BIOREMEDIATION OF HEAVY METAL ($HgCl_2$) FROM WATER BY *SPIRULINA PLATENSIS*

[1]Akshita Sharma, [2]Manjari, [3]Kamalpreet Kaur, [4]Anshu Sibbal Chatli

[1,4]Assistant professor, [2,3]Research Scholar
[1, 2, 3] Department of biotechnology, [4]Department of Microbiology
Guru Nanak Girls College, Model Town, Ludhiana, India

***Abstract*: Spirulina Platensis is non toxic blue green algae which is filamentous cyanobacteria taken by the human dietary supplement and use it as a food. Spirulina platensis is a biomass which is dried form of Arthrospira platensis. These Blue-green algae are the primary diet of human, animals and aquatic life because it is easily digested due to lack of the cell wall. Spirulina platensis has been found naturally in ponds and lakes (Lake Chad). Spirulina platensis has been demonstrated for its ability to absorb heavy metal from water sample [1]. The present study was focused on using fresh and aged Spirulina platensis in absorbing Mercuric chloride from the self-adulterated water sample. Spirulina platensis was cultivated for two weeks, fresh Spirulina platensis was isolated along with a nutrient deprived Spirulina platensis. The analysis was made by comparing the activity of Spirulina platensis on the basis of Mercuric absorption from self adulteration of – (a) fresh versus aged strains. Their efficacy in absorbing Mercuric Chloride was studied by ICP-MS (Inductively Coupled Plasma Mass Spectroscopy. The result demonstrated- appreciable amount of Mercuric Chloride was reduced from the water sample rendering bioremediation capacity to Spirulina platensis.**

***Keywords*: Bioremediation, Mercury, Bio-removal, Spirulina platensis.**

INTRODUCTION

Without the advancement and expansion of industrial sector, rapid economic progress cannot be achieved. When industries released the waste materials into the environment, it amends the environmental conditions in an adverse manner (Kalpan 2013). Certain Industries have heavy metals as their major ingredient, and their discharge in the environment is an element of considerable concern today (Disyanwongs 2002). Heavy metals, refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at even low concentration (Kumar *et al*. 2015). Heavy metals are released into waterways due to various anthropogenic activities and natural processes (Figure- 1) and remain in the environment because they are non-biodegradable; so their treatment is of special importance (Inthorn *et. al*. 2002). Heavy metals, based on their toxicity, can be categorized into two groups. The first group contains the heavy metals that are required for the nourishment at very low concentration, say in traces, for majority of micro-organisms but potentially hazardous when they are present in higher amounts. This group includes heavy metals like As, Cr, Co, Ni, Se, Va and Zn. The second group contains the heavy metals that are highly hazardous and do not have any nutritional value. This group contains the heavy metals like Pb, Hg, Cd, Ur, Ag and Be (Inthorn 2001).

Water bodies across the world have been severely polluted with toxic metal polishing with Mercuric poisoning being one of the most common. It has been responsible for causing Minimata disease and long term frequently exposure has been proven to cause various kidney ailment, mental retardness (Reilly *et. al*. 2010) amongst others (Table-2). The major cause of Mercuric poisoning has been attributed to the very nature of it becoming a part of the food chain by bioaccumulation and bio magnification in fishes, Spirulina has shown promising results in bio absorption of Mercury from water sample (Dolatabadi and Hosseini 2016). The present study has been aimed to utilize ability of *Spirulina platensis* to absorb Mercury (Saudi 2013) by utilizing fresh culture (Kapoor *et. al*. 1998) at high concentration.

Removal of contaminants like heavy metals from soil and water bodies has been a crucial challenge for the biotechnologists and it cannot be overlooked. Biotechnology based approaches have the potential to contribute significantly to the achievement of this goal (Murali *et. at.* 2014) (Nriagu and Pacyna 1988). Using Microalgae for the removal of heavy metals has the potential to achieve greater performance at a lower cost than conventional wastewater treatment technologies (Bakkaloglu *et. al*. 1998). *Spirulina platensis* can be grown on the wastewater to better the water quality and the heavy metals contaminated in the wastewater can be separated (Disyawongs 2002).

PRINCIPLE OF BIOREMEDIATION

Bioremediation involves biological system to enhance the break-down or transformation of certain chemicals to less harmful forms (Yati *et.al.*2016). Bioremediation encourages the growth and development of micro flora or microbial consortia at the contamination sites which then performs various activities (Agarwal 1998).The natural process of Bioremediation relies on bacteria, fungi and plants (Phytoremediation) to transform the pollutant or heavy metals from more complex form to less complex form, because they can utilize the contaminants as their energy source in their biochemical processes or metabolic pathways (Salem *et.al.* 2012) Bioremediation is very cost effective and eco-friendly as they involve the use of micro-organisms. Bioremediation presents the feasibility to demolish or distribute harmless contaminants using natural biological activity (Vidali 2001).

PRINCIPLE OF BIO-SORPTION

Bio-sorption is the physio-chemical process which occurs in the biomass naturally and binds the contaminants on its cellular structure by allowing it to concentrate. Bio-sorption is a process which involves the use of micro-organisms like bacteria, fungi, algae etc., to discharge and purify heavy metals from the aqueous solution. The cellular components of the micro-organism interacts with metals ions and lead to the uptake of heavy metals from the aqueous solution (Kapoor and Viraraghavam 1998). The process of the interaction of metal ions with micro-organism is very diverse. There are some factors that influences the mechanism of metal bio-sorption (Volesky 1990) (Veglio and Beolchini 1997) (Wang *et.al.*2000) such as-

- The status of biomass (living or non-living).
- Types of biomaterials.
- Properties of metal solution chemistry.
- Optimum environment condition such as pH, temperature etc.

ALGAE AS BIOSORBENT

Bio treatment with microalgae is particularly attractive because of their photosynthetic capabilities, converting solar energy into useful biomasses and incorporation nutrients such as nitrogen and phosphorus causing eutrophication (Nove and Pauw 1988). Because of certain advantages of algal, the use of algal biomass as a bio-sorbent is emerging as an attracted, economical and effective proposition (Holaz and Volesky 1994) (Singh *et. al.* 2001).

Unlike other microbes like bacteria and fungi, algae produces large biomass as they are photosynthetic in nature and also have low nutritional requirement. Various factors govern the binding of metals ions on algal surface like ionic charge on metals, algal species and chemical composition of the metal ion solution (Holaz and Volesky 1994) (Khoo and Ting 2001).

MATERIAL AND METHODS: -

SAMPLE PREPARATION-

Spirulina platensis mother culture was obtained from OfERR Nallayan Research Centre for Sustainable Development, Tamil Nadu, and India. *Spirulina platensis* was cultivated in an open tank with a nutrient media at a temperature of 26- 30° C for 14 days under sunlight and continuous agitation.

MEDIA USED-

Zarrouk media (Table-1 (A) (B)) was used to cultivate *Spirulina platensis*. This media include Micronutrients and macronutrients. For cultivating *Spirulina platensis*, high pH and temperature plays an important role. High alkalinity (pH-9) of the media will inhibit the growth of other contaminating micro-organisms. The optimal temperature for *Spirulina platensis* culture is in the range of 35-38° C.

HARVESTING OF SPIRULINA PLATENSIS-

Fresh Spirulina grows on the surface of the culture media. To harvest *Spirulina platensis*, muslin cloth was used. Above layer of *Spirulina platensis* was removed from the medium and allowed to dry completely at room temperature.

Spirulina platensis sample was scraped out from the foil or muslin cloth after drying completely at room temperature. Then, the sample was grinded by using pestle mortar to make it a fine powdered. The dried form of *Spirulina platensis* was stored in eppendorf tubes or vials.

INOCULATION OF Hg INTO SPIRULINA PLATENSIS CULTURES-

- After the optimum growth of *Spirulina Platensis* in the fresh culture and aged culture, 15 ml of the *Spirulina platensis* sample were taken into two separate 100ml conical flasks.
- In the first conical flask (Liquid-1), sample taken from the fresh (Figure- 2(B)) *Spirulina platensis* culture, was not diluted, but in the second conical flask (Liquid-2), the sample was taken from the aged *Spirulina platensis* culture (Figure- 2(A)) and was diluted by adding 4ml of distilled water into 11ml of *Spirulina platensis* culture.
- Afterwards, in the two separate conical flasks which contain fresh and aged *Spirulina platensis* culture, added 1g of Mercuric Chloride (Figure- 3).
- The sample was incubated at room temperature for 2-3 days.
- Then the sample was analyzed by the technique inductively coupled plasma mass spectroscopy (ICP-MS)

RESULTS AND DISSCUSION-

Self-adulterated water sample containing fresh *Spirulina platensis* was analyzed for Mercuric Absorption. 1.0gm of Self adulterated sample of *Spirulina platensis* was digested with the combination of 5 ml 30% Hydrogen peroxide. After the sample was completely digested, it was quantitatively transferred and diluted with Milli-Q ICP-MS grade water into 50 ml volumetric flask and made the volume up to the mark. Afterwards we analyzed the samples of *Spirulina Platensis* for the amount of Mercuric chloride absorbed.

In Case of Liquid sample-1: 15 ml of water sample was taken and 1 gm of Mercuric chloride was added. Earlier, this sample depicted the presence of **6.06%** of Mercuric Chloride. But when the Fresh *Spirulina platensis* was added to the water sample and incubated at room temperature for 72hrs, it was observed that Fresh *Spirulina platensis* culture absorbed mercuric chloride and the amount present earlier was reduced to **3.41%**.

In Case of Liquid Sample-2: Similarly with the second sample, it was depicted that the amount of Mercuric chloride present was **6.06%**. But when Aged *Spirulina platensis* was introduced in the same sample and incubated at room temperature for 72hrs, the Mercuric chloride was absorbed by the Aged *Spirulina platensis* culture and the original concentration was reduced to **2.41%**.

Upon analyzing the Sample for Mercury absorption, it was observed that Aged culture had better absorption capacity as compared to the fresh culture of *Spirulina platensis*.

CONCLUSION-

The present study indicates that *Spirulina platensis* has potential in absorbing heavy metals like mercuric chloride from water. This study and findings could be used for remediation and purification of water bodies by inoculating them with *Spirulina platensis*. Since mercuric poisoning is one of the leading causes of Minamata Diseases affecting fishes and later humans, present study provides a cheap and easy way of dealing with water pollution. Further studies can help in understanding the actual mechanism of Phytoremediation and uptake of mercuric chloride by *Spirulina platensis*.

FIGURES: -

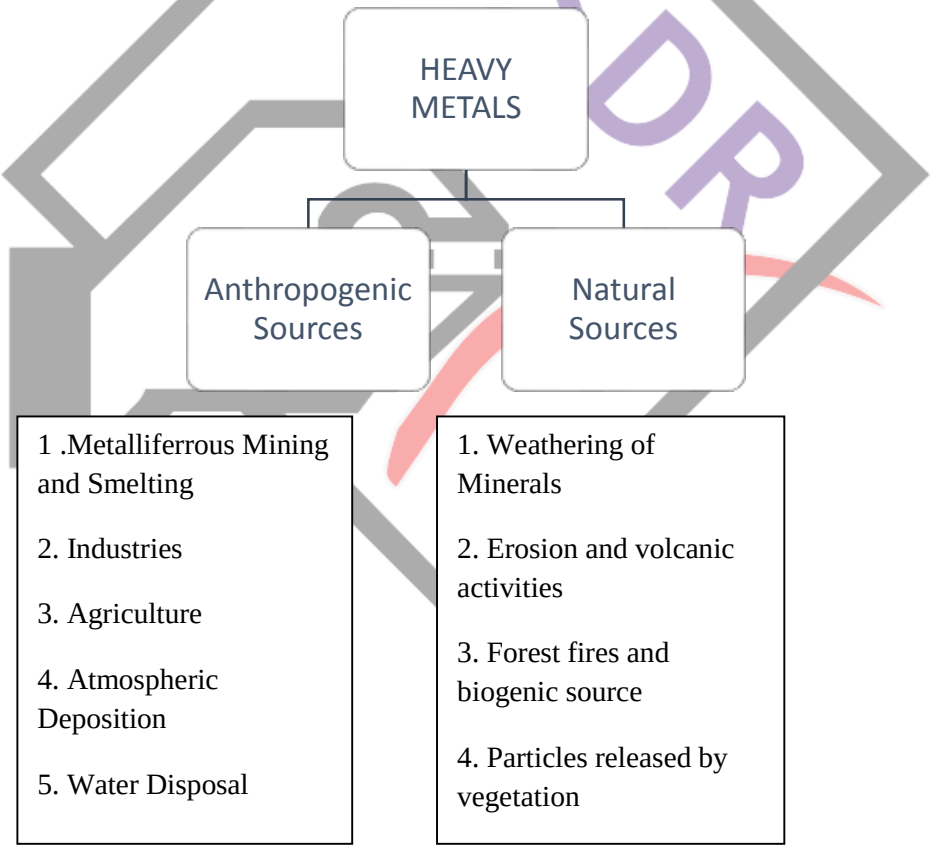

Figure 1: Sources of heavy metals in the environment.

(A) **(B)**

Figure 2 : (A) Aged Culture and (B) Fresh culture of Spirulina platensis.

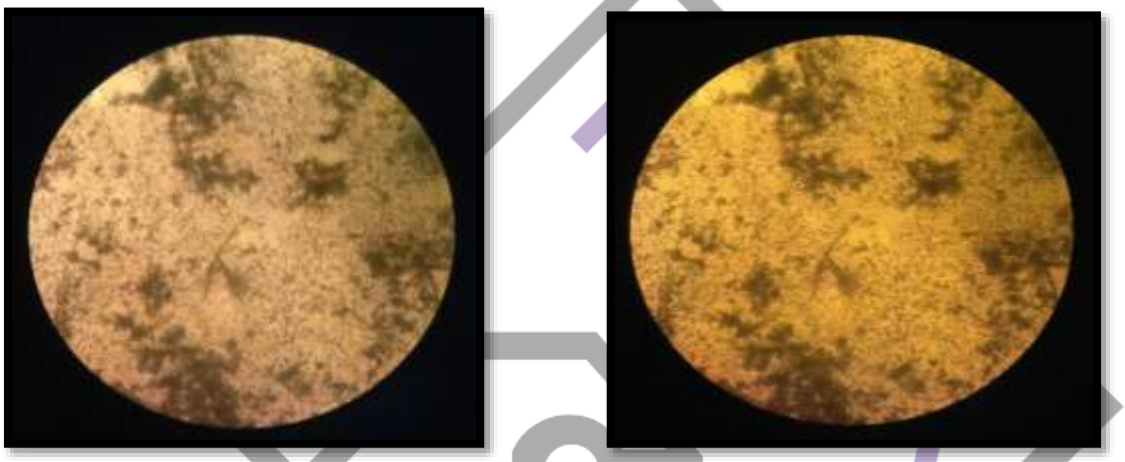

Figure: 3:- Microscopic views of Spirulina platensis after adding Mercuric Chloride**.**

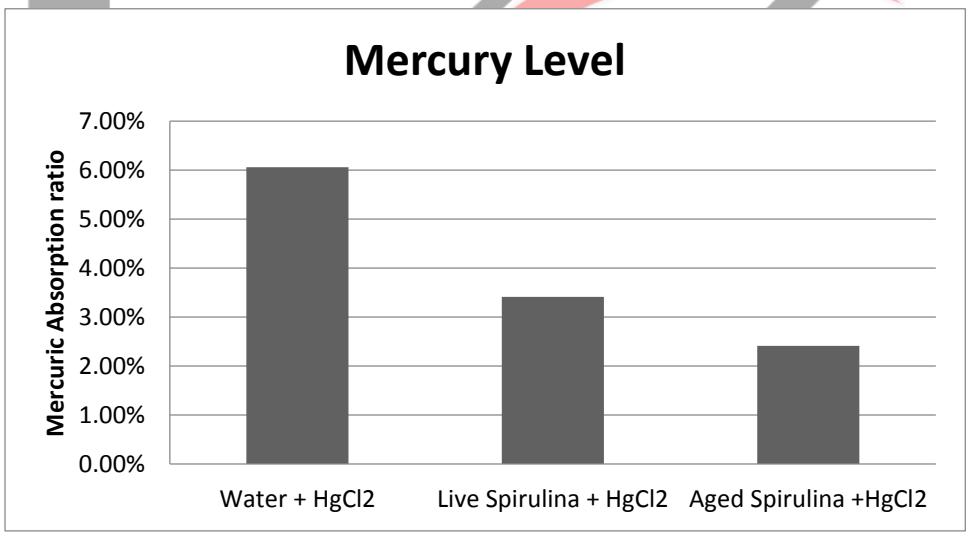

Figure: 4:- Comparison of Mercuric Absorption by Fresh and Aged *Spirulina platensis*

Tables:-

Zarrouk's Medium:

Constituent	Amount to be added (g/L1)
Sodium Bicarbonate	18.0
Sodium Nitrate	2.5
Di-potassium phosphate	0.5
Potassium Sulphate	1.0
Sodium Chloride	1.0
Calcium Chloride Dihydrate	0.04
Ehylene di amine tetre acetic acid di sodium salt	0.08
Magnesium Sulphate	0.2
Ferrous Sulphate Hepta hydrate	0.01
A_5 micronutrient solution	1 ml

A_5 Micronutrients: -

Constituents	Amount to be added (g/l)
Boric acid	2.86
Manganese Chloride tetra-hydrate	1.81
Zinc Sulphate heptahydrate	0.22
Copper Sulphate	0.08
Ammonium Molybdate	0.39

(B)

Table-1: (A) (B) Composition of Zarrouk media for the cultivation of *Spirulina platensis* (Morais *et. al.* 2015)

Metal	Health Hazards	Major Sources
Mercuric chloride	Corrosive to skin, eyes and muscle membrane. Dermatitis, nervous and kidney damage, anorexia, protoplasm poisoning, severe muscle pain, wildlife destruction.	Pesticides, batteries, paper and leather industry, thermometers, electronics, amalgam in dentistry, pharmaceuticals.

Table-2: Major sources and associated health hazards of Metal Mercury (Garbisu *et. al.* 1997)

BIBILIOGRAPHY: -

[1] A.Kapoor and T. Viraraghavan,(1998) Biosorption of heavy metals on Aspergillus niger, effect of pre-treatment. Biores. Technol, Vol.63, pp. 109.

[2] Aggarwal S.K. (1998), Environmental Biotechology, (1st edition) APH publishing corporation, New Delhi, India, pp. 267289.

[3] Base- O' Reilly, S; McCarty, KM; Steckling, N; Lettmeier, B (2010), Mercury exposure and children's health, 40(8):186-215.

[4] De La Nove, J. and De Pauw, N. (1988), the potential of microalgae Biotechnology. A Review of production and uses of Microalgae. Biotechnology advances, Vol.6, pp.725-770.

[5] Drora Kalpan 2013, Absorption and Adsorption of Heavy Metal by microalgae.

[6] Duangrat Inthorn, Nalin Sidtitoon, Suthet Silapanuntakul and Aran Incharoensakdi, Sorption of mercury, cadmium and lead by microalgae, Science Asia 28(2002); 253-261.

[7] Gannikar Disyanwongs 2002 ,Accumulation of copper, mercury and lead in Spirulina platensis studied in Zarrouk medium, J. KMITNB, Vol.12, No.4, Oct-Dec.

[8] Garbisu and Alkorta, (1997), S. Gang, S. Weixing, Sunflower Stalks as absorbents for removal of metal, Ind. Eng. Chem.Res. , 37(4), pp. 1324-1328.

[9] Holaz Z R and Volesky B,(1994), Biosorption of lead and nickel by bimass of marine algae, Biotechnol. Bioeng. Vol.43, pp.1001-1009.

[10] I. Bakkaloglu, T.J Butter, L.M Evison, F.S. Holland, I.C. Hancockt (1998), Screening of various types biomass for removal and recovery of heavy metals (Zn, Cu,Ni) By biosorption,sedimentation and desorption, Wat. Sci. Technol Vol.38, Issue. 6: 269-277.

[11] Inthorn D (2001), Removal of heavy metal by using microalgae. Photosynthectic microorganism in environmental biotechnology. Springer, Verlag Hong Kong Ltd.310:111-169.

[12] Jerome O. Nriagu and Jozef M.Pacyna (1988), Quantitative assessment of worldwide contamination ofair, water and soil by trace metals, Nature 333, 134-139.

[13] K. Suresh Kumar, Hans-Uwe Dahms, Eun-Ji Won, Jae-Seong Lee, Kyung-Hoon Shin, Microalgae- A promising tool for heavy metal remediation, Vol. 113, March 2015.

[14] Khoo K M and Ting Y P, (2001), Biosorption of gold by immobilized fungal biomass, Biochem. Eng. J. Vol.8, pp.51-59.

[15] Michele Greque De Morais, Cleber Klasener Da Silva, Adriano Arruda Henrard and J Orge Alberto Vieira Costa (2015), Carbon-di-oxide mitigation by microalga in a vertical tubular reactor with recycling of the culture medium, Vol.9 (33), pp. 1935-1940.

[16] Murali O. and Santosh Kumar Mehar, Bioremediation of heavy metal using Spirulina 2014, Internation journal of Geology, Vol.4(1), Jan-Apr, pp 244-249, ISSN: 2277-2081.

[17] Naif Abdullah AI-Dhabi, (2013), Heavy metal analysis in Commercial Spirulina products for human consumption,Saudi J Biol Sci.20(4): 383-388.

[18] Salem, I.B.: Sghaier, H.; Trifi, H, Heni, S.; Khwaldia, K.; Saidi, M. and Landoulsi, A. (2012). Isolation and Characterization of a novel micrococcus strain for bioremediation of Strntium in radioactive residues. Afr. J. Microbiol. Res. 64: 851-858.

[19] Somayeh Dalatabadi, Seyyed Abalfazl Hosseini, (2016), Wastewater treatment using Spirulina platensis, ISSN: 2249-1929, Vol.6, No.4, pp. 1239-1246.

[20] Veglio F. and Beolchini F., Removal of metals by biosorption: A review on hydrometallurgy. 44(1997) pp.301-316.

[21] Vidali M., (2001) Bioremediation, An overview, Pure App. Chem., 73(7): 1163-1172.

[22] Volesky B., (1990), Biosorptiona and biosorbent in Biodorption of heavy metals, edited by B. Volesky (CRC press, Boca Raton, Flouride) pp. 3-5.

[23] Wang J L, Han Y J and Qian Y,(2000), Progress in metal biosorption by microorganisms,Microbiology,Vol.27,pp.449-452.

[24] Yati Prabha, S.K Soni, Sharmita Gupta and Sonal (2016), Potential algae in Bioremediation of wastewater: Current Research. Int. J. curr. Microbiol. App.Sci, 5(2): 693-700.