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Studies on the effect of sodium arsenate & cadmium chloride on *Pithophora oedogonia* (Mont.) Wittrock 1877

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ABSTRACT

Cadmium and Arsenic are heavy metals although not common in our environment, its threat in certain places are aggravated due to anthropogenic factors. To know its critical role on plants the investigation was made using Na_2HAsO_4 and CdCl_2 treatment on *Pithophora oedogonia* (Mont.) Wittrock 1877. The observations were made after 14 days of treatment. The changes were noted. In both cases, the treated cells exhibited gradual disruption of cell wall and cell membrane. The chlorophyll content initially increased and finally decreased due to the notable destruction of chloroplasts in both treated cells. A profuse number of akinetes were observed at 100 ppm and 150 ppm of Na_2HAsO_4 and CdCl_2 treated media. Decrease in protein content was started at 100 ppm in both cases. The lipid content initially decreased at 50 ppm and at 100 ppm lipid profile increased in terms of toleration to the Na_2HAsO_4 and CdCl_2 stress. *Pithophora oedogonia* (Mont.) Wittrock 1877 exhibited more sensitivity to CdCl_2 stress & showing abrupt changes in chlorophyll-a and chlorophyll-b production. The carotenoid production shown more sensitivity in Na_2HAsO_4 stress. Total phenol production was decreased initially and at 200 ppm CdCl_2 stress had shown significant enhancement than the control set but at the 200 ppm of Na_2HAsO_4 shown inhibitory effect.

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INTRODUCTION

Heavy metals are having high atomic weight. They are 5 times denser than water (Tchounwou *et al.*, 2012). Nowadays, it is a serious issue to keep safe environment from two important biologically hazardous heavy metals viz. Arsenic (As) and Cadmium (Cd). Since heavy metals are non-biodegradable and hence can be separated out through different physical or chemical process (Jung *et al.*, 2017). Arsenic can be found in different forms depending upon its oxidation state or valency which ranges from +3 to +5. Arsenite with oxidation state +3 is more toxic than arsenate, which contains the oxidation state of +5 (Gupta, 2007). According to the World Health Organization (WHO, 2011), arsenic exhibit high toxicity in its inorganic form, that can be found in contaminated drinking water, food preparations from contaminated water, contaminated food crops etc. whereas cadmium is a byproduct mainly of mining origin, extracting and refining the zinc and least amount from lead and copper ores. Arsenic is a potent carcinogenic agent and can cause conjunctivitis, skin lesions and hard patches on the palms and feet; on the other hand, cadmium can damage the kidneys, lungs and livers by deposition and can exhibit chronic & acute effects respectively (Rashid & Mridha, 1998). Most

importantly, cadmium deposition shows the longer half-lives in human body that sustains throughout lifetime (Andreae & Klumpp, 1979). According to WHO's guideline value for arsenic and cadmium in drinking water should be lower than 10 $\mu\text{g/L}$ and 3 $\mu\text{g/L}$ respectively.

Algae exhibit high accumulative ability in case of arsenic and cadmium that accumulate it from nearby environment and can synthesize water-lipid soluble compounds (Bernard, 2008). Freshwater algae contain comparatively low amount of arsenic than marine algal species. A study on growth of *Chlorella vulgaris* in a pure culture showed that the growth is unaffected in 100 ppm of arsenic (Maeda *et al.*, 1983) and inhibits growth in cadmium at concentration of 7 ppm (Cheng *et al.*, 2016). The photosynthetic efficiency is hampered due to metal ion which effects on photosynthetic pigments & photosynthetic enzymes etc (Singh *et al.*, 2018). Küpper *et al.* (1995) proved that cadmium can damage the photosynthetic ability by incorporating it in chlorophyll and thus prevent photosynthetic light harvesting. In *Dunaliella salina*, it has been observed that β -carotene, water extractable carbohydrate & fatty acids are increased with the rising concentration of arsenic in medium (Yamaoka *et al.*, 1992). During the heavy metal stress, amount

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of phenol was observed higher in algae that recover or tolerate stress with the metal chelating ability and act as antioxidants (Michalak, 2006; Mira *et al.*, 2002).

In the present study, an easily available Chlorophycean member *Pithophora oedogonia* has been tested for the effect of different concentrations of arsenic and cadmium stress. The alga was collected from the farm house of Burdwan University in Purba Bardhaman district. For this study, focus was made on changes of protein content, lipid content, pigment content and total phenol content.

MATERIAL & METHODS

Collection & Identification of Algal Sample

Algal sample was collected from the farm house of Burdwan University campus, Purba Bardhaman district in West Bengal. The geological location is 23°13'02.7"N & 87°50'58.4"E taken by GARMIN GPSMAP 78S GPS location tracker device. Ecological parameters were analyzed from the water sample following standard protocol. The collected sample was washed properly in gentle running water and purity was checked microscopically. Depending upon the observation of morphological characteristics under microscope the sample was identified as *Pithophora oedogonia* (Mont.) Wittrock 1877.

Preparation, Preservation and Media Culturing of the Sample

After the purification of the sample, little amount of the sample *Pithophora oedogonia* was fixed in 4% formalin. Different grades of arsenic and cadmium were prepared in modified CHU 10 media. The arsenic and cadmium concentrations of 50 ppm, 100 ppm, 150 ppm, 200 ppm were made up by Sodium Arsenate (Na_2HAsO_4) & Cadmium Chloride (CdCl_2) respectively. 5 gm of algal sample was weighted and put in each modified CHU 10 media of different arsenic and cadmium concentration for 14 days. Only CHU 10 media with 5 gm of algal sample was treated as control and each experiment was repeated thrice. After the given period of time the *Pithophora* samples from different media were collected & little amount of *Pithophora* sample was allowed to dry in air flow of normal room temperature for further study. Slides were prepared from different culture media of arsenic and cadmium concentration for changes of morphological study. Images were taken by Zeiss Axiostar Plus Microscope with Nikon DS 60 camera.

For permanent slides preparation, standard procedure was followed. Small portion of the sample was taken on a clean slide then it was covered by GFW solution as a mountant. After putting cover glass it was sealed with synthetic paint and then kept for drying.

Ecological Data

Ecological data of the studying sample *Pithophora oedogonia* at the time of collection was taken with the help of Multi-Parameter

PCSTestr™ 35 for p^H , electrical conductivity, total dissolved solute (TDS), salinity, temperature & with the help of Hi-Media Laboratories Pvt. Ltd. supplied test kits for other tests. Ecological data is shown in Table 1.

Table 1: Ecological data of the collection spot

1.	PH	8.06
2.	Temperature	32.3°C
3.	Total Dissolve Solute (TDS)	438 ppm.
4.	Salinity	252 ppm.
5.	Electrical conductivity	606 $\mu\text{S}/\text{cm}$.
6.	Total dissolve Oxygen	9 ppm.
7.	Alkanity	110 ppm.
8.	Sulphate (SO_4)	50 ppm.
9.	Arsenic (As)	Nil.
10.	Nitrite	0.02 ppm.
11.	Nitrate	10 ppm.
12.	Fluoride	0.05 ppm.
13.	Free Chlorine	Nil.
14.	Zinc (Zn)	Nil.

Chlorophyll and Carotenoids Extraction & Estimation

0.5 gm of algal sample was taken to study and grinded with the help of motor & pestle in 10 ml. of HPLC grade methanol. The homogenate was centrifuged for few minutes with REMI RM-12C micro centrifuge machine and the supernatant was kept in a measuring cylinder. This washing was repeated 3-4 times until the pellet lost its greenish colouration. Absorbances of algal extract were taken with the help of single beam L1-721 Microprocessor Visible Spectrophotometer (Lasany) at 663 nm, 646 nm & 470 nm. Estimation of chlorophyll a, chlorophyll b and carotenoids were done by using following formulae (Lichtenthaler & Wellburn, 1983).

$$\text{Chlorophyll a } (\mu\text{g/ml}) = 12.21 (A_{663}) - 2.81 (A_{646})$$

$$\text{Chlorophyll b } (\mu\text{g/ml}) = 20.13 (A_{646}) - 5.03 (A_{663})$$

$$\text{Carotenoid } (\mu\text{g/ml}) = (1000 A_{470} - 3.27 [\text{chl a}] - 104 [\text{chl b}]) / 229$$

Extraction & Estimation of Total Soluble Protein

Protein extraction was done by Barbarino & Laurencio (2005) described method. In brief, 1gm of dried algal sample was immersed in 4ml. of distilled H_2O for 12 hours and grinded with potter homogenizer. Washing and centrifugation at 4°C (for 20 minutes at 15,000g) was done with another 4ml. of distilled water where supernatant was collected and pellet was mixed with 1ml. of assay grade 0.1N NaOH with 0.5% β -mercaptoethanol purchased from Hi-Media Laboratories Pvt. Ltd. The centrifugation was done with the help of REMI RM-12C micro centrifuge machine. Pellet mixture was again centrifuged at 21°C and supernatant was collected to combine with the previously collected supernatant for protein study. Protein estimation was done following Lowry *et al.* (1951) described method with the help of Folin-Ciocalteu. Absorbance was taken with single beam L1-721 Microprocessor Visible Spectrophotometer (Lasany) at 660nm.

Total Lipid Extraction & Estimation

Lipid extraction & estimation was done according to Bligh & Dyer (1959) described method. In brief, 1 gm of dried algal sample was immersed in HPLC grade methanol-chloroform mixture which was 20 times greater (w/v). Equal amount of 0.2 parts of the volume of crude extract, 0.9% NaCl solution was added and allow it to separate into bi-phasic form of the mixture by standing. Upper phase was removed by siphoning in such a way that lower phase was not disturbed & thus rinsing was done thrice with small amount of pure solvent (chloroform: methanol: NaCl). At last, methanol was added to it and allowed the solution into monophasic. Finally, the mixture was evaporated with hot water bath & the amount of lipid was calculated Wensar weighing machine.

Total Phenol Content Determination

0.5 gm of dried algal sample was immersed with 10 ml methanol for 24 hours. The methanol extract was filtered in another amber coloured vial, washed thrice & then evaporated. Serial dilution of gallic acid was prepared up to concentration of 200 μ g/ml from 0 μ g/ml 0.2 ml. phenol extract was mixed with 0.8 ml distilled water and 1ml Folin-Ciocalteu reagent (1:1). After 5 minutes 2ml of 7.5% Sodium carbonate was added to the sample mixture. Total phenol content was measured as described by Singleton et al. (1999) described method at 765 nm.

RESULTS & DISCUSSION:

Observation of Morphological Changes

After the fourteen days exposure to the different concentrations (50 ppm, 100 ppm, 150 ppm and 200 ppm) of sodium arsenate and cadmium chloride few morphological changes in cell wall, cell membrane, pigmentation, akinete formation etc. were observed under microscope in the algal sample. It was compared to the samples of control set. Both the sodium arsenate and cadmium chloride treated cells exhibited gradual disruption of cell wall and cell membrane. Cadmium Chloride had shown more efficiency than the sodium arsenate. The cell size gradually decreased with the increase of the concentrations of sodium arsenate and cadmium chloride in the culture media. At 200 ppm cadmium chloride the cells were found remarkably died.

The chlorophyll content initially increased and finally decreased due to the notable destruction of chloroplasts in both the cadmium chloride & sodium arsenate treated cells.

Both the intercalary and terminal akinetes formation starts at 50 ppm concentration of sodium arsenate and cadmium chloride. A profuse number of akinetes were observed at 100ppm, and 150 ppm concentrations of sodium arsenate and cadmium chloride. But 200ppm cadmium chloride solution shows gradual destruction of the akinetes.

So, it is clear from the above mentioned observations that the alga shows sensitivity to both of sodium arsenate and cadmium

chloride. 200 ppm concentration of cadmium chloride solution showed more toxic effects than the rest of the concentrations. Figure 1 shows the morphological changes under different stress conditions.

Observation of Chlorophyll and Carotenoids Change

After fourteen days treatment with sodium arsenate and cadmium chloride solution, the obtained results are shown in Figures 2-4 for chlorophyll-a, chlorophyll-b & carotenoids respectively.

In sodium arsenate treated samples chlorophyll-a, chlorophyll-b and carotenoid contents were increased rapidly at 50 ppm concentration & then gradually decreased from 100 ppm to 200 ppm which is below the control set. In case of cadmium chloride treated samples chlorophyll-a and chlorophyll-b slightly increased at 50 ppm concentration and increased rapidly at 100 ppm concentration showing the highest value. After gradual decrease at 150 ppm concentration, suddenly chlorophyll-a concentration falls at 200 ppm which shows much lower value

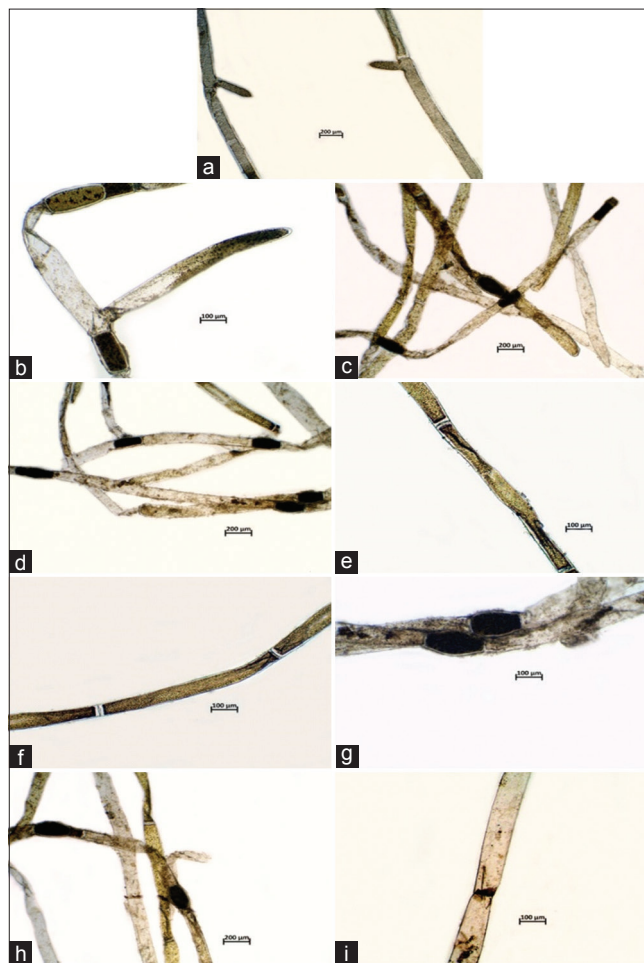


Figure 1: Observation of morphological changes : a- Control sample, b- 50 ppm of Sodium arsenate stress, c- 100 ppm of Sodium arsenate stress, d- 150 ppm of Sodium arsenate stress, e- 200 ppm of Sodium arsenate stress, f- 50 ppm of Cadmium chloride stress, g- 100 ppm of Cadmium chloride stress, h- 150 ppm of Cadmium chloride stress, i- 200 ppm of Cadmium chloride stress

than the control set. Carotenoid value also decreased which is slightly lower than the control one.

Observation of Total Soluble Protein Changes

After fourteen days of treatment, changes in protein content were noted. In Sodium arsenate treated samples showed highest protein content at 50 ppm concentration which is

even greater than the protein content of control sets. 100 ppm, 150 ppm and 200 ppm sodium arsenate concentration shows gradual decrease of protein content. In case of Cadmium chloride treated samples also shows higher protein content, but here sudden and rapid fall of the protein content takes place. Figure 5 shows the changes of total soluble protein with the treatments.

Observation of Total Soluble Lipid Changes

In both sodium arsenate and cadmium chloride treated samples at 50 ppm concentration lipid content decreases, and this decrease of lipid content is lower in sodium arsenate treated samples than cadmium chloride treated samples. Both the 100 ppm concentration shows highest lipid content which is greater than control set. Lipid content gradually decreased from 150 ppm concentrations and 200 ppm shows much decreased value than the control set. Figure 6 shows the changes of total lipid content with different treatment.

Observation of Total Phenol Changes

In sodium arsenate treated samples 50 ppm concentration showed sudden decrease in phenolics contents; 100 ppm and 150 ppm concentrations shows gradual increase and 150 ppm

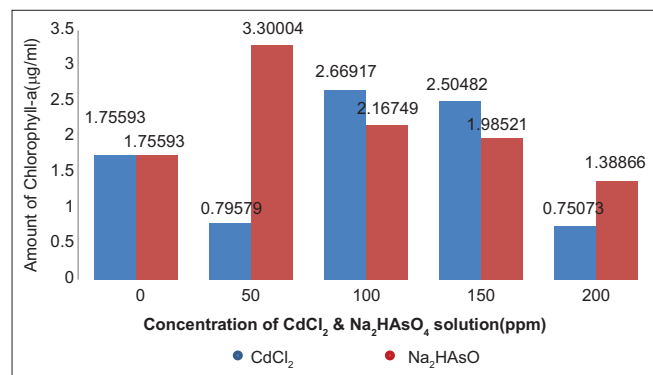


Figure 2: Chlorophyll-a content changes in *Pithophora oedogonia* treated with different grades of CdCl₂ & Na₂HAsO₄ solution

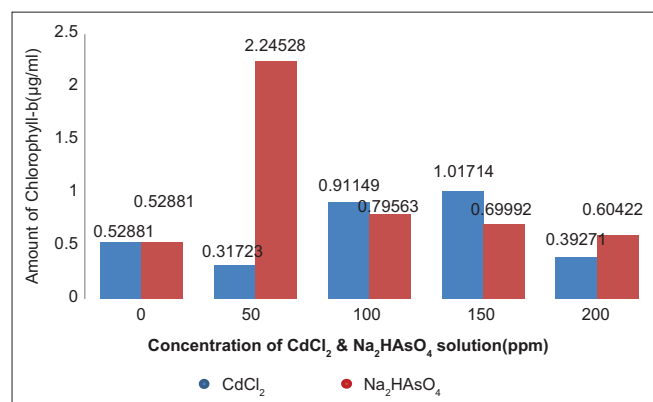


Figure 3: Chlorophyll-b content changes in *Pithophora oedogonia* treated with different grades of CdCl₂ & Na₂HAsO₄ solution

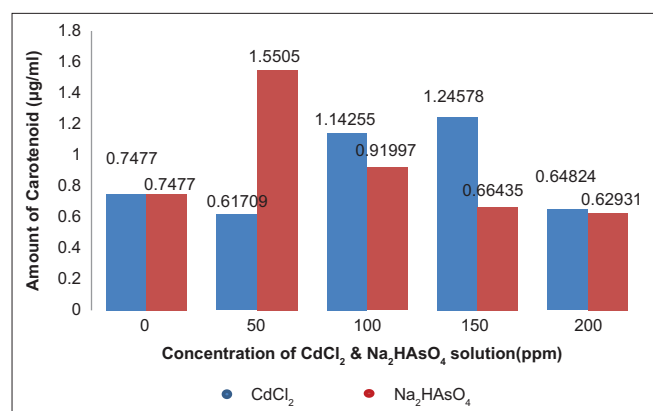


Figure 4: Carotenoid content changes in *Pithophora oedogonia* treated with different grades of CdCl₂ & Na₂HAsO₄ solution

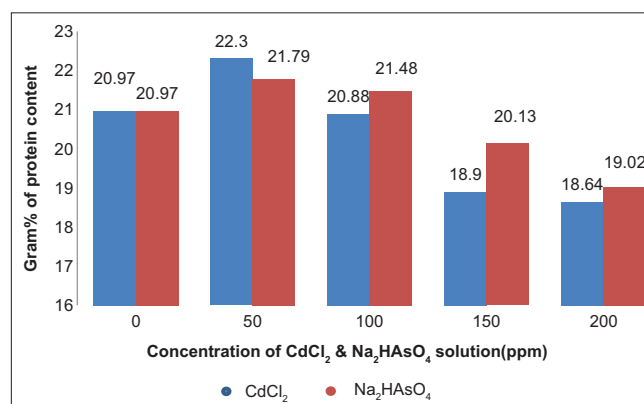


Figure 5: Protein content changes in *Pithophora oedogonia* treated with different grades of CdCl₂ & Na₂ HAsO₄ solution

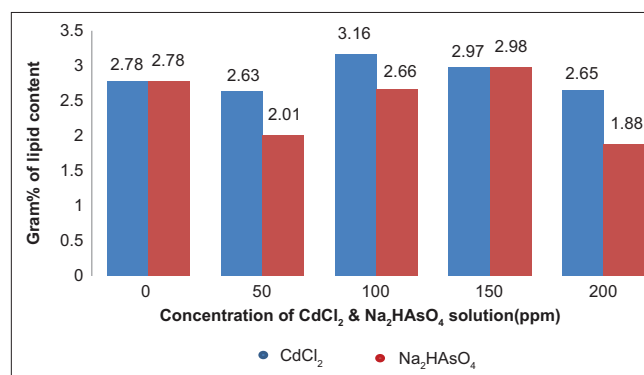


Figure 6: Lipid content changes in *Pithophora oedogonia* treated with different grades of CdCl₂ & Na₂ HAsO₄ solution

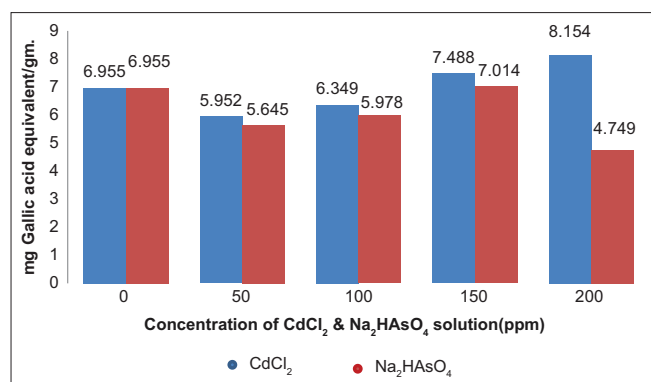


Figure 7: Phenol content changes in *Pithophora oedogonia* treated with different grades of CdCl₂ & Na₂HAsO₄ solution

concentration shows highest phenolics content which is more than the control set. Phenolics content suddenly falls at the 200 ppm concentration. Figure 7 shows the changes of phenolics in different stress conditions.

CONCLUSION

After fourteen days sodium arsenate and cadmium chloride treatment on *Pithophora oedogonia* (Mont.) Wittrock 1877, the different changes in protein, lipid, pigments, total phenolics contents were noted, where, protein content decreasing starts at 100 ppm concentration in both cases and the result shows that Cadmium chloride is more sensitive for protein synthesis than that of the sodium arsenate. The lipid content shows initially decreased a 50 ppm concentration, at 100 ppm lipid profile increasing to tolerate the As and Cd stress, where response rate noticed was more or less same in both the cases. *Pithophora oedogonia* (Mont.) Wittrock 1877 shown more sensitivity to Cadmium chloride stress showing abrupt changes in chlorophyll- a and chlorophyll-b production. The carotenoid production shows more sensitivity to As than the Cd stress. The total phenolics production was decreased initially at 50 ppm conc. At 200 ppm Cd stress had shown significant increase in phenolics content than the control set. But the 200 ppm of sodium arsenate shows inhibitory effect on the production of phenolics. This study shows the interactive role of the naturally occurring alga with hazardous chemicals. It forecasts the bioremediation or biosensor role of alga for next studies.

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