Metal biosorption onto dry biomass of *Arthrospira* (*Spirulina*) *platensis* and *Chlorella vulgaris*: Multi-metal systems

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ABSTRACT

Binary and ternary systems of Ni^{2+}, Zn^{2+}, and Pb^{2+} were investigated at initial metal concentrations of 0.5, 1.0 and 2.0 mM as competitive adsorbates using *Arthrospira platensis* and *Chlorella vulgaris* as biosorbents. The experimental results were evaluated in terms of equilibrium sorption capacity and metal removal efficiency and fitted to the multi-component Langmuir and Freundlich isotherms. The pseudo second order model of Ho and McKay described well the adsorption kinetics, and the FT-IR spectroscopy confirmed metal binding to both biomasses. Ni^{2+} and Zn^{2+} interference on Pb^{2+} sorption was lower than the contrary, likely due to biosorbent preference to Pb. In general, the higher the total initial metal concentration, the lower the adsorption capacity. The results of this study demonstrated that dry biomass of *C. vulgaris* behaved as better biosorbent than *A. platensis* and suggest its use as an effective alternative sorbent for metal removal from wastewater.

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1. Introduction

Heavy metal pollution is one of the most severe environmental problems in the XXI century. Toxic metals such as lead, zinc and nickel are of great concern for human health and natural environment [1,2]. Their removal from industrial wastewaters is usually done through expensive and not environmentally friendly physicochemical techniques; therefore, searching for low-cost alternative methods for metal removal, like biosorption, has become a challenge worldwide [3,4].

Cyanobacteria and microalgae are photoautotrophic microorganisms that require only water, sunlight and carbon dioxide to grow. Their low-cost production on inorganic media implies the replacement of conventional nutrient sources by alternative ones, such as nitrates and ammonia from wastewaters, and carbon dioxide from effluent gases [5]. The use of photobioreactors in the production of these microorganisms can lead to high productivities [6].

Additionally, due to their relatively high sorption capacity, their biomasses are increasingly used to develop new biosorbents [2]. These microorganisms have cell walls constituted of peptidoglycan, teichuronic and teichoic acids, polysaccharides and proteins, which carry charged groups such as carboxyl, phosphate, hydroxyl or amine [6]. So, depending on pH, cell surface possesses cationic or anionic sites [7,8] available for biosorption. In particular, carboxyl and amino groups were reported to be very effective to this purpose [9–11].

Biomasses of the cyanobacterium *Arthrospira platensis* and the microalga *Chlorella vulgaris* were studied in the removal of toxic metals such as lead [12,13], zinc [13,14], nickel [13,15], copper [16,17], cadmium [18,19] and chromium [20,21]. Unfortunately, however, most of the available information regards only single component systems. Taking into consideration that many industrial effluents contain more than one metal, and that the biosorption of one metal considerably changes in the presence of another, the study of multi-component systems is of utmost importance.

Biosorption mechanism in the presence of two or more metals is very complex and has not yet been fully understood. It depends on pH, temperature, number of solutes and their concentrations [4], and type of interaction among different ions as well as between ions and cell binding sites. A mixture of metals can show three possible behaviors [7,22,23]: antagonism, synergism and no-interaction.

To investigate the interactive effects of metals and the potential of *A. platensis* and *C. vulgaris* biomasses as metal biosorbents, binary and ternary systems containing Ni^{2+}, Zn^{2+} and Pb^{2+} were compared to single systems [13]. The adsorption results were evaluated in terms of equilibrium sorption capacity and metal removal efficiency, and modeled by the pseudo second order kinetic model of Ho and McKay [24]. An adsorption isotherm study was also performed, in which adsorption data were processed by the

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multi-component Langmuir and Freundlich models. To elucidate and deepen knowledge on metal biosorption, FT-IR spectroscopy was finally applied to both dry *A. platensis* and *C. vulgaris* metal-bound biomasses.

2. Materials and methods

2.1. Biosorbents production and preparation

As previously described in more detail [13], *A. platensis* UTEX1926 (University of Texas Culture Collection, Austin, TX, USA) and *C. vulgaris* CCAP211 (Culture Collection of Algae and Protozoa, Argyll, UK) were grown batch-wise in Schlösser [25] and Bold's Basal [26] media, respectively, in tubular photobioreactor at $82 \pm 5\,\mu$mol photons m^{-2} s^{-1} through the daily addition of pure CO_2. Once the stationary phase had been reached, cells were centrifuged (ALC 4226 centrifuge, Milan, Italy) for 15 min at 3500 rpm, washed until reaching pH 7.0, dried at 40 °C for 24 h and ground to a fine powder, which was sieved through a 120 mesh (0.125 mm) sieve. The undersized fraction was then used for the adsorption tests.

2.2. Biosorption tests

Stock metal solutions (100 mM) were prepared by dissolving known amounts of high-purity Ni(NO$_3$)$_2$·6H$_2$O, Zn(NO$_3$)$_2$·6H$_2$O and Pb(NO$_3$)$_2$ (Sigma–Aldrich, Milan, Italy) in deionized water (1 L) and stored in amber flasks at room temperature (20 ± 1 °C).

Adsorption tests were carried out with 2 g L^{-1} of each biosorbent in metal solutions with different initial concentrations ($C_0 = 0.5$, 1.0 and 2.0 mM), in 250-mL Erlenmeyer flasks agitated at 150 rpm, pH 5.0–5.5 and room temperature. Samples were withdrawn at fixed times (5–120 min) and filtered through 0.45 μm-pore diameter membrane filters (Millipore, Vimodrone, Italy). The filtrate was analyzed for metal concentration using an atomic adsorption spectroscope, model AA240FS (Varian, Milan, Italy). All tests were carried out in triplicate, and the results expressed as mean values. Standard deviations between data and mean values never exceeded 6.4%.

2.3. Sorption capability

Sorption capability of both biomasses was evaluated either in terms of sorption capacity at equilibrium (q_{eq}), expressed in mmol g^{-1}:

$$q_{eq} = (C_0 - C_{eq})\frac{V}{m} \tag{1}$$

or metal removal efficiency (Y_R), expressed in %:

$$Y_R = \left(\frac{C_0 - C_{eq}}{C_0}\right) \times 100 \tag{2}$$

where C_{eq} (mM) is the equilibrium metal concentration, V (L) the solution volume and m (g) the biomass dry weight.

Preference of a biosorbent for one of the two metals (1 and 2) of a binary system was expressed in terms of separation factor α_2^1 [27,28]:

$$\alpha_2^1 = \frac{q_{eq,1}C_{eq,2}}{q_{eq,2}C_{eq,1}} \tag{3}$$

being $\alpha_2^1 > 1$ when 1 is preferred and vice versa [28].

2.4. Sorption kinetics

The integrated and rearranged pseudo second order rate expression of Ho and McKay [24], based on solid adsorption capacity, was used to analyze sorption kinetics:

$$\frac{t}{q_t} = \frac{1}{k_2 q_c^2} + \frac{1}{q_c}t \tag{4}$$

where q_t (mmol g^{-1}) is the sorption capacity at a given time, k_2 (g mmol^{-1} min^{-1}) the pseudo second order rate constant and q_c the theoretical sorption capacity at equilibrium.

Plots of t/q_t versus t allowed estimating k_2 and q_c.

2.5. Equilibrium isotherms

Multi-component Langmuir [29] and Freundlich [30] isotherms were applied to elaborate dual metal tests results.

The former is based on the use of single component adsorption data to describe the behavior of sorption mixtures. Owing to full competition among metals for the binding sites on cell surface, theoretical values of sorption capacity are given by the equation:

$$q_{L,i} = \frac{q_{max,i}K_{L,i}C_{eq,i}}{1 + \sum_{j=1}^{n}K_{L,j}C_{eq,j}} \quad n = 2 \text{ for binary systems} \tag{5}$$

where $q_{L,i}$ (mmol g^{-1}) is the sorption capacity at equilibrium for sorbate "i", and $C_{eq,i}$ and $C_{eq,j}$ (mM) are the equilibrium concentrations of sorbates "i" and "j". The theoretical maximum sorption capacity for sorbate "i", $q_{max,i}$ (mmol g^{-1}), and the Langmuir equilibrium constants for sorbates "i" and "j", $K_{L,i}$ and $K_{L,j}$ (L mmol^{-1}), were taken from previous work performed on single-component systems, carried out under the same conditions as this one [13].

The multi-component Freundlich model proposed by Sheindorf et al. [30], assuming that each component individually obeys the Freundlich isotherm, is given as:

$$q_{eq,i} = K_{F,i}C_{eq,i}\left(\sum_{j=1}^{n}a_{ij}C_{eq,j}\right)^{1/n_i - 1} \quad n = 2 \text{ for binary systems} \tag{6}$$

where the Freundlich constant $K_{F,i}$ (mmol$^{1-1/n}$ L$^{1/n}$ g^{-1}) and $1/n_i$ (dimensionless) were taken from single metal tests, carried out under the same conditions as this one [13], and a_{ij} is the competition coefficient in multi-component system.

The competition coefficient was calculated as the intercept on the ordinate axis of the straight line obtained plotting $C_{eq,i}/C_{eq,j}$ versus $\beta_i/C_{eq,j}$, being:

$$\beta_i = \left(\frac{K_{F,i}C_{eq,i}}{q_{eq,i}}\right)^{1/(1-(1/n_i))} \tag{7}$$

2.6. Characterization studies

At the end of biosorption tests, biomasses were recovered and submitted to FT-IR analysis as previously described [13]. Weighted, dry biomass powders were thoroughly mixed with KBr and pressed to obtain homogeneous disks to be analyzed in a Thermofisher FT-IR instrument. All the reported spectra are presented in common scale.

3. Results and discussion

3.1. Separation factor of binary systems

Preference of adsorbents for one of the two metal ions in Ni^{2+}/Zn^{2+}, Ni^{2+}/Pb^{2+} and Zn^{2+}/Pb^{2+} binary mixtures was evaluated in terms of separation factor (α_2^1) as defined in Eq. (3). In general,
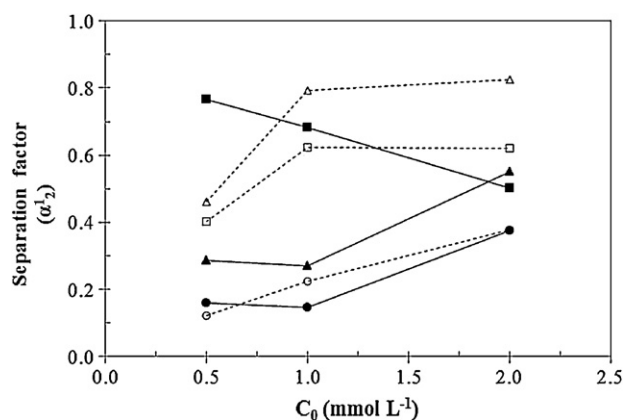
Fig. 1. Separation factor (α_2^1) as function of initial metal concentration ($C_0 = 0.5$, 1.0 and 2.0 mM) in binary systems: Ni/Zn (squares), Ni/Pb (circles) and Zn/Pb (triangles), using *Chlorella vulgaris* (full symbols) or *Arthrospira platensis* (empty symbols) as biosorbents.

was $\alpha_2^1 < 1$ (Fig. 1), which means that both exhibited the following metal affinity: $Pb^{2+} > Zn^{2+} > Ni^{2+}$. This behavior probably resulted from the different outer electronic configurations of the ions ([Xe] $4f^{14}\,5d^{10}\,6s^2$ for Pb, [Ar] $3d^{10}\,4s^2$ for Zn, [Ar] $3d^8\,4s^2$ for Ni). In other words, the decrease in the number of electrons lying in very diffused and distant orbitals (d and f), which are more available than those closer to the nucleus to establish interactions with functional groups, could have resulted in a decrease in affinity between such ions and the biosorbents [13]. This metal affinity order is also in accordance with the ionic radius that progressively decreased passing from Pb^{2+} (1.19 Å) to Zn^{2+} (0.74 Å) and Ni^{2+} (0.69 Å) [31].

Comparing the results of experiments at metal equimolar levels, to avoid any interference due to different concentration ratios, the separation factor did in fact vary in the ranges $0.40 \leq \alpha_{Zn}^{Ni} \leq 0.77$, $0.12 \leq \alpha_{Pb}^{Ni} \leq 0.38$ and $0.27 \leq \alpha_{Pb}^{Zn} \leq 0.82$, and the values increased with the metal level. These results are in accordance with those expressed by the Langmuir equilibrium constants (Supplementary Material Table 1) obtained in single metal tests [13].

Supplementary material related to this article found, in the online version, at doi:10.1016/j.jhazmat.2012.03.022.

At the highest C_0, i.e. under conditions favoring the statistical probability of sorbent to bind a metal based on its affinity, α_{Pb}^{Zn} was significantly lower with *C. vulgaris* (0.55) than with *A. platensis* (0.82), highlighting higher preference of the former biosorbent for all three metals, especially for Pb^{2+}. Such different relative adsorption power of biosorbents, consistent with different values of the Langmuir equilibrium constants (K_L) especially for Pb^{2+} alone (Supplementary Material Table 1), was likely due to the difference in the composition of *A. platensis* and *C. vulgaris* cell walls and primarily to the average dissociation degree of functional groups present on the surface of their cells.

As is well known, the outer cell wall of eukaryotic cells is in fact mainly composed of acidic mucopolysaccharides, in addition to glycolipids and glycoproteins, whereas that of prokaryotic cells of peptidoglycan. The mucopolysaccharides present in the cell wall of eukaryotic cells like those of *C. vulgaris* are long unbranched polysaccharides consisting of a repeating negatively charged disaccharide unit consisting of a hexose or a hexuronic acid linked to a hexosamine. On the other hand, the peptidoglycan of prokaryotes as *A. platensis* is mainly a copolymer of N-acetylglycosamine and N-acetylmuramic acid, both provided with amidic groups [32]. In this case, the only dissociable groups possibly available for metal removal are the carboxylic ones present in the branched lateral polypeptidic chains. The same mureinic material composes the outer sheath of cyanobacteria that covers the sequence of cells

to give the so-called trichomes [33]. However, such a filamentous structure remarkably reduces the surface available for metal binding and, in combination with the aforementioned factors, it should be recognized as the principal cause of the lesser *A. platensis* ability to remove metal ions compared to *C. vulgaris*.

At the lowest C_0, α_{Pb}^{Zn} decreased to 0.27 with *C. vulgaris* and 0.46 with *A. platensis*, pointing out a generalized increase in preference for Pb^{2+}, which suggests that, under these conditions of likely diffusion limitation, the higher affinity for Pb^{2+} became the prevailing phenomenon.

3.2. Dual adsorption of nickel/zinc

In Ni^{2+}/Zn^{2+} adsorption tests (Tables 1 and 2), an increase in the initial concentration of one metal (for instance $C_{0,Zn}$) at a given level of the competing metal ($C_{0,Ni}$) led to a generalized increase in the equilibrium sorption capacity of the former ($q_{eq,Zn}$) and a corresponding decrease in that of the latter ($q_{eq,Ni}$). Thinking the saturated biomass–metal cluster as a salty polyelectrolyte with low solubility, such behavior seems to contrast with what one would expect from the common ion effect of the competing ion, which suggests a simple statistical effect. Accordingly, the highest $q_{eq,Zn}$ values were obtained with both *A. platensis* (0.380 mmol g^{-1}) and *C. vulgaris* (0.524 mmol g^{-1}) at the highest $C_{0,Zn}$ (2.0 mM) and lowest $C_{0,Ni}$ (0.5 mM); oppositely for $q_{eq,Ni}$ (maximum values of 0.275 and 0.255 mmol g^{-1} with *A. platensis* and *C. vulgaris*, respectively).

Another interesting aspect deals with the biosorbent saturation state, which can be evaluated by comparing results obtained at equimolar levels, i.e. under conditions of no interference of the statistical effect. When total initial metal concentration ($C_{0,T}$) was increased from 2.0 mM (i.e. $C_{0,Ni} = C_{0,Zn} = 1.0$ mM) to 4.0 mM, both $q_{eq,Ni}$ and $q_{eq,Zn}$ (Tables 1 and 2) increased to a less extent than a free adsorption would suggest, indicating incipient biosorbent saturation due to excess metal in relation to the number of adsorption sites. Contrary to what expected from the smaller size of cyanobacteria cells, the saturation state appeared more advanced in the case of *A. platensis* biomass, which can be ascribed to different functional groups able to link metals. This aspect will be investigated later through IR-FT analysis. Finally, for both biomasses, it was mostly $q_{eq,Zn} > q_{eq,Ni}$, which can be justified by the higher affinity of both biosorbents for Zn^{2+} compared to Ni^{2+} [13] as well as by the separation factor results (Section 3.1).

Since the metal removal efficiency (Y_R) not only increases with biosorbent capacity, but is also a decreasing function of C_0, its response to an increase in C_0 was completely different from that of q_{eq}. For this reason, the best compromise between these opposite influences, ensuring the highest Y_R values for both Ni^{2+} (34.0% with *A. platensis* and 54.2% with *C. vulgaris*) and Zn^{2+} (56.3% with *A. platensis* and 64.7% with *C. vulgaris*), was obtained at the same intermediate C_0 values (Tables 1 and 2).

As previously suggested [13], the kinetic study of dual metal systems was made by comparing the theoretical sorption capacities (q_c) and pseudo second order rate constants (k_2) calculated by the pseudo second order model of Ho and McKay [24], whose values (Table 3) were in excellent agreement with the experimental ones (Tables 1 and 2), and the same applies also to the other binary systems.

But some exceptions, k_2 decreased with increasing $C_{0,T}$. In particular, the highest values for Ni^{2+} were obtained at $C_{0,Ni} = C_{0,Zn} = 0.5$ mM with *C. vulgaris* (2.37 g mmol^{-1} min^{-1}) and $C_{0,Ni} = 0.5$ mM and $C_{0,Zn} = 1.0$ mM with *A. platensis* (1.85 g mmol^{-1} min^{-1}), respectively. However, limiting the comparison to equimolar levels, one can observe that this decrease occurred with *A. platensis* within the whole experimental dominion, indicating fast saturation, but was almost negligible with *C. vulgaris* at $C_{0,T} = 2.0$ and 4.0, which suggests no saturation and

Table 1
Dual metal adsorption results: nickel(II), zinc(II) and lead(II) concentration at the start (C_0) and equilibrium (C_{eq}), adsorption capacity at equilibrium (q_{eq}) and metal removal efficiency (Y_R) of *A. platensis*.

$C_{0,Ni}$ (mM)	$C_{0,Zn}$ (mM)	$C_{0,Pb}$ (mM)	$C_{eq,Ni}$ (mM)	$q_{eq,Ni}$ (mmol g^{-1})	$Y_{R,Ni}$ (%)	$C_{eq,Zn}$ (mM)	$q_{eq,Zn}$ (mmol g^{-1})	$Y_{R,Zn}$ (%)	$C_{eq,Pb}$ (mM)	$q_{eq,Pb}$ (mmol g^{-1})	$Y_{R,Pb}$ (%)
0.5	0.5	–	0.333	0.083	33.4	0.223	0.138	55.4	–	–	–
0.5	1.0	–	0.330	0.085	34.0	0.437	0.282	56.3	–	–	–
0.5	2.0	–	0.357	0.071	28.6	1.28	0.360	36.0	–	–	–
1.0	0.5	–	0.713	0.144	28.7	0.230	0.135	53.9	–	–	–
1.0	1.0	–	0.705	0.148	29.5	0.598	0.201	40.2	–	–	–
1.0	2.0	–	0.816	0.092	18.4	1.24	0.380	38.0	–	–	–
2.0	0.5	–	1.45	0.275	27.5	0.287	0.107	42.7	–	–	–
2.0	1.0	–	1.58	0.208	20.8	0.616	0.192	38.4	–	–	–
2.0	2.0	–	1.69	0.155	15.5	1.54	0.228	22.8	–	–	–
0.5	–	0.5	0.421	0.040	15.8	–	–	–	0.197	0.152	60.7
0.5	–	1.0	0.421	0.039	15.8	–	–	–	0.435	0.282	56.5
0.5	–	2.0	0.415	0.043	17.0	–	–	–	1.24	0.378	37.8
1.0	–	0.5	0.807	0.096	19.3	–	–	–	0.185	0.158	63.1
1.0	–	1.0	0.806	0.097	19.4	–	–	–	0.483	0.258	51.7
1.0	–	2.0	0.807	0.096	19.2	–	–	–	1.27	0.366	36.6
2.0	–	0.5	1.49	0.256	25.6	–	–	–	0.203	0.148	59.4
2.0	–	1.0	1.49	0.256	25.6	–	–	–	0.570	0.215	43.0
2.0	–	2.0	1.63	0.184	18.4	–	–	–	1.25	0.373	37.3
–	0.5	0.5	–	–	–	0.236	0.132	52.9	0.146	0.177	70.7
–	0.5	1.0	–	–	–	0.270	0.115	45.9	0.430	0.285	57.0
–	0.5	2.0	–	–	–	0.314	0.093	37.3	1.23	0.382	38.2
–	1.0	0.5	–	–	–	0.474	0.263	52.6	0.147	0.177	70.6
–	1.0	1.0	–	–	–	0.555	0.223	44.5	0.497	0.252	50.3
–	1.0	2.0	–	–	–	0.633	0.183	36.7	1.23	0.384	38.4
–	2.0	0.5	–	–	–	1.27	0.365	36.5	0.146	0.177	70.7
–	2.0	1.0	–	–	–	1.29	0.355	35.5	0.518	0.241	48.2
–	2.0	2.0	–	–	–	1.34	0.327	32.7	1.258	0.371	37.1

provides further proof of the better ability of this biomass as biosorbent. With both biomasses, Zn^{2+} was more rapidly adsorbed than Ni^{2+} ($k_{2,Zn} > k_{2,Ni}$), as expected by the higher affinity of both biosorbents for it.

Comparing the results of mono- and dual-component systems, Ni^{2+} and Zn^{2+} seemed to behave as antagonists, because their simultaneous adsorption (Tables 1–3) was less effective than that of each metal alone (Supplementary Material Table 1). At the lowest $C_{0,Ni}$, Ni^{2+} Y_R values were almost the same as that obtained only with this metal, whereas those for Zn^{2+} were 16–66% lower, which means

that, at low metal level, only Zn^{2+} adsorption was strongly affected by the co-metal presence.

On the other side, $C_{0,T}$ proved to exert a great influence on the adsorption of both ions. For instance, with *A. platensis* biomass, Ni^{2+} and Zn^{2+} removal efficiencies at $C_{0,Ni} = C_{0,Zn} = 2.0$ mM (15.5% and 22.8%) (Table 1) decreased by about 40–45% compared to the corresponding mono-component systems at $C_0 = 2.0$ mM (28.5% and 38.4%) (Supplementary Material Table 1).

Sorption capacity at equilibrium suffered exactly the same reductions as Y_R, being these two parameters related to one

Table 2
Dual metal adsorption results: nickel(II), zinc(II) and lead(II) concentration at the start (C_0) and equilibrium (C_{eq}), adsorption capacity at equilibrium (q_{eq}) and metal removal efficiency (Y_R) of *C. vulgaris*.

$C_{0,Ni}$ (mM)	$C_{0,Zn}$ (mM)	$C_{0,Pb}$ (mM)	$C_{eq,Ni}$ (mM)	$q_{eq,Ni}$ (mmol g^{-1})	$Y_{R,Ni}$ (%)	$C_{eq,Zn}$ (mM)	$q_{eq,Zn}$ (mmol g^{-1})	$Y_{R,Zn}$ (%)	$C_{eq,Pb}$ (mM)	$q_{eq,Pb}$ (mmol g^{-1})	$Y_{R,Pb}$ (%)
0.5	0.5	–	0.233	0.134	53.5	0.200	0.150	60.0	–	–	–
0.5	1.0	–	0.229	0.136	54.2	0.353	0.323	64.7	–	–	–
0.5	2.0	–	0.374	0.063	25.3	0.953	0.524	52.4	–	–	–
1.0	0.5	–	0.535	0.232	46.5	0.195	0.153	61.1	–	–	–
1.0	1.0	–	0.551	0.224	44.9	0.456	0.272	54.4	–	–	–
1.0	2.0	–	0.682	0.159	31.8	1.08	0.460	46.0	–	–	–
2.0	0.5	–	1.49	0.255	25.5	0.246	0.127	50.7	–	–	–
2.0	1.0	–	1.49	0.254	25.4	0.493	0.254	50.7	–	–	–
2.0	2.0	–	1.52	0.238	23.8	1.23	0.384	38.4	–	–	–
0.5	–	0.5	0.234	0.133	53.2	–	–	–	0.062	0.219	87.6
0.5	–	1.0	0.241	0.130	51.9	–	–	–	0.152	0.424	84.8
0.5	–	2.0	0.312	0.094	37.5	–	–	–	0.781	0.610	61.0
1.0	–	0.5	0.562	0.219	43.8	–	–	–	0.061	0.219	87.7
1.0	–	1.0	0.557	0.222	44.3	–	–	–	0.156	0.422	84.4
1.0	–	2.0	0.690	0.155	31.0	–	–	–	0.801	0.599	59.9
2.0	–	0.5	1.17	0.415	41.5	–	–	–	0.068	0.216	86.4
2.0	–	1.0	1.17	0.413	41.3	–	–	–	0.193	0.403	80.7
2.0	–	2.0	1.38	0.310	31.0	–	–	–	0.906	0.544	54.4
–	0.5	0.5	–	–	–	0.153	0.173	69.4	0.056	0.222	88.8
–	0.5	1.0	–	–	–	0.188	0.156	62.5	0.150	0.425	85.0
–	0.5	2.0	–	–	–	0.249	0.125	50.1	0.775	0.613	61.3
–	1.0	0.5	–	–	–	0.355	0.323	64.5	0.059	0.220	88.1
–	1.0	1.0	–	–	–	0.384	0.308	61.6	0.144	0.428	85.6
–	1.0	2.0	–	–	–	0.475	0.262	52.5	0.773	0.613	61.3
–	2.0	0.5	–	–	–	0.913	0.543	54.3	0.060	0.220	88.0
–	2.0	1.0	–	–	–	0.911	0.545	54.5	0.143	0.424	85.7
–	2.0	2.0	–	–	–	1.07	0.465	46.5	0.775	0.613	61.3

Table 3

Theoretical sorption capacity, q_c, and pseudo second order rate constant, k_2, obtained from linear fit by Eq. (4) of the experimental data of nickel(II), zinc(II) and lead(II) dual biosorption by *A. platensis* and *C. vulgaris* biomasses, at different initial metal concentrations (C_0).

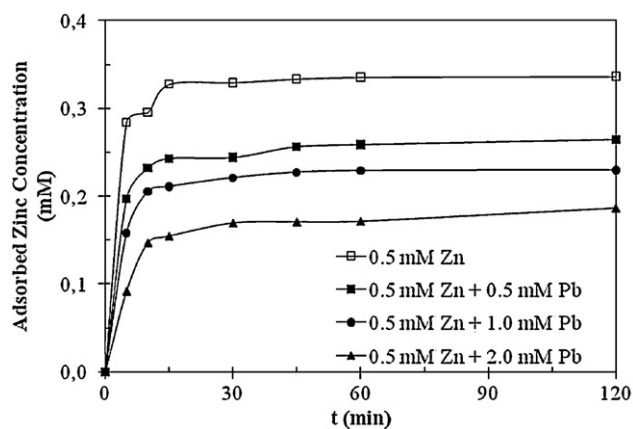| $C_{0,Ni}$ (mM)	$C_{0,Zn}$ (mM)	$C_{0,Pb}$ (mM)	*Arthrospira platensis*						*Chlorella vulgaris*					
			$q_{c,Ni}$ (mmol g^{-1})	$k_{2,Ni}$ (g mmol^{-1} min^{-1})	$q_{c,Zn}$ (mmol g^{-1})	$k_{2,Zn}$ (g mmol^{-1} min^{-1})	$q_{c,Pb}$ (mmol g^{-1})	$k_{2,Pb}$ (g mmol^{-1} min^{-1})	$q_{c,Ni}$ (mmol g^{-1})	$k_{2,Ni}$ (g mmol^{-1} min^{-1})	$q_{c,Zn}$ (mmol g^{-1})	$k_{2,Zn}$ (g mmol^{-1} min^{-1})	$q_{c,Pb}$ (mmol g^{-1})	$k_{2,Pb}$ (g mmol^{-1} min^{-1})
0.5	0.5	–	0.092	1.64	0.137	4.75	–	–	0.131	2.37	0.154	2.89	–	–
0.5	1.0	–	0.084	1.85	0.281	4.25	–	–	0.141	1.37	0.323	3.50	–	–
0.5	2.0	–	0.097	0.531	0.367	1.19	–	–	0.082	0.576	0.519	1.04	–	–
1.0	0.5	–	0.149	1.72	0.135	5.05	–	–	0.235	2.33	0.149	2.20	–	–
1.0	1.0	–	0.146	1.48	0.209	1.29	–	–	0.236	0.728	0.291	0.579	–	–
1.0	2.0	–	0.101	1.24	0.384	1.17	–	–	0.178	0.379	0.470	0.716	–	–
2.0	0.5	–	0.277	0.741	0.106	2.02	–	–	0.264	0.935	0.119	2.61	–	–
2.0	1.0	–	0.178	0.755	0.187	1.81	–	–	0.270	0.962	0.252	1.42	–	–
2.0	2.0	–	0.148	0.325	0.206	0.530	–	–	0.277	0.788	0.386	0.654	–	–
0.5	–	0.5	0.049	1.33	–	–	0.155	4.14	0.135	2.55	–	–	0.221	6.07
0.5	–	1.0	0.047	1.14	–	–	0.287	2.98	0.131	1.92	–	–	0.429	3.18
0.5	–	2.0	0.052	0.796	–	–	0.389	1.25	0.102	1.40	–	–	0.614	0.977
1.0	–	0.5	0.093	1.58	–	–	0.161	3.67	0.226	1.42	–	–	0.222	5.97
1.0	–	1.0	0.104	1.44	–	–	0.263	1.97	0.223	1.40	–	–	0.424	3.20
1.0	–	2.0	0.109	0.994	–	–	0.374	1.15	0.144	1.52	–	–	0.605	0.607
2.0	–	0.5	0.270	0.700	–	–	0.149	4.36	0.426	0.808	–	–	0.218	6.08
2.0	–	1.0	0.277	0.787	–	–	0.223	1.94	0.421	0.601	–	–	0.408	3.86
2.0	–	2.0	0.188	0.765	–	–	0.375	1.28	0.322	0.598	–	–	0.561	0.847
–	0.5	0.5	–	–	0.133	4.49	0.178	4.01	–	–	0.178	2.09	0.222	6.01
–	0.5	1.0	–	–	0.118	4.20	0.290	3.01	–	–	0.163	2.01	0.430	2.93
–	0.5	2.0	–	–	0.091	3.38	0.378	1.63	–	–	0.117	1.90	0.628	0.628
–	1.0	0.5	–	–	0.260	3.73	0.181	3.88	–	–	0.328	2.48	0.221	5.81
–	1.0	1.0	–	–	0.224	3.42	0.251	2.89	–	–	0.315	2.18	0.435	2.29
–	1.0	2.0	–	–	0.189	1.81	0.381	1.14	–	–	0.269	1.54	0.657	0.324
–	2.0	0.5	–	–	0.378	1.40	0.176	3.69	–	–	0.546	1.09	0.221	5.79
–	2.0	1.0	–	–	0.361	1.39	0.243	2.64	–	–	0.553	0.868	0.436	2.30
–	2.0	2.0	–	–	0.331	1.37	0.352	1.84	–	–	0.484	0.733	0.650	0.424
R^2			0.736–0.999		0.987–0.999		0.998–1.000		0.936–0.999		0.989–0.999		0.998–1.000	

Fig. 2. Zinc(II) adsorption onto *A. platensis* as function of time: $C_0 = 0.5$ mM zinc(II) without [12] and with lead(II) at different concentrations.
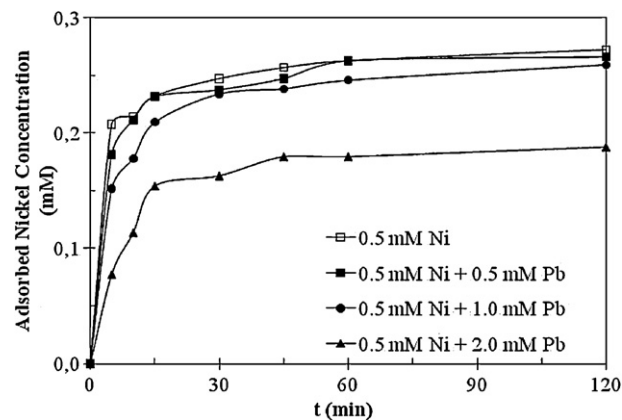

Fig. 3. Nickel(II) adsorption onto *C. vulgaris* as function of time: $C_0 = 0.5$ mM nickel(II) without [12] and with lead(II) at different concentrations.

another. As said reductions increased with the competing metal level, the above-mentioned statistical effect on adsorption seemed to be confirmed. Interestingly, the corresponding decreases in k_2 with *A. platensis* were more marked (by 80% for Ni^{2+} and 67% for Zn^{2+}) than for Y_R. With *C. vulgaris* such reductions were only a bit higher for Y_R and lower for k_2.

These results taken together demonstrate that the biosorption kinetic constants suffered always greater reductions than the equilibrium parameters (removal efficiency and adsorption capacity), suggesting a kinetic control of the binding of the metal ions onto the surface of the cells.

3.3. Dual adsorptions of nickel/lead and zinc/lead

The other binary systems showed qualitatively the same behaviors as Ni^{2+}/Zn^{2+}. However, whereas Pb^{2+} interference on Ni^{2+} and Zn^{2+} biosorption was significant in terms of all sorption parameters, that of the other two metals on Pb^{2+} biosorption was very little and sometimes negligible at equilibrium (Y_R and q_{eq}), but significant at the beginning of runs (k_2), consistently with the above-supposed kinetic control, especially with *C. vulgaris*.

As for Ni^{2+}/Zn^{2+}, in Ni^{2+}/Pb^{2+} and Zn^{2+}/Pb^{2+} sorption tests carried out either with *A. platensis* (Table 1) or *C. vulgaris* (Table 2), an increase in initial Pb^{2+} concentration ($C_{0,Pb}$) at a given level of the competing metal led to a generalized increase in Pb^{2+} equilibrium sorption capacity ($q_{eq,Pb}$) and a corresponding decrease in those of the competing metals. However, whereas such a decrease in both $q_{eq,Ni}$ and $q_{eq,Zn}$ was present in Zn^{2+}/Pb^{2+} under all conditions investigated (Fig. 2, for example), it was negligible at low metal concentrations in Ni^{2+}/Pb^{2+} (Fig. 3) and became significant with *A. platensis* only at $C_{0,Pb} = C_{0,Ni} = 2.0$ mM and with *C. vulgaris* at $C_{0,Pb} = 2.0$ mM irrespective of the Ni^{2+} level. The stronger Pb^{2+} interference on Ni^{2+} biosorption by *C. vulgaris* compared to *A. platensis* is consistent with the higher affinity of the former biosorbent for Pb^{2+} [13].

As a result of the higher affinity for Pb^{2+}, the highest $q_{eq,Pb}$ values were obtained at the highest $C_{0,Pb}$, almost irrespectively of both the type and concentration of the competing metal, either with *A. platensis* (0.366–0.384 mmol g^{-1}) or *C. vulgaris* (0.544–0.613 mmol g^{-1}). The higher $q_{eq,Pb}$ values obtained with the latter biosorbent confirm its larger capability of biosorbing the three metals at the beginning of runs. With regards to the saturation state of biosorbents in the presence of Ni^{2+}/Pb^{2+} and Zn^{2+}/Pb^{2+}, when $C_{0,T}$ was increased from 2.0 to 4.0 mM under equimolar conditions, once again the sorption capacities of all three metals increased less than expected likely because of incipient saturation. Finally, for both biomasses, it was $q_{eq,Pb} > q_{eq,Zn} > q_{eq,Ni}$, i.e. this

parameter followed the same affinity order observed with single metals [13].

Pb^{2+} Y_R values in binary systems were higher with *C. vulgaris* (86.4–88.8%) than with *A. platensis* at the lowest $C_{0,Pb}$, almost irrespective of the type and concentration of the competing metal. On the other hand, using *A. platensis* it was, in combination with Zn^{2+} (about 71%), substantially higher than with Ni^{2+} (about 59–63%). These results suggest that the high affinity of *C. vulgaris* for Pb^{2+} practically masked any interference of the competing metal (Tables 1 and 2). Such an interference was evident with *A. platensis*, in that, Y_R at the lowest $C_{0,Pb}$ was reduced, compared to the mono-component system, by only 9% in the presence of Zn^{2+}, but no less than 24% in the presence of Ni^{2+}. If from the one hand this result is consistent with the highest affinity of both biosorbents for Pb^{2+}, from the other it appears to be in contradiction with their higher affinity for Zn^{2+} than for Ni^{2+}. However, one should take into account that the interference of a metal in the binding of another onto the surface of cells is a complex phenomenon depending also on electric interaction, complexation, etc.

As for the Ni^{2+}/Zn^{2+} equimolar system, also in the other two binary systems k_2 decreased with increasing $C_{0,T}$ (Table 3), apparently with no appreciable influence of the type of competing metal. The highest $k_{2,Pb}$ values obtained at $C_{0,Pb} = C_{0,Zn} = C_{0,Ni} = 0.5$ mM were in fact almost coincident for the two binary systems containing Pb^{2+}, either with *C. vulgaris* (6.0–6.1 g mmol^{-1} min^{-1}) or *A. platensis* (4.0–4.1 g mmol^{-1} min^{-1}). Consistently with the established affinity order, it was once more $k_{2,Pb} > k_{2,Zn} > k_{2,Ni}$ with both biomasses.

Likewise Ni^{2+}/Zn^{2+}, also the other two binary systems exhibited antagonism when compared with mono-component systems. However, as expected by the highest affinity of both biosorbents for Pb^{2+}, whereas the Pb^{2+} ability of reducing both Y_R and q_{eq} for the other metals was always significant (by 29–35% for Ni and 15% for Zn) almost irrespectively of the type of biosorbent, those induced by Zn^{2+} and Ni^{2+} on Pb^{2+} removal were little or even negligible (Tables 1 and 2). With *A. platensis* k_2 decreased more markedly for Ni^{2+}/Pb^{2+} (by 53% for Ni^{2+} and 44% for Pb^{2+}) than for Zn^{2+}/Pb^{2+} (by 14% for Zn^{2+} and 20% for Pb^{2+}), consistently with the higher affinity of this biosorbent for Zn^{2+} than for Ni^{2+}. As expected by the better performance of *C. vulgaris* as a biosorbent [13], all k_2 values decreased, as a result of metal competition, more strongly than with *A. platensis*.

Concluding, although Pb^{2+} removal efficiency and sorption capacity at equilibrium were not remarkably influenced by Ni^{2+} and Zn^{2+} with both biosorbents, the rate at which Pb^{2+} biosorption took place was, consistently with a kinetic control of the process.
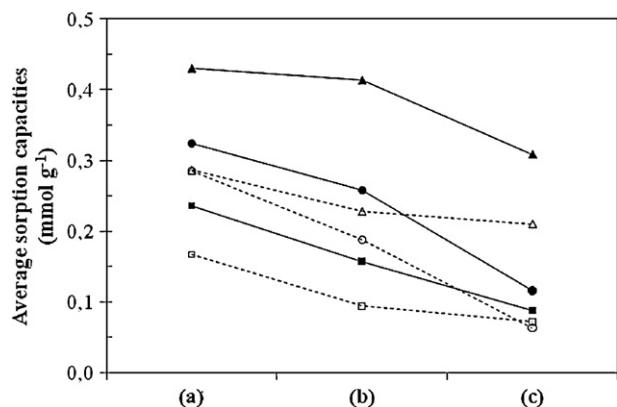
Fig. 4. Sorption capacity of nickel(II) (squares), zinc(II) (circles) and lead(II) (triangles) onto *A. platensis* (full symbols) and *C. vulgaris* (empty symbols) in single (a) [12], dual (b) and ternary (c) systems. All metals were present at concentration of 1.0 mM.

3.4. Binary systems isotherms

To evaluate the fitting of multi-component isotherms, the equilibrium sorption capacity was also calculated by the competitive Langmuir (Eq. (5)) and Freundlich (Eq. (6)) models. The estimated values (data not shown) were very close to the experimental ones for Ni^{2+} and Zn^{2+} adsorption (Tables 1 and 2), the maximum standard deviation for the Langmuir model being 9% and that for the Freundlich one 12%. These results indicate that, also in binary systems, Ni^{2+} and Zn^{2+} biosorption by *A. platensis* and *C. vulgaris* followed an intermediate behavior between mono- and multilayer mechanism, already shown with single metals [13]. Contrarily, the same models overestimated Pb^{2+} sorption capacity up to 19% and 13%, respectively, which suggests that they are not able to describe well the competitive biosorption when metals have a too large difference in affinity.

Maximum sorption capacity (q_{max}) estimated by the competitive Langmuir model for Ni^{2+} biosorption by *A. platensis* decreased with increasing the initial co-metal concentration (Table 4), while that for Zn^{2+} was not significantly influenced by either the co-metal type or concentration. With *C. vulgaris*, Ni^{2+} and Zn^{2+} q_{max} seemed to decrease with increasing initial co-metal concentration only when Pb^{2+} was not among them.

The Langmuir constant (K_L) decreased in all cases with increasing the initial co-metal concentration, which demonstrates that biomass affinity for one metal is highly dependent on the co-metal level. As expected, the affinity order found for binary systems was the same already found for single metal adsorption, as a result from the outer electronic configuration of the metal [13]: $Pb^{2+} > Zn^{2+} > Ni^{2+}$, and again *C. vulgaris* showed, in most cases, higher affinity than *A. platensis*.

The constant K_F of the competitive Freundlich model for Ni^{2+} and Zn^{2+} was, in general, dependent on the type of co-metal, but not on its initial concentration (Table 4). On the other hand, an increase in Pb^{2+} concentration remarkably affected the affinity of biomass for Zn^{2+}, but not for Ni^{2+}. More interestingly, taking the K_F values at different concentrations as an average, a comparison with the mono-component system shows that *A. platensis* affinity for Pb^{2+} was not affected by the presence of the other two metals, whereas that of *C. vulgaris* for Ni^{2+} and Zn^{2+} decreased by only 11% and 6%, respectively. As expected by the previous considerations, the largest decreases in K_F induced by Pb^{2+} were observed with *A. platensis* for Zn^{2+} (22%) and *C. vulgaris* for Ni^{2+} (31%).

The parameter n of the Freundlich model that expresses the adsorption intensity was always >1, which means that the selected metals were effectively biosorbed by both biomasses [7,13].

Table 4
Isotherm parameters and constants calculated at equilibrium by the multicomponent models of Langmuir (K_L, q_{max}) and Freundlich (K_F, n) for nickel(II), zinc(II) and lead(II) removal using *A. platensis* and *C. vulgaris* biomasses as biosorbents.

		Langmuir							Freundlich						
		Arthrospira platensis			*Chlorella vulgaris*				*Arthrospira platensis*			*Chlorella vulgaris*			
		q_{max} (mmol g^{-1})	K_L (L mmol^{-1})	R^2	q_{max} (mmol g^{-1})	K_L (L mmol^{-1})	R^2		K_F (mmol$^{1-1/n}$ L$^{1/n}$ g^{-1})	n	R^2	K_F (mmol$^{1-1/n}$ L$^{1/n}$ g^{-1})	n	R^2	
Ni(II)	+0.5 mM Zn	0.633	0.154	0.998	0.741	0.402	0.999		0.195	1.20	0.999	0.319	1.32	0.999	
	+1.0 mM Zn	0.531	0.104	0.972	0.672	0.280	0.980		0.195	1.22	1.000	0.336	1.41	1.000	
	+2.0 mM Zn	0.380	0.047	0.989	0.590	0.148	0.980		0.188	1.20	0.999	0.345	1.43	0.999	
	+0.5 mM Pb	0.708	0.196	0.999	0.745	0.170	0.970		0.214	1.34	1.000	0.257	1.14	0.999	
	+1.0 mM Pb	0.569	0.133	0.996	0.311	0.042	1.000		0.214	1.35	1.000	0.266	1.22	0.999	
	+2.0 mM Pb	0.414	0.044	0.991	0.790	0.037	0.915		0.210	1.31	0.999	0.235	1.11	0.999	
Zn(II)	+0.5 mM Ni	0.576	0.588	0.999	0.906	1.04	1.000		0.338	1.97	0.996	0.512	1.59	0.999	
	+1.0 mM Ni	0.580	0.525	0.999	0.866	0.805	0.999		0.299	1.54	0.996	0.533	1.74	0.999	
	+2.0 mM Ni	0.599	0.400	1.000	0.838	0.550	0.999		0.317	1.65	0.998	0.489	1.55	0.999	
	+0.5 mM Pb	0.600	0.569	1.000	0.920	0.509	0.999		0.333	1.60	0.998	0.530	1.71	0.999	
	+1.0 mM Pb	0.612	0.472	0.999	0.601	0.151	0.958		0.287	1.30	0.998	0.485	1.52	0.999	
	+2.0 mM Pb	0.603	0.207	1.000	0.760	0.076	0.997		0.240	1.24	0.999	0.355	1.18	0.999	
Pb(II)	+0.5 mM Ni	0.507	0.611	0.999	0.662	3.64	1.000		0.380	3.20	1.000	0.564	2.37	0.995	
	+1.0 mM Ni	0.495	0.468	0.999	0.655	2.83	0.999		0.381	3.03	0.999	0.564	2.33	0.994	
	+2.0 mM Ni	0.475	0.282	0.999	0.662	1.66	0.999		0.380	2.76	0.998	0.513	1.90	0.993	
	+0.5 mM Zn	0.537	0.758	1.000	0.668	3.69	1.000		0.380	3.14	1.000	0.594	2.95	0.998	
	+1.0 mM Zn	0.531	0.666	1.000	0.668	2.97	1.000		0.380	3.12	0.999	0.568	2.46	0.996	
	+2.0 mM Zn	0.525	0.543	1.000	0.661	2.22	1.000		0.381	3.12	0.999	0.577	2.44	0.995	
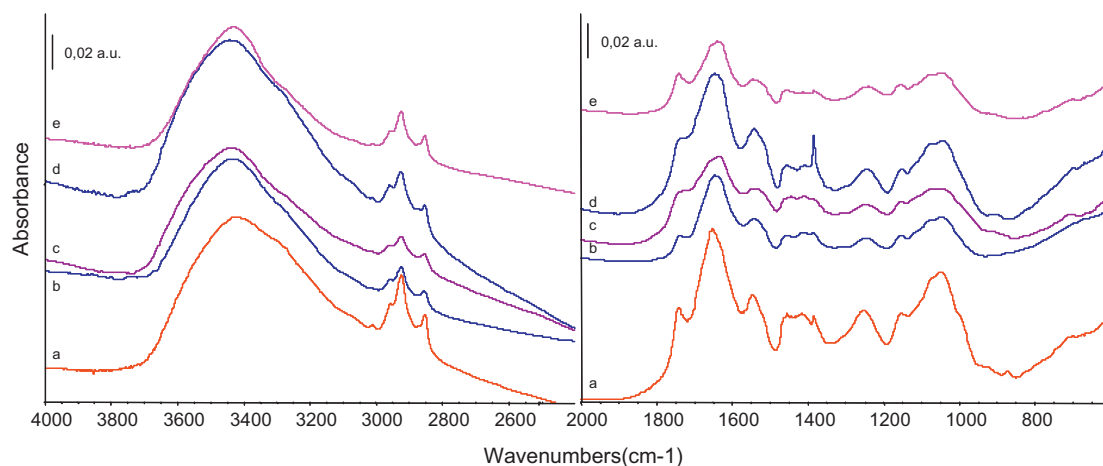
Fig. 5. FT-IR spectra of *C. vulgaris*, fresh (a) [12] and metal-loaded ((b) Ni^{2+}/Zn^{2+}; (c) Ni^{2+}/Pb^{2+}; (d) Zn^{2+}/Pb^{2+}; and (e) $Ni^{2+}/Zn^{2+}/Pb^{2+}$). Spectra in common scale.

However, as expected, such intensity was higher for Pb^{2+} (1.90–3.20) than for Zn^{2+} (1.18–1.97) or Ni^{2+} (1.11–1.43). Similar *n* values for Ni^{2+} and Zn^{2+} suggest that these metals bound to both biomasses with very similar intensities. Finally, the highest intensity reductions with respect to single metals were exerted by Pb^{2+} on Zn^{2+} biosorption onto *A. platensis* (43%) and by Ni^{2+} onto Pb^{2+} biosorption onto *C. vulgaris* (40%).

3.5. Ternary biosorption

The adsorption mechanism involving three sorbates is much more complex than in dual systems, due to chemical interactions among metal ions and with biomass, and also depends on ionic radii and electronegativities [34]. Nevertheless, an additional test was carried out at $C_0 = 1.0$ mM of each metal with both biosorbents.

Fig. 4 compares the different q_{eq} behaviors in three-component system with those in single and dual ones. All biosorption capacities somewhat decreased with increasing the number of co-metals. For instance, Zn^{2+} adsorption capacities of *A. platensis* were 0.285 mmol g^{-1} in single solution, 0.201 and 0.223 mmol g^{-1} in the presence of Ni^{2+} and Pb^{2+}, respectively, and 0.063 mmol g^{-1} in the simultaneous presence of all three metals. Further investigation is needed to clarify the mechanisms of metal adsorption in ternary systems.

3.6. FT-IR analysis

The main FT-IR bands of fresh *C. vulgaris* and *A. platensis* biomasses (Figs. 5 and 6, spectra "a") were previously discussed [13], and some common features are summarized here. The main peaks in the high frequency region, centered at 2923 and 2852 cm^{-1} were assigned to CH stretching modes of alkyl chains. The weak component at 3010 cm^{-1} characterizes the unsaturated CH groups belonging to lipids and phospholipids fractions in *C. vulgaris* biomass. Broad and weak shoulders at 3300 and 3080 cm^{-1} can be assigned to NH stretching modes of amine-amide containing groups. At lower frequencies, other absorption bands are detected at 1742 cm^{-1} (C=O stretching mode of ester functional group, belonging to glyceride component), around 1650 cm^{-1} (amide I), 1548 cm^{-1} (amide II) and 1280 cm^{-1} (amide III), and 1416 cm^{-1} (COO— symmetric stretching mode of carboxylates, whose asymmetric stretching mode is masked by the strong amide I absorption at 1650 cm^{-1}). The first band was more pronounced in *C. vulgaris* spectrum, according to the higher lipid content of eukaryotic (triglycerides) than prokaryotic (phosphoglycerides) cells. Complex and broad absorption bands can also be detected in the range 1200–1050 cm^{-1}, assigned to CC and CO stretching mode of the polysaccharides hydroxyl groups.

Spectra "b" to "d" in Fig. 5 correspond to the metal-loaded *C. vulgaris* biomass. In this case, metal biosorption in binary systems lowered the broad shoulder at 3300 cm^{-1}, with respect to the main absorption centered at 3420 cm^{-1}, due to cation interaction with
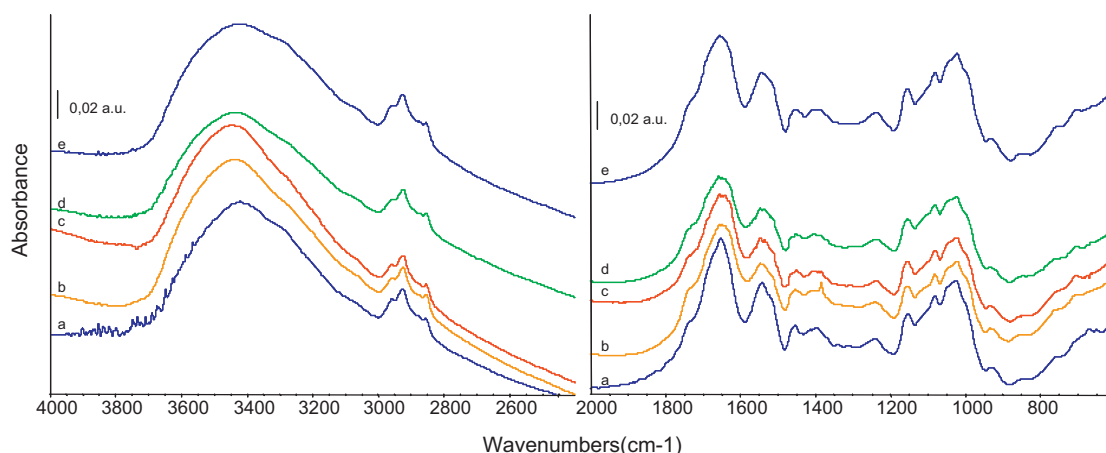

Fig. 6. FT-IR spectra of *A. platensis*, fresh (a) [12] and metal-loaded ((b) Ni^{2+}/Zn^{2+}; (c) Ni^{2+}/Pb^{2+}; (d) Zn^{2+}/Pb^{2+}; and (e) $Ni^{2+}/Zn^{2+}/Pb^{2+}$). Spectra in common scale.

electron rich amide and/or amino groups. A slight shift of the broad band due to OH stretching modes can also be noticed. Zn^{2+}/Pb^{2+} seemed to affect to lower extent the NH band, possibly due to the previously discussed antagonistic effect of Pb^{2+} on Zn^{2+} biosorption. Several effects can be noticed in the low frequency region of spectra: a general reduction of the main bands intensities, which is especially evident for Ni^{2+}/Pb^{2+}; a decrease of the 1742 cm^{-1} carbonyl band, relative to the amide main band, and a shift of the same band to lower frequencies, which leads to a partial overlapping with the amide I/carboxylate band at 1650 cm^{-1}. Once again this effect was more pronounced in the Ni^{2+}/Pb^{2+} spectrum. Carboxylate symmetric stretching band at 1416 cm^{-1} was also reduced in intensity in comparison with the adjacent absorption due to CH deformation modes (around 1430 cm^{-1}).

Three metals adsorption (Fig. 5, spectrum "e") enhanced some of the effects described above: a further decrease in the overall IR bands intensities, related to metal adsorption, a selective decrease in the relative intensities and a broadening of the amide band at 1548 cm^{-1}, which we can tentatively assign to a further interaction of metal ions with the amide component. On the other hand, detection of the C=O IR band almost unperturbed suggests that the lipid fraction was not significantly involved in the overall biosorption.

Comparison with *C. vulgaris* mono-component systems shows that the competitive sorption of two and three metals affected strongly amino and amide groups, acting as ligands, while hydroxyl groups of the saccharide component were somehow less involved. Complexation of carboxylate anions that coordinate metal ions was also detected. Possibly, preferential interaction of the lead-containing binary system with the ester fraction of the lipid component (see shift of the C=O band) can be proposed; however, this effect could not be detected in the ternary system.

Spectra of *A. platensis* after two and three metals sorption are also depicted (Fig. 6). However, very little difference can be noticed compared to those reported for single metals [13]. The same functional groups were clearly involved, and no preferential biosorption could be remarked [35,36].

4. Conclusions

A. platensis and *C. vulgaris* dry biomasses were employed as biosorbents in Ni^{2+}, Zn^{2+} and Pb^{2+} multi-component systems at different initial metal concentrations (C_0). The separation factor indicated that both biomasses have the following affinity order: $Pb^{2+} > Zn^{2+} > Ni^{2+}$. *C. vulgaris* behaved as better biosorbent because of higher equilibrium sorption capacities (q_{eq}) and removal efficiencies (Y_R). The difference in biosorption could have resulted from the difference in cell wall composition of the eukaryotic cells of *C. vulgaris* and the prokaryotic ones of *A. platensis*. High q_{eq} values were obtained when C_0 of the selected metal was higher than that of the co-metal. In tests with one or more metals, q_{eq} somewhat decreased with increasing the number of co-metals.

In all binary systems, metals seemed to behave as antagonists. However, whereas the Pb^{2+} ability of reducing Y_R and q_e of the other metals was always significant, those of Zn^{2+} and Ni^{2+} on Pb^{2+} removal were almost negligible. The pseudo second order model of Ho and McKay showed excellent fit of the experimental data, and the presence of a co-metal lowered the rate constant for a given metal. The multi-component Langmuir and Freundlich models represented well Zn^{2+} and Ni^{2+} biosorption, highlighting an intermediate behavior between mono- and multilayer mechanism, but overestimated the Pb^{2+} sorption capacity owing to too large difference in affinity.

Spectroscopic data indicated that competitive biosorption of two and three metals required the interaction of metals with electron-rich biomass functional groups, i.e. amino, amide, esters and, to lower extent, carboxylate groups, found on the surface of the cells. These findings are in agreement with data reported for single metal adsorption.

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