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BIOREMEDIATION OF HEAVY METALS IN THE AQUATIC BIOTA USING MICRO ALGAE

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***Abstract*:** In the present study it has been found that algae have immense capacity to absorb metals, and there is considerable potential for using them to treat wastewater. Concentration of metal and biomass of algae, pH, temperature, biochemical activities of the organism affect the rate of metal absorption. Algae can effectively remove metals from water bodies. In the case of test spices, *Diatoms* can be used for removal of Arsenic from aquatic biota. The present investigation was under taken to study the removal of Arsenic from the metal treated medium in vitro. Arsenic is a carcinogenic and highly toxic heavy metal existed in two valiancy state such as As (V) and As (III). The toxicity of metal can be reduced in alkaline condition. So the sea water containing medium were used for the present study. The parameters of productivity, photosynthesis rate, respiration, pigmentation, end product of photosynthesis bioactive phenol and proline were studied in different concentration of As (III) and As (V). The maximum removal of the metal from the aqueous medium was observed at the exponential growth phase and also increase in the amount of proline and phenol in the test species under stress condition. So algae are used for biosorption of Arsenic from the aquatic biota.

Key words: **Bioremediation, Arsenic removal, Diatoms, Productivity, Proline.**

I. INTRODUCTION

Environmental pollution is one of the greatest life threatening problem of this century. It has been damaging to aquatic ecosystem due to the discharge of heavy metals, pesticides, and salts from agricultural, urban and industrial wastes (Fatma T et al, 2007). Industrial activities introduce novel chemicals into the environment in addition to altering the natural flow of materials (Faisal and Hasnain, 2004). Metals, including heavy metals, occur in solution in almost all natural waters (Wong et al, 1978). The major sources of heavy metals in water bodies including mining, municipal sewage / waste water, industrial processing of metals and ores, industrial wastes, and solid waste dumps (Forstner and prosi, 1979). The presence of heavy metals in the environment is of major concern because of their toxicity, biomagnification tendency and threat to human life and the environment (Hoesfall and Spiff, 2005).Various studies have been carried out to show the role of algae in the bioremediation of heavy metals and heavy metals can be incorporated in to food chain or release into water bodies which are sources of drinking water supplies (Forstner and Prosi, 1979). Arsenic (As) is a component of many industrial raw materials, products and wastes. Elevated levels of arsenic in drinking water have been implicated in human diseases and mortality (Wang S, Zhao X, 2009). Chronic exposure to arsenic causes neurological and hematological toxicity (Bayron. W. R et al, 1967). The most common Arsenic species observed in the environment are the trivalent form As (III) and pentavalent form Arsenic As (V) in which As (III) is more toxic than As (V) (Cullen. W. R and Reimer. K. J, 1989). Because Arsenic readily changes valence state and reacts to form species with varying toxicity and mobility, effective treatment of arsenic can be challenging. Arsenic treatment technologies requires peroxidation step to form As (V) from As (III) (Kim. M. J and Nriagu. J, 2000). But the cost and secondary product formation during other conventional method reduce the practical's (Wang. S and Mulligan. C. N, 2006). Use of biological processes provides a means for cost effective removal for the treatment of metal contaminated waters (Sibi. G, 2014). Microalgae are known to sequestrate heavy metals due to their cell wall constituents which act as binding sites for metals (Davis et al, 2003). Bioaccumulation of metals by algae may create a feasible method for remediating water contaminated with metals (Darnal. D. W et al, 1986). It is well established that several marine and fresh water algae are able to take up various heavy metals selectively from aqueous media and to accumulate these metals within their cells (Kumar. D, Gaur. J. P, 2011). Microalgae have been shown to accumulate arsenic and could potential remediate through adsorption and biotransformation of inorganic arsenic (Maeda. S et al, 1985). Biological materials can thus be developed into a very good system for the removal of metallic pollutants. It can be very cost effective and seems to be a very promising field of research (Meenal Kowshik and Sarita Nazareth, 1999). These are considerable studies made over few decades on biological removal of Arsenic and their biotransformation in the aquatic environment as Arsenic compound have been established as carcinogenic substances (Manju. M. R and Jyothi Krishna, 2013).

II. INNOCULATION OF ALGAE

For the present study *Diatom Navicula* was used as the test spices. For the uniformity of the experiment, a definite amount of the inoculum was used throughout the experiment. The light necessary for the growth was provided by using fluorescent tubes having 1200 lax light intensity for 12-14 hours a day. The experiment was carried out in duplicate. The pH was determined in all culture. At every alternate day biomass was determined. Productivity was also determined in every alternate days. Different pigments such as chlorophyll a, chlorophyll b, chlorophyll c, carotenoid, were determined by Larzen and parkings equation. Protein content was determined at the end of growth phase by Lowry's method. Proline content also determined at the end of growth phase.

III. METERIALS AND METHODS

Diatoms are single cell eukaryote microalgae which are found in all habitat where water is present. For the present study Diatom *Navicula* was used as the test species. In this study, is the emphasis has been laid to know the efficiency of algal species in removing the arsenic from aqueous solutions. The effect of As (III) and As (V), in different valences on the growth parameters such as biomass, productivity, chlorophyll, protein, proline content of algae Diatom *Navicula.* For the present study algae were cultivated in the laboratory and were grown in Guillards F/2 medium. This is a common and widely used general enriched sea water medium designed for growing coastal marine algae, especially diatoms [18]. From the preliminary trial experiment, it was observed that Diatom *Navicula* thrive well at an alkaline pH 7.8. Medium was prepared by dissolving necessary nutrients in 1000 ml sea water. The medium was autoclaved at 1210C for 20 minutes. As salinity is an important factor for marine forms, a salinity of 35ppm was maintained throughout the experiment for Diatom. The selected valances of Arsenic were As (III) and As (V) were 0.001ppm, 0.01ppm, 0.1ppm, 0.5ppm, 1ppm, 2ppm, 5ppm, 10ppm. A control was maintained in each experiment for comparison studies. The alternate dark and light was provided for the growth.

IV. GROWTH PARAMETERS

- Biomass Determination: On the basis of the number of cells with the help of haemocytometer.
- Determination of productivity by modified Winkler's method.
- Determination of the quantitative variation in the pigments by spectrophotometry.
- Estimation of protein by Lowry's method.
- Proline content determined by Spectrophotometry.

V. RESULT AND DISCUSSION

Biosorption of Arsenic (III) and Arsenic (V) from aqueous solution by microalgae was investigated.PH plays an important role in its metal uptake capacity and the biological process of Arsenic removal [17]. As (III) and As (V) dissolved in sea water medium in alkaline condition was maintained throughout the experiment. The Diatom *Navicula* thrive well in alkaline pH. The algae was grown in Guillards F/2 medium having alkaline pH for the better growth of the algae.

Growth response: Growth pattern of Microalgae Diatom in relation to different concentration of Arsenic (V) and Arsenic (III) has been studied. By preliminary screening experiment, the range of toxicity of test species was determined. The different concentration selected for the study for Arsenic (V) and Arsenic (III) were 0.001ppm, 0.01ppm, 0.1pm, 0.5ppm, 1ppm, 2ppm, 5ppm, 10ppm respectively.

Growth pattern of test species in relation to different concentration of As (III) and As (V) has been shown in (Fig 1 and 2).

The growth rate of algae was stimulated by the presence of Arsenic in the culture medium and similar is the case with the environment [17].

In As (V) and As (III) supplement medium algae showed decrease in the growth rate with increase of As concentration. In the case of biomass in As (V) was found to be having maximum growth at a concentration of 0.5 ppm and As (III) in 2ppm respectively. (Fig 1 and 2).

In the case of productivity, Arsenic is found have stimulatory effect on the growth of test species (Fig 3 and 4).

The pigmentation chlorophyll a, chlorophyll b, chlorophyll c, carotenoids more than that of control As (V) and As (III). Chlorophyll c and carotenoid pigments are greater than that of chlorophyll a and chlorophyll b. (Fig 5 and 6).

The protein content in As (III) and As (V) gradual decrease with increase in concentrations (Fig 7 and 8) (Fig 9 and 10).

Among the pigments carotenoid was found to be more than that of other chlorophyll pigments compared. That may be reason for the yellowing of culture in the presence of As compared with the control. Thus it was concluded that by the effect of As, there was increase in carotenoid pigment when compared with other chlorophyll pigment chlorophyll c and carotenoid pigments shows an adjacent values in As (V) and As (III). In nature of algae subjected to a variety of stresses due to fluctuating environmental conditions. In response, they have developed elaborate protective mechanisms to ensure their survival [1]. Accumulation of proline has been reported to be an important index for stress tolerance capacity in plants, bacteria, protozoa, algae and marine invertebrates [19] due to its function as a stabilizer [20]. Present study shows increase the biomass with gradually increase the proline content in the test species.

The increase in proline may be due to the adaption of algae to the stress condition. Proline accumulation in algae under heavy metal stress has reported by many workers [21]. Wu et al, 1998 reported increase proline in chlorella under salt stress condition. The present study focused on the significant role of proline in the pollution stress adaptation mechanism in Diatoms and it helps to resist adverse environmental conditions (heavy metal, salt). The p^H had pronounced effect on growth of test species. Maximum biosorption was observed at alkaline p^H. Thus increase in the growth was reflected in the increase in the rate of biosorption. Chlorophyll content decreased with increasing concentration of carotenoid pigments. The medium was alkaline and is treated with Arsenic (V) and As (III). While proline content was increased with heavy metal and salt stress.

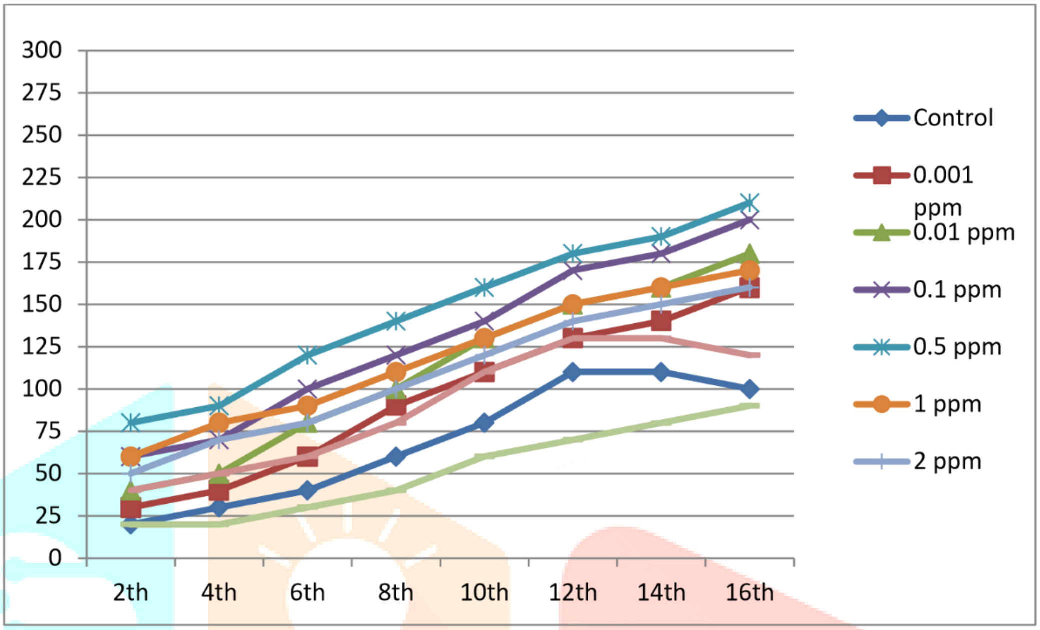

Fig.1. Effect of arsenic (v) on biomass of diatom. (express the biomass in number of cells per liter)

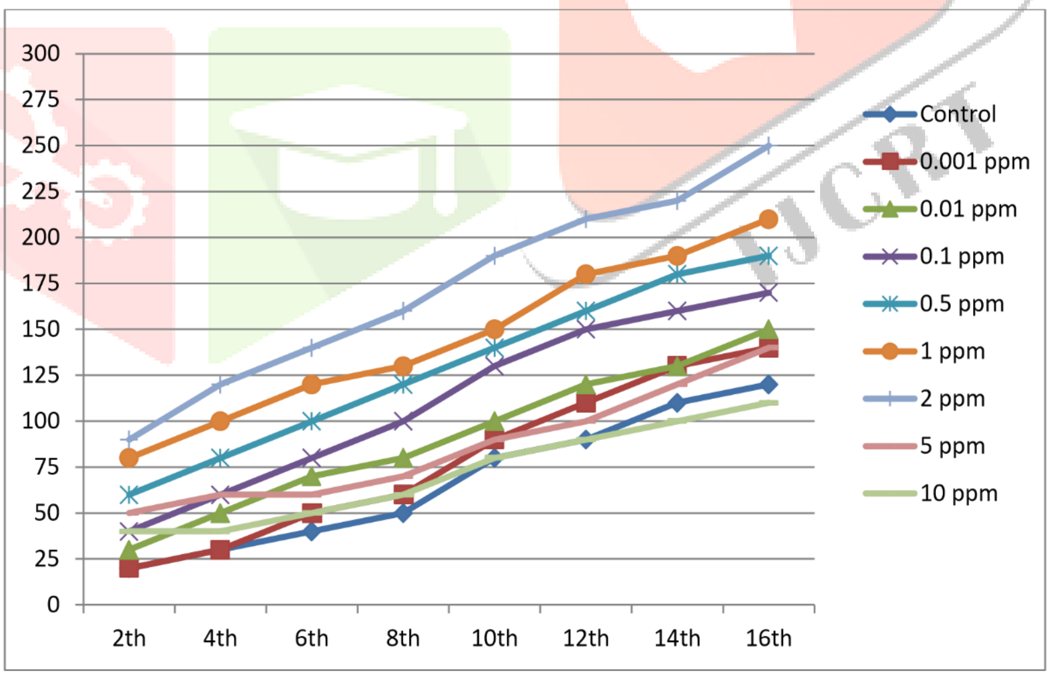

Fig. 2. Effect of arsenic (iii) on biomass of diatom. (express the biomass in number of cells per liter)

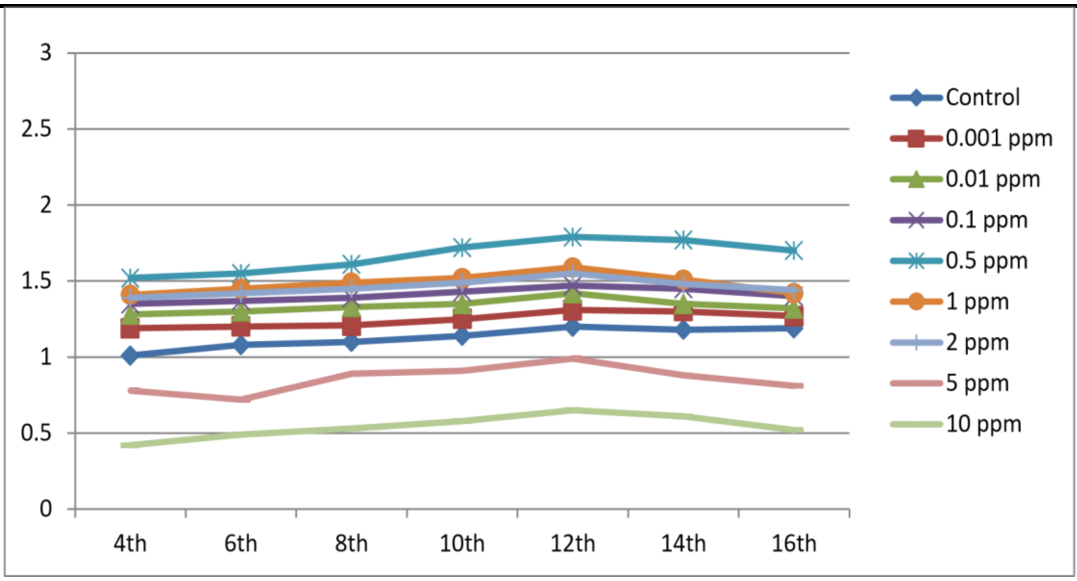

Fig.3. Effect of arsenic (v) on productivity of diatom.

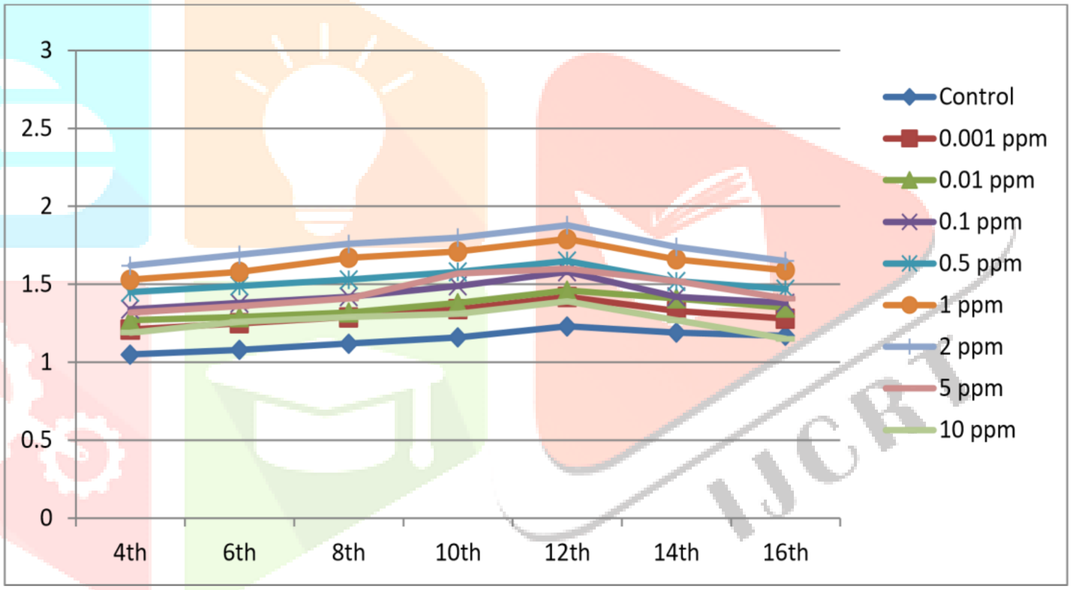

Fig.4. Effect of arsenic (iii) on productivity of diatom.

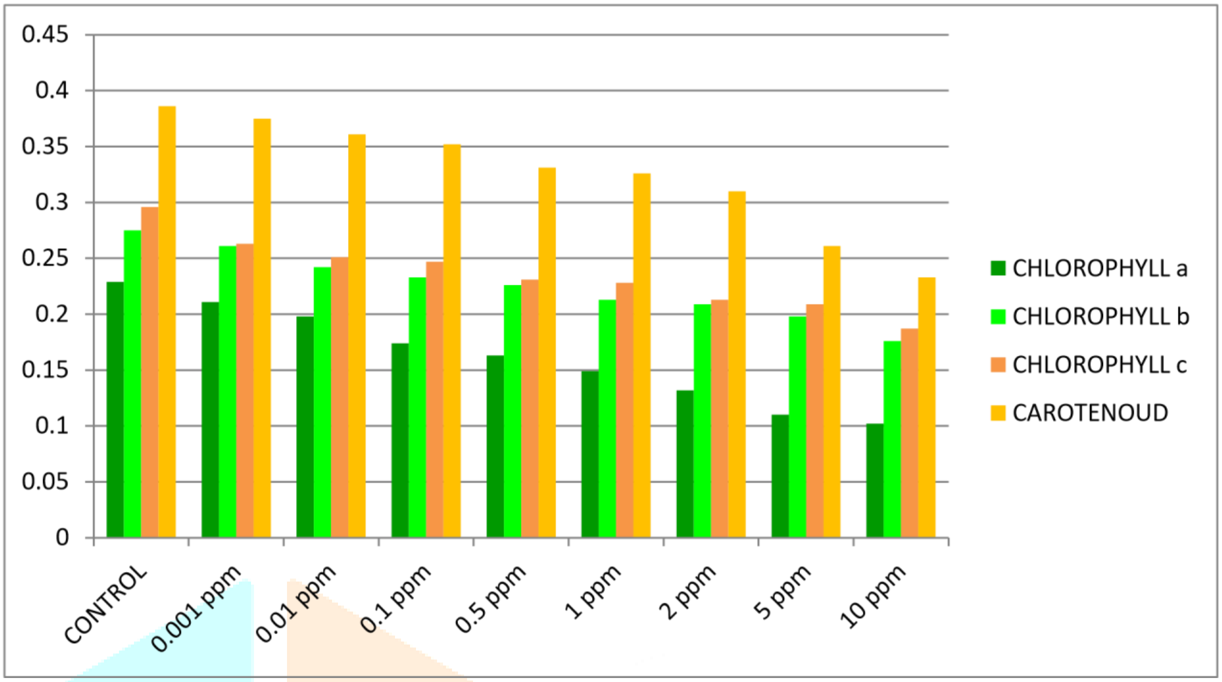

Fig.5. Estimation of pigments in different concentration of arsenic (v)

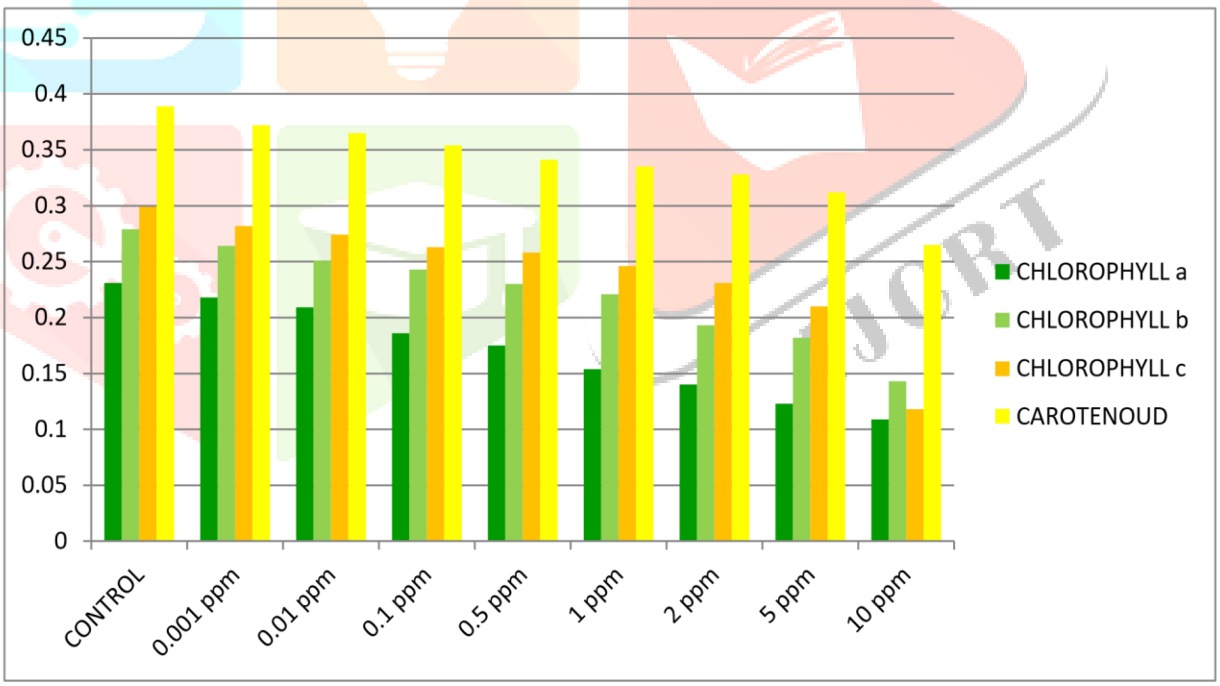

Fig.6. Estimation of pigments in different concentration of arsenic (iii).

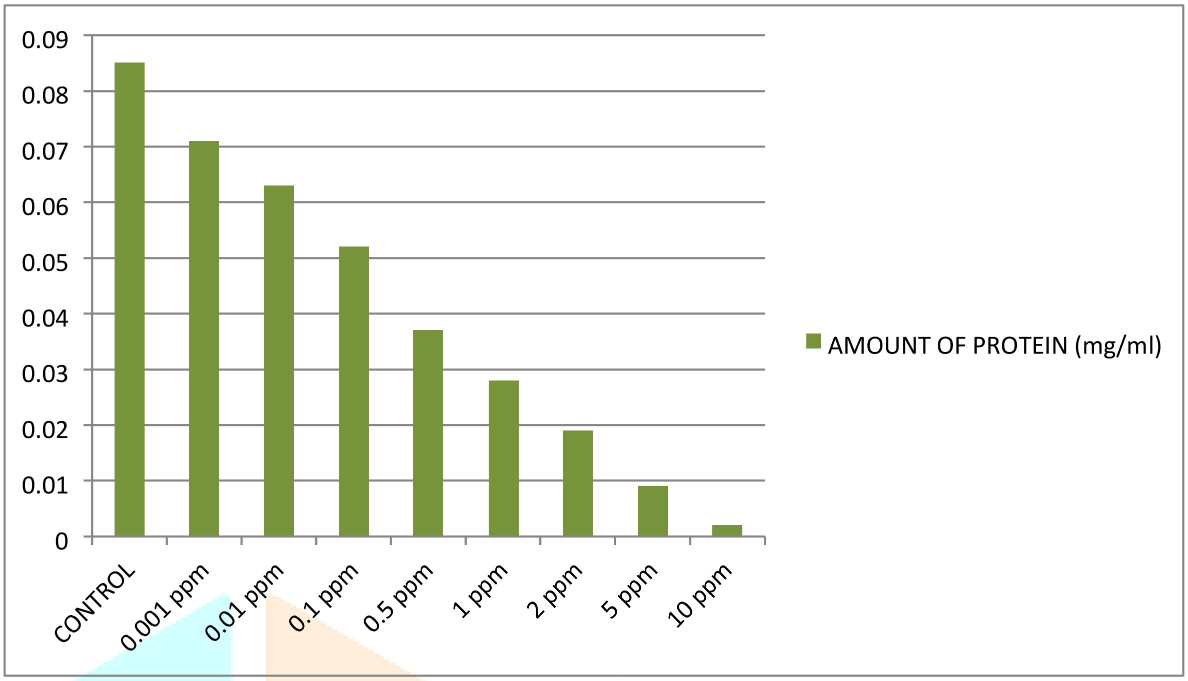

Fig.7. Protein content of diatoms on different concentration of arsenic (v).

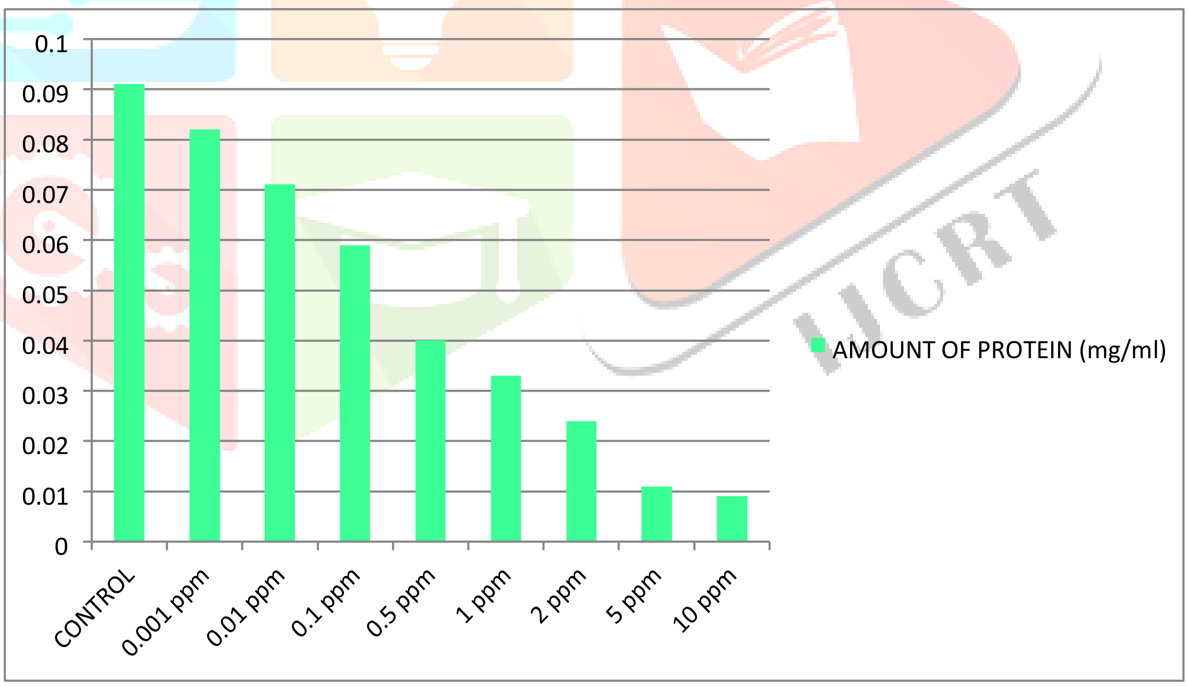

Fig.8. Protein content of diatoms on different concentration of arsenic (iii).

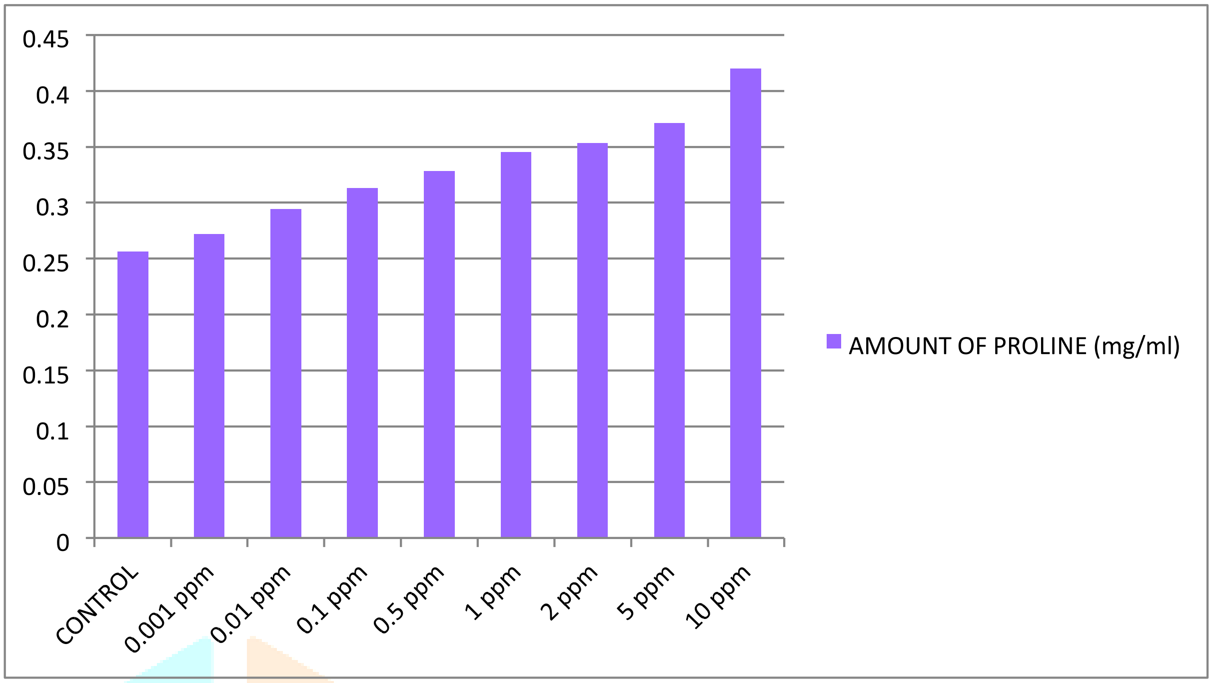

Fig.9. Proline content of diatoms on different concentration of arsenic (v).

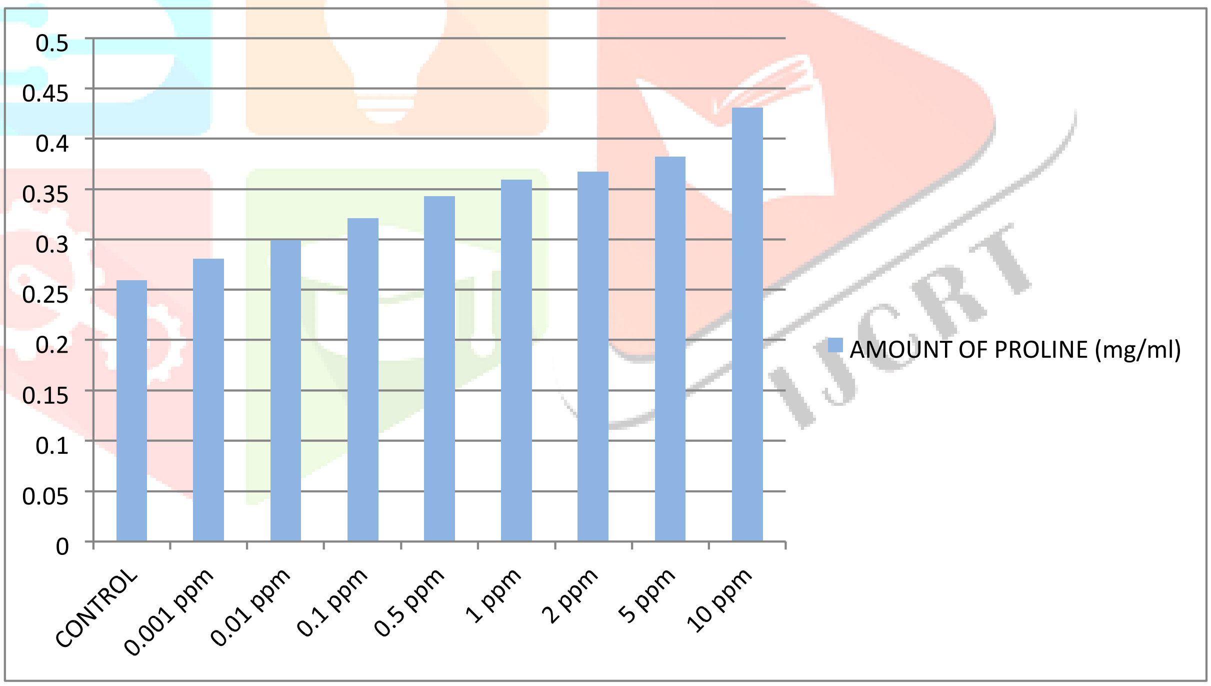

Fig.10. Proline content of diatoms on different concentration of arsenic (iii).

VI. CONCLUSION

From the above findings, it can be concluded that microalgae Diatom can be effectively used for removing Arsenic from the water bodies. Among the two valency states As (III) and As (V), Arsenic (V) was more toxic in test species. This was reflected in biomass, productivity, protein, and proline content. By the effect of Arsenic, there is increase in carotenoid pigment when compared with other chlorophyll pigments Alkaline p^H promote better growth of Diatoms. The proline appears to be a stress induced substance in the Diatoms and that helps to resist adverse conditions (heavy metals and salt). Thus Diatoms with the potential of synthesizing more proline, may prove to be a promising candidate for the bio-remediation of aquatic pollution. This study emphasizes the role of algal communities as indicators as well as agents of bioremediation that can be sustainably used for waste water treatment economically.

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REFERENCES

[1] Bayron W R, Bierbower J B, Hansen Wtt, Pathologic changes in rats and dogs from two-year feeding of arsenite or sodium arsenate. Toxicology and applied pharmacology, Vol. 10, pp. 132-147, 1967.

[2] Cullen. W. R and Reimer. K. J, Arsenic Speciation in the environment. Chemical Reviews, Vol. 89, pp. 713-764, 1989.

[3] Darnal D W et al, Selective recovery of gold and other metal ions from an algal biomass. Environmental science and technology. 20, pp. 206-208, 1986.

[4] Davis. T A, Volesky. B, Mucci. A, A review of the biochemistry of heavy metal biosorption by brown algae. Water Research, Vol. 37 pp. 4311-4330, 2003.

[5] Delauney. A, Verma. D. P. S, Proline biosynthesis and osmoregulation in plants. Plant Journal, Vol. 4, pp. 215-223, 1993.

[6] Faisal. M, Hasnein. S, "Microbial conversion of Cr (VI) into Cr (III) in industrial effluent", African journal of Biotechnology, Vol. 33, pp. 610-617, 2004.

[7] Fatma. T. et al, "Impact of environmental pollution on cyanobacterial proline content", Journal of Applied phycology, Vol. 19, pp. 625-629, 2007.

[8] Forstner. U, Prosi. F, "Heavy metal pollution in fresh water ecosystem in: Ravera, O. (ed)", Biological aspects of fresh water pollution Pergamon, Press Oxford, pp. 129-161, 1979.

[9] Guillard R.R.L, Ryther J.H, Studies of marine planktonic diatoms. I. Cyclotella Hustedt and Detonula confervacea Cleve, Journal of Microbiol. Vol. 8, pp. 229-239, 1962.

[10] Horstall. M Jnr, Spiff. A. I, Effects of temperature on the sorption of pb^{2+} and cd^{2+} fromaquous solution by caladium bicolor (wild cocoyam) biomass. Eletronic Journal of Biotechnology Vol. 8, pp. 1-4, 2005.

[11] Kim. M. J and Nriger. J, Oxidation of arsenate in ground water using ozone and oxygen. Science of Total Environment, Vol. 247: pp. 71-79, 2000.

[12] Kumar D, Gaur J P, Metal biosorption by two Cyanobacterial mats in relation to biomass concentration, pretreatment and reuse. Bioresource technology, Vol.102 pp. 2529-2535, 2011.

[13] Maeda S, et al, Bioaccumulation of arsenic by fresh water algae and the application to the removal of inorganic arsenic from an aqueous phase. Part II, By Chlorella vulgaris isolated from arsenic polluted environment. Separation science and technology. 20, pp. 153- 161, 1985.

[14] Manju. M. R, Jyothi Krishna, Biosorption of Arsenic in the aquatic food chain using microalgae, Journal of Aquatic biology and fisheries. Vol. 2, pp. 329-333, 2013.

[15] Meenal Kowshik, Sarita Nazareth, Biosorption of metals by Fusariumsolani. Asian journal of Microbiology, Biotechnology and Environmental Science. Vol. (1-2): pp. 57-61, 1999.

[16] Shah. K and Dubey. R. S, Effect of cadmium on proline accumulation and ribonuclease activity in rice seedlings: role of proline as a possible enzyme protectant. Biol. Plant.Vol. 40, pp. 121-130, 1998.

[17] Sibi. G, Biosorption of Arsenic by Living and Dried biomass of fresh water microalgae potentials and Equilibrium studies. Journal of Bioremediation and Biodegradation. Vol. 5, pp. 249-257, 2014.

[18] ang. S and Mulligan. C. N, Natural attention process for remediation of arsenic contaminated soils and ground water. Journal of hazard mater.Vol. 138, pp. 459470, 2006.

[19] Wong S, Zhao X, "On the potential biological treatment of arsenic contaminated soil and ground water", Journal of environment management, Vol. 90, pp. Z 367-Z 376, 2009.

[20] Wong. P T, Chau. Y. K, Lukon. P. L, "Toxicity of a mixture of metals on fresh water algae", Journal of Fish Resource. 35, pp. 479-481, 1978.

[21] Wu J T, Hsieh M T, Kow L C, Role of proline accumulation in response to toxic copper in Chlorella sp. (Chlorophyceae) cells. Journal of Phycology. 34, pp.113-117, 1998.