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NEW BIOREMEDIATION TECHNIQUES OF HEAVY METALS FROM AQUEOUS SOLUTIONS USING BIOSORPTION AND PHYTOEXTRACTION METHODS

PhD THESIS ABSTRACT

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Keywords: heavy metals, bioremediation, biosorption, fir tree sawdust (*Abies alba*), macrofungus *Agaricus bisporus* and *Lactarius piperatus*, *Saccharomyces cerevisiae*, linear and non-linear regression analysis, kinetics, thermodynamics, phytoremediation, phytoextraction, *Vallisneria spiralis*, stress proteins.

INTRODUCTION

Amplification of industrial activity as well as a notable demographic outburst has resulted in serious environment pollution, with dramatic consequences on atmosphere, soil and water.

Currently, pollutant dispersion and production rate have exceeded the natural biodegradation process. In the quest of technological solutions for environment pollution, physical and chemical processes are usually seen as essential. However, biological processes can provide valuable insight as well.

Heavy metals are regarded as impactful, non-degradable toxic pollutants of natural water sources. Not only they persist within the environment but they also pose serious risks as far as population health is concerned since they can be accumulated by living organisms (Mehmet et al., 2007).

The heavy-metal pollution issue, together with a strong urge of heavy metal retrieval from residual water has led to the development of various conventional technologies (chemical precipitation, solvent extraction, reverse osmosis and use of ionic changes). An alternative to all those mentioned before would be *bioremediation*, a process whereby biological organisms are used in solving environmental issues, such as water and soil purification (Vidali, 2001).

The present doctoral thesis is focused on setting for the results obtained in the studies of bioremediation of a series of heavy metals from watery solutions through biosorption and phytoextraction techniques.

Biosorption can be defined as the ability of a series of biomaterials to accumulate heavy metals from aqueous solutions through a mechanism metabolically controlled or via physiochemical processes. Biosorption methods feature some advantages when compared with the conventional ones. Some of them would be: lower costs, increased efficiency and the possibility to recuperate both biosorbent and heavy metals etc. (Ahalya et al., 2003).

Phytoremediation consists of the direct use of green plants and microorganisms associated with the bioremediation processes *in situ*. It consists on the uptake, degradation, stabilization of various pollutants from the contaminated soils, surface or subterranean waters.

Phytoextraction can be regarded as a sub process of phytoremediation, in which the dangerous and toxic elements or compounds from soil or water can be removed with the help of plants. Phytoextraction processes enable heavy-metal extraction from soil and water through root absorption and transportation in the superior parts of the plant, which can be harvested later on (Adesodun et al., 2010; Sakakibara et al., 2011).

Phytoextraction efficiency in the bioremediation process relies on the plants ability to extract and transport the metal itself. In order to achieve some notable results in heavy-metal absorption, transportation and accumulation, a detailed study of the process, influencing factors and knowledge of the biochemical process is required. Thus, new perspectives arise through the development of some processes based on transgenic plants that are capable to perform pollutant hyper-accumulation.

The biosorption studies have been made with the purpose of obtaining some new biosorbent types, which feature an increased absorption capacity and can contribute to the facilitation of the absorption mechanisms.

In this study, the phytoremediation research of heavy metals from watery solutions is concentrated on phytoextraction studies based on aquatic plants. The phytoextraction process has been correlated with the plants biochemical responses to the stress effect produced by the heavy metals toxicity.

The present work contains two main parts, which include first, a biosorption (A) and secondly a phytoextraction (B) part of heavy metals. Phytoextraction was realized taking into consideration biochemical responses of the studied plant.

1. Biosorption

1.1. Biomasses used for biosorption studies

For the biosorption studies we focused on easy available, green and eco-friendly with low operational cost materials, namely: fir tree sawdust (*Abies alba*), two types of macrofungus, *Lactarius piperatus* and *Agaricus bisporus*, and two types of yeast contain the same strain *Saccharomyces cerevisiae* (residual waste brewery and DSM 1333 pure strain yeast), used in batch system in different form (chemically treated and immobilized (based biocomposites).

a. Fir tree sawdust (*Abies alba*) biomass

Sawdust is a waste by-product of the timber industry, easy available in large quantities and inexpensive from the economical point of view. In Romania, the wood processing is largely extended, especially fir wood, whereof large quantities of sawdust are annually produced. *Abies alba* fir is a common tree from some forest areas in Transylvania, Romania.

The fir tree sawdust was obtained from a local sawmill in Huedin, Cluj County, Romania. Prior to its utilization the considered biomass was washed several times with distilled water in order to eliminate surface impurities, and finally dried at 105°C for 24 h. The dried biomass was grinded and sieved (400-600 µm). The sieved sawdust was then stored in an airtight box before its utilization. Humidity, apparent density and elemental analysis of the considered biosorbent were determined according to the general procedures.

Figure 1. Raw (native) fir tree sawdust *Abies alba*

In order to enhance the biosorption capacity, fir tree sawdust biomass was subject to NaOH and H_2O_2 treatments, thereafter the obtained biomass were used for the removal of Cd (II) ions from aqueous solutions.

b. Macrofungi: *Agaricus bisporus* and *Lactarius piperatus* biomass

Commercial fruit bodies of *A. bisporus* were purchased from a local commercial company from Nuşfalău, Sălaj County, Romania. A special substrate for cultivation is produced from nitrogen-amended cereal straw, which is subjected to an aerobic solid-state fermentation, or composting, that replaces easily available carbon and nitrogen compounds with humic-rich complexes. This highly selective medium is then pasteurized and inoculated with *A. bisporus* mycelium.

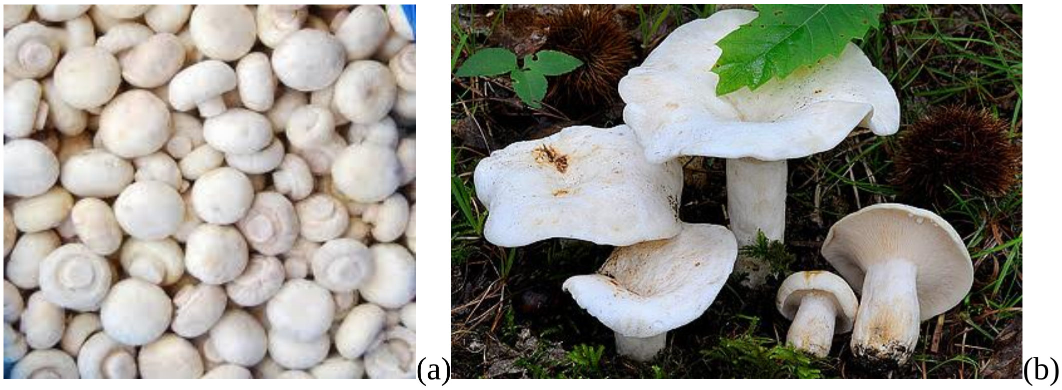


Figure 2. *A. bisporus* macrofungus (a) harvested form, (b) prepared for experiments.

L. piperatus biomass was collected from a local woodland area near Cluj-Napoca, Cluj County, Romania.

Both macrofungus were used as adsorbent in suspended form for the biosorption of Cd (II) and Zn (II) ions from aqueous solutions, in batch conditions.

The cross-linking procedure with Na-alginate described in literature (Zhao and Duncan, 1997) was used for biomass immobilization in order to obtain the biocomposite.

c. *Saccharomyces cerevisiae* yeast biomass

S. cerevisiae is an important biosorbent widely investigated for its abilities in the removal of heavy metal ions form aqueous solutions. Two types of biosorbents that contain the same strain, namely *S. cerevisiae* were used for experiments:

Residual brewery waste biomass (RBW) was collected from CIUC brewery (Miercurea-Ciuc, Romania) after being used in fermentation processes and transported to the laboratory in airtight special containers. RBW samples were then washed with bi-distilled water and separated by vacuum filtration, dried in a hot air oven at 80°C for 24 hours.

DSM 1333 pure strain yeast (DSM) was provided from University of Pécs, Hungary, Medical School, Department of Medical Microbiology and Immunology, in the lyophilized form. The composition of growth medium was Müller-Hinton substrate (3% glucose, peptone, yeast-extra, NaCl, pH=7).


(a)


(b)

Figure 3. *Saccharomyces c.* yeast (a) residual brewery and (b) DSM 1333 pure strain (lyophilized form).

The aim of study was to investigate the biosorption potential on cadmium removal of these two types of yeast biomasses, unmodified and microwaves and ultrasound modified forms.

1.2. Preparation of heavy metals solutions

In this thesis the removal of two toxic metals, Cd (II) and Zn (II) ions was considered. The stock solution, 1 g/L of cadmium (II) and zinc (II) was prepared by dissolving $Cd(NO_3)_2 \cdot 4H_2O$ and $ZnSO4 \cdot 7H_2O$ salt in distilled water. The required concentrations were obtained by diluting the stock solution to the desired concentrations, in 45-365 mg/L range. HCl (0.1 M) and NaOH (0.1 M) volumetric solutions were used to adjust the solution pH. All chemicals used were of analytical grade.

1.3. Biosorption experiments

The biosorption of Cd (II) and Zn (II) ions onto previously mentioned biomasses in different forms (suspended and immobilized) was investigated in batch system. Biomasses were contacted with 100 mL aqueous solution of Cd (II) and Zn (II) ions for 240 minutes (until equilibrium was reached), in all cases. In order to establish the evolution of the removal process, samples of 100 µL were collected at different time intervals. Samples collected at predetermined time intervals were filtered (microfilter ME cellulose 0.45 µm) and the remaining concentration in aqueous phase was determined using an Atomic Absorption Spectrometer (SensAA Dual GBS Scientific Equipment, Australia).

In order to evaluate the amount of cadmium and zinc ions retained per unit mass of biomasses, the adsorption capacity was calculated using the following equation:

$$q_e = \frac{(C_0 - C_e) \cdot V}{m} \tag{1}$$

where, q_e is the amount adsorbed at equilibrium (mg/g), C_0 is the initial metal ions concentration (mg/L), C_e is the equilibrium metal ions concentration (mg/L), V is the volume of the aqueous phase (L), and m is the biosorbent mass.

Removal efficiency, E (%), was calculated as a ratio between Cd (II) or Zn (II) biosorbed at time t (mg/L) and the initial metal ions concentration (mg/L):

$$E, (\%) = \frac{C_0 - C_t}{C_0} \times 100 \tag{2}$$

Experimental data were used to determine the equilibrium time, equilibrium concentrations, amounts adsorbed at equilibrium, effect of stirring rate, biomass quantity, optimum initial pH, and temperature influence over the biosoprtion process. Also the experimental data were used to establish isotherm (linear and nonlinear regression), kinetics models and to calculate thermodynamic parameters. All the experiments were repeated three times, the values presented were calculated using averaged concentration values.

1.4. Results and discussion

All experiments were conducted in batch conditions. Batch experiments usually focus on the study of parameter influencing biosorption, which are important in the evaluation of the full biosorption potential of any biomaterial. The studied parameters are including:

- biomass quantity
- effect of the initial metal ion concentration
- contact time
- initial pH of the solution
- temperature
- stirring rate
- effect of ultrasound and microwave irradiation

Biomass quantity

In order to determine the optimal biomass quantity for sawdust and macrofungi experiments, various amount of biomass between 1-5 g were contacted with Cd (II) and Zn (II) 50 mg/L solutions. In other case, on the immobilized (based biocomposites) experiments, an amount of biomass in 0.5-2.5 g interval was used.

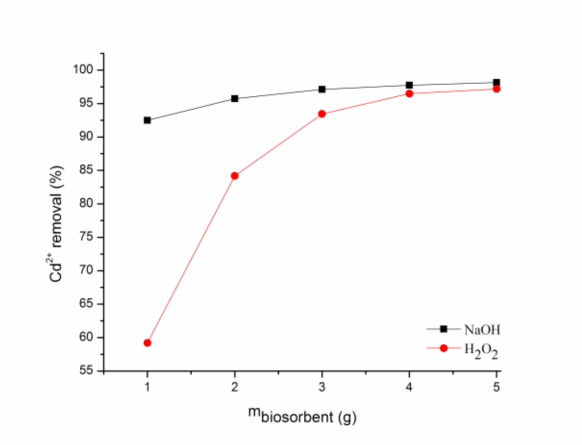


Figure 4. The effect of the treated fir tree biomass quantity on Cd (II) biosorption; C_i = 50 mg/L, d = 0.4-0.6 mm, 296 K, pH 5.5, 700 rpm.

As figures shows, as the biosorbent quantity increase, the removal efficiency will also increase due to the number of sites available and exchangeable ions available for biosorption (Nagy et al., 2013b).

This tendency is observed for both types of biomass and for both form (treated/immobilized), but the obtained adsorption capacities and removal efficiencies were higher in case of NaOH treated fir tree sawdust. Following these studies, we establish the optimum biomass quantity for each type of biomasses for the further experiments.

Effect of initial metal ions concentration

The influence of initial metal ion concentration in the solutions on the adsorption capacity and removal efficiency, for all studied biomasses was studied. The experiments were carried out using five different initial metal ion concentrations (Cd (II) and Zn (II)) in range of 50-365 mg/L at fixed adsorbent quantity (5 g for natural and 2 g for immobilized experiments, respectively), 700 rpm stirring rate and room temperature. Romanian law normative shows the the maximum allowed values in wastewaters are 0.2 and 0.5 mg/L for Cd (II) and Zn (II) ions, respectively.

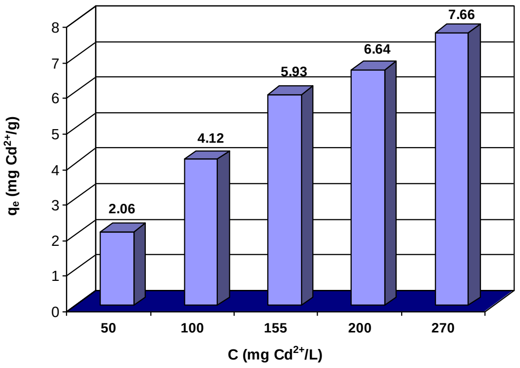


Figure 5. Influence of the initial Cd (II) concentration over the adsorption capacity on the *Lactarius piperatus* macrofungus; C_i = 50-270 mg/L, 0.6 <d < 1.2 mm, 296 K, 5.72 pH, 700 rpm.

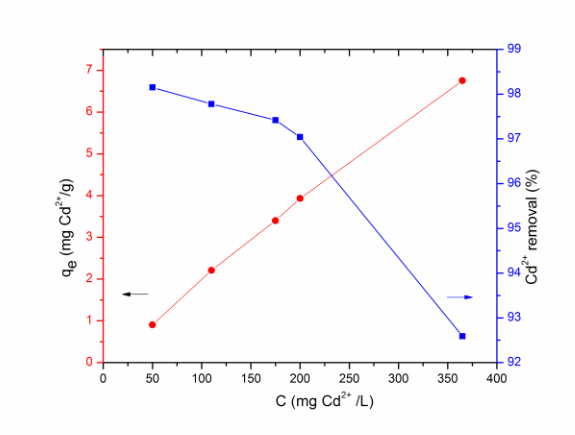


Figure 6. Influence of the initial Cd (II) concentration over the adsorption capacity and removal efficiency on NaOH treated fir tree sawdust (*Abiesalba*) biomass; C_i = 50-365 mg/L, 5g biomass,d = 0.4-0.6 mm, 296 K, pH 5.5, 700 rpm.

All experiments showed that the adsorption capacities increase with the increase of concentration up to a limit, however, a decrease in removal efficiency was observed

This fact indicates that if the metal ions concentration in solution increases, the difference in concentration between bulk solution and surface also increases, intensifying the mass transfer processes. Accordingly a higher quantity will be adsorbed proving that theused biomasses have a high adsorption capacity for metal ions. Further increase of the initial metal ion concentration does not lead to an increase of the adsorption capacity.A further increase in initial metal ions concentration did not lead to a significant improvement in biosorption capacity due to the saturation of the active sites presents on biosorbent surface.

Contact time

In order to establish the equilibrium time for Cd (II) and Zn (II) biosorption onto studied biomasses, contact time influence over the process evolution was investigated until equilibrium was reached.

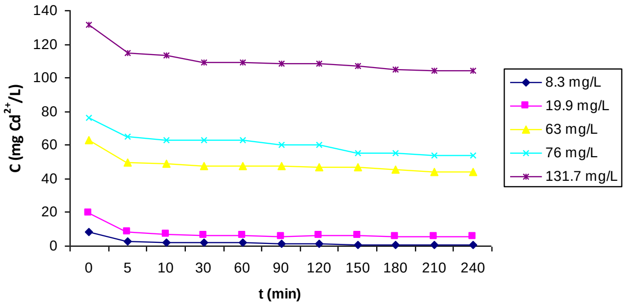


Figure 7. Effect of contact time and initial concentration on the biosorption of Cd (II)on RBW biomass; 23±2^{o}C, pH=6.5, 150 rpm.

It was observed that the adsorption capacities and removal efficiencies increase rapidly (in the first 80-100 min) due to the abundant availability of active binding sites on the adsorbent, and with gradual occupancy of these sites (max 240 min), the adsorption became less efficient in the later stages.

Initial pH of the solution

The initial pH experiments were conducted with the initial metal ion concentration of 50 mg/L Cd (II) and Zn (II), on case, at room temperature of 23°C by varying the initial pH of Cd (II)

Zn (II)solution from 2 to 9.5 for a constant biomass quantity of 5 and 2 g/100 mL solution (immobilized experiments).

Results for untreated fir tree sawdust (*Abies alba*) and *Lactarius piperatus* macrofungus are presented in Fig. 8,9.

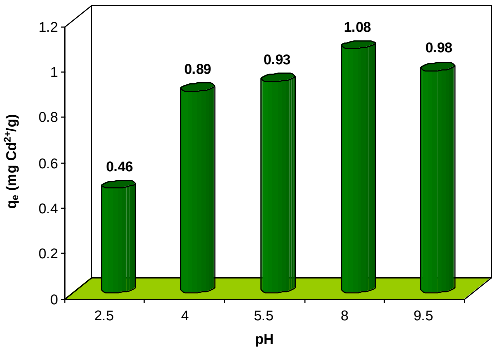
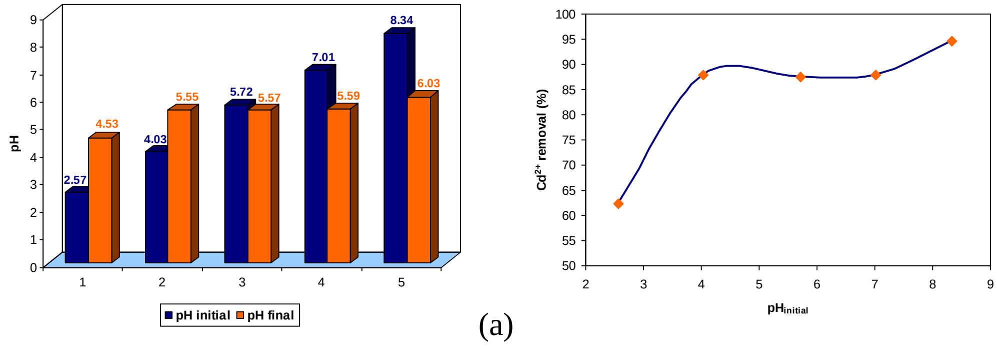


Figure 8. The effect of initial pH values on Cd (II) biosorption using fir tree sawdust (*Abies Alba*); C_i = 60 mg Cd^{2+}/L, 5g biosorbent, 296 K, 5.5 pH.



(a) (b)

Figure 9. (a) Initial and final pH values and (b) the effect of initial pH values on removal efficiency values for Cd (II) biosorption using *Lactarius piperatus* macrofungus; C_i = 50 mg/L, 0.6 <d < 1.2 mm, 296 K, 700 rpm.

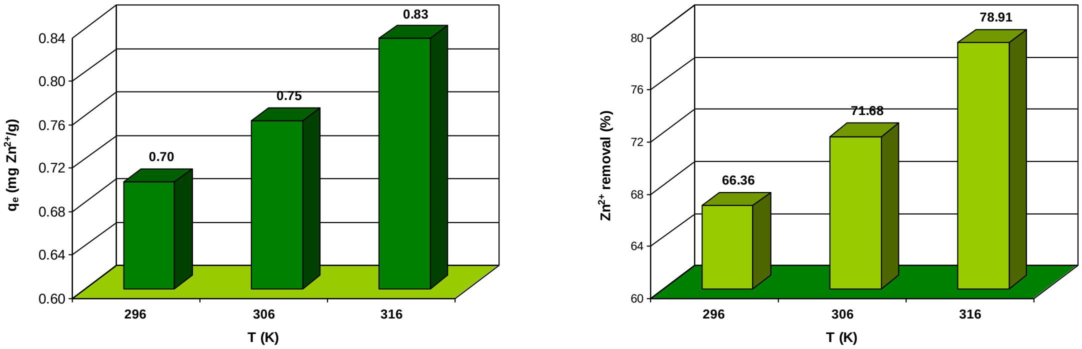
Interesting changes on pH were observed. Modifying the initial pH values of the metal solutions, it was observed that the biosorbent reset the solution pH from the initial value to 6.3 in all cases (after around 80 min) and this value is maintained to the end of experiments.

As the initial pH increases, the adsorption surface becomes less positive and therefore electrostatic attraction between the metal ions and sawdust surface is likely to be increased. The optimum pH that provides maximum removal of cadmium was an initial 8 pH. Similar results were reported using an indian deciduous wood at pH 6.7 process (Rahman and Islam, 2009). At higher pH values (9.5), Cd (II) ions begin to precipitate, therefore the biosorption studies are significantly influenced.

Temperature

All experiments were made with an initial metal concentration of 50 mg/L Cd (II) and Zn (II) at initial pH of the solution (5.8 and 5.6 pH), of 5 g/100 mL solution by varying the temperatures in 23-45°C range.

The effects of temperature on biosorption of Cd (II) and Zn (II) ions onto *L. piperatus* were conducted 23°C, 33°C, 43°C. As can be observed,an increase in temperature led to an increase of the adsorption capacity (efficiency), suggesting that the biosorption process is endothermic (Fig. 10).



(a) (b)

Figure 10. Temperature influence over the (a) adsorption capacity and (b) removal efficiency of Zn (II) on *Lactarius piperatus* macrofungus; C_i = 50 mg/L, 0.6 <d < 1.2 mm, 5.49 pH, 700 rpm.

Higher temperatures were not considered (for all biomasses studied) due to the fact that preliminary studies showed that after 50ºC desorption process begin.It is always desirable to conduct/evaluate biosorption at room temperature, as this condition is easy to replicate.

Stirring rate

The effect of stirring rate is an important parameter, which affects the adsorption capacity and removal efficiency; therefore, an optimal speed should be used in wastewater treatment. Stirring rates ranging between 100 and 700 rpm were tested.

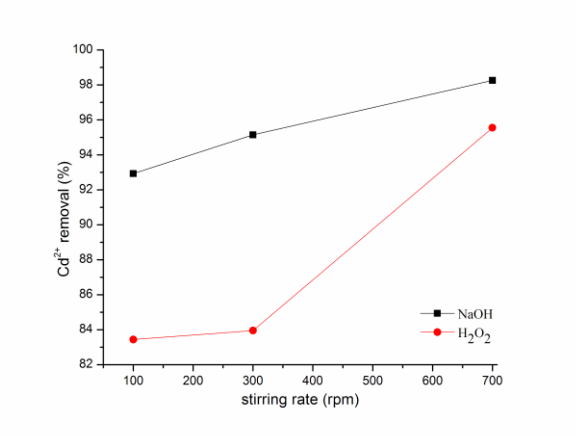
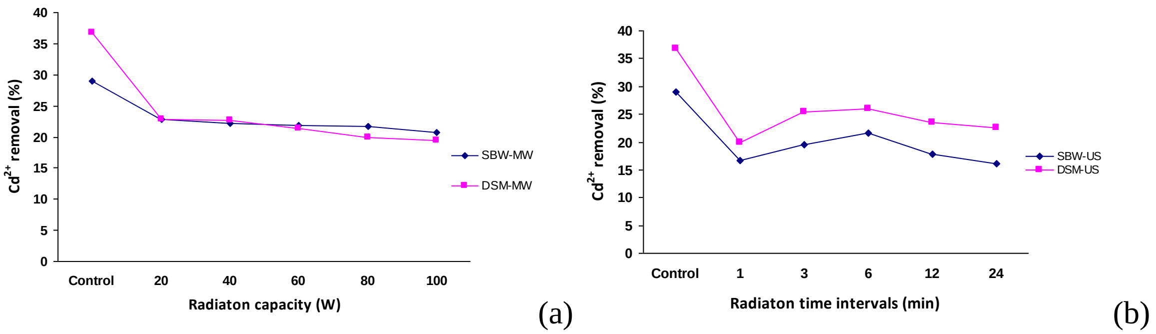


Figure 11. The effect of stirring rate over the removal efficiency for treated fir tree sawdust (*Abies alba*) on Cd (II) biosorption; C_i = 50 mg/L, 5g biomass, d = 0.4-0.6 mm, 296 K, pH 5.5.

Higher stirring rate ensure the availability of all surface binding sites for Cd (II) ions uptake while lower speeds cause inefficient dispersion of the biomass particles in solution that led to particles agglomeration. Therefore, a stirring speed of 700 rpm was selected for further experiments.

Effect of ultrasound and microwave irradiation

In order to follow the biosorption potential, of the studied yeast cells, these were subjected to different types of ultrasound and microwave treatments. The removal capacity of cadmium ions decreases with the increase of ultrasound exposure in comparison with the control sample (absence of ultrasound).



(a) (b)

Figure 12. Microwave and ultrasound irradiation effect over (a) removal efficiency and (b) adsorption capacity of Cd (II) ions biosorption on RBW and DSM biomass; C_i = 8-132 mg/L, 23±2°C, pH=6.5, 150 rpm.

By increasing the time of ultrasound irradiation (12-24 min), a major decrease on cadmium uptake was observed. This behavior can be explained by the cavitation processes that affects directly the damages on yeast cell wall.

By microwave irradiation of RBW and DSM biomass cell suspension, a major degradation of complex structure was observed. These cells modifications were observed in reduction of cadmium ions uptake, shown in Fig. 12.

As a result of the microwave irradiation, the number of viable yeast cells was rapidly reduced or destroyed, decreasing the active sites number on the cell walls. These active sites play an important role in the binding of cadmium ions.

1.5. Biosorption isotherm models

In our study, two parameters isotherm models, Langmuir and Freundlich were used for linear regression analysis to describe (coefficient of determination, R^2) the equilibrium of Cd (II) and Zn (II) adsorbed onto the untreated and chemically treated fir tree sawdust. Isotherms parameters provide good opportunity to compare de adsorption capacities for the used biomasess.

Langmuir model is frequently used for adsorption of heavy metal, dyes etc. (Garg et al., 2007; Yang and Al-Duri, 2005) this is applicable for a monomolecular layer adsorption at specific homogenous sites on the adsorbent surface (Langmuir,1918).

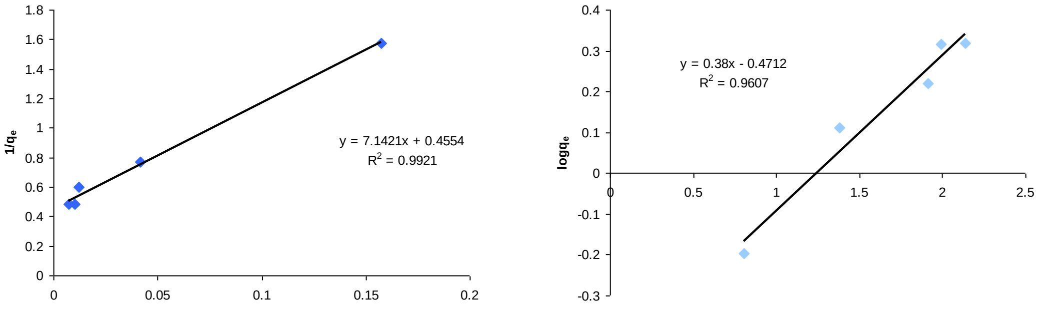
Freundlich isotherm is an empirical model that takes into account the heterogeneity of the surface of the adsorbent (Freundlich, 1906).

Table 1. Langmuir and Freundlich coefficients calculated using linear regression analysisfor Cd (II) biosorption on untreated and chemically treated fir tree sawdust (*Abiesalba*) biomass.

	Langmuir coefficients			**Freundlich coefficients**		
	K_L (L/mg)	**q_{max} (mg/g)**	**R^2**	**n**	**K_f ($mg^{(1-1/n)}L^{1/n}/g$)**	**R^2**
Untreated	3.25	2.20	0.9921	2.63	0.31	0.9607
NaOH	0.096	8.84	0.9999	1.73	1.21	0.9443
H_2O_2	0.35	2.67	0.9971	4.15	1.01	0.9218

The maximum adsorption capacity of Cd (II) ions for untreated fir tree sawdust, $q_m$was calculated to be 2.1958 mg/g. Chemically treated fir tree sawdust biomass show an enhancement in adsorption capacity where, q_m was found to be 8.84 mg/g for NaOH treated and 2.67 mg/g for H_2O_2treated biomass. Langmuir and Freundlich coefficients, calculated using linear regression (for untreated and treated biomass), are presented in Table 1.

The linear plots of the two considered isotherms for Cd (II) biosorption by untreated fir tree sawdust are showed in Fig. 13.



(a) (b)

Figure 13. Langmuir and Freundlich isotherm model for Cd (II) biosorption on fir tree sawdust (*Abies Alba*).

From the linearity of the two plots (Table 1), it can be concluded that as the Langmuir plot is closer to the linearity (higher R^2 values), the metal ions biosorption on untreated and both chemically treated fir tree sawdust, this model better describes the considered process. This may be due to the homogenous distribution of active sites of bothstudied biomass.

Nonlinear (optimization) equilibrium modeling

Using two and three parameters adsorption isotherm models, adsorption capacity values, q_e can be calculated as a function of equilibrium concentrations, C_e, which can be determined experimentally. The goal is the identification of the model parameters which gives the minimal differences between the measured and calculated value of q_e. Traditional way to realize parameter estimation is linearization and linear regression of the isotherm models

Nonlinear regression can be performed in different ways. In this case a least square based mathematical optimization was applied by using a global optimization algorithm CMA-ES (Covariance Matrix Adaptation Evolution Strategy) (Hansen et al., 2003).

The results obtained for Cd (II) biosorption onto *Lactarius piperatus*, when nonlinear regression analysis was applied to the equilibrium isotherms were presented in Table 2 (isotherm parameters, standard deviation and coefficient of determination).

Table 2. Adsorption isotherm models and their coefficients calculated using nonlinear regression analysis for Cd (II) biosorption on *Lactariuspiperatus* biomass.

Isotherm	Calculated parameters			S_D	R^2
Langmuir	q_{max} = 9.0066	K_L = 0.0468	-	0.2471	0.9845
Freundlich	K_F = 1.357	n = 2.6848	-	0.5491	0.9232
Dubinin-Radushkevich	q_s = 6.9031	k_{ad} = 1.807×10^{-5}	-	0.5458	0.9241
Tempkin	b_T = 1206.18	A_T = 0.4161	-	0.2909	0.9785
Hill	q_{sH} = 7.9316	n_H = 4.5484	K_D = 6.4492	0.1987	0.9900
Redlich-Peterson	K_R = 0.3761	a_R = 0.0282	g = 1.0812	0.2333	0.9862
Sips	**K_s = 0.2087**	**β = 1.2949**	**a_S = 0.0259**	**0.1955**	**0.9903**
Toth	K_T =15.397	a_T = 27.804	t = 0.9086	0.2389	0.9855
Khan	q_s = 11.0202	b_K = 0.0360	a_K = 1.1006	0.2389	0.9855
Radke-Prausnitz	a_{RP} = 0.4215	r_R = 9.007	β_r = 0	0.2471	0.9862

Results show that comparing the standard deviation values obtained for each of the considered isotherms, the fitting degree follows the sequence: Sips (best fit) > Hill > Redlich-Peterson > Toth = Khan > Langmuir = Radke-Prausnitz > Tempkin > Dubinin-Radushkevich > Freundlich (Fig. 14).

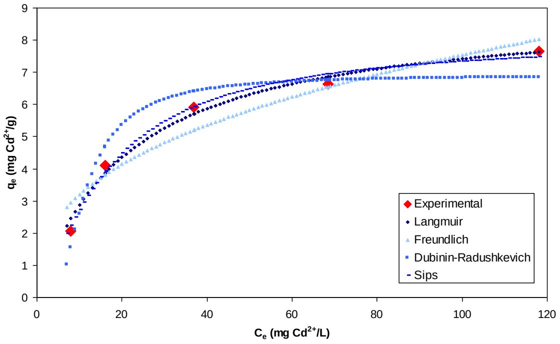


Figure 14. Representation of the Langmuir, Freundlich, Dubinin-Radushkevich and Sips plots in nonlinear regression analysis by comparison with experimental data, for Cd (II) biosorption using *Lactarius piperatus* macrofungus; C_i = 50-270 mg/L, 0.6 <d < 1.2 mm, 296 K, 5.72 pH, 700 rpm.

Moreover, comparing the coefficient of determination values obtained for each of the considered isotherms, the fitting degree follows a similar sequence: Sips (best fit) > Hill > Redlich-Peterson = Radke-Prausnitz > Toth = Khan > Langmuir > Tempkin > Dubinin-Radushkevich > Freundlich.

1.6. Biosorption thermodynamics

In order to describe the thermodynamic behavior of metal ions biosorption, thermodynamic parameters were calculated.

Experimental results were used to calculate the thermodynamics parameters for the untreated and treated fir tree sawdust (*Abies alba*) and *Agaricus bisporus* macrofungusand are presented in Table 3.

Table 3. Termodynamic parameters for the adsorption of Cd (II) on treated fir tree sawdust (*Abies Alba*) at various temperatures.

Biomass type	**ΔS° (kJ/K·mol)**	**ΔH° (kJ/mol)**	**ΔG°, (kJ/mol)**		
			296 K	**306 K**	**316 K**
Untreated	-3.10×10^{-3}	5.36	6.28	6.31	6.34
NaOH	-3.4×10^{-3}	-10.21	-0.146	0.194	0.534
H_2O_2	6.4×10^{-3}	19.87	0.926	0.286	-0.354

The positive value of the enthalpy (5.36 kJ/mol) confirmed that Cd (II) biosorption on untreated fir tree sawdust is an endothermic process (Fig. 15), where higher temperature makes the adsorption easier (Bhattacharyya and Gupta, 2008).

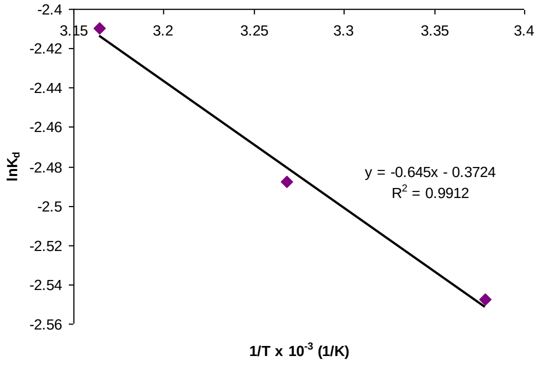


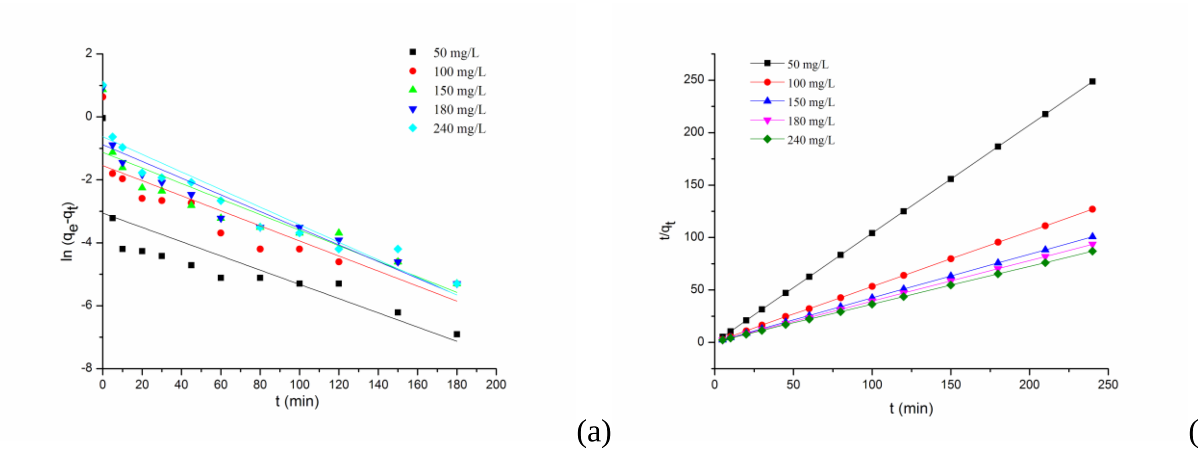
Figure 15. Plot of lnK$_d$ versus 1/T for the estimation of the thermodynamic parameters for Cd (II) biosorption on fir tree sawdust (*Abies Alba*).

1.7. Adsorption kinetics

To analyze the adsorption rates of metal ions in our studied biomasses, pseudo-first- and pseudo-second-order, intra-particle and liquid film diffusion were used to test the experimental data. Linear regression was used to determine the best fitting correlation coefficients.

Calculated adsorption capacity values (q_e) are much lower in comparison to experimental (q_{exp}) ones and also R^2 (regression coefficient) values are small, suggesting that pseudo-first-order model cannot describe the studied system (none of the biomassstudied).

Application of the pseudo-second-order kinetic model for Cd (II) biosorption on chemically treated fir tree sawdust is presented in Figs. 16 and Table 4.



(a)

(b)

Figure 16. Plots of the pseudo-first-order (a) and pseudo-second-order (b) kinetic models for Cd (II) biosorption using H_2O_2treated fir tree sawdust (*Abies alba*) biomass; C_i = 50-240 mg/L, 5g biomass,d = 0.4-0.6 mm, 296 K, pH 5.5, 700 rpm.

Table 4. Pseudo-first-order and pseudo-second-order rate constants, calculated and experimental q_e values for Cd (II) biosorption on treated fir tree sawdust (*Abies alba*) biomass using different initial concentrations.

C (mg/L)	**q_e (exp) (mg/g)**	**Pseudo-first-order**			**Pseudo-second-order**		
		k_1 (1/min)	**q_e (calc) (mg/g)**	**R^2**	**k_2 (g/mg·min)**	**q_e (calc) (mg/g)**	**R^2**
NaOH							
50	0.903	1.95×10^{-2}	0.03	0.5011	358.25×10^{-2}	0.903	1
100	2.21	1.5×10^{-2}	0.19	0.5353	38.88×10^{-2}	2.21	0.9999
175	3.40	2.33×10^{-2}	0.15	0.6205	88.35×10^{-2}	3.40	1
200	3.93	2.26×10^{-2}	0.20	0.6437	58.37×10^{-2}	3.93	1
365	6.75	2.97×10^{-2}	0.60	0.8048	24.03×10^{-2}	6.77	1
H_2O_2							
50	0.965	2.26×10^{-2}	0.05	0.6211	256.19×10^{-2}	0.966	1
100	1.89	2.39×10^{-2}	0.21	0.7623	50.22×10^{-2}	1.90	0.9999
150	2.38	2.47×10^{-2}	0.32	0.8046	34.35×10^{-2}	2.39	1
200	2.57	2.65×10^{-2}	0.41	0.8486	27.31×10^{-2}	2.58	0.9999
250	2.76	2.79×10^{-2}	0.53	0.8727	20.89×10^{-2}	2.78	1

As tables shows the linearized pseudo-second-order kinetics model provide much better R^2 values (0.995-1) than those for the pseudo-first-order model (0.5011-0.8727). The theoretical q_e (cal) values were also found in concordance with the experimental values (exp), therefore it can be considered that the adsorption system follows pseudo-second-order kinetics.

Studied shown that the pseudo-second-order kinetic model descriebes better the biosorption process in all cases.

1.8. Biomasses characterization in the biosorption processes

Infrared analysis allows identification of some characteristic peaks that belongs to organic functional groups, including those implied in the biosorption process. Some chemical groups have been proposed to be responsible for the adsorption heavy metal ion. In order to determine the functional groups involved in Cd (II) biosorption onto sawdust, a comparison between the FTIR spectra before (raw) and after biosorbtion (used) of cadmium was realized, Figure 17.

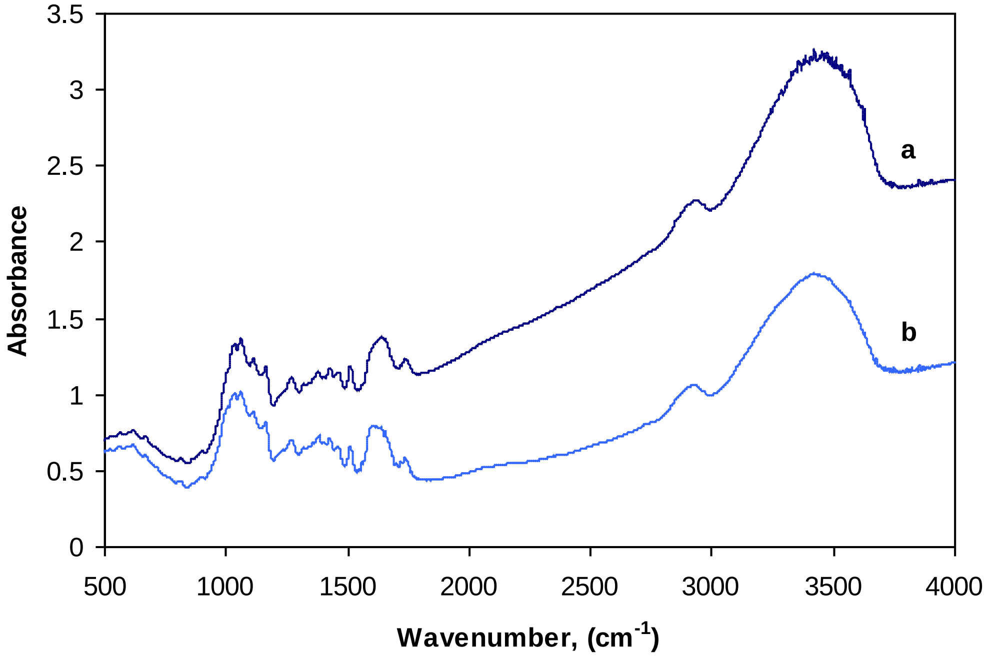


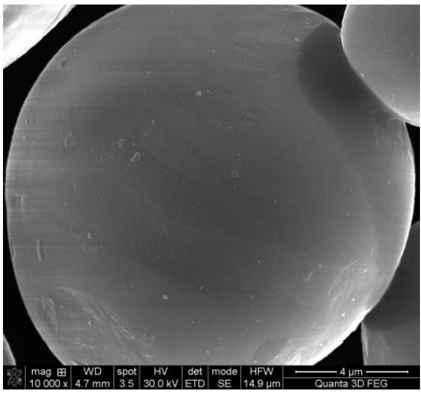
Figure 17. FTIR spectra of fir tree sawdust (*Abies Alba*) before (a) and after (b) Cd (II) biosorption.

First change was observed in case of the strong peak which corresponds to O-H bond stretching of hydroxyl group from cellulose and lignin, and water, recorded at 3421 cm^{-1} and 3416 cm^{-1} respectively. A change was also observed in the 2937 cm^{-1} peak (C-H stretch), which shifted to 2924 cm^{-1} this indicating an ion exchange process between protons.

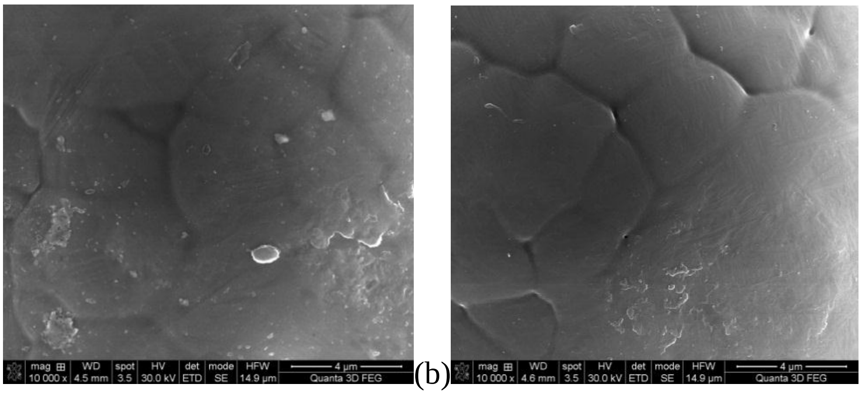
An intense changes on band at 1635 and 1604 cm^{-1} for raw and used biosorbent respectively, attributed to the N-H bend stretching from the amidesgroup was also identified. In the 1500 cm^{-1} region, at 1512 cm^{-1} and 1508 cm^{-1} another shift of peak was observed, which belongs to the nitro groups ($-NO_2$ stretching).

FTIR studies revealed that several functional groups present in the fir tree sawdust are able to bind the heavy metal ions, in our case Cd (II) ions.

Scanning electron microscopy is utilized for characterizing surface microstructures, porosity and fundamental physical properties of different adsorbents.The surface morphology of untreated fir tree (*Abies Alba*) sawdust, *Agaricus bisporus* and *Lactarius piperatus* macrofungus was determined using a scanning electron microscope JEOL JSM 5510 LV (Jeol, Japan).



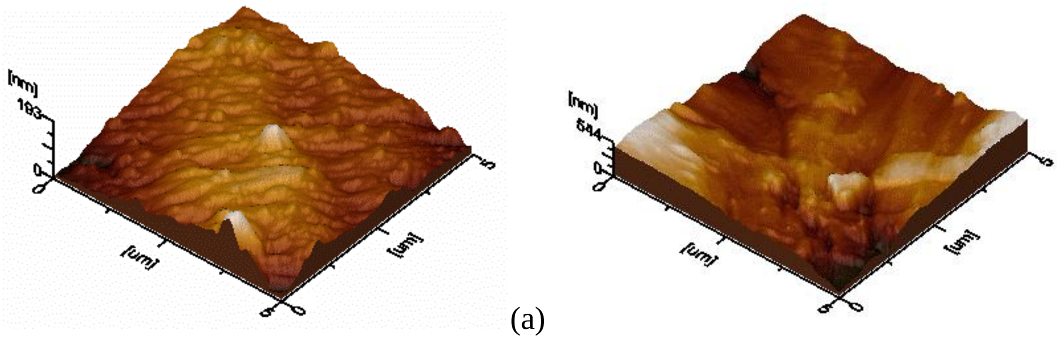
(a)



(b) (c)

Figure 18. SEM images of the *Agaricus bisporus* macrofungus (a) unloaded, (b) Cd (II) loaded and (c) Zn (II) loaded.

AFM technique shows high sensitivity to small height variations in the film surface. The three dimensional (3D) topography obtained from untreated fir tree sawdust (*Abies alba*), and chemically treated, with NaOH are presented in Figs. 19.



(a) (b)

Figure 19. AFM images of (a) untreated and (b) NaOH treated fir tree sawdust film, obtained by spreading of dried powder on adhesive plastic band: 3D-topography, scanned area: 5 µm x 5 µm.

2. Phytoextraction

The aim of the second part of this thesis was to study the accumulation capacity of *Vallisneria spiralis* and the plant biochemical responses under metal stress conditions. Beside the biochemical responses, we studied the behavior of proteins in aquatic plant generated by different heavy metals toxicity.

Vallisneria spiralisis a submerged perennial freshwater aquatic plant; commonly known as **Straight Vallisneria, Tape grass** and **Eel grass** belong to families of *Hydrocharitaceae*.

It has narrow, linear leaves that range on color from a pale-green to reddish up to 30-60 cm long and up to 0.75 inches broad (Roe and Colin, 1967).

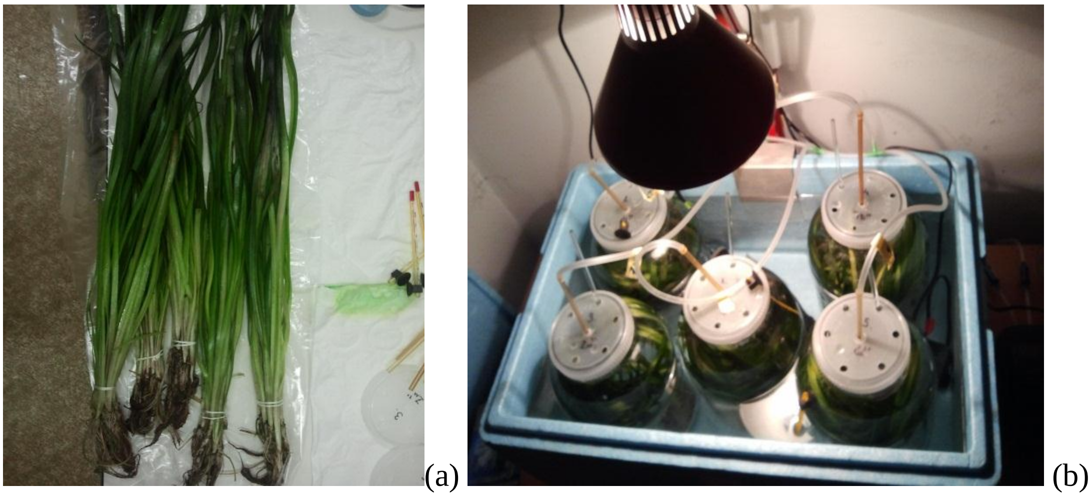

(a) (b)

Figure 20. (a) aquatic plant *Vallisneria spiralis* (b) plant under metals exposure.

Phytoextraction experiments

Experiments were carried out, using commercially available tape grass, was rinsed several times with distilled water and placed into the glass bottles containing the modified Hoagland nutrient solution. It has the following chemical composition: 1 mM KNO_3; 1 mM $Ca(NO_3)_2$; 1 mM $NH_4H_2PO_4$; 1 mM $MgSO_4$; 25 mM KCl; 12.5 mM H_3BO_3; 1 mM $MnSO_4$; 1 mM $ZnSO_4$; 0.25 mM $CuSO_4$; 0.25 mM H_2MoO_4 (85% MoO_3) with Fe(III)-citrate as iron source (Taiz and Zeiger, 2002).

Before exposure to heavy metals, plants were acclimatized in the initial solution for 4 days, then transferred into another solution containing Cu, Pb, Cd and Zn, as follows: 157 µM Cu^{2+} (10 mg/L), 48 µM Pb^{2+} (10 mg/L), 89 µM Cd^{2+} (10 mg/L) and 152 µM Zn^{2+} (10 mg/L).Control plants were placed into a Hoagland solution without heavy metals. Metal exposure experiments were followed for 6 days.

Water samples of 1-2 ml were also collected from the aqueous solution in order to determine the heavy metal content by atomic absorption spectroscopy (Perkin-Elmer 2380 AAS). Also, during the experiments (6 days), samples of 1-2 leaves were collected at regular 24 hour intervals, rinsed with distilled water, frozen in liquid nitrogen and stored at -20°C until protein extraction. After 6 days exposure, leave fragmentations, necrotic symptoms began to appear.

All reagents of analytical grade were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Metal accumulation and plant physical changes

Heavy metal stress of *Vallisneria spiralis* applying Cu, Pb, Cd and Zn in a aqueous solution was investigated at a constant temperature during a six-day cycle. Changes in metal concentration during the 6 days exposure are presented in Fig. 21.

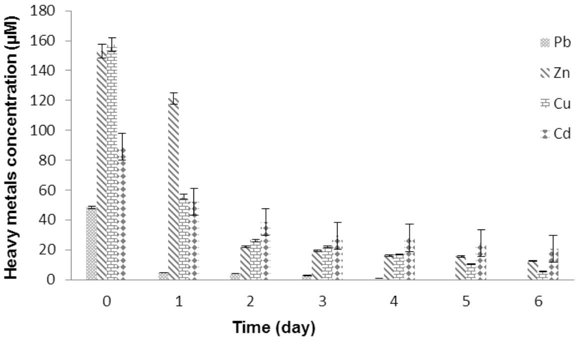


Figure 21. Heavy metal concentrations in the growth medium of *V. spiralis*, with an initial concentrations of 157 µM Cu, 89 µM Cd, 152 µM Zn and 48 µM Pb, pH 6.5, T= 26°C, during a six-day exposure. The error bars show the standard deviation values of measurments (n=3).

The most significant change occurred in the case of Cu, which otherwise was present in the highest concentration among the metals applied. At the first day, concentration decreased from 157 to 55.4 µM that is an uptake of about 70%. The next 5 days of the experiment a decreased copper uptake was observed.

Pb uptake showed similar tendency to the copper uptake, where the initial concentration was decreased from 48 µM to 4.6 µM during the first day. After 4 days of treatment, this metal could no longer be detected in the growth medium.

Cadmium provided a significant metal uptake during the second day, with nearly 50 % decrease in the metal concentration. This was followed by a constant decay in the growth medium.

The Zn concentration decreased slightly during the first day in the aqueous solution. This was followed by a more significant decrease during the second day. No other significant changes occurred during the last four days of the experiment.

During the treatments, various physical modifications of the plants were observed. By the second day several chlorotic symptoms appeared, followed by the meltdown and finally necrosis of the primary leaves by the sixth day.

Our results are in agreement with earlier observations with other types of aquatic plants showing high metal binding capacities (Schiewer and Volesky, 2000), whereas numerous studies deal with the effect of Cd, Cu, Zn or Pb (El-Khatib et al., 2011; Singh et al., 2010) on aquatic plants.

Protein analysis results

The protein profile of the control, as well as the exposed plant material was analysed with microchip electrophoresis by using a molecular sieving environment. Appearance/disappearance and quantitative changes in protein profile were observed. Protein groups of 9.5, 12.4, 15, 25, 37 and 47 kDa molecular mass respectively were detected in both, the control and the treated plants.The appearance and dissapearence of these proteins for each day of exposure are presented in Table 5.

The effect of heavy metals on protein profiles has resulted in the appearance of new proteins of 11.2 kDa, 30.8 kDa, 60 kDa, 85 kDa, 95.5 kDa, and 127 kDa molecular mass range, whereas these proteins were not detectable in the control plants.

The effect of Cu caused the appearance of five (11.2; 30.8; 60; 85; 127 kDa), the Pb that of three (30.8; 60; 95.5 kDa) and Cd that of one (30.8 kDa) proteins, respectively.

On the other hand, the disappearance of 13.4 kDa and 55.5 kDa molecular mass proteins in the case of Cu, and of the 18.6 kDa one in the case of Pb treatment, was also observed. These proteins could not be detected after the third or fourth day. Cd did not cause these kinds of changes.

Zn caused only quantitative changes in the protein profile when plant material was exposed to the applied doses for the entire duration of the experiment.

Table 5. The protein profile of control and each individual plant exposed to different heavy metals in *Vallisneria spiralis*, after a six-day treatment.

	9.5 kDa	11.2 kDa	12.4 kDa	13.4 kDa	15 kDa	18.6 kDa	25 kDa	30.8 kDa	37 kDa	47 kDa	55.5 kDa	60 kDa	67 kDa	85 kDa	95.5 kDa	127 kDa
Cu	+	$\uparrow^{2,3}$	+	$\downarrow^{1\text{-}3}$	+	+	+	$\uparrow^{2,3}$	+	+	$\downarrow^{1\text{-}3}$	$\uparrow^{2,3}$	-	$\uparrow^{2,3}$	-	$\uparrow^{2,3}$
Pb	+	-	+	+	+	$\downarrow^{1\text{-}3}$	+	$\uparrow^{2\text{-}4}$	+	+	+	$\uparrow^{2\text{-}4}$	+	-	$\uparrow^{2\text{-}4}$	-
Cd	+	-	+	+	+	+	+	$\uparrow^{3,4}$	+	+	+	-	$\uparrow^{3,4}$	$\uparrow^{3,4}$	-	-
Zn	+	-	+	+	+	+	+	-	+	+	+	-	+	-	-	-
Control	+	!	+	+	+	+	+	!	+	+	+	!	+	!	!	!

Symbols: + present in control and/or heavy metal treated plants during the experiment; ! Not present in control plant**s**; - no values ,$\downarrow^{1\text{-}3}$disappear after 1-3 day of treatment; $\uparrow^{2,4}$appear after 2-4 day oftreatment.

In addition to the above mentioned heavy metal-induced qualitative alterations in the protein patterns, clear quantitative differences were also recorded for each kind of exposure. Such changes were observed not only because of the appearance and/or disappearance of some proteins, but were registered on the amounts of the 9.5; 12,4; 15; 25; 37; 47 kDa proteins present during the entire duration of the experiment.

The quantitative changes were followed for each metal in case, but the most important changes were appearing after Pb treatments. Alterations in the presence of Pb on normalized microchip electrophoresis electropherogram are presented in Fig. 22 during 6 days Pb treatment.

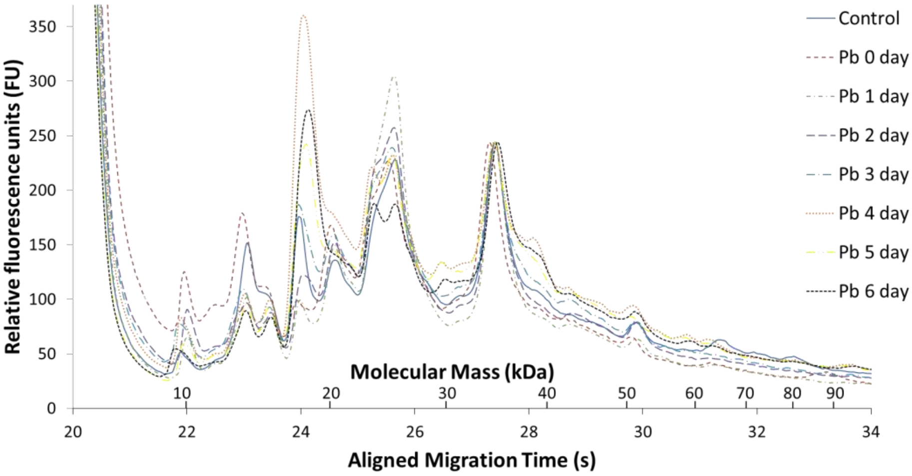


Figure 22. Microchip electrophoresis normalized electropherogram profile of Pb treated *Vallisneria spiralis* with an initial concentration of 48 µM. Experimental conditions: HSP 250 Protein Chip; SLB buffer (Tris-HCl pH 8.5); fluorescence detection with 630 nm excitation and 650 emission wavelengths; injection, 80 s at 1000 V; substances migrate toward the anode. The proteins are visualized by covalently bound fluorescent dye.

The 15 kDa, 18.6 kDa, 25 kDa and 37 kDa molecular mass proteins showed the most important changes in concentration. This kind of quantitative modification was followed over all six-day treatments.

General Conclusions

The aim of this thesis was to investigate the bioremediation of heavy metals using new biosorption and phytoextraction techniques.

During biosorption processes it was studied the removal of Cd (II) and Zn (II) ions from aqueous solutions using new types of natural biomasses as biosorbents. The potential removal capacity of the following natural biomasses: fir tree sawdust (*Abies alba*), commercially avaible *Agaricus bisporus* and wild (natural) *Lactarius piperatus* macrofungi and *Saccharomyces cerevisiae* yeast cells was studied. Our study is focused on easy available, cheap by-products provided from different type of industries (wood, fermentation and food).

In order to enhance the adsorption capacities of these biomasses, different methods were applied, namely: chemically treatments over the sawdust and immobilization techniques for macrofungi.

It was developed and optimized the experimental biosorption procedures in batch monometallic system.

The influence of various parameters (biomass quantity, effect of initial metal ion concentration, contact time, stirring rate, initial pH of the solution and temperature) was followed evaluating and calculating the adsorption capacities and removal efficiencies.

Equilibrium isotherm model parameters were successfully calculated using linear (Langmuir and Freundlich) and non-linear regression (ten isotherm models). Covariance Matrix Adaptation Evolution Strategy nonlinear optimization algorithm was used to determine the best fitted isotherm data. It was concluded that nonlinear regression analysis has better performances, with Sips model describing the biosorption process best for all studied biomasses.

To analyse the adsorption rates, pseudo-first- and pseudo-second-order, intra-particle and liquid film diffusion were used to test the experimental data. Linear regression was used to determine the best fitting correlation coefficients. Studied shown that the pseudo-second-order kinetic model describes better the biosorption process in all cases.

In order to describe the thermodynamic behaviour of metal ions biosorption, thermodynamic parameters were calculated.

To obtain valuable information about functional groups which have an importand role on metal ions binding and to examine the morphological structure of biomass surfaces FTIR, SEM and AFM analysis were taken.

In the second part of this thesis it was studied the phytoextraction potential of Cu, Pb, Cd and Zn using aquatic plant *Vallisneria spiralis* in monometallic system. The process were followed by the changes on heavy metal ions concentration against the 6 days exposure and were characterized the phytoextraction capacity.

The phytoextraction levels were correlated with the plant biochemical responses generated by the heavy metals toxicity. Beside the biochemical responses, we studied the behavior of proteins on proteomic profile.

The microchip electrophoresis measurements were used for the first time in the detection on proteomic changes in aquatic plants. Comparing the protein profiles in plants we succeeded to determine the appearance and disappearance of the proteins, as well some quantitative changes caused by metals exposure.

Our developed new bioremediation methods provide a good opportunity on practical application in the removal of heavy metals from aqueous solutions.

Published scientific papers

Nagy, B., Măicăneanu, A., Indolean, C., Burcă, S., Silaghi-Dumitrescu, L., Majdik*, C., **2013a**. Cadmium (II) ions removal from aqueous solutions using Romanian untreated fir tree sawdust- a green biosorbent .Acta Chim.Slov. 60, 263-273.

Nagy, B., Măicăneanu, A., Indolean, C., Mânzatu, C., Silaghi-Dumitrescu, L, Majdik*, C., **2013b**. Comparative study of Cd (II) biosorption on cultivated *Agaricus bisporus* and wild *Lactarius piperatus* based biocomposites. Linear and nonlinear equilibrium modeling and kinetics. J. Taiwan. Inst. Chem. E., 10.1016/j.jtice.2013.08.013.

Nagy, B., Tonk, Sz., Indolean, C., Măicăneanu, A., Majdik*, C., **2013c**. Biosorption of Cd (II) ions by unmodified, microwave and ultrasound modified brewery and pure strain yeast biomass. Am. J. Anal. Chem., 4, 63-71.

Articles submitted for publication

Nagy, **B**., Török, A., Makszin, L., Majdik, C., Valasek, A., Kerepesi, I., Kilár*, F., Heavy metal induced protein profile changes in aquatic plant *Vallisneria spiralis*. Send to South Afr. J. Bot. 2013.

Nagy, **B**., Szilágyi, B., Majdik, C., Katona, G., Indolean*, C., Măicăneanu*, A., Metal ions biosorption on *Lactarius piperatus* macrofungus. Linear and nonlinear equilibrium modelling and kinetics. Under Revision, Environ. Prog. Sust. Energ., 2013.

Nagy, **B**., Mânzatu, C., Măicăneanu, A., Indolean, C., Barbu-Tudorean, L., Majdik*, C., Linear and nonlinear regression analysis for heavy metals removal using *Agaricus bisporus* macrofungus. Send to Arab. J. Chem., 2013.

Nagy, **B**., Mânzatu, C., Măicăneanu, A., Indolean, C., Silaghi-Dumitrescu, L., Majdik*, C., Effect of alkaline and oxidative treatment on sawdust capacity to remove Cd (II) from aqueous solutions. FTIR an AFM study. Send to J. Wood Chem. Technol., 2013.

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