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Biosorption of Technologically Valuable Metal Ions on Algae Wastes: Laboratory Studies and Applicability

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Abstract: In the context of circular economy that recommends the most efficient use of waste, algae wastes have a huge potential for valorisation. In this study, algae wastes obtained after alkaline extraction of active compounds from two types of marine algae (green algae – *Ulva sp*. and red algae – *Callithamnion sp*.), were used as biosorbents to remove metal ions from aqueous effluents. Their efficiency in the biosorption processes was tested for Zn(II), Cu(II) and Co(II) ions, considered technologically valuable metal ions. The batch mono-component experiments performed under optimal conditions (pH = 5.0; 4.0 g biosorbent/L; 22 ± 1°C), showed that more than 75 % of the metal ions are removed when their initial concentration is less than 1.25 mmol/L. The very good fit of the experimental data with the pseudo-second order kinetic model and Langmuir isotherm model, and the high values obtained for the maximum biosorption capacity (q_{max}: Cu(II) (0.52 mmol/g) > Zn(II) (0.41 mmol/g) > Co(II) (0.39 mmol/g) for G-AWB, and q_{max}: Cu(II) (1.78 mmol/g) > Zn(II) (1.72 mmol/g) > Co(II) (1.66 mmol/g) for R-AWB) highlights the potential use of these biosorbents to remove such technologically valuable metal ions from industrial wastewater. This possibility was tested using industrial wastewater samples obtained from the metal coating industry. Quantitative removal (> 91 %) of Zn(II), Cu(II) and Co(II) ions was obtained when their initial concentration was adjusted to 50 mg/L. Also, the rapid and efficient desorption of these metal ions from loaded-biosorbents, by simple treatment with small volumes of HNO_3 (10^{-1} mol/L), further emphasizes the possibility of their recovery and reuse in the technological circuit. The results included in this study indicate that algae wastes have the potential to be used in industrial effluents decontamination processes and opens new perspectives for the implementation of circular economy principles.

Keywords: algae wastes; biosorption; technologically valuable metal ions; wastewater; decontamination processes

1. Introduction

The development of the industrial sectors brings, in addition to important benefits on the quality of life, and a number of disadvantages related to climate change, as a consequence of environmental pollution. The growing needs for raw materials, as well as industrial emissions of pollutants into air, water or soil, are significantly contributing to the decline in environmental quality [1]. Therefore, environmental pollution due to industrial emissions still remains a global issue that requires increased attention.

The most important problems of environmental pollution are due to the discharge of improperly treated industrial effluents into water sources [2,3]. Such effluents often contain high concentrations of heavy metals, which not only pollutes the environment, but is also a major source of public health problems due to their mobility, persistency and accumulation tendency [4]. Therefore, technical solutions must be sought for the removal of metal ions from industrial effluents, and these must be environmentally friendly, efficient and adaptable to certain industrial situations.

Numerous chemical, physical or biological methods have been reported in the literature for the removal of heavy metal ions from aqueous media [5,6]. Unfortunately, most of these methods, although technologically efficient, have some disadvantages that limit their practical applicability. In general, chemical methods (such as chemical precipitation, oxidation, flocculation, coagulation, etc.) are considered expensive and non-ecological, due to the high consumption of chemical reagents and the large amounts of sludge that are generated during the treatment process [6,7]. Physical methods (such as membrane processes, osmosis, etc.) require expensive equipments and are inefficient when the concentration of metal ions is higher than 100 mg/L [8,9]. Biological methods (such as bioaccumulation or phytoremediation), although characterized by high selectivity, have a modest efficiency in removing heavy metals and a high risk of contamination [10,11]. Compared to these, physico-chemical methods (such as ion exchange or adsorption/biosorption) are much more advantageous to be used in the treatment of industrial effluents, because: (i) are considered environmental-friendly methods, as long as they do not generate toxic sludge, (ii) allow the treatment of large volumes of effluents, (iii) have a high efficiency in a wide range of metal ions concentrations (from a few mg/L to a few hundred mg/g), (iv) allow the quantitative recovery of removed metal ions, and (v) require a short working time and simple operating conditions [12–14]. Moreover, the cost of the treatment process can be significantly reduced if inorganic (adsorption processes) or biological (biosorption processes) materials are used instead of ion exchange resin (ion exchange process) for the removal of heavy metals [15]. Therefore, the removal of metal ions using natural biologic materials, which are available, easy to prepare and safe to store, can be an environmentally friendly solution to this problem. Numerous agricultural wastes (such as fruits peels, cereal straw and shells, peanut shells, etc.) [16–18], aquatic and terrestrial plant residues (like micro and macro-algae, leaves of plants or trees, plant stems, tree bark, etc.) [19,20], or agri-food industrial waste and by-products (such as tea and coffee waste, fermentation waste, sugar waste, etc.) [21,22] have been tested in the literature as biosorbents for the removal of various metal ions from aqueous media. Unfortunately, many of these materials have already a number of other established used, which significantly reduces their widespread applicability in metal ions removal processes.

This is also the case of marine algae, whose active compounds (such as alginate, fucoidan, sugars, starch, colour pigments, etc.) are increasingly used in the food, pharmaceutical and cosmetic industries [23]. However, in order to avoid the use of organic solvents for the extraction of such active compounds, it is preferred to treat the algae biomass with concentrated alkaline solutions (NaOH or KOH). Concentrated alkaline solutions solubilise the polysaccharides in the cell walls and ionize most of the molecules inside the algae cells, thus allowing the extraction of these active compounds [24]. Algae wastes obtained after the extraction are often incinerated or stored, because they contain traces of alkaline solutions in their composition (even after several washing steps), which limits the possibilities of use for other purposes. However, such algae wastes have some important characteristics such as: (i) high specific surface area (due to the rupture of the cell walls during the extraction process), (ii) a large number of functional groups that are dissociated (due to alkaline treatment) and thus available to interact with metal ions in aqueous media, and (iii) minimal risk of secondary pollution due to the fact that most soluble organic compounds have already been removed by extraction, that qualify them for use as biosorbent materials. Due to these particularities, studies in the literature [25,26] have shown that biomass wastes obtained after alkaline extraction are, in most cases, much more efficient biosorbents in metal ions retention processes than the initial raw biomass. In addition, because algae biomass is used first in the extraction stage, many of mechanical operations required to prepare the biosorbent (washing, grinding, sieving, etc.) are already done. Therefore, algae waste can be considered low-cost materials, and their use in the decontamination processes of aqueous effluents can open new perspectives for their use in accordance with the principles of the circular economy.

In order to test this possibility, Zn(II), Cu(II) and Co(II) ions were chosen for experimental studies. The selection of these metal ions was made on the basis of two criteria, namely: (i) industrial importance – all these metal ions are considered technologically valuable due to their numerous industrial used [27], which make their recovery from wastewater an important goal in increasing the viability of technological processes, and (ii) the effects on environmental pollution – the accumulation of high concentrations of such heavy metals in water sources significantly affects the quality of ecosystems and had severe consequences for human health [28].

In this study, the feasibility of using two types of algae wastes (green algae – *Ulva sp*. (G-AWB) and red algae – *Callithamnion sp*. (R-AWB)) as biosorbents for the removal of Zn(II), Cu(II) and Co(II) ions from aqueous effluents was examined. The biosorption and desorption characteristics of these materials for considered metal ions was evaluated using aqueous solutions of metal ions, in batch and mono-component systems, under optimal conditions (pH = 5.0; 4.0 g biosorbent/L; 22±1°C). The biosorptive performance of G-AWB and R-AWB biosorbents was compared with that of the initial raw algae biomasses. The biosorption processes was studied trough isotherm and kinetic experiments, and the obtained results were modelled. The possibility of recovery of retained metal ions was verified by desorption studies. In order to asses the biosorptive potential of these biosorbents (G-AWB and R-AWB) under real conditions, samples of industrial washing wastewater, obtained from metal coating industry, were also used in the biosorption experiments. The results included in this study could be relevant to highlight a new and environmental friendly alternative to the removal of technological valuable heavy metals from wastewater.

2. Materials and Methods

2.1. Materials and reagents

Two types of algae wastes, obtained from green algae – *Ulva sp*. (G-AWB) and red algae – *Callithamnion sp*. (R-AWB), were used as biosorbents. After alkaline extraction (Soxhlet extractor, 1N NaOH solution, 70 °C, 6 h), both materials were washed until a neutral pH, dried in air (room temperature, 48 h) and mortared. The waste samples were stored in desiccators to keep the humidity constant. Stock solutions (0.01 mol/L) of metal ions (Zn(II), Cu(II) and Co(II)) were prepared from metal nitrate (Chemical Company, Iaşi, Romania). These solutions were used to prepare all working solutions. HNO_3 solution (10^{-1} mol/L) were used for desorption experiments and to adjust pH. Distilled water (obtained from a commercial distillation system) was used in all experiments. Wastewater samples were obtained from a local metal coating company. After sampling, the samples were filtered (quantitative filter paper) to remove solid impurities, and stored in the refrigerator until use (maximum 4 days).

2.2. Characterization of biosorbents and wastewater samples

FTIR spectrometry (Bio-Rad Spectrometer, 400–4000 cm^{-1} spectral domain, 4 cm^{-1} resolution, KBr pellet technique, ACD/Spec Manager software) was used to identify the nature of chemical bonds and functional groups on the biosorbents surface. SEM microscopy (SEM/EDAX Hitach S3000N, 20 kV) was used to highlight particle morphology. Chemical composition of biosorbents particles was determined by EDAX (SEM/EDAX Hitach S3000N, 20 kV).

The most important qualitative parameters of wastewater samples (pH, TSS, chloride, sulphate, CCO-Cr, Ca(II) and Mg(II)) were analyzed according with standard methodology [29].

2.3. General batch biosorption procedure

Batch studies of metal ions biosorption (Zn(II), Cu(II) and Co(II)) were carried out in 100 mL Erlenmeyer flasks at different initial metal ions concentration and contact time, under optimal conditions (pH = 5.0; biosorbent dosage = 4.0 g/L; temperature = 22 ± 1°C), established in previous study [30]. The experiments were performed in mono-component systems, by mixing 0.1 g of biosorbent (G-AWB or R-AWB) with a constant volume of metal ions solution (25 mL), for a given period of time. The initial concentration of metal ions was varied between 0.2 and 2.0 mmol/L, while the variation interval of contact time was between 5 and 180 min. The solution was then stirred (150 rpm) to reach the equilibrium, and filtered (quantitative filter paper). The concentration of each metal ion in the filtrated solution was measured by Atomic Absorption Spectrometry (AAS NovaA400 spectrometer, in acetylene/air flame, at characteristic wavelength). The removal percent (R, %) and biosorption capacity (q, mmol/g) were calculated using experimental data and the following equations:

$$R = \frac{c_0 - c}{c_0} \cdot 100 \qquad (1)$$

$$q = \frac{(c_0 - c) \cdot V}{m} \qquad (2)$$

where: c_0, c are initial and equilibrium concentration of metal ions in solution (mmol/L), V is volume of solution (mL), and m is the mass of biosorbent (g).

All experiments were carried out in triplicate, and mean values of measurements were used in figures. A significance level of $p < 0.05$ was used throughout the study.

2.4. Data analysis

The equilibrium experimental results were analyzed using three isotherm models, namely Freundlich (eq. (3)), Langmuir (eq. (4)) and Temkin (eq. (5)) models [31,32].

$$\ln q = \ln K_F + \frac{1}{n} \ln c \qquad (3)$$

$$\frac{1}{q} = \frac{1}{q_{\max}} + \frac{1}{q_{\max} \cdot K_L} \cdot \frac{1}{c} \qquad (4)$$

$$q = B \cdot \ln A_T + B \cdot \ln c \qquad (5)$$

where: q is biosorption capacity (mmol/g), q_{max} is the maximum biosorption capacity (mmol/g), K_L is Langmuir constant (L/g), c is equilibrium concentration of metal ions in solution (mmol/L), n is heterogenity factor, K_F is Freundlich constant (L/g), A_T is Temkin equilibrium constant (L/g), B is a constant related to the head of biosorption process (J/mol).

The Langmuir constant was used to calculate the Hall parameter (R_L) (eq. (6)) [31] and the Gibbs free energy (ΔG) (eq. (7)) [32], according with the relations:

$$R_L = \frac{1}{1 + K_L \cdot c_0} \qquad (6)$$

$$\Delta G = -RT \cdot \ln K_L \qquad (7)$$

where: c_0 is the highest initial concentration of metal ions in solution (mmol/L), R is the universal gas constant (8.314 J/mol K), T is temperature (K).

For the mathematical analysis of the kinetic data, three kinetic models (pseudo-first order model (eq. (8), pseudo-second order model (eq. (9) and intra-particle diffusion model (eq. (10)) were used [33,34]. These models allow the identification of the rate-controlling step, which is important in evaluating the efficiency of biosorption processes.

$$\lg(q_e - q_t) = \lg q_e - k_1 \cdot t \qquad (8)$$

$$\frac{t}{q_t} = \frac{1}{k_2 \cdot q_e^2} + \frac{t}{q_e} \qquad (9)$$

$$q_t = k_{diff} \cdot t^{1/2} + c \qquad (10)$$

where: q_e, q_t are the biosorption capacities at equilibrium and at time t (mmol/g), k_1 is the rate constant of pseudo-first order model (1/min), k_2 is the rate constant of pseudo-second order model (g/mmol min), k_{diff} is the intra-particle diffusion rate constant (mmol/g $min^{1/2}$), c is the concentration of metal ions in solution at equilibrium (mmol/L).

The isotherm and kinetic model that best describe the experimental biosorption data was chosen based in the value of the regression coefficients (R^2) and root-mean-square deviation (RMSD, eq. (11)) , calculated from the statistical analysis (ANOVA).

$$RMSD = \sqrt{\frac{\sum_{i=1}^{n} (q_{\exp} - q_{calc})^2}{n-1}} \qquad (11)$$

where: q_{exp}, q_{cal} are experimental and calculated values of biosorption capacity at equilibrium (mmol/g), n is number of replicates ($n = 3$).

2.5. Desorption of metal ions from loaded biosorbents

In desorption experiments, 0.5 g of dried biosorbent loaded with metal ions (G-AWB and R-AWB) was added to 10 mL of 10^{-1} mol/L HNO_3 solution, and mixed for 60 min, at 150 rpm. After filtration (on quantitative filter paper), the amount of desorbed metal ions was analyzed by AAS spectrometry. The percent of metal ions desorbed (D, %) was calculated according with the equation:

$$D,\% = \frac{c_d \cdot 100}{q_e \cdot m} \tag{12}$$

where: c_d is the concentration of metal ion in desorbing solution (mmol/L); q_e is the biosorption capacity (mmol/g) and m is the mass of biosorbent used in desorption experiments (g).

Next, the biosorbents were washed with distilled water, air-dried at room temperature, and used in another biosorption/desorption cycle. Five biosorption/desorption cycles were tested, and biosorption and desorption efficiencies were determined in each cycle.

2.6. Wastewater experiments

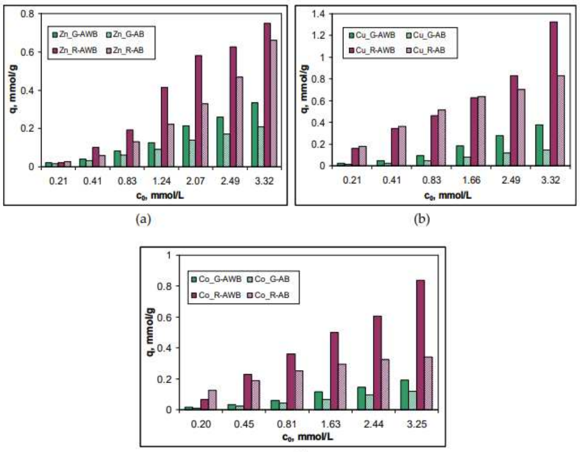
Wastewater samples (250 mL) were used for the removal on metal ions in mono-component systems and batch experiments. In each sample the initial solution pH and the concentration of metal ions was adjusted at 5.0 and 50 mg/L respectively, at constant temperature (22 ± 1°C), and a constant amount from each biosorbent (1.0 g) was added. After stirring 3 hours at 150 rpm, the samples were filtered (quantitative filter paper) and analyzed. The concentration of metal ions in the filtered samples was determined by AAS spectrometry, while others parameters (pH, TSS, chloride, sulphate, CCO-Cr, Ca(II) and Mg(II)) of wastewater samples (before and after biosorption) were determined according with standard methodology [29].

3. Results and discussion

3.1. Biosorptive potential of algae waste biomass

The biosorptive potential of the two algae wastes (G-AWB and R-AWB) was examined in comparison with the raw algae biomass (G-AB and R-AB), under optimal experimental conditions, at different initial concentrations of each metal ion (Zn(II), Cu(II) and Co(II)). The values of the biosorption capacities (Figure 1) indicate that both algae wastes are more effective in the removal of each metal ions compared to raw algae biomass. Thus, regardless of the nature of metal ions (Zn(II), Cu(II) or Co(II)) and their initial concentration (c_0, mmol/L), the biosorption capacities increase in the order: R-AWB > G-AWB > R-AB > G-AB (see Figure 1).

The differences in biosorption capacity are significant, especially at high values of the initial concentration of metal ions (Table 1), which suggest that algae wastes are much more efficient biosorbents compared to the raw algae biomass. On the other hand, if in the case of Zn(II) and Co(II) ions, R-AWB has a biosorption capacity more than twice higher compared with R-AB, in the case of Cu(II) ions, such increase was obtained for G-AWB (see Table 1).



(a) (b)

(c)

Figure 1. Variation of the biosorption capacity as a function of initial metal ions concentration for Zn(II) – a, Cu(II) – b and Co(II) – c.

Table 1. The values of the biosorption capacities obtained at the highest initial concentration of metal ions.

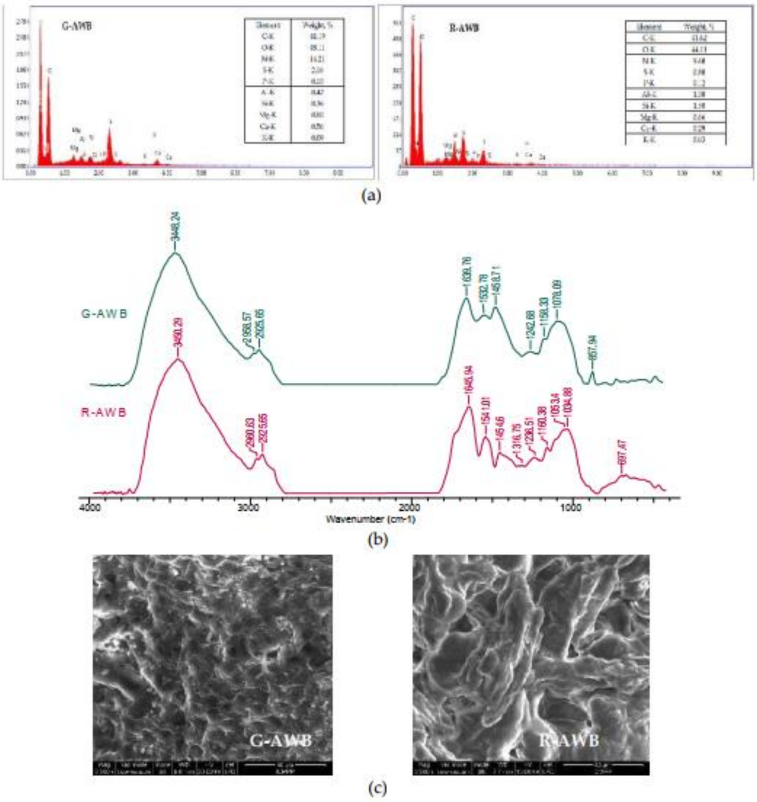
Metal ion	**q, mmol/g**		Δq, %	**q, mmol/g**		Δq, %
	G-AWB	G-AB		R-AWB	R-AB	
Zn(II)	0.34	0.21	61.90	1.05	0.49	114.28
Cu(II)	0.38	0.15	153.33	1.33	0.83	60.24
Co(II)	0.19	0.12	58.33	0.83	0.34	144.12

Therefore, both algae wastes (G-AWB and R-AWB) have the potential to be used as biosorbents in the removal of metal ions from aqueous media, and a detailed analysis of their biosorptive performances will allow us to highlight possible practical applications.

3.2. Characterization of G-AWB and R-AWB biosorbents

Figure 2a illustrates EDX spectra and elemental composition of G-AWB and R-AWB. The analysis of these data shows that both algae wastes contain in their composition large amounts of C (> 41 %), O (44-48 %), S (0.8-2.16 %) and N (9.60-14.21 %), elements that can enter in the structure of many functional groups, but also Ca (0.29-0.56 %), Mg (0.66-0.80 %) or K (0.03-0.09 %) (see Figure 2), which are mobile elements, and can be involved in ion exchange processes. These characteristics are important from the application point of view, and highlight the possibility of using these wastes as biosorbents to remove metal ions from aqueous media.

It is well known that the presence of functional groups on the surface of biosorbents is essential for the evaluation of their biosorptive properties. This is due to the fact that superficial functional groups are mainly responsible for the binding of metal ions in aqueous solution, and their number and nature determine the efficiency of biosorption processes [34,35]. The identification of the superficial functional groups of G-AWB and R-AWB was performed using FTIR spectra, presented in Figure 2b.



(a)

(b)

(c)

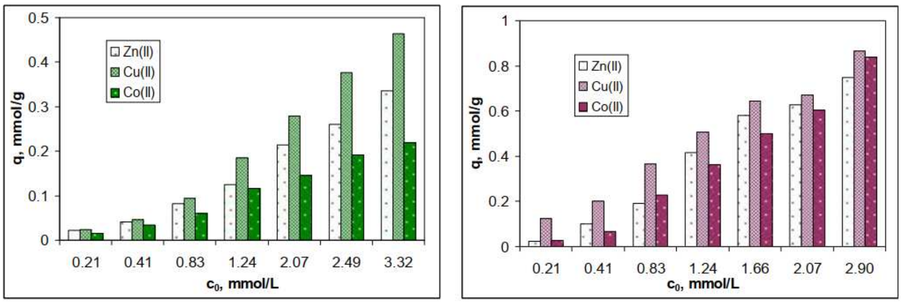
Figure 2. EDX spectra (a), FTIR spectra (b) and SEM images (c) for G-AWB and R-AWB.

The intense band at 3448-3450 cm^{-1} corresponds to O–H stretching vibrations from alcohols, acids and carbohydrates. The bands from 2925-2960 cm^{-1} can be attributed to the presence of asymmetric and symmetric stretch C–H bonds from saturated hydrocarbon radicals. The band at 1639-1645 cm^{-1} indicate the presence of C=O bonds from carbonyl and carboxyl compounds. The band at 1532-1541 cm^{-1} can be attributed to the asymmetric stretching of C–O from carboxyl groups. The peaks at 1454-1458 cm^{-1}, 1236-1242 cm^{-1}, 116-1158 cm^{-1} and 1034-1078 cm^{-1} indicate the presence of asymmetric stretching vibrations of C–H, C–N, C–C and C–O bonds mainly from polysaccharides. Therefore, the presence of O-donor functional groups (such as OH, C–O, C=O, etc.) on the surface of G-AWB and R-AWB highlights the potential applications of these materials as biosorbents. The surface morphology of G-AWB and R-AWB was examined by SEM microscopy. SEM images (Figure 2c) show that both algae waste (G-AWB and R-AWB) have macro- and micro-pores on the entire surface, and their distribution is quite uniform. These characteristics ensure efficient contact between the biosorbent surface and the metal ions solution favouring the biosorption process.

Therefore, both algae wastes (G-AWB and R-AWB) have numerous superficial functional groups and porous surface, and are expected to achieve a high efficiency in the removal of considered metal ions (Zn(II), Cu(II) and Co(II)) from aqueous effluents.

3.3. Biosorption efficiency and isotherm modelling

The biosorption efficiency of Zn(II), Cu(II) and Co(II) ions on G-AWB and R-AWB was examined at different initial metal ions concentration (0.2 – 3.2 mmol/L), at pH of 5.0 and biosorbent dosage of 4.0 g/L, contact time of 24 h and room temperature (22 ± 1 °C). The obtained values of the adsorption capacity at different initial metal ions concentration are presented in Figure 3.



(a) (b)

Figure 3. Variation of the biosorption capacity as a function of initial concentration of Zn(II), Cu(II) and Co(II) ions for G-AWB (a) and R-AWB (b).

As can be seen from Figure 3, the efficiency of biosorption processes depends on the nature of the biosorbent and the nature of the metal ion. The removal percents (R, %) varies from 86.01-81.09 % for Zn(II) ions, from 94.06-90.92 % for Cu(II) and from 74.07-45.62 % for Co(II) in the case of G-AWB, and from 84.53-79.20 % for Zn(II), from 96.09-84.86 % for Cu(II) and from 82.57-47.81 % for Co(II) in the case of R-AWB, on entire studied metal ions concentration range.

The analysis of these experimental data highlights three important aspects in the evaluation of biosorption processes. The first aspect is related to the fact that with the increase of the initial concentration of metal ions, the removal percents decrease. This variation can be explained considering the ratio between the number of functional groups on the biosorbent surface and the number of metal ions in aqueous solution [35,36]. At lower initial metal ions concentrations this ratio is high, the interactions between functional groups of biosorbent and metal ions occurs with high probability, and therefore the efficiency of the biosorption processes is high. When the initial concentration of metal ions increases, this ratio decreases, the superficial functional groups of biosorbents become saturated, and consequently the efficiency of biosorption processes decreases. Therefore both biosorbents (G-AWB and R-AWB) have maximum biosorption efficiency at low initial concentrations of metal ions (up to 1.25 mmol/L), when the removal percent are higher than 82 % for Zn(II), 91 % for Cu(II) and 75 % for Co(II), respectively. The second aspect is related to the nature of biosorbent. As can see in Figure 3, R-AWB allows higher biosorption capacities than G-AWB, regardless of the nature of metal ion. These differences in biosorption capacity can be explained by taking into account the differences in the chemical composition of the two types of algae. Thus, compared to green algae, red algae cells contain colour pigments, lipids (in fairly large quantities), etc., compounds that are easily extracted in alkaline media [37,38]. Their extraction in alkaline medium increases the degree of cell walls breakage, and the surface of R-AWB becomes much more uneven compared to G-AWB. This can be easily seen in Figure 2c. Consequently, the number of superficial functional groups available for interaction with metal ions is higher in the case of R-AWB compared with G-AWB (see Figure 2b), which explains the differences between the biosorption capacities obtained experimentally. The third aspect concerns the efficiency of biosorption processes depending on the nature of metal ions. It can be seen from Figure 3 that, both in the case of R-AWB and in the case of G-AWB, the efficiency of biosorption processes follows the order: Cu(II) > Zn(II) > Co(II). This order may be justifies by the geometric characteristics of these ions and the values of their electronegativity [39]. All these observations highlight the biosorption potential of G-AWB and R-AWB for metal ions in aqueous media and outline the possible practical applications in the remediation processes.

Quantitative evaluation of the biosorption processes of Zn(II), Cu(II) and Co(II) ions on G-AWB and R-AWB was performed by modelling the experimental isotherms using Langmuir, Freundlich and Temkin isotherm models. The isotherm model parameters are summarized in Table 2, while the linear representations of each isotherm model are illustrated in Figure S1 (Supplementary materials).

Table 2. Isotherm parameters for Zn(II), Cu(II) and Co(II) ions biosorption on G-AWB and R-AWB.

Isotherm parameter	G-AWB			R-AWB		
	Zn(II)	Cu(II)	Co(II)	Zn(II)	Cu(II)	Co(II)
Langmuir model						
R^2	0.9856	0.9934	0.9993	0.9791	0.9789	0.9813
RMSD	1.04	1.56	2.11	1.16	2.01	2.12
q_{max}, mmol/g	0.41	0.52	0.39	1.72	1.78	1.66
K_L, L/mmol	1.87	4.87	1.55	1.56	1.67	1.44
R_L	0.04	0.05	0.09	0.18	0.17	0.19
ΔG, kJ/mol	-14.35	-13.88	-10.75	-10.91	-12.58	-10.08
Freundlich model						
R^2	0.9778	0.9722	0.9687	0.9597	0.9222	0.9073
RMSD	2.03	1.87	1.98	1.89	2.01	2.08
1/n	0.95	0.88	0.70	0.52	0.61	0.81
K_F, L/mmol	0.54	0.99	0.12	3.42	1.37	1.18
Temkin model						
R^2	0.9317	0.9082	0.9641	0.9112	0.9508	0.9190
RMSD	1.76	1.59	2.03	3.02	2.76	2.59
A_T, L/mol	0.18	0.21	0.16	0.65	0.26	0.53
B_T, J/mol	12.93	18.99	12.10	14.93	25.03	14.68

As can be seen from Table 2, the experimental isotherm data are best described by the Langmuir isotherm model. Therefore, the retention of metal ions takes place predominantly on the surface of the two biosorbents, until the formation of complete monolayer coverage. In addition, the surface of G-AWB and R-AWB can be considered homogeneous and contains active sites for the biosorption of metal ions that are relatively uniformly distributed [33]. The maximum biosorption capacity (q_{max}, mmol/g) increase in the order Cu(II) (1.78 mmol/g) > Zn(II) (1.72 mmol/g) > Co(II) (1.66 mmol/g) in the case of R-AWB (Table 2), while in the case of G-AWB, the values of this parameter are lower, and follow the same order (see Table 2). These values are comparable with those previously reported in the literature for other types of waste (Table 3) and highlight the practical applicability of these biosorbents in the removal of metal ions from aqueous media.

Table 3. Maximum biosorption capacity (q_{max}, mmol/g) for the biosorption of Zn(II), Cu(II) and Co(II) ions on different waste, under similar experimental conditions.

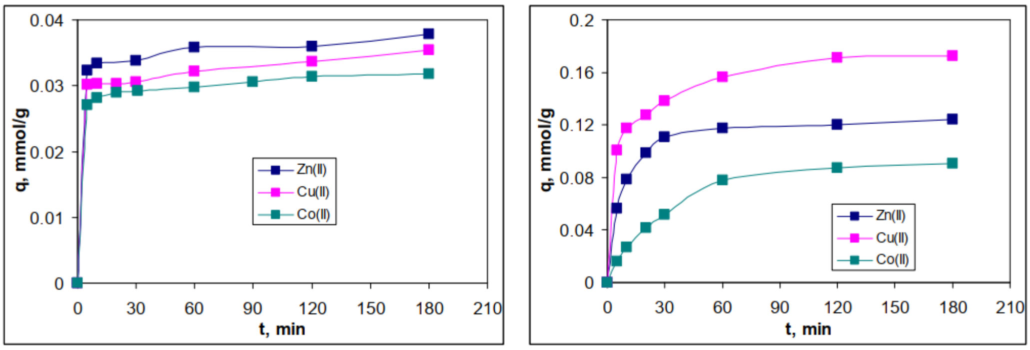
Biosorbent	**Zn(II)**	**Cu(II)**	**Co(II)**	**References**
Orange peel	1.22	1.12	-	40
Corn Cob	1.16	1.01	-	41
Rice husk	0.01	0.02	0.01	42
Neochloris sp.(green microalgae)	1.20	1.23	-	43
Alginate	0.57	1.01	0.32	44
Ulva lactuca (green algae)	0.34	0.47	0.22	This study
Callithamnion sp. (red algae)	0.75	0.87	0.64	This study
G-AWB	0.41	0.52	0.39	This study
R-AWB	1.72	1.78	1.68	This study

Langmuir constant values (K_L) show that strong interactions between metal ions and superficial functional groups are involved in the biosorption processes, regardless of the nature of metal ions or biosorbent (Table 2). In addition, the separation factor (R_L) and Gibbs free energy variation (ΔG), calculated based on the Langmuir constant (see eqs. 6 and 7), indicate that all biosorption processes are spontaneous (ΔG < 0) and favourable (R_L < 1). For both biosorbents, the R_L values increase in the order: Cu(II) > Zn(II) > Co(II), but the values of this parameter do not significantly depend on the nature of metal ion (Table 2).

The 1/n parameter in the Freundlich model is smaller than unit in all the cases (Table 2), which suggests that all biosorption processes are favourable. Moreover, the values obtained in the case of R-AWB are lower than those obtained in the case of G-AWB, which indicates that R-AWB has better biosorptive performances for these metal ions, than G-AWB. These observations are also supported by the values of the parameters of the Temkin model (Table 2), which show that in the biosorption process, the retention of metal ions is achieved by strong interactions, which occur more easily in the case of R-AWB than in the case of G-AWB. These results confirm the trend of removal efficiency illustrated in Figure 3 and indicates that G-AWB and R-AWB could be a promising option for removing Zn(II), Cu(II) and Co(II) ions from aqueous effluents.

3.4. Influence of contact time and kinetic modelling

The influence of contact time on the biosorption of Zn(II), Cu(II) and Co(II) ions onto G-AWB and R-AWB is presented in Figure 4. At the initial concentration of 0.4 mmol M(II)/L, the time require to reach equilibrium (initial stage) was 30 min for G-AWB and 60 min for R-AWB, respectively (Figure 4). The efficiency of removing metal ions at these values of the contact time, follows the order: Zn(II) (69 %) > Cu(II) (68 %) > Co(II) (64 %) for G-AWB, and Cu(II) (79 %) > Zn(II) (75 %) > Co(II) (71 %) for R-AWB, respectively.



(a) (b)

Figure 4. Variation of the biosorption capacity as a function of contact time for the retention of Zn(II), Cu(II) and Co(II) ions on G-AWB (a) and R-AWB (b).

As is expected, in the initial stage, the biosorption rate is high, due to the large number of functional groups available on the surface of the two biosorbents, which can interact with metal ions. Once the equilibrium time was attained, the biosorption rate decreases significantly, due to the decrease in the number of functional groups left free. Such a variation is frequently reported in the retention of metal ions [41–44] and highlights the fact that the biosorption processes involves the existence of chemical interactions between metal ions and the superficial functional groups of the biosorbent. Much more important for this study are the differences in kinetic behaviour for the two biosorbents. Thus, if in the case of G-AWB, the equilibrium state is reached quickly (30 min) and the differences between the biosorption capacities of metal ions are rather small (Figure 4), in the case of R-AWB, the time to reach equilibrium is longer (60 min), and the values of the biosorption capacities depend by the ionic radius and the electronegativity of each metal ion (Figure 4). These results show that, while in the case of G-AWB most of functional groups are found on the surface of the biosorbent, in the case of R-AWB such functional groups re also found inside of the cracks of the biosorbent particles and that they are available to participate in the biosorption processes. These observations are confirmed by the morphological structure of the two biosorbents (Figure 2c) and are a direct consequence of the extraction of active compounds from algae biomass in alkaline medium.

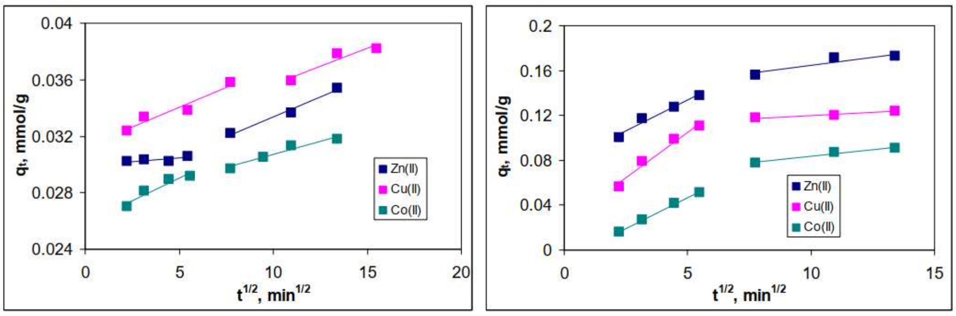
The kinetic evaluation of the studied biosorption processes was done by modelling the experimental data (Figure 4) using pseudo-first order kinetic model, pseudo-second order kinetic model and intra-particle diffusion model (eqs. 8-10). The kinetic parameters obtained in each case are summarized in Table 4, while the linear representations of the pseudo-first order kinetic model and pseudo-second order kinetic are illustrated in Figure S2 (Supplementary materials) and in Figure 5 for the intra-particle diffusion model.

Table 4. Kinetic parameters for Zn(II), Cu(II) and Co(II) ions biosorption on G-AWB and R-AWB.

	Kinetic parameter	G-AWB			R-AWB		
		Zn(II)	Cu(II)	Co(II)	Zn(II)	Cu(II)	Co(II)
	q^e_{exp}, mmol/g	0.035	0.038	0.032	0.124	0.173	0.091
	Pseudo-first order model						
	R^2	0.9765	0.8231	0.9421	0.8517	0.9854	0.9916
	q^e_{calc}, mmol/g	0.059	0.053	0.043	0.045	0.089	0.0832
	$k_1 \cdot 10^2$, 1/min	0.440	0.360	0.590	1.061	1.473	1.192
	Pseudo-second order model						
	R^2	0.9986	0.9989	0.9995	0.9998	0.9991	0.9992
	q^e_{calc}, mmol/g	0.035	0.038	0.032	0.127	0.178	0.106
	k_2, g/mmol min	8.326	10.753	13.158	1.365	0.847	0.324
	Intra-particle diffusion model						
I	R^2	0.6256	0.9348	0.9242	0.9696	0.9771	0.9987
	c, mmol/L	0.030	0.031	0.026	0.079	0.023	0.008
	$k^I_{diff,}$ mmol/g min$^{1/2}$	$9 \cdot 10^{-5}$	$6 \cdot 10^{-4}$	$6 \cdot 10^{-4}$	0.017	0.017	0.011
II	R^2	0.9863	0.8976	0.9431	0.8657	0.9793	0.9626
	c, mmol/L	0.028	0.031	0.027	0.135	0.109	0.061
	$k^{II}_{diff,}$ mmol/g min$^{1/2}$	$6 \cdot 10^{-4}$	$6 \cdot 10^{-4}$	$6 \cdot 10^{-4}$	0.003	0.001	0.002

The results presented in Table 4 show that the regression coefficients (R^2) of the pseudo-second order model have highest values (close to unity), and the biosorption capacities calculated (q^e_{calc}, mmol/g) from this model are close to experimental values, for all metal ions and both biosorbents. Therefore, the biosorption of Zn(II), Cu(II) and Co(II) ions on G-AWB and R-AWB is best described by the pseudo-second order model, which assumes the existence of chemical interactions (through electron sharing) between metal ions and the functional groups of the biosorbents. In addition, to achieve biosorption, two functional groups are required, which must be located at a suitable geometric distance to be able to bind the metal ions [45]. This observations explains the calculated values of q_e and k_2 (Table 4) in the case of Zn(II), Cu(II) and Co(II) ions biosorption on G-AWB and R-AWB. This, if in the case of G-AWB, the smaller number of functional groups and their predominant localization on the surface of the biosorbent (Figure 2 b and c), lead to obtaining small values of q_e and high values of k_2 (Table 4), in the case of R-AWB, a great number of functional groups and their localization also in the cracks of the biosorbent particles (Figure 2 b and c), makes the values obtained for q_e to be higher and the values of k_2 to be lower (Table 4), for all metal ions. Therefore, it can be said that in the case of G-AWB, the binding interactions of metal ions on the surface of the biosorbent are achieved much faster than in the case of R-AWB, and this is clearly highlighted in Figure 4.

To better understand the importance of elementary diffusion steps in the retention of Zn(II), Cu(II) and Co(II) ions on G-AWB and R-AWB, the intra-particle diffusion model was also used to analyzing experimental kinetic data. As shown in Figure 5, all biosorption processes can be considered as occurring according to a two-step transport mechanism, regardless of the nature of the biosorbent and the nature of metal ions.



(a) (b)

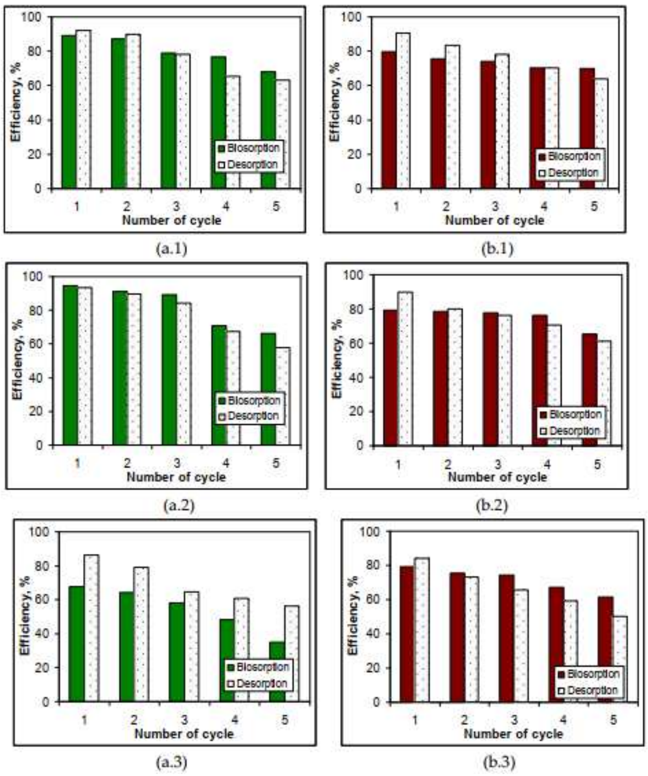
Figure 5. Linear representations of the intra-particle diffusion model for the biosorption of Zn(II), Cu(II) and Co(II) ions on G-AWB (a) and R-AWB (b).

The first region is the film diffusion step (or surface biosorption), which can be attributed to the diffusion of metal ions through the solution to the external surface of the biosorbents, while the second region, corresponds to the transport of metal ions from the surface of the biosorbents into the interior of the biosorbent particles [33,46]. Analyzing the values of the kinetic parameters obtained for this model (Table 4), it can be seen that the elementary diffusion steps are much more important in the case of R-AWB (where the number of functional groups is greater) that in the case of G-AWB (when the number of functional groups is smaller). But, for none of the biosorbent and none of the metal ions, elementary diffusion processes are not the rate-limiting step in the biosorption, since the linear representations (Figure 5) do not pass through the origin. However, elementary diffusion processes have an important contribution to the achievement of biosorption processes. Thus, in the case of G-AWB, there is no significant difference in the rate constant for the two regions (Table 4), precisely due to the relative uniform morphology of the surface of this biosorbent (Figure 2c). In the case of R-AWB, the rate constants of the two regions differ significantly, and the much slower diffusion of metal ions inside the pores of the biosorbent particles (the second region), makes the contact time required to reach the equilibrium in this case to be higher (Figure 4).

On the other hand, if in the case of G-AWB, all metal ions have similar affinities for the functional groups of the biosorbent (concentrations of the metal ions are close, in both regions) (Table 4), in the case of R-AWB it can be observed that the Co(II) ions bind more easily to functional groups of the biosorbent, followed by Cu(II) ions and Zn(II) ions (as the concentration of these ions increases in this order, in both regions) (Table 4). This demonstrates once again that R-AWB has a much more heterogeneous surface compared with G-AWB (as can be seen in Figure 2c), and that the ease with which metal ions penetrate into surface cracks depends, mainly, by their structural characteristics. Thus, the larger the ratio of electronegativity to ionic radius (Co(II) > Cu(II) > Zn(II)), the easier metal ions penetrate inside R-AWB particles to interact with functional groups. Consequently, their concentration in the second diffusion zone is lower (Co(II) < Cu(II) < Zn(II)) (Table 4). This behaviour significantly influences metal ions recovery and biosorbent regeneration, which will be discussed in detail in the next section.

3.5. Desorption of metal ions and biosorbents regeneration

Desorption of metal ions and regeneration of G-AWB and R-AWB was further examined using 10^{-1} mol/L HNO_3 solution as eluent. The results obtained for the desorption of Zn(II), Cu(II) and Co(II) ions, on loaded G-AWB and R-AWB are illustrated in Figure 6.



(a.1) (b.1)

(a.2) (b.2)

(a.3) (b.3)

Figure 6. Desorption/biosorption efficiency of Zn(II) - 1, Cu(II) - 2 and Co(II) - 3 ions on G-AWB (a) and R-AWB (b) in five consecutive cycles.

Two observations emerge for the analysis of the experimental data presented in Figure 6. The first refers to the biosorptive performances of the two algae wastes decrease with the increase in the number of cycle of use (from cycle 1 to cycle 5), regardless of the nature of metal ion. This decrease is more pronounced in the case of G-AWB (20.97 % for Zn(II), 28.66 % for Cu(II) and 32.77 % for Co(II)) than in the case of R-AWB (10.04 % for Zn(II), 14.16 % for Cu(II) and 17.87 % for Co(II)), and is evident only after the third biosorption/desorption cycle (Figure 6). The decrease of the biosorption efficiency with the increase of the number of cycles is frequently mentioned in the literature [45,46], and it is caused by the degradation of the functional groups of the biosorbent upon repeated use. In addition, after desorption with mineral acids, protonated functional groups can interact with each other, via hydrogen bonds, and these formed bonds can no longer be broken when adjusting the pH for a new biosorption cycle. Therefore, some functional groups become unavailable to interact with the metal ions in the aqueous solution, leading to a decrease in biosorption efficiency. If in the case of R-AWB, the large number of functional groups (Figure 2b) makes this decrease less important, in the case of G-AWB, when the number of functional groups is smaller (Figure 2b), this causes a much more obvious decrease in the biosorption efficiency. However, the experimental results indicated that both algae wastes (G-AWB and R-AWB) have good reusability (up to three cycles), and this highlights the potential applications of these biosorbents in the treatment of industrial effluents.

The second observation is related to the possibility of recovery of metal ions, through desorption. The experimental data presented in Figure 6 shows that desorption efficiency decrease with the increase in the number of cycles of biosorbent use (from cycle 1 to cycle 5), and this decrease is somewhat greater in the case of R-AWB than in the case of G-AWB, for all studied metal ions. Thus, if for Zn(II) ions, desorption efficiency decreases by 26.66 % in the case of G-AWB and by 28.79 % in the case of R-AWB, from cycle 1 to cycle 5, for Cu(II) ions this decrease is 28.77 % in the case of G-AWB and 30.18 % in the case of R-AWB, while for Co(II) ions the decrease in desorption efficiency is the highest (31.50 % for G-AWB and 34.24 % for R-AWB) (Figure 6). These differences in desorption efficiency are due, on the one hand, to the more heterogeneous morphology of R-AWB compared to G-AWB) (Figure 2c) and, on the other hand, to the geometrical dimensions and affinity of the metal ions to interact with the functional groups of the biosorbent. For example, Co(II) ions, which have the highest ratio of electronegativity to ionic radius, will bind to the functional groups on the outer surface of the biosorbent, but will easily penetrate the cracks of the R-AWB particles. During of desorption, the Co(II) ions on the outer surface of the biosorbent are released first, and the protonated functional groups will block the release of ions from inside the wrinkles. Consequently, part of Co(II) ions cannot longer be desorbed, which leads to the decrease of desorption efficiency. The same processes take place in the case of Zn(II) and Cu(II) ions, with the mention that the share of ions trapped in the wrinkles of the biosorbent particles depends on the ratio between electronegativity and ionic radius. Therefore, the recovery of metal ions by desorption with mineral acids (10^{-1} mol/L HNO_3 in this case) is quantitative only in the first 2-3 desorption cycles, after which the process is no longer efficient. However, the use of a small volume of eluent (10 mL of 10^{-1} mol/L HNO_3 for 0.5 g of exhausted biosorbent) represents a significant advantages for the use of these algae wastes (G-AWB and R-AWB) in the recovery processes of Zn(II), Cu(II) and Co(II) ions.

3.6. Evaluation of the applicability in the treatment of industrial effluents

To evaluate the applicability of these biosorbents (G-AWB and R-AWB) in the treatment of industrial effluents, samples of washing water (wastewater) from metal coating industry were used, in which the concentration of Zn(II), Cu(II) and Co(II) ions was adjusted to 50 mg/L. This type of wastewater was selected for the experimental studies because: (i) it has a low content of organic compounds, therefore the blocking of the functional groups of the biosorbents with such compounds is minimal, (ii) it has a rather acidic pH (2.9 – 3.4), which makes Zn(II), Cu(II) and Co(II) ions to be found predominantly as free ions available for biosorption, and (iii) the content of metal ions is quite high, reaching up to 45-60 mg/L (data not shown). Thus, for each metal ion, a wastewater sample (250 mL) was prepared, in which the pH was adjusted to 5.0 and the concentration of metal ions to 50 mg/L, and which was contacted with 2.0 g of each biosorbent, for 3 hours at 150 rpm. After filtration, the concentration of Zn(II), Cu(II) and Co(II) was analyzed, as well as some quality parameters of industrial effluents (pH, TSS, chloride, sulphate, CCO-Cr, Ca(II) and Mg(II)), mentioned in the national regulations [47]. The obtained results and summarized in Table 5.

Table 5. The values of some parameters for wastewater samples, before and after Zn(II), Cu(II) and Co(II) ions biosorption on G-AWB and R-AWB.

Parameter	**Recommended values [47]**	**Before biosorption**	**After biosorption**	
			G-AWB	**R-AWB**
pH*	6.5 – 8.5	5.00	5.78	6.14
Zn(II), mg/L	1.0	50.00	2.36	1.85
Cu(II), mg/L	0.2	50.00	0.64	0.53
Co(II), mg/L	1.0	50.00	4.01	2.79
Ca(II)*, mg/L	300	91.40	92.03	92.38
Mg(II)*, mg/L	100	32.20	31.89	32.03
Chloride*, mg/L	500	127.21	131.14	129.02
Sulphate*, mg/L	600	567.25	548.31	550.87
CCO-Cr*, mg O_2/L	125	41.57	43.58	42.93
TSS*, mg/L	-	873.11	881.04	883.32

* Average of three determinations.

Analyzing the results presented in Table 5, it can be seen that: (i) after biosorption, the treated effluents still have a slightly acid pH, and therefore require and additional neutralization step, (ii) the concentration of Zn(II), Cu(II) and Co(II) ions is significant reduced (over 91 % for G-AWB and over 94 % for R-AWB), but it is still higher than the maximum permissible limit (Table 5), which means that the biosorption process must be coupled with another metal ions removal step so that the treated effluents reach the imposed quality requirements, and (iii) the values of other quality parameters vary insignificantly after biosorption (Table 5), so the use of algae wastes as biosorbents does not contribute to additional pollution of treated effluents (secondary pollution is minimal). All these observations suggest that the algae wastes (G-AWB and R-AWB) have practical potential to be used for the removal of Zn(II), Cu(II) and Co(II) ion from industrial effluents.

However, the practical applicability of such a metal ion removal process is much easier to highlight if its evaluation is carried out taking into account some performance criteria. Table 6 presents the proposed evaluation criteria and the scores obtained by the two algae wastes (G-AWB and R-AWB) in the removal processes of Zn(II), Cu(II) and Co(II) ions from aqueous media. The score for each criterion, from 1 to 3 (1 – lowest, 2 – medium, 3 – highest), was assigned based on the results obtained in biosorption experiments (in mono-components systems).

The high score of studied biosorption processes (Table 6) suggests the possibility of their widespread use in the treatment of industrial effluents. However, two clarifications should be made. The first is related to the fact that there are no differences between the scores obtained for G-AWB and R-AWB. Therefore, even if these two wastes are mixed, the performances of the biosorption processes do not change significantly. The second clarification is related to the rather general nature of the criteria mentioned in Table 6. These criteria need to be formulated in much more detail and the scores need to be assigned on a much wider scale to highlight the rather small differences between the biosorption performances of some such materials for certain metal ions. But, all these aspects will be presented and discussed in a further study.

Table 6. Criteria for evaluation the practical applicability of Zn(II), Cu(II) and Co(II) ions removal by biosorption on G-AWB and R-AWB.

Criteria		**Ideal**	**G-AWB**			**R-AWB**		
			Zn(II)	**Cu(II)**	**Co(II)**	**Zn(II)**	**Cu(II)**	**Co(II)**
Biosorbent obtaining	Biomass purchase	3	2	2	2	2	2	2
	Preparation steps	3	3	3	3	3	3	3
	Stability over time	3	3	3	3	3	3	3
Technical performances	Ease of achieving optimal conditions	3	2	2	2	2	2	2
	Biosorption efficiency	3	2	3	1	2	3	1
	Desorption efficiency	3	3	3	2	3	3	2
	Number of usage cycles	3	2	2	2	2	2	2
	Recovery/recycling costs	3	2	2	2	2	2	2
Quality of treated effluents	Final content of metal ions	3	2	2	1	2	2	1
	Secondary pollution	3	3	3	3	3	3	3
Total score		**30**	**24**	**25**	**21**	**24**	**25**	**21**

4. Conclusions

The use of biomass wastes as biosorbents for the removal of metal ions from aqueous media is one of the ways to valorize these wastes, which respects the principles of the circular economy. Therefore, in this study, algae wastes obtained after alkaline extraction of active compounds from two types of marine algae (green algae – *Ulva sp*. and red algae – *Callithamnion sp*.), were used for the removal of Zn(II), Cu(II) and Co(II) ions from aqueous effluents. The selection of these metal ions for the experimental studies was done considering their technological importance. The experimental results performed in batch mono-component systems, have shown that under optimal conditions (pH = 5.0; 4.0 g biosorbent/L; 22 ± 1°C), more than 75 % of the metal ions are removed (76 % for G-AWB and 79 % for R-AWB), when the initial concentration is lower than 1.25 mmol/L. The experimental data are best fit by the pseudo-second order kinetic model and Langmuir isotherm model, and the maximum biosorption capacities follow the order: q_{max}: Cu(II) (0.52 mmol/g) > Zn(II) (0.41 mmol/g) > Co(II) (0.39 mmol/g) for G-AWB, and q_{max}: Cu(II) (1.78 mmol/g) > Zn(II) (1.72 mmol/g) > Co(II) (1.66 mmol/g) for R-AWB. Also, desorption of metal ions is simple (using as eluent 10^{-1} mol/L HNO_3) and is quantitative in the first three cycles. These arguments highlight the potential use of these biosorbents to remove such technologically valuable metal ions from industrial wastewater. This possibility was tested using industrial washing wastewater samples obtained from the metal coating industry, when more than 91 % of metal ions (Zn(II), Cu(II) and Co(II)) are removed. These experimental results indicate that algae wastes (G-AWB and R-AWB) have the potential to be used in industrial effluent decontamination processes, and opens new perspectives for their valorisation.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: Linear representations of Langmuir (a), Freundlich (b) and Temkin (c) isotherm models for Zn(II), Cu(II) and Co(II) ions biosorption on G-AWB (1) and R-AWB (2).; Figure S2: Linear representations of pseudo-first order (a) and pseudo-second order (b) kinetic models for Zn(II), Cu(II) and Co(II) ions biosorption on G-AWB (1) and R-AWB (2).

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