ORIGINAL PAPER

Comparison of the efficiency of duckweed in heavy metal removal from aqueous solutions in combined and separate forms

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ABSTRACT

Today, heavy metal contamination is a major environmental concern across the world. Some of the common heavy metal pollutants in industrial wastewater include lead, copper, cadmium, and nickel. This study aimed to compare the efficiency of duckweed in the removal of heavy metals from aqueous solutions in combined and separate forms. This applied, fundamental research was conducted based on empirical studies. Heavy metal solutions were prepared at the concentrations of 5, 10, and 25 mg/l, and duckweed (weight: 0.2, 0.4, 0.8, and 1.2 g) was added to the prepared solutions (100 ml). Plant weight in the combined solution was four times higher than the separate solution. After the contact time of five, 10, and 15 days, heavy metal residues in the solutions was measured using ICP-OES. According to the results, heavy metal removal from the separate solutions differed with the combined solutions. Both systems were compared in terms of the contact time, initial heavy metal concentration, and removal order. The obtained results indicated that the removal efficiency of heavy metals was higher in the combined solutions compared to the separate solutions. Increased initial concentration also reduced removal efficiency in the separate solutions, while the removal rate remained constant in the combined solutions. Moreover, the heavy metals in the combined solutions were removed within a shorter time. The removal sequence of the heavy metals from separate solutions was lead>cadmium>nickel>chromium at the maximum removal time. In the combined heavy metal solutions, such removal rate sequence was not observed.

Keywords: Aqueous solutions, Duckweed, Heavy metals

Introduction

Today, heavy metal pollution is considered to be a major environmental concern across the world. Unlike most organic materials, heavy metals cannot be converted by microorganisms and only aggregate in water, soil, living organisms, and bottom sediments.[1] These pollutants enter the environment due to the use of natural components or as a result of agricultural and industrial activities, such as mining operations, smelting, and metal refineries, which have been reported to be the main sources of heavy metal release into the environment.[2, 3]

Heavy metals such as lead, copper, cadmium, and nickel are the most common pollutants in industrial sewage. Even at low concentrations, these elements could cause toxicity in living organisms, including humans.[4] Each year, various industries release large quantities of heavy metals into the environment through the production of sewage containing heavy metals; some of these industries include mining, metal extraction, automotive plants, chemical and electronic industries, electroplating metals, metal plating, and battery factories.[5] The plating industry is considered to be one of the most hazardous chemical

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industries as it releases substantial amounts of contaminated metals into sewage.[6] Therefore, in order to protect the environment and public health, heavy metals must be removed from wastewater and sewage.

Several methods have been proposed for the removal of heavy metal ions, the most common of which include precipitation, flocculation, reduction, ion exchange, evaporation, and membrane processes.[7] Each of these methods has specific limitations despite their advantages. Some of these limitations include the low efficiency in the removal of heavy metal ions, over-consumption of reagents and chemicals, production of toxic sludge, high costs, and issues regarding the safe disposal of the residual materials. With this background, there is an urgent need for novel technologies that could effectively minimize the concentration of heavy metals in a cost-efficient manner and at acceptable environmental levels.[8]

Recently, use of aquatic plants has been proposed for the removal of heavy metals from water bodies.[9] Duckweed is scattered in fresh and stagnant waters of the northern, western, southern, and other regions in Iran. This plant is found abundantly in Iran. Some of the main benefits of duckweed include rapid twofold growth within a short time and resistance to harsh environmental conditions due to the multi-layered structure of the plant and its advanced, strong root system, which enables duckweed to grow well in winter.[10]

Several studies have been focused on the use of duckweed for various purposes, such as wastewater treatment and removal of dyes and heavy metals, confirming its efficiency in this regard.[10] Duckweeds (Wolffia, Lemna, Wolfiella, and Spirodela) are divided into freshwater and brackish estuaries. Duckweed grows easily *in-vitro* and is commonly used in ecotoxicological research. Furthermore, many studies have indicated that duckweed has notable potential for the removal of heavy metals.[11] For instance, in the study conducted by Mishra and Tripathi entitled "Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes", three aquatic plants, including duckweed, were able to remove heavy metals (iron, copper, zinc, chromium, and cadmium) by more than 90% within 15 days. Moreover, the mentioned research demonstrated that the maximum elimination of heavy metals occurred within 12 days after the experiment, followed by a gradual decline in the removal rate.[12]

In another study in this regard, Reinhold reported that aquatic plants were directly and indirectly effective in the removal of new organic pollutants from wetland systems.[13] Similarly, Kilic *et al.* investigated brilliant blue R removal from a culture media, reporting that the addition of the triacontanol hormone could increase the biomass and dye removal rate.[14] In addition, the findings of the mentioned research indicated that duckweed can be used effectively to remove dye from contaminated wastewater. In this regard, Li *et al.* reported that duckweed powder could remove organic and inorganic mercury from aqueous solutions.[15]

The present study aimed to compare the efficiency of duckweed in the removal of heavy metals (lead, cadmium, nickel, and chromium) from aqueous solutions in combined and separate forms.

Materials and Methods

Initially, the duckweed plant was obtained from a fish breeding site and transferred to the laboratory. Before placing the plants in each sample container, they were washed several times with distilled water in order to eliminate possible contamination. To prepare the heavy metal solutions, we used lead nitrate, cadmium nitrate, chromium nitrate, and nickel sulfate. In order to prepare the aqueous solutions of the heavy metals, a stock solution (100 ppm) was obtained for the preparation of solutions with the concentration of 5, 10, and 25 mg/l. Afterwards, the four mentioned heavy metals were combined at the concentration of 5 mg/l. The same process was also carried out for the other two concentrations in order to obtain the combination of all the heavy metals at three different concentrations. In addition, the heavy metals were individually placed in containers to compare their separate removal efficiency with their combination.

In order for the plant to have contact with the aqueous solutions of the heavy metals, we used disposable containers with the volume of 100 milliliters. The containers were washed with acid and tap water in three steps, as well as distilled water. Following that, the heavy metals solutions were transferred to the disposable containers, and duckweed was added to the containers with the weights of 0.2, 0.4, 0.8, and 1.2 grams. It is notable that the weight of the duckweed containing the four heavy metals in the combined solutions was considered to be four times the weight of the separate solutions. As such, the weight of duckweed was determined to be 0.8 and 1.2 grams for the combined solutions and 0.2 and 0.4 gram for the separate solutions.

Prior to the addition of duckweed to the sample containers, the plants were washed with distilled water. Afterwards, the plants were placed on a paper towel so as to remove the excess moisture and weighed by the balance immediately afterwards. At the next stage, the light exposure required for the plant was supplied using a fluorescent lamp. The heavy metal containers were evaluated at the concentrations of 5, 10, and 25 mg/l without the plant to detect the evaporation or absorption of the heavy metals in the disposable containers.

In preliminary studies, the highest removal efficiency of the heavy metals was observed in the combined solutions within the initial five days. Accordingly, the contact time was determined to be five and 10 days for the combined solutions. Furthermore, the preliminary studies also indicated that the separate solutions required more time for removal at the concentrations of 10 and 25 mg/l. Therefore, the contact time was determined to be 10 and 15 days for these solutions.

Heavy metal solutions were passed through filter papers after the contact times of five, 10, and 15 days, and the filtered solution was transferred to polyethylene bottles (volume: 100 ml) to measure the residual heavy metals in the solutions using the ICP device (model: OES-ICP model). Data analysis was performed in Excel software (2007).[10]

Results and Discussion

Figs. 1-6 show the results of the study regarding the comparison of the removal efficiency of heavy metals by duckweed from aqueous solutions in combined and separate forms.

Effect of the contact time

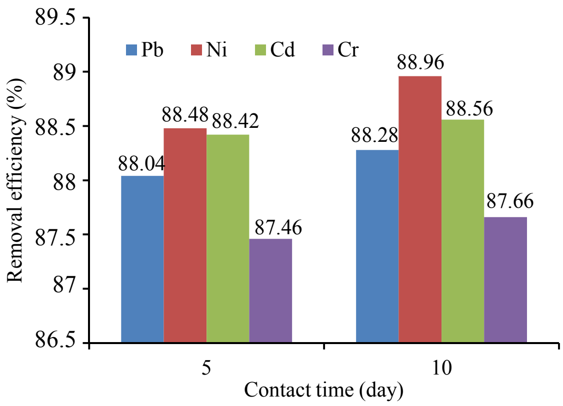

Fig. 1. Duckweed efficiency in combined heavy metal removal at concentration of 5 mg/l (duckweed weight: 0.2 g)

Fig. 1 depicts the removal efficiency of the heavy metals in the combined solutions. As is observed, at the contact times of five and 10 days, the removal efficiency of all the heavy metals slightly increased at the interval of five and 10 days. In addition, the concentration of the heavy metals in water decreased as a function of time, while its changes were imperceptible with the increased contact time. This resulted from the absorption of the heavy metals by duckweed. However, it is notable that depending on chemical properties and pH, each element caused changes in the experiments in water as a function of the contact time.

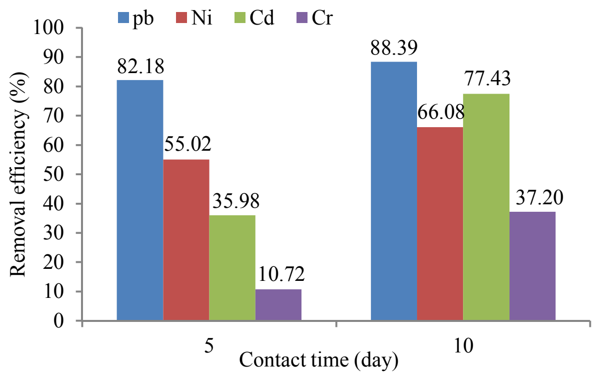

Fig. 2. Duckweed efficiency in separate heavy metal removal at concentration of 5 mg/l (duckweed weight: 0.2 g)

Fig. 2 shows the removal efficiency of heavy metals in the separate solutions. As can be seen, with the contact times of five and 10 days, the removal efficiency of all the heavy metals increased at the interval of five and 10 days. In the present study, the appropriate contact time was determined to be five and 10 days for the combined and separate solutions, respectively.

After 10 days in a separate sample, the removal efficiency decreased slightly, which is consistent with the findings of Mishra and Tripathi. In the mentioned study, the simultaneous removal of heavy metals using three aquatic macrophytes indicated that the aquatic plants, including duckweed, could remove more than 90% of heavy metals (iron, copper, zinc, chromium, and cadmium) within 15 days. Furthermore, the obtained results demonstrated that the highest removal was attained at the contact time of 12 days, followed by the reduction of the removal rate. In this study, the concentrations of heavy metals were 1, 2, and 5 mg/l.[12] Therefore, the removal efficiency of heavy metals in the combined sample was observed to be higher and occurred within a shorter contact time.

Effect of the initial concentration of the heavy metals

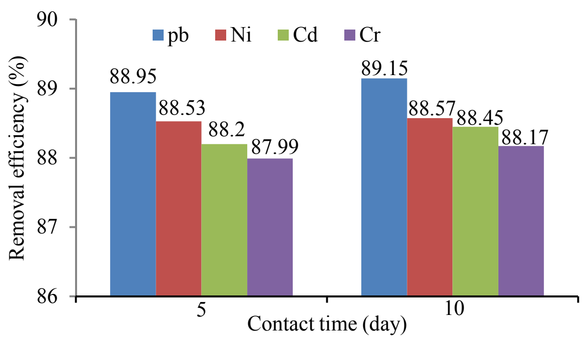

Fig. 3. Duckweed efficiency in combined heavy metal removal at concentration of 10 mg/l (duckweed weigh: 1.2 g)

Fig. 3 depicts the removal rate of the heavy metals in the combined solutions at the concentration of 10 mg/l. According to the findings, the removal efficiency of lead, nickel, cadmium, and chromium was 88.95%, 88.53%, 88.2%, and 87.99% at the contact time of five days, and 89.15%, 88.57%, 88.45%, and 88.17% at the contact time of 10 days, respectively at the concentration of 5 mg/l. As for the heavy metal removal in the combined form, the removal efficiency of lead, nickel, cadmium, and chromium was estimated at 88.04%, 88.48%, 88.42%, and 87.46% at the contact time of five days, and 88.28%, 88.96%, 88.56%, and 87.66% at the contact time of 10 days, respectively at the concentration of 5 mg/l (Fig. 1). Comparison of Figs. 1 and 3 (Figs. 1 and 3 shows both combined solutions at different concentration) shows that the contact time remained constant, while the removal efficiency of the heavy metals in the combined form remained almost unchanged by increasing the concentration from 5 mg/l to 10 mg/l. This could be due to the interference of the heavy metals in the combined solutions, and further investigations may be required in this regard.

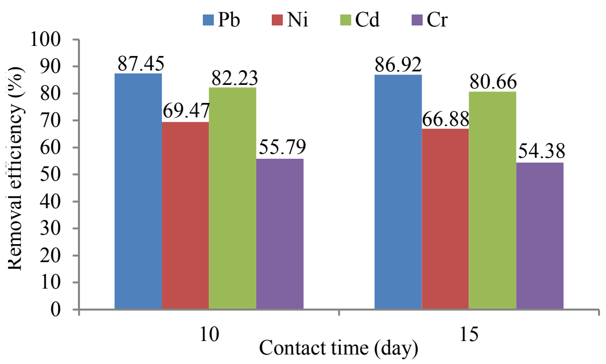

Fig. 4. Duckweed efficiency in separate heavy metal removal at concentration of 10 mg/l (duckweed weight: 0.4 g)

Fig. 4 illustrates the removal rate of the heavy metals in the separate solutions at the concentration of 10 mg/l. Comparison of Figs. 2 and 4 shows that in the separate solutions and with constant contact time, increasing the concentration of the heavy metals and duckweed weight resulted in higher removal efficiency.

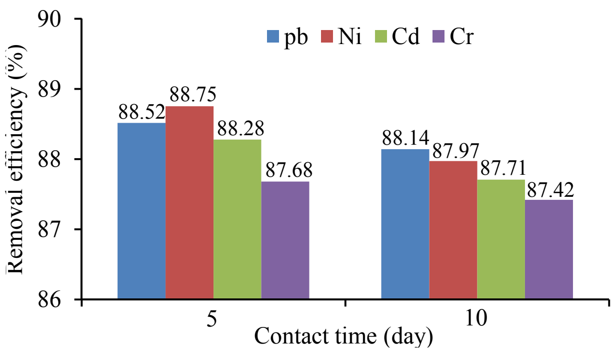

Fig. 5. Duckweed efficiency in combined heavy metal removal at concentration of 25 mg/l (duckweed weight: 1.2 g)

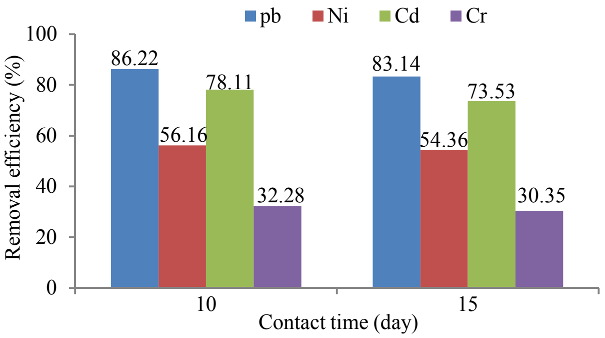

Fig. 6. Duckweed efficiency in separate heavy metal removal at concentration of 25 mg/l (duckweed weight: 0.4 g)

Fig. 5 shows the removal efficiency of heavy metals in the combined solutions at the concentration of 25 mg/l. Fig. 6 depicts the removal efficiency of the heavy metals in the separate solutions at the concentration of 25 mg/l. Comparison of Figs. 4 and 6 shows that in the separate solutions and with the constant contact time, the increased concentration of heavy metals and constant duckweed weight led to the reduction of the removal efficiency. Therefore, it could be concluded that the weight of duckweed was also important in this case. The difference in this regard could be due to the toxic effects of the heavy metals at the high concentrations of duckweed.

According to the current research, the accumulation of the four heavy metals at high concentrations caused damage to the plant within a shorter time. Heavy metals cause oxidative stress, which in turn leads to the production of reactive oxygen species (ROS). ROS exert variable toxic effects on plants, such as growth retardation, reduction of the chlorophyll content and photosynthesis, inhibition of enzymatic activity, biological molecule damage (e.g., lipids, proteins, and nucleic acids, especially DNA), and cell membrane peroxidation, which leads to the loss of ions.[12] In the present study, cell membrane peroxidation resulted from the removal of heavy metals and diminished removal efficiency within the time interval of 5-10 days at the concentration of 25 mg/l.

According to the results of the present study, the removal efficiency of the heavy metals decreased at the higher initial concentration of the heavy metals in the combined and separate solutions. This is consistent with the results obtained by Axtell *et al.*, which indicated that the nickel removal efficiency of *Lemna minor* duckweed at the nickel concentrations of 1, 3, and 5 mg/l was 100%, 100%, and 95%, respectively.[16] Moreover, Diyanati *et al.* conducted a research regarding the removal of phenol using duckweed, and the removal efficiency was observed to reduce at higher phenol concentrations.[10] This could be attributed to the toxicity of heavy metals at high concentrations in duckweed.

Effects of the type of heavy metals

As is depicted in Fig. 6, another important influential factor in the removal rate of heavy metals is the type of heavy metals. In the present study, the highest removal rate was observed in the separate solutions of lead, as well as the combined heavy metal solutions at the nickel concentrations of 5 and 25 mg/l and lead concentration of 10 mg/l. On the other hand, the lowest removal rate in all the solutions was observed with chromium. These findings are in agreement with the results of the Kaur *et al.* research, only at concentration 10 mg/l. In the mentioned research, the lead removal efficiency was higher compared to nickel using duckweed at various pHs.[17] However, comparison of the removal rate of four heavy metals using duckweed was not performed under the same conditions. This could be due to the various mechanisms of heavy metal absorption by duckweed. In addition, the presence of some heavy metals may affect the removal of other heavy metals.[18]

The mechanism of contaminant absorption by duckweed is known as rhizofiltration.[19] Rhizofiltration is precipitation or adsorption onto the plant roots or absorption into and segregation in the contaminant roots, which are considered in the solution circumambient the root region to clear up wastewater by the constructed wetland.[20, 21] This approach could be used for heavy metals such as lead, cadmium, nickel, zinc, and chromium, which are generally accumulated in the roots of plants.[22] Although lead does not have a specific function in the physiological reactions of plants, it could be absorbed by plants due to its chemical similarity to essential elements.[23]

In a research in this regard, Vardanyan and Ingole investigated the concentration of heavy metals in various tissues of salty soils in Armenia and India, reporting that the essential metals in the tissues had accumulated more frequently, and the least concentration of the metals related to the elements was unnecessary.[24] Similar to lead, cadmium has no specific function in the metabolic processes of plants; nevertheless, it is absorbed by plants due to its chemical similarity to calcium.[25] Moreover, soluble cadmium could enter plant roots through cell wall movements (apoplastic) and via the symplastic pathway. After entering the roots, cadmium could be moved from the cellular pathway and finally enter the transpiration stream of the raw sap.[26]

Nickel is an element that is involved in the nitrogen metabolism of plants, and plants absorb nickel for this purpose.[27] Nickel is currently regarded as an essential element with extremely low utilization, and its only defined role is to participate in the metabolism of urea. It is notable that this process plays a pivotal role in the plants that use urea as a source of nitrogen.[28]

Chromium has no specific absorption mechanism. Therefore, the absorption of this heavy metal occurs with the occupation of the carriers of the essential elements of plants. The toxic effects of chromium on plants (absorption, transfer, and accumulation) mainly depend on its metallic species. Furthermore, chromium competes with iron and phosphorus for binding to the carriers.[29] In a research in this regard, Wallace investigated the mechanism of chromium absorption in the three- and six-valence compounds in barley, where the major part of chromium(6) is actively absorbed, while chromium(3) is mainly absorbed inactively. The radioactivity studies in the mentioned study indicated that chromium is mainly motile in wood vessels.[29]

The results obtained by Shanker *et al.* demonstrated that chromium is mainly accumulated in the roots of plants, which is also due to the immobilization of chromium in the root vacuoles, which reduces its toxicity in the plant.[30] H+ATPase plasma membrane function is a disorder which contributes to the decreased absorption of chromium-fed plants. ATPase plays a key role in compensating for the toxicity of heavy metals, and the reduction of ATPase activity occurs with the breakdown of the membrane bonds, which is often caused by the formation of free radicals. The reduced activity of ATPase also diminishes proton secretion, thereby preventing active transfer in the membrane of the root cells and nutrient absorption.[31]

Effects of heavy metal solutions in combined and separate forms

As is shown in Fig. 6, the combined and separate heavy metal solutions were effective in the removal of heavy metals. In the present study, the separate heavy metal solutions led to the following heavy metal removal sequence: lead>cadmium>nickel>chromium at the maximum removal time, respectively. However, no clear sequence was denoted in the combined heavy metal solutions in terms of the removal rate, which differed at various concentrations. Nonetheless, the lowest removal rate was observed with chromium in both the separate and combined solutions.

The sequence of heavy metal removal in the combined solutions at the concentration of 5 mg/l was as follows: nickel>cadmium>lead>chromium, while the removal of the heavy metals at the concentrations of 10 and 25 mg/l was as follows: lead > nickel > cadmium> chromium and nickel >lead>cadmium>chromium, respectively. The difference in this regard could be due to the interactions of heavy metals with their removal rate in the combined solutions. Jain *et al.* have confirmed the effects of heavy metals on the removal rate of each other.[18] Accordingly, the presence of the copper ion in the solution alone increases the removal rate by duckweed more significantly compared to the same amount of iron ions in the solution.

The removal of heavy metals is possible using duckweed. Some of the main influential factors in this regard include proper time, type of heavy metals, the initial concentration of heavy metals, and use of the combined or separate solutions of heavy metals.

Conclusion

The present study aimed to compare the efficiency of duckweed in the removal of heavy metals from aqueous solutions in combined and separate forms. According to the results, the removal of heavy metals in the separate solutions differed from the combined solutions. Furthermore, the removal efficiency of heavy metals was higher in the combined solutions, and the removal rate of heavy metals in these solutions was closer to the separate solutions. The findings of the research indicated that increasing the initial concentration of the heavy metals was associated with reduced removal efficiency in the separate solutions, while the removal rate remained constant in the combined solutions. In addition, the removal of heavy metals from the combined solutions required less time. The sequence of the removal of heavy metals from the separate solutions was as follows: lead>cadmium>nickel>chromium at the maximum removal time, respectively. However, no such clear sequence was observed in the removal rate of the heavy metals in the combined solutions. In conclusion, it is recommended that in further investigations, the experiments be repeated within a wide range of pH values at the optimal pH. Moreover, the addition of nutrients to heavy metal solutions could augment the growth of duckweed so as to avoid plant growth reduction.

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