

Research Article Volume 33 Issue 1 - December 2023 D0I: 10.19080/IJESNR.2023.33.556353



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# Evaluation of *Spirulina subsalsa* Photosynthetic Efficiency under Industrial Sewage Treatment



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Submission: December 02, 2023; Published: December 19, 2023

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#### Abstract

On the earth's surface three parts are covered with brackish water and one part is covered with fresh water. The population expansion and industrial development have deteriorated the quality of freshwater reservoirs around the world and have caused freshwater shortages in certain areas. The discharge of industrial effluents containing toxic heavy metals (cadmium (Cd) mercury (Hg), and chromium (Cr)) into the environment has a serious impact on human, animal, and aquatic life. Hence, *Spirulina subsalsa* is a photosynthetic filamentous prokaryotic cyanobacterium and have the ability for the reduction of metal pollutants. So in this study is more useful to assess the basic photosynthetic efficiency parameters and the toxicity mitigation possibilities of *S.subsalsa* under wastewater sewage treatment. The results (growth of cyanobacterial species, lipid peroxidation and photosynthetic pigments) are concluded that have the ability to reduce the wastewater sewage pollutants of Kurnool, Andhra Pradesh area.

Keywords: Spirulina subsalsa; Industrial wastewater; Photosynthetic pigments

# Introduction

Nowadays, pollution is a major threat, both water and air polluted with contamination of different types of pesticides and industrial waste are evaporated lot of carbon (C) products through anthropogenic and natural sources in environment. Cyanobacteria are photosynthetic prokaryotes used as a food supplement for humans & aquatic ecosystem. This cyanobacterial species (*S subsalsa*) contribute about 40% of present day global photosynthetic biomass production and its convert solar energy into biomass-stored chemical energy [1]. The various researchers are also recognized as an excellent source of biomolecules such as vitamins (66%), fatty acids and proteins and this biomass has been employed to produce bioenergy, bioactive compounds, antimicrobial and antiviral compounds, biofuels, biofertilizers, and food additives [2]. There are various cyanobacterial species are benefit wastewater treatment by producing oxygen  $(0_2)$  it allows aerobic bacteria to breakdown organic contaminants in the water and taking up excess nitrogen (N) and phosphorus (P) in the process. Depending on the source's wastewater, this water composition is broadly classified into three categories

which can come from all the above-mentioned sources. There are mainly two kinds of wastewater, i.e. municipal wastewater, and industrial wastewater. Municipal wastewater consists of domestic wastewater originating from households, sewer overflows and storm drains. Industrial wastewater contains a wide range of contaminants, the quantities and concentrations of these substances depend upon their source. Wastewater is a by-product of domestic, industrial, agricultural and commercial waste. Wastewater includes substances such as human waste, food scraps, oils, soaps and chemicals. In homes, this includes water from sinks, showers, bathtubs, toilets, washing machines and dishwashers. Wastewater is 'used water from any combination of domestic, industrial, commercial or agricultural activities, surface runoff or storm water [3,4].

Pollutants or metals are typically categorized as physical, chemical and biological, which are include complex organic materials (N and P rich compounds), pathogenic organisms (bacteria, viruses, and protozoa) and also synthetic organic chemicals, inorganic chemicals, microplastics, sediments,

radioactive substances, oil, heat, heavy metals and many other pollutants may also be present in wastewater [5]. There are several cyanobacterial species that have good bioremediation agents and mitigating the toxic pollutants. The heavy metals are metallic element which is toxic and has a high density (5mg/cm<sup>3</sup>), specific gravity or atomic weight and which are fall into 2 groups; the 1st group essential metals (Fe, Zn and Cu), these metals are functioning as micronutrients for plants and cyanobacteria and another group include nonessential metals (Cd, Lead (Pb), Mercury (Hg) and Arsenic (As), this nonessential metals are more toxic even at low concentrations. The essential metals are involved in numerous physiological processes and photosynthetic reactions in cyanobacteria and plants, but at high concentrations non-essential metals strongly impair the growth of plants and cyanobacterial biomass [6]. In the environment is susceptible to toxic metals and various pollutants because of their persistence and toxicity [7-9]. Many industries discharge toxic metals waste and polluted soil or water resources. These non-biodegradable pollutants accumulate in the food web and disturb the ecological balance by magnifying up the trophic levels. There are various methods used for wastewater purification i.e., evaporation, ion exchange, electroplating, membrane processes or precipitation, to eliminate persistent toxic metals, particularly from liquid waste [10]. Cyanobacteria have the ability to emerge as a potentially cost-effective method to remediate toxic pollutants through the mechanism of biosorption, bioaccumulation, and intracellular degradation. Due to the stress ability of cyanobacteria to survive extreme environments and metabolise a variety of compounds, which are promising in degrading peculiar pollutants and removing heavy metals from wastewater [11]. In the last 12 years back, arsenic ('As') contamination occurred in borewells of Bangladesh and Karnataka regions. Some researchers are worked on this aspect using Spirulina platensis as an experimental material and reduced the 'As' toxicity in ground water wells of Bangladesh. Based on various scientific articles, heavy metals are causing toxicity in cyanobacterial species and disturbed the photosynthetic energy process in Spirulina [12,13]. Spirulina subsalsa is a photolithotrophic, prokaryotic trichomatous, nonnitrogen fixing, multicellular and filamentous cyanobacterium. The trichomes consist of a number of cells (6-10) covered by a mucilagenous sheath and are spirally twisted. Spirulina has been studied for Single Cell Protein (SCP) (14), edible source of vitamins, minerals, proteins and polyunsaturated fatty acids (gamma-linolenic acid) [15], therapeutic properties [16], antioxidant activity [17].

*Spirulina* consist of two important light-harvesting pigments, such as phycocyanin (PC) and Allophycocyanin (APC). The PC is a fluorescent antioxidant; soluble in water, whereas APC is a bridging pigment between PBSs and photosynthetic lamellae. In addition, a blue pigment of PC is also very useful for medicinal, therapeutics studies, and also *Spirulina* is very useful for bioremediation agent. <u>PBSs</u> are attached to the cytosol (stromal) face of the cyanobacterial thylakoid membrane. PBSs

are large water-soluble protein complexes which usually consist of a tri cylindrical APC core and six PC rods attached to the core [18]. PBSs are structurally related to mammalian bile pigments. El-Bestawy [5] studied the role of *Anabaena variabilis, Anabaena oryzae* and *Tolypothrix ceylonicain* the improvement of water quality in domestic-industrial wastewater. Phytochelatins (PCs) and Metallothioneins (MTs) are different classes of heavy metalbinding protein molecules. PCs are enzymatically synthesized peptides, whereas MTs are gene-encoded polypeptides, which are played an important role in the detoxification of cadmium (Cd<sup>2</sup> <sup>+</sup>) [19,20] demonstrated in *Cylindrothica fusiformis* and also produce carbohydrate as a defense mechanism against copper toxicity in algal stationary phase.

Highest reduction in Biochemical Oxygen Demand (BOD), Total Dissolved solids (TDS) and Chemical Oxygen Demand (COD) (89.29 %, 38.84 %, and 73.68 % respectively) was recorded with Anabaena species. Photosynthesis, an important process for plant growth and biomass production is negatively affected due to increasing levels of heavy metals in air emissions or soil environment [21]. Heavy metals are generally inhibiting normal physiological processes and also disturbances the photosynthetic process in cyanobacteria and higher plants, it has previously shown that different heavy metals can interfere with various steps of the photosynthetic electron transport chain. Inhibitions were found on both the donor and acceptor sides of photosystem II (PS II), at the cytochrome b<sub>c</sub>f complex and in photosystem I (PS I) also. In the photosynthetic electron transport chain, photosystem (PS) is the site most sensitive to metal ions [22]. Reduction of photosynthetic pigments by the heavy metals also indirectly influences the photosynthesis [6]. Heavy metal toxicity causes reduction of leaf growth and disorganization of chlorophyll structure [23]. Interaction of heavy metals with light harvesting pigments (chlorophyll, carotenoids) reduces their content by Bertrand & Poirier, [24] and disrupts energy transfer in lightharvesting antennae. Metals damage the structure and function of the chlorophyll a [25]. Photosynthetic pigments were found to be reduced under the excessive concentrations of Hg, Ni [26]. The photosynthetic apparatus including PS II is particularly sensitive to heavy metals [27]. Though both Cu at equimolar concentration to PSII RC [28]. Electron transport between Pheo and Q, was impaired in PSII core and RC particles by Cu (80 mM) treatment, Yruela et al. [29] showed that <230 Cu (II) or PSII RC, which inhibited O<sub>2</sub> evolution and variable chlorophyll a fluorescence around 50%, did not affect the polypeptide composition of PSII, and only higher copper concentration caused the release of OEC polypeptides. Several recent studies have shown successful in removal of P and nitrogenous compounds from nutrient-rich wastewaters [30]. In these conditions Spirulina protect cellular and sub cellular system from the cytotoxic effects of active oxygen radicals with antioxidative and non-enzymatic components such as SOD, CAT and Ascorbic acid, Glutathione. This research study mainly focuses on industrial wastewater sewage treatment on Spirulina of photosynthetic efficiency (Chl, PBPs) studies.

## **Materials and Methods**

# Cultivation of *Spirulina subsalsa* and Industrial wastewater sewage treatment (IWST)

The mother cultures of Spirulina subsalsa strain (BDU -141021) were obtained from the National Facility for Marine Cyanobacteria (NFMC), Bharathidasan University, Tamil Nadu, India, it is cultivated autotrophically using new Spirulina nutrient medium [31]. After growth screening of two cyanobacterial species, i.e., Chlorella and Spirulina subsalsa, S.Subsalsa was selected for this study. Stock cultures were maintained in a culture room separately at 10 hours dark and 14 hours cool fluorescent light (20Wm<sup>-2</sup>) at 25 ± 2°C. All the culture flasks were continuously moving with shakers. The cultures were maintained in a bacteria free state by regularly transferring the exponentially growing cultures to fresh sterile growth medium. Both species cultures were examined under microscope to check the growth and bacterial contamination of all. The biomass of Spirulina culture inoculated 20 to 25 days in new Spirulina nutrient media and cells were collected by filtration using filter paper 8mm pore size for further inoculation. Seven day old Spirulina culture was inoculated in Spirulina nutrient medium this is called Living biomass (S.subsalsa + NS medium).

# Maintenance of Industrial wastewater sewage treatment (IWST) with Spirulina

The collected wastewater sewage from near Kurnool industrial area, Andhra Pradesh, this IWS incubated at laboratory conditions  $(28\pm4^{\circ}C)$  and filtered for the treatment. After filtration of this IWS maintained with *Spirulina* nutrient media for the treatment with *S.subsalsa*, After 7 days incubation, the appropriate amounts of waste water were added i.e. Mid log phase *S. subsalsa* cells (7<sup>th</sup> Day – OD = 0.8452) were treated with different concentrations of waste water pollutants,5, 10, 20, 40 and 60ppm/ L incubated 4 days. The both IWS and *S.subsalsa* mixed well with appropriate volumes and incubated at shaker for four days [1]. The *Spirulina* was treated with wastewater at different 6 different concentrations (5, 10, 20, 40 and 60ppm/ L) for 48 and 96hrs intervals. In control experiments the investigated pollutant was absent; it's contain only living biomass. Later we perform various experiments such as growth of *Spirulina*, chlorophyll PBPs content etc.

## Determination of malondialdehyde (MDA)

Malondialdehyde (MDA), a major end-product and an index of lipid peroxidation, was measured using the thiobarbituric acid method. The treated algal cells will be collected and incubated with 2ml of 10% (w/v) trichloroacetic acid for 1h at 25°C, and then 1 ml of 0.6% (w/v) 2-thiobarbituric acid (in 10% trichloroacetic acid) was added. The mixture was heated for 15min at 100°C in a water bath and cooled quickly to stop the reaction [35]. The mixture was centrifuged at 5000rpm for 15min and the supernatant was collected. The absorbance was read at 532, 600 and 450nm. MDA level was calculated by

 $MDA \ content \ \left(nMole / 10^7\right): \left\{ \ 6.45 \times \left(OD_{_{532}} - OD_{_{600}}\right) - 0.56 \times OD_{_{450}} \right\} \ \times \ 3 \ \left(reaction \ volume\right) / \ total \ cell \ number \ \left(10^7 \ cells\right) + 10^7 \ cells \ s = 10^$ 

# Determination of the photosynthetic pigment levels (*Chlorophyll* and Phycobiliproteins)

After completion of incubation period (48 & 96hrs), the growth of industrial waste water treated *S.subsalsa* was observed under microscope and recorded the absorption at 560nm using UV-Visible spectrophotometer. The photosynthetic pigments e.g., total *Chlorophyll* (Chl), were analyzed in 5ml of chilled 80% acetone followed by sonication and centrifuzed at 3000rpm for 10min at 4°C. The absorbance of the resulting supernatant was taken at 480, 645 and 663nm.

#### Chlorophyll

*Chlorophyll* content was measured by Deng et al. [32]. In brief, at 96hrs, triplicates of 15mL of well-blended cultures were centrifuged at 3000rpm for 10min and the pellets were homogenized with 5mL of high-performance liquid chromatography (HPLC)-grade methanol for pigment extraction. Then, the mixtures were vigorously shaken with a vortex and placed in the dark at 4°C for 24h. The methanol-extracted samples were centrifuged at 10000rpm for 5min to remove the pellets, and the supernatants were transferred into 96-well plates and measured for *Chl*orophyll at 750, 665, and 652nm using a multimode microplate reader. All absorbance values were corrected using HPLC-grade methanol as control. Concentrations of *chl* a ( $\mu$ g/3.66x 10<sup>7</sup>) (*Ca*) were calculation described in Porra, [33].

## Estimation of total phycobiliproteins content

The PBPs of all treated samples industrial wastewater sewage (5, 10, 20, 40 and 60ppm/ L) at 96hrs, *S.subsalsa* biomass was harvested through centrifuge at 3000rpm for 15mins, and dried for 14hrs (50°C) for PBPs extraction. It was extracted in 0.1M potassium phosphate buffer (pH-6.8) till coloured supernatant obtained from the centrifuged partially dried pellet through repeated freezing (-20°C) and thawing (4°C) method. The absorbencies of PBPs containing supernatant were measured at 615, 652 and 562nm using micro plate reader. Equation for estimation of PBPs described in Siegelman & Kycia [34] and expressed in mg/cell.

### Statistical analysis

All experiments were run in triplicate and the results were presented as mean ± standard deviation. The differences between the control and the treatments were analyzed by using Excel, Origion 8.5 version. Statistical analysis of the experimental data utilized Independent-Samples T Test. Each of the toxicity data was compared to its corresponding control. Statistical significance (\*) was accepted when the probability of the result assuming the null hypothesis (p) was less than 0.05.

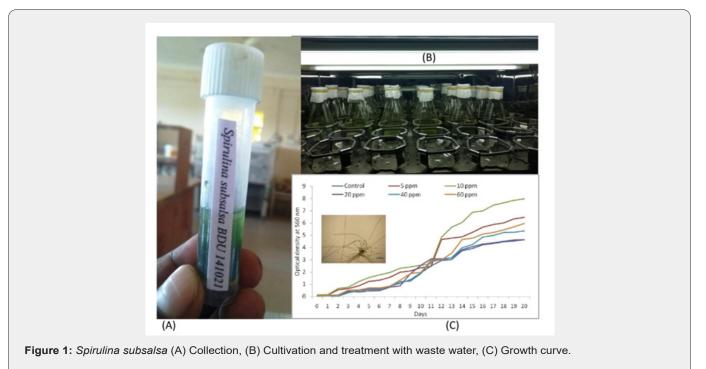
## Results

# Growth characteristics of S.subsalsa

Detoxification is the process of neutralizing or elimination of toxic metals from the ecosystem or body, in wastewater treatment systems, the bioremediation of the pollutants using Spirulina subsalsa occurs via two mechanisms; bioaccumulation and biosorption, followed by biodegradation. Macro or micro metals are very important for the growth of living organisms, and high amount of metal concentrations leads to toxic ('Hg' 'As' and 'Pb'). Some heavy metals are one of the toxic metals from industrial wastewater, these metals are releasing severe impact on environmental system, and many of the metals are toxic even at very low concentrations. Heavy metal detoxification is the removal of metallic toxic substances from the environment. Figure 1 shows effects of wastewater sewage on the growth characteristics of S.subsalsa. Figure 2 a clear dose-response relationships were observed after 48 and 96hrs of exposure. After 48 and 96h exposure, the growth of S.subsalsa was significant increase in the treatments relative to the controls, although there was no significant difference between the controls and the treatments

with 20ppm  $L^{-1}$  wastewater pollutants. Moreover, it is interesting to find that the growth inhibition rate (*IR*, %) decreased with increasing wastewater pollutants concentrations at the same exposure time.

In the thylakoid membrane of algae, there are a series of enzymes and other pigment-protein complexes called PS II and PS I. PS II is the more useful pigment complex (Chl, PBPs, (PC & APC)) during the photosynthetic process in Spirulina (3). Figure 3 shows algal absorption intensity (a.u) was decreased under Industrial wastewater treatment with Spirulina (IWST). The Chl a absorption peaks in the red region and blue region were observed at 660 and at 470nm, respectively. A wide around 430nm was indicative of the presence carotenoid. The shoulder absorption peak at about 415nm implied the presence cytochrome *b*-559 and pheophytin in the complex. It showed that the absorption spectra of the PSII particles from algae treated by IWST were the same as that of the control. However, the absorption intensity from algal treated was higher than that of the control. *Chl a* and *b* are two main absorption bands denoted the Soret and the Q bands [36] (Figure 4).



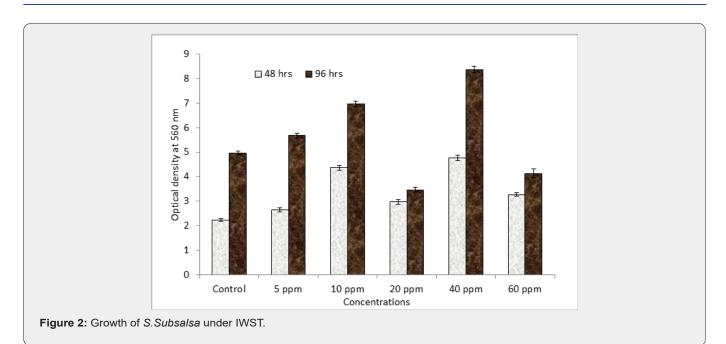
### Lipid peroxidation

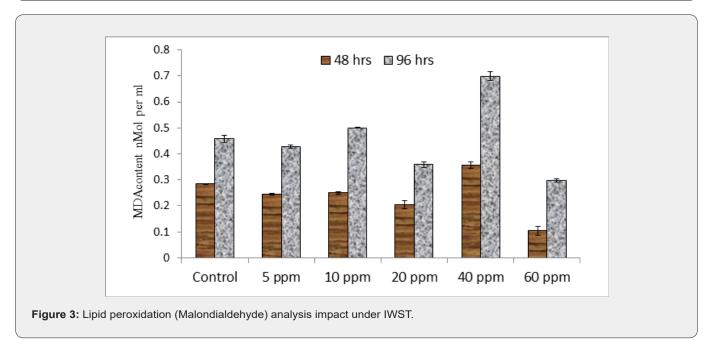
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Malondialdehyde (MDA) is a final product of polyunsaturated fatty acids peroxidation in the cells. The thiobarbituric acid reactive substance (TBARS) assay was used to measure the MDA content oxidative Stress. As shown in Figure 3, the MDA contents of the *Spirulina* cells increased with increasing IWST concentration (5, 10, 20, 40 and 60ppmL<sup>-1</sup>), it indicates that there is no damage

at plasma membrane of *Spirulina subsalsa* cells. It interestingly showing improvement at concentrations of 10 and 40ppmL<sup>-1</sup> and but other concentration of IWST slight significantly improvement of 5, 20 and 60ppmL<sup>-1</sup> at 48 and 96hrs (Figure 3). The significant, dose-dependent decrease in the accumulation of MDA in the algal cells after exposure to the IWST was clearly indicative there is no cellular lipid peroxidation.

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## Photosynthetic pigment (Chl, PBPs) levels of S. Subsalsa

## **Chlorophyll**

The *Chl a*, is an efficient indicator of the growth situation of cyanobacterial cells. The *Chl* a content of *S. Subsalsa* exposed to different IWST is shown in Figure 5. This fig showing the *Chl* a content of *S.Subsalsa* was significantly increased, especially at high concentrations of IWTS (5, 10, 20, 40 and 60ppm L<sup>-1</sup>) during 48 and 96h exposure. The *Chl* a content was 0.0166, 0.0229, 0.1291, 0.0683, 0.1547 and 0.1088µg/ml at 48hrs and at 96hrs *Chl* a activity significantly enhanced 0.0633, 0.1067, 0.2292, 0.1427, 0.2472 and 0.1583µg/ml of *Spirulina subsalsa*. Between both exposure times at 96hrs more significantly increased *Chl* a.

#### Impact of IWST on total PBPs content:

The PBSs are a brilliantly colored group of disc-shaped proteins bearing covalently attached open-chain tetra pyrroles known as phycobilins. The PBSs absorbs light in the 500-650nm wavelength range, transferring energy to membrane bound *Chl* for photosynthesis. PC and APC were composed of two polypeptides, such as  $\alpha$  and  $\beta$  polypeptides. The PC is an antioxidant, fluorescent protein and major constitute of the PBSs while, APC holds the bridging pigments between PBS and photosynthetic lamellae. After 48hrs of incubation of IWST, in Figure 5 showing, lowest concentration of IWST (5ppmL<sup>-1</sup>) increased the PBPs content in *S.Subsalsa* as compared to control but further increase in IWTS concentration resulted in gradual decrease. The PBPs content significantly showed 4% increase at 5ppmL<sup>-1</sup>, 47% increase at 10ppmL<sup>-1</sup>, 6% increase at 20ppmL<sup>-1</sup>, 64% increase at 40ppmL<sup>-1</sup> and 26% increase at 60ppmL<sup>-1</sup> at 96hrs (Figure 6). It has

been suggested that rapid entry of IWST ions might result in detachment of PBSs from the thylakoid membranes that lead to reduction in photosynthesis.

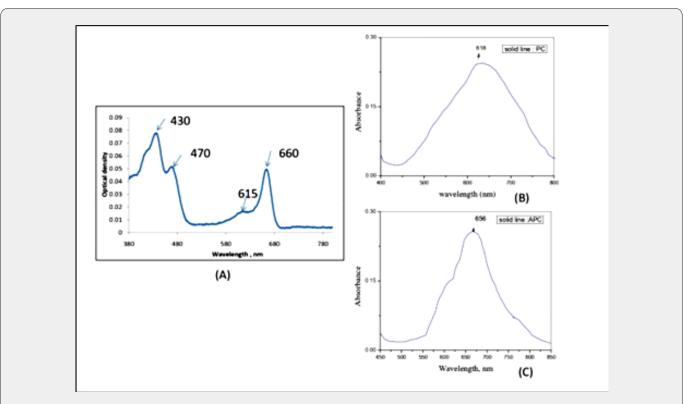
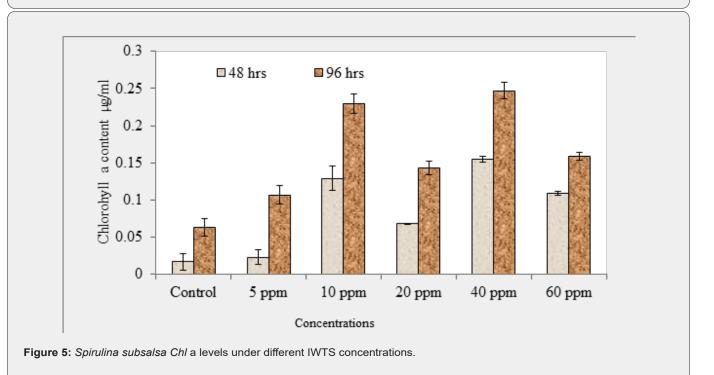
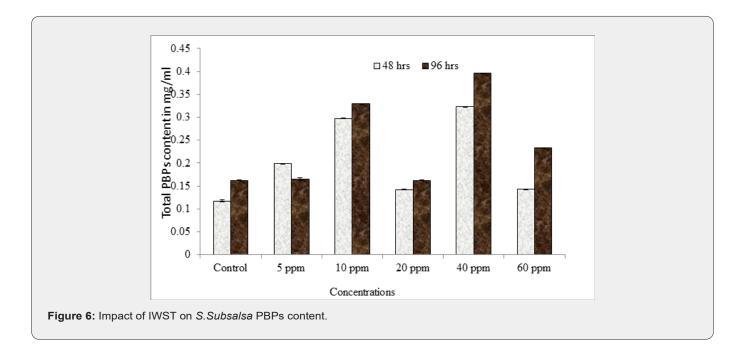


Figure 4: Spectral properties of S. Subsalsa photosynthetic pigments (a, b & c).



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### Discussion

Biological processes such as suspended cultivation are very effective against nitrogenous compound removal; several species such as Oscillatoria, Phormidium, Aphanocapsa and Westiellopsis have been found to take up P and N compounds very efficiently from the effluents which in turn reduce the pollution loads of environment. There are several limitations of using suspending microalgae, such as perfect operating conditions that are hard to maintain, and it's difficult to maintain monospecific. Secondly, there are not many effective processes available to separate microalgae from effluents before they can be discharged in the environment. Because of this sole reason, a very limited number of stabilization ponds and high-rate algal ponds are in use now. In wastewater treatment systems designed to remove nutrients, mainly dissolved N and P, is becoming an important step of treatment. Discharge of these nutrients into sensitive water bodies leads to eutrophication by stimulating the growth of unwanted plants such as algae and aquatic macrophytes. Higher concentrations of metal pollutants produce toxicity and enhance the generation of reactive oxygen species, which results in the disruption of the cellular Spirulina metabolism. Physicochemical methods of sewage treatment are mostly environmentally degrading and require heavy machinery, increasing the cost of the process. Consequently, the center of attention has shifted to bioremediation. Microorganisms utilize the organic and inorganic nutrients from the sewage, thereby reducing the pollutant load. Photosynthetic cyanobacteria, such as Spirulina Subsalsa, can thrive and reproduce in lower bioavailability. Because of their simple growth requirements such as sunlight, carbon dioxide; easiness in their genome manipulations and well-documented applications, there is a need

and a good chance for optimal utilisation of cyanobacteria. Hence, *Spirulina subsalsa* is an organism of choice for bioremediation. With several bioactive compounds and catalytic enzymes, this cyanobacterium has proven to degrade heavy metals as well as biological contaminants. Moreover, the biomass generated during sewage treatment can be utilised to extract commercially valued products (bioactive compounds) or nutrient-enriched food supplements for humans and animals.

# Conclusion

The present study clearly indicated that *Spirulina subsalsa* is an important part of aquatic ecosystem and is dominant in most contaminated habitats. This is attributed to their inherent potential to survive in such eutrophic and adverse habitats and resilience to environmental extremes. Reports on their wastewater treatment potential reflect that *S. subsalsa* is efficient in treating various wastewaters sewage treatment and increasing of *Chl* and PBPs content it is indicated that enhancement of *S. subsalsa* biomass production because of more photosynthetic process going into the cells of *S. subsalsa*. And also, nutrient removal, water quality improvement as well as heavy metal/toxic pollutant mitigation. However, these areas still need more in-depth understanding. The indigenous differences in the characteristics and composition of various wastewaters are the main factors responsible for varied response of cyanobacteria in terms of their remediation potential.

## Acknowledgement

This work was supported by the Dr. D.S. Kothari Post-Doctoral Fellowship (September 2021 to September 2024) Scheme [Sanctioned letter No: F .4-2/2006 (BSR)/BL/20-21/0020)], University Grants Commission (UGC), New Delhi.

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