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Phytoremediation study of water polluted with heavy metals using floating macrophytes: *Lemna minor* and *Pistia stratiotes*

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Abstract. This work aims to highlight the capacity of *Lemna minor* and *Pistia stratiotes* macrophytes to decontaminate waters polluted with heavy metals, through a process of phytoextraction and phytoaccumulation. The purpose of the study was to elaborate an *in-situ* bioremediation technology for the waters of the decantation ponds originated from the mining industry. Experiments were conducted on water samples collected from Aurul tailings management facility, in Baia Mare region, northern Romania. The materials deposited on this facility resulted from the re-processing of tailings containing residual gold. The capacity of phytoextraction and bioaccumulation of heavy metals in the plants tissues have been assessed. Three types of samples have been used in the laboratory experiment: a blank sample consisting of bottled drinking water, a water sample taken from the drainage ditch, and a sample taken from the tailings pond. The phytoextraction of heavy metals from the analyzed water samples (μg L^{-1}) and the bioaccumulation of heavy metals in the tissues of the plants (mg kg^{-1} d.m.) were monitored. The heavy metal concentrations have been measured before and after the phytoextraction process, and an accumulation of heavy metals (Cd, Cu, Ni, Zn, Pb, Fe) in *L. minor* and *P. stratiotes* plant tissues has been noticed.

Key Words: bioremediation, phytoextraction, heavy metals, *Lemna minor*, *Pistia stratiotes.*

Introduction. Bioremediation of contaminated waters with heavy metals is a very extensively study since the globally trend towards sustainable development is to depollute a historically contaminated site with fewer resources and more effectively.

Technologie's development for the remediation of biological and ecological reconstruction is based on research on bioremediation of polluted sites (Barbu & Sand 2004; Buta et al 2011; Elekes et al 2010; Glick & Stearns 2011; Malschi 2009; Oros 2002, 2011; Pivetz 2001).

For the remediation of industrial wastewater and leachate from landfills it was used constructed wetlands (Jing et al 2001; Kuschk et al 2005; Masi et al 2002; Sim et al 2008). The main processes that take place are the degradation, transformation, and phytoextraction plan biosorption pollutants with aquatic species (Dickinson et al 2009; Juang & Chen 2007). By using these biological systems consisting of water plants, green algae, cyanobacteria and various bioaccumulating species, it is possible to eliminate or reduce various heavy metals and other pollutants (Jing et al 2001; Kuschk et al 2005; Malschi 2009; Malschi et al 2012; Oprea et al 2013). The most common indicators in laboratory studies or parameters, affecting the bioaccumulation of metals in plant tissue, are: pH, metal concentration, temperature, duration of exposure, evaporation, solar radiation (Buta et al 2011).

Polluted waters with heavy metals can be treated effectively by building wetlands. Remediation of heavy metals using bioreactors and constructed wetlands has been the subject of many researchers (Dickinson et al 2009; Jing et al 2001; Juang & Chen 2007; Kuschk et al 2005).

Lemna minor (duckweed) and *Pistia stratiotes* (water lettuce) have been studied and many results underlined their effect in phytoremediation through bioaccumulation of pollutants, accumulation of metals and wastewater treatment (Dosnon-Olette et al 2011; Malschi et al 2012; Rahmani & Sternberg 1999; Rahman et al 2007; Willey 2007).

Aurul tailings pond is located in the eastern part of Baia Mare, a distance of approx. 2900 meters from the limit of the municipality built. The area where the tailings pond is built has a slope from northeast to southwest (Report site 2012). It was operated in 1999 and closed in 2006, and is currently in a state of preservation. Being a relatively new pond, Aurul tailings meet the requirements of Best Available Techniques (BAT). The amount of tailings that can be deposited on gold tailings pond is approx. 15 million tons, and so far have been deposited approximately 5,436,000 tons. By exploiting Central Pond and storage of approx. 8 milions tons resulting from the processing of tailings, tailings gold will have a maximum height of 17 m on the south-west.

The water grid surface is very rich in Baia Mare area, because of the rain falling during the year and are grouped in two catchments: Somes and Lăpuşul.

Săsar River drains from east to west the city of Baia Mare, collecting and Firiza Chiuzbaia rivers, the rivers Sfântul Ioan, Racoş and Borcea and is a right tributary of the river Lăpus.

At depths between 4-15 m is the upper aquifer (water table) with a medium flow regime prevailing direction S-SV.

The existing geology shows the presence of Neogene magmatic activities by intrusive bodies and particularly effusive andesitic rocks. This type of mineralization, is found in the hydrothermal deposits and it is known as “low sulfidation” with specific metallic elements as: Au, Ag, Pb, Zn, Cu with economical value. Also, the alluvial deposits are present stratigrafically, specific to the paleo-terraces as well as the dejection cones.

Pheylonian deposits were considered for a long period the most important metallic concentration because of their high metal content. The deposits from Baia Mare region have a characteristic vertical zonality (Modoi 2010).

Material and Method. Five samples were taken from Aurul tailing in 24 may 2013 and for the laboratory experiment we have had single samples as follows: s5 sample and s1 (S1) containing water from the tailing pond and s2, s3 and s4 (S2) sample containing water from the drainage ditches of the tailing (Figure 1).

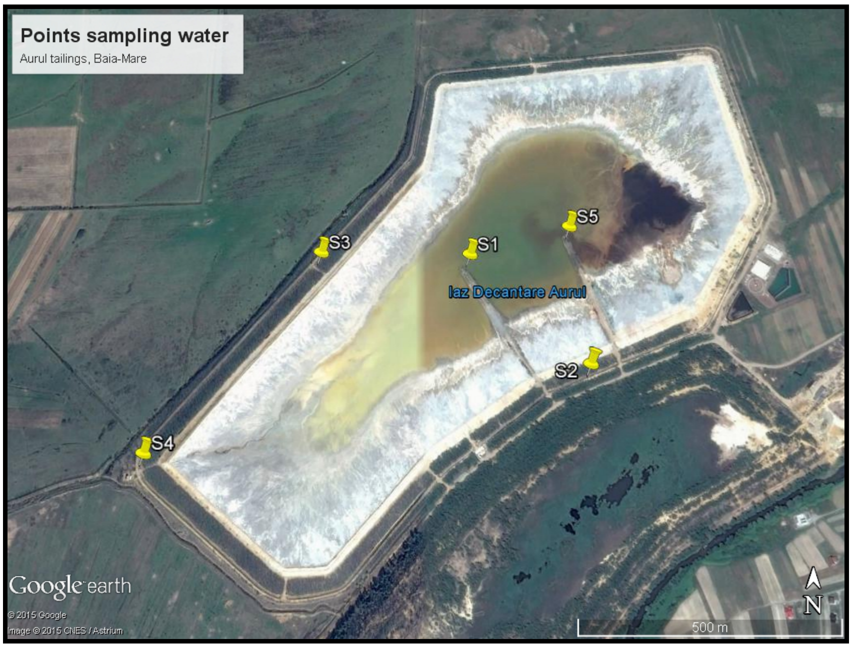


Figure 1. Points sampling water from perimeter Aurul tailings, Baia-Mare (Google Earth).

The work method included the analysis of quality parameters and heavy metal content of the samples before and after phytoextraction process. During the 14 days, the plant species were monitored and mortality rate was calculated. To experiment with *L. minor* and *P. stratiotes* three types of samples have been used in the laboratory: a blank sample consisting of bottled drinking water, a water sample taken from the drainage ditch, and a sample taken from the tailings pond.

Plants *L. minor* and *P. stratiotes* were harvested from Greenhouse Botanical Garden - Cluj Napoca. In the experiment, 500 mL of water from each sample were placed in plastic containers. Each version had three pieces of plants were *P. stratiotes* and the plant *L. minor* evenly covered the surface of water. Because of the acid from the water samples drainage ditch and pond, both plant species have begun to dry from the first days of the experiment.

The quality of water parameters was analysed with the WTW 720 Multiparameter. The heavy metals (Pb, Cu, Zn, Ni, Fe) have been determined with atomic absorption spectrometry (AAS) (ZEEnit 700 Analytik Jena), the method used for determination of trace elements from solution (flame method, mg L^{-1}). Before analyse, the water samples were acidified with HNO_3 65% at a pH between 2 and 3 and filtered with qualitative filter paper of 125 mm.

The bioaccumulation of heavy metals (Cd, Cu, Ni, Zn, Pb, Fe, Cr) in the green tissue samples (mg kg^{-1} dry matter) have been measured after the phytoremediation process. The heavy metals concentration was determined by the flame atomic absorption spectrometer ZEEnit 700 (Analytic Jena). The detection limit for the methods was between 1 to 5 ppb for Cr, Cd, Zn, Cu, Ni and Pb. The relative standard deviations are between 0.3 and 1% for absorbances of 0.1 to 0.2. The error of the methods was of maximum ±5%.

The samples were prepared and mineralized according to SR ISO 11466 protocol (SR ISO 11466, 1999), as follows: 100 grams of sample were dried in an oven (Memmert UNB 400, Germany) at 105°C for 24 hours. After drying, it was placed into the desiccator, and then was cooled. The samples were manually crushed and passed through the sieve (250 µm). Three representative samples of 3 g each were weighed (precision 0.1 mg) and mixed with aqua regia (3:1 HCl 37% : HNO_3 65%) and left over night for 16 hours at room temperature to allow the slow oxidation of the organic matter from the sample.

The samples were then transferred into the reaction vessels and mineralized with aqua regia according to SR ISO 11466 (1999) protocol. The mineralization consisted in gradually rising the temperature until the reflux and maintaining the reflux temperature for 2 hours. After cooling, the supernatant was filtered (0.45 µm) and analyzed by atomic absorption spectrometry.

Results and Discussion. The biomonitoring and phytoextraction testing with *L. minor* and *P. stratiotes* were performed in the Biotechnologies Laboratory within the Faculty of Environmental Sciences and Engineering, Babeş-Bolyai University of Cluj-Napoca.

During the experiments the quality of water parameters was improved (Table 1). High values of parameters (EC and TDS) indicates a charge of salt and this is proved by the high salinity. The high values of the oxidation-reduction potential are correlated with a highly oxidizing environment.

Table 1

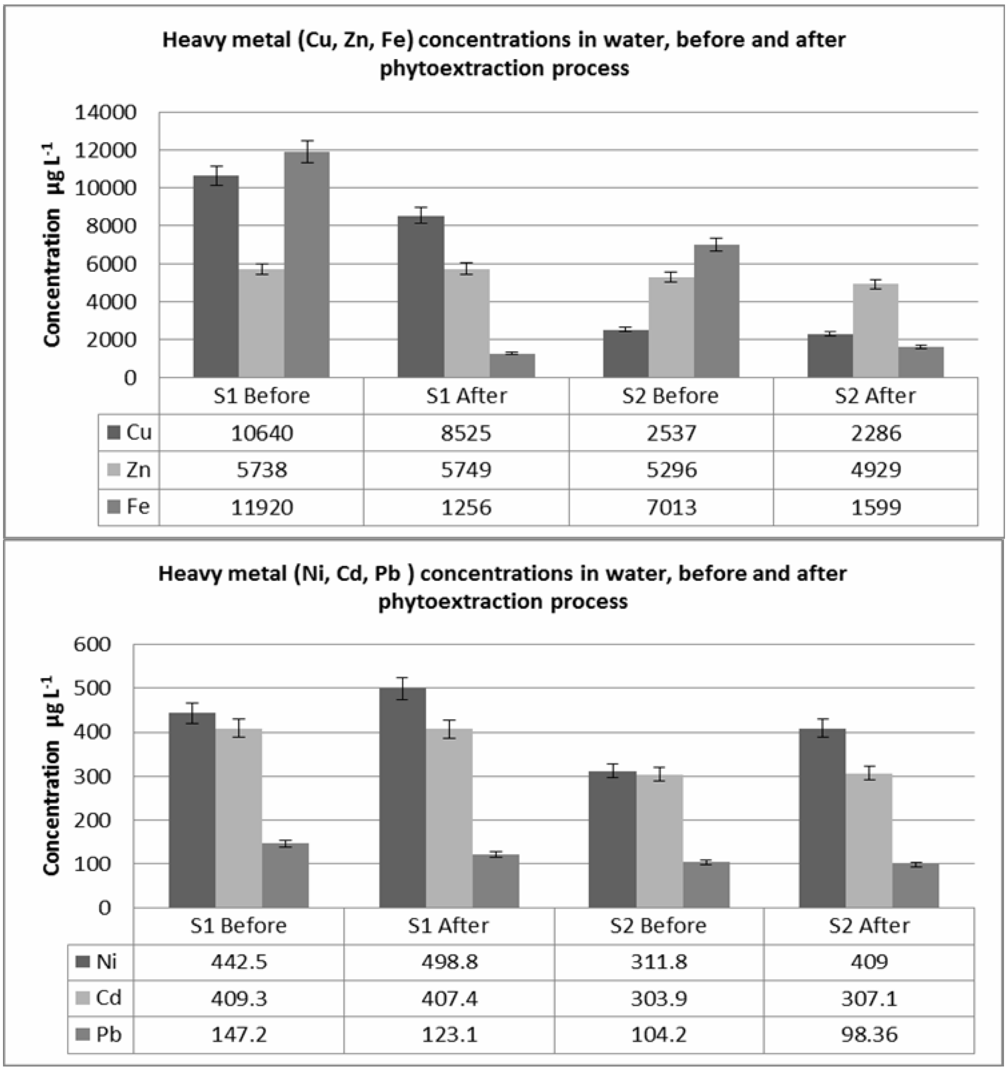
Analysis of physico-chemical parameters before and after phytoextraction process

	BS Before	*BS After*	*S1 Before*	*S1 After*	*S2 Before*	*S2 After*
pH	6.74	7.5	3.8	4.7	3.4	4.4
T (^{o}C)	20.9	21.6	20.9	21.6	20.9	21.6
Eh (mV)	-3.2	-46.6	159.4	110.9	181.5	126.4
EC (µS cm^{-1})	83,1	127	4570	4700	4170	4300
TDS (mg L^{-1})	41.55	63.5	2285	2350	2085	2150
Salinity ($^{o}/_{oo}$)	0	0	2.4	2.5	2.2	2.3

BS – blank sample (bottled drinking water), S1 – water from the tailings pond, S2 – water from the drainage ditch.

Heavy metals and metalloids bioaccumulations were noticed in leaves and stalks of *Lemna minor* grown on polluted waters from Aurul tailing pond for Cu, Fe, Pb (Figure 2).

The Figure 3 indicates the percentage concentration of heavy metals extracted by plants during the experiment.



	S1 Before	S1 After	S2 Before	S2 After
Cu	10640	8525	2537	2286
Zn	5738	5749	5296	4929
Fe	11920	1256	7013	1599

	S1 Before	S1 After	S2 Before	S2 After
Ni	442.5	498.8	311.8	409
Cd	409.3	407.4	303.9	307.1
Pb	147.2	123.1	104.2	98.36

Figure 2. Heavy metal concentrations in water, before and after phytoextraction process (S1 – water from the tailings pond, S2 – water from the drainage ditch).

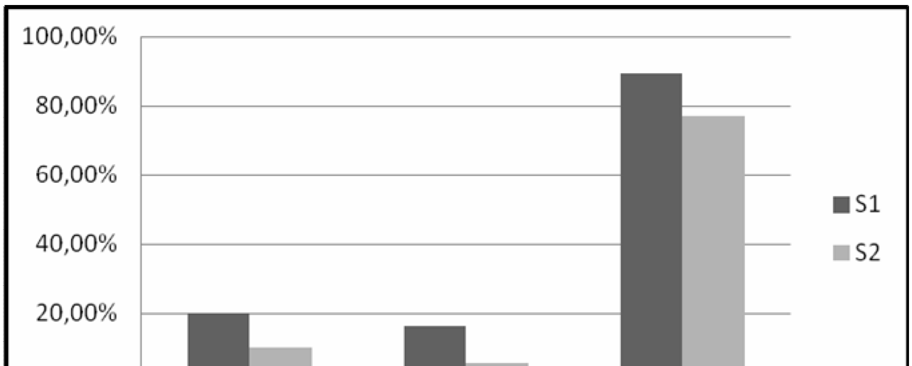


Figure 3. Concentration of heavy metals after phytoextraction plan process (S1 – water from the tailings pond, S2 – water from the drainage ditch).

The Figure 4 indicates the heavy metal concentrations in plant tissues after the experiment.

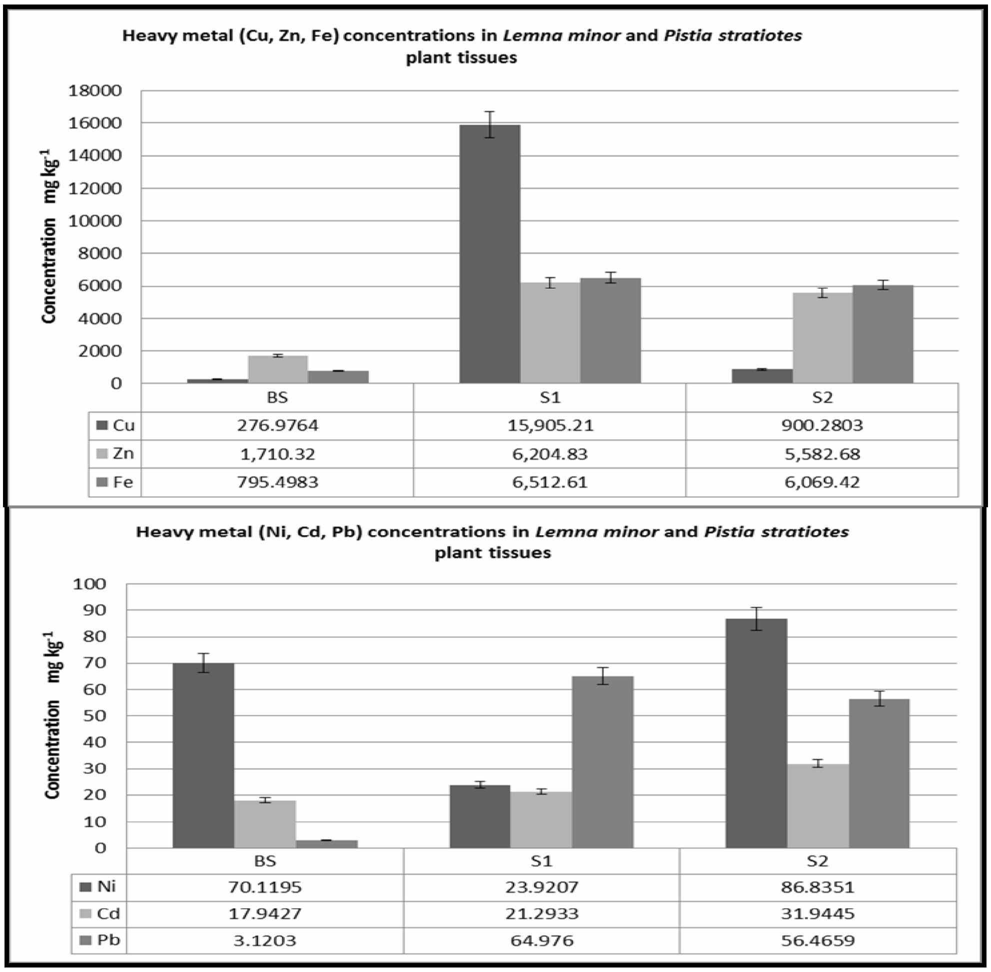


Figure 4. Heavy metal concentrations in *L. minor* and *P. stratiotes* plant tissues (BS – blank SAMPLE - bottled drinking water, S1 – Water from the tailings pond, S2 – water from the drainage ditch).

L. minor plants were harvested and included dried plants and green plants, to calculate mortality rates (Figure 5). For each variant were counted in box 2 cm^2, green plants and dry. There were three replications for each variant.

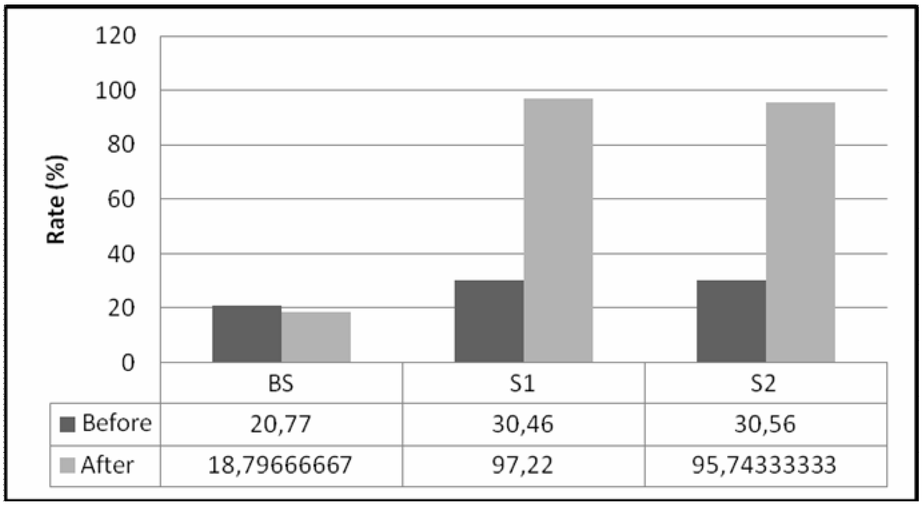


Figure 5. Mortality rate of *L. minor* plant after phytoextraction process (BS – blank sample - bottled drinking water, S1 – water from the tailings pond, S2 – water from the drainage ditch).

In Figure 6 it can observe the evolution of plant *L. minor* and *P. stratiotes* during the two-week experiment.

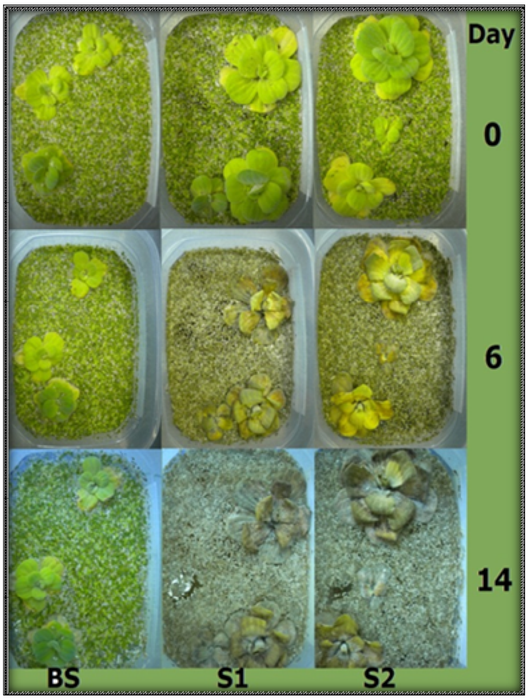


Figure 6. The evolution of plant *L. minor* plant and *P. stratiotes* in biomonitoring and phytoremediation process (BS – blank sample - bottled drinking water, S1 – water from the tailings pond, S2 – water from the drainage ditch).

Conclusions. The aim of this paper was to highlight the ability of plants to improve water quality parameters and bioaccumulation of heavy metals in their tissues. Analysis of physico-chemical parameters showed a significant improvement thereof. After analyzing the heavy metals before and after the phytoextraction process, a certain amounts of metals as Fe, Cu, Zn, Pb, Cd has been noticed at the *L. minor* and *P. stratiotes* plant. A very high rate of mortality for the two plant species has been identified during the laboratory experiment especially for the ones on the tailings pond water and also in the water from the drainage ditch, compared with the blank sample, bottled drinking water.

The results from the laboratory experiment confirmed that associating plants as *L. minor* and *P. stratiotes* is a viable method and a mind-set notion looking for decontamination and improvement of the physical and chemical parameters of polluted water. Moreover this method is an *in-situ* bioremediation technology for the waters of the decantation ponds originated from the mining industry.

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