Removal of Heavy Metal Contaminants from Wastewater by Using Chlorella vulgaris Beijerinck: A Review

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Abstract: Removal of heavy metals is very important in wastewater treatment process, due to their abundant hazardous effects. There are various chemical and physical methods including ion exchange, reverse osmosis, electrodialysis, and ultrafiltration for removing heavy metals from wastewater, but biological treatment has attracted researchers for years as it is cheap and efficient. Microalgae have a significant capability of absorbing and eliminating heavy metals from wastewater. One of the most attractive microalgae species for this application is the *Chlorella vulgaris* Beijerinck. The current study takes a literature review of using microalgae species, especially *C. vulgaris*, with the aim of wastewater heavy metal treatment. In this regard firstly, various methods of eliminating heavy metals using microalgae were investigated, and then the application of *C. vulgaris* in the process of eliminating heavy metals from wastewater is fully presented. It became obvious that the use of *C. vulgaris* application is more helpful in the case of Copper, Lead, Zinc, Cadmium, and Nickel. Moreover, the main factor affecting heavy metal treatment using *C. vulgaris* is the pH of media, and the second effective parameter is temperature that is often considered about 25°C. The appropriate time period for the treatment was 5-7 days. Generally, *C. vulgaris* presented a very favorable efficiency in eliminating various heavy metals and is capable of removing heavy metals from wastewater to more than 90% on average.

Keywords: Adsorption, Chlorella vulgaris, heavy metals, microalgae, pollution, ultrafiltration.

1. INTRODUCTION

Environmental pollution has always been a challenge for human beings, but it has become main concern during the last century. Water pollution is probably the most important and dangerous among all pollution types, because it seriously affects human life in all aspects, directly or indirectly. The existence of the pollutants in wastewater is considered a result of residential, agricultural and industrial use of water. During the two first stages of water treatment, solid particles which are easily separable and some organic compounds are washed from water, but this apparently clean water still includes some inorganic nitrogen compounds such as nitrates, phosphorus, heavy metals and some toxic organic compounds, which make the third stage of treatment necessary [1-3]. Heavy metal removal is very important due to their hazardous effects. There are various chemical and physical methods including ion exchange, reverse osmosis, electrodialysis and ultrafiltration for cleaning heavy metals from wastewater, but two parameters of economic efficiency and treatment operation efficiency have attracted researchers to another approach named biological treatment for years [4, 5].

Biological treatment is the process of using specific microorganisms to convert soil or harmful pollutants present in water to harmless products [3, 6]. Microalgae seem to be promising due to
their favorable capabilities, including the substantial capability of absorbing heavy metals and eliminating them from wastewater [7-9]. Cheap cultivation, effective biological treatment and production of valuable biomass of the microalgae made it appropriate method with many advantages. One of the most ideal microalgae species for this purpose is *Chlorella vulgaris* [10-12]. The current study takes a look at research literature of using *C. vulgaris*, with the aim of wastewater heavy metal removal. In this regard firstly, basic concepts of this field including various methods of eliminating heavy metals and using microalgae for this target was addressed, and then the application of *C. vulgaris* in the process of eliminating heavy metals from wastewater was fully presented.

### 1.1. Heavy Metal Treatment from Wastewater

Heavy metals, most notably lead, mercury, copper, cadmium, nickel, and arsenic, are the dangerous toxins around us. These toxics are found in air, drinking water and soil [13]. So they are quickly imported to human body directly or through the food cycle. Some of these metals are necessary for our body in low quantities, but their excessive presence in our body leads to acute poisoning. The main disadvantage of heavy metals is that they are not metabolized in the human body. Actually, heavy metals are not disposed of our body and they are aggregated after entering. This leads to several illnesses and effects including neurological disorders, cancers, hormonal imbalance, cardiovascular and respiratory disorders, etc. they increase and develop the virus, bacterial and fungal infections [14].

According to statistics, the global environmental pollution level through heavy metals has been multiplied by 4000 during the past 150 years. Increased concern about the rising risk of contamination of the aquatic environment and drinking water supplies with heavy metals such as Cadmium, Lead, Nickel, Chromium, Copper, Mercury, and Cobalt is caused by the accumulation of heavy metals in the food chain and sustainability in nature. Some heavy metals such as Mercury, Lead, Cadmium, Nickel, and Chromium are toxic even in small amounts, and their presence in drinking water endangers the health of all the living. Therefore, treatment and removal of heavy metal contaminations before being discharged to water resources is essential [15].

### 1.2. Heavy Metal Treatment Methods

There are various chemical and physical methods to remove heavy metals from water sources [4, 5, 16-18]. Any of the physical and chemical methods have their own problems. This encouraged researchers to develop a clean, cheap and secure approach as biotreatment. Eslami and Nemati (2015) in a review study surveyed various methods of removing heavy metals from wastewater. The results of their work are illustrated in Table 1 [3]. Given the above and according to this table, physical and chemical methods used for heavy metal removal are not often favorable due to high costs and low efficiency while biotreatment, due to the low investment costs and high efficiency can be very promising.

In the following, we describe the biological treatment process, its mechanism and influencing factors in detail.

### 2. TREATMENT OF HEAVY METALS FROM WASTEWATER USING MICROALGAE

#### 2.1. Heavy Metal Bioremediation

Bioremediation is the capability of some microorganism biomasses to remove heavy metals from wastewater through bioabsorption and indirect metabolic activities. Algae, molds, yeasts, bacteria, and fungi are some of these absorbents. Each one of the various physical and chemical methods briefly discussed in the last chapter, has its own disadvantages such as incomplete metal separation, requiring expensive equipment and monitoring systems, high energy consumption and leaving residue contaminants that must be removed. On the other hand, the above methods do not have the adequate ability to remove heavy metals when the concentration of metal ions is in the range of 10 to 100 mg per liter. Using biological methods for heavy metal removal from wastewater can fix some of the limitations and problems of chemical and physical methods, and it is considered a more economical solution. Bioremediation is the process of using special microorganisms to convert harmful contaminators present in water or soil to harmless products [6, 19].

#### 2.2. Bioremediation of Heavy Metals using Microalgae

Among microorganisms, microalgae have the potential to remove heavy metals, so they have a special place in the biological treatment. Research
has shown that some heavy metals are essential for the normal functioning of microalgae, including Iron and Manganese (for photosynthesis), Chrome (for metabolism), Zinc (affecting the performance of Chlorophyll and proteins) and Cobalt (for intracellular reactions and production of essential vitamins). About 75 years ago, the idea of using microalgae for wastewater treatment came to the researchers’ mind. But the use of microalgae for the purification and removal of heavy metals was proposed firstly by Oswald and Gootas (1957), and then the first serious studies in this field were started by Oswald (1988), continuing increasingly until now [20, 21].

Field experiments reported by Gale (1986), showed that photosynthetic microalgae play an effective role in removing toxic metals from wastewater and with the use of Cyanobacteria in ponds, they can absorb and remove up to 99% of the metal particles. Earlier Soeder et al. (1978) showed that a microalgae species called Coelastrium proboscideum can absorb 100% of Lead with 1 ppm concentration, dissolved in water in 24 hours at 23°C, and it can also absorb around 90% of it only after 1.5 hours at 30°C. Mac Hardy (1990) found that these microalgae have a high ability to absorb Zinc metal in its cell, by studying the ability of microalgae Cladophora glomerata [22]. Inthorna et al. (2002) investigated the ability of three green microalgae for the removal of Mercury, Cadmium, and Lead [23].

Also, Dwivedi (2012) stated that among the various species of microalgae, three species of Scenedesmus, Chlorella and Spirulina are the most common species used to remove heavy metals [6].

Metal ion absorption by microalgae comes along with releasing protons or other connected cations and an ion exchange process occurs in practice. Ion exchange capacity between different algae is very different. Single-cell algae such as Chlorella have a higher ion exchange capacity than string formed species, and this is due to the surface to volume ratio is higher in unicellular algae. By increasing the pH, the ion exchange capacity of algae is increased, because the competitive effects of H⁺ ions for binding to the cation binding sites is reduced. The important point here is that one metallic ion may bind to one alga through ion exchange, and a different metallic ion does it very differently, like complex formation with carboxyl groups or electrostatic bonds. Thus, we may see differences in methods and mechanisms of different ions [24, 25].

### 2.3. Influential Factors of Heavy Metal Removal using Microalgae

Several factors such as biomass concentration, environment pH, temperature, ionic strength, the initial concentration of the metal ions and various forms of them influence the adsorption of heavy metals by algae.

#### 2.3.1. The Initial Concentration of Metal Ions

Increasing concentration of metal ions in solution firstly increases the amount of metal adsorption by algae and then it saturates in a certain concentration. Compared to the adsorption of metal ions, the intracellular absorption of these ions by cells with increased concentrations of dissolved ions is reduced.

<table>
<thead>
<tr>
<th>Treatment Process</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical sedimentation</td>
<td>Low initial costs, easy utilization, less sedimentation time and improved sludge sedimentation</td>
<td>High sludge production, very high utilization and sludge disposal costs</td>
</tr>
<tr>
<td>Flotation</td>
<td>Low hydraulic retention time, relatively low costs</td>
<td>Further treatments are required to improve the heavy metal removal yield</td>
</tr>
<tr>
<td>Ion exchange</td>
<td>Lack of sludge production, low required time</td>
<td>High investment and current costs, resins are not suitable for all metals</td>
</tr>
<tr>
<td>Biological treatment</td>
<td>Low investment and utilization costs, high efficiency</td>
<td>The need to a specific microbe, special environmental conditions, requiring a substrate for microorganisms</td>
</tr>
</tbody>
</table>

Table 1. Some heavy metal treatment methods comparison [3].
2.3.2. pH

Most studies have shown that metal ion absorption is under influence of ion concentration in the environment, so determining the optimal pH for maximum metal removal by the algae is required. The optimal pH for each metal ion is different depending on the microalgae type, and there are also differences in case of different ions. The measure of this difference is the isoelectric point. At pH lower than the isoelectric point, cells are positively charged in the surface, which inhibits the binding of metal ions. Chen et al. (1993) reported that by increasing pH, there will be more negative places available for absorption of Copper ions on the surface of microalgae. Copper removal increases by increasing pH. Also, Crist et al. (1994) stated that the lower the pH, the lower is the number of binding sites, and also pH is increased during metal ion absorption. In general, the acidic pH of 4 and 6 are suitable for metal ion absorption. In very acidic environments, pH<2, the decrease in metal ion absorption by microalgae is significant because the high concentration of H⁺ ions competes with metal ion binding to cellular ligands [6, 26, 27].

2.3.3. Various Metal Ion Compounds

Metals exist in wastewater in several forms (free ions to intricate complexes with organic compounds and ligands, and also ions adsorbed on the surface of the particles); however, their toxic effects on the cells and even absorption amount depends only on the concentration of free ions. The concentration of free ions is determined by complex reactions between metal ions, ligands, pH and hydrolysis constant of complexes. In most cases, decreasing pH increases free ions concentration. Therefore, in appropriate and adequate absorption of metal ions by the microalgae, the free ions to total metal concentration ratio must be preserved at a high level [28, 29].

2.3.4. Biomass Concentration

The amount of collected metal ions from the environment is also dependent on the biomass concentration. Increasing biomass concentration by one gram decreases metal ion adsorption because it changes the present equilibrium in the adsorption process. On the other hand, increasing the concentration of biomass decreases the available metal ions and increases the electrostatic reactions, interactions between the binding sites, and reduces the amount of mixing. Electrostatic interactions between cells in the adsorption of metal ions are important because when the distance between the cells is higher, more metal ions are absorbed by the cells. However, increasing biomass concentration raises the number of metal ions absorbed into the cell, because the number of binding places is increased this way [30, 31].

2.3.5. Temperature

Generally, present studies in the field of effect of temperature on heavy metal removal using microalgae do not show decisive and identical results and the results do not match in most cases. According to one argument, the adsorption of metal ions by microalgae rises by increasing the temperature, because the adsorption of metal ions by the cells is an endothermic process, although this rule may have exceptions; for example, the absorption of Cadmium ion by Sargassum (macroalgae) is exothermic and decreases with the temperature rise. Another reason for the increased absorption of metal ions with increasing temperature is that rising temperature increases the number of active sites available for bonding metals. In intracellular absorption of metal ions by living cells, the maximum absorption occurs in an optimal temperature and every temperature change leads to a reduction in ion absorption. Totally, it seems that the effect of temperature on the removal of heavy metals by microalgae can be different according to the type of the considered metal and microalgae specie. In most researches done on heavy metal removal from wastewater using microalgae, the temperature is considered to be in 25-30 range, which is the optimal temperature for microalgae growth [32, 33].

2.3.6. Ion Strength

The amount of metal ion absorbed by algae is affected by the presence of anions and cations in the bulk. Increasing the ionic strength reduces the adsorption because of a competition between light metals and a variety of heavy metals to bind to active sites. Besides, increasing the number of anions reduces the number of metal ions available by the formation of insoluble complexes with metal cations. Chen et al. (1997) studied the effect of ion strength on the adsorption of metal ions. They used Sodium Chlorite in order to regulate the amount of ionic strength in amounts of 0.5, 0.05 and 0.005 mol/L and found that when the ionic strength is reduced from 0.5 to 0.05 mol/l,
efficiency of removal increases from 80% to 95%. This observation convinced them that during adsorption, there is a competition between metal ions and other functional groups. At fixed pH, the number of functional groups is constant, so available locations for metal ion absorption are reduced by increasing the ionic strength [33, 34].

In this part, biological treatment of heavy metals by microalgae, the general mechanism of treatment including adsorption and intracellular absorption and their influencing factors such as pH, temperature, ionic strength, and concentration of biomass are addressed. In the next section, we will discuss heavy metal removal especially using microalgae C. vulgaris.

3. HEAVY METAL TREATMENT USING MICROALGAE C. VULGARIS

Chlorella vulgaris is a photosynthetic microorganism from Chlorellaceae family, which was discovered by the Dutch researcher Willen Beijrenick in 1980. This microorganism is a unicellular green microalga, having spherical cells with a diameter of 2 to 10 micrometers, in which a mother cell delivers 4 daughter cells, so its growth rate is high (cellular bulk doubling time is about 19 hours). Rapid growth, easy and flexible terms of culture and resistance to unfavorable factors are some advantages that make these microalgae appropriate for various applications in food industry, agriculture, cosmetics, pharmaceutical, wastewater treatment, and biofuel production. Japan, Germany, and Taiwan are the main Chlorella producers [10-12].

About optimal conditions of C. vulgaris growth, much research has been done. Factors affecting C. vulgaris growth come in two categories of nutritional factors and environmental factors. Among the nutritional factors, type and amount of carbon source and nitrogen source are the most important. And the most important environmental factors include the medium pH value, temperature, and light intensity. Based on extensive researches, glucose and nitrate are respectively the most appropriate sources of carbon and nitrogen for C. vulgaris growth. However, these microalgae are capable of nurturing from inorganic carbonic compounds, such as carbonates, but the most important inorganic carbonic source that relates this biological species to biological treatment is its ability to remove or consume carbon dioxide. According to studies, the optimal concentration of glucose for C. vulgaris is about 6 g/L, and optimal concentration of nitrate is about 1 g/L, and also optimal content of carbonate is 8% in form of carbon dioxide-enriched air. The environmental optimal conditions are pH = 9, at 30°C and light intensity of 5 Klux [35, 36].

3.1. Application of C. vulgaris in Industrial Wastewater Treatment

As it was mentioned, C. vulgaris due to rapid growth and high metabolism, easy and flexible culture conditions, and resistance to unwanted factors, is considered important in various applications including wastewater treatment. The flexibility of culture conditions makes its growth possible in different wastewater environments and that is the reason for vast investigations on this field aiming to use C. vulgaris in wastewater treatment. What has been mostly in researchers’ mind is the capability of this microalgae in nutrition and cleaning nitrogen sources present in wastewater, which is one of the most important and dangerous pollutants in wastewater [37-39].

In 1996, Lau et al. studied the ability of C. vulgaris in removing inorganic materials in wastewater, it became clear that the microalgae are capable of removing up to 86% of inorganic nitrogen and up to 70% of inorganic phosphorus [40]. Kim et al. also reported that 93.5% of nitrogen and 96% of phosphorus in animal waste has been removed by the C. vulgaris during 4 days [41]. Gonzalez et al. (1997) investigated treating ammonia and phosphorus from agricultural wastewater in a cylindrical and triangular bioreactor using C. vulgaris. The results showed that cylindrical bioreactor was the best for ammonia removal and triangular bioreactor had a higher efficiency for phosphorus [42]. Mamun et al. (2012) studied the effect of C. vulgaris on removing nitrogen and phosphorus content from residential wastewater in pH = 7. After 5 days, the highest removal for nitrogen and phosphorus was observed to be respectively 83.1% and 91.2% [43]. Farooq et al. (2013) used C. vulgaris to remove nitrate with an initial concentration of 18 mg/L and phosphate with an initial concentration of 28 mg/L in a wastewater and after 8 days they reported the efficiency over 99.9% in both cases [44]. Salgueiro et al. (2016) approved the elimination of over 99% of phosphorus in a wastewater using C. vulgaris in pH = 9.8 and temperature of 26°C [45].
3.2. Heavy Metal Treatment from Wastewater using C. vulgaris

Studies confirmed the high ability of *C. vulgaris* in heavy metal removal from wastewater. A serious research in this field was started by Greene in New Mexico university of United States in 1990. Greene studied the performance of *C. vulgaris* in removing metal ions of Nickel, Cadmium, Cobalt, Copper, Chrome, Lead, Aluminum, Zinc, Mercury and even Uranium oxide and investigated the effect of factors like pH and ion strength on the amount of metal ion absorbed by the *C. vulgaris*. In his study, the main contents of the microalgae culture medium consisted of 0.3 g/L of Potassium Phosphate, 0.3 g/L of Magnesium Sulphate heptahydrate, 2 g/L of Potassium nitrate and 0.6 g/L of Urine, and the environment pH was fixed to 5.5 by carbon dioxide injection. Also, the initial concentration of metal ions was considered to be 0.1 mM [1].

Brinza et al. (2007) have referred to the use of microalgae *Chlorella* genus, particularly *C. vulgaris*, in order to remove heavy metals such as Co, Cu, Pb, Cd, Zn and Ni in their study [46]. Hameed and Ebrahimi (2007) examined 14 different species of microalgae that have great potential for the removal of heavy metals and introduced *C. vulgaris* as one of the most effective forms of microalgae for biological removal of a wide range of heavy metals, because of having alginate compounds in the cell wall [47]. Doshi (2007) et al. stated that a lot of researches have been conducted on the biological removal of Copper with a focus on *C. vulgaris* specie (in form of alive culture or dead biomass) at pH = 4-7 [48]. The studies also demonstrated that Copper biological absorption mechanism by *C. vulgaris* is based on electrostatic interactions between Copper ions and microalgae cell wall compounds [49]. Rajamani (2007) stated that the adsorption in *C. vulgaris* occurs by its porous cell wall. Moreover, the cell wall of this microalgae has ligands that are capable of forming bonds with various metal groups [50].

Peralez-Vela et al. (2008) pointed out the high capacity of genus *Chlorella* in removing heavy metals Cu, U and Cd [51]. Khan et al. (2008) have reported that *C. vulgaris* has a high potential for Cadmium removal [52]. Gonzalez et al. (2011) stated that *C. vulgaris* is capable of removing bivalent (such as Hg, Cd, Pb, Ni, Co, Zn), trivalent (Cr, Fr) and hexavalent (Cr (VI)) heavy metals [53]. Kumar et al. (2014), examining different microalgal species and the previous researches done on them in their review study, came to the conclusion that two species of *Chlorella* and *Scenedesmus* are the most important microalgal options for heavy metal removal [54]. *C. vulgaris* shows an average performance in case of Mercury, but in the case of two other metals, especially Lead; its performance is very good compared to other species. And also *C. vulgaris* potentially efficient for the removal of heavy metals in Acid Mine Drainage (AMD) [55].

Mehta et al. (2001) managed to remove 70% of Nickel and 80% of Copper using *C. vulgaris* in a wastewater with an initial concentration of 5.2 milligrams per liter. A year later, these researchers managed to reduce the Copper content of a wastewater from 32 mg/L to 14.48 mg/L in operational conditions of pH = 3.5 and temperature = 25°C, using *C. vulgaris* [56]. Also, Inthorn et al. (2002) succeeded in decreasing the Mercury, Cadmium and Lead content of a wastewater by respectively 94%, 89% and 88%, using *C. vulgaris* and in pH = 7.5 [23]. Tien et al. (2005) managed to remove 4 mg/g of Cu$^{2+}$ by cultivating *C. vulgaris* in pH = 4.5 [57]. Al-Rub et al. (2004) investigated the *C. vulgaris* performance in both cultivating biomass and dry biomass in the same conditions of pH = 5 for Nickel. The results showed that *C. vulgaris* has the same capabilities in metal removal in these two states so that Nickel removal for cultivating biomass was obtained as 15.4 mg/g and for dry biomass, it was obtained as 15.6 mg/g. They also investigated the *C. vulgaris* performance in another study (2006), in order to bioremediation of Copper and showed that microalgae are able to absorb 58.5 mg of Copper in 1 gram of its biomass in conditions of pH = 5 and temperature of 25°C [58]. Mehta et al. (2001) showed that *C. vulgaris* ion absorption for Nickel and Copper is respectively 70% and 80% in a solution with a concentration of 5.2 mg/L, while if the concentration reaches 10 mg/L, the microalgae absorb only 37% and 42% of Nickel and Copper, respectively [10, 59].

Aksu and Donmez (2006) worked on the removal of two Cadmium and Nickel metals with an initial concentration of 150 mg/L using *C. vulgaris* biomass with a concentration of 1 g/L. in this study, in 25°C, absorption of Cadmium in pH = 4 equaled to 86.6 mg/g and for Nickel in the pH = 4.5 equaled to 58.4 [60]. Ferril et al. (2008) reported the absorption of Nickel, Lead and zinc, respectively as 29, 131 and 43 mg by *C. vulgaris* in
terms of pH = 5 and 20°C [61]. Vogel et al. (2010) cultivated C. vulgaris for 4 days with a concentration of 0.76 g/L and managed to absorb 14.3 mg/g of uranium metal. In this study, the initial concentration of uranium was 23.8 mg/L and pH was considered as 4.4 [50].

Ferreira et al. (2012) reported removing 131.36 mg/g of Lead and 43.41 mg/g of Zinc in pH = 5, using C. vulgaris dry biomass [61]. Fathi et al. (2013) removed 70% of Mercury content of a wastewater with an initial concentration of 20 mg/L using C. vulgaris. Chan et al. (2014) reduced the Copper and Zinc content of a wastewater by 81.7% and 94.1%, respectively after 10 days of cultivating C. vulgaris [62]. Savari et al. (2015) investigated the ability of C. vulgaris in the elimination of Lead, Cadmium and Nickel heavy metals from industrial wastewater. They reduced the amount of Lead, Cadmium, and Nickel respectively up to 94, 86 and 94.9% during 5 days, initial concentrations being 112, 28 and 203 µg/L [63].

Eidizadeh et al. (2015) compared the performance of two microalgae species, C. vulgaris s, and Nanocloropsis oculata, in removing Lead and Nickel contents from an industrial wastewater. Culture period was 5 days, the temperature was 25°C and the initial concentration of Lead and Nickel ions were respectively considered as 112 and 203 µg/L in this study. C. vulgaris has a higher capability in case of Lead metal, so that the maximum Lead removal after 5 days is 94% for C. vulgaris and 88.3% for N. oculata. But in the case of Nickel, the performance of the two microalgae are very close so that the maximum efficiency of Nickel removal for C. vulgaris was measured to be 94.9% and for N. oculata equaled to 93.4% [64].

Hammouda et al. (2015) cultivated combinational species of C. vulgaris in order to remove heavy metals in 30°C, 4 Klux of light intensity and pH = 4. At the end of the 20 days period of culture, they managed to remove 99.5% of Nickel, 73.2% of Mn, 56.3% of Cr, 54.5% of Cu and 51.4% of Zn from a wastewater, which was a mixture of residential and industrial wastewater [65].

3.3. Factors Affecting Heavy Metal Removal from Wastewater using C. vulgaris

We studied the factors which have an impact on the treatment of heavy metals by microalgae though the last chapter are also true about C. vulgaris, but among them, four factors i.e. pH value, temperature, the initial concentration of metals and contact time have been under more attention in researches in case of C. vulgaris. Therefore, we address the impact of these factors on heavy metal removal from waste water using C. vulgaris.

3.3.1. pH Effect

Greene’s (1990) study on the effect of pH on the adsorption of metal ions by C. vulgaris showed that reducing the pH, leads to desorption of metal ions and could adversely affect the absorption of metals. Also, the optimal range for absorption of metal ions, Cadmium, Zinc, Chromium, Copper, Nickel, and Cobalt was 6-7, and it is 4-5 for Lead and Aluminum. The results of his work are illustrated in Fig. (1). In this figure, the vertical axis represents the amount of metal ion absorption, and its maximum can be 0.1 mMolar, which is the initial concentration. As can be seen, Zinc has the highest absorption and then Lead and Cadmium are at the second and third places [1].

Aung et al. (2013) investigated the effect of pH in 3-6 range in 30°C on the absorption of the Lead metal ion by C. vulgaris, the initial concentration being 0.1 molar. The results implied that the maximum removal efficiency, 99.4%, occurs at pH = 6. The Lead absorption efficiencies in various pH values are shown in Fig. (2) [13].

Yang et al. (2014) investigated the effect of pH on removing four metals of Mn, Zn, Cd, and Cu using C. vulgaris culture. The culture period was 7 days, the temperature was set to 28°C, pH was in 6 levels in 2-10 range and initial concentration of heavy metals was considered to be 5 mmol in this study. The results are illustrated in Fig. (3), as we can see in the figure, increasing pH to an identified value (pH = 6) leads to an increase in removing heavy metal, but the removal percentage decreases for pH values above 6 [66].

Malakotian et al. (2015) also evaluated the effect of pH value in the range of 3-8 on removal percentage of Zinc ion using C. vulgaris with biomass concentration of 2 g/L, metal initial concentration of 50 mg/L, temperature and contact time being respectively 25°C and 0 minutes, and came to the conclusion that increasing pH to lower than 7, continuously increases the absorption efficiency and increasing the pH higher than 7 decreases the removal efficiency of this metal. The maximum removal efficiency of Zinc was recorded as 90.23% in pH = 7 [67].
Fig. (1). Effect of pH on various metal ions absorption by C. vulgaris [1].

Fig. (2). Lead removal efficiency in different pH values using C. vulgaris [13].
3.3.2. Temperature Effect

As it was pointed out in the previous chapter, in examining factors affecting heavy metal removal by microalgae part, the effect of temperature parameter can be different according to the microalgae and heavy metal type. In *C. vulgaris* case, Aksu (2002) observed the increase of adsorption of the Zinc ion by *C. vulgaris* dry biomass, with rising temperature, but he reported a decrease in Cadmium adsorption from 83.3 mg/g (in 20°C) to 51.2 mg/g (in 50°C) under the influence of temperature raise in another experiment [68].

Yang *et al.* (2014) investigated the effect of temperature on Cu, Cd, Mn and Zn heavy metals removal using Chlorella culture. Culture period was considered to be 7 days, temperature was set to 28°C and pH = 6. They investigated the effect of initial concentration on the amount of metal ions removal, applying three different levels of initial concentrations for each metal ion. The results showed that increasing the concentrations significantly reduces removal efficiency, so that the maximum removal efficiency of the considered metals with an initial concentration of 2 mM was 62.05 and 83.68 respectively for Zn and Mn, and it was 83.6% and 74.34% for Cu and Cd, with initial concentrations of 0.2 mM. The results are shown in Table 2. In this table, IC and RE respectively represent the initial concentration of metal ions and the removal efficiency [66].

Researches show that the initial concentration of metal ions has an important impact on their adsorption by microalgae. We can investigate this considering the metal and microalgal type used. In other words, each microalgal species has a specific threshold for an initial concentration of each metal ion, and in case of excessive presence of a metal ion, we observe a disorder in microalgal performance in removing the metal, and the removal efficiency significantly decreases [69].

In the case of *C. vulgaris*, Yang *et al.* (2014) studied Cu, Cd, Mn and Zn metal ions removal through culturing a type of Chlorella. The culture period was considered to be 7 days, temperature was set to 28°C and pH = 6. They investigated the effect of initial concentration on the amount of metal ions removal, applying three different levels of initial concentrations for each metal ion. The results showed that increasing the concentrations significantly reduces removal efficiency, so that the maximum removal efficiency of the considered metals with an initial concentration of 2 mM was 62.05 and 83.68 respectively for Zn and Mn, and it was 83.6% and 74.34% for Cu and Cd, with initial concentrations of 0.2 mM. The results are shown in Table 2. In this table, IC and RE respectively represent the initial concentration of metal ions and the removal efficiency [66].
In another study, Malakotian et al. (2015) investigated the effect of initial concentration of Zinc metal on the amount of its removal using \textit{C. vulgaris} dry biomass. In this study, microalgae concentration was 2 g/L, and temperature and pH were set to 25°C and 7, respectively. Results showed that with an increasing initial concentration of metal ion from 50 to 250 mg/L, its removal decreases by 44.9% [67].

### 3.3.3. Contact Time Effect

Contact time is different according to the form of the treatment process, which can be in the form of complete cultivation of microalgae in wastewater medium or merely using microalgae biomass as a biological absorber. In the first case, which was formerly discussed, the microalgae is often cultivated for 5 to 10 days in the wastewater medium and the absorption process takes place during growth, but in the latter case purification process only includes adsorption and it occurs in a shorter time compared to the first case [2]. In the case of biological adsorption, Aung \textit{et al.} (2013) examined the effect of contact time on the absorption of this metal in their study on Lead absorption using \textit{C. vulgaris}, with an initial concentration of 1 mM and with the temperature of 30°C and pH = 6. According to their observations, the minimum time required to achieve the highest removal efficiency of Lead is about 100 minutes [13].

Malakotian \textit{et al.} (2015) also investigated the effect of contact time of biomass and the pollutants in order to remove the Zinc metal using \textit{C. vulgaris} biomass. In this study, a biomass with 2 g/L concentration, T = 25°C, pH = 7 and initial concentration of Zinc \textit{i.e.} 50 mg/L was used [67]. The time required to reach the maximum removal percentage of Zinc is about 60 minutes. Also, Rodrigues \textit{et al.} investigated the presence and removal of more than one metal by \textit{C. vulgaris}. It is the potential sorbents of Ni$^{2+}$, Zn$^{2+}$ and Pb$^{2+}$ mixtures which exhibit the following affinity order: Pb$^{2+} >$ Zn$^{2+} >$ Ni$^{2+}$. Zn$^{2+}$ and Ni$^{2+}$ compete somewhat equally in \textit{C. vulgaris} biosorption. Pb$^{2+}$ biosorption by \textit{C. vulgaris} is mostly not affected by Zn$^{2+}$ and Ni$^{2+}$ [70].

At last, a brief history of the most important works done is presented in Table 3 in order to compare the results of different studies conducted for the performance of \textit{C. vulgaris} in removing heavy metals from wastewater.
various heavy metals. IC and Temp respectively represent the initial concentration and operational temperature.

**CONCLUSION**

The removal of heavy metals from wastewater using *C. vulgaris* is studied in this research. Various methods of removing heavy metals from wastewater are investigated and the biologic approach is highlighted. Mechanism of heavy metal removal by *C. vulgaris* microalgae and factors affecting biological heavy metals removal, including medium pH, temperature, ion strength and the concentration of biomass are discussed. It is clear that the use of *C. vulgaris* in removing Copper, Lead, zinc, Cadmium, and Nickel is higher compared to the other metals. Highest metal removal was for lead equal to 99.4% after that nickel removal efficiency was 99%. The best pH of the culture medium was between 6-7 and also optimum temperature was between 25-30°C. Also, the results showed that increasing the concentrations significantly reduces removal efficiency.

**CONSENT FOR PUBLICATION**

Not applicable.

**FUNDING**

None.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest, financial or otherwise.

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Table 3. A brief history of the most important works on the performance of *C. vulgaris* in removing heavy metals [67].

<table>
<thead>
<tr>
<th>Metal</th>
<th>IC (mg/L)</th>
<th>pH</th>
<th>Temp (°C)</th>
<th>% Removal</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg-Cd-Pb</td>
<td>1</td>
<td>7</td>
<td>28</td>
<td>94-89-88</td>
<td>[23]</td>
</tr>
<tr>
<td>Cd-Ni</td>
<td>150</td>
<td>4.4</td>
<td>25</td>
<td>57.3-38.9</td>
<td>[60]</td>
</tr>
<tr>
<td>U</td>
<td>23.8</td>
<td>4.4</td>
<td>-</td>
<td>60.1</td>
<td>[50]</td>
</tr>
<tr>
<td>Cu</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>70</td>
<td>[62]</td>
</tr>
<tr>
<td>Pb</td>
<td>112</td>
<td>6</td>
<td>30</td>
<td>99.4</td>
<td>[13]</td>
</tr>
<tr>
<td>Zn–Cu–Mn–Cd</td>
<td>5 (mmol)</td>
<td>6</td>
<td>28</td>
<td>98-93-96-94</td>
<td>[66]</td>
</tr>
<tr>
<td>Pb–Cd-Ni</td>
<td>112-28-203 (µg/L)</td>
<td>-</td>
<td>-</td>
<td>94-86-94.9</td>
<td>[63]</td>
</tr>
<tr>
<td>Pb-Ni</td>
<td>112-203 (µg/L)</td>
<td>-</td>
<td>25</td>
<td>94-94.9</td>
<td>[34]</td>
</tr>
<tr>
<td>Ni–Mn–Cu–Zn</td>
<td>-</td>
<td>7.8</td>
<td>30</td>
<td>99-73-54-51</td>
<td>[65]</td>
</tr>
<tr>
<td>Zn</td>
<td>50</td>
<td>7</td>
<td>25</td>
<td>90.2</td>
<td>[67]</td>
</tr>
</tbody>
</table>


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