

BIOSORPTION OF CADMIUM AND LEAD USING MICROALGAE *SPIRULINA SP.* ISOLATED FROM KOYA CITY (IRAQ)

ABDULKAREEM, P. M.^{1*} – ANWER, S. S.²

¹*Department of Biology, Health and Sciences Faculty, Koya University, Erbil, Iraq*

²*Department of Clinical Biochemistry, College of Health Sciences, Hawler Medical University,
Erbil, Iraq
(phone: +964-750-494-3730)*

**Corresponding author*

e-mail: parween.mohsin@koyauniversity.org; phone: +964-750-441-2402

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Abstract. The alternative to the traditional method for heavy metal uptake is biosorption. The potential of *Spirulina sp.* for biosorption of waste effluent was examined. In this study, morphology and molecular features of microalgae *Spirulina sp.* isolated from Koya city were identified on. The rate of biosorption of cadmium (Cd²⁺) and lead (Pb²⁺) were studied by using a spectrophotometer. The optimum uptake for both metals Cd²⁺ and Pb²⁺ showed at low metal concentration of 100 mg l⁻¹ 88.8% and 93.6%, respectively. *Spirulina sp.* showed the ability to remove metals at all pH values 38.1% and 85.8% for Cd²⁺ and 34.6% and 88.4% for the Pb²⁺ with maximum uptake at 30 °C 85.7% for Cd and Pb optimum uptake was 88.4%. Optimum removal showed at agitation rate 150 rpm 87.1) in Cd²⁺, and Pb²⁺ optimum adsorption at 150 rpm was 89.9%. Both metals showed optimum adsorption at 1.5 g l⁻¹ of algal biomass, Cd²⁺ removed 86.7%, and Pb²⁺ 89.2%. Pb²⁺ showed the best performance for metal biosorption.

Keywords: metal uptake, algal biomass, temperature, agitation rate, pH

Introduction

Microalgae include those chlorophyll-bearing organisms that are thalloid (Vashishta et al., 2002). Nearly 75% of algal species are microalgae that contribute 30-40% of oxygen to the atmosphere (Ponuswamy et al., 2013). They are the primary producers who switch water and carbon dioxide into carbohydrates and oxygen in the presence of the sun (Chisti, 2008). Microalgae biomass is often measured with a concentration of chlorophyll and can provide a useful index of potential production.

Cadmium (Cd²⁺) is a chemical element that involves different machinery to induce its harmful effect on many biological activities in humans, animals, and various other organisms. In humans, the adverse effect of Cd²⁺ is not only limited to the kidney and bone, but it includes nearly every organ and tissue where it accumulates, which argues the need for public health measures aimed at reducing exposure. There are many ways in which this heavy metal can be suppressed in its activities, forming the prospect for reduced metal toxicity involving Cd²⁺ (Hogan, 2010; Sharma et al., 2015).

Lead (Pb²⁺) is number two on the “Top 20 List” of toxic and hazardous substances of the ATSDR. Pb²⁺ accounts for the majority of pediatric heavy metal poisoning (Roberts, 1999). Pb²⁺ occurs in many forms worldwide in natural sources and is one of the most commonly and uniformly distributed trace metals (Jackson et al., 2005). Pb²⁺ creates several adverse effects in both males and females on the reproductive system. Common impacts shown in males involve decreased libido, unusual spermatogenesis (decreased motility and number), chromosomal harm, infertility, abnormal prostatic function, and

serum testosterone alterations. Women, on the other hand are more prone to infertility, miscarriage, premature membrane rupture, pre-eclampsia, pregnancy hypertension, and premature delivery (Flora et al., 2011). Also, during gestation, the direct influence of Pb^{+2} on the developmental stages of the fetus was registered (Saleh et al., 2009; Flora et al., 2012). Hasson (2018) measured concentrations of some heavy metals in various imported and locally produced vegetable crops in Baghdad city, including root crops and the daily intake of four main heavy metals (Cd, Cu, Zn, and Pb) had been estimated, which revealed a high consumption of Cd^{+2} (310 and 372 μg per day) for imported and local vegetables that affect human health. Because of multiple anthropogenic activities, heavy metals are released into the fairway. Their therapy is of particular significance since heavy metals are non-biodegradable and cause environmental damage due to their poisoning to many crops, livestock, and microorganisms in the aquatic environment (Sadettin and Donmez, 2007; Kaplan, 2013; Salih et al., 2017). Traditional methods for the removal of dissolved heavy metal ions involve chemical precipitation, electrochemical treatment, chemical oxidation and reduction, ion exchange, filtration and evaporative recovery (Aksu et al., 2002). Biosorption of heavy metals by microalgae is generally a biphasic process involving adsorption by materials associated with extracellular cells, such as polysaccharides and cell wall components (Roy et al., 1993; Das et al., 2008).

Materials and methods

Sampling and cultivation

Microalgae sample was isolated along Taq-Taq river in Koya city during May-September 2018, Water samples were taken from a depth between 10 and 50 cm from the bottom (Prasad et al., 2013) and plated Blue-green 11 medium (BG11) as described by Allen (1968) and Oswaled (1988). The cultures were incubated at pH 7.8 and 28 °C under constant light 800Lx. Two weeks later, following the growth of colonies on the agar media, the colonies were removed with pasture micropipettes and gently blown into the liquid medium, then incubated at 28 °C, 800 Lx, and pH 7.8.

Morphological and molecular identification of algae

After a 15-days single colony removed and examined under a light microscope for identification purposes based on their morphology as described by Prescott (1963), Desikachary (1968), Rippka (1988) and Holt et al. (1994). Further identification was performed by polymerase chain reaction (PCR) to confirm algal genera.

Extraction of DNA

Genomic DNA extraction was performed using a commercial extraction kit (Thermofisher, USA) according to the manufacture's instruction. DNA samples were then checked for their quantity and quality using Bio-photometer (Eppendorf, German).

PCR amplification and DNA sequencing

The targeted region of 16S rRNA of algae amplified by PCR using universal primers, forward primer A8F (5- AGAGTTTGATCCTGGCTCAG-3), and the reverse primer A1492R (5- TACGGCTACCTTGTTACGACTT- 3). Primers were previously used by

Ponnuswamy et al. (2008). A total of 50 µl volume of the reaction mixture was prepared, which contained 40–55 ng DNA, 100 mM of each primer, 0.05 U/µl Taq DNA polymerase, 4 mM MgCl₂, and 0.4 mM of each dNTP.

ABI 3130XL genetic analyzer (USA) was employed to find the order of the nucleotides of 16S rRNA for the algae sample. The PCR products were cut from the agarose gel and utilized as a base of DNA template for sequence-specific PCR amplification and sent for nucleotide sequencing analysis to the private medical genetic laboratory in Intergene Genetic Center, Ankara, Turkey.

Preparation of biomass

Spirulina sp. biomass initially dried from moisture on an aluminium tray, kept in the oven at 80 °C for 24 h, after cooling, this material was subjected to sieve analysis.

Chemicals

Cadmium nitrate tetrahydrate (Cd(NO₃)₂•4H₂O) and lead nitrate (Pb(NO₃)₂) were used to prepare 1000 mg l⁻¹ inventory metal ion solutions that were diluted to produce metal ion alternatives for adsorption. Before the microorganisms were mixed, the pH value of each metal solution was adapted with 1 mol l⁻¹ NaOH to the appropriate value (Fan et al., 2008).

Batch experiments

Batch experiments were performed at different initial metal concentrations 100-500 mg l⁻¹, pH 6.5-8.5, temperature 20-40 °C, agitation speed from zero-200 rpm, and biosorbent dosage 0.3-1.5 g l⁻¹ according to Aksu (2005). The rate of biosorption was read using spectrophotometer at 540 nm for Cd⁺² and 530 nm for the Pb⁺². Metal containing medium was used as blank (control medium contained both metals and solution without biosorbent to observe any reaction of the solution with metals). Each experiment was carried out in triplicate. The yield of biosorption was determined (*Eq. 1*) by the formula below (Blanco et al., 1999; Aksu, 2005):

$$\text{Biosorption \%} = (C_0 - C) \cdot 100 / C_0 \quad (\text{Eq.1})$$

C₀ - Initial metal concentration, C - Final metal concentration.

Statistical analysis

The test results were statistically analyzed with a one-way variance analysis (ANOVA) followed by a Tukey post hoc test ($P \leq 0.5$) to assess significant differences between the experimental classes.

Results

Morphological and molecular identification of algal strain

After observation under the microscope at different magnifications and according to the guide of taxonomic, in the isolated and purified samples trichomes width without akinetic and heterocyst, cell length, pointed calyptas, type of coil and helix shape (*Fig.*

1) were observed and identified as *Spirulina* according to Ciferri (1983), Rippka (1988), Tomasseli (1997) and Prasal et al. (2013).

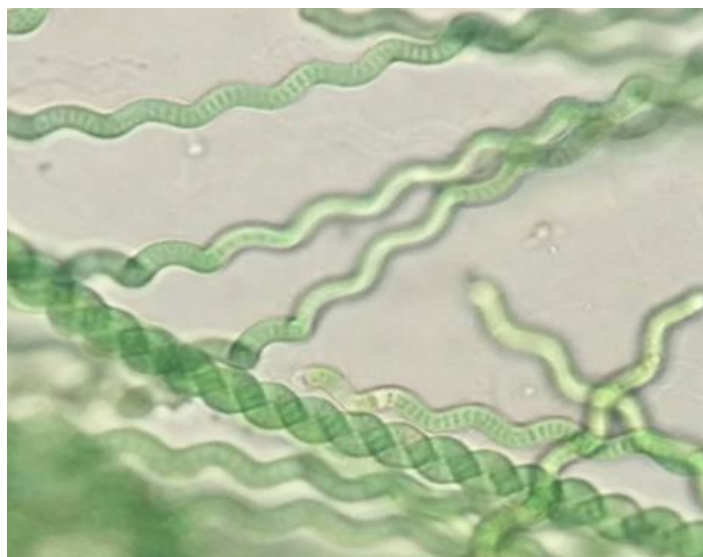


Figure 1. Microscopic observation of *Spirulina* sp.

By molecular examination, the sequence from 16S rDNA of algae specimen was made of 1200-1400 bp (amplified fragment was 1468 bp, also after testing some non-nucleotides were removed, linked to the character of the sequencing assessment) and then placed to BLAST compared to other stored algae sequence genus from the Gene Bank database. The primary sequence analysis using a universal primer of the observed specimen disclosed that algae from Koya city belong to the *Spirulina* sp. genus. Its rDNA conforms to the same rDNA sequence fragment symbol required at the Gene Bank in the National Center for Biotechnology Information (NCBI), pairwise analysis of the algae specimen is shown in *Figure 2*.

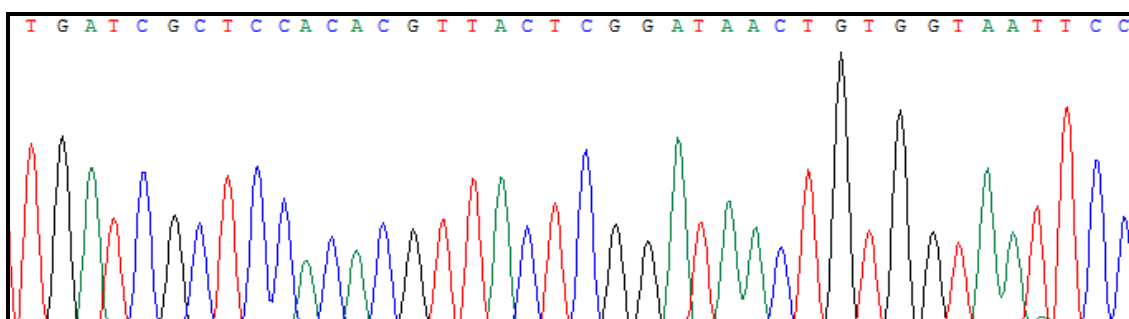


Figure 2. The partial sequencing result of 16S rDNA of *Spirulina* sp.

Effect of Cd and Pb concentrations on biosorption

Figures 3 and 4 showed the effect of initial metal ion concentration (Cd^{+2} and Pb^{+2}) ranging from (100-500 mg l^{-1}). Optimum removal showed during 15 h, and the rapid removal showed at hour 3; by increasing metal concentration, the biosorption process decreased. In the case of Cd^{+2} , the optimum adsorption (88.8%) was recorded at a

concentration of 100 mg l⁻¹, while high removal of Pb⁺² was (93.6%) at the same concentration. Increasing concentration of both metal ions led to a decline in adsorption, indicating saturation of all the binding sites on the algal surface. The result showed that there were no significant differences between heavy metal concentration with Cd⁺² (*p*-value = 0.0936) and Pb⁺² (*p*-value = 0.0981).

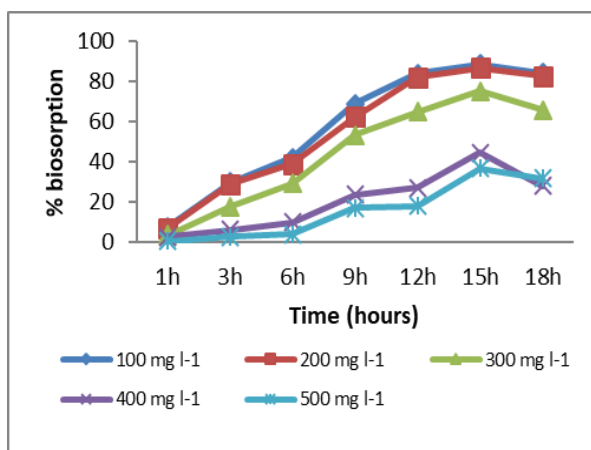


Figure 3. Effect of metal Cd⁺² concentration on biosorption process (pH 7, 35 °C, 100 rpm)

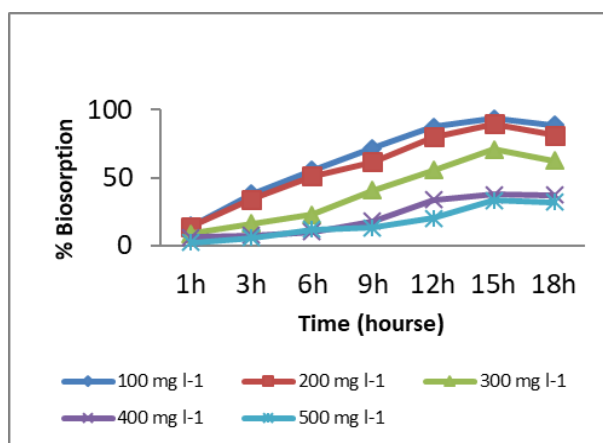


Figure 4. Effect of metal Pb⁺² concentration on biosorption process (pH 7, 35 °C, 100 rpm)

Effect of pH on biosorption of Cd and Pb by *Spirulina* sp.

The pH is an important factor in biosorption processes. To find an appropriate pH for *Spirulina* sp. efficient heavy metal biosorption, studies were conducted at five distinct original pH values (6.5, 7, 7.5, 8, 8.5), *Spirulina* sp. showed ability to remove metals at all pH values 38.1% and 85.8% for Cd⁺² and 34.6% and 88.4% for the Pb⁺². The Statistical analysis showed significant differences among pH and adsorption percentage of both Cd⁺² and Pb⁺² ions. A significant correlation had been recorded among pH degree change with both Cd⁺² (*p*-value = 0.0039) and Pb⁺² (*p*-value = 0.0065), which means with increasing pH, the adsorption percent increased, and the peak of adsorption percent was reached to 85.8 for Cd⁺² and 88.4 for Pb⁺² ion. The optimum removal showed at pH 8 (Cd⁺² 85.8% and Pb⁺² 88.4%) as shown in *Table 1*.

Table 1. Effect of pH on biosorption of Cd^{+2} and Pb^{+2} by *Spirulina* sp. 200 mg l⁻¹, 35 °C, 100 rpm

| pH | <i>Spirulina</i> sp. (Cd^{+2}) | | | <i>Spirulina</i> sp. (Pb^{+2}) | | |
|-----|--------------------------------------|---------------------------------------|-------------|--------------------------------------|---------------------------------------|-------------|
| | C ₀ (mg l ⁻¹) | CC ₀ (mg l ⁻¹) | %Adsorption | C ₀ (mg l ⁻¹) | CC ₀ (mg l ⁻¹) | %Adsorption |
| 6.5 | 167.9 | 63.9 | 38.1 | 162.7 | 56.3 | 34.6 |
| 7 | 178.6 | 122.2 | 68.4 | 172.7 | 108.7 | 62.9 |
| 7.5 | 188.6 | 157.2 | 83.3 | 178.7 | 147.4 | 82.5 |
| 8 | 214.3 | 183.9 | 85.8 | 192 | 169.7 | 88.4 |
| 8.5 | 202.1 | 169.6 | 83.9 | 183.3 | 156.6 | 85.5 |

Effect of temperature on biosorption of Cd^{+2} and Pb^{+2} by *Spirulina* sp.

To find an appropriate temperature for *Spirulina* sp.'s on efficient heavy metal adsorption, studies were conducted at five distinct temperature values (20, 25, 30, 35, and 40 °C) as shown in Figures 5 and 6. Rapid removal showed in first hour at 20 °C. Optimum removal of Cd^{+2} showed at a temperature of 30 °C (85.7%), and for Pb^{+2} , optimum removal was (88.4%) at the same temperature. The effect of temperature showed significant differences among temperature and adsorption percentage of both Cd^{+2} and Pb^{+2} ions. Significant differences had been stated related to temperature change in both Cd^{+2} (*p*-value = 0.025) and Pb^{+2} (*p*-value = 0.0248) biosorption.

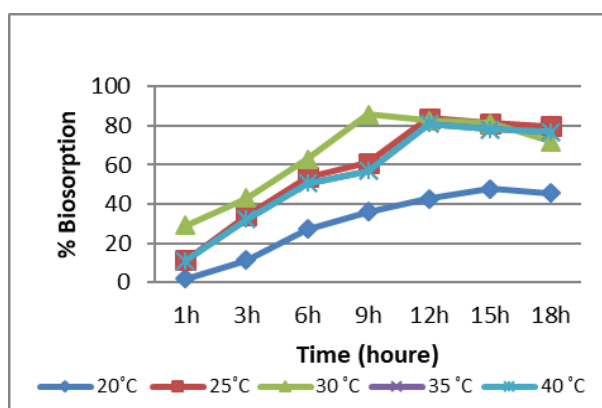


Figure 5. Effect of temperature on Cd^{+2} adsorption C₀- 200 mg l⁻¹, pH 8, 100 rpm

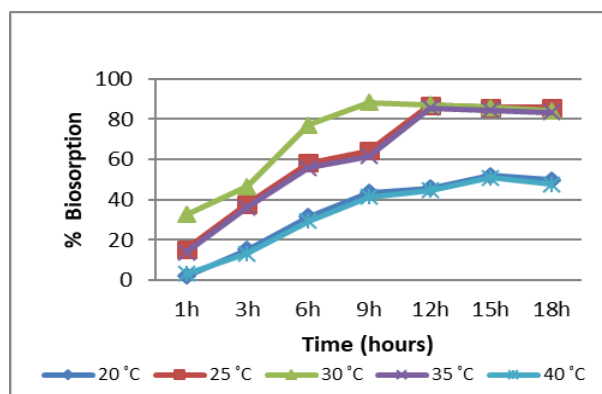


Figure 6. Effect of temperature on Pb^{+2} adsorption by *Spirulina* sp. C₀- 200 mg l⁻¹, pH 8, 100 rpm

Effect of agitation rate (rpm) on biosorption of Cd⁺² and Pb⁺² by *Spirulina* sp.

The effect of agitation rate on Cd⁺² and Pb⁺² adsorption by *Spirulina* sp. is shown in Table 2. At equilibrium, metal uptake increases with an increase of agitation rate - rpm (unshaken to 150 rpm), and the optimum removal showed at rpm 150 (87.1%) in Cd⁺² and Pb⁺² showed optimum adsorption at 150 rpm (89.9%). The adsorption process decreased with 200 rpm. The *p*-value of Cd⁺² (*p*-value = 0.469) and Pb⁺² (*p*-value = 0.5031) that showed no significant correlation between rpm, in spite of increasing rpm, the adsorption percent of Cd⁺² and Pb⁺² ions were not changed significantly.

Table 2. Effect of agitation rate on biosorption of Cd⁺² and Pb⁺² by *Spirulina* sp. (200 mg l⁻¹ initial metal concentration (C₀), pH 8, 30 °C)

| Agitation rate | <i>Spirulina</i> sp. (Cd ⁺²) | | | <i>Spirulina</i> sp. (Pb ⁺²) | | |
|-------------------------|--|---------------------------------------|-------------|--|---------------------------------------|-------------|
| | C ₀ (mg l ⁻¹) | CC ₀ (mg l ⁻¹) | %Adsorption | C ₀ (mg l ⁻¹) | CC ₀ (mg l ⁻¹) | %Adsorption |
| Unshaken (non-agitated) | 122.1 | 26.1 | 21.3 | 186 | 59.0 | 31.7 |
| 50 rpm | 127.9 | 76.5 | 59.8 | 197.3 | 102.6 | 52.0 |
| 100 rpm | 141.4 | 119.6 | 84.6 | 208 | 179 | 86.1 |
| 150 rpm | 146.4 | 124.6 | 87.1 | 212 | 185 | 89.9 |
| 200 rpm | 132.1 | 106.0 | 80.3 | 204 | 174.3 | 85.5 |

Effect of algal biomass on biosorption of Cd⁺² and Pb⁺² by *Spirulina* sp.

The effect of biomass on Cd⁺² and Pb⁺² ion removal is indicated in Figures 7 and 8. At equilibrium, metal uptake increases with an increase in biomass from 0.3 to 1.5 g l⁻¹. Optimum removal showed during 6 h in a biomass of 1.5 g l⁻¹, rapid removal showed at 1 h in a biomass of 0.3 g ml⁻¹. Both metals showed optimum adsorption at 1.5 g l⁻¹ of algal biosorbent 86.7%, of Cd⁺² and 89.2% of Pb⁺² were removed.

The result showed significant differences among algal biomass and adsorption percent of both Cd⁺² and Pb⁺² ions. The *p*-value for both Cd⁺² (*p*-value = 0.0095) and Pb⁺² (*p*-value = 0.0036), means with increasing of algal biomass, the adsorption percent increase and the peak of adsorption percent was reached to 86.7 for Cd⁺² and 89.2 for Pb⁺² ion.

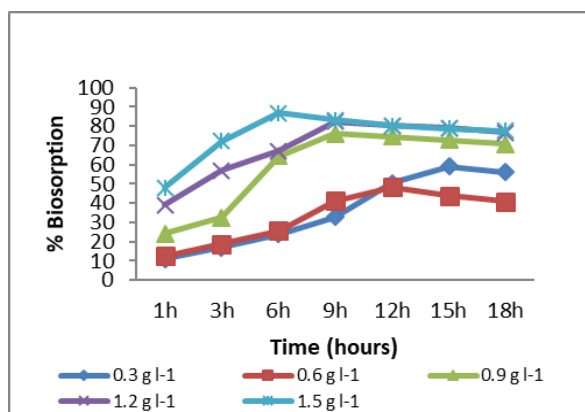


Figure 7. Effect of biosorbent dosage on Cd⁺² adsorption (200 mg l⁻¹ initial metal concentration, pH 8, 30 °C, 150 rpm)

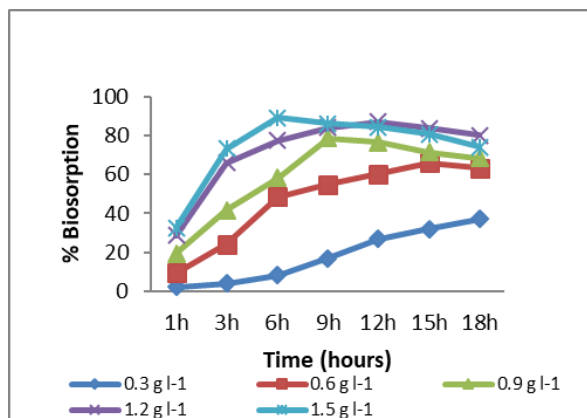


Figure 8. Effect of biosorbent dosage on Pb^{+2} adsorption (200 mg l^{-1} initial metal concentration, pH 8, $30\text{ }^{\circ}\text{C}$, 150 rpm)

Scanning electron microscopy (SEM) analysis

The biosorbent morphology, which is cell/filamentous algae-binding- Pb^{+2} and Cd^{+2} , was examined using SEM with 200x magnification to demonstrate the porous places of cell/filamentous algae as seen in (Fig. 9a, b, c) illustrate the efficient binding with Cd^{+2} and Pb^{+2} , respectively. However, in composites with a greater content of Cd^{+2} and Pb^{+2} , the particles are often abnormal in form and circular in the preparing of irregular surface, this distinctive shift in the analyzed SEM pictures highlights the existence of Cd^{+2} .

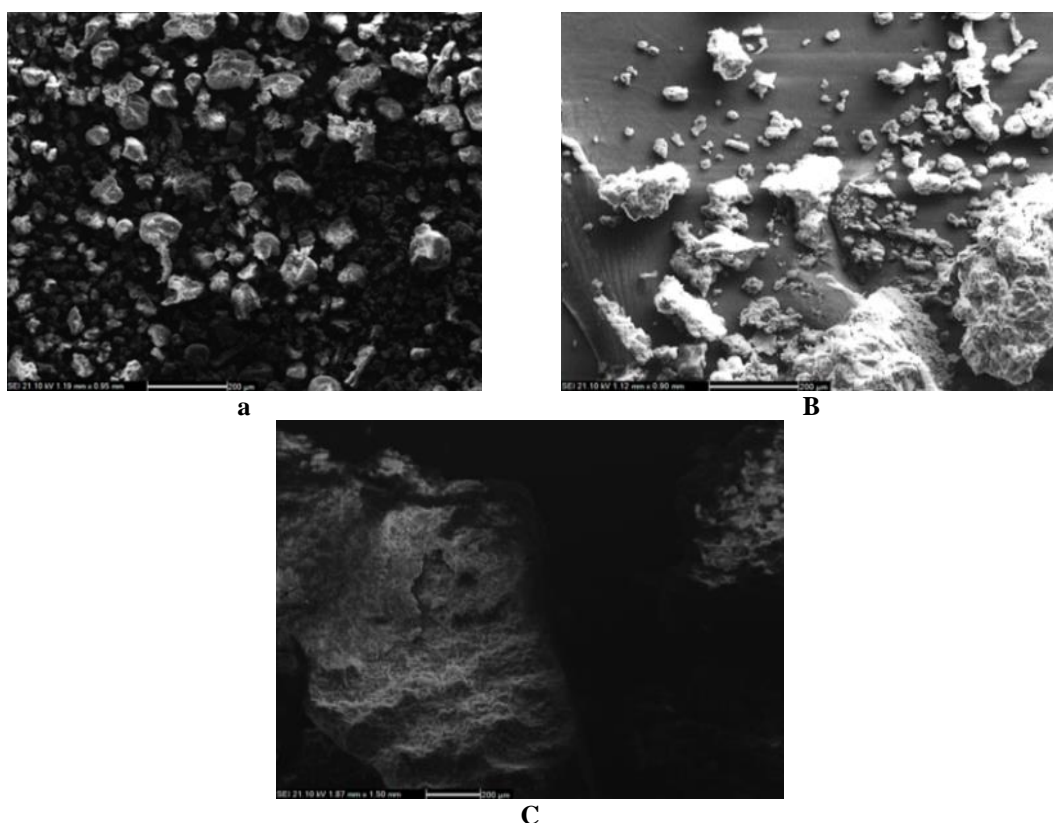


Figure 9. SEM image *Spirulina* (a) before adsorption, (c) and (c) binding Cd^{+2} and Pb^{+2} and on the cell/filamentous algae surface

Discussion

Isolated and purified microalgae strain observed under microscope and culture showed filamentous spiral-shaped microalgae that were identified as *Spirulina* sp. and the strain was confirmed by using molecular identification. The same characteristics showed by isolated *Spirulina* from (Koya city). In many cases, the selection of efficient organism is known to be essential tools in the successful treatment of wastewater effluents rich in toxic compounds such as Cd (II) and Pb (II) in textile industry effluents. Microalgae were proposed as perfect candidates for the wastewater treatment system. In the present research, *Spirulina* strain was used as biomass for Cd⁺² and Pb⁺² adsorption process under various initial metal concentrations, pH, biosorbent dosage, temperature, and agitation velocity. In the experiments, it was showed that there are different factors affecting the biosorption of heavy metals by *Spirulina* sp. The present results were in agreement with Gupta and Rastogi (2008), König-Péter et al. (2015). Al-Homaidan et al. (2015) and Palaniswamy and Veluchamy (2017). As shown in the results, Pb⁺² biosorption was more prominent than Cd⁺² biosorption. Similar results were obtained by Domínguez-Bocanegra et al. (2013), who reported that Pb⁺² biosorption was more prominent than Cd⁺² and nickel. By increasing the original metal concentration, the proportion of biosorption of metal ions reduced (Figs. 3 and 4) same result was obtained by Abdel-Aty et al. (2013).

The fast adsorption of the metals shows that the sorption method may be ionic where the acidic (anionic) dye molecules bind to the multiple strongly loaded organic functional groups present on the biomass surface (Gulnaz et al., 2004).

pH is one of the most significant parameters affecting original adsorption frequency and ability; the optimum pH was 8 (Table 1). This phenomenon is due to the nature of the chemical interaction of each heavy metal with algal cell walls. Hydrogen ion also functions as a bridging ligand between the cyanobacterial cell wall and the heavy metal molecules, and the results were in agreement with Abdel-Aty et al. (2013) who confirmed that the biosorption of Cd (II) and Pb (II) was increased with increasing the pH value and disagreed with König-Péter et al. (2015) who stated that the biosorption process was successful at pH 5–6 for Pb(II) and pH 4–6 for Cd(II) adsorption. Metals biosorption on *Spirulina* sp. has been researched at various temperatures of 20-40 °C, and the optimum temperature for adsorption of metals showed at 30 °C and the lowest adsorption at 20 °C (Figs. 5 and 6). However, temperature variation in comparisons had an important impact on the removal of Cd⁺² and Pb⁺² of *Spirulina* sp. The results of this investigation were in agreement with Donmez et al. (1999), Dhargalkar (2004) and König-Péter et al. (2015). To determine the impact of agitation velocity on metal adsorption, a series of studies at distinct agitation degrees (unagitated – 200 rpm) were conducted and shown in Table 2 indicating that the degree of agitation positively affected the sorption process rise in agitation rate biosorption of metals improved optimum adsorption at 150 rpm with agitation levels greater than 200 rpm. This relates to the weakening interaction between the sorbent locations and the metal ion described by the Van-der Waals interaction Anwer and Abdullah (2017) when they study the effect of agitation rate on direct Blue nine adsorption using baker's yeast.

Figures 7 and 8 showed the effect of biosorbent dosage to remove heavy metal, Optimum removal showed in biomass 1.5 at 6 h, fast removal showed in biomass 0.3 at 1 h. By increasing biosorbent dosage adsorption of biomass increased due to increase in the region and the accessibility of more biosorption locations (Mane et al., 2007). After

biosorption process ended the algal biomass accumulated and used for further study which was desorption experiments.

However, in composites with a higher content of Cd^{+2} and Pb^{+2} , the particles are mainly abnormal in form and circular in the preparation of a rougher surface, this distinctive shift in the analyses SEM pictures highlights the existence of Cd^{+2} and Pb^{+2} in the cell/filamentous algae surface this result is agreed with Salih et al. (2017).

Conclusion

Biosorption of heavy metals (Cd^{+2} and Pb^{+2}) increased by increasing biosorbent (*Spirulina* sp.). This study shows that isolated *Spirulina* sp. deserves attention as biosorbent, which can be utilized in the treatment of wastewater effluents containing heavy metals before the treatment, and the technique can be modified further to make it applicable on a large scale to clean up heavy metal contamination using dried biomass of *Spirulina* sp. In most of the studies that done previously by researchers spirulina cells were purchased in the dried form while in our research we isolated spirulina from different site of Koya city which was not conducted before, and cultured *Spirulina* sp. by using different parameters to obtain high rate of biomass in lab.

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