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Phycoremediation of carton box industry effluent using consortia of green microalgae *Chlorella* sp. and *Scenedesmus* sp. and phytotoxicity assessment

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Industrialization, in the environmental context, has accumulated heavy metal concentrations considerable and it is adversely affecting biological system. In order to regulate heavy metal levels from effluents before their release into the environment, treatment of the wastewaters to reduce the content is mandatory. Phycoremediation is recognized as a cost effective technology for treating industrial effluents. The present study was carried out with the carton box manufacturing industry effluent contaminated with heavy metals collected from Peenya industrial area, Bangalore to determine the phycoremediation potential of algal consortium comprising of *Chlorella* and *Scenedesmus.* Microalgal species grown in wastewater with two different concentrations (50 and 100 %) were analyzed for their influence on algae growth, biomass and protein contents. Algae treated effluent showed the reduction of total solids, total dissolved solids and total suspended solids and heavy metal levels. These results indicate the efficacy of algal consortium for phycoremediation of carton box manufacturing industry effluent.

Keywords: Heavy metals, Industrial effluents, Pollution

Current rapid industrialization, population, and urbanization have added huge loads of pollutants in the water bodies. These organic pollutants are introduced into the environment through industrial discharges, agricultural uses, or other waste disposal practices[1]. Most of the heavy metals are water soluble and they cannot be removed by normal physical removal methods. Heavy metals, such as chromium, nickel, copper, lead, and zinc in wastewater are hazardous to health. The nature of heavy metals polluted wastewater effluents on humans may be toxic, neurotoxic, carcinogenic, and mutagenic[2]. It is reported that each metal exhibit specific sign of their toxicity and the signs associated with cadmium, lead, chromium, arsenic, zinc, mercury, copper and aluminium poisoning are gastrointestinal disorders, diarrhea, stomatitis, tremor, ataxia, paralysis, vomiting, depression and pneumonia[3].

Due to the significant disadvantages associated with chemical methods, such as more energy requirements, incomplete metal removal, toxic waste products generation, there is an increasing demand for eco-friendly techniques using low cost[4]. In recent years there has been an increase in demand for microbial biomass for remediation purpose. Phycoremediation is the use of macroalgae or microalgae for the removal or biotransformation of pollutants, including nutrients and xenobiotics, from wastewater and CO_2 from air[5]. Algae is considered as a renewable source of natural biomass having potential towards metal removal from the aqueous solutions or effluents because of their main constituents such as carbohydrates, proteins and phenolic compounds consisting of metal bonding groups such as amines, carboxylates and hydroxyls[6,7]. Algal species are relatively easy to grow, adapt and manipulate within a laboratory[8].

Algae can absorb different types of pollutants from solutions or effluents using mechanism of biosorption. It incorporates a series of independent metabolic processes, including physical and chemical adsorption, electrostatic interaction, ion exchange, complexation, chelation and microprecipitation[9]. Bioaccumulation occurs in two stages, in the first, biosorption, the contaminant is retained on the surface of the cell wall and in the second, the contaminant undergoes via active transport into the cell[10].

The carton boxboard manufacturing industry generates wastewater during production. These wastewaters comprise of high levels of glue and inks. Hence, the total and suspended solids concentrations are also high. In addition, significant concentrations of metals, namely aluminum, chromium, iron, sodium, magnesium, lead and zinc are evident due to the

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presence of various types of inks used[11]. It is necessary that heavy metals are removed from the wastewater before it is discharged it into water bodies. The present work is focused on biological treatment to reduce the heavy metals from a carton box effluent through treatment with algal consortium.

Materials and Methods

Algae cultivation

Chlorella sp. and *Scenedesmus* sp. were isolated using serial dilution, standard plating, colony isolation and culture techniques. The algal genus was identified with the help of standard monographs and recent available literature[12]. The algal species were grown in BG-11 growth media[13] under controlled conditions in a growth chamber at a temperature of 27°C and light intensity of 16-30 W/m^2. After two weeks, the biomass was separated from the media by centrifugation. The pellets were washed twice with distilled water before being used for the studies.

Physicochemical analysis of the wastewater

The wastewater used in the present investigation was obtained from a Carton boxboard manufacturing industry, Peenya industrial area, Bangalore. The wastewater samples were analyzed for pH, total solids and total dissolved solids, total suspended solids according to standard methods of analysis of wastewater (American Public Health Association, 1998). Heavy metals chromium (Cr), Arsenic (Ar) and Nickel (Ni) were measured by Atomic Absorption Spectroscopy (SIMADZU, Model-AA6401F AAS).

Experiment design

Experiments were set up to investigate chromium, arsenic and nickel removal by growing microalgae *Chlorella* sp. and *Scenedesmus* sp. consortium in various concentrations of wastewater. The experiment was carried out in 250 mL sterile Erlenmeyer flasks covered with cotton plugs coated with the plastic. The wastewater was diluted to different concentrations of test solutions viz., 50 and 100% using distilled water, while BG-11 medium prepared in distilled water served as control.

Flasks were inoculated with 5 mL of *Chlorella*/*Scenedesmus* sp. consortium, taken from the stock algal cultures. The concentration of algae at the beginning of the experiment was 38×10^3 cells/mL. Algae were counted in Haemocytometer chamber using light microscope. The flasks were incubated at 16:8 day-night cycle with the average 16-30 W/m^2. The control and effluent-treated algal consortium were subjected to growth, biochemical analysis and heavy metal analysis. Growth curve of algae, total chlorophyll and total protein contents were measured as described below at different day intervals (Day 1, 3, 6, 9 and 12). All experiments were carried out in triplicate and analyzed statistically.

Growth analysis and Biomass estimation

Growth curve of *Chlorella* sp. and *Scenedesmus* sp. consortium in effluent was measured by turbidometrically at 680 nm using a spectrophotometer. Biomass content was estimated using the formula (weight [g/L] =OD primary∗0.238[14].

Biochemical analysis

Total protein and Total chlorophyll content

Protein measurement was performed according to Lowry's method[15]. Total chlorophyll content was determined according to the method of Parson and Strickland[16].

Heavy metal analysis

Heavy metals, chromium, nickel and arsenic were analyzed before and after algae treatment by atomic absorption spectrometric analysis by APHA standard methods[17].

Phytotoxicity studies

To achieve the purpose of phycoremediation study, a bioassay was performed as a parallel monitoring system according to method of Kagalkar *et al.*[18]. Assay was performed in order to assess toxicity of wastewater containing heavy metals after its phycoremediation process. The effect of wastewater before and after its phycoremediation was recorded. Tests were carried out with seeds of higher plant *Phaseolus mungo.* Ten individual seeds were used for the germination in each set and watered separately with 10 mL of wastewater and cell free wastewater obtained after phycoremediation. The samples were watered every day for 8 days. A control set was also performed with same number of seeds watered with 10 mL of water. The percentage of seed germination along with root and shoot lengths of the seedlings were recorded in each case separately. Germination inhibition (%), reduction in length of shoot (%) and reduction in length of root (%) was calculated for comparative study of the effect of wastewater before and after phycoremediation.

Statistical analysis

Data were analyzed statistically using the Graph pad software. Experiments were carried out in triplicates (n=3). The results were reported as mean±SE.

Results

Physicochemical analysis

The collected carton box industry effluent was analyzed for physicochemical characteristics like pH, color, odor, total solids (TS), total suspended solids (TSS) and total dissolved solids (TDS). The results of physicochemical properties of wastewater after phycoremediation process are presented in Table 1. Results from this experiment showed that at the 12th day of the phycoremediation, pH of the wastewater significantly increased from 5.8 to 7.2 and the amount of TDS was reduced efficiently from the wastewater. After phycoremediation of wastewater, the color of wastewater changed from the light black color to the green colour.

Table 1—Physicochemical characteristics of carton box industry wastewater before and after phycoremediation treatment

Parameters	Before treatment	After treatment
pH	5.8	7.2
Colour	Light black	Green
Odour	odour	odourless
Total Solids (TS) mgL^{-1}	146±0.23	136±.27
Total Suspended Solids (TSS) mgL^{-1}	6±0.08	4±0.12
Total Dissolved Solids (TDS) mgL^{-1}	140±.14	132±.15

Each value represents the mean ± standard error values (n=3)

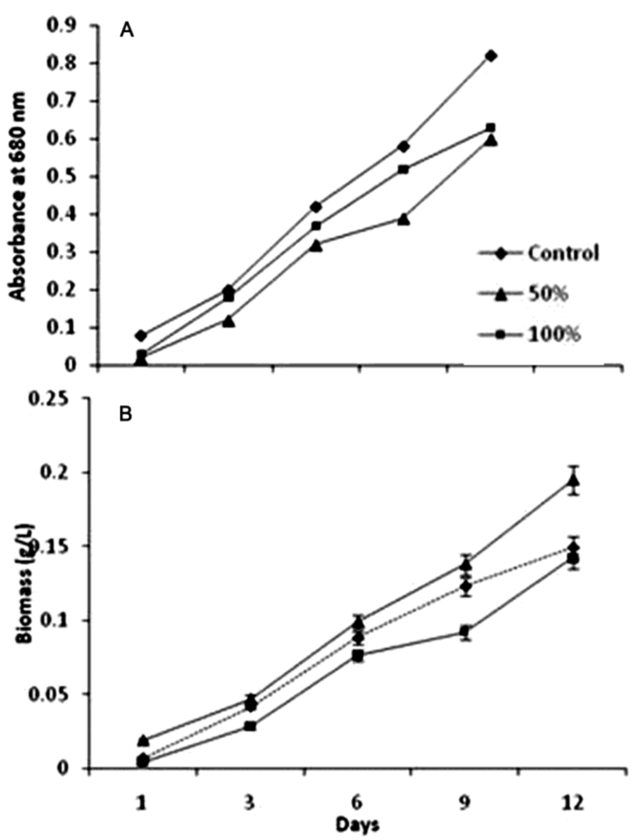

Fig. 1—Growth curve of (A) *Chlorella*/*Scenedesmus* sp.; and (B) Biomass content of algal consortium treated with 50 % and 100 % concentrations of effluent

Growth analysis and Biomass analysis

The growth capability of *Chlorella* sp. and *Scenedesmus* sp. against heavy metals such as chromium, arsenic and nickel present in the carton box industry effluent has been checked by growing the species in 50 and 100% effluent. There was increase in growth of algal cells with increasing concentration of effluent as compared to control (Fig. 1A).

The entire waste water medium (100%) is suitable for growth of algae. Biomass contents of algal consortium were obtained as 72.82% in 50% effluent and 76.41% in 100% effluent when compared to the control (Fig. 1B).

Biochemical analysis

The total protein content of algal consortium was 64% in 50% effluent and 96% in 100% effluent treatment when compared with the control (Fig. 2A).

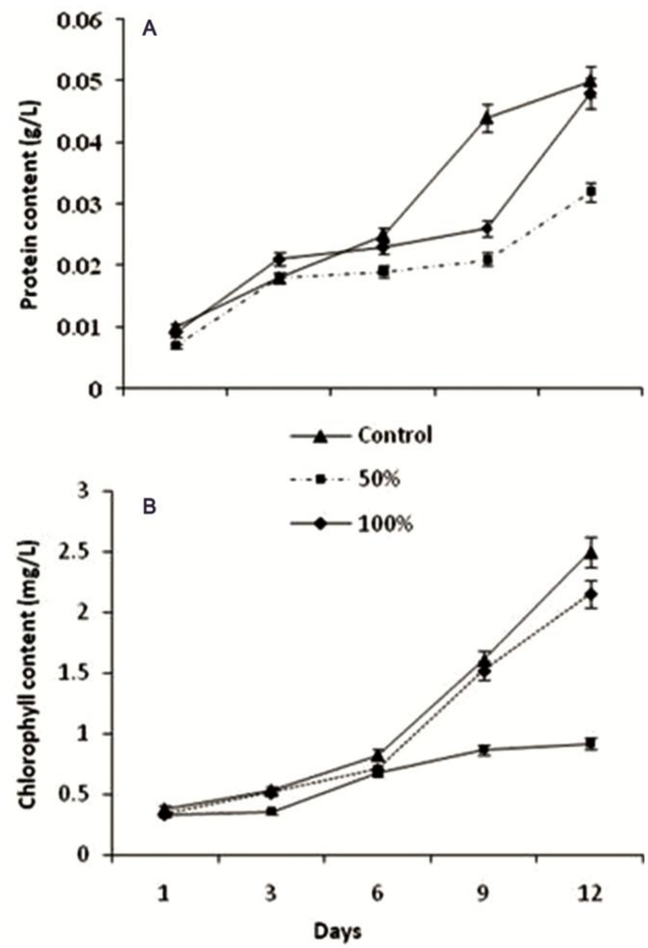

Fig. 2—(A) Protein content; and (B) Chlorophyll content of algal consortium treated with 50 % and 100 % concentrations of effluent

In the present study, we found increase in the protein content with increase in duration. Effect of heavy metals on total chlorophyll content of algal consortium is shown in Fig. 2B. Total chlorophyll content in effluent-treated algal cells was less when compared to the control. There is dose dependent increase in total chlorophyll content was observed at different day intervals. The total chlorophyll content of algal consortium was 76% in 50% effluent and 84% in 100% effluent, when compared with the control.

Heavy metals removal efficiency of algal consortium

The presence of chromium, arsenic and nickel were analyzed by atomic absorption spectroscopy (AAS), and it was found to be 0.2 mg/L, 7.59 µg/L, and 64.03 mg/L, respectively in 100% effluent. However, after algal consortium treatment, heavy metals contents of effluent were significantly reduced as 0.03 mg/L, 1.2 µg/L, 0.59 mg/L for chromium, arsenic and nickel, respectively. The maximum metal removal occurred in algal consortium 85% for Cr, 84.19% for As and 99.17% for Ni in 100% effluent treatment (Fig. 3).

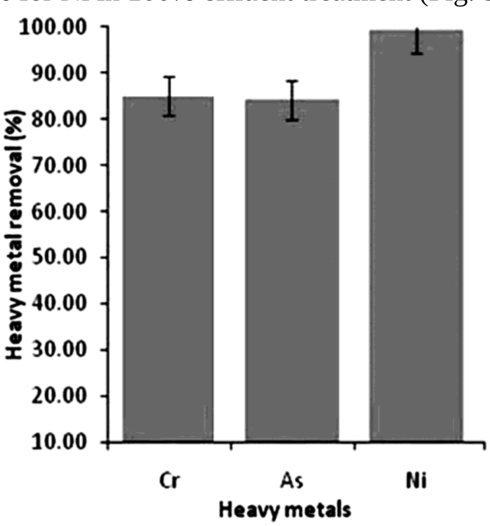

Fig. 3—Heavy metal removal percentage of algal consortium treated with effluent

Phytotoxicity studies

Phytotoxicity test were performed in order to assess the toxicity of industry wastewater and treated wastewater obtained after its phycoremediation. Toxicity tests in view of seed germination and root growth have been proposed by government agencies to study the potential for possibility of removal of contaminated effluents disposed into the environment directly. Phytotoxicity study revealed the toxic nature of industry effluent to the higher plant *Phaseolus mungo* (Fig. 4). Germination (%) of plant was less in industry effluent treatment as compared to treated wastewater after its phycoremediation and normal water (Table 2).

Discussion

The algae *Chlorella* sp. and *Scenedesmus* sp. have been widely used in wastewater treatment due to fast growth rates and remediation ability. After phycoremediation of wastewater, pH of the wastewater significantly increased from 5.8 to 7.2. The microalgae, through photosynthesis, reduces dissolved CO_2 concentrations which, in turn, raise the pH level[19]. Total dissolved solids content of the effluent decreased upon treatment, which is due to the utilization of nutrients from effluent by algae. It is found that there is decrease in physicochemical parameters which is due to the binding of negatively charged uronic acids, present in microalgae to cations in heavy metals[20].

Table 2—Phytotoxicity study of wastewater and treated wastewater after its phycoremediation

Observations	I	II	III
Germination (%)	80 %	00	60%
Shoot length (cm)	7.5±0.08	0.0±0.0	6.56±0.2
Root length (cm)	6.5±0.26	0.0±0.0	5.75±0.17

[Each value represents the mean ± standard error values (n=3). I = Seeds treated with water.; II= Seeds treated with waste water; III = Seeds treated with treated wastewater after its phycoremediation

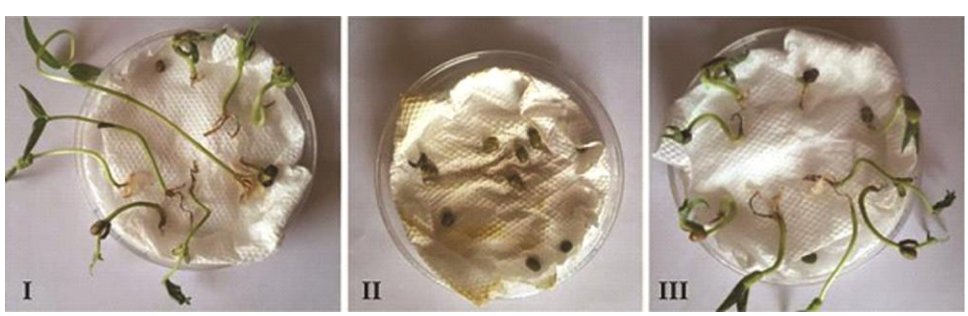

Fig. 4—Phytotoxicity of industry effluent to the germination of higher plant *Phaseolus mungo*. [I = Seeds treated with water. II= Seeds treated with wastewater. III = Seeds treated with treated wastewater after its phycoremediation]

When wastewater was used as growth media for algae, there was a dose dependent increase in biomass concentration. While Balaji *et al.*[21] observed reduction in biomass contents of *Arthrospira platensis* in tannery effluent as 67.88% in 50% effluent and 49.32% in 100% effluent. Ajayan *et al*.[22] found reduction in biomass content of *Scenedesmus* sp. in tannery effluent. It may be due to the higher amount of heavy metals and other toxic pollutants present in the effluent which reduced the growth of microalgae[23].

In the present study, we found increase in the protein content with day intervals, which is similar to the results of previous studies on seaweed *Ulva faciata* and *Chaetomorpha antennina* which showed that the effluent had positive effect on the protein content of the alga with increase in 35% of protein level to soda ash industry effluent exposure[24]. The cell wall of microalgae consists mainly of polysaccharides, proteins, which provide various functional groups. These functional groups offer a net negative charge to the cell surface and binds with heavy metal ion with strong affinity[25]. Algae can incorporate toxic effects of heavy metals by accumulating protein at low heavy metal level or by increasing respiration leading to the utilization of carbohydrate in favor of protein accumulation[26]. The intracellular metal detoxification mechanisms comprise binding of metal with metal binding peptides and proteins within the cytoplasm and sequestration of metals in the form of electron-dense polyphosphate granules[27]. Change in total chlorophyll content in algal cells as compare to control could be attributed due to disorganization of chloroplast structure, peroxidation of chloroplast membranes and increased production of free radicals[28,29]. El-Naggar *et al.*[30] reported inhibition of chlorophyll biosynthesis in *Monoraphidium minutum* and *Nitzchia perminuta*by Co^{2+} treatment. The mechanism proposed is the replacement of magnesium in the chlorophyll molecule and blocking of chlorophyll synthesis, this may be due to inhibition of reduction step in the biosynthetic pathways of this pigment leading to lower chlorophyll contents.

The algae biomass is considered as ideal biosorbent for the selectively removing and reducing the concentration of heavy metals due to some features which includes availability in either live or dead form, low nutrient requirement, large biomass production, high bio sorption capacity, high tolerance to heavy metals, regeneration potential[31,32] . Shashirekha *et al.*[33] reported that blue green algae are potential organisms having capacity to remove metals and other toxic components from tannery wastewaters. The mechanism of removing heavy metals from wastewater by microalgae is related to their large surface area and binding capacity[34]. The toxic effect of heavy metals may be related to production of reactive oxygen species (ROS) and the resulting unbalanced cellular redox status. Algae have developed extracellular and intracellular mechanisms to cope up with heavy metal toxicity. At lower levels, algae can accumulate heavy metals[35]. It has been reported that cell wall is the main barrier for metal uptake, and surface adsorption is an essential system that allows microalgae to endure high levels of harmful metals in their encompassing medium[36]. Tripathi *et al*.[37] reported use of *Scenedesmus* sp. ISTGA1 for municipal wastewater treatment. Also Wang *et al.*[38] reported removal of Al, Ca, Fe, Mg, and Mn from local municipal wastewater treatment plant (MWTP) using *Chlorella* sp. Kumar *et al.*[39] checked efficiency of different wastewater viz., domestic sewage, industrial and aquaculture wastewater for heavy metal removal using marine algae *Chlorella marina*.

During phytotoxicity study, the heavy metal content in industry effluent was significantly reduced the length of shoot and root than the treated wastewater after its treatment. The probable reason is that, heavy metals have some impacts on plant includes decreased seed germination and lipid content, inhibition of photosynthesis, decreased enzyme activity and plant growth[40]. Phycoremediation process removes significant quantity of heavy metals, and hence treated wastewater would be less toxic to plants.

Conclusion

It is evident from the present study that microalgal consortium could remediate carton box industry wastewater and offers promising alternative to traditional technologies in the treatment of heavy metals. Algal consortium was effective in detoxifying heavy metals that leads to the reduction in total solids, total dissolved solids and total suspended solids in effluent. *Chlorella-Scenedesmus* showed dose dependent increase in growth rate, total chlorophyll and total protein contents during effluent treatment. The algal consortium also effectively removed heavy metals, there by exhibiting bioremediation potential of carton box industry effluent. The algal treatment system is offering an eco-friendly technology in the industrial wastewater treatment.

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Conflict of interest

The authors declare that they have no conflict of interest.

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