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Use of the microalga *Scenedesmus obliquus* (Turpin) Kutzing to remove some heavy metals from the industrial wastewaters

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ABSTRACT

Aquatic ecosystms may receive hazardous heavy metals resulting from the overconsumption of petrochemical products. Chemical and physical analysis of wastewater of certain petrochemical plant was studied; and pointed for the existence of Cd, Pb, Zn, Ni, Cu, Co, Fe and Mn. The objective of this work is to study the effectiveness of immobilized *Scenedesmus obliquus* to remove such pollutants. Both fresh and dry biomass of the micro-green alga *S. obliquus* in immobilized form was used as low cost, efficient, and eco-friendly biosorbent for some heavy metals' removal. According to the results, the biosorption removal efficiency in general with all heavy metals was around 50% after 15 min. of treatment, and it reached 95% after 60 min, while the efficiency rate remained stable or was slightly lowered after 120 min. Moreover, the cited results clarify that *S. obliquus* as biosorbent was more powerful in removing Pb, Cd and Fe followed by Ni, Cu, Mn, Co, and Zn from the aqueous solution of petrochemical wastewater. For further verification, at the end of the treatment process, the algae cells were examined under the scanning electron microscope (SEM), which showed large clusters of heavy metals appeared adsorbed on the surfaces of the cells, which reflects the high efficiency of *S. obliquus* in removing heavy metals from industrial wastewaters. These results motivate us to apply the use of live or dry algae mass as an effective and safe method for the disposal of heavy metals and industrial effluents.

INTRODUCTION

Since the beginning of creation, water has been the mainstay of life, hence clean water resources are essential to all living organisms for their growth, reproduction and even survival. Domestic, agricultural and industrial wastewater especially mining, manufacturing and power generation had a negative impact on water quality and consequently on biota and Human health (**Mosleh *et al.*, 2012**). Heavy metals (HMs) are released into the water environment mainly through, agricultural (pesticides, fertilizers) and industrial activities (painting, petroleum refining, mining activities, smelting, car exhausts, battery manufacturing and pigments, (**Lesmana *et al.*, 2009; Mosleh and Mofeed, 2014; Ardila *et al.*, 2017**). Heavy metals are elements with atomic weights ranged from (63.5 - 200.6) and a basic gravity greater than 5.0. Industrial wastewater is the main source of heavy metals contamination (**Shanab *et al*., 2012; Mofeed and Mosleh, 2013**). In recent years, pollution by heavy metals has become a big concern. This anxiety about heavy metals is due to they are not biodegradable but it has the ability to accumulate in living tissues, resulting in long-term sustainability in their negative impact on human health (**Deng *et al*., 2008; Metwali *et al*., 2014*;* Mosleh *et al*., 2014*;* Ibrahim, *et al*., 2016**).

Moreover, HMs can endanger ecosystems and public health because of their high mobility, lack of degradation, and high capacity to accumulate within all living beings. Also, it can participate in biological reactions that ruin vital processes within cells, tissues and organs promoting disease even if present with its low concentration in the environment (**Topcuoglu *et al*., 2003; Mosleh *et al.,* 2006; Mosleh, 2013**). However, some of these metals are essential micronutrients for plant growth (e.g., zinc, copper, manganese, nickel, and cobalt), whereas others may have unknown biological functions and are poisonous such as Cd, Pb and Hg (**Gaur and Adholeya, 2005**). Mercury, cadmium, copper, zinc, lead, and nickel, among other harmful heavy metals, are the most common contaminants in both marine and freshwater (**Travieso *et al*., 1999; Yu *et al*., 1999; Mehta and Gaur 2005; Singh *et al*., 2007; Mosleh, *et al*., 2014**).

The conventional treatment methods for extracting biologically polluted heavy metals or effluents of wastewater include ions exchange, reduction of contaminants, chemical precipitation, membranes filtration, treatment of nanotechnology, electrochemical removal and advanced oxidation (**Chalivendra, 2014**). Additionally, there are several biological processes that use dead or alive, free or immobilized cells of algae or plant tissues, as cells have carbohydrates and polypeptides in their walls with, aldehydes, hydroxyl, amines ketones, carboxyl groups and phosphates responsible for metal caption adsorption and chelation (**Wang, 2009**). Unfortunately, using of these conventional processes is limited due to significant disadvantages, such as low selectivity, incomplete removal, high energy consumption, high cost or high toxic waste generation. Therefore, the world is constantly in need to find safe, low-cost and more effective alternative methods for extracting heavy metals from contaminated water.

Bioasorption is using the dried organisms as adsorbents (**Mofeed and Mosleh, 2013; Chalivendra, 2014; Mofeed, 2017**). Considering the biosorption mechanism; there are many ways for metal uptake by microbial cells (**Ahalya *et al*., 2003; He and Paul, 2014**), which can be classified according to different criteria depending on the cell structure according to the location where metal is removed from by extracellular accumulation. Where biological materials may have physiochemical properties that help to remove some heavy metals from wastewater by covalent bonding or ionic (**He and Chen 2014; Zeraatkar *et al*., 2016; Salama *et al*., 2019**). Metal biosorption from aqueous solutions has the potential to be a useful wastewater treatment strategy. It focuses on biological material's ability to absorb heavy metals ions from wastewater through metabolically mediated or physicochemical uptake pathways (including living or dead microbial biomass and their components, seaweeds, and so on) (**Fard**

et al*., 2011; Chalivendra, 2014**). Biosorption is considered as an alternative powerful technique for extracting heavy metals from wastewater due to its great advantages such as low cost and high efficiency (**kumar *et al*., 2006; Handojo *et al*., 2016**), in addition, it can compensate for the drawbacks of commercial resins, which reduce the efficiency of adsorption in wastewaters to the lower metal concentrations (**Eccles, 1999**), this motivates its production in large quantities and in economical ways (**Abdel-Aal and Mofeed, 2020)**. Also, algae are ideally suited for evaluating water quality and have been shown to be effective bioindicators because they have rapid reproduction rates, very sensitive responses to chemical changes, eutrophication and pollution, and receive a wide variety of typically specific species (**Larson and Passy, 2012; Mofeed, 2020**). Therefore, this study was aimed to evaluate the feasibility of using both dry and fresh biomass of the microalga ***S. *obliquus* in removing some heavy metals from industrial wastewaters.

MATERIALS AND METHODS

Biological material

Microalgal biomass and stock solution

Scenedesmus obliquus (Turpin) Kutzing (SAG 276-3a; Gottingen, Germany cultures; formerly *S. acutus*; **Schlosser, 1994**) was maintained in batch cultures containing 100 mL of Bold basal medium (BBM) (**Bischoff and Bold,1963**), under continuous aeration, at 22 ±1 ^{o}C, illumination of µmol.m^{-2}s^{-1} and placed on an orbital shaker (150 rpm). Every week the subcultures were made by adding 20 mL of one-week-old suspension into 100 mL of fresh growth medium.

Harvesting of microalgae

Harvesting of the algal biomass culture was done by centrifugation at 4000 rpm for 10 min. The recovered biomass was washed in deionized water before being centrifuged again. Samples were dried in oven at 60 °C until the humidity reached the lowest level, and finally stored at 4°C for further use.

Immobilization of *S. obliquus* in alginate beads and its application in heavy metal removal.

Immobilization of microalgae have been used to facilitate evaluation of its biosorption capacity to heavy metals from aqueous solution, by using separating funnel packed with immobilized *S. obliquus* cells in sodium alginate beads. 4% sodium alginate solution was made by dissolving 4 g sodium alginate (Sigma-Aldrish) in 100 ml distilled water and vigorously mixing for 30 minutes at 60 °C with continuous stirring for improved solubility. (**Kumar *et al*., 2018**). After cooling, 1 g (1 % W/V) was added and stirred for 5 minutes at room temperature. The beads were made by dropping the cell suspension and sodium alginate combination into a cold sterile 2.5% $CaCL_2$ solution at room temperature in sterile conditions under gentle stirring. The beads were spherical with an average diameter of 1.5 mm 0.2 mm and a diameter of 1.5 mm 0.2 mm. The resulting spherical beads were rinsed multiple times with autoclaved distilled water to remove unreacted $CaCL_2$ from the surface, and then kept overnight at 4°C in autoclaved distilled water to stabilize and harden the beads by the same procedure, sodium alginate beads without incorporation of the *S. obliquus* biomass are also prepared and used as control. The beads were immersed in 0.2 M HCl buffer (pH 7.2) for storage and kept at 4 °C until needed. The experiment was carried out in a 100 mL separating funnel, containing alginate algal beads. The separating funnel's effluent wastewater was removed. The effluent was collected in 5 ml at a flow rate of 3 $ml.min^{-1}$ on a regular basis (every 30 minutes for up to 2 hours) and examined using inductively coupled plasma-atomic emission spectroscopy. The absorption of *S. obliquus* for metals ions was determined by the difference in the concentration of the heavy metals before and after absorption.

Industrial wastewater sample.

Collection and preparation

Water samples were collected from industrial drainage of Petrochemicals plant; the samples were kept in the dark and transported to the laboratory, where the chemicals investigations were done. Industrial wastewater samples were mixed well, and filtrated through millipore filtration system (Millipore Comp. 0.22) and stored at 4°C to be used for chemicals analysis.

Physico-chemical analysis

The filtered industrial wastewater sample was taken in a polyethylene bottle in order to directly determine the physico-chemical parameters (Temperature, pH, Nitrite –N, Nitrate-N, Ammonia-N, and total dissolved salts, Chlorides, Sulfate, Reactive Silica, Hardness, Total Carbon, Alkalinity and Ortho-phosphate) as soon as reaching the laboratory, according to standard methods followed by American Public Health Association APHA, (1995). The concentrations of heavy metals in the samples were determined using atomic absorption spectrophotometry (**kumar *et al.*, 2008**). Standard operating parameters were set and the hollow lamps for Cd, Zn, Cu, Mn, Pb, Fe, Co and Ni (Analytikjena Model Nova350) were used as radiation source and fuel was air acetylene. All the standard and samples were run in triplicate (**Kunkel, 1973**).

Biosorption Experiment

The scientific objective of this experiment was to evaluate the adsorption efficiency of *S.obliquus* as algae biosorpent depending on contact time and concentrations of the algal biosorpent. The biosorption experiments were carried out in batch mode, were, in different concentrations (0.2, 0.4, 0.6 0.8, and 1 $g.L^{-1}$) of the dried crushed algae biosorpent which were added to 100 ml of the industrial wastewater in 250 ml conical flask for designed intervals period of 15, 30, 60 and 120 min on an orbital shaker at 150 rpm. The temperature adjusted at 23 ±1°C and pH 7.5 before adding adsorbent. The suspended solids were separated out with GF/C filter. Heavy metal ion concentrations were then measured in the filtrate water. The residual concentrations of the tested heavy metals (Cd, Zn, Cu, Mn, Pb, Fe, Co and Ni) in the filter were determined using atomic absorption spectrophotometry (**Kunkel, 1973**). The percentage removal and metal uptake efficiencies (Biosorption %) of all adsorbents were determined with following expressions (**Hashim and Chu, 2004**).

$$\text{Biosorption } (\%) = (C_i - C_f)/ C_i \times 100$$

The amount of metal adsorbed, Q (mg metal/gram absorbent) was computed using the following equation (**Chen**, 2005):

$$Q = (C_i - C_f)\, V/m$$

Where, Q= Amount of metal adsorbed ($mg.g^{-1}$) C_i= Initial metal concentration in solution ($mg.L^{-1}$) C_f = Final metal concentration in supernatant after adsorption ($mg.L^{-1}$) V = Volume of solution (ml) m = Mass of the adsorbent (g).

Scanning electron microscope examination:

At the end of the experiment the algal biosorpent, *S. obliquus,* in both the control and the treated with heavy metals were harvest and then fixed using 2.5% glutaraldehyde for 12 h at 4^oC. The fixed cells were dehydrated and washed by using a series of ethanol and then embedded in epoxy resin, following the method described by **Sadiq *et al.,* (2017**), then observed using JEOL JSM 6510 model scanning electron microscope (SEM).

Statistical analysis

All the biosorption experiments were conducted in triplicates to substantiate the results. The data shown are the mean ± standard deviations. Data were analyzed by Student's T test for independent samples. Analysis was performed using the SPSS 14.0 for Windows (SPSS, Michigan Avenue, Chicago, IL, USA), and the minimum significant level was set at 0.05. The multi-dimensional analysis; Cluster analysis was achieved by MVSP program using UPGMA cluster (**Legendre and Legendre, 1998**) to clarify the similarity in heavy metals removal efficiency.

RESULTS

Inspection of (Table 1) revealed that, total alkalinity values fluctuated between 6 $mg.L^{-1}$ in industrial effluent wastewater before treatment and 4.4 $mg.L^{-1}$ after treatment. While the total hardness value after treatment (3.86 $mg.L^{-1}$) was higher than before treatment process, where it reached to 1.48 $mg.L^{-1}$ (Table 1). The mean concentration of total dissolved solid (TDS) in industrial effluent is presented in (Table 1), where it was recorded as 180 $mg.L^{-1}$ for industrial effluent wastewater before treatment and 112 $mg.L^{-1}$ after treatment. Also**,** the pH of the samples varied from 6.2 to 7.8, while the values of the free dissolved carbon dioxide during the whole period of investigation showed up 7.25 $mg.L^{-1}$ in industrial effluent before discharge. However after industrial effluent wastewater discharged total carbon level reached to 6.5 $mg.L^{-1}$. While chloride concentration fluctuated in narrow range between the untreated (16.76 $mg.L^{-1}$) and the treated (17.11 $mg.L^{-1}$) industrial effluent wastewater also other parameters such as; Phosphate, Sulphate, Nitrite, Nitrate, before treatment/and after treatment of industrial effluent wastewater as shown in Table (1).

Biochemical analysis.

The data pertaining biochemical analysis revealed that, moisture content of *S. obliquus* was 10.2%, as well as the ash content of *S. obliquus* was 57.13%. While carbohydrates, protein and fat component of algal cells was 17.75, 16.80 and 0.36 % respectively (Table 2)

Table 1: Physico-chemical parameters of the discharged petrochemical industrial effluent wastewater before and after treatment.

Parameters	Industrial wastewater ($mg.L^{-1}$)	
	Before treatment	**After treatment**
pH (Unit)	6.20 ± 0.1	7.80 ± 0.89
Temperature ^{0}C	24.00 ± 1.00	24.4 ± 1.20
Sulphate $mg.L^{-1}$	1.40 ± 0.003	0.20 ± 0.16
O. Phosphate $mg.L^{-1}$	0.015 ± 0.002	0.022 ± 0.04
TDS ($mg.L^{-1}$)	180.5 ± 12.7	112.30 ± 4.8
Ammonia ($mg.L^{-1}$)	5.50 ± 0.027	1.32 ± 0.08
Nitrate ($mg.L^{-1}$)	3.80 ± 0.003	2.20 ± 0.01
Nitrite ($mg.L^{-1}$)	0.20 ± 0.003	0.027 ± 0.01
Silica	1.30 ± 0.002	0.027 ± 0.0
Chloride ($mg.L^{-1}$)	16.76 ± 0.81	7.11 ± 0.51
Total alkalinity (mg $CaCO_3.L^{-1}$)	6.00 ± 0.12	4.00 ± 0.14
Total carbon ($mg.L^{-1}$)	7.25 ± 0.32	6.50 ± 0.11
Hardness (mg $CaCO_3.L^{-1}$)	1.48 ± 0.07	3.86 ± 0.08

Table 2:- Biochemical analysis of *S. obliquus* biomass.

Component %	moisture content	Fat	Protein	Carbohydrates	Ash
	10.20 ± 0.12	0.36 ±0.01	16.80 ±0.25	17.75 ± 0.35	57.13± 0.42

Data presented are mean (±SD).

Efficiency removal of heavy metals by the dried algal biomass

The experiment was conducted to study the effect of dry *S. obliquus* on removal of some heavy metals (Cd, Pb, Zn, Ni, Cr, Cu, Fe and Mn ($mg.L^{-1}$) from the petrochemicals industrial effluent wastewater. Results of heavy metals removal from the petrochemicals industrial effluent were depended up on, both biomass amount and contact time.

Dry *S. obliquus* adsorbed Mn according to concentrations and contact time presented in (Fig. 1). After 60 min of treatment the amount adsorbed of Mn at concentration 1 $g.L^{-1}$ reached to 74.2 %. While the best removal efficiency for Co by *S. obliquus* was at concentrations 1 $g.L^{-1}$ of dry biomass after 60 and 120 min of treatment with adsorbed capacity reached to 63 and 68.22 % respectively (Fig. 1). Data presented in (Fig. 1) showed that, with 1 $g.L^{-1}$ *S. obliquus'* biomass and at contact time 30 and 60 min, the maximum removal percentage of Cu was 78 and 89.33% respectively. Also, data in (Fig. 1) showed that, the dry *S. obliquus* ability to removal Pb from the petrochemicals industrial effluent after 120 min of treatment with the concentration of 0.4 $mg.L^{-1}$ was 81.4%. While the Pb removal after

120 min reached to 95.8% after the treatment of dry algal at concentration 1 g.L^{-1} of dry algae. Also data in (Fig. 1) showed that the %removal of Zn metal ion by *S. obliquus* after 15 min of dry biomass at concentration 0.2 g.L^{-1} was 37%. On the other hand the removal of Cu reached to 54.4% with the concentration 1 g.L^{-1} respectively after 60 min. While data in (Fig. 1) presented the removal efficiency by *S. obliquus* for Ni metal ion from petrochemicals industrial effluent after 30 and 60 min with concentration 1 g.L^{-1} of dry algal was 77.8 and 89% respectively. The removal percentage of Cd by dry *S. obliquus* presented at (Fig. 1) at concentration 0.2 g.L^{-1} after 15 min the adsorbed capacity reached to 55.2%, while after 60 min of treatment with concentration 1 g.L^{-1} the adsorbed capacity reached to 95.15%.While the data presented in (Fig. 1) showed that after 15 min %removalof Fe reachedto 63.5% for Fe metal ion by dry *S. obliquus* at concentration 1 g.L^{-1}. While after 60 min of treatment with the same concentration the removal percentage reached to 91%.

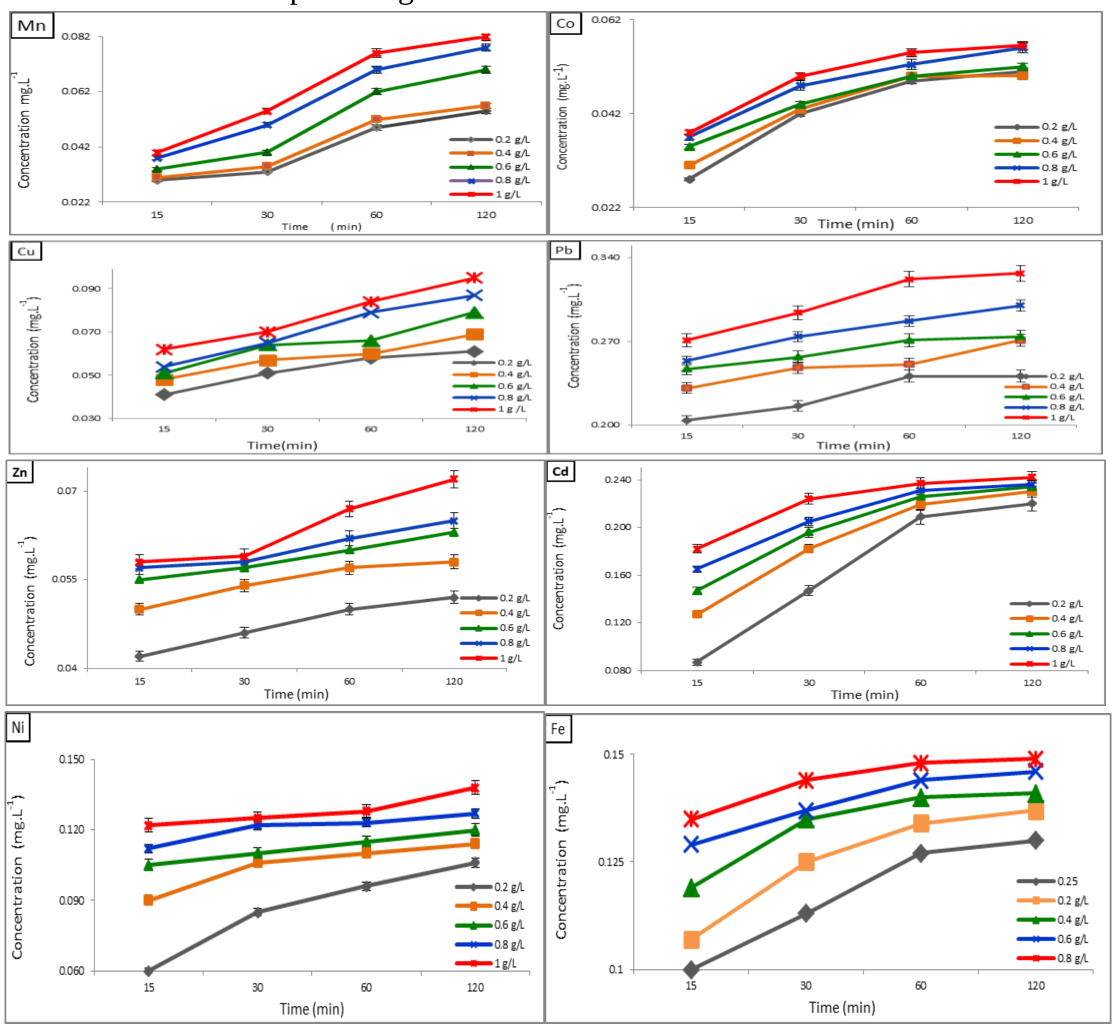

Fig. 1: The adsorbed amount of some heavy metals (mg.L^{-1}) by dry *S. obliquus* after different time of treatment with dry algae.

Results of removing of metal ions by immobilized green microalgae *S. obliquus*.

Data listed in (Fig. 2) according to biomass concentration the immobilized of *S.obliquus* showed the efficiency of Ni removal was 85.7% at concentration 0.4 $g.L^{-1}$ of algal solution beads after 120 min and it was 82.2% at concentration 0.8 g/L after the same time.Also date presented (Fig. 2) indicated that the treatment of industrial wastewater containing immobilized *S. obliquus* beads theremoval % of Zn was 48.3 and 52% after 60 min of treatment according to concentration 0.4 $g.L^{-1}$ and 1 $g.L^{-1}$ of immobilized biomass respectively. Also (Fig. 2) presented the removal efficiency of *S. obliquus* beads for Mn metal ion at concentration 0.8 $g.L^{-1}$ of fresh algal biomass was 59.7% after 120 min in like manner at concentration 1 $g.L^{-1}$ of fresh algal biomass the percentage of removal reached to 68.2 % within the same time. Data in (Fig. 2) showed alginate beads of *S. obliquus* was able to adsorb Pb after 60 min with efficiency reached to 88.6% at the concentration 0.4 $g.L^{-1}$ of fresh immobilized. While at concentration 0.8 $g.L^{-1}$ the efficiency was 92.71% after the same time. While date presented (Fig. 2) indicated that the treatment of industrial wastewater containing immobilized *S. obliquus* beads, the removal % of Co was 48.3 and 65% after 60 min of treatment according to concentration 0.4 and 1 $g.L^{-1}$ of immobilized biomass respectively. While the bioremoval of Cu was shown in the removal percentage reached to 57.7% and 66.6% of fresh immobilized atconcentration 0.8 $g.L^{-1}$ after 60 and 120 min for *S. obliquu* respectively. Also data in (Fig. 2) showed that the initial concentration of Cd was 0.075 $mg.L^{-1}$ the bioremoval efficiency by immobilized *S.obliquus* was 81.33% at concentration 0.2 $g.L^{-1}$ of algal solution after 60 min, and ended up to 89.33% at concentration 0.8 $g.L^{-1}$ of fresh immobilized after the same time. While the data presented in (Fig. 2) showed that the treatment of industrial wastewater containing Fe with immobilized *S. obliquus* the biomass in sodium alginate-beadsremoved 84.8% of metal with concentration 1 $g.L^{-1}$ of algal biomass after 60 min. Nevertheless the removal percentage was 21.2% at concentration 0.2 $g.L^{-1}$ of algal biomass after 15 min by immobilized *S.* o*bliquus*.

Regarding data in (Figs. 3-A, B and 4-A, B) reflect the similarity in biosorption efficiency of both fresh and dry *S. obliquus* biomass to the tested heavy metals from the aqueous solution of petrochemical plant, where it clarifies that it was more powerful in removing Pb and Cd followed by Fe which were grouped in one minor subgroup with a noticeable high similarity. On the other, in case of dry biomass; Ni and Cu were grouped in minor subgroup which related with less similarity with Mn, Co and Zn, however, all those heavy metals were grouped together but with high dissimilarity in case of immobilized fresh *S. bliquus* cells.

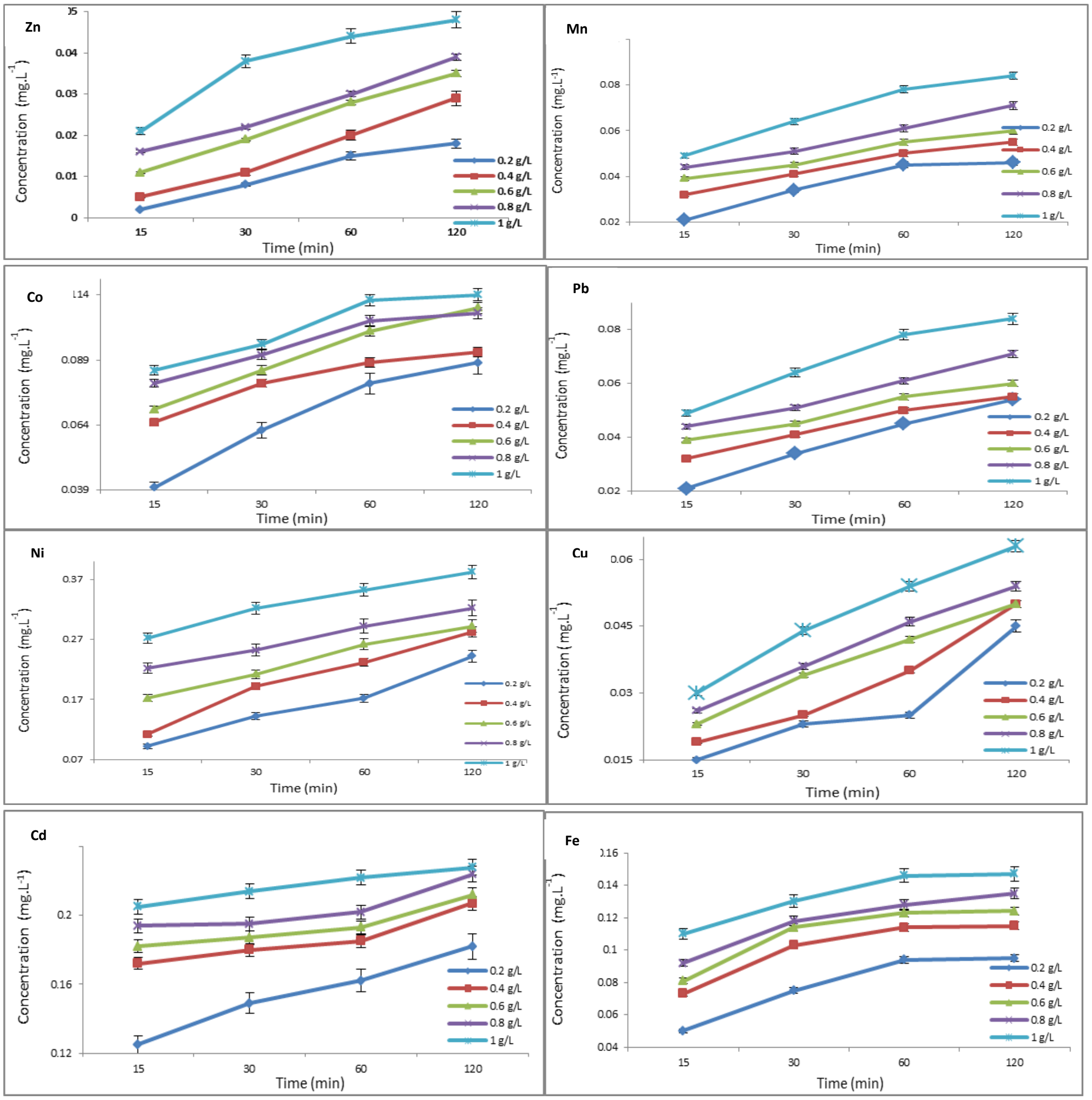

Fig. 2: Immobilization of *S. obliquus* biomass in alginate beads and its application in heavy metals removal from industrial wastewater.

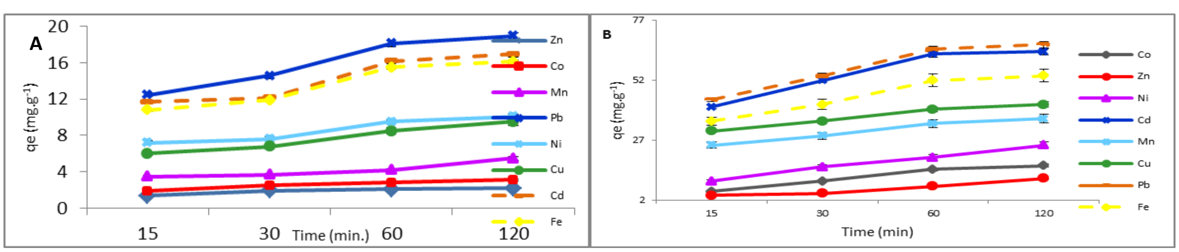

Fig. 3. Metal uptake capacity as a function of time using (A) dry algae *S.obliquus* and (B) immobilization of *S. obliquus* biomass in alginate beads.

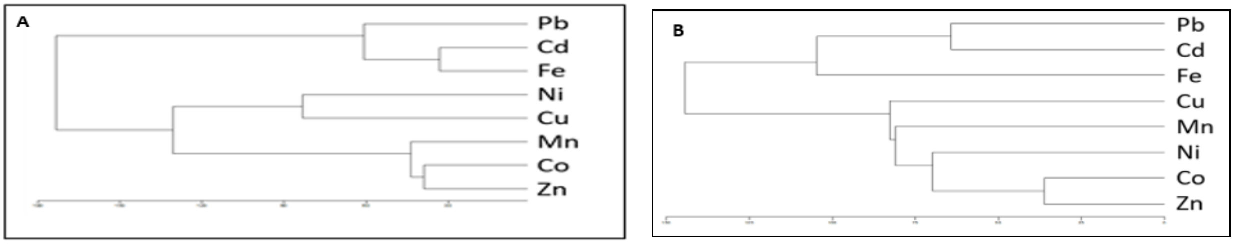

Fig. 4: Dendrogram produced by the Cluster analysis of the tested heavy metals adsorbed by (A) dry and (B) fresh biomass of *S. obliquus;* using UPGMA cluster.

Scanning electron microscope examination of *S. obliquus* cells.

S. obliquus cells were examined using scanning electron microscope (SEM) after exposure to the aqueous solution of petrochemical plant as well as the normal cell in the BBM medium. The resulted SEM images showed changes in the treated *S. obliquus* cells in compared to control cells (Fig. 5).The cells have lost the characteristic regular shape of *S. obliquus* cellsand aggregate in as irregular clustersaround heavy metal particles. Where, the fusion between cells is no longer limited to fusion with the lateral walls only, but some cells appeared to fuse with each other from the apical wall.The cells became smaller in width and more spindle in shape.

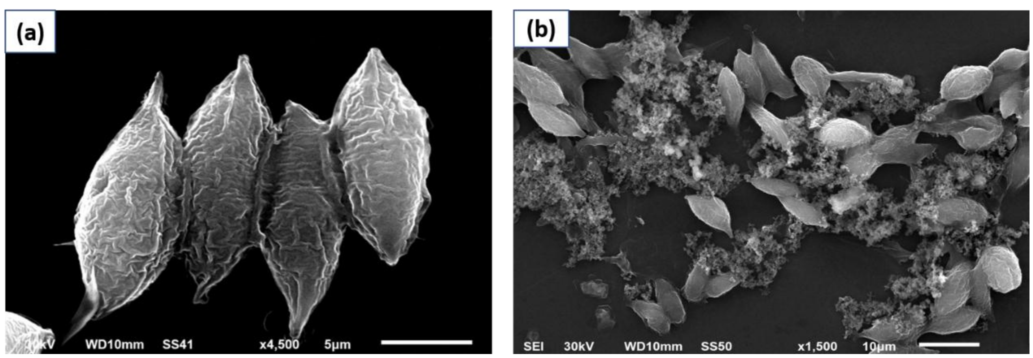

Fig. 5: Cellular structure of *S.obliquus* using scanning electron microscope (SEM), where (a) control and (b) after exposed to heavy metals aquas solution.

DISCUSSION

Increasing urbanization and industrialization have caused heavy metals to reach dangerously harmful levels in the environment, implying that heavy metals enrichment in many ecosystems is directly linked to human activity (**Lasat, 2000; Estrella and Garcia, 2009; Harabawy and Mosleh, 2014**). Mercury, cadmium, copper, zinc, lead, and nickel and other harmful heavy metals, are well-known freshwater and marine contaminants (**Travieso *et al.*, 1999; Yu *et al.*, 1999; Mehta and Gaur 2005; Singh *et al*., 2007**). In an ecological way, however, any metal or metalloid that pollutes the environment or cannot be decomposed biologically is considered a pollutant (and is therefore bio accumulated) it's possible to classify it as heavy metals (**Estrella and Garcia, 2009**).

During our investigation the value of TDS in industrial effluent water before treatment was (180.5 ± 12.69 mg.L^{-1}) higher than obtained value after treatment (112.3 ± 4.79 mg.L^{-1}), in which explained that the water had previously been treated. On the other hand, TDS isn't usually thought of as a major pollutant, it is not deemed to be associated with health effects. It's used to determine the aesthetic qualities of drinking water as well as an overall measure of the existence of a wide range of chemical pollutants (**Ogemdi and Gold, 2018**). While chloride levels have been shown to rise in direct proportion to pollution levels (**Drakare *et al.*, 2003; Gao and Song, 2005; Campbell *et al.*, 2015**). Petrochemical industrial effluent water of company had high chlorosity values ranged from 16.76 mg.L^{-1}in water before discharge while it was 17.11 mg.L^{-1}in water after discharge. It's appeared that chloride value became higher after the treatment than before. That result had the compliance to (**WHO, 1990**) which reported that water chloride may be increased by treatment processes in which chloride or chlorine is used. In similar studies (**Aziz *et al.*, 1996**) investigated that Damietta estuary had a relatively high chlorosity values (9-20.5 g.L^{-1}) higher sodium and chloride concentrations are known to cause plant toxicity.While carbon dioxide levels are predicted to nearly double in the next century (**Muylaert *et al.*, 2005; Mofeed, 2017**) free and total carbon dioxide concentrations increased by increasing pollution (**Schippers *et al.*, 2004**). The result recorded for carbon dioxide within industrial effluent after water discharged into the sea was lower than in industrial effluent of factory before water discharge. It was reported by (**Aziz *et al.*, 1996**) that agricultural and urban effluents both cause CO_2 concentrations in water to rise, whereas industrial discharge affects total CO_2 concentrations depending on the chemicals used. While the total alkalinity values in present study was 6 mg.L^{-1} in wastewater before treatment and reached to 4.4 mg.L^{-1} in wastewater after treatment so the result in our investigation observed high level of eutrophic conditions, according to study by **(Campbell *et al.*, 2015)** who reportedthat the values of total alkalinity greater than 1.4 m equ.l indicate eutrophic conditions. The parameters that determine the amenability of waste water to biological treatment are alkalinity and pH **(Jingxi Ma *et al.*, 2020**).

Nitrate, a nitric acid compound, is a photosynthetic autotroph and in some cases, a growth-limiting nutrient. Algae and other aquatic plants use it to make plant protein, which can then be used by animals to make animal protein. The nitrate levels in wastewater effluent were higher before treatment than after treatment. Nitrate is an important component of farm fertilizers because it is needed for plant uptake and development. Nitrate in the aquatic environment is the most heavily oxidized source of nitrogen (**Atul and Narang, 2018; Kumar *et al.*, 2018**).Only inorganic forms of silica are used in the main cycle of silica, which includes the use of dissolved silicon and its degradation following the death of species (**Raymont, 1980**). Generally the dissolved reactive silica value in the present study area discharged from two points in the petrochemicals industrial effluent wastewater the first one was before treatment and it was higher than the second point which was after treatment. Agriculture activities such as mineral application, fertilizer application, extensive irrigation, and increased weathering were linked to an increase in silicate concentration **(Juttner *et al*., 1996**).

Algae are a wide category of eukaryotic organisms ranged from unicellular like *Chlorella* to multicellular like giant kelp and to huge brown algae that can grow up to 50 meters. Several reports had been showed that algae can absorb phosphorus, nitrate and some heavy metals leading to an enhancement quality of water (**Davis *et al*., 2003; Li and Tao 2015**). The benefits of algal biosorbent can compensate for the drawbacks of commercial resins, which have to reduce sorption efficiency at lower metal concentrations in wastewaters (**Eccles, 1999**). The majority of studies focus on the removal efficiency of metals by dry algal biomass, meaning that dead cells may absorb more metals than living cells (**Mehta and Gaur, 2005**). Bacteria or (bacterial exo polysaccharides), land plants or (their products), aquatic plants, algae, fungi, and peat moss have all gotten a lot of attention recently for their ability to remove heavy metals from the surrounding media (**Sandau *et al.*, 1996; Iyer *et al*., 2004**). The discharge of unaffected tannery wastewater containing toxic substances of heavy metals in the ecosystem is one of the most important environmental and health challenges in our society **(Mofeed *et al.*, 2021)**. Therefore there is a rapidly need for the development of novel, eco-friendly, cost-effective and efficient, methods for the remediation of inorganic metals (Hg, Cd, Cr and Pb) released into the environment. Biosorption is a physiochemical property of biological material that causes contaminants, primarily HMs, to be removed from industrial wastewater by covalent bonding (**He and Chen 2014; Zeraatkar *et al*., 2016; Salama *et al*., 2019**). Metal biosorption from aqueous solutions has the potential to be a useful wastewater treatment system. It focuses on biological materials ability to absorb heavy metals ions from wastewater through metabolically mediated or physicochemical uptake pathways (which can include dead or living algae, seaweeds and so on (**Fard *et al*., 2011; Chalivendra, 2014**). Protons compete with metal cations for binding sites because ion exchange is the mechanism of biosorption, and pH is the most important regulatory parameter that determines the availability of the site to the sorbate. The existence of the biosorbent and the availability of binding sites, as well as the biomass content, are two other significant variables in biosorption.

During the entire investigation period of present study the biosorption performance affected by two factors; contact time and biomass amount/weight the following was evident from data presented within the study. According to the contact time especially in first 15 min the biosorption efficiency was 50%, however after about 60 min the efficiency reached to 95.8 and 92.7% or more for dry and fresh algae, after 120 min, the efficiency rate remained stable or was slightly lower than 60 min at biomass weight 1 $g.L^{-1}$, the biosorption capacity reached to the maximum while at biomass weight 0.2 $g.L^{-1}$ the capacity of biomass for binding metal ions was the lowest. Several reports had been showed that algae can absorb phosphorus, heavy metals and nitrateleading to an enhancement of the water quality (**Davis *et al*., 2003**). Metal biosorption experiments have been conducted with freshwater green algae (*Chlamydomonas reinhardtii, Cladophora spp., Chlorella spp., and Scenedesmus spp.*)where at low quantities of metals in aqueous solutions, algal biosorbents retain a high metal sorption effectiveness

(**Mehta and Gaur, 2005**). Algal biosorbents have the potential to compensate for the deficiencies of commercial resins, which have reduced sorption effectiveness at lower metal concentrations in wastewaters. (**Eccles, 1999**). *S. obliques* immobilized in alginate beads may be used for cadmium bioremediation processes at low concentrations of the metals, since the presence of viable micro-algae biomass increases the alginate's removal capability **(Liu *et al*., 2020)**. Immobilizedcellsmoreeffective than free cells formetal removal by biomass due to increase inthecellwallpermeability **(Dwivedi,1989).** It is clear that, the biosorption capacity of immobilized beads of *S. obliquus* for Pb, Cd, Fe and Cu metal ions was higher than immobilized beads for Mn, Co and Zn during the entire period of our work.The treatment corresponding to alginate beads with immobilized *S. obliquus*biomass is estimated to be the best for Pb elimination. At 60 min, it was discovered that the micro-algae improves its metal removal potential at low metal concentrations, achieving a removal percentage of 59.67 % with *S. obliquus*.The biosorption efficiency for Pb metal ion was in a small scale when compared to other metal ions of the present study within effluent waste water. The result by (**Ardila *et al*., 2017**) investigated the immobilized *S. obliquus* had the ability to adsorb Cr metal ions from tannery wastewater, with removal levels up to 35.3 % (**Dwivedi, 1989**). Another study by (**Aksu, 1998**) reported that immobilization appears to increase the amount of metal accumulated by biomass. it's worth mentioning that biosorbtion efficiency for Pb, Zn and Co metal ions by two immobilized beads of green microalgae *S. obliquus* within petrochemical industrial effluent had the lowest capacity/or efficiency of biosorption in comparison to the overall biosorption potential of other metal ions (Fe, Mn, Cu and Cd) after the same time, during the whole period of investigation.Consequently as obvious from presented results and data the immobilized alginate beads of *S. obliquus* had the superiority in adsorbtion capacity for different metal ions.

CONCLUSION

Many studies have tried various methods for removal of heavy metal from industrial wastewater. Through the obtained results, testing the green micro-alga *S. obliquus* as a biosorbent proved its superior ability to remove heavy metals from aquas industrial wastewater that negatively adversely affect the environment. Where, during the first 15 min contact time the biosorption efficiency was about to 50%, however after 60 min. only the efficiency reached to 95.8%. The results proved differences in the ability to remove different heavy metals by bio-accumulation in algal cells, where the highest results were for lead, cadmium and iron, followed by copper and nickel, then came the rest of the heavy metals (manganese, cobalt and zinc, in order). Which makes us take into account types of heavy metals to be removed from wastewater so that we can choose the appropriate adsorbent in order to obtain the best removal efficiency. So, we recommend using*S. obliquus* as low-cost, eco-friendly and efficient biosorpent to eliminate some heavy metals from the petrochemical's industrial effluent wastewater.

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