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Influence of clinoptilolite on the efficiency of heavy metal removal from wastewater by *Chlorella vulgaris*

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ABSTRACT

Heavy metals are the main pollutants in electroplating wastewater. Currently, mainly chemical methods, which generate huge amounts of toxic sludge, are used in industrial wastewater treatment. To achieve environmental sustainability, there is a need to introduce an eco-friendly treatment of such contaminated wastewater. The present study was carried out in order to: (i) examine the hypothesis that a mixture of clinoptilolite and *Chlorella vulgaris* would enhance the efficiency of the removal of heavy metals, (ii) evaluate the potential application of sediments containing *C. vulgaris* and clinoptilolite after N-NH_4 assimilation for heavy metal removal, (iii) assess the selectivity of *C. vulgaris* and clinoptilolite for heavy metal removal and (iv) verify if there was potential applicability of *C. vulgaris* and clinoptilolite as effective and eco-friendly biomaterial for developing the Fe(III), Zn(II) and Pb(II) removal procedure. The influence of Pb(II) on Fe(III) and Zn(II) uptake was also studied. The study was conducted in a multimetallic system. The results demonstrated that all the sorbents were sufficient to remove >97.5% of Fe(III) and Pb(II) and 75% of Zn(II) from the investigated wastewater. The maximum uptake of lead and iron (99.9%) was more efficient than the uptake of zinc (94.8%). The mixture of unicellular algae and zeolites enhanced the efficiency of the removal of heavy metals since ion-exchange resins, such as zeolites, have mono-functional sites, whereas the algal cell walls have multi-functional sites, thereby complementing each other.

Keywords: Microalgae; Clinoptilolite; Bioremoval; Fe(III), Zn(II) and Pb(II); Industrial wastewater

1. Introduction

Heavy metals are the main pollutants present in electroplating wastewater. Zinc-plating wastewater usually contains many heavy metals, such as Fe(III), Zn(II) and Pb(II). Chemical precipitation, chemical oxidation or reduction, electrochemical treatment, evaporative recovery, filtration, ion exchange and membrane technologies are used to remove heavy metal ions from industrial wastewater. In wastewater containing 1–100 mg/L of dissolved heavy metal

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ions, such physico-chemical methods show low efficiency for metal removal [1,2].

Many studies have focused on the use of biological methods for the industrial wastewater treatment [3–5]. The use of microalgae in bioremoval of several pollutants from wastewater provides a promising alternative to physico-chemical methods, mostly owing to their high metal binding capacity and the source of this raw material. Bioaccumulation (i.e., active biosorption) of metals by algae depends on several processes such as metal binding on intracellular compounds, intracellular precipitation, methylation and other mechanisms [6].

One of the most ubiquitous microalgae is *Chlorella vulgaris.* Owing to its high growth rates at minimum environmental requirements and the high ability to remove pollutants, *C. vulgaris* is known as one of the most useful microalgae with many potential applications [7,8]. The removal of heavy metals from electroplating wastewater by *C. vulgaris* has numerous advantages, for example, low cost and non-toxic treatment without any chemicals [9]. *Chlorella vulgaris* could be cultivated in photo-bioreactors [10–12].

Clinoptilolite can absorb many ions and is selective towards heavy metals [12,13]. Clinoptilolite removes ions by means of ion exchange and adsorption processes [14,15].

The processes of adsorption, biosorption and bioaccumulation may be tools for remediation technologies as well as for wastewater treatment. There is a need to find more selective sorbents which can be used in multimetallic systems [16].

Zabochnicka-Świątek [4,12,17] investigated the heavy metal removal by *C. vulgaris* and clinoptilolite from synthetic wastewater. However, performance of a combined industrial wastewater treatment including biomass from sediments after N-NH_4 assimilation in bioremoval of heavy metals from electroplating wastewater has not been previously investigated. Therefore, this study aims to fill this gap in the literature by investigating the recycling of sediments containing a mixture of *C. vulgaris* and clinoptilolite for N-NH_4 removal and then reusing the (recycled) sediment in heavy metal removal from electroplating wastewater. The investigations are in line with Patent P.412966 [18] on the method for biological treatment of industrial sewage to remove nitrogen and heavy metals.

This study was carried out in order to: (i) examine the hypothesis that the addition of clinoptilolite would enhance the efficiency of the removal of heavy metals by *C. vulgaris*, (ii) evaluate the potential application of sediments containing a mixture of *C. vulgaris* and clinoptilolite after N-NH_4 assimilation for heavy metal removal, (iii) assess the selectivity of *C. vulgaris* and clinoptilolite for heavy metal removal and (iv) to verify if there was potential applicability of *C. vulgaris* and clinoptilolite as effective and eco-friendly biomaterial for developing Fe(III), Zn(II) and Pb(II) removal procedure. The influence of Pb(II) on the Fe(III) and Zn(II) uptake was also studied. The study was conducted in a multimetallic system.

2. Materials and methods

2.1. Characteristics of the sorbents

Chlorella vulgaris was used as the study material in experiments on bioaccumulation of Fe(III), Zn(II) and Pb(II) from electroplating wastewater. Algae were cultured in 250 mL flasks at the irradiance of 80 ± 5 μmol photons m^2/s in a climatic chamber with the light/dark period of 12/12 h and at temperature 29°C ± 0.5°C during the day and 25°C ± 0.5°C at night in order to increase the biomass production. Cells in the exponential growth phase were used. The algae were harvested by centrifugation. The concentration of living biomass in the experimental samples equaled 1%.

Natural zeolite (clinoptilolite) in the concentration of 1% was used for the experiments. Second, a mixture of *C. vulgaris* (1%) and clinoptilolite (1%) was prepared. Third, the sediment after 1-month N-NH_4 assimilation from industrial wastewater using a mixture of *C. vulgaris* (1%) and clinoptilolite (1%) in the previous stage was used in the concentration of 1%. The sediment contained 1% of a mixture of the *C. vulgaris* biomass and clinoptilolite (grain size of 0.5–1.0 mm) which had been originally mixed in 1:10 ratio. During the experiment, 2% of molasses were added every day at the beginning of the night phase to support the algal growth.

2.2. Batch sorption experiments

Electroplating wastewater was used. The wastewater was taken after a pre-treatment process in which emulsion of anionic polymers and alkaline aqueous solution of chelating agent were used. The wastewater was polluted with the following heavy metals: Fe = 20 mg/L, Zn = 12 mg/L and Pb = 10 mg/L. These heavy metals were dissolved in the wastewater as salts: $Fe(NO_3)_3{\cdot}9H_2O$, $Zn(NO_3)_2{\cdot}6H_2O$ and $Pb(NO_3)_2$. Due to its low tendency to form metal complexes, nitrate was used as a counter ion [19]. The following parameters were determined in the wastewater: pH = 8.09, dissolved oxygen concentration (DO) = 38.8%, N-NH_4 concentration = 22.4 mg/L, N-NO_3 = 1.4 mg/L and total N = 25.2 mg/L.

The following sorbents were added to 500 mL samples of electroplating wastewater containing Fe(III) and Zn(II) (No: 1–4) or Fe(III), Zn(II) and Pb(II) (No: 5–8):

- A sample – algae (*C. vulgaris)* 1% (No. 1 and No. 5),
- AZ sample – mixture of algae (*C. vulgaris)* 1% and zeolite (clinoptilolite) 1% (No. 2 and No. 6),
- Z sample – zeolite (clinoptilolite) 1% (No. 3 and No. 7),
- S sample – sediment after N-NH_4 assimilation 1% (No. 4 and No. 8).

1 mL of growth medium was added to each sample containing the living algal biomass. Based on the previous investigations, the Bioflorin growth medium was used. The Bioflorin is an inexpensive, multi-purpose concentrate of nutrients designed for the nourishment of plants. Bioflorin contains 5.9% of nitrogen (N), 3.0% of phosphate (P_2O_5), 6.5% of potassium (K) and other macro- and micronutrients, including B, Cu, Mn, Mo, Zn.

The experiment in triplicates lasted 96 h. The relative standard deviation was between 5% and 10%.

2.3. Analytical methods

The pH, DO, optical density, N-NH_4, dry mass, chlorophyll *a* and heavy metal concentrations were measured. The pH was checked with a pH meter and the oxygen content was measured with an oxygen meter. A standard densitometer was used to determine optical density. Heavy metal concentrations were determined by emission spectrometry with inductively coupled plasma optical emission spectrometry. Bioaccumulation is a very rapid process. According to the literature, the optimal time to follow the bioaccumulation kinetics and to reach equilibrium conditions should be short: <65 min. This is because physiological changes in algae or physico-chemical changes in the medium could modify metal speciation. Particularly, according to the physico-chemical changes in the solution, the following mechanisms could occur: a decrease in metal concentrations, an increase of the complexation with extracellular ligands or the production of exudates [19].

However, exposure lasting a few days could be helpful to determine the growth rates under stress conditions in the wastewater contaminated with heavy metals [20–22].

Hence, during the current investigations, the measurements were performed after 1, 12, 24, 36, 48, 72 and 96 h of contact time. Before the measurements, the samples were agitated for 1 h in a rotary shaker.

The N-NH_4 content was measured by titration every 24 h. After 96 h of contact time, the measurements of dry mass and chlorophyll *a* were performed in the samples containing only *C. vulgaris*. The analysis of the dry residue was done in accordance with the Polish Standard. To determine chlorophyll *a*, the absorbance measurements were taken at wavelengths of 664, 647, 470 and 740 nm, using a reference solution of acetone 90%.

3. Results and discussion

3.1. Effects of pH

The main metal binding sites in algae are carboxyl and sulphate groups. These groups are acids and their availability is pH dependent. These groups generate a negatively charged surface at pH in the range of 3.5–5.5. Metal biosorption is dependent on the electrostatic interactions between the cationic species and this surface [23].

pH is an important parameter for the sorption process. Heavy metals appear in different forms at various pH values. This influences their ability of being accumulated by microorganisms or adsorbed on clinoptilolite.

Iron, which most often occurs in the trivalent form, is insoluble at neutral pH [23]. Algae can absorb this metal and bind it in the form of cystic spores, which are located on the cell wall [24].

The free Zn(II) ion coordinates water molecules to form octahedral $Zn(H_2O)_6^{2+}$ in the environment where inorganic and organic ligands are absent. In freshwater, Zn(II) at pH = 4–7 exists as a positively charged ion Zn^{2+} while $ZnSO_4$ and free ions dominate at pH = 6 [25]. At pH > 9, anionic forms $Zn(OH)_2$, $Zn(OH)_3^-$ and $Zn(OH)_4^{2-}$ are prevalent [26].

The accumulation of lead by algae is dependent on water pH [20]. With increasing pH, the solubility of lead decreases [27]. Pb(II) removal by clinoptilolite is an ion-exchange process with very strong specific binding of this metal [28].

Fig. 1 presents changes in pH values with time during removal of heavy metals.

In each system, only a small change in the pH values was observed during the experiment. After the first hour, pH decreased in the samples containing *C. vulgaris*. Next, a slight pH increase was observed, which persisted until the end of the experiment. The increase of pH could be attributed to the dissolution of some cytoplasmic components or ions (e.g., carbonates) that are released into the solution [1].

The highest pH was found in the samples containing sediment and the lowest in the ones containing *C. vulgaris.* The maximum pH = 7.73 was observed after 12 h of contact time in the multimetallic system containing sediment as the sorbent. The lowest pH = 6.62 was found after 12 h of contact in the binary metal system containing algal biomass.

It was also observed that pH in the samples containing *C. vulgaris* and clinoptilolite was higher than in the samples

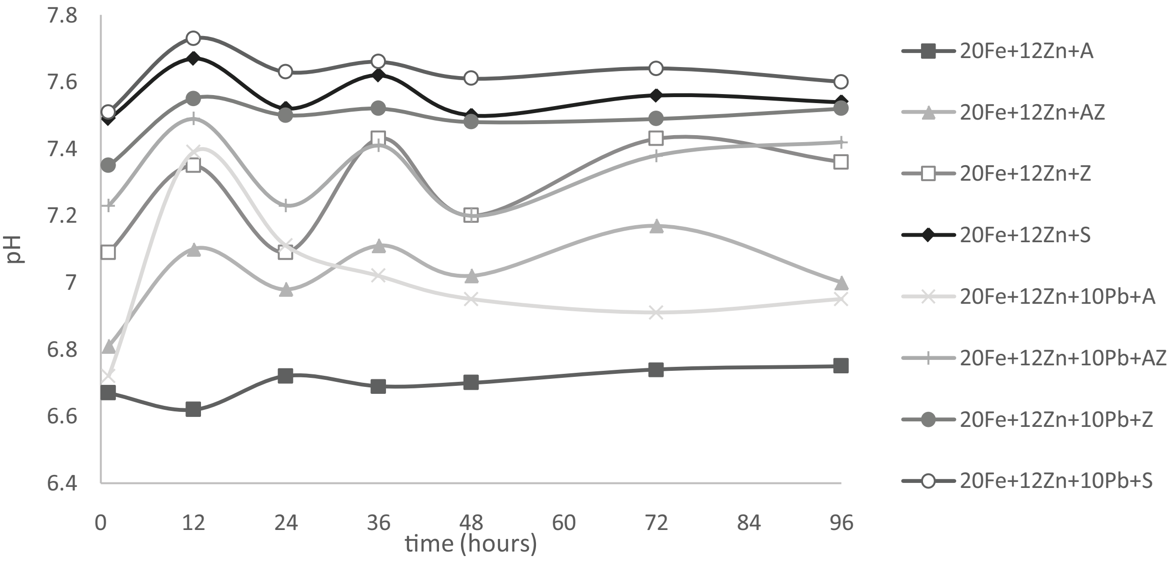

Fig. 1. pH values observed during the sorption experiment.

with *C. vulgaris* alone because the use of clinoptilolite increases pH. In the water solution of clinoptilolite and *C. vulgaris*, the pH values of 8.02 and 7.03, respectively, were found.

3.2. Cell activity

The oxygen concentration is an important factor for the indication of cell activity. In general, the solubility of gas in water depends on the water nature, pressure, temperature and the physico-chemical and biological composition of water. The solubility of gases in liquid decreases with the increasing temperature. Oxygen in water and wastewater comes mainly from the atmosphere and from the process of photosynthesis by aquatic plants (seaweed, algae and plankton) [29]. Fig. 2 presents the oxygen concentration changes with time during the removal of heavy metals.

A decrease in the concentration of oxygen after 1 h was observed. Then, the oxygen concentration varied with time. The oxygen content was lower after 96 h than at the beginning, which could have been caused by the reduced photosynthetic activity due to the toxicity of heavy metals towards algae.

After 36 and 48 h of contact time, the highest increase in the oxygen concentration was observed in samples containing the *C. vulgaris* biomass. The lowest value of 50.2% was found after 48 h of contact time in samples containing the sediment in the binary metal system.

The optical density of a cell measured during the experiment is presented in Table 1.

During the experiment, only a small change in the values of optical density was observed. The optical density was higher in the multimetallic system than in the binary metal system. For the samples with *C. vulgaris* biomass, the maximum optical density was 282 NTU after 1 h for the investigated wastewater with Fe(III), Zn(II) and Pb(II). It was also observed that the optical density varied in the day/night phase. Every light phase caused a little increase in density.

After 96 h of contact time, the chlorophyll *a* and dry mass values were measured (Table 2).

Chlorophyll indicates the intensity of algae life processes. It is, therefore, a useful indicator in assessing whether biomass is still alive. The highest chlorophyll *a* content was observed in the multimetallic rather than in the binary metal system. This implies that the cell activity of algae was greater in the presence of Pb(II). There was an almost 3-fold increase in the chlorophyll *a* content and a 1.2-fold increase in dry mass between the binary and multimetallic systems.

After the experiment, the quantity of biomass was higher in the multimetallic system than in the binary metal system. This confirms the better cell activity of algae in the presence of Pb(II).

3.3. Ammonium nitrogen removal from the investigated wastewater

Nitrogen is one of the substances contained in the investigated wastewater which can be removed by both algae and clinoptilolite. Nitrogen in wastewater occurs in the form of NO_3^- and NH_4^+ and it can be released into the gaseous form. Ammonia ions dominate below pH = 9.0. The most common nitrogen compounds assimilated by microalgae are ammonium (NH_4^+) and nitrate (NO_3^-) [30]. However, nitrate is consumed after ammonia has been utilized by *C. vulgaris* [31].

Table 1
Optical density measurements, NTU

Contact time, h/light phase	20Fe + 12Zn + A	20Fe + 12Zn + 10Pb + A
1/day	202	282
12/night	200	268
24/day	201	272
36/night	200	270
48/day	202	271
72/night	200	280
96/day	201	278

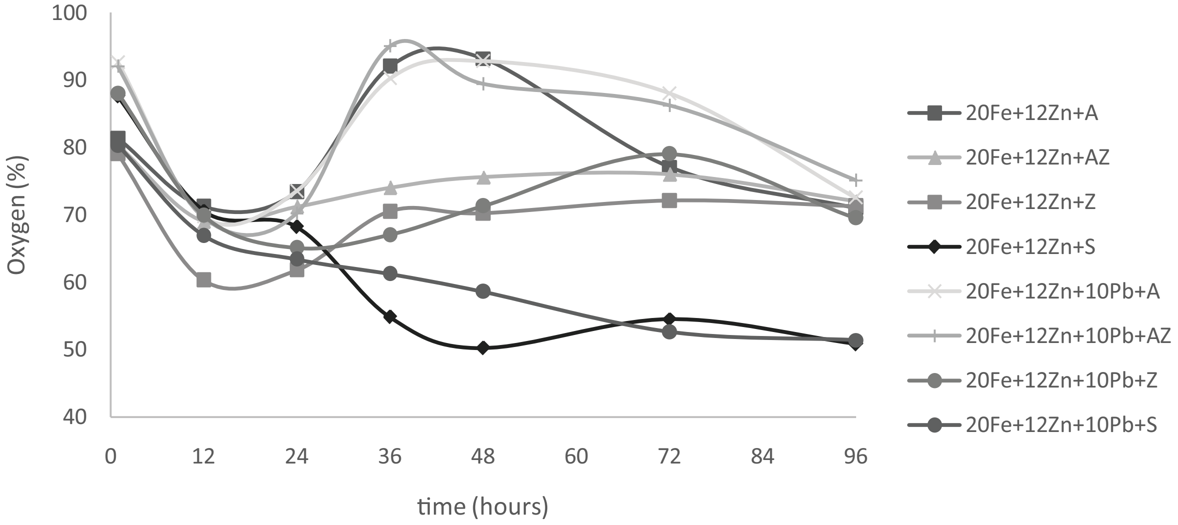

Fig. 2. Oxygen concentration values observed during the sorption experiment.

After 24 h of contact time, the final concentration of ammonium nitrogen in almost each sample reached values below the maximum permissible concentration of N-NH_4 in the investigated wastewater (Fig. 3) and the N-NH_4 concentration was lower than 20 mg/L.

In the sample 20Fe + 12Zn + 10Pb + AZ, the N-NH_4 concentration decreased below 20 mg/L after 48 h of contact time and the ammonium nitrogen content decreased steadily during the next days.

The highest decrease in the ammonium nitrogen content with time was observed in AZ samples. It could have been due to the double amount of sorbents (a mixture of algae 1% and zeolite 1%) added to the investigated wastewater.

3.4. Study of bioremoval

There are differences in the mechanisms involved in metal accumulation by cells. The main parameters that affect the performance of a living biosorbent are pH, temperature and the presence of co-ions [1]. The presence of lead as a potentially competing ion has the influence on the uptake of iron and zinc (Fig. 4).

The removal of the investigated heavy metals was most effective after 1 h. The concentration of all heavy metals was below the maximum permissible concentration after 1 h of contact time until the end of the experiment in A and AZ samples. This is reflected in a study by Sprynskyy et al. [32], which shows that the best sorption on clinoptilolite occurs after a 0.5-h contact [32]. Hence, the application of sorbents such as *C. vulgaris* and a mixture of *C. vulgaris* and clinoptilolite is an effective method for rapid removal of iron, zinc and lead from the investigated wastewater.

Table 2
Results of chlorophyll *a* and dry mass measurements

Sample	Chlorophyll *a*, mg/L	Dry mass, mg/L
20Fe + 12Zn + A	2.2	39.9
20Fe + 12Zn + 10Pb + A	6.2	48.1

It was observed that the addition of lead affected the efficiency of the sorption of other investigated metals. The final concentrations of iron and zinc with and without lead were slightly different. Unlike Z and S samples, the final concentration of zinc in A and AZ samples was lower in the multimetallic system than in the binary system. However, the presence of lead as well as the time contact influenced the final concentration of iron.

Removal of Zn(II) in Z and S samples was not as effective as in A sample or in the mixture of AZ samples. The concentration of zinc was above the maximum permissible concentration in Z samples after 24 h of contact time and in S samples during the whole experiment.

Functional groups related to biosorption, for example, carboxyl, imidazole, sulphydryl, amino, phosphate, sulphate, thioester, phenol, carbonyl, amide, hydroxyl, are bonded to polysaccharides and proteins. Gardea-Torresdey et al. [33] found that algal biosorption of lead(II) is mainly due to the ion-exchange process with positively charged metal cations competing for negatively charged surface of the cell membranes [33]. In metabolically active cells, bioaccumulation is the second step in the removal process of heavy metals. During bioaccumulation, the transport of contaminants to the cell and the accumulation of metals inside the cell occur.

A samples: The accumulation of Pb(II) by algae proceeds most efficiently at a pH close to the value of 8.0 [11]. During the experiment, the pH in A sample was of 6.62–7.39. The lowest Pb(II) concentration, equal to 0.02 mg/L, was found after 12, 24 and 96 h of contact time. The lowest Fe(III) content of 0.06 mg/L was observed after 96 h of contact time in the binary metal system. The Zn(II) concentration was the lowest after 1 h of the experiment, when it equalled 0.73 mg/L in the multimetallic system.

AZ samples: In AZ samples, the lowest Fe(III) content of 0.06 mg/L was observed after 96 h of contact time in the

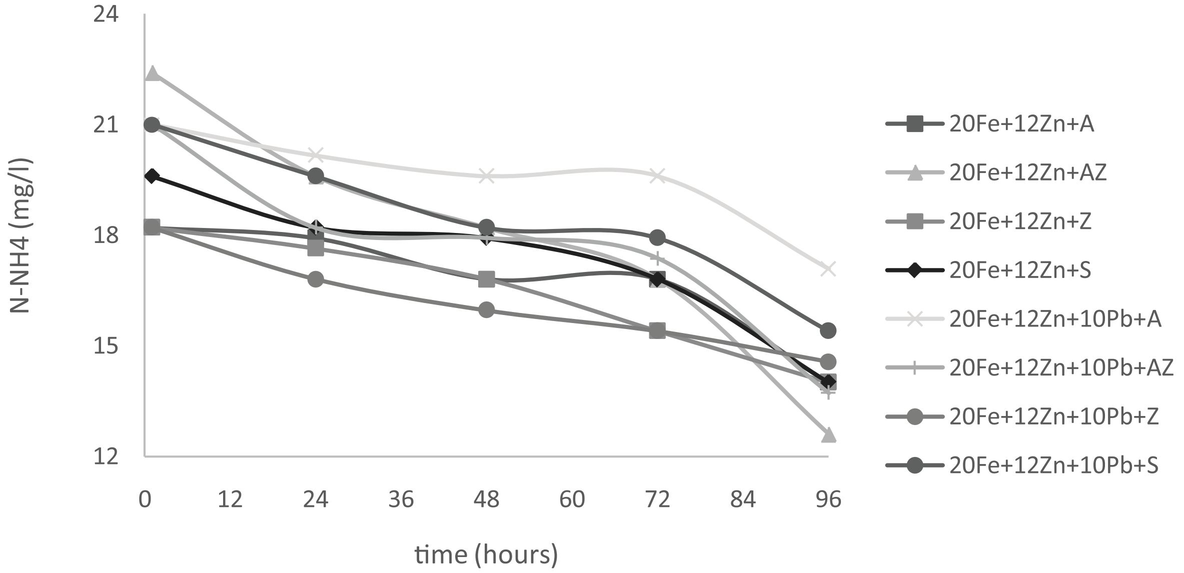

Fig. 3. N-NH_4 values observed during the experiment as a function of time.

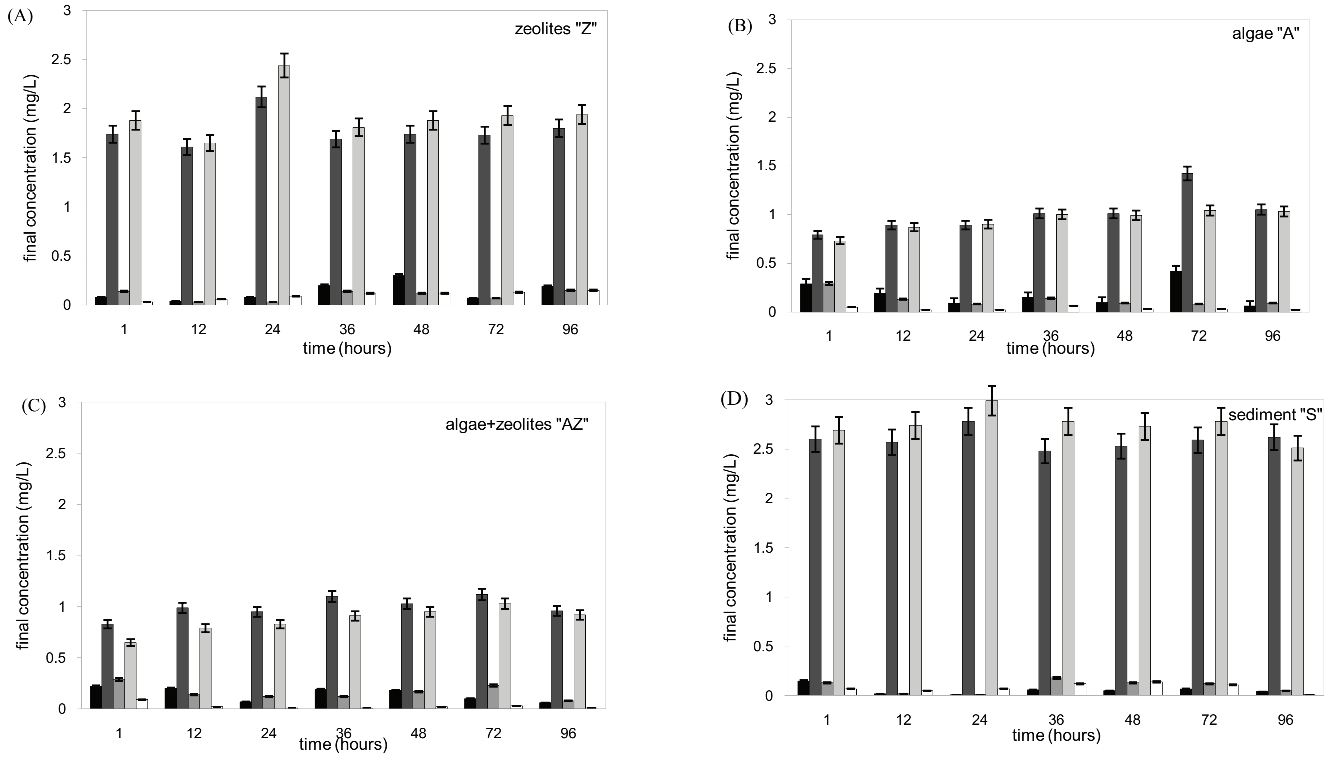

Fig. 4. Final concentration of heavy metals in the binary and the multimetallic systems as a function of time after addition of sorbents : (A) algae (*C. vulgaris)* 1%, (B) mixture of algae (*C. vulgaris)* 1% and zeolite (clinoptilolite) 1%, (C) zeolite (clinoptilolite) 1% and (D) sediment after N-NH_4 assimilation 1%. (■Fe(III) from the mixture of Fe(III) and Zn(II), ■Zn(II) from the mixture of Fe(III) and Zn(II), ■Fe(III) from the mixture of Fe(III), Zn(II) and Pb(II), ■Zn(II) from the mixture of Fe(III), Zn(II) and Pb(II); □Pb(II) from the mixture of Fe(III), Zn(II) and Pb(II).)

binary metal system. The Zn(II) concentration was the lowest after 1 h of the experiment, when it equalled 0.65 mg/L in the multimetallic system. The lowest Pb(II) concentration was found after 24, 36 and 96 h of contact, when it equalled 0.01 mg/L.

Z samples: In Z samples, the lowest Fe(III) content of 0.03 mg/L was observed after 12 and 24 h of contact for the investigated wastewater containing the multimetallic system. The Zn(II) concentration was the lowest after 12 h of the experiment, when it fell to 1.61 mg/L in the binary metal system. The lowest Pb(II) concentration after 1 h of contact was of 0.03 mg/L.

S samples: In S samples, the concentration of Zn(II) decreased from C_0 = 12.30 mg/L to 2.99 mg/L–2.51 mg/L. However, it did not decrease below the limit value of 2 mg/L. In S samples, the lowest Fe(III) content of 0.01 mg/L was observed after 24 h of contact time in the binary metal system. The lowest Pb(II) concentration was found after 96 h of contact, when it fell to 0.01 mg/L.

3.5. Uptake

The uptake of heavy metals is shown in Fig. 5.

Very high sorption efficiency of all the investigated heavy metals was observed. However, the accumulation of zinc was not as high as that of iron and lead. The removal of zinc was more efficient in all samples with *C. vulgaris* as the sorbent. This implies that the sediment was a less suitable sorbent for this metal than algae. The results demonstrated that all the sorbents were sufficient to remove >97.5% of Fe(III) and Pb(II) and >87.5% for A, >90% for AZ, 80% for Z and 75% for S samples of Zn(II) from the investigated wastewater.

A samples: In A samples, the highest uptake in the binary metal system was of 99.7% for Fe(III) and 93.6% for Zn(II). In the multimetallic system, the highest uptake was of 99.6% for Fe(III), 94.1% for Zn(II) and 99.8% for Pb(II). Guo et al. [24] observed that iron creates extremely stable complexes with algal cells. Iron bonds to specific receptor sites on the algal cell surface and forms cystic spores. In the biological processes, organic ligands can transfer heavy metals into algae.

AZ sample: In the binary metal system, the samples with *C. vulgaris* and clinoptilolite as the sorbent showed the highest uptake of 99.7% for Fe(III) and 93.2% for Zn(II). In the multimetallic metal system, the highest uptake was of 99.6% for Fe(III), 94.8% for Zn(II) and 99.9% for Pb(II).

Z sample: For the investigated wastewater containing the binary metal system, the highest uptake was of 99.8% for Fe(III) and 86.9% for Zn(II). In the multimetallic metal system, the highest uptake was of 99.9% for Fe(III), 86.6% for Zn(II) and 99.7% for Pb(II).

In the samples with *C. vulgaris* as well as with clinoptilolite as the sorbent, it was observed that the zinc uptake was not as high as that of iron and lead. However, the sorption of zinc occurred more efficiently when *C. vulgaris* or the mixture of *C. vulgaris* and clinoptilolite was applied as the sorbents.

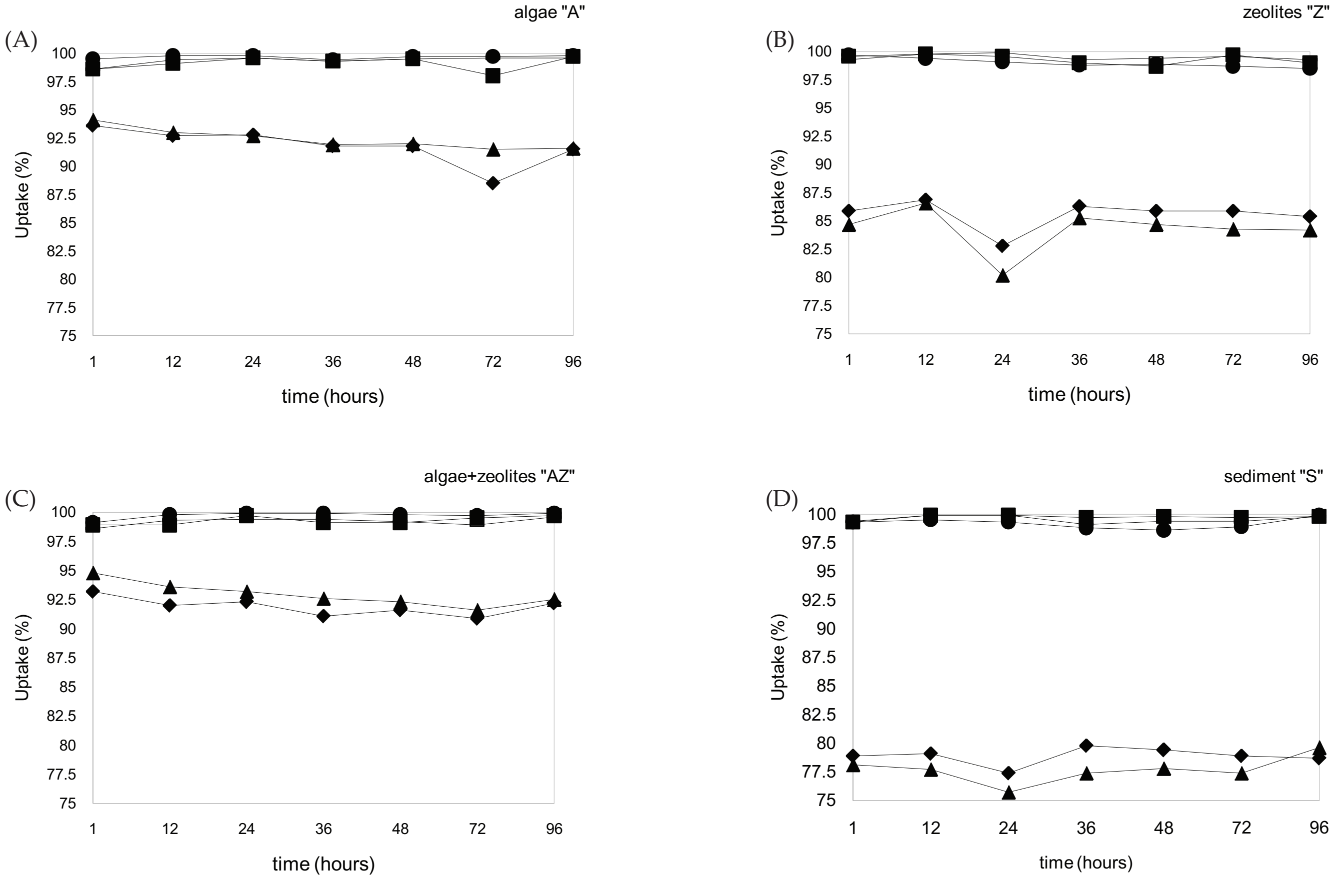

Fig. 5. Uptake of heavy metals in the binary and multimetallic systems as a function of time after addition of sorbents: (A) algae (*C. vulgaris)* 1%, (B) mixture of algae (*C. vulgaris)* 1% and zeolite (clinoptilolite) 1%, (C) zeolite (clinoptilolite) 1% and (D) sediment after N-NH$_4$ assimilation 1%. (■Fe(III) from the mixture of Fe(III) and Zn(II), ♦Zn(II) from the mixture of Fe(III) and Zn(II), ▬Fe(III) from the mixture of Fe(III), ▲Zn(II) and Pb(II) Zn(II) from the mixture of Fe(III), Zn(II) and Pb(II); ●Pb(II) from the mixture of Fe(III), Zn(II) and Pb(II).)

Table 3
Selectivity series of sorbents towards Fe(III), Zn(II) and Pb(II) in the multimetallic systems

Contact time, h	1	12	24	36	48	72	96
A	Pb > Fe > Zn	Pb > Fe > Zn	Pb > Fe > Zn	Pb > Fe > Zn	Pb > Fe > Zn	Pb > Fe > Zn	Pb > Fe > Zn
AZ	Pb > Fe > Zn	Pb > Fe > Zn	Pb > Fe > Zn	Pb > Fe > Zn	Pb > Fe > Zn	Pb > Fe > Zn	Pb > Fe > Zn
Z	Pb > Fe > Zn	Fe > Pb > Zn	Fe > Pb > Zn	Fe > Pb > Zn	Fe > Pb > Zn	Fe > Pb > Zn	Fe > Pb > Zn
S	Fe ≥ Pb > Zn	Fe > Pb > Zn	Fe > Pb > Zn	Fe > Pb > Zn	Fe > Pb > Zn	Fe > Pb > Zn	Fe ≥ Pb > Zn

This may be attributed to the higher affinity for Zn(II) by algae than by clinoptilolite.

S sample: In the binary metal system with the sediment consisting of *C. vulgari*s and clinoptilolite (in a 1:10 ratio), the highest uptake of 99.9% for Fe(III) and 79.8% for Zn(II) was found. In the multimetallic metal system, the highest uptake was of 99.9% for Fe(III), 79.6% for Zn(II) and 99.9% for Pb(II).

The results also indicated the selectivity series for the removal of the investigated heavy metals from the investigated wastewater (Table 3).

The selectivity series for A and AZ samples was identical within the investigated contact time. The contact time to some extent influenced the selectivity series. Certain differences between Z and S sorbents in the selectivity of the removal of heavy metals were found. Clinoptilolite and sediments consisting of clinoptilolite influenced the selectivity series. The selectivity series for the binary metal system was Fe > Zn during the experiment.

Inglezakis et al. [34] found that the selectivity sequence for clinoptilolite can be given as Pb^{2+} > Fe^{3+} > Cr^{3+} ≥ Cu^{2+} [34]. Zabochnicka-Świątek [14] observed that the sorption of Pb^{2+} by clinoptilolite is more effective than the sorption of any other metal ions, for example, Cd^{2+}, Cu^{2+}, Ni^{2+}, Cr^{3+} or Fe^{3+}. The affinity of clinoptilolite towards cationic elements obtained by Erdem et al. [35] was as follows: Pb^{2+} > Fe^{3+} > Zn^{2+} [35]. Wang and Chen [36] investigated the affinity sequence of microalgae *Chlamydomonas reinhardtii* for the following metal ions, which was: $Pb^{2+} > Hg^{2+} > Cd^{2+}$, with the maximum biosorption capacity for Pb of 96.3 ± 0.86 mg g^{-1} dry biomass. Klimmek et al. [37] found the maximum biosorption capacity of *C. vulgaris* for Pb^{2+} and Zn^{2+} of 0.46 and 0.18 mmol g^{-1}, respectively [37]. This is in agreement with the current study and explains the most efficient removal of lead and iron in the present experiment.

4. Conclusions

For all of the investigated heavy metals, very high sorption efficiency of each sorbent was observed. The mixture of unicellular algae and zeolites enhanced the efficiency of heavy metal removal since ion-exchange resins, such as zeolites, have mono-functional sites. Multi-functional sites of algal cell walls, including carboxyl, imidazole, sulphydryl, amino, phosphate, sulphate, thioester, phenol, carbonyl, amide and hydroxyl, enhanced the efficiency of heavy metal removal. Furthermore, sediments containing a mixture of *C. vulgaris* and clinoptilolite after N-NH_4 assimilation could serve as effective and eco-friendly biomaterial for the development of Fe(III), Zn(II) and Pb(II) removal procedure.

The results indicate that the most significant decrease in metals concentrations was observed after the first hour of contact time. During the experiment, Fe(III) and Pb(II) concentrations were below the maximum permissible level after 1 h of contact time. The results demonstrated that all the sorbents were sufficient to remove >97.5% of Fe(III) and Pb(II) and 75% of Zn(II) from the investigated wastewater. The maximum uptake of lead and iron (99.9%) for both metals was more efficient than the uptake of zinc (94.8%).

The maximum uptake of Pb(II) was observed by S and the mixture of AZ sorbents and the maximum uptake of Fe(III) by Z and S sorbents.

The removal of zinc was not as high as that of iron and lead. The uptake of zinc was the most efficient when A or the mixture of AZ sorbents were used.

The selectivity series of A and AZ sorbents was as follows: Pb(II) > Fe(III) > Zn(II) and for series Z and S (after 12 h of contact time) selectivity turned to be Fe(III) > Pb(II) > Zn(II). The selectivity sequence for the binary metal system for all the sorbents was Fe > Zn during the experiment. The contact time only slightly influenced the selectivity series for Z and S sorbents.

The addition of lead affected the efficiency of sorption of the other investigated metals (iron and zinc).

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