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Eco-Friendly Biosorption of Heavy Metals by *Chlorella vulgaris*: A Green Solution for Wastewater Remediation

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ABSTRACT

This study highlighted the environmental and health threats posed by heavy metal pollution in wastewater and explored the use of *Chlorella vulgaris* as a sustainable biosorbent for metal removal. The dried algal biomass was found to contain moisture, carbohydrates, protein, fats, and ash in specific proportions. Key water quality parameters such as pH, conductivity, turbidity, total dissolved solids (TDS), and chloride levels were analyzed before and after treatment. The results showed that metal removal efficiency varied by type and conditions. The smallest mesh size (0.063µm) provided the highest iron removal (16.5–27.5%). Lead removal was highly effective, reaching 93.3% at 4mg/ L concentration. Nickel and zinc also showed significant reductions. Statistical analysis indicated strong adsorption affinities for copper and zinc, while iron and cobalt were less effectively adsorbed. A cluster analysis revealed distinct groupings based on adsorption behavior, with iron and copper showing similarities, while lead and cadmium formed a separate cluster with lower removal efficiency.Overall, the findings demonstrate the potential of *C. vulgaris* as a cost-effective biosorbent for heavy metal removal, supporting its application in sustainable water treatment and environmental remediation efforts.

INTRODUCTION

Heavy metals are materials with atomic weights among 63.5 and 200.6, having a particular gravity above 5.0. Industrial wastewater accommodates the biggest supply of heavy metals pollutants **(Mosleh, *et al*., 2006)**. In ecological terms, any steel or metalloid that contaminates the surroundings and cannot be biologically decomposed is classed as a pollutant-and such is described as heavy metals **(Estrella & Garcia, 2009; Mosleh, 2013)**. Over the latest years, wastewater pollutants via way of means of heavy metals have grown to be a motive for principal concern. Heavy metals were assumed to be added to aquatic environments through business strategies (painting, mining, smelting, vehicle exhaust, battery manufacturing, petroleum refining, pigments), agriculture (fertilizers and pesticides), and waste disposal (**Lesmana, *et al*., 2009; Ardila, *et al*., 2017**). The mobility, non-degradability, and bioaccumulation capacity of specific heavy metals regularly improve alarm regarding its feasible danger to the atmosphere and human health, thereby interfering with vital organic strategies even at a decrease attention and inflicting diseases. While a number of those metals are taken into consideration vital micro-vitamins for plant growth (such consists of zinc, copper, manganese, nickel, and cobalt), others are without regarded organic features and pitifully poisonous, inclusive of Cd, Pb, and Hg **(Gaur & Adholeya, 2004; Mosleh & Omar Almagrabi, 2012)**. Such dangerous heavy metals consist of mercury, cadmium, copper, zinc, lead, and nickel that are famous freshwater and marine pollutants **(Travieso, *et al*., 1999; Mehta & Gaur, 2005; Singh, *et al*., 2007; Mofeed & Mosleh, 2013; Mofeed, 2017)**.

The traditional techniques for the elimination of heavy metals from biologically infected wastewater consist of ion exchange, contaminant reduction, membrane filtration, chemical precipitation, activated carbon adsorption, nanotechnology treatment, superior oxidation, and electrochemical elimination **(Pugazhenthiran, *et al*., 2015)**. While effective, those techniques have a tendency to be high priced and feature numerous shortcomings, which include low selectivity for metallic ions, excessive electricity consumption, incomplete elimination, and technology of poisonous waste. This ignites the world`s hobby in cheaper, safer, and greater green techniques for heavy metals extraction. Biosorption is composed withinside the use of those organisms to interrupt down or detoxify environmental pollution like heavy metals, to a much less poisonous shape both *in situ* or *ex situ* **(Wang & Chen, 2009; 2014)**. There are unique mechanisms for the elimination of heavy metals with the aid of using microorganisms which include biosorption, biomineralization, and biotransformation **(Mosleh *et al*., 2021, 2023)**. It is a biosorption technique, wherein residing organisms like algae, bacteria, fungi, and yeast are used as biosorbents. This technique appears promising with reference to its price-effectiveness and performance **(El-Sayed *et al.*, 2024)**. Biosorption operates primarily based totally on organic substances with their physicochemical homes resulting in the elimination of contaminants like heavy metals from wastewater through ionic or covalent bonding **(He & Chen, 2014; Salama *et al*., 2019; El-Sayed *et al*., 2024)**. This manner takes benefit of the capacity of residing or useless microorganisms, seaweeds, and different organic substances to take in heavy metallic ions from wastewater, with blessings which include low price and excessive performance, much less chemical and organic sludge, simpler biosorbent regeneration, and the ability for metals recovery **(Fard *et al*., 2011; El-Naggar, 2018)**.

Aquatic plants and microalgae are the spine of the aquatic meals chain affecting the better trophic degree and water excellent. Algae represent a huge organization of eukaryotic organisms starting from unicellular paperwork like chlorella, via massive kelp, to huge brown algae developing as much as 50 meters long. They are one of the maximum effective organizations of photosynthetic organisms according to unit place in comparison with different organisms. Contributions of algae to the development of the quality of water include taking over phosphorus, nitrate, and heavy metals **(Davis *et al*., 2003; Rollin, 2011, Mosleh *et al*., 2014; 2021; 2023; El-Sayed, *et al*., 2024)**. Most research centered at the elimination performance of metals with the use of dried algal biomass; it's miles advised that dwelling cells might additionally provide absolutely special attributes of absorption **(Mehta & Gaur, 2005)**. The primary focus of most research is on the metal removal efficiency of dried algal biomass. In general, non-living (dead) cells tend to absorb more metals than living ones **(Mehta & Gaur, 2005)**. This study specifically aimed to evaluate the effectiveness of dried *Chlorella vulgaris* in removing selected heavy metals from wastewater. The objectives were as follows:

1. To assess the quality of wastewater and treated effluent,
2. To evaluate the growth of *C. vulgaris* for heavy metal removal, and
3. To investigate the factors affecting recovery processes using both dried and living algal materials in bioremediation—such as treatment duration, wastewater concentration, and biomass quantity used.

MATERIALS AND METHODS

The strain of green microalgae *C. vulgaris* was obtained from the National Institute of Oceanography and Fishery, Egypt, and its biomass was cultivated using Bold Basal Medium (BBM). Collection and processing of water samples took place in the mornings of July 2023 and 2024 at the industrial drainage site of a petrochemical facility. The samples were kept in a dark environment and transported promptly to the laboratory. Upon arrival, they were thoroughly mixed, and 10 liters of each were filtered through a Millipore filtration system. The first liter was discarded, while the remaining filtrate was stored at 4°C in darkness for subsequent chemical analysis. All chemicals used were of analytical grade, with solutions prepared using distilled water. For physico-chemical analysis, 250mL of the filtered sample was transferred to a polyethylene bottle for testing parameters such as chlorides, total alkalinity, biochemical oxygen demand (BOD), total hardness, ammonia-nitrogen (NH4-N), nitrite-nitrogen (NO2-N), reactive silica, ortho-phosphate, total dissolved phosphorus, sulfate concentration, and hardness (Ca^{2+} and Mg^{2+}), following the methods outlined by **APHA (1985)**. While dissolved oxygen and BOD were analyzed immediately upon arrival, other chemical analyses were conducted the next day. Free dissolved carbon dioxide (CO2) was determined using a modified titration method, where 0.05 N NaOH was added to a 100mL sample containing phenolphthalein indicator until a faint pink color appeared. If the sample turned pink upon adding the indicator, titration continued using 0.05 N HCl instead.

Experimental procedure

Green algae have been gathered from aqueous answers with the aid of using centrifugation at 5000rpm for 10min. Cells have been washed 3 instances with deionized water and dried in an oven at a 100˚C for twenty-four hours or till gaining a regular weight. Samples were cooled in a desiccator for forty five minutes earlier than dry weight determination. For the fats evaluation, about 2g of algal pattern was changed into macerated with water, methanol, and chloroform. After centrifugation, the chloroform layer was evaporated to dryness to determine the fat content **(Pearson, 1981)**. Protein analysis was performed using the method described by **Harold *et al.* (1981)**. In simple terms, calcination was used to determine ash content by applying high temperatures in a muffle furnace until complete carbonization of the organic matter occurred. Moisture content was determined by weighing 5g of the powdered sample, which was then dried in an oven at 70°C for 4–5 hours. The reduction in weight indicated the moisture content.

Biosorption experiments

After incubation, the dried algal biomass was filtered and dried at 75°C for 48 hours, then sieved using mesh sizes of 250, 125, and 63µm. The dried microalgae were mixed with 1L of effluent wastewater and incubated at room temperature with shaking at 250 rpm. Various weights of dried algae (0.25, 0.50, 1.0, 2.0, and 4.0 g) were mixed with 1L of effluent water for different contact times (15, 30, 60, and 120 minutes). Heavy metal concentrations were measured using an ICP Spectrometer (ICAP 6000 series, Thermo Scientific), and the values were calculated using standard calibration curves. The physicochemical parameters of the analyzed water samples—including pH, conductivity, turbidity, total dissolved solids (TDS), color, and salinity—were measured using standard methods outlined by **APHA (1995)**.

The results are summarized in the tables. Before treatment, the total alkalinity of the industrial effluent water was 6mg/ L, which decreased to 4.4mg/ L after treatment. Total hardness increased from 1.48mg/ L before treatment to 3.86mg/ L after treatment. The TDS concentration was reduced from 1,180 to 112mg/ L. The pH increased from 6.2 to 7.8, while the concentration of free dissolved carbon dioxide decreased from 7.25 to 6.5mg/ L. Chloride concentration changed slightly from 16.76mg/ L before treatment to 17.2mg/ L after treatment. Several other parameters, including phosphate, sulfate, nitrite, nitrate, ammonia, and silica, also showed variations before and after treatment, as presented in Table (2).

Statistical analysis

All biosorption experiments were conducted in triplicate to ensure the reliability of the results. The data presented represent mean values with standard deviations. Statistical analysis was performed using Student's T-test for independent samples, utilizing SPSS

14.0 for Windows (SPSS, Michigan Avenue, Chicago, IL, USA), with a significance threshold set at 0.05.

RESULTS

The study examined the composition of *Chlorella vulgaris* biomass, revealing that it contained 9% moisture, 11.38% carbohydrates, 34.24% protein (including 16.80% crude protein), 0.53% fat, and 44.7% ash. Various water quality parameters—including pH, conductivity, turbidity, total dissolved solids (TDS), color, vegetable oil, and salinity—were measured using standard methods.

Physicochemical analysis showed that treatment lowered total alkalinity from 6 to 4.4mg/ L, while total hardness increased from 1.48 to 3.86mg/ L. TDS decreased from 180 to 112mg/ L, and pH ranged between 6.2 and 7.8. Dissolved carbon dioxide concentrations also dropped from 7.25 to 6.5mg/ L. Chloride levels changed slightly, from 16.76 to 17.11mg/ L, alongside alterations in phosphate, sulfate, nitrite, nitrate, ammonia, and silica concentrations.

Heavy metal removal efficiency was heavily influenced by biomass concentration and mesh size. Iron (Fe) elimination reached 16.5–27.5%, with smaller mesh sizes yielding better results. Lead (Pb) showed high removal efficiency, reaching 93.3% at a concentration of 4 mg/L with a 0.063-µm mesh. Nickel (Ni) removal improved with finer meshes, achieving up to 11 mg/L. Zinc (Zn) was efficiently removed, with concentrations rising from 14.5 to 18.8mg/ L as mesh size decreased.

Statistical analysis showed that *C. vulgaris* had the strongest adsorption affinity for copper (Cu) and zinc (Zn), whereas iron (Fe) and cobalt (Co) were less effectively adsorbed. Lead, nickel, manganese, and cadmium exhibited lower removal efficiencies. Cluster analysis grouped iron and copper together, while lead and cadmium formed a separate group with lower adsorption rates. Nickel and zinc also clustered together, indicating similar removal behaviors.

Overall, *C. vulgaris* demonstrated strong biosorption potential, particularly for copper and zinc, supporting its application as a cost-effective, eco-friendly method for heavy metal removal from wastewater.

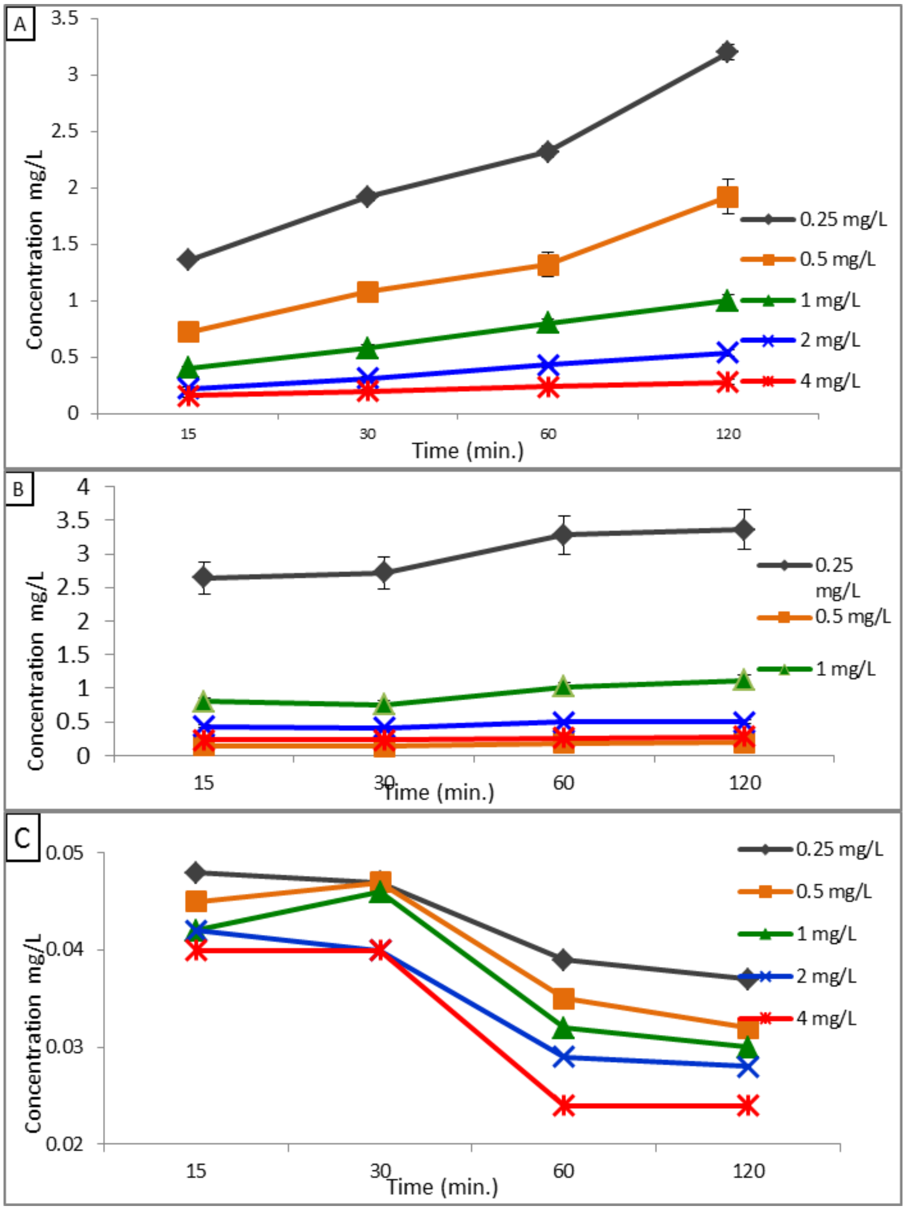

Fig. 1. The concentration of Cu (mg/L) adsorbed by dried *C. vulgaris* at various biomass mesh sizes: A: 0.25µm, B: 0.125µm, and C: 0.063µm

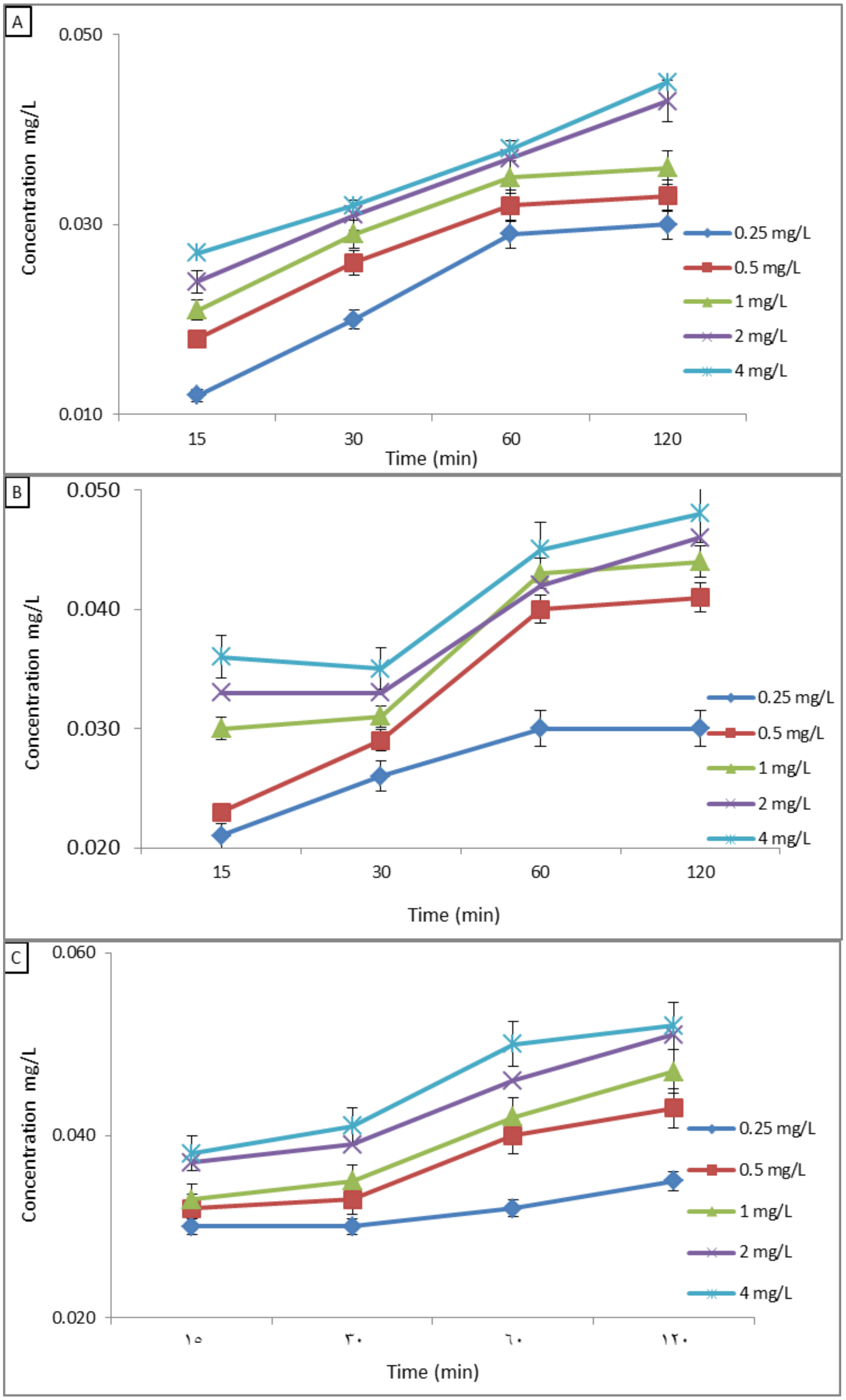

Fig. 2. The Concentration of Cd (mg/L) adsorbed by *C. vulgaris* according to different mesh sizes of biomass A: 0.25µm, B: 0.125µm and C: 0.063µm

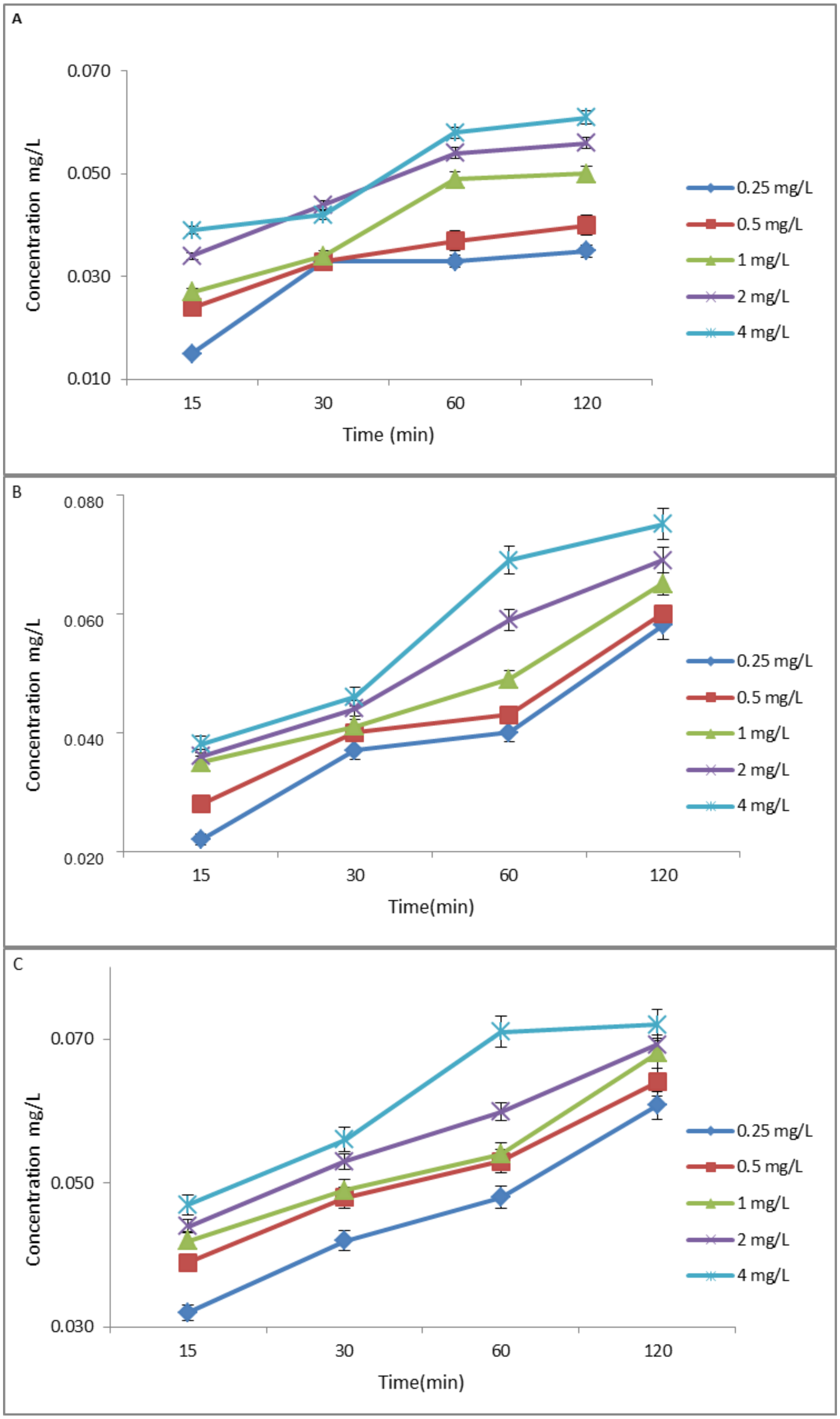

Fig. 3. The cocentration of Mn (mg/L) adsorbed by *C. vulgaris* according to different mesh sizes of biomass A: 0.25µm, B: 0.125µm and C: 0.063µm

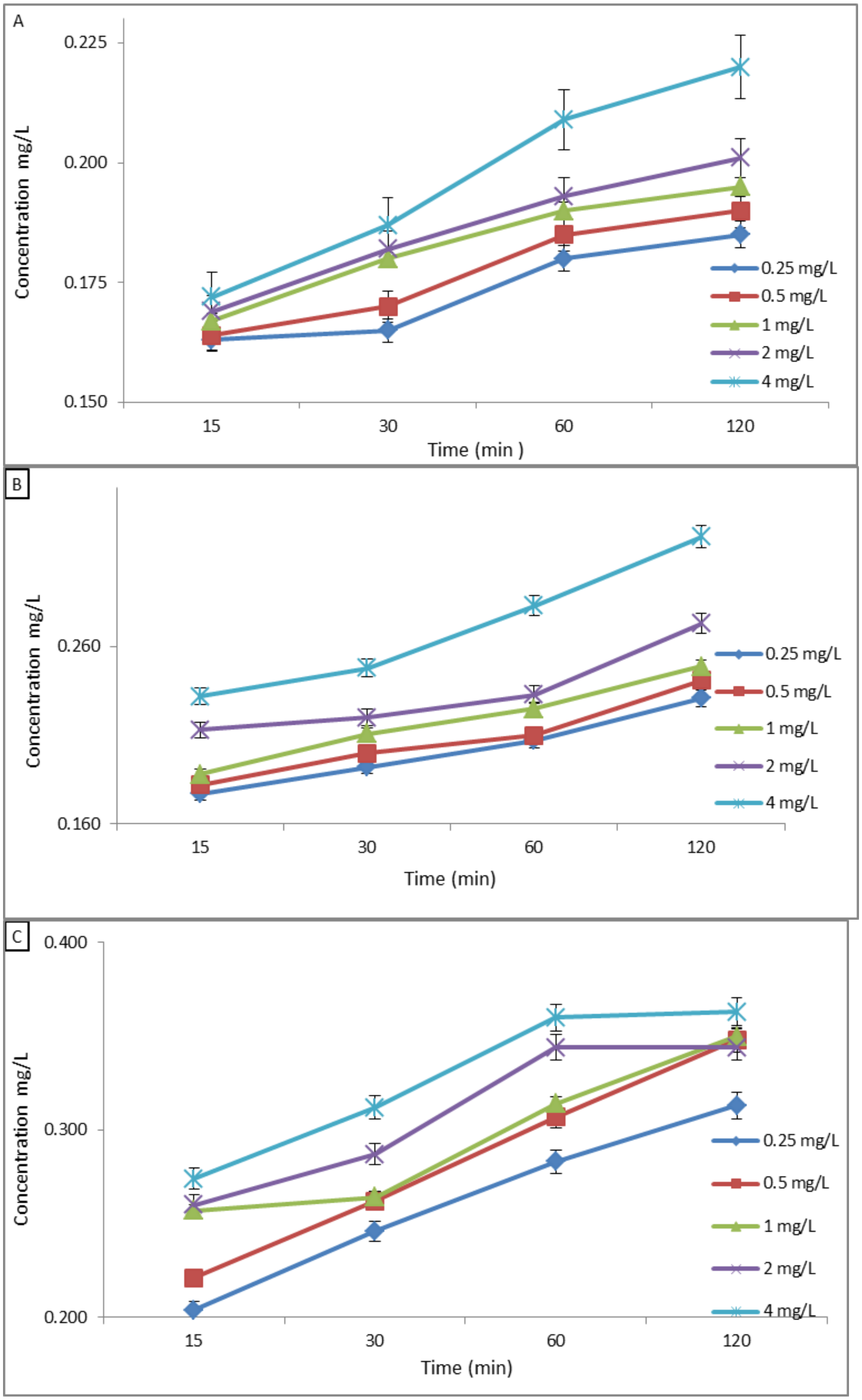

Fig. 4. The concentration of Fe (mg/L) adsorbed by *C. vulgaris* according to different mesh sizes of biomass A: 0.25µm, B: 0.125µm and C: 0.063µm

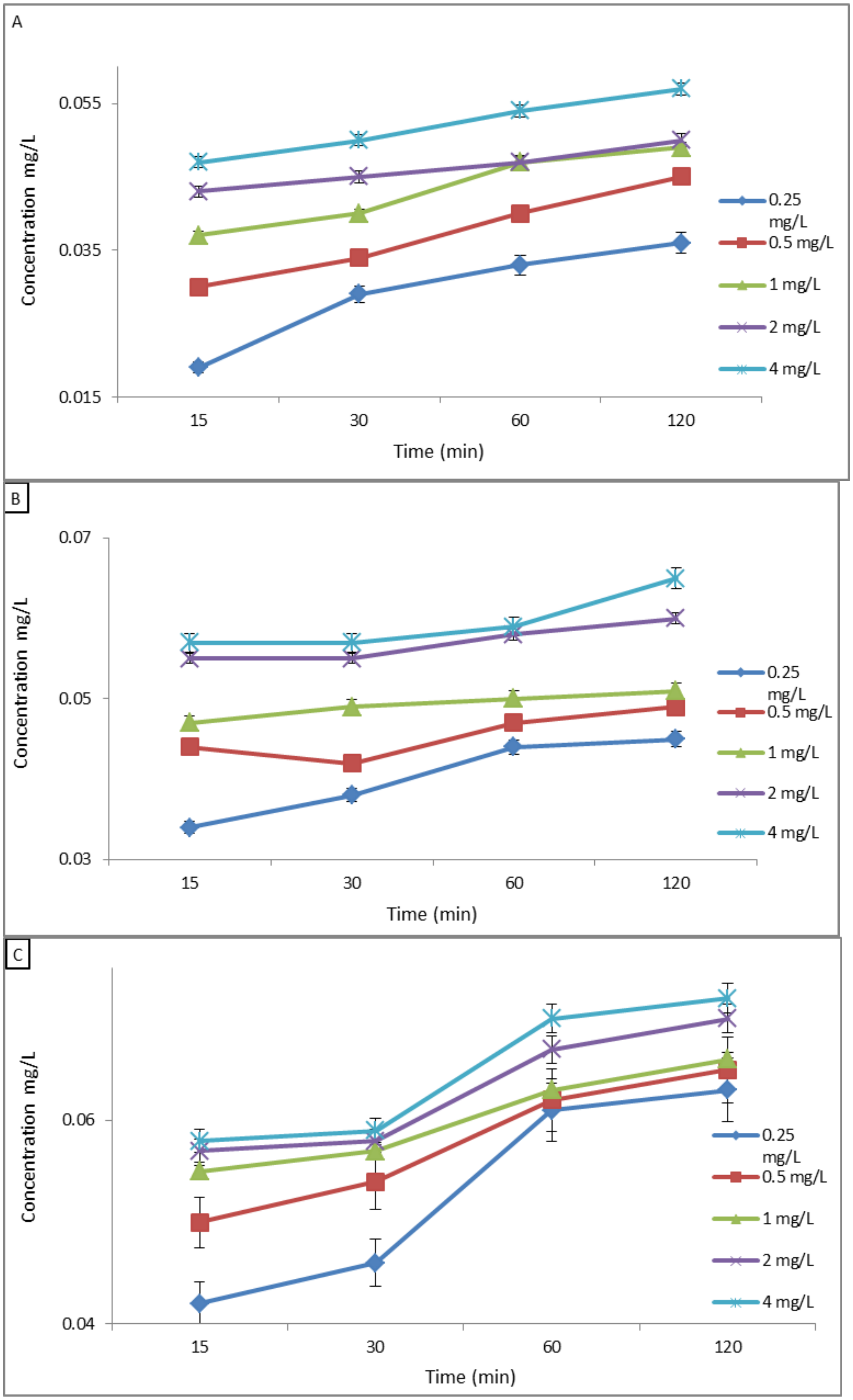

Fig. 5. The concentration of Co (mg/L) adsorbed by dry *C. vulgaris* according to different mesh sizes of biomass A: 0.25µm, B: 0.125µm and C: 0.063µm

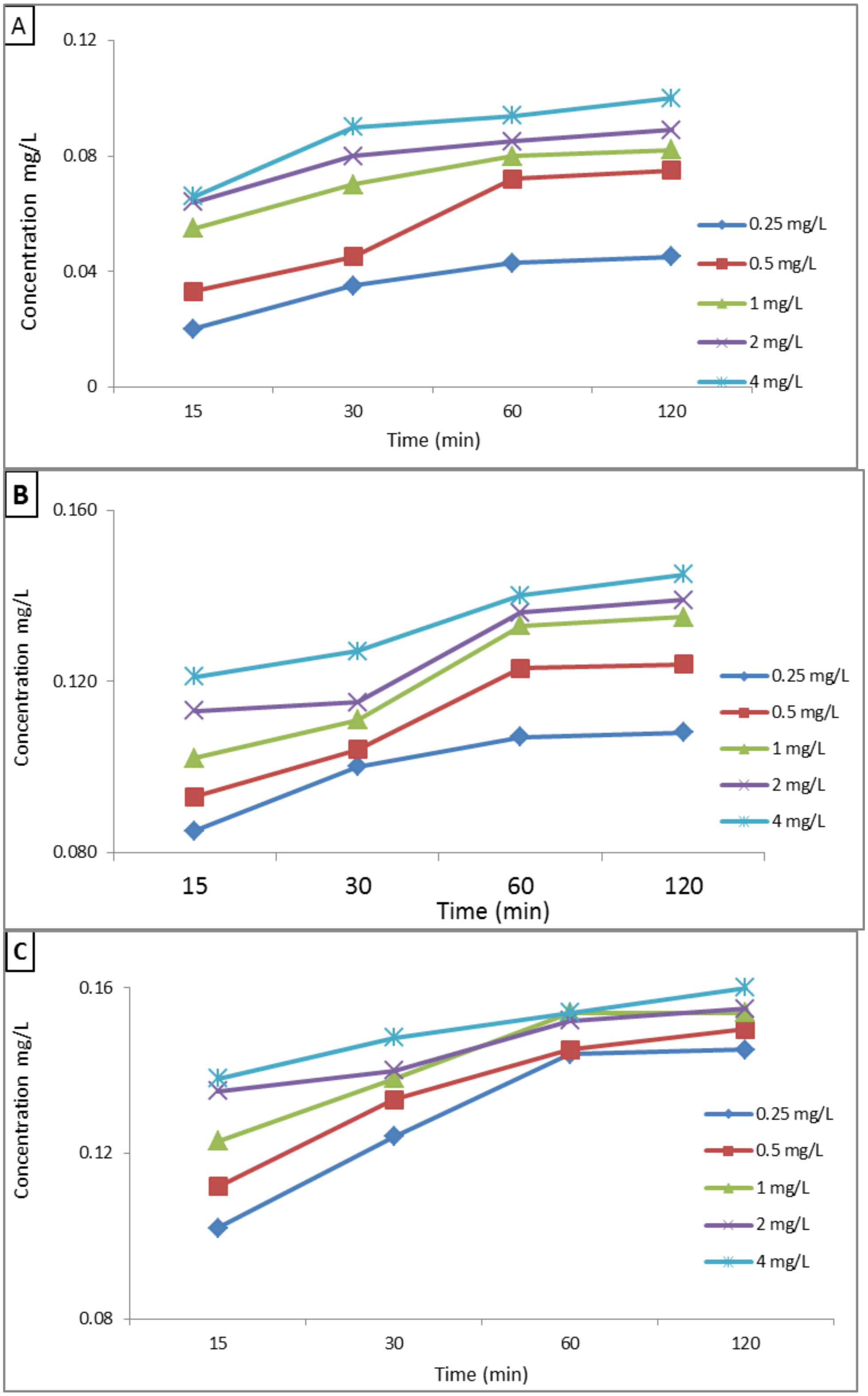

Fig. 6. The concentration of Pb (mg/L) adsorbed by *C. vulgaris* according to different mesh sizes of biomass A: 0.25µm, B: 0.125µm and C: 0.063µm

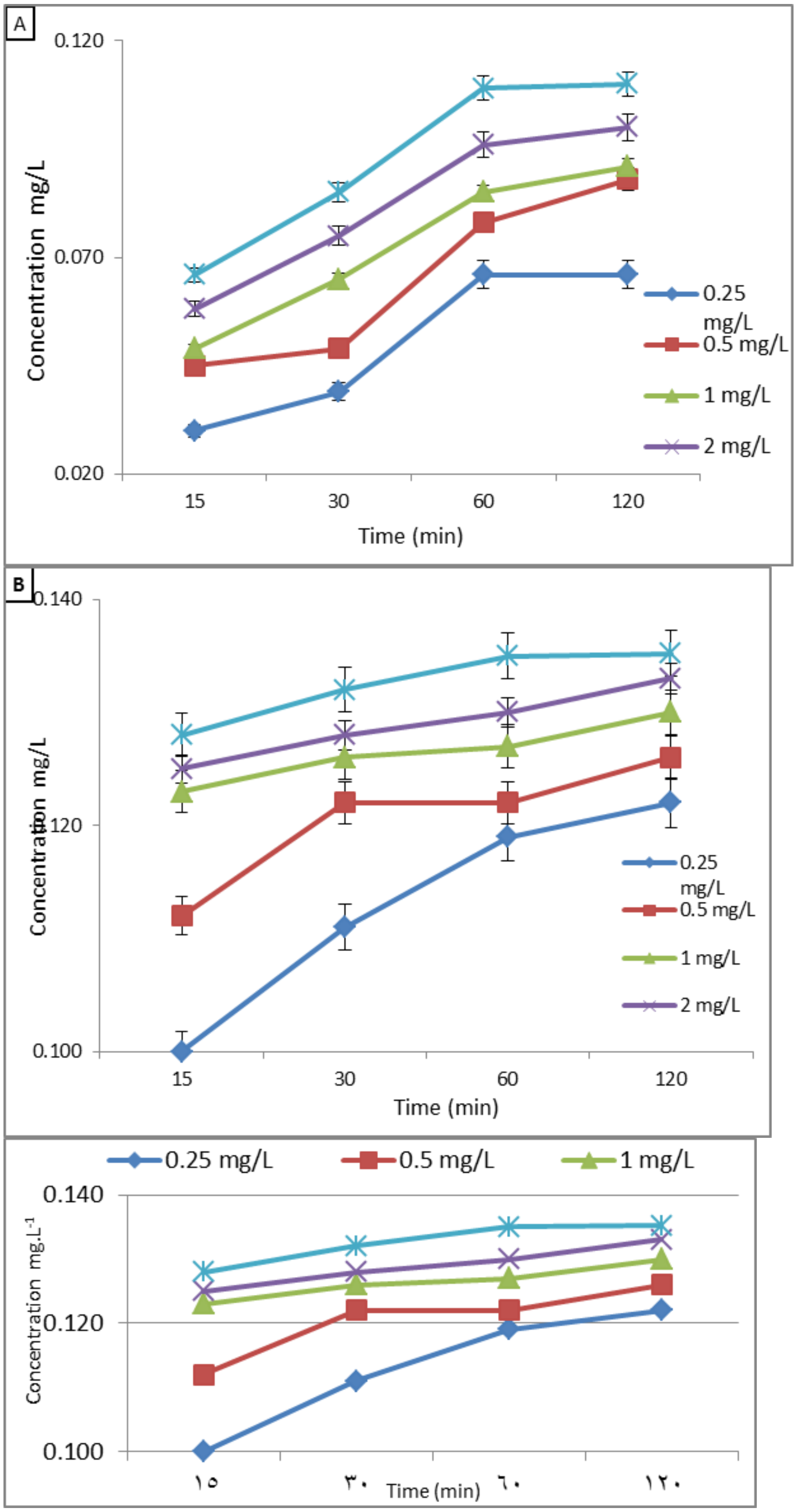

Fig. 7. The concentration of Ni (mg/L) adsorbed by *C. vulgaris* according to different mesh sizes of biomass A: 0.25µm, B: 0.125µm and C: 0.063µm

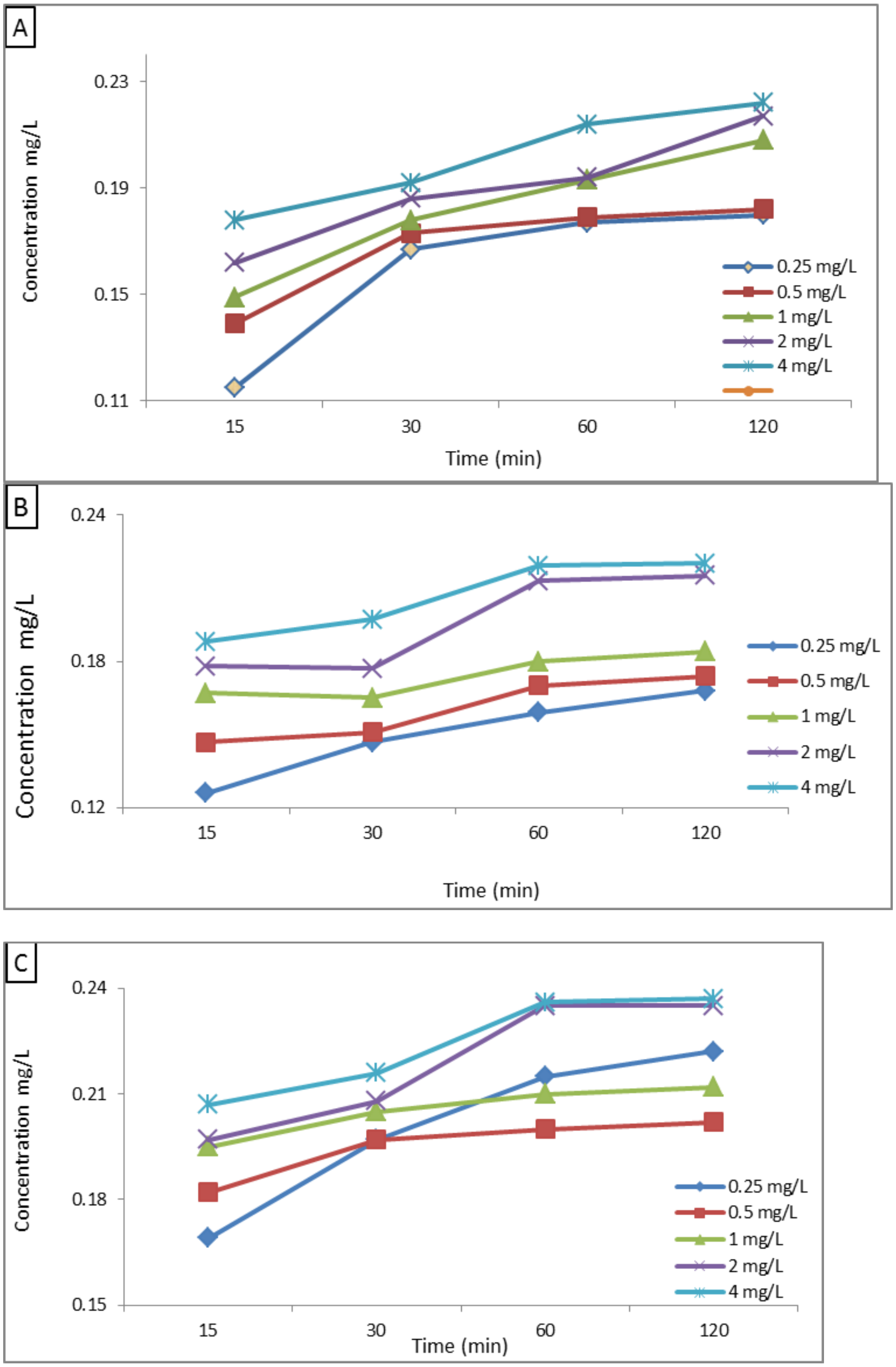

Fig. 8. The concentration of Zn (mg/L) adsorbed by *C. vulgaris* according to different mesh sizes of biomass A: 0.25µm, B: 0.125µm and C: 0.063µm

Table 1. Physico-chemical parameters in petrochemical industrial effluent wastewater before and after discharge

Parameter	Value of industrial wastewater (mg/L).	
	Before treatment	After treatment
pH (Unit)	6.2 ± 0.1	7.8 ± 0.89
Temperature ^{0}C	24 ± 1	24.4 ± 1.2
Sulphate (mg/L)	1.4 ± 0.0026	0.2 ± 0.160
O. Phosphate (mg/L)	0.015 ± 0.003	0.022 ± 0.04
TDS (mg/L)	180.5 ± 12.69	112.3 ± 4.79
Ammonia (mg/L)	5.5 ± 0.0265	1.32 ± 0.081
Nitrate (mg/L)	3.8 ± 0.0028	2.2 ± 0.006
Nitrite (mg/L)	0.2 ± 0.0028	0.027 ± 0.01
Silica	1.3 ± 0.0017	0.027 ± 0.0
Chloride (mg/L)	16.76 ± 0.81	7.11 ± 0.51
Total alkalinity (mg $CaCO_3$/L)	6 ± 0.12	4 ± 0.14
Total carbon	7.25 ± 0.32	6.5 ± 0.11
Hardness (mg $CaCO_3$/L)	1.48 ± 0.07	3.86 ± 0.08

Table 2. Biochemical analysis of *C. vulgaris*

%Component	moisture content	Fat	Protein	Carbohydrates	Ash
C. vulgaris.	11.2 ± 0.2	0.41 ± 0.01	17.90 ± 0.25	17.30 ± 0.35	57.23 ± 0.42

Data presented are mean (±SD).

DISCUSSION

The physico-chemical parameters of business effluent were investigated according with the numerous popular methods **(APHA, 1995)**. The key findings are as follows:

pH: The pH of business effluents remained inside the WHO variety of 6.5 - 8.5. Total dissolved solids degrees reduced post-remedy indicating preliminary infection however powerful remedy. Suspended solids: Large quantities of suspended debris may want to spoil aquatic existence and modulate the quality of water. However, the improved chloride degrees post-remedy correlate properly with research displaying that remedy can now and again boom chloride degrees. Carbon dioxide concentrations post-remedy had been lower, steady with findings that business discharge reasons adjustments to CO_2 concentrations. A decline in alkalinity post-remedy may also suggest predominant changes in water chemistry, as may also arise for positive eutrophic conditions.

Hardness, on the opposite hand, reduced post-remedy that may have an effect on the toxicity of metals. Low phosphate concentrations post-remedy might mitigate the hassle of eutrophication. While stages of sulfate stay exceptionally inside fee recommendations, better stages are connected to business waste. Ammonia stages reduced notably after remedy, whilst nitrate stages had been nonetheless the best earlier than remedy. That once more meditated the overall profile for business wastewater. Algal biosorption of heavy metals freshwater algae inclusive of *Chlamydomonas reinhardtii*, Cladophora spp., *Chlorella* spp., and *Scenedesmus* spp. have presented wonderful biosorbents for metallic elimination from aqueous solution, and their capacity regularly surpasses that of industrial resins **(Eccles, 1999, Mehta & Gaur, 2005; El-Sayed *et al.*, 2024)**. Theoretically, the uptake of metals is higher with lifeless algal biomass than with residing cells, each of which has positive regions of growth. Generally, inexhaustible biomaterials show off excessive capability and selectivity for metallic uptake, because of purposeful organizations found in their mobileular walls **(Rayson & Williams, 2011, Mosleh *et al.*, 2021)**. There has been popularity of intense adaptability of metallic absorption in fungal systems **(Siegel *et al.*, 1990)**. Macroalgae and microalgae function bio-remediators in soaking up toxins, bio-collecting bio-contaminants, this which includes immobilization of risky substances **(El-Sayed, *et al.*, 2024)**. *C. vulgaris*, the inexperienced micro-alga, is resistant and powerful in metallic uptake; it's been developing for billions of years in lots of habitats, and as a consequence has emerged as a substantial candidate for wastewater remedy.

Physico-chemical evaluation of industrial effluents

The biosorption performance of algae for metal removal was evaluated using *Chlorella vulgaris*. A key observation was that the duplication of experiments was overlooked in this study, although *C. vulgaris* demonstrated efficient removal of metal ions. Biosorption showed a gradual increase within the first 60 minutes—removal efficiency ranged from 85 to 95% within the first 15 minutes and plateaued or slightly decreased by 120 minutes.

However, inconsistencies were observed. Higher biomass concentrations generally yielded better removal results. For instance, among all tested biomass levels, 4mg/ L achieved the highest removal efficiency. Additionally, smaller mesh sizes (0.063µm) exhibited superior biosorption performance compared to larger mesh sizes (0.250µm).

C. vulgaris effectively removed heavy metals such as Ni, Zn, Co, Pb, and Cd, achieving a lead biosorption capacity of up to 120 mg Pb/g dry weight. These findings align with earlier studies, differing only in specific experimental conditions **(Inthorn *et al.*, 2002; Goher *et al.*, 2016; Mosleh *et al.*, 2021, 2023; El-Sayed *et al*., 2024)**. In contrast, copper and manganese removal was evaluated by other researchers, suggesting the need for context-specific optimization **(Mofeed, 2017)**. Overall, *C. vulgaris* shows strong potential for use in environmental bioremediation systems for heavy metal removal.

The efficacy of living versus dead biomass has been widely discussed. **Rayson and Williams (2011)** reported that non-living biomaterials possess several advantageous properties, including high capacity, rapid binding, and strong selectivity for heavy metals. Similarly, *Datura innoxia* has shown that functional groups from lipids, carbohydrates, and proteins within cell walls are critical for metal ion uptake. On the other hand, **Siegel *et al.* (1990)** demonstrated that fungal systems are highly adaptable in binding metal cations such as Fe, Ni, Cu, Zn, Ag, Cd, and Pb.

Algae, including *C. vulgaris*, tend to absorb hazardous substances from their environment, bioaccumulate and biotransform organic compounds, and immobilize inorganic metals, thereby reducing their toxic effects **(Saleh, 2015)**. Numerous studies have reported physiological responses of algae to chemical pollutants **(Collén *et al.*, 2003; Torres *et al.*, 2008; Unal *et al.*, 2010; Jiang *et al.*, 2013)**.

A prominent example is *Chlorella vulgaris*, a unicellular freshwater microalga that has existed since the pre-Cambrian era, approximately 2.5 billion years ago. Comparative studies of three algal species showed a rapid decrease in metal ion concentrations within the first 15 minutes, with approximately 80% removed. Continued removal reached up to 95% by 60 minutes, while at 120 minutes, the rate either stabilized or slightly declined.

The amount of metal ions adsorbed varied across algal species and was influenced by biomass concentration. A biomass dose of 4mg/ L of dried algal cells resulted in the highest removal efficiency among the three species, while the lowest efficiency was observed at 0.25mg/ L. Biosorption was also tested across three particle sizes: 0.250, 0.125, and 0.063µm. The 0.063µm mesh size provided the most effective biosorption, while the 0.250µm size showed the lowest efficiency.

This study specifically focuses on the biosorption of heavy metals from dried *C. vulgaris* in untreated tannery wastewater. Such wastewater contains highly toxic heavy metals and poses serious environmental and public health challenges. Therefore, there is an urgent need for the development of newer, more efficient, eco-friendly, and cost-effective technologies to remove inorganic pollutants—particularly Cr, Hg, Cd, and Pb—responsible for environmental degradation **(Igiri *et al.*, 2018)**.

Biosorption—a key physicochemical property of biomass—enables the removal of contaminants, particularly heavy metals, from wastewater through ionic or covalent bonding **(He & Chen, 2014; Zeraatkar *et al.*, 2016; Salama *et al.*, 2019)**. This method is considered one of the most promising approaches for wastewater treatment, as it facilitates metal ion uptake through both metabolically mediated and physicochemical pathways. These pathways utilize both living and non-living microorganisms, including seaweeds and similar biological materials **(Chubar *et al.*, 2003; Fard *et al.*, 2011)**.

Several parameters significantly influence biosorption performance, including temperature, pH, dissolved oxygen, and contact time **(Park *et al.*, 2010)**. Among these, pH is especially critical, as it controls the availability of binding sites and affects ion exchange mechanisms **(Vijayaraghavan & Yun, 2008)**. Additional important factors include the availability of biosorbent material, the nature of active binding sites, and biosorbent dosage **(Li & Tao, 2015)**.

Three major variables were identified as key influencers of biosorption efficiency: time, mesh size, and biomass concentration. The initial removal efficiency reached nearly 50% within 15 minutes, increasing to 85% or higher by 60 minutes. After 120 minutes, the efficiency either stabilized or slightly declined. The maximum removal efficiency was observed at a biomass dose of 4 mg/L, whereas the lowest binding capacity was noted at 0.25 mg/L.

In terms of granule size, the smallest mesh (0.063 mm) demonstrated the highest metal binding efficiency, whereas medium (0.125 mm) and large granules (0.250 mm) exhibited comparatively lower performance.

C. vulgaris exhibited strong biosorption capability for heavy metals such as Ni, Zn, Co, Pb, and Cd. **Inthorn *et al.* (2002)** reported a maximum lead adsorption capacity of 127 mg Pb/g dry weight, with other studies showing adsorption ranges between 31 to 90 mg Pb/g. However, biosorption efficiencies for Fe, Mn, and Cu were significantly lower—72.8%, 71%, and 73.3%, respectively. In comparison, another algal species, *Ulva lactuca*, showed greater effectiveness in removing Fe, Mn, and Cu **(Mofeed, 2017)**.

The role of carboxyl functional groups in Cu binding on *C. vulgaris* was further emphasized by **Mehta *et al.* (2001)**. Additionally, the biosorption of Cu(II) using Ca-alginate and agarose-immobilized *C. vulgaris* was investigated as early as 1998.

In terms of Cd removal, **Raikova *et al.* (2016)** and **El-Sayed *et al.* (2019)** found that *C. vulgaris* removed 56% of Cd from wastewater. In contrast, the current study reported a significantly higher removal efficiency of 83%. **Igiri *et al.* (2018)** also highlighted *C. vulgaris* as a highly effective biosorbent for Cd, Cu, and Pb, with removal efficiencies of 95.5%, 97.7%, and 99.4%, respectively—findings consistent with our study, especially in the case of Pb, where up to 93% removal was achieved in petrochemical effluent.

Goher *et al.* (2016) confirmed that dried cells of *C. vulgaris* can effectively adsorb Cu, Cd, and Pb, with efficiency dependent on pH, dosage, and contact time. **Mehta and Gaur (2005)** also documented biosorption of Cu and Ni by *C. vulgaris*, with Cu removal peaking at 73.3% and Ni at 96% after 120 minutes—figures slightly deviating from other reports but still within expected ranges.

Overall, *C. vulgaris* was especially effective in removing Pb, Ni, Zn, Co, and Cd, in alignment with previous findings **(Goher *et al.*, 2016; Igiri *et al.*, 2018)**.

Mechanisms of heavy metal biosorption by *Chlorella vulgaris*

The biosorption of heavy metals by *C. vulgaris* involves multiple complex mechanisms, including:

1. **Surface adsorption and functional group interaction**

The cell wall of *C. vulgaris* contains polar functional groups such as carboxyl (-COOH), hydroxyl (-OH), amino ($-NH_2$), and sulfate ($-SO_4$). These groups facilitate metal binding via electrostatic attraction and covalent bonding. Metals such as Pb^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}, and Ni^{2+} adhere effectively to the algal surface **(Joo *et al.*, 2021)**.

2. **Ion exchange mechanism**

Metal ions in solution replace naturally occurring ions like Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} within the algal biomass, thereby enhancing biosorption efficiency **(Joo *et al.*, 2021)**.

3. **Complexation and chelation**

C. vulgaris secretes extracellular polymeric substances (EPS), which engage in complexation and chelation, forming stable complexes with metal ions. This mechanism is particularly effective for Cu^{2+} and Zn^{2+} due to their high affinity for protein-based chelating sites **(Joo *et al.*, 2021)**.

4. **Intracellular uptake and accumulation**

Some metal ions are actively transported across the algal cell membrane via specific transport proteins and stored in vacuoles or incorporated into metabolic pathways, further reducing their bioavailability **(Abreu *et al.*, 2014)**.

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