Plant Archives Vol. 19, Supplement 2, 2019 pp. 839-842 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

DETERMINE THE ABILITY OF *SCENEDESMUS ACUMINATUS* TO UPTAKE OF ARSENIC BY USING SCANNING ELECTRON MICROSCOPE TECHNIQUE

Siham N. Lefta
*Biology Department, Collage of Science, Al-Mustansiryih University, Iraq.
Email: Siham.neamah@uomustansiriyah.edu.iq

Abstract

In the present study the *Scenedesmus acuminatus* was isolated from Tigris River in Baghdad city. The isolated strain was identified according to its morphological characteristics as *Sc. acuminatus* which able to grow in broth medium in the presence of heavy metals. Initially the Scanning electron microscopy techniques was used to determine if this strain is able to uptake Arsenic which has been detected in Tigris River as a toxic pollutant. The results showed that *Sc. acuminatus* has affinity for Arsenic, and this technique could be very useful for the identification process in contaminated Rivers. The result indicated the ability of *Sc. acuminatus* to uptake the Arsenic with various concentration and it was between (3.2, 3.9, 5.1 and 6.0) µg /mg for survival cells while it was (3.3,4.3,7.4 and 8.5) µg /mg for mortality cells in concentration of Arsenic (4,8,16 and 32) µg /ml respectively. The result demonstrated that the mortality cells have high affinity to uptake Arsenic from the aqueous solution than the survival cells .

Keywords: SEM, Arsenic, *Scenedesmus acuminatus*, Microalgae

Introduction

Negative effects of presence heavy metals in aquatic ecosystems has become series problem due to their harmful effects on human health and on the flora and fauna of receiving water bodies (**Sahoo**, and **Seckbach**, 2015. Although low concentrations of some heavy metals are metabolically important to many survival organisms, at higher levels they can potentially be toxic (Wang and Chen 2009). It is recognized that finding methods for removal of heavy metals from aqueous water is of great importance Fu and Wang, (2011), Garg and Manoj (2008) and Alpana, *et al.* (2007).

Arsenic (As) is the most toxic heavy metal ion (carcinogenic metal) affecting the environment that raises much concern from the both environmental and human health (IPCS 2001). Nowadays, high arsenic levels have been found in freshwater ecosystems such as lakes and rivers around the world due to anthropogenic inputs such as combustion of fossil fuels, many industrial raw materials, mining and smelting, and the application of pesticide and preservatives and additives to livestock feed ATSDR. 2007 and Wang and Zhao 2009.

Inorganic (As) over a long period can lead to chronic arsenic poisoning (Arsenicosis). (IPCS 2001, ATSDR. 2007 and Wang and Zhao 2009) During long-term exposure to high levels of inorganic arsenic (e.g. through drinking-water), the first changes are usually seen in the skin: pigmentation changes and then skin lesions and hard patches on the palms of the hands and soles of the feet IPCS 2001, ATSDR. 2007. Other effects include peripheral neuropathy, gastrointestinal symptoms, conjunctivitis, diabetes, renal system effects, enlarged liver, bone marrow depression, destruction of erythrocytes, high blood pressure and cardiovascular disease. Biosorption utilizes the ability of biological materials to accumulate heavy metals from aqueous solution by either metabolically mediated, or physicochemical pathways of uptake Sulaymon; Mohammed and Al-Musawi (2013). The aim of finding more efficient and cost-effective metal-removal biosorbent Chen, *et al.* (2012). Among them, algae have proved to possess high metal binding capacities due to the presence of polysaccharides, proteins or lipid on the surface of their cell walls containing some functional groups such as amino, hydroxyl, carboxyl and sulphate, which can act as binding sites for metals Chen, *et al.* (2012). and Bayo (2012). The biosorbent was characterized by employing instrumental techniques, viz., Fourier transform infrared spectroscopy (FTIR), thermo gravimetric analysis (TGA) and scanning electron micro-scope (SEM). Despite the extensive information available on the genus *Scenedesmus acuminatus* about its ability to biosorbent heavy metals, the objective of this study was to determine whether *Sc. acuminatus* which isolated from Tigris River in Baghdad city has this ability. For this purpose we used electron microscopy techniques in combination and use algal bio mass to study the uptake of Arsenic from the aqueous solution .We select Arsenic among heavy metal because it is very toxic and has no biological functions and because it has been found high concentration in the river ATSDR. 2007 and Bayo (2012) .

Materials and Methods

Microalgal isolate

Green algae *Scenedesmus acuminatus* specimens was isolated from Tigris river in Baghdad city, Iraq, which located on longitude 33°36'01.94"N and latitude 44°20'19.41"E, during summer 2011 from a polluted region. Prepare axenic cells culture by using method was described by Hamdy (2000), Cells were grown in Jaworski's Medium (JM) was obtained from the Culture Collection of Algae and Protozoa (CCAP) held by University of LJMU. Arsenic stock solution was prepared with As (NO_3) in deionized water and was sterilized by filtration with 0.45µm Millipore filters paper. Initial to test whether *Scenedesmus acuminatus* could grow in the presence of Arsenic. As stock solution was added to JM liquid medium to reach a final concentration of 2µg/ml. Cultures were incubated at 27°C for 72h in illumination incubator provided by cool white fluorescent lamps set on 14:10 h light : dark photo-period also another culture without Arsenic as control.

2.2 Technique of Scanning Electron Microscopy (SEM)

Samples of *Scenedesmus acuminatus* cultures were transfer in to the Central service laboratory in college of education for pure science (Ibn–Al-Haitham). Samples of *Scenedesmus acuminatus* cultures were fixed in 2.5% glutaraldehyde for 4 h and washed four times with sterile distilled water. Finally, all samples were mounted on metal stubs and coated with gold. An Inspects' Model Scanning Electron Microscope (FEI , Netherlands, Eindhoven) was used to view the images INCA system software and X- Act Detector, operated at 20 kV coupled to SEM was used.

2.3 The Uptake of Various Concentration of Arsenic By Using Survival and Mortality Algal Biomass.

Culture of *Scenedesmus acuminatus* specimens were grown in JM (Jaworski's Medium) then take 50 ml of isolate culture and added 1L of media broth in conditions (pH 6.7, TEM 27±2Cº and 2000 lux illumination density14 hours light : 10hours dark) with shaking at 100rpm to 18-20 day to get heavy culture of algae. It has been prepared (4, 8, 16 and 32µg/ml) of Arsenic. Used three replicates for each concentration. Had been got it 0.05g of isolate. Then suspended 50 mg of this pellet culture strain in 50 ml of media with various heavy metal concentrations, While The mortality cells obtained by heat killing process by placing the algal cultures on water bath at 50Cº for 2h, and then centrifuged to get a palette which then suspension in 50ml of each heavy metals concentration Duangrat *et al.* (2002). Each sample incubate in (pH 6.7, TEM 27±2Cº and 2000 lux illumination density14 hours light : 10hours dark) for 3 days. After 3day make a centrifuge to the samples (3000rpm for 15 min) to get the pellet again this pellet treated with mix with10ml of 9:1 mixture of sulfuric acid : perchloric acid the mixture digests the palette algae till it turned colourless, then cooled and make up to known volume using distilled water to be ready for measuring Arsenic by using the ICPMS technique which found in chemistry department in LJM University.

The metal uptake (M)then estimate in unit of µg/mg using the formula

M = C V/W

M= Metals uptake estimated in units of µg/mg.

C= Spectrophotometer reading of concentration of the sample estimated in units µg/ml.

V= Volume of extraction of the sample estimated in units ml.

W= Dry weight of the algae estimated in units mg.

Results and Discussion

The result showed that the presence of Arsenic in the cell which grow in broth medium with 3µg/ml. The Division of Chlorophyta composed of different classes; most of them had the ability to uptake heavy metal by either biosorption or bioaccumulation. There are many reports and reviews on the sorption of Arsenic metal ion on marine algae, green seaweed, and freshwater green algal species with varying removal efficiencies. This capacity of uptake Arsenic may be due to the functional groups which found on the surface of cell wall which can act as binding sites for metal or by accumulation the metal intracellular Dwaish *et al.* (2011).

Over the last decades, several studies on the ability to uptake heavy metal by either biosorption or bioaccumulation Sulaymon *et al.* (2013), Bayo (2012), Macek, & Mackova, (2011) and Mehta and Gaur, (2005). The current study explore the effect of Arsenic on *Scenedesmus acuminatus* in order to determine the capacity of this microorganism to sorption heavy metal (As) by using SEM technique.

The *Scenedesmus acuminatusis* was isolated from a region of Tigris River through Baghdad city, that has been polluted region and cultured using streaking on agar method Stein (1973).

The micro alga used in this study *Scenedesmus acuminatusis* which is the most common freshwater genera of colonial-celled green algae, belonging to the phylum Chlorophyta. It is more often found floating in the creek water and not attached to rocks on the bottom, roughly cylindrical in shape and usually more than 2 times long as broad, colonies of usually single, but sometimes double rows of 4-16 cells that are joined along the long axis and it is a very common and sometimes abundant genus. Some species bear spines, others ridges and others no ornamentation .cell sizes vary greatly from spices to species Prescott, (1982).

The results are summarized in (Fig. 1). In this work *Scenedesmus acuminatus* cultures were prepared with a 4, 8, 16, 32 µg/ml of Arsenic concentration with the aim of determining whether *Scenedesmus acuminatus* can uptake metals from the polluted culture.

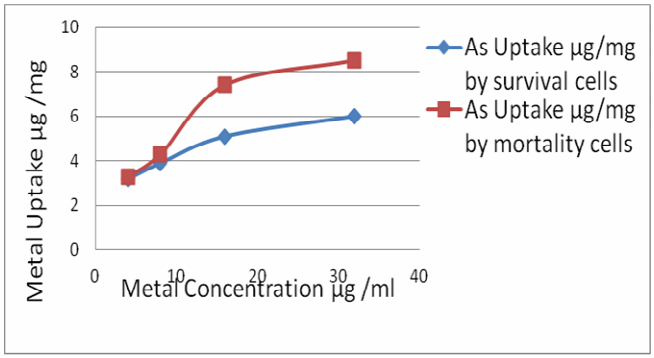

Figure 1: The metal uptake by the isolate which grown in media with various concentration of Arsenic.

Unpolluted and polluted cultures were prepared to SEM (Figs. 2, 3). The Division of Chlorophyta composed of different classes; most of them had the ability to uptake heavy metal by either biosorption or bioaccumulation. There are many reports and reviews on the sorption of Arsenic metal ion on marine algae, green seaweed Mehta & Gaur (2005) and freshwater green algal species with varying removal efficiencies. This capacity of uptake As may be due to the functional groups which found on the surface of cell wall which can act as binding sites for met-al or by accumulation the metal intracellular Alpana *et al* (2007).

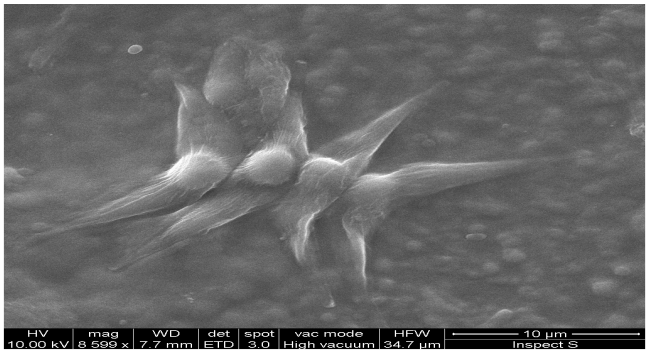

Fig. 2: *Scenedesmus acuminatus* cell grown in the unpolluted growth medium by using SEM.

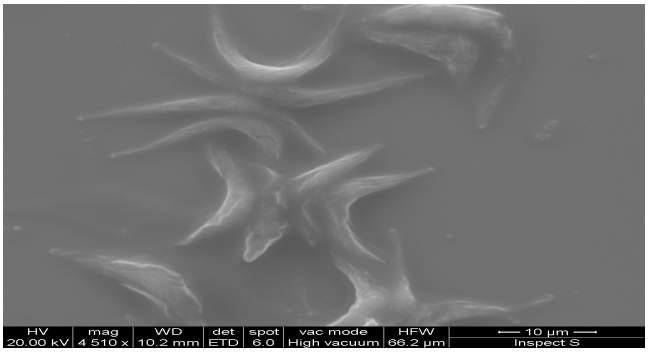

Fig. 3: *Scenedesmus acuminatus* cell grown in the polluted growth medium by using SEM.

The isolation of some strains from polluted environments evidences the capacity of these organisms to tolerate the presence of toxic compounds that confirm the ability of cell to sorption the metal Sulaymon *et al.,* 2013 and Duangrat *et al.,* 2002. Cell from As-polluted cultures exhibit morphological changes (Fig. 3). The SEM image shows cell that are more deformed when compared with the unpolluted solution figure (2) , may be that is because binding happened between heavy metal and cell wall of the cell, and that same to the results which found by Olal (2016). Using these technique allowed us to analyze the microorganisms morphostructurally and evaluate the presence of the Arsenic. The metal uptake by the isolate which grown in media with various concentration of Arsenic by *Scenedesmus acuminatus* is found to be in the range of 3.2-6.0 µg/mg for the survival cells and to be 3.3-8.5 µg /mg for the mortality cells (Fig. 1).

The result shown that metal uptake was a measure at unit concentration for Arsenic in the case of the survival and the mortality of *Scenedesmus acuminatus* had large affinity between Arsenic and the mortality algal cells, that is agree with the result of Azari *et al.* (2017) and Sibi (2014). It is clear from the results that the uptake of As by survival and mortality cells of the algae isolate *Scenedesmus acuminatus* depended on the metal concentration and their physiological status, and that agree with the study of Azari *et al.* (2017), Dwaish *et al.* 2011 and Kizilkaya *et al.* 2012. They investigated that the metal uptake by several microorganisms including the algae *Scenedesmus vacuolatus* and it was observed that the predominant constituents of micro-algal cell walls polymeric carbohydrates which therefore reflected a matrix build of monosaccharid unit that are cross-linked in a specific manner of the cells to heavy metals.

Conclusion

The *Scenedesmus acuminatus* which isolated from polluted site in Tigris River in Baghdad city is able to uptake Arsenic, the uptake of Arsenic is depended on the metal concentration and their physiological status therefore the mortality cells had more affinity to uptake the Arsenic from aqueous solution than the survival cells The result have also shown that initial SEM constitutes with a set of methodologies have proven to be a useful technique that allow a quick diagnosis of whether a microorganism can uptake a metal or not.

Acknowledgements

I would like to thanks Dr. Ahmed sahi and Dr Dina Yousif Mohammed to joining me in many of the experiments and in the development of ideas expressed in this paper.

Reference

Sahoo, D.S.J. (2015). The Algae World. Cellular Origin, Life in Extreme Habitats and Astrobiology 597

Wang, J. and Chen, C. (2009) Bio sorbents for heavy metals removal and their future. Biotechnol Adv., 27: 195-226.

Fu, F. and Wang, Q. (2011). Removal of heavy metal ions from wastewaters: a review. J. Environ. Manage. 92: 407–441.

Garg, M.C. (2008). Removal of Heavy Metals from Aqueous Solution by Waste Biomass. A Thesis Submitted in partial fulfillment of the requirement for the award of the degree of Master of technology in Environmental Science and Technology. Department of Biotechnology and Environmental Sciences Thapar University.

Alpana, S.; Mehta, S.K. and Gaur, J.P. (2007). Removal of heavy metals from aqueous solution by common freshwater filamentous algae. World J. Microbiol. Biotechnol., 23: 1115-1120.

IPCS (2001). Arsenic and arsenic compounds, 2nd ed. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 224; http://whqlibdoc.who.int/ehc/WHO_ EHC_ 224.pdf).

ATSDR (2007). Toxicological Profile for Arsenic. Agency for Toxic Substances and Disease Registry. http://www.atsdr.cdc.gov/toxprofiles/tp2.pdf.

Wang, S. and Zhao, X. (2009). On the potential of biological treatment for arsenic contaminated soils and groundwater. J Environ Manage, 90: 2367-2376.

Sulaymon, A.H.; Mohammed, A.A. and Al-Musawi, T.J. (2013). Removal of lead, cadmium, copper, and arsenic ions using biosorption: equilibrium and kinetic studies. Desalination and Water Treatment. 51(22-24): 4424-4434.

Chen, C.Y.; Chang, H.W.; Kao, P.C.; Pan, J.L. and Chang, J.S. (2012). Biosorption of cadmium by CO(2)-fixing microalga *Scenedesmus obliquus* CNW-N. Bioresour.

Bayo, J. (2012). Kinetic studies for Cd (II) biosorption from treated urban effluents by native grapefruit biomass (*Citrus paradisi* L.): the competitive effect of Pb (II), Cu (II) and Ni (II). Chem Eng J. 191: 278–287.

Macek, T. and Mackova, M. (2011). Potential of biosorption technology. In: Microbial Biosorption of Metals (eds P. Kotrba, M. Mackova & T. Macek), 7–17. Springer, Dordrecht

Hamdy, A.A. (2000). Biosorption of heavy metals by marine algae. Curr Microbiol. 41: 232-238.

Duangrat. Ia, Nalin. Sa, Suthep. Sa and Aran. I. (2002). Sorption of mercury, cadmium, Arsenic and lead by microalgae. Science Asia 28: 253-261.

Mehta, S.K. and Gaur, J.P. (2005). Use of alga for removing heavy metal ions from wastewater: progress and prospects. Crit. Rev. Biotechnol. 25: 113–152.

Stein (1973). Handbook of Phycological Methods. Culture methods and growth measurements. Cambridge University Press.

Prescott, G.W. (1982). Algae of the western Great Lakes Area. Brown, W.M.C.com. publishers, Dubuque, Lowa, 16th printing, 977.

Edward, G.B. and David, C.S. (2010). Fresh water algae. printed and bound in Barcelona, Spain by GRAFOS S.A.

Alpana, S.; Mehta, S.K. and Gaur, J.P. (2007). Removal of heavy metals from aqueous solution by common freshwater filamentous algae. World J. Microbiol. Biotechnol., 23: 1115-1120 .

Olal, F.O. (2016). Biosorption of Selected Heavy Metals Using Green Algae, Spirogyra Species. Journal of Natural Sciences Research. 6(14): 22-34.

Kizilkaya, B.; Turkera, G.; Akgulb, R. and Doganc, F. (2012). Comparative Study of Biosorption of Heavy Metals Using Living Green Algae *Scenedesmus quadricauda* and *Neochloris pseudoalveolaris*: Equilibrium and Kinetics. Journal of Dispersion Science and Technology. 33: 410-419

Dwaish A.S., Mohammed, D.Y.; Jawad, A.M. and AL-kubaicy, A.A. (2011). Determine the uptake of lead in *Chlorella vulgaris* isolated from Tigris River in Baghdad city. Int. Jou. of Scie & Engi. Rese., 2(9).

Azari, A.; Rezakazemi, M. and Ansarpour, M. (2017). Removal of Heavy Metals from Industrial Wastewaters: A Review. Chem. Bio. Eng. Rev., 4(1): 37–59

Sibi, G. (2014). Biosorption of Arsenic by Living and Dried Biomass of Fresh Water Microalgae-Potentials and Equilibrium Studies. J Bioremed Biodeg 5: 249.