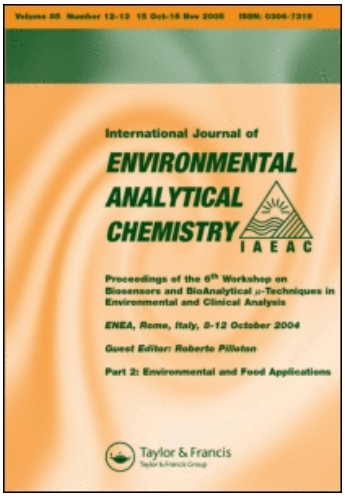

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Cleansing contaminated seawaters using marine cyanobacteria: evaluation of trace metal removal from the medium

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The use of microbial biomass in biosorption is already being studied as a potential alternative to (or combined with) conventional processes, where several algae and microorganisms have already shown promising ability to uptake metals. Cyanobacteria (blue-green algae) are widespread organisms, with specific properties, such as high nutrient removal capacity and tolerance to highly variable conditions which make them well-suited for wastewater and remediation purposes. The main aim of this work was to evaluate the use of a marine cyanobacterium LEANCYA 21 (*Synechocystis sp.*), collected from the Portuguese southern border, for the removal of selected trace metals when in natural seawater culture medium. It was observed, for the first time, that this particular strain is capable of uptaking Pb, Ni and Zn (at nM levels) from seawater solutions using small amounts of biomass. Uptake values for Pb were up to 90% (0.75 mg g^{-1} biomass) in 6 h. The specific biosorption curves of Ni and Zn showed that these metals follow a first order kinetics biosorption in batch conditions. Solutions containing multimetals have revealed that Ni uptake is affected by the presence of Pb and Zn. The calculated specific absorption values were high enough to predict a possible application in aquaculture where such low levels of metals may inhibit microalgae growth.

Keywords: heavy metal removal; trace metal speciation; seawater; cyanobacteria; biosorption

1. Introduction

Large and rapid industrial development carried serious environmental problems such as the presence of heavy metals in waters. Numerous industries and processes contribute to the occurrence of these elements in water bodies as a result of anthropogenic activities with further quantities produced by daily domestic effluents [1,2].

Much interest has arisen concerning heavy metals as contaminants, since they tend to persist permanently in the environment. Due to their atomic structure, they cannot be further mineralised to a completely innocuous form – this contributes to their hazard potential [3,4]. Even knowing that some of these metals are non toxic at low concentrations, environmental researchers have focused on their accumulation through the food chain, with what may have serious ecological and health problems [5,6].

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Thus, many attempts are being made to deal with the reduction/elimination of the contamination by these polluting agents. Generally, effluents containing trace metals are treated through physical and chemical processes. However, these conventional clean-up processes show limitations when applied to effluents containing metals at low concentrations (10 to 100 mg L^{-1} of dissolved metal) or even for large volumes [7]. Moreover, these methods have been shown to be inefficient for the complete removal of trace metals and often become extremely expensive due to the high cost of equipment and reagents and high energy requirements [8,9].

In the past two decades, the development of alternative technologies for water cleansing, especially using biotechnological processes has been observed [2,8–11]. These new methods have already shown advantages, compared with traditional methods, such as easy implementation, low operating cost (through the use of naturally abundant renewable biomaterials), minimisation of chemical and/or biological sludge to be disposed of, high efficiency in detoxifying very diluted effluents, rapid kinetics of metal removal, easy adaptation to the physico-chemical parameters of the effluent, possibility to treat waters containing multiple metals, and selectivity to remove only the desired metals [9,12–14].

Biosorption, a promising biotechnological process, consists of heavy metal retention, removal or recovery from a liquid environment using solids of vegetal origin or microorganisms [3]. It has already been studied for heavy metal removal from aqueous solutions through the use of bacteria, algae and fungi [3,4,9,12,15,16] and also by plant root tissues [12,17]. In this process, the metal removal rates from solution will depend on the chosen biosorbent.

The ability of algal biomass for metal accumulation was proven to be similar, and even sometimes higher, than chemical sorbents [15]. Metal biosorption experiences using algal biomass as sorbents have already been carried out using, for example, freshwater green algae (*Chlorella sp.*, *Cladophora sp.*, *Scenedesmus sp.*, *Chlamydomonas reinhardtii*) and brown algae (*Sargassum natans*, *Fucus vesiculosus*, *Ascophyllum nodosum*, *Laminaria japonica*) [15]. Nevertheless, just a few of the thousands of algal species known have been studied for metal sorption and successive use in wastewater treatment [15,16].

Among the several organisms that can be used in biosorption, cyanobacteria show several advantages in metal removal that justify their utilisation: low nutritional requirement, trophic independence in relation to carbon and nitrogen and also high tolerance to adverse conditions that characterise heavily polluted effluents [12,18]. Some studies of heavy metal removal using freshwater cyanobacteria have already been made. *Gloeothece magna* [4] and *Tolypothrix tenuis* [12], two different strains of freshwater cyanobacteria, have shown ability for cadmium removal and *Microcystis aeruginosa* demonstrated a very high nickel sorption capability [15]. Besides these reports and their strong potentiality in biosorption, their has been very little scientific investigation of the use of marine cyanobacteria in the bioremediation process.

The possibility of removing these pollutants from seawaters through biosorption using marine cyanobacteria is an emerging filed of study. Moreover, these organisms have already shown their ability to grow in laboratory scale conditions using natural mimetic culture media, as described elsewhere.

The main aim of this work was to study, for the first time, the possibility of using a marine cyanobacterium, from the *Synechocystis* sp. strain, collected near the

Portuguese southern border, for the removal of selected trace metals added to natural seawater. In order to predict its application on a large scale, preliminary results of the removal rates of Zn, Pb and Ni, and the optimisation of some parameters for the metal removal, by the speciation of the culture medium were obtained.

2. Experimental

2.1 *Decontamination of materials and culture media*

All plastic material including polycarbonate bottles, filtration system, falcons, culture flasks and filters were acid cleaned. After decontamination, culture media containers were microwave-sterilised through a time-power sequential programme (250 W 1 min, 500 W 2 min and 700 W 2 min). Culture media were sterilised by filtration (0.1 μm pore-size filter). All sample manipulations were carried out using gloves in a laminar flow hood.

2.2 *Reagents*

Natural seawater was collected at 5 Km from Matosinhos coast (north western Portugal) in April 2007 and placed directly in decontaminated 20 l HDPE containers. At the laboratory, seawater was immediately filtrated, first through 0.45 μm pore-size filter and then through 0.1 μm polycarbonate membrane. Sterilised seawater was sub-sampled and stored in the dark. The determined concentrations of the selected trace metals were: 29.5 nM for Zn, 10.5 nM for Pb and 4.2 nM for Ni.

The metal standard solutions used in voltammetric determinations were prepared by dilution of the atomic absorption spectrometry standard solutions (BDH, Spectrosol grade). Dimethylglyoxime (DMG, Sigma) 0.1 M was prepared in NaOH. Boric acid (Aldrich) 1 M and ammonia 0.35 M (borate buffer) were also used; 100 μl of this buffer in 10 mL seawater gave a pH of 8.3. Ligand, buffer and all metal solutions were stored at 4°C. The water used for reagent preparation and rising was deionised (conductivity $< 0.1\,\mu S\,cm^{-1}$).

2.3 *Cyanobacteria attainment*

2.3.1 *Cyanobacteria isolation and cultures in Z8 medium*

The marine cyanobacterium used in this work was from the *Synechocystis* sp. strain, named LEANCYA 21, and was collected at the Luz beach (southern region of Portugal), being supplied by the Centre of Marine and Environmental Research (CIIMAR). After being collected, LEANCYA 21 isolation and culture were performed in Z8 medium supplemented with NaCl at a concentration of $20\,g\,L^{-1}$. The Z8 medium consisting of: (per litre) 31 mg K_2HPO_4, 21 mg Na_2CO_3, 16 mg $FeCl_3$, 3 mg EDTA, 3.75 g $MgSO_4 \cdot 7H_2O$, 8.75 g NaCl, 0.003 mg $Na_2WO_4 \cdot 2H_2O$, 0.009 mg $(NH_4)_6Mo_7O_{24} \cdot 2H_2O$, 0.012 mg KBr, 0.008 mg KI, 0.029 mg $ZnSO_4 \cdot 7H_2O$, 0.016 mg $Cd(NO_3) \cdot 4H_2O$, 0.015 mg $Co(NO_3)_2 \cdot 6H_2O$, 0.012 mg $CuSO_4 \cdot 5H_2O$, 0.020 mg $NiSO_4(NH_4)_2SO_4 \cdot 6H_2O$, 0.004 mg $Cr(NO_3)_3 \cdot 9H_2O$, 0.0009 mg V_2O_5, 0.047 mg $Al_2(SO_4)_3K_2SO_4 \cdot 24H_2O$, 0.31 mg H_3BO_3 and 0.22 mg $MnSO_4 \cdot 4H_2O$. Strain was grown for 4 weeks in 41 medium, at 25°C, at a light

intensity of 10 mmol photons $m^{-1}s^{-1}$ provided by cool white fluorescent tubes and with a light/dark cycle of 14 h/10 h.

2.3.2 *Cyanobacteria biomass*

The cyanobacteria biomass used in this work was supplied by the Centre of Marine and Environmental Research (CIIMAR). Biomass was obtained by harvesting the cyanobacteria cells after the growth period by centrifugation and then frozen at −20°C and freeze-dried. Lyophilised material was stored at −20°C and then used in the sorption experiments. The procedure is described in detail elsewhere [19].

2.4 *Controls and elutriates*

2.4.1 *Effect of contact time*

In order to evaluate the metal removal kinetics, known concentrations −50 nM – of each one of the studied metals (zinc, lead and nickel) were added (from the corresponding nitrate salt) to natural seawater (50 mL) containing 1.25 mg of cyanobacteria biomass. Suspensions were manually stirred (each 15 min) and samples analysed up to 24 h.

Control solutions containing only seawater with each one of the metals, at the same concentrations described above and without cyanobacteria biomass were also carried out in parallel for comparison. The determined pH of the solutions was 8.05 (Lab pH meter, GLP 22 Crison).

All experiments were carried out in triplicate.

2.4.2 *Effect of biomass concentration*

Metal ions (Zn, Pb and Ni) were added individually (from the nitrate salts) at a known concentration (50 nM) to 50 mL of natural seawater. The pH of the solutions was 8.05 (Lab pH meter, GLP 22 Crison).

In order to evaluate the effect of the amount of cyanobacteria biomass, experiments were performed using three different concentrations of biomass: 1.25, 2.50 and 12.50 mg per 50 mL of solution. Each one of the suspensions containing the metal ions were kept in contact with the biomass for 6 h, being manual stirred every 15 min. Control solutions containing just metal ions (without cyanobacteria biomass) were also tested for comparison. Experiments were carried out in triplicate.

2.4.3 *Synergistic and/or antagonist effects on the biosorption*

The interaction of the studied metals with each other and its possible effect on the observed remotion was also evaluated. Thus, multiple metal ion solutions were prepared by the addition of 50 nM of each of the metals – Zn, Pb and Ni, from the nitrate salt – to 50 mL of natural seawater. LEANCYA 21 cyanobacterium biomass was added to the solutions at two different concentrations: 2.50 and 12.50 mg per 50 mL. Experiments were performed as described in 2.4.2. Control solutions containing all the studied metals (without cyanobacterium biomass) were also analysed in parallel for comparison. The determined pH of the solutions was 8.05 (Lab pH meter, GLP 22 Crison). Experiments were carried out in triplicate.

2.4.4 *Metal release/uptake from/to cyanobacterium*

The amount of trace metals released to seawater by LEANCYA 21 cyanobacterium biomass was determined for suspensions containing 12.50 mg of biomass in 50 mL of seawater. Suspensions were manually stirred (each 15 min) and samples analysed up to 24 h. Experiments (including controls without biomass) were performed in triplicate.

2.5 *Determination of trace metal contents*

Prior to analysis, suspensions and solutions were filtered (0.45 μm pore-size filter) in a vacuum filtration system at 0.1 bar. The filtrates collected were acidified (adding 1% of HCl concentrated) and then microwave-digested (250 W) for 8 min. All solutions obtained were stored at 4°C.

Metal concentrations were determined by anodic stripping voltammetry (ASV) for Zn, Cd and Pb. Ni was determined by cathodic stripping voltammetry (CSV) after sample pH neutralisation with ammonia (6 M). The voltammetric equipment consisted of an Autolab voltammeter (Ecochemie) connected to a Metrohm 663-VA electrode stand provided with a hanging mercury drop electrode (HMDE). Operational details are described in the literature [20].

3. Results and discussion

3.1 *Effect of contact time*

The use of marine cyanobacteria for trace metal removal from contaminated seawaters receives scant coverage in the literature. No research in the biosorption field was found for the chosen strain in this work, *Synechocystis sp.*

In order to evaluate the possibility of using this cyanobacterium for seawater treatment (namely in fishfarming ponds), we decided to commence our studies by determining the metal removal kinetics of selected trace metals – Zn, Pb and Ni – present in known concentrations from seawater solutions as a function of contact time.

It was observed, for all the studied metals, that LEANCYA 21 cyanobacterium was able to remove high amounts of the cations from solution in a relatively short time (Figure 1).

In all cases a similar shape trace is observed, characterised by a strong increase in metals sorption initially (up to 30 min), followed by a slow increase until equilibrium is reached. The necessary time for achieving the equilibrium was 2 hours for Ni and Zn. For Pb, the steady state was achieved after 1h, showing a slight increase until a second plateau was reached, after 6 h. These two sorption stages observed are in agreement with the reported data in literature [15,21], where the first stage, due to the metal biosorption, is very fast and then is followed by a slower second phase where the system achieves equilibrium.

The amounts of metals sorbed at equilibrium are: 0.83 mg g^{-1} of dry biomass for Zn; 0.70 mg g^{-1} of dry biomass for Ni and 2.38 of dry biomass for Pb, achieving a maximum sorption of 3.27 mg g^{-1} of Pb after 24h.

LEANCYA 21 cyanobacterium have been shown to be capable of removing the selected trace metals from marine medium even for small amounts of biomass. In fact, the cyanobacteria concentration used in these experiments was 10 times lower than the ones described for other biosorbents [8,21].

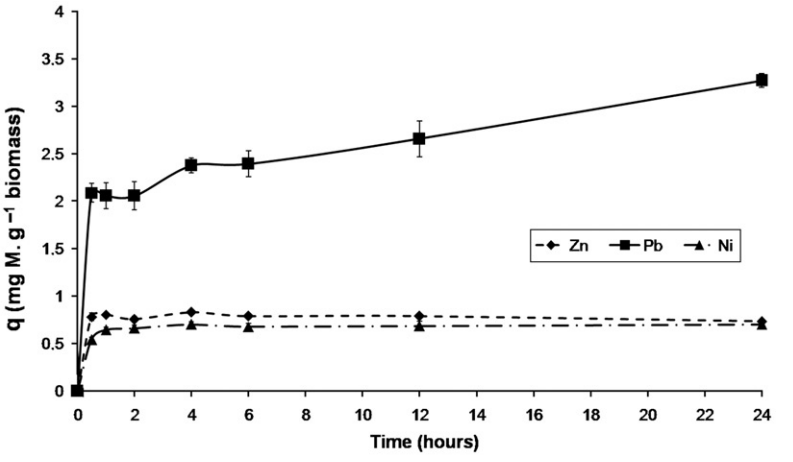

Figure 1. Specific biosorption (q) of Zn, Pb and Ni in seawater solutions as a function of time. LEANCYA 21 cyanobacterium biomass used was 1.25 mg per 50 ml of solution. Initial concentrations of metals were 69.4 nM for Zn, 42.6 nM for Pb and 45.0 nM for Ni. Standard deviations ($n = 3$) are also given.

3.2 *Effect of the biomass concentration on the biosorption*

As it was observed, the marine cyanobacterium LEANCYA 21 was able to remove Zn, Pb and Ni from natural seawater solutions. Nevertheless, many other parameters must be optimised prior to its application in industrial plants.

In order to determine the optimal removal/costs relationship we evaluated the effect of the LEANCYA 21 biomass concentration on the removal of Zn, Pb and Ni from natural seawater solutions. The contact time used was 6 h (selected from the results obtained in Figure 1), which guarantees that the steady state was already achieved for all metals.

The results were expressed in terms of the specific metal removal, a useful parameter which allows the comparison of different kind of sorbents. Nevertheless, the total amount of metal removed was also calculated. In fact, this parameter has practical implications – an increase in the amount of biomass used in order to enhance the amount of metal removed also means an increase in the costs for producing the biomass.

Figure 2 shows the specific absorption determined for each metal when different cyanobacteria biomasses were used. From the results, it can be observed that the profile shown for the metals uptake was very similar.

It can be observed, in all cases, that the maximum specific absorption is obtained for the smaller amount of cyanobacteria biomass. The amounts of metals sorbed for 1.25 mg of biomass are: 0.80 mg g^{-1} for Zn; 0.58 mg g^{-1} for Ni and 3.06 mg g^{-1} for Pb. An increase in the amount of biomass used led to a reduction in the metal specific sorption, in all cases.

On the other hand, if the amount of Pb removed from solution is calculated as the percentage removed as a function of the initial value, an increase in the Pb removal proportional to the biomass used, which achieved up to 90% for 12.50 mg in 50 mL of solution, is observed. These results are very similar to the ones described previously for the cyanobacterium *Spirulina sp* [8]. The authors have observed that the use of 12.50 mg of *Spirulina sp*. biomass was able to remove up to 95% of Pb from a 50 mL sample of an effluent collected in a copper smelter and refinery. This increase in the metal removal for

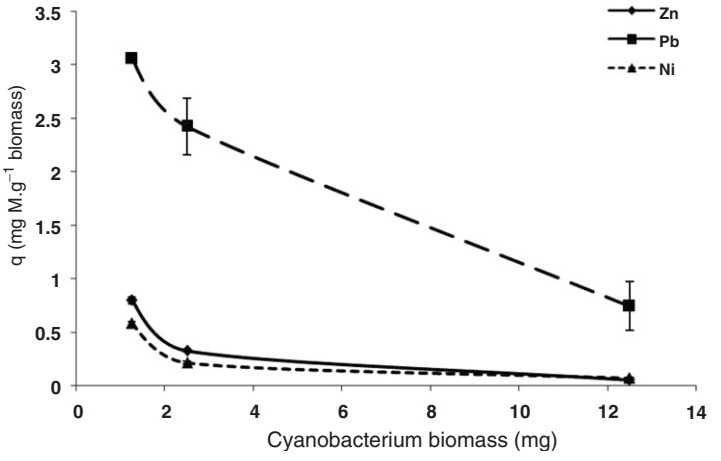

Figure 2. Specific biosorption (q) of Zn, Pb and Ni from seawater solutions as a function of the amount of LEANCYA 21 cyanobacterium biomass. Standard deviations ($n = 3$) are also given.

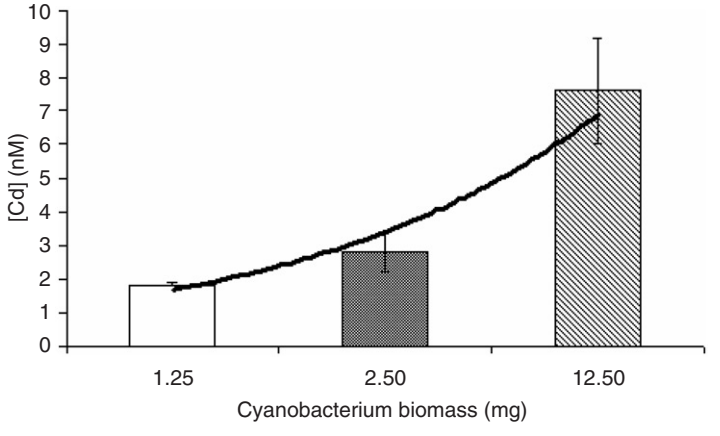

Figure 3. Amount of Cd released by LEANCYA 21 cyanobacterium biomass to Zn single solution of natural seawater (50 nM) as a function of the amount of biomass used. Standard deviations ($n = 3$) are also given.

higher biomass concentrations could be basically due to a greater availability of the metal specific binding sites [15]. However, in order to validate this statement, further experiments with different initial concentrations of the metals are necessary. Therefore, at higher concentrations, an increase of the algal biomass could be seen as a strategy to increase the amount of the metal removed [20].

Additionally, during the experiments it was observed that cyanobacterium LEANCYA 21 was able not only to uptake but also to release other metals, namely Cadmium to the medium.

Figure 3 shows the representative results regarding to the Cd concentration determined in Zn single solution (50 nM). Results obtained for the other systems are not presented

Table 1. Uptake values and respective specific absorption (q) of Zn, Pb and Ni from seawater in single and multiple metal ion solutions when different amounts of LEANCYA 21 cyanobacterium biomass were used. Standard deviations ($n = 3$) are also given.

		Single metal solution		Multiple metal solution	
	Biomass (*g*)	Amount uptaken from solution ($x10^{-3}$ mg)	*q* (mg M × g^{-1})	Amount uptaken from solution ($x10^{-3}$ mg)	*q* (mg M × g^{-1})
Zinc	0.00250	0.82	0.328	0.52	0.208
	0.01250	0.70	0.056	0.86	0.069
Lead	0.00250	6.06	2.424	4.80	1.92
	0.01250	9.31	0.745	6.45	0.516
Nickel	0.00250	0.54	0.216	0.96	0.384
	0.01250	0.88	0.070	1.1	0.088

because the observed behaviour was always the same, independently of the metal solution tested (individually or in a mixture). It can be seen that the cadmium concentration showed an exponential raise proportional to the amount of biomass used, indicating its dependency.

3.3 *Synergistic and/or antagonist effects on the biosorption*

It is well known that the simultaneous presence of several metals may lead to effects on biochemical processes such as the metal biosorption [15]. We have studied the removal of the selected metals when in multiple metal ions solutions in order to ascertain whether there was any kind of effect on the biosorption, namely due to competition for the binding sites.

Table 1 presents the results obtained for the different systems, containing known concentrations of the metals, individually or in a mixture. The cyanobacterium biomass amounts were chosen from the results in Figure 2.

Results show different effects associated with the presence of the selected trace metals in seawater, which affected the biosorption rate.

The comparison between the Pb concentrations in single and multiple metal solutions shows a lower uptake by LEANCYA 21 in the latter case, up to 28% for 12.5 mg, suggesting that the presence of others metals in solution had an antagonist effect on Pb biosorption. This behaviour was observed independently of the amount of biomass used. The same behaviour is observed for Zn.

On the other hand, it was observed that the Ni removal uptake increased when multiple metal solutions were tested. The amount of Ni removed was around 15% higher (for 2.50 mg) than for single metal solutions, indicating a tendency for a synergistic effect in this case.

3.4 *Metal release/uptake from/to cyanobacterium*

The LEANCYA 21 biomass capacity of removing selected metals from seawater at relatively toxic concentrations has been shown. However, as this cyanobacterium was

Table 2. Concentration of Zn, Cd, Pb and Ni in natural seawater as a function of time. LEANCYA 21 cyanobacterium biomass used was 12.50 mg per 50 ml of solution. Standard deviations ($n = 3$) are also given.

	Metal concentration in seawater (nM)			
Exposure time (h)	Zinc	Cadmium	Lead	Nickel
Natural seawater	29.5 ± 1.8	–[a]	10.5 ± 1.7	4.2 ± 1.0
6	15.4 ± 0.4	6.6 ± 0.6	10.0 ± 0.2	7.4 ± 0.4
12	17.7 ± 0.4	7.8 ± 0.8	10.9 ± 0.1	6.6 ± 0.1
24	17.7 ± 0.2	7.0 ± 0.2	9.9 ± 0.1	7.4 ± 0.6

Note: [a]Below the detection limit.

grown in a metal enriched medium (Z8), some of these metals might have been carried by the wall cells when the biomass was obtained. In order to validate the results obtained, the amount of metals released/uptake from/to the cyanobacterium biomass was determined.

The concentrations of the selected metals in natural seawater were determined as a function of the time and results are shown in Table 2.

Distinctive behaviours were observed, depending on the metals. There was an uptake of Zn from the seawater, leading to a reduction of its concentration up to 50% of the initial concentration. This variation occurred mainly in the first 6 hours of contact, being unaltered for the remaining 18 hours. We decided to compare these results with the ones shown in Figure 2 for Zn removal, through the number of mols of Zn which were uptaken in both cases. Removal of Zn from natural seawater, in Table 2, was 0.70×10^{-9} mol, which is very similar to the value obtained in Figure 2 (with solutions containing additional 50 nM of Zn) which is 0.66×10^{-9} mol. These results corroborate the previous observation that the removal of Zn is independent of the amount of cyanobacterial biomass used.

For Ni, the concentrations determined in seawater have shown an increase up to 41% of its initial concentration. The same behaviour was observed for Cd, where the results in natural seawater are similar to the ones obtained for solutions containing added amounts of metals (Figure 3). The observed desorption in these cases could be due to the presence of the metals on the cyanobacteria cells surface, once the biomass was obtained from a grown culture medium rich in metallic species (*Z8 medium*).

No alterations were observed regarding Pb concentration in seawater. This result indicates that the capacity of LEANCYA 21 for uptaking Pb may be a result of detoxifying mechanisms which are activated only at higher concentrations.

4. Conclusions

The marine cyanobacterium LEANCYA 21 was able to remove significant amounts of Zn, Ni and Pb from seawater solutions. This remotion was effective for really low metal levels present in seawater, in order to simulate natural conditions found in fishfarming. The determined uptake for Pb was up to 90%, while for Zn and Ni the depletion was 30% from their initial concentration. It was also observed that Zn and Ni removal were not affected by the biomass concentration.

Ni uptake was enhanced in the presence of other metals in solution, indicating some tendency for synergistic effects on its removal, while multimetal systems revealed an antagonistic effect on Pb and Zn removal, probably due to a competition mechanism to the binding sites onto the cyanobacterium cell wall.

Results obtained so far point out that the use of the *Synechocystis sp*. cyanobacterium biomass for trace metal removal of contaminated seawater might be very promising, especially in aquaculture. Nonetheless, further investigation is needed concerning not only the kind of interaction between the biomass and the metals but also concerning the optimal parameters for the sorption of the metal ions which may include: comparison with other cyanobacteria strains, initial concentration of metal, pH, temperature, interfering cations and anions, among others.

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