*Chlamydomonas moewusii*, *Auxenochlorella pyrenoidosa*, *Scenedesmus* sp. *Bioremediation Journal*, 1-20.

Vinodhini, M., and Soundhari, C. (2019). Phycoremediation of dairy effluent by using microalgal consortium. *Journal of Pharmaceutical Innovation*, 8, 128-133.

Von Moos, N., and Slaveykova, V.I. (2014). Oxidative stress induced by inorganic nanoparticles in bacteria and aquatic microalgae–state of the art and knowledge gaps. *Nanotoxicology*, 8, 605-630.

Von Sperling, M. (2007). Wastewater characteristics, treatment and disposal. IWA publishing.

Wada, Y., Flörke, M., Hanasaki, N., Eisner, S., Fischer, G., Tramberend, S., Satoh, Y., Van Vliet, M.T.H., Yillia, P., Ringler, C., and Burek, P. (2016). Modeling global water use for the 21st century: The Water Futures and Solutions

(WFaS) initiative and its approaches. *Geoscientific Model Development*, 9(1), 175-222.

Wagner, H., Liu, Z., Langner, U., Stehfest, K., and Wilhelm, C. (2010). The use of FTIR spectroscopy to assess quantitative changes in the biochemical composition of microalgae. *Journal of Biophotonics*, 3, 557-566.

Wahidin, S., Idris, A., and Shaleh, S.R.M. (2013). The influence of light intensity and photoperiod on the growth and lipid content of microalgae *Nannochloropsis* sp. *Bioresource Technology*, 129, 7-11.

Wan Mohamad Apandi, W.A., and Matias Peralta, H.M. (2015). Removal of nutrients and selected heavy metals in wet market wastewater by using microalgae *Scenedesmus* sp. In *Applied mechanics and materials* (Vol. 773, pp. 1210-1214). Trans Tech Publications Ltd.

Wang, H., Hu, Z., Xiao, B., Cheng, Q., and Li, F. (2013). Ammonium nitrogen removal in batch cultures treating digested piggery wastewater with microalgae *Oedogonium* sp. *Water Science and Technology*, 68(2), 269-275.

Wang, J.H., Zhang, T.Y., Dao, G.H., Xu, X.Q., Wang, X.X., and Hu, H.Y. (2017a) Microalgae-based advanced municipal wastewater treatment for reuse in water bodies. *Applied Microbiology and Biotechnology*, 101, 2659-2675.

Wang, L., Li, Y., Chen, P., Min, M., Chen, Y., Zhu, J., and Ruan, R.R. (2010). Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. *Bioresource Technology*, 101, 2623-2628.

Wang, Q., Li, H., Shen, Q., Wang, J., Chen, X., Zhang, Z., Lei, Z., Yuan, T., Shimizu, K., Liu, Y., and Lee, D.J. (2022). Biogranulation process facilitates cost-efficient resources recovery from microalgae-based wastewater treatment systems and the creation of a circular bioeconomy. *Science of the Total Environment*, 828, 154471.

Wang, Z., Dong, J., and Li, D. (2012). Conformational changes in photosynthetic pigment proteins on thylakoid membranes can lead to fast non-photochemical quenching in cyanobacteria. *Science China Life Sciences*, 55(8), 726-734.

WBSCD, (2019). *Indian Water Tool. World Business Council for Sustainable Development*. Available online at: https://www.indiawatertool.in/ (accessed June 02, 2019).

Wei, Y.Y., Zheng, Q., Liu, Z.P., and Yang, Z.M. (2011). Regulation of tolerance of *Chlamydomonas reinhardtii* to heavy metal toxicity by heme oxygenase-1 and carbon monoxide. *Plant and cell physiology*, 52, 1665-1675.

Wensel, P., Helms, G., Hiscox, B., Davis, W.C., Kirchhoff, H., Bule, M. (2014). Isolation, characterization, and validation of oleaginous, multi-trophic, and haloalkalinetolerant microalgae for two-stage cultivation. *Algal Research*, 4, 2–11.

Werner, T.P., Amrhein, N., and Freimoser, F.M. (2007). Specific localization of inorganic polyphosphate (poly P) in fungal cell walls by selective extraction and immunohistochemistry. *Fungal Genetics and Biology*, 44, 845-852.

Whitton, R., Ometto, F., Pidou, M., Jarvis, P., Villa, R., and Jefferson, B. (2015). Microalgae for municipal wastewater nutrient remediation: mechanisms, reactors and outlook for tertiary treatment. *Environmental Technology Reviews*, 4, 133-148.

WHO & UNICEF, (2010). Progress on Sanitation and DrinkingWater–2010 Update. World Health Organization, Geneva, Switzerland, p. 60.

WHO and UNICEF Joint Monitoring Programme (2000). Global water supply and sanitation assessment report. New York: UNICEF and Geneva: WHO

Wirtz, M., Birke, H., Heeg, C., Müller, C., Hosp, F., Throm, C., Konig, S., Salit, A.F., Rippe, K., Petersen, G., Wade, R.C., Rybin, V., Schffzek, K., and Hell, R. (2010). Structure and function of the hetero-oligomeric cysteine synthase complex in plants. *Journal of Biological Chemistry*, 285, 32810–32817.

Wong, Y.K., Ho, Y.H., Ho, K.C., Leung, H.M., and Yung, K.K.L. (2017). Maximization of cell growth and lipid production of freshwater microalga *Chlorella vulgaris* by enrichment technique for biodiesel production. *Environmental Science and Pollution Research*, 24, 9089-9101.

Wu, L.F., Chen, P.C., and Lee, C.M. (2013). The effects of nitrogen sources and temperature on cell growth and lipid accumulation of microalgae. *International Biodeterioration and Biodegradation*, 85, 506-510.

Wu, Y., Gong, W., and Yang, W. (2017). Shade inhibits leaf size by controlling cell proliferation and enlargement in soybean. *Scientific Reports*, 7, 9259.

Xia, S., Wan, L., Li, A., Sang, M., and Zhang, C. (2013). Effects of nutrients and light intensity on the growth and biochemical composition of a marine microalga *Odontella aurita*. *Chinese Journal of Oceanology and Limnology*, 3, 1–6.

Xie, X., He, Z., Chen, N., Tang, Z., Wang, Q., and Cai, Y., (2019). The roles of environmental factors in regulation of oxidative stress in plant. *BioMed research international*.

Xin, L., Hong-Ying, H., and Yu-Ping, Z. (2011). Growth and lipid accumulation properties of a freshwater microalga *Scenedesmus* sp. under different cultivation temperature. *Bioresource Technology*, 102, 3098-3102.

Xing, C., Li, J., Yuan, H., and Yang, J. (2022). Physiological and transcription level responses of microalgae *Auxenochlorella protothecoides* to cold and heat induced oxidative stress. *Environmental Research*, 211, 113023.

Xiong, J.Q., Kurade, M.B., Kim, J.R., Roh, H.S., and Jeon, B.H. (2017). Ciprofloxacin toxicity and its co-metabolic removal by a freshwater microalga *Chlamydomonas mexicana*. *Journal of Hazardeous Material,* 323, 212–219.

Xu, J., Zhao, Y., Zhao, G., and Zhang, H. (2015). Nutrient removal and biogas upgrading by integrating freshwater algae cultivation with piggery anaerobic digestate liquid treatment. *Applied Microbiology and Biotechnology*, 99, 6493-6501.

Xu, K., Zou, X., Wen, H., Xue, Y., Qu, Y., and Li, Y. (2019). Effects of multi-temperature regimes on cultivation of microalgae in municipal wastewater to simultaneously remove nutrients and produce biomass. *Applied Microbiology and Biotechnology*, 103, 8255-8265.

Yaakob, M.A., Mohamed, R.M.S.R., Al-Gheethi, A., Ravishankar, G.A., and Ambati, R.R. (2021). Influence of nitrogen and phosphorus on microalgal growth, biomass, lipid, and fatty acid production: an overview. *Cells*, 10, 393.

Yabuta, Y., Mieda, T., Rapolu, M., Nakamura, A., Motoki, T., Maruta, T., Yoshimura, K., Ishikawa, T., and Shigeoka, S. (2007). Light regulation of ascorbate biosynthesis is dependent on the photosynthetic electron transport chain but independent of sugars in Arabidopsis. *Journal of Experimental Botany*, 58, 2661-2671.

Yadav, G., and Sen, R. (2017). Microalgal green refinery concept for biosequestration of carbon-dioxide vis-à-vis wastewater remediation and bioenergy production: Recent technological advances in climate research. *Journal of CO_2 Utilization*, 17, 188-206.

Yana, S., Nizar, M., and Mulyati, D. (2022). Biomass waste as a renewable energy in developing bio-based economies in Indonesia: A review. *Renewable and Sustainable Energy Reviews*, 160, 112268.

Yang, J., Cao, J., Xing, G., and Yuan, H. (2015). Lipid production combined with biosorption and bioaccumulation of cadmium, copper, manganese and zinc by oleaginous microalgae *Chlorella minutissima* UTEX2341. *Bioresource Technology*, 175, 537-544.

Yang, X., Xu, H., Shao, L., Li, T., Wang, Y., and Wang, R. (2018). Response of photosynthetic capacity of tomato leaves to different LED light wavelength. *Environmental and Experimental Botany*, 150, 161-171.

Yeh, K.L., Chang, J.S., and Chen, W.M. (2010). Effect of light supply and carbon source on cell growth and cellular composition of a newly isolated microalga *Chlorella vulgaris* ESP- 31. *Engineering in Life Sciences*, 10(3), 201-208.

Yilancioglu, K., Cokol, M., Pastirmaci, I., Erman, B., and Cetiner, S. (2014). Oxidative stress is a mediator for increased lipid accumulation in a newly isolated *Dunaliella salina* strain. *PLoS One*, 9(3).

Yin, Z., Zhu, L., Li, S., Hu, T., Chu, R., Mo, F., Hu, D., Liu, C., and Li, B. (2020). A comprehensive review on cultivation and harvesting of microalgae for biodiesel production: environmental pollution control and future directions. *Bioresource Technology*, 122804.

Yong, W.K., Sim, K.S., Poong, S.W., Wei, D., Phang, S.M., and Lim, P.E. (2018). Interactive effects of temperature and copper toxicity on photosynthetic efficiency and metabolic plasticity in *Scenedesmus quadricauda* (Chlorophyceae). *Journal of Applied Phycology*, 30(6), 3029-3041.

Yousuf, P.Y., Hakeem, K.U.R., Chandna, R., and Ahmad, P., (2012). Role of glutathione reductase in plant abiotic stress. *In Abiotic stress responses in plants* (pp. 149-158). Springer, New York, NY.

Yu, Z., Song, M., Pei, H., Han, F., Jiang, L., and Hou, Q. (2017). The growth characteristics and biodiesel production of ten algae strains cultivated in anaerobically digested effluent from kitchen waste. *Algal Research*, 24, 265-275.

Yun, H.S., Kim, Y.S., and Yoon, H.S. (2020). Characterization of *Chlorella sorokiniana* and *Chlorella vulgaris* fatty acid components under a wide range of light intensity and growth temperature for their use as biological resources. *Heliyon*, *6*, 04447.

Zamani-Ahmadmahmoodi, R., Malekabadi, M.B., Rahimi, R., and Johari, S.A. (2020). Aquatic pollution caused by mercury, lead, and cadmium affects cell growth and pigment content of marine microalgae *Nannochloropsis oculata*. *Environmental Monitoring and Assessment*, 192(6), 1-11.

Zarrinmehr, M.J., Farhadian, O., Heyrati, F.P., Keramat, J., Koutra, E., Kornaros, M., and Daneshvar, E. (2019). Effect of nitrogen concentration on the growth rate and biochemical composition of the microalga, Isochrysis galbana. *Egyptian Journal of Aquatic Research*, 46, 1687–4285.

Zhang, H.C., Ma, K.X., Yang, Y.J., Shi, C.Y., Chen, G.W., and Liu, D.Z. (2018). Molecular cloning, characterization, expression and enzyme activity of catalase from planarian *Dugesia japonica* in response to environmental pollutants. *Ecotoxicology and Environmental Safety*, 165, 88-95.

Zhang, L., Wang, N., Yang, M., Ding, K., Wang, Y.Z., Huo, D., and Hou, C. (2019). Lipid accumulation and biodiesel quality of *Chlorella pyrenoidosa* under oxidative stress induced by nutrient regimes. *Renewable Energy*, 143, 1782-1790.

Zhang, M., Pan, L., Su, C., Liu, L., and Dou, L. (2021). Simultaneous aerobic removal of phosphorus and nitrogen by a novel salt-tolerant phosphate-accumulating organism and the application potential in treatment of domestic sewage and aquaculture sewage. *Science of the Total Environment*, 758, 143580.

Zhang, R., and Sharkey, T.D. (2009). Photosynthetic electron transport and proton flux under moderate heat stress. *Photosynthesis Research*, 100, 29-43.

Zhang, R., Kong, Z., Chen, S., Ran, Z., Ye, M., Xu, J., Zhou, C., Liao, K., Cao, J., and Yan, X. (2017). The comparative study for physiological and biochemical mechanisms of *Thalassiosira pseudonana* and *Chaetoceros calcitrans* in response to different light intensities. *Algal research*, 27, 89-98.

Zhang, X., and Liu, Y. (2022). Circular economy is game-changing municipal wastewater treatment technology towards energy and carbon neutrality. *Chemical Engineering Journal*, 429, 132114.

Zhao, L.S., Li, K., Wang, Q.M., Song, X.Y., Su, H.N., Xie, B.B., Zhang, X.Y., Huang, F., Chen, X.L., Zhou, B.C., and Zhang, Y.Z. (2017). Nitrogen starvation impacts the photosynthetic performance of *Porphyridium cruentum* as revealed by chlorophyll a fluorescence. *Scientific reports*, 7, 1-11.

Zhen-Guo, S., Xian-Dong, L., Chun-Chun, W., Huai-Man, C.H., and Hong C.H. (2002). Lead Phytoextraction from contaminated soil with high biomass plant species. *Journal of Environmental Quality*, 31, 1893-1900.

Zhou, G.J., Ying, G.G., Liu, S., Zhou, L.J., Chen, Z.F., Peng, F.Q., 2014. Simultaneous removal of inorganic and organic compounds in wastewater by freshwater green microalgae. *Environmental Science: Processes & Impacts*, 16, 2018-2027.

Zhou, H., Zhao, X., Kumar, K., Kunetz, T., Zhang, Y., Gross, M., and Wen, Z. (2021). Removing high concentration of nickel (II) ions from synthetic wastewater by an indigenous microalgae consortium with a Revolving Algal Biofilm (RAB) system. *Algal Research*, 59, 102464.

Zhu, L. (2015). Microalgal culture strategies for biofuel production: a review. *Biofuels, Bioproducts and Biorefining*, 9(6), 801-814.

Zhu, L., Gao, N., Cong, and R.G. (2017). Application of Biotechnology for the production of biomass-based Fuels. BioMed research international.

Znad, H., Naderi, G., Ang, H.M., and Tade, M.O. (2012). CO_2 biomitigation and biofuel production using microalgae: photobioreactors developments and future directions. *Advances in Chemical Engineering*, 230-244.

Zobayed, S.M.A., Afreen, F., and Kozai, T. (2005). Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St. John's wort. *Plant Physiology and Biochemistry,* 43, 977–984.

Zouari, M., Hassena, A.B., Trabelsi, L., Rouina, B.B., Decou, R., and Labrousse, P. (2019). Exogenous proline-mediated abiotic stress tolerance in plants: Possible mechanisms. In *Osmoprotectant-mediated abiotic stress tolerance in plants* (pp. 99-121). Springer, Cham.

Zuka, Z., McConnell, B., and Farag, I. (2012). Comparison of freshwater and wastewater medium for microalgae growth and oil production. *Journal of American Science*, 8, 392-8.

Publications and Conferences

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Research Papers

- **Singh, D.V.,** and Singh, R.P., 2022. Algal consortia based metal detoxification of municipal wastewater: Implication on photosynthetic performance, lipid production, and defense responses. Science of The Total Environment, 814, p.151928.
- **Singh**, **D.V.,** Upadhyay, A.K., Singh, R., and Singh, D.P. 2022. Implication of municipal wastewater on growth kinetics, biochemical profile, and defense system of *Chlorella vulgaris* and *Scenedesmus vacuolatus*. Environmental Technology & Innovation, p.102334.
- **Singh**, **D.V.,** Upadhyay, A.K., Singh, Singh, R., and Singh, D.P., 2021. Microalgal competence in urban wastewater management: phycoremediation and lipid production. International Journal of Phytoremediation, 1-11.
- Upadhyay, A.K., Singh, R., **Singh, D.V.,** Singh, L. and Singh, D.P., 2021. Microalgal consortia technology: A novel and sustainable approach of resource reutilization, waste management and lipid production. Environmental Technology & Innovation, p.101600.
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- Singh, R., Upadhyay, A.K., **Singh, D.V.,** Singh, J.S., Singh, D.P., 2019. Photosynthetic performance, nutrient status and lipid yield of microalgae *Chlorella vulgaris* and *Chlorococcum humicola* under UV-B exposure. Current Research in Biotechnology 1: 65-77.

Book chapters

- **Singh, D.V.,** Upadhyay, A.K., Singh, R. and Singh, D.P., 2022. Persistent Organic Pollutants: Sources, Impacts, and Their Remediation by Microalgae. In Environmental Biotechnology (pp. 57-86). Apple Academic Press.

- **Singh, D.V.,** Upadhyay, A.K., Singh, R. and Singh, D.P. Health benefits of bioactive compounds from microalgae. In Phytomedicine (pp. 291-319). Academic Press.
- **Singh, D.V.,** Upadhyay, A.K., Singh, R. and Singh, D.P., 2020. Eco-friendly and eco technological approaches in treatment of wastewater by different algae and cyanobacteria. In Algae and sustainable technologies (pp. 43-64). CRC Press.

Conferences

- Oral presentation on "**Effect of municipal wastewater on growth profile, pigment content and biofuel production of algal consortia"** in National conference on "Sustinable Environment: Challenges and opportunities" (29-30 August, 2022) organized by School of Libral Arts and Sciences ERA University, Lucknow.
- Oral presentation on "**Algal Consortia as the competent and alternative bioresource for wastewater remediation"** in National conference for Innovation and technology for sustainable rural development (1-2nd July, 2022) organized by Babasaheb Bhimrao Ambedkar Central University, Lucknow.

Appendix

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Algal consortia based metal detoxification of municipal wastewater: Implication on photosynthetic performance, lipid production, and defense responses

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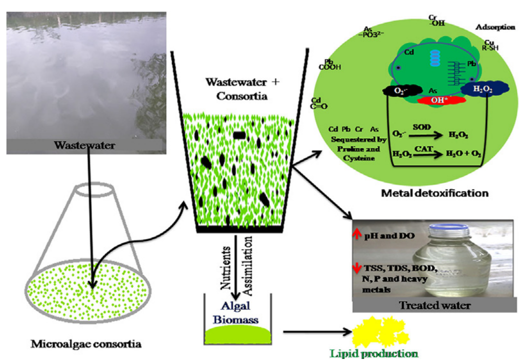
HIGHLIGHTS

- Consortia removed nutrients (83–98%) and detoxified heavy metals (50–94%) from WW.
- Different WW concentrations amplified photosynthetic performance of consortia 2.
- FTIR spectroscopy showed WW triggered neutral lipid accumulation in consortia 2.
- Antioxidant responses indicated that consortia 2 was competent to tolerate heavy metals.
- Consortia of two microalgae seem feasible for WW remediation and lipid production.

GRAPHICAL ABSTRACT



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ABSTRACT

The present investigation was carried out to assess the competence of artificially engineered microalgal consortia i.e. consortia 1 (*Scenedesmus vacuolatus* + *Chlorococcum humicola*), consortia 2 (*Tetradesmus sp.* + *Scenedesmus vacuolatus*), and consortia 3 (*Chlorococcum humicola* + *Scenedesmus vacuolatus* + *Tetradesmus sp.*) for municipal wastewater treatment and lipid production under laboratory conditions. The purpose of the present study was to screen the competent microalgae consortia based on wastewater remediation, photosynthetic performance, and antioxidant defense responses. The outcome based on nutrient reutilization (78.98–98%), metal detoxification (50–94%), and biomass production (1.43–1.65 folds) reflected greater adaptability and tolerance of consortia 2 against different concentrations of wastewater. The photosynthetic performance parameters such as active photosystem II reaction centre, the quantum yield, and photosynthetic performance index were increased by 1.20–2.35 folds in consortia 2 after treatment with different concentrations of wastewater. Additionally, Fourier transform infrared spectroscopy peak showed at 1750 cm^{-1} confirmed neutral lipid accumulation in consortia 2 at 100% concentration of wastewater. The measurement of oxidative stress markers such as thiobarbituric acid reactive species and hydrogen peroxide showed considerable decline in consortia 2 as compared to consortia 1 and 3. Interestingly, increased non–enzymatic (1.02–2.44 folds) and enzymatic antioxidant (1.05–4.14 folds) activity in consortia 2 reflected that oxidative stress was attenuated by the amplified activity of ascorbic acid, proline, cysteine, superoxide dismutase, catalase, and glutathione reductase. Overall, photosynthetic performance, lipid production, and antioxidants activity represented that the consortia 2 can be effectively used for sustainable wastewater treatment and lipid production. Thus, the synergistic association of two microalgae may be the superior and neoteric paradigm with multilevel benefits such as sustainable nutrient resource utilization, metal detoxification, and lipid production.

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Implication of municipal wastewater on growth kinetics, biochemical profile, and defense system of *Chlorella vulgaris* and *Scenedesmus vacuolatus*

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ABSTRACT

In the present investigation, the growth pattern, cellular modification, oxidative stress markers, and defense responses in *Chlorella vulgaris* and *Scenedesmus vacuolatus* were analyzed after treatment with municipal wastewater (25%–100%). The main aim was to compare the *Chlorella vulgaris* and *Scenedesmus vacuolatus* on account of remediation efficiency, lipid production and defense responses under different concentrations of wastewater. The results revealed that the nutrients (93.28%) and heavy metal (92.08%) removal was highest at 25% of wastewater concentration while maximum specific growth rate (0.0708 day^{-1}), total chlorophyll (0.118 mg g^{-1} fw), and carbohydrate content (24.06 mg L^{-1} fw) was observed with *Scenedesmus vacuolatus* at 50% concentration of wastewater. Additionally, fourier transform infrared spectroscopy showed 100% concentration of wastewater enhanced lipid accumulation in *Scenedesmus vacuolatus* than *Chlorella vulgaris*. The lesser accumulation of thiobarbituric acid reactive species and hydrogen peroxide while increment in antioxidants activity (1.87–15.63 folds) may be responsible for imparting tolerance to *Scenedesmus vacuolatus* against different concentrations of municipal wastewater. Overall, the study revealed that 50% and 100% wastewater concentration was suitable for excellent growth and lipid accumulation in *Scenedesmus vacuolatus*, respectively. Thus, due to high nutrients reutilization potential, metal removal rate, growth profile, lipid production, and anti-oxidative responses, *Scenedesmus vacuolatus* seems more effective and tolerant than *Chlorella vulgaris*. Therefore, exploiting *Scenedesmus vacuolatus* may be the successful step towards sustainable management of wastewater and lipid production.

1. Introduction

Globally, water pollution is the major issue that is increasing due to simultaneous increase in population, urbanization (Singh et al., 2020), industrialization, and impromptu management of wastewater (Singh and Singh, 2021). The problem of wastewater management is more severe in developing countries like India where various technological and economic constraints are the major hurdles in proper wastewater treatment (Upadhyay et al., 2021). Wastewater of different origins differs greatly in physical and chemical properties. Wastewater rich in organic matter, nutrients, salts, and heavy metals not only deteriorate water quality but also disturb the equilibrium of the recipient water bodies (Rajasulochana and

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Microalgal competence in urban wastewater management: phycoremediation and lipid production

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Microalgae in aquatic environs: A sustainable approach for remediation of heavy metals and emerging contaminants

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ABSTRACT

Water pollution has grown to be a grave concern in the world. Direct discharge of wastewater poses risks to the aquatic ecosystems by causing eutrophication and degrades their physico-chemical characteristics. Moreover, wastewater is mainly enriched with recalcitrant toxic substances that pose detrimental impacts on the receiving environments. Conventional treatment approaches are mostly applied to remove nuisance pollutants from aquatic systems but are expensive and inefficient. Exploring microalgae has been found to be an efficient and ecofriendly technique for purification of aquatic environs. Furthermore, microalgae can effectively remove N (90–98.4%), P (66%–98%), Pb (75%–100%), Zn (15.6–99.7%), Cr (52.54%–96%), Hg (77%–97%), Cu (45%–98%) and Cd (2–93.06%) from contaminated aquatic systems. Microalgae play a pivotal role in degrading the complex pesticides (α-endosulfan, lindane, isoproturon and glyphosate) and emerging concerned contaminants (triclosan, bisphenol A, 17α-ethinylestradiol, tramadol and diclofenac) in elegant manner from disturbed environs. Apart from toxic pollutant removal, microalgae produce biomass, thereby acts as the efficient source of additional products like biofuel, carbohydrates, lipids and proteins which can make phycoremediation more frugal and sustainable.

Contents

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Microalgal consortia technology: A novel and sustainable approach of resource reutilization, waste management and lipid production

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ABSTRACT

The present study was conducted to assess the efficiency of naturally growing competent microalgal consortia (*Chlorella vulgaris*, *Chlorococcum humicola* and *Nannochloropsis* sp.) in the waste remediation of sewage water and lipid production. Results showed that microalgal consortia during wastewater (WW) treatment efficiently utilized the inorganic nutrients (N and P) and exhibited higher growth, biomass and photosystem II activity (Fv/Fo, Fv/Fm, Mo, ETo/RC and Pi_{Abs}) as compared to respective individual algal species. SEM data showed clumped morphology and 16.21% increases in the cell size treated with WW. The wastewater treated with microalgal consortia showed reduction in physicochemical parameters viz., conductivity (19.24%), NO_3^{-1} (68.55%), PO_4^{-3} (55.99%), TS (41.31%), TSS (35.48%), TDS (53.32%) and BOD (66.85%) after 15d of treatment. The FTIR spectra (4000–3400 cm^{-1}) of algal biomass generated in BG-11 nutrient solution (NS) and wastewater showed higher absorption representing –OH compounds as compared to tap water. Further, the high IR spectral peak length ratio of L/C in microalgal consortia treated with WW exhibited greater lipid accumulation which could be used in the bioenergy production in sustainable way. Thus, species richness and complementary association of algae may be a better solution with multiple benefits of waste reutilization, phycoremediation and lipid yield.

1. Introduction

The unprecedented population growth on the earth has become a serious cause of various socioeconomic apprehensions like the fresh water scarcity, massive waste load, land shortage and energy (Dong et al., 2018; Enamala et al., 2020). These paradigms are directly associated with mismanaged water practices, developmental priorities, fast industrial insurgency and high living standards. In developing countries like India, high population growth significantly contributes to direct discharge of waste water into the river without any proper management due to limited facilities, large area requirement and high cost of water treatment operation and maintenance. To reduce the waste, focus should be directed towards application of wastewater as source of nutrients and energy for micro-organisms (Perry et al., 2011). Among different bio-remediation methods, phycoremediation by microalgae is currently exploited as the flexible and ecofriendly approach for management of contaminated environments. Thus, coupling of algal phycoremediation of wastewater with

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Photosynthetic performance, nutrient status and lipid yield of microalgae *Chlorella vulgaris* and *Chlorococcum humicola* under UV-B exposure

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ABSTRACT

The present study was conducted to examine the photosynthetic performance, nutrient status and lipid yield in microalgae *Chlorococcum humicola* and *Chlorella vulgaris* under different durations of UV-B exposure (1/2 h, 1 h, 2 h, 3 h and 4 h). Results revealed that UV-B reduces the photosynthetic performance of photosystem II by altering photosynthetic performance index (Pi $_{ABS}$), maximum quantum yield (Fv/Fm), net closing rate of reaction centre (Mo), trapping flux (TRo/RC) and effective antenna size with their respective control. Scanning Electron Microscopy (SEM) exhibited variegated structure and increased cell size by ~15–65% which was more pronounced in the case of *C. humicola* at 4 h of UV-B. The Energy Dispersive Spectroscopy (EDS) data showed that the content of microelements (C, O, Na, and K) in terms of atomic weight % was found to be significantly increased in *C. vulgaris* while with *C. humicola*, it was restricted to carbon (C) only. Further, the high antioxidant (ascorbic acid, cysteine and proline) potential and carotenoid/chl a photoprotection response reflects protection against UV-B in the both algae. In the case of lipid, comparative greater increase in cell size of *C. humicola* correlated with high lipid yield as compare to *C. vulgaris* at 2 h of UV-B could be employed in the production of biofuel in sustainable manner. Thus, algae *C. humicola* could be a best alternative feed stock of lipid and biofuel production in the area receiving high solar radiation.

1. Introduction

Since time immemorial, energy is the fundamental prerequisite for the existence of lives on the earth. The major source of energy is fossil fuel which continuously dwindling due to overexploitation and anthropogenic interferences (Gasparatos et al., 2017). This might have cause a paradigm shift towards a search for renewable source to meet the demand of energy. The renewable sources includes solar energy, agricultural crops (Maize, Sunflower), non-agricultural crops (*Jatropha* sp.) and algae (Voloshin et al., 2016). The cultivation of microalgae are more ecofriendly and cheaper as it can be cultivated in waste water as well as in non-arable land. Over the past few years, microalgae- a third generation biofuel acts as a bridge in the production of bio-energy against limited water, land and environmental stress (Stockenreiter et al., 2016). Off the environmental stressors, solar ultraviolet radiation (UVR) emission has profound effect on the chemical composition of the atmosphere, flora and fauna (Madronich et al., 2018). It has also been reported that UVR play significant role in the generation of photochemical smog, photo damage, change in precipitation pattern, coral bleaching, vegetation loss and agricultural escalation and altering ecosystem services etc. (Jansen et al., 1998; Danovaro et al., 2008; Comont et al., 2013; Kataria et al., 2014; Williamson et al., 2014).

The UV-B irradiance (5% of total UV light from the sun) supplied by the sun is a serious hazard to lives on the earth (Herndon et al., 2018). Algae being dominant photosynthetic microorganisms in upper layer of aquatic surface are more prone to high UVR exposure leads to bring about photo bleaching coupled reduced upwelling of nutrients and oxidative damage (Williamson et al., 2019). In addition, UVR causes photosynthesis inhibition in algae, damage the efficiency of the biological pumps and fixation of CO_2 for their subsequent transfer into the organic matter (Mata et al., 2010). However, microalgae have shown tendency to acquire high degree of adaptation against UVR during the course of evolution (Holzinger et al., 2018). The high adaptability of microalgae against UVR is not only valuable for lipid production but also beneficial for accumulation of nutrients, carbon sequestration, environmental remediation, nutrient cycling and production of oxygen in the environment (Brennan and Owende, 2010; Mata et al., 2010).

The UV- B (280–320 nm) is known to have both detrimental and beneficial effects on plants animals and microalgae (Hockberger, 2002). In animals, UV-B causes skin diseases, dermatitis, DNA damage, mutation,

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Dig Vijay Singh/Ph.D. Thesis/ DES/BBAU/2022 1 Chapter-1 Introduction 1.0 Introduction Water is imperative and necessary resource for the survival of organisms and underpins the functioning of different ecosystem (Mushtaq et al., 2020). Inspite of being essential component of the life, water is one of the most poorly managed natural resource today. The most pervasive problem that affects the global population is inadequate access to clean water. Shrinking lakes, rapidly declining ground water table, deteriorating water quality and inadequate treatment methods has increased the scarcity of freshwater (Hoekstra, 2014). Water crises and insufficient clean water has become the world's most persistent problem now (Hanikel et al., 2020). The scarcity of clean water will more worsen in near future even in the regions currently containing adequate quantity of water for human use, if not managed properly. At world economic forum, Ban Ki-moon urged leaders from the all over the world to keep water crisis as the top agenda to prevent emerging conflicts over the freshwater. The inadequate freshwater availability and supply will hamper economic growth and accelerate the regional disputes for the water (Hightower and Pierce, 2008). Schewe et al., (2014) have reported that climate driven changes can leads to increment of 40% people living in regions with water availability of less than 500 m 3 yr –1 . By 2050, the population will reach to 9.5 billion globally and approximately 66% of total population will shift from rural to urban area (UN, World Urbanization Prospects: The 2014 Revision). Such population growth and migration towards urban areas will not only increase water scarcity but also leads to vast generation of wastewater

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1 & 2 July 2022

Society for Environmental Sustainability
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Centre for Innovation, Incubation, Entrepreneurship (CIIE);
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and delivered oral presentation on

Algal consortia as the competent and alternative bioresource for wastewater remediation

at
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