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ORIGINAL ARTICLE

Biochemical Effects of Lead (II) on Algae Collected From East Calcutta Wetlands

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ABSTRACT

Exposure to the heavy metal Lead is a global public health concern due to its critical negative health effects, especially in Children. Lead makes its way into the environment through various anthropogenic processes like automobile exhaust, effluents from leather and paint and ceramic industries. East Calcutta Wetlands, situated at the eastern fringes of the metropolitan city Kolkata, is the major waste treatment and recycling site of Kolkata. It is receiving the municipal sewage and the industrial waste generated in the city for more than 150 years. The area is well known for its waste utilization through sewage-based agriculture and pisiculture and has been incorporated in the list maintained by the Ramsar Buraeu established under the Ramsar Convention and is recognised as a 'Wetland of International Importance'. The wetland has been reported to be contaminated with heavy metals including lead. Four Algal Samples were collected from a fishery pond in East Calcutta Wetlands. This place receives the municipal sewage and industrial waste of the city for about 150 years. Protein, Chlorophyll, Lipid and Non-protein Thiol content was estimated at 0.5 mg/l, 5 mg/l and 10 mg/l Lead concentration.

Key Words: *Lead, East Calcutta Wetlands, Algae, Protein, Chlorophyll, Non-protein thiol, Bioremediation*

INTRODUCTION

Exposure to the heavy metal lead is a global public health concern due to its toxicity and persistent nature. Lead occupies the second position in the priority list of most hazardous chemicals prepared by the Agency of Toxic Substances and Diseases Registry (ATSDR). Main sources include effluents from industries like leather, paint, electric, battery, pulp, paper, ceramic, kitchen wares, pottery, insecticidal and mosquito coils, automobile exhausts [1]. The U.S. Environmental Protection Agency (EPA) recommends lead in air not to exceed 1.5µg/m^3 and in drinking water to15 µg/L. According to the U.S. Consumer Product Safety Commission (CPSC), blood-lead level above 10 g /100 ml is a health concern [2].

East Calcutta Wetlands, located approximately between latitudes 2225' to 2240' N to longitudes 8820' to 8835' E covers 12,500 hectares area. Situated between the levee of river Hoogly on the west and river Kulti Gong at the east the region acts as the sewage treatment and resource recovery system of Kolkata. The resource recovery takes place mainly by three processes sewage-fed fisheries, agriculture on the garbage substrate and paddy cultivation using pond effluent. Of these, sewage-fed fisheries play the most important role. The Kolkata Municipality generates 600 million litres of wastewater and 2,500 tonnes of garbage daily. The wastewater is received by 286 sewage-fed fisheries and local farms. The wetland has been incorporated in the list maintained by the Ramsar Buraeu established under the Ramsar Convention and is recognised as a 'Wetland of International Importance' [3, 4, 5]. It is the only site in West Bengal that is registered in the Ramsar list.

The cumulative efficiency of the wetland in reducing the Biological Oxygen Demand (BOD) of the sewage water is more than 80 per cent. The sewage-fed fisheries act like solar reactors. The solar energy is trapped by a dense population of plankton which is again consumed by fish. A study conducted by the Institute of Environmental Studies and Wetland Management has reported 30 genera of phytoplankton.

The wetland has been found to be contaminated with heavy metals. Presence of mercury has been reported in marketable fishes and vegetables grown in this area by a group of researchers [6, 7]. Another group has reported toxic metals like arsenic, chromium and lead in vegetables cultivated in the East Calcutta Wetland [3]. As the algae and other phytoplankton of this area are growing in presence of heavy metals they are expected to have a tolerance against

heavy metals and can be useful for the end of the pipe treatment for removal of heavy metals from industrial effluents. Purpose of the study was to check the biochemical effect of lead (II) on green algae collected from the East Calcutta Wetlands and also, to check the potential of the algae for bioremediation of lead (II).

MATERIALS AND METHODS

Sample Collection :

Algal samples were collected from two fisheries of East Calcutta Wetlands that receive waste water for pisciculture. The ponds were designated as Pond 1 and Pond 2. Algae were collected from the following sources: i) Water samples Pond 1 and Pond 2 and (Sample 1 and 3) ii) a piece of thermocol found at the bank of Pond 2 containing an algal film (Sample 2).

Acclimatization, growth and maintenance of algae in laboratory:

Samples 1 and 3 were transferred to conical flasks in the laboratory. Sample 2 was scrapped from the thermocol and transferred to pond water. The samples were maintained in the laboratory at 30C at 12 hour day-night cycle for 3 days. After 3 days, the samples were transferred to a mixture of pond water and modified Chu-10 media (Composition for 1 L: $NaNO_3$ 0.232g; K_2HPO_4 0.01g; $MgSO_4$ 0.025g; Na_2CO_3 0.02g; Fe(III)-citrate 3.5g; Na-silicate 0.044g; citric acid 3.5g) in 3:1 ratio. The increased of media was increased gradually after 15 days of growth and the algae were finally grown in the modified Chu-10 media after three successive transfers. Sample 1 showed a considerable increase in cell mass. During the final transfer in Chu-10 media, Sample 1 was diluted in 1:100 ratio and was transferred separately to another conical flask containing the media and was designated as Sample 1A. Therefore, at the end of the final transfer, 4 samples were obtained. Sample 1: Pond water of Pond 1; Sample 1A: sample 1 diluted in 1:100 ratio in the laboratory after acclimatization; Sample 2: Scrapped from a thermocol sheet containing an algal film from Pond 2; Sample 3: Pond water of Pond 2. The algae were maintained in the media for one month with one transfer after 15 days before starting the experiments. During the study, stock culture of algae was maintained in the above medium. Algae were grown in 12-hour day-night cycle and the temperature was maintained at 35C.

Cell harvest:

Algae were inoculated into modified Chu-10 media in presence of 0.5, 5 and 10 mg/L initial concentrations of lead (II). Lead (II) was added to the media in form of lead acetate at 35C 12-hour day-night cycle. The algae were allowed to grow for 15 days. Algal cells were then harvested using glass filter (Himedia, equivalent to Gf/F grade) by filtration under high pressure.

Estimation of protein:

Algal cells were homogenized in 5% NaOH (0.5 ml) and 5% Na-deoxycholate (0.5 ml). Protein content was measured in the homogenate by Folin-Lawry method [8].

Estimation of chlorophyll:

Algal cells were lysed by ultrasonication for 30 minutes in presence of 90% acetone (1 ml) and kept for 24 hours at 4C. The cells were then homogeinsed and chlorophyll estimation was done by spectrophotometric method following the protocol of Arnon et al [9]. The OD values were taken at 664nm, 647nm and 630nm.

Estimation of non-protein thiol:

Cells were homogenized in 6% TCA and centrifuged at 5,000 rpm for 10 minutes to separate the proteins. Non-protein thiol was estimated in the supernatant using 5,5'di-thio-bis [nitro-benzoic acid] (DTNB) reagent in presence of 0.3M phosphate buffer at pH 7.4. Spectrophotometric analysis was done at 440 nm with the yellow colouration produced [10].

Estimation of lipid:

Lipids were extracted from the by n-hexane. Total amount of lipid was estimated gravimetrically following Bigh-Dyer method after complete evaporation of the solvent [11].

Photomicrography:

Algal cells were spread on grease-free glass slide with an inoculation needle and dried under air at room temperature. Photomicrographs of the algae were taken using bright field microscopy (Olympus BX 51) with 400 times magnification.

Estimation of residual lead in growth media:

After separation of cells, media were digested in an acid chamber with concentrated nitric acid for 2 hours followed by concentrated hydrochloric acid for 30 minutes to remove all organic carbonaceous or proteinaceous residual matter. Residual lead was estimated in the digest by Atomic Absorption Spectroscopy (AAS) (Varian AA 240).

RESULTS AND DISCUSSION

Acclimatization of algae in laboratory:

Algae were acclimatized successfully in laboratory in modified Chu-10 media. Considerable growth was observed after 15 days when algae were transferred in Chu-10 media from pond water after successive increase in media proportion to pond water. Growth of algae increased in laboratory when they were kept in media for 1 month with one

transfer after 15 days. The extent of growth remained the same after 6 months. Sample 1A, which was prepared diluting Sample 1, was also found to grow in a similar extent to the other samples.

Growth of algae with varying concentrations of lead (II):

Algae were found to grow in presence of lead (II) with initial concentrations of 0.5 g/L and 5 g/L. Growth was retarded in presence of lead (II) when the initial concentration was 10 mg/L.

Protein content:

Protein content decreased when lead (II) was present in the media at the initial concentration of 0.5 mg/L and 10 mg/L. An increase in protein content was observed with the initial concentration of lead (II) of 5 mg/L, for all the samples except Sample 1. In sample 1, change in protein content was statistically insignificant in presence of 5 mg/L lead (II) in the media. Initial decrease in protein content may be due to an inhibitory effect of lead (II) on cellular metabolism. However, as the samples succeeded to grow in presence of 0.5 mg/L of lead (II), it can be inferred that this concentration is not highly toxic for algae. Synthesis of some special proteins like metallotionein may be induced by lead (II) with the initial concentration of 5mg/L which resulted in increased protein content of algal cells. It is evident that the concentration 0.5 mg/L can induce stress but is not high enough to induce synthesis of stress-proteins. On the other hand, reduction in protein content by 10 mg/L of lead (II) may be due to the toxic effect of lead (II) on cellular metabolism. The growth was also found to be retarded at 10 mg/L concentration of lead (II) and this supports the possibility of having toxic effects of lead (II) at this concentration (Fig. 1).

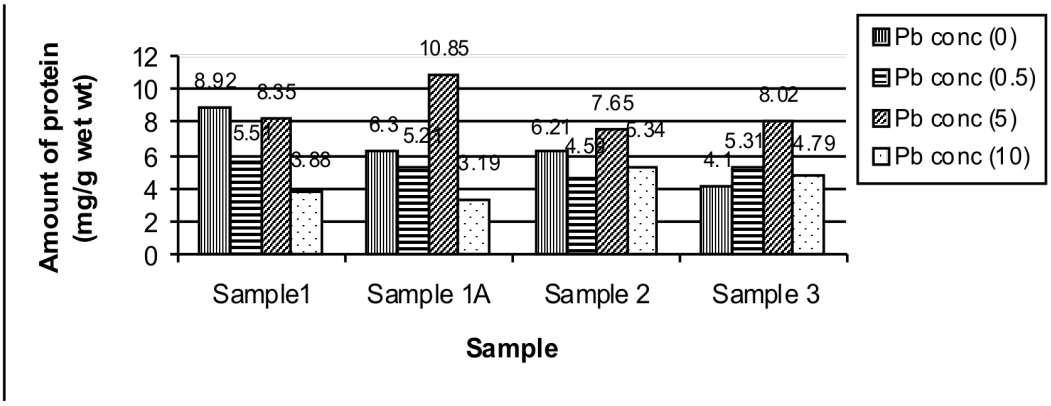

Fig. 1: Protein content of algae samples grown in Chu 10 media with different concentrations of lead (II) (0, 0.5, 5, 10 mg/L)

Chlorophyll content:

Chlorophyll a, b and c decreased in presence of lead with the initial concentrations of 0.5 and 5 mg/L, and were almost nil with 10 mg/L. (Fig. 2, 3, 4). Total chlorophyll content followed the same trend as the individual components (Fig. 5). The decrease was more with the initial concentration of 0.5 mg/L than 5 mg/L. It indicates that the algae have developed adaptive mechanism against the stress induced by lead (II) at the concentration of 5 mg/L. The lower concentration is not sufficient to induce the adaptive mechanism. The trend for change in chlorophyll is similar to that of the total protein content. Reduced chlorophyll content due to Pb (II) in different plant species has been well documented [12]. Stiborova et al [13] have reported lower chlorophyll content in barley leaves due to lead (II) absorption. A decrease in chlorophyll content has been reported in *Triticum sativum* and *Lens esculata* on treatment with lead nitrate [14]. Decrease in chlorophyll concentration and photosynthetic ability have been observed in *Synechococcus leopoliensis* (Cyanobacteria). Decrease in chlorophyll content was decreased with increased concentration of lead (II) from 25 to 200 mg/L [15]. The enzyme delta-amino levulinic acid synthetase has been found to be reduced in mung bean seedlings by lead treatment. The enzyme is located in chloroplast and plays a role in chlorophyll synthesis [16]. Result of the present study is in accordance with the observations made by other researchers. There is possibility that reduced chlorophyll content is due to an inhibition of delta-amino levulinic acid synthetase in the present algae.

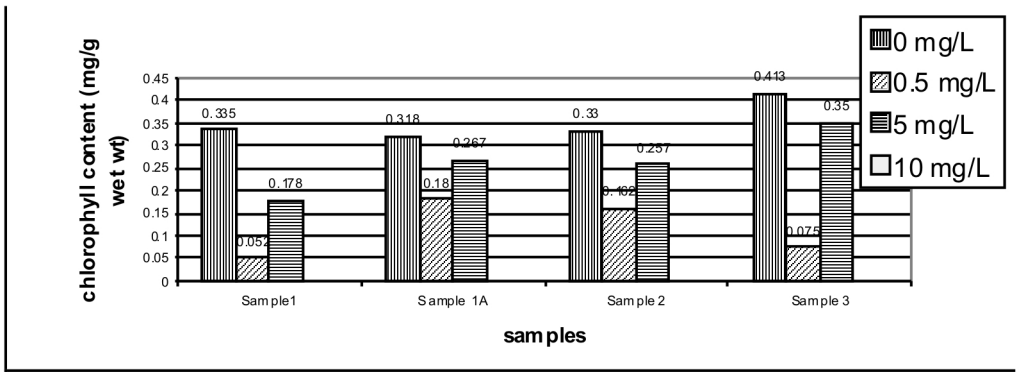

Fig. 2: Chlorophyll a content of algal samples grown in Chu 10 media with various concentrations of lead (II) (0, 0.5, 5, 10 mg/L)

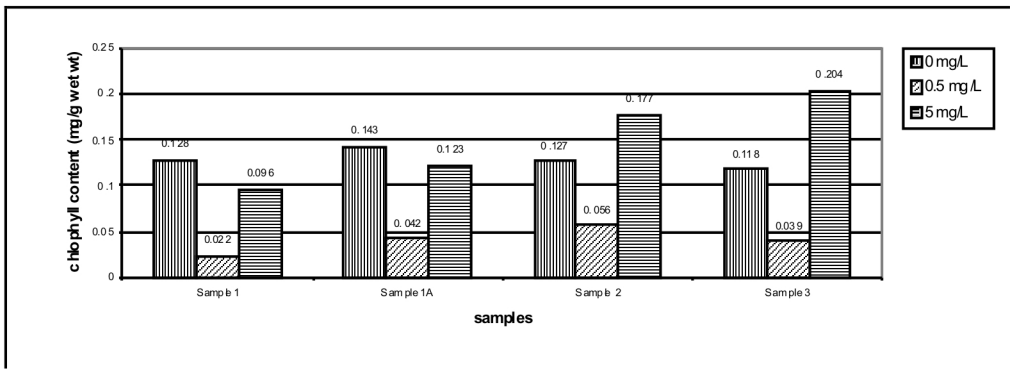

Fig. 3: Chlorophyll b content of algal samples grown in Chu 10 media with various concentrations of lead (II) (0, 0.5, 5, 10 mg/L)

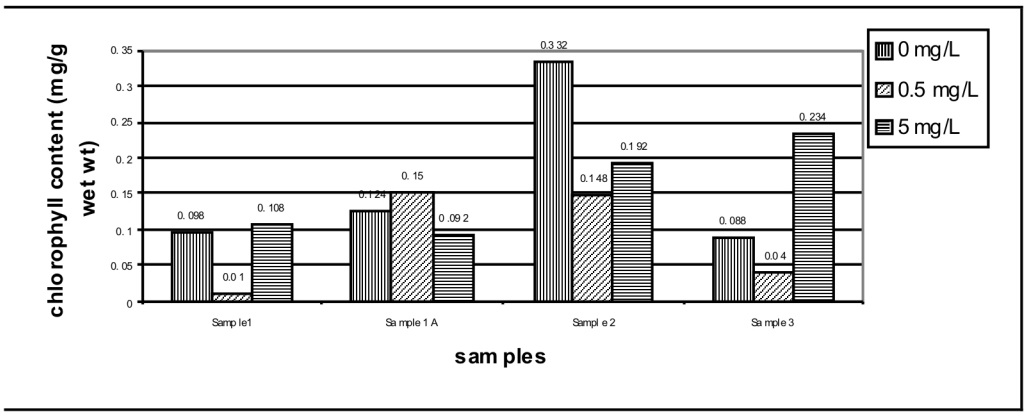

Fig. 4: Chlorophyll c content of algal samples grown in Chu 10 media with various concentrations of lead (II) (0, 0.5, 5, 10 mg/L)

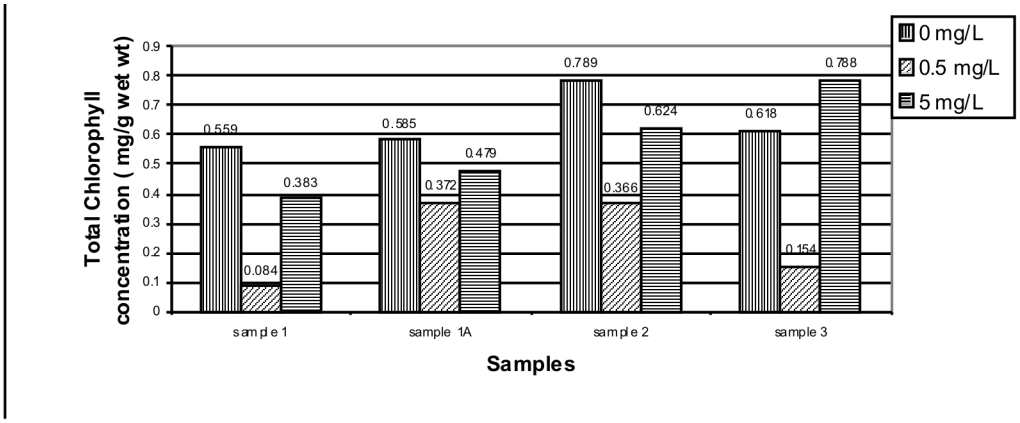

Fig. 5: Total chlorophyll content of algal samples grown in Chu 10 media with various concentrations of lead (II) (0, 0.5, 5, 10 mg/L)

Non-protein thiol:

Non-protein thiol content of algae was found to be decreased in presence of lead (II). The observation is in accordance to the observation of protein and chlorophyll thiol concentration decreased in presence of 0.5 mg/L lead (II) and then increased with 5 mg/L. However, with 10 mg/L of lead (II), thiol concentrations were similar to that with 5 mg/L for Samples 1 and 1A and decreased in Samples 2 and 3 (Fig. 6). This may be due to the possibility that 0.5 mg/L lead (II) is not enough to trigger the adaptive response and increase the concentration of thiol compounds. Like protein and chlorophyll content, increase in thiol content was triggered at the 5 mg/L concentration of lead (II). Lead (II) and mercury (II) are known to form strong complexes with the thiol group [17]. Heavy metals are known to induce oxidative damage in plants. The effect includes lipid peroxidation, DNA damage, depletion of sulfhydyrls and altered calcium homeostasis [18]. The observation of depletion of non-protein thiol content due to lead (II) exposure was similar to the effect of cadmium (II). A transient decrease in glutathione level along with the anti-oxidant defence enzyme glutathione reductase was observed in plants [19] resulting in oxidative damage. There are two possibilities for having lower levels of non-protein thiols, including GSH, in presence of lead (II). The first possibility is that the free thiol groups are not available due to direct binding of lead (II). The alternative is that most of the glutathione is utilized for synthesis of metallothionein, the algal component similar to phytochelatin of higher plants. Phytochelatin, a polymer of glutathione, is known to be induced by heavy metal stress [20]. Though phytochelatin is best demonstrated in presence of cadmium and it removes cadmium very efficiently [21], its role in lead (II) tolerance cannot be ruled out as lead (II) has a high affinity for SH groups.

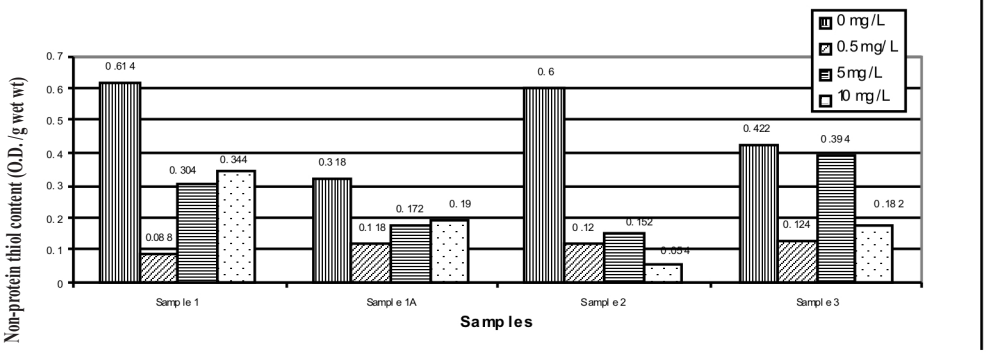

Fig. 6: Non-protein content of algal samples grown in Chu 10 media with various concentrations of lead (II) (0, 0.5, 5, 10 mg/L)

Total lipid:

Total lipid content was measured with the initial concentration of 5 mg/L as at this concentration, less deviation from the control was observed compared to the other concentrations. Therefore, this concentration may be considered effective to induce adaptive changes in algae. Total lipid content reduced in presence of lead (II) at the initial concentration 5 mg/L (Fig. 7). Detailed study has not been carried out with lipid content, but this decrease can be related to the reduction of thiol level. Since thiol is an important part of anti-oxidant defence mechanism, it can be

expected that a short-term depletion of thiol may result in oxidative stress which in turn results in lipid peroxidation and lower content of lipid [22].

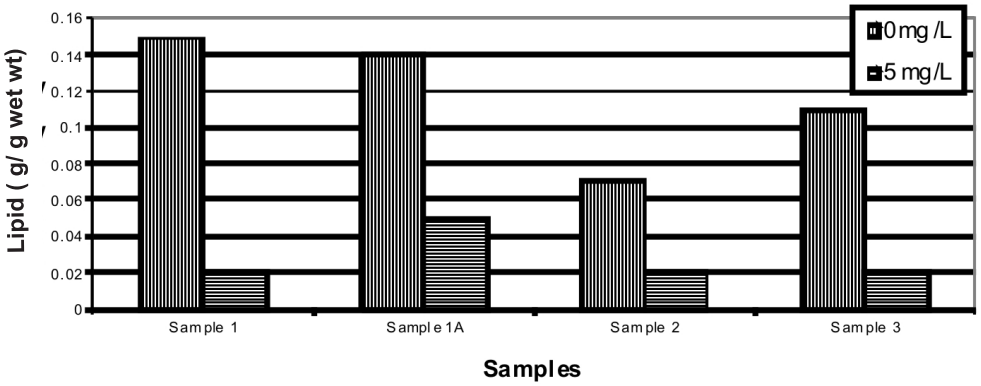

Fig. 7: Lipid content of algal samples grown in Chu 10 media in absence and presence of lead (II) (5 mg/L)

Photomicrography:

From the morphological study, all the samples seem to have *Oedogonium* as the major algal component. Samples 1, 1A and 2 showed less filamentous structures under the microscope due to exposure to lead (II).

Removal of lead (II):

All the samples reduced lead (II) from the growth media. Initial concentration of lead (II) was 5 mg/L and algal biomass was 4 g and 6 g respectively at the beginning and after 15 days (Fig. 8). Temperature was maintained at 30C. Maximum removal (91.42%) was obtained with Sample 2 followed by sample 1A (86.86%). The other samples were also found to remove more than 60% of lead (II). From the result, it can be stated that algal samples collected from the east Calcutta wetlands can remove lead (II) from simulated solution under laboratory condition. They have the potential for use in the finishing step for treatment of industrial waste for removal of lead.

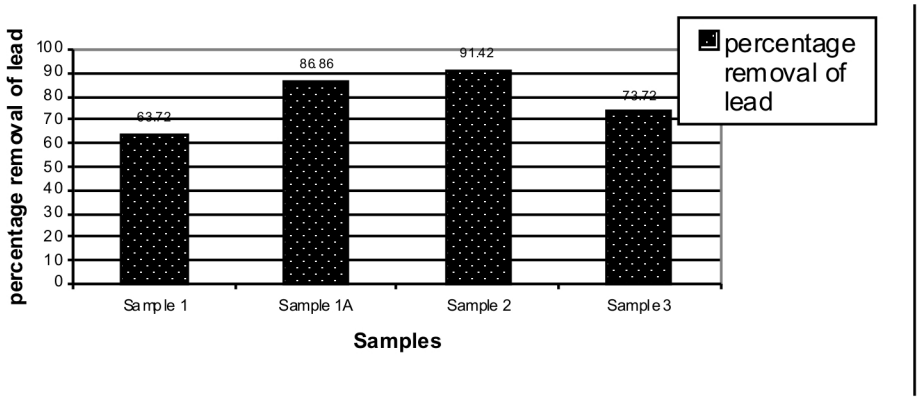

Fig. 8: Percentage removal of lead (II) after 15 days by algal samples grown in Chu 10 media; initial concentration of lead (II): 5 mg/L

CONCLUSION

The algal samples collected from the East Calcutta Wetlands demonstrated adaptive mechanism against heavy metal stress induced by lead (II). All of them reduced lead concentration in the media by more than 60% after 15 days under laboratory condition. The results indicate that the samples could be used for bioremediation of lead (II) and for the end-of-the-pipe treatment of industrial effluent.

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