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Determine the uptake of lead in *Chlorella vulgaris* isolated from Tigris River in Baghdad city

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Abstract—Chlorella vulgaris was isolated from Tigris River in Baghdad city. The isolated strain was identified according to its morphological characteristics as Chlorella vulgaris. The isolate was able to grow in broth medium in the presence of heavy metals. In this paper we present initially the Scanning electron microscopy techniques coupled to Energy Dispersive X-ray will be used to determine if this strain is able to uptake lead which has been detected in Tigris River as a toxic pollutant. The results indicate that Chlorella vulgaris has affinity for lead, and this technique could be very useful for the identification process in contaminated Rivers. The result shown the ability of chlorella vulgaris to uptake the lead with various concentration and it was between(2.2 ,2.7 ,3.2 ,3.7) µg /mg for living cells while it was between (2.3 ,4.1 ,6.8 ,7.9) µg /mg for non living cells in concentration of lead (3,6,12,18) µg /ml respectively, the result shown that the non-living cells have high affinity to uptake lead from the aqueous solution than the living cells .

Index Terms—Tigris river ,Baghdad city,heavy metals ,chlorella vulgaris

1 INTRODUCTION

The presence of heavy metals in aqueous water streams has become a problem due to their harmful effects on human health and on the flora and fauna of receiving water bodies. It is recognized that finding methods for removal of heavy metals from aqueous water is of great importance. Lead is among the most toxic heavy metal ion affecting the environment [1]. It comes into water through the combustion of fossil fuels and the smelting of sulphide ore, and into lakes and streams by acid mine drainage. Process industries, such as battery manufacturing and metal plating and finishing are also prime source of Pb pollution. The current EPA and WHO drinking water standard for lead is 0.05 mg/L and 10 µg/L, respectively. Lead accumulates mainly in bones, brain, kidney and muscles and may cause many serious disorders like anemia, kidney diseases, nervous disorders and sickness even death [3]. It is therefore, essential to remove Pb(II) from wastewater before disposal.Biosorption utilizes the ability of biological materials to accumulate heavy metals from aqueous solution by either metabolically mediated, or physico-chemical pathways of uptake [3] the aim of finding more efficient and cost-effective metal-removal biosorbent. Among them, algae have proved to possess high metal binding capacities [4] 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 [5,6]. The biosorbent was characterized by employing instrumental techniques, viz., Fourier transform infrared spectroscopy (FTIR), thermo gravimetric analysis (TGA) and scanning electron microscope (SEM). Despite the extensive information available on the genus Chlorella vulgaris about its ability to biosorbent heavy metals, the objective of this study was to determine whether Chlorella vulgaris which isolated from Tigris River in Baghdad city has this ability. For this purpose we used electron microscopy techniques in combination with an electron dispersive X-ray detector and use algal bio mass to study the uptake of lead from the aqueous solution.We select lead among heavy metal because it is very toxic and has no biological functions and because it has been found high concentration in the river

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2 MATERIALS AND METHODS FOR

2.1 MICROALGAL ISOLATE

The Chlorella vulgaris was isolated from a region of Tigris river through Baghdad city, that has been polluted region .after getting axenic cells culture by using method

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was described by [7], Cells were grown in JM(Jaworski's Medium) was obtained from the Culture Collection of Algae and Protozoa (CCAP) held by University of LJMU . Lead stock solution was prepared with Pb (NO3) in deionized water and was sterilized by filtration with 0.45µm Millipore filters paper. Initial to test whether Chlorella vulgaris could grow in the presence of lead. Pb 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 we prepared another culture without treated with lead as control.

2.2 Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) microanalysis For SEM analysis.

samples of Chlorella vulgaris. 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 (F E I , Netherlands, Eindhoven) was used to view the images and An energy dispersive X-ray s (Oxford Instruments, Bucks, England) INCA system software and X- Act Detector, operated at 20 kV coupled to SEM was used.

2.3 Study the uptake of various concentration of lead by using living and non-living algal biomass.

Take 50 ml of culture of the isolate were grown in JM(Jaworski's Medium) and added 1L of media broth in conditions (pH6.7 ,tem 27±2C^o 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 (3,6,12 and18 µg/ml) of lead Used three replicates for each concentration. Had been got it 0.05g of isolate. Then suspended 50mg of this pellet culture strain in50 ml of media with various heavy metal concentrations, While The nonliving cells obtained by heat killing process by placing the algal cultures on water bath at 50C^o for 2h,and then centrifuged to get a palette which then suspension in 50ml of each heavy metals concentration (8). Each sample incubate in (pH6.7 ,tem 27±2C^o 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 mixtuer digests the palette algae till it turned colourless, then cooled and make up to known volume using distilled water to be ready for measuring lead by using the ICPMS technique which found in chemistry department in LJMUniversity.

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.

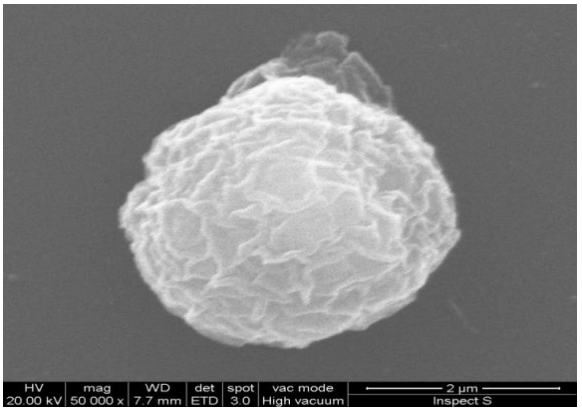
V= Volume of extraction of the sample estimated in units ml.

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

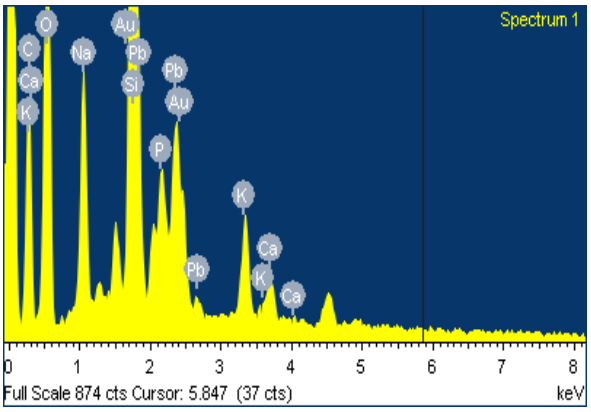
3 Results

As We used SEM and EDX to study the effect of lead on Chlorella vulgaris. in order to determine the capacity of this microorganism to sorption heavy metal(pb). Tigris River through Baghdad city, that has been polluted region by using streaking on agar method [9]..

Chlorella is a genus of single-celled green algae, belonging to the phylum Chlorophyta. It is spherical in shape, about 2 to 10 µm in diameter, and is without flagella[10],that clear in (Fig. 1a and 1b). In this work Chlorella vulgaris cultures were prepared with a 2µg/ml of lead concentration with the aim of determining whether Chlorella vulgaris can uptake metals from the polluted culture. Unpolluted andpolluted cultures were prepared and analyzed by EDX coupled to SEM (Fig.1a, 1b).



(1A)

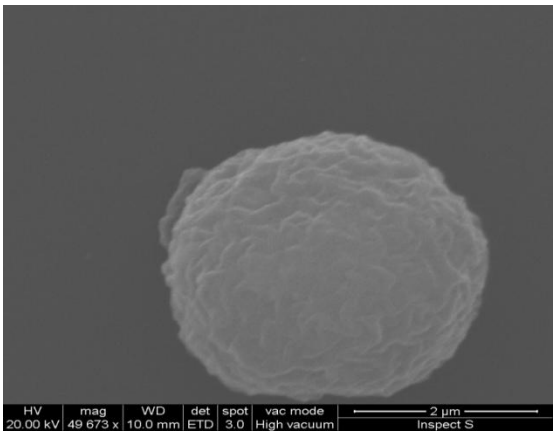


(1B)

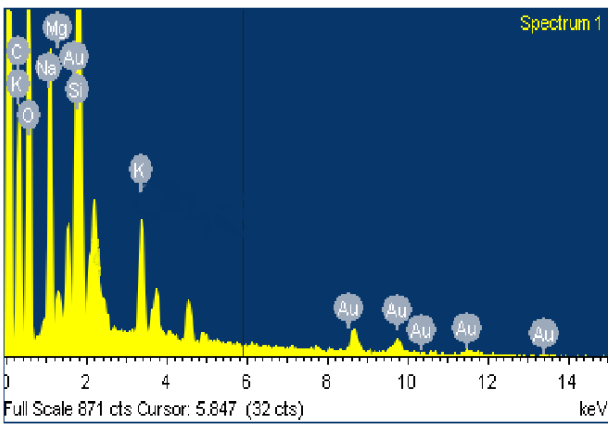
FIG.1: CELL OF CHLORELLA VULGARIS GROWN IN THE PRESENCE OF 2µG/ML OF LEAD (1A) IMAGE OF CELL (2A) LEAD IS DETECTED IN THE CELL AND X-RAY ANALYSIS SPECTRA COUPLED TO SEM SHOW A PB PEAK.

If The result Shown in fig (1) that the EDX analysis evidences the presence of lead in the cell which grow in broth medium with 2µg/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 lead metal ion on marine algae , green seaweed [11], and freshwater green algal species with varying removal efficiencies. This capacity of uptake Pb 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 [6].

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 [5]. Cell from Pb-polluted cultures exhibit morphological changes (Figs. 1). 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 [12] Gold were detected in the spectra of the unpolluted and polluted cell because the specimen coated with Au before scan by SEM, The metal Zn which found in unpolluted and polluted cell because the media had this metal in composition .To determine whether the Chlorella vulgaris strain could uptake lead, two high resolution microscopy techniques, SEM , was employed coupled with an energy dispersive X-ray technique. Using these techniques together allowed us to analyze the microorganisms morphostructurally and evaluate the presence of the lead.



(2A)

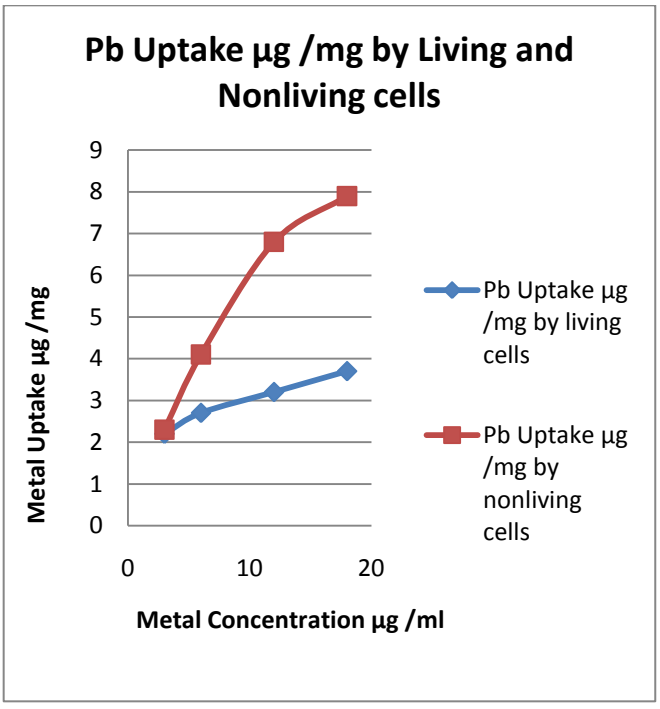


(2B)

FIG 2: UNPOLLUTED CULTURES OF CHLORELLA VULGARIS CELLS ARE ANALYZED BY SEM-EDX INDIVIDUAL CELL IS VIEW IN SEM IMAGE (FIG. 2A). THE EDX ANALYSIS OF THE CELL SHOWS THAT LEAD IS NOT DETECTED IN THE CELLS (FIG. 2B).

The metal uptake by the isolate which grown in media with various concentration of lead by chlorella vulgaris is found to be in the range of 2.2-3.7 µg/mg for the living cells and to be 2.3- 7.9 µg /mg for the non living cells.

The result shown that metal uptake was a measure at unit concentration for lead in the case of the living and the nonliving of chlorella vulgaris had large affinity between lead and the non living algal cells, that is agree with the result of (13)that studied the bioaccumulation of cadmium and zinc in a diatom Cosinodiscus granii and found that the heat -killed cells accomplished more metals than did the living cells.



It is clear from the results that the uptake of Pb by living and nonliving cells of the algae isolate chlorella vulgaris depended on the metal concentration and their physiological status, and that agree with the study of (14) investigated that the metal uptake by several microorganisms including the algae C.caladium, and it was observed that the predominant constituents of microalgal 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 .

4 Conclusions

The initial SEM with EDX 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. The result have also shown that. The Chlorella vulgaris which isolated from polluted site in Tigris River in Baghdad city is able to uptake lead, the uptake of lead is depended on the metal concentration and their physiological status therefore the non-living cells had more affinity to uptake the lead from aqueous solution than the living cells.

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