

## Phytoremediation of polluted water from Roşia Montană mining area

<sup>1</sup>Alexandru Dan Petaca, <sup>1</sup>Dana Malschi, <sup>2</sup>Erika Levei, <sup>2</sup>Claudiu Tanaselia, <sup>1</sup>Carla Nicoară, <sup>3</sup>Elena Rînba

<sup>1</sup>Babeş-Bolyai University, Faculty of Environmental Sciences and Engineering, 30 Fântânele Str., 400294 Cluj-Napoca, Romania; <sup>2</sup>INCDO INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath Str., 400293, Cluj-Napoca; <sup>3</sup>Babeş-Bolyai University, Botanical Garden, 42 Republicii Str., Cluj-Napoca, Romania. Corresponding author: A. D. Petaca, dan\_petaca@yahoo.com

**Abstract.** Phytoremediation tests for heavy metals removal with aquatic species *Lemna minor*, *Pistia stratiotes*, *Vallisneria spiralis*, on contaminated acidic waters collected from Rosia Montana mining area, in 2016, were performed. The study was conducted in micro containers with contaminated water using constructed wetlands systems. Three types of experimental waters for each species have been used: drinking plain water as blank, drainage waters collected from Abrudel river downstream of Gura Rosiei tailings dump and from Saliste Valley downstream of Saliste tailings dump. Heavy metals concentrations were measured by inductively coupled plasma mass spectrometry using an ELAN DRC II (Perkin Elmer) spectrometer, and the water quality parameters (pH, Eh - oxidation-reduction potential, EC - electrical conductivity, TDS – total dissolved salts and S - salinity) were analyzed using a 320i multiparameter (WTW), before and after the phytoremediation process. During two weeks, the phytoextraction experiment with aquatic species (*L. minor*, *P. stratiotes* and *V. spiralis*) showed a significant decreases of heavy metals concentrations, with an efficiency of 6.54-25.80% for Pb; 9.85-66.29% for Al; 100% for Cd; 59.54-78.63% for Ni and 32.91-48.37% for Zn, in case of Abrudel river water, while in case of the water from Saliste Valley with significant decrease of Al (30.40%), Ni (21.10%), Pb (60.86%) and Fe (88.71%). *Vallisneria spiralis* has shown high phytoremediation efficiency by increasing pH (24.13%) and EC (3.36%). The promising results on phytoremediation of contaminated acidic drainage waters using aquatic species in constructed wetlands indicates the possibility of implementing this biotechnology.

**Key Words:** heavy metals, phytoremediation, *Lemna minor*, *Pistia stratiotes*, *Vallisneria spiralis*.

**Introduction.** The work was carried out to investigate the current situation of the environment and pollution of the Rosia Montana mining area, to the identification of an effective phytoremediation method involving low cost, lack of large equipment, minimal power consumption, high bioremediation etc. Environmental reconstruction is a measure to be applied in all degraded sites because they can be reintegrated into the biological circuit. Regardless of the methods used for remediation, ecological restoration, restoring the natural aspect of a site after anthropic degradation is required by law and is part of the integrated environmental management for closing any mining activity. Phytoremediation uses a number of techniques, among which the phytoextraction, the phytostabilization, the bioaccumulation, and the rizoextraction.

The heavy metals from polluted waters may be efficiently treated by using artificial tailing dams or specially provided ecosystems in humid areas. The removal of heavy metals in bioreactors and built humid areas has represented the objective of different researches (Jing et al 2001; Vidali 2001; Juang & Chen 2007; Cayuela et al 2007; Dickinson et al 2009; Oros 2002, 2011), and numerous results highlighting the effect of *L. minor* in phytoremediation by bioaccumulation of pollutants from contaminated waters (Rahmani & Sternberg 1999; Rahman et al 2007; Dosnon-Olette et al 2011; Malschi 2009, 2014; Malschi et al 2013, 2015, 2018; Brahaita 2015).

*L. minor* is known as pollution bioindicator for the biomonitoring and ecotoxicity tests on the aquatic environment (ISO20079 standard: 2005).

This work aims to highlight the capacity of macrophytes *Lemna minor*, *Pistia stratiotes* and *Vallisneria spiralis* 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. The experiments were conducted on water samples collected from Rosia Montana, in Alba region, of north-western Romania. As a laboratory method were used the bio monitoring and phytoremediation, the phytoextraction and bioaccumulation of heavy metals, with *Lemna minor*, *Pistia stratiotes* and *Vallisneria spiralis*.

**Material and Method.** The work method included the analysis of quality parameters and heavy metal content of the samples before and after phytoextraction process. During the two week experiment (14 to 28 March 2016), the plant species were monitored and the mortality rate calculated. Three types of samples have been used in the laboratory, in the experiment with *L. minor*, *P. stratiotes* and *V. spiralis*: drinking plain water as blank, drainage water from Abrudel river of Gura Rosiei tailings dump and the water from Salistei Valley downstream of Saliste tailing dump.

The water quality parameters (pH, Eh - oxidation-reduction potential, EC - electrical conductivity, TDS – total dissolved salts, and S – salinity) of samples analyzes were performed using multiparameter WTW multi 350 i. The data regarding the phytoextraction and bioaccumulation of heavy metals were obtained by inductively coupled plasma mass spectrometry using an ELAN DRC II (Perkin Elmer) spectrometer, at the Research Institute for Analytical Instrumentation, ICIA Cluj – Napoca.

**Results and Discussion.** The bioremediation effect of aquatic species has been highlighted in many research studies (Masi et al 2002; McCutcheon & Schnoor 2004; Romero Nunez et al 2011; Pivetz 2001; Sim et al 2007). Therefore, it has been demonstrated the effect of some species like *V. spiralis* L. (Hydrocharitaceae) for metal hyperaccumulation; *L. minor* L. and *P. stratiotes* L. (Araceae) for accumulating metals and wastewater treatment (Malschi et al 2013, 2015, 2018).

The biomonitoring and phytoextraction testing with *L. minor* (Figure 1), *P. stratiotes* (Figure 2) and *V. spiralis* (Figure 3), were performed in the Biotechnologies Laboratory within the Faculty of Environmental Sciences and Engineering of Babeş-Bolyai University Cluj-Napoca. After the second week (Figure 1), the color of *L. minor* is changed, depending on the degree of water pollution. In the waters with high pollution (variants 2 and 3), the plant dies or is getting darker compared to variant 1 of micro tank which is blank.

From the data in Table 1, it can be observed that the mortality in water from Salistei Valley downstream tailings dump increased to 50% in the first 6 days during the experiment and after two weeks increased to 90.29%. In water from Abrudel river downstream tailings dump Gura Rosiei the mortality increase to 35% during the experiment. It can be observed from the result of mortality that the water from Abrudel river is not very polluted.

Table 1

The mortality of *Lemna minor* in the biomonitoring tests (14.03-28.03.2016)

Variants	Mortality (%) (mean values of 3 repetitions)					
	14.03 (initial mortality)	17.03	20.03	23.03	25.03	28.03 (after 2 weeks)
Drinking plain water as blank	14.28	20.6	30.76	31.13	28.93	32.52
Water from Abrudel river downstream GuraRosiei tailings dump	9.09	20.7	35.39	32.33	29.45	34.37
Water from Salistei Valley downstream Salistei tailings dump	6.84	28.4	50.00	56.98	80.58	90.29

Regarding the *P. stratiotes* and *V. spiralis*, each variant had five pieces of plants. It can be observed that in the third variant of water from Salistei Valley the plants died from the early days of the experiment (Figures 2 and 3).

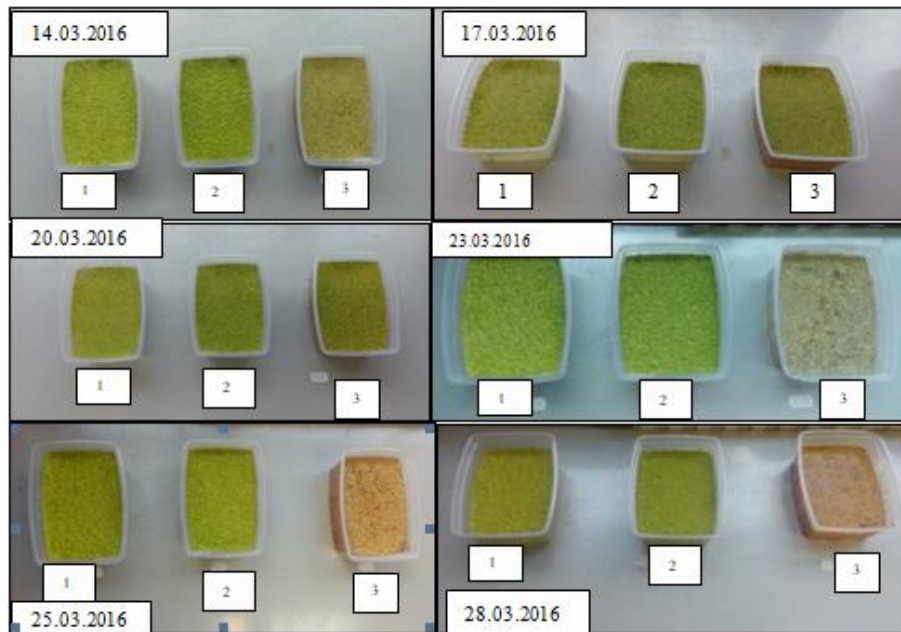


Figure 1. Images on laboratory monitoring with *Lemna minor* during the experiment (14.03.2016-28.03.2016). Variants: 1 = drinking plain water as blank; 2 = water from Abrudel river downstream of Gura Rosiei tailings dump; 3 = water from Salistei Valley downstream of Salistei tailings dump (photos by Dan Petaca).

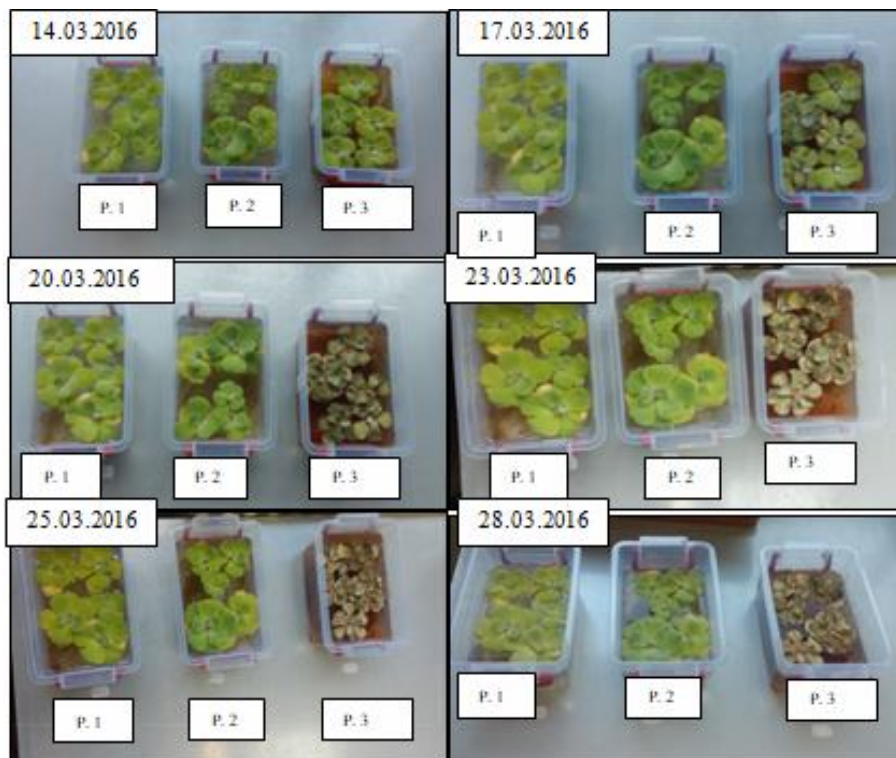


Figure 2. Images of the experiment with *Pistia stratiotes*. Variants: 1 = drinking plain water as blank; 2 = water from Abrudel river downstream Gura Rosiei tailings dump; 3 = water from Salistei Valley downstream Salistei tailings dump (photos by Dan Petaca).

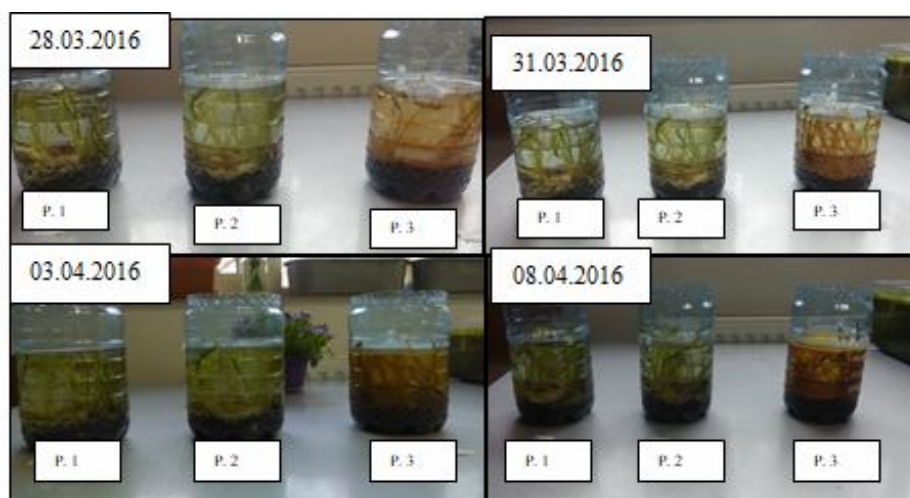


Figure 3. Images of the experiment with *Vallisneria spiralis*. Variants: 1 = drinking plain water as blank; 2 = water from Abrudel river downstream Gura Rosiei tailings dump; 3 = water from Salistei Valley downstream Salistei tailings dump (photos by Dan Petaca).

During the experiments the quality of water parameters was improved when *L. minor* was used. EC ( $\mu\text{S}/\text{cm}$ ) of the water from Abrudel river downstream tailings dump Gura Rosiei and of the water from Salistei Vallei downstream tailings dump had increased after two weeks with *L. minor* phytoremediation, with an efficiency  $E = 14.72\%$ . *L. minor* had TDS increasing of the water from Abrudel river downstream tailings dump Gura Rosiei and from the water of Salistei Valley downstream tailings dump. In water from Abrudel river downstream tailings dump Gura Rosiei, *L. minor* had an efficiency on the decreasing of REDOX potential of  $E = 94.28\%$ . In the water from Abrudel river downstream tailings dump Gura Rosiei, *L. minor* had an effect in reducing acidity from the pH 7 to 7.5, with an efficiency of 7.14% (Table 2).

Table 2

Quality parameters and metal analysis of water collected from tailings dumps at Rosia Montana and Salistei Valey and subject bioremediation experiment with *Lemna minor* (14.03.2016-28.03.2016)

Variants		EC ( $\mu\text{S}/\text{cm}$ )	TDS (mg/L)	Salinity (‰)	Eh (mV)	pH
Drinking plain water as blank	A	373	236	0.1	-18.9	6.9
	B	388	248	0.1	-54	7.4
Water from Abrudel river downstream GuraRosiei tailings dump	A	258	166	0	-24.5	7
	B	287	184	0	-47.6	7.5
Water from Salistei Valley downstream Salistei tailings dump	A	3570	2258.71	1.8	209.2	2.9
	B	4220	2697.31	2.2	230.8	2.6

A = before bioremediation (14.03.2016); B = after bioremediation (28.03.2016).

During the experiments the physico-chemical parameters of water were improved when *P. stratiotes* was used. EC ( $\mu\text{S}/\text{cm}$ ) of the water from Salistei Valley downstream tailings dump had increased after two weeks with *P. stratiotes* (with the efficiency  $E = 35.52\%$ ). *P. stratiotes* had TDS increasing of the water from Salistei Valley downstream tailings dump. In the water from Abrudel river downstream tailings dump Gura Rosiei, *P. stratiotes* had efficiency on the decreasing of REDOX potential of  $E = 64.34\%$ . In the water from Abrudel river downstream tailings dump Gura Rosiei, *P. stratiotes* had an effect in reducing acidity from the pH 7 to 7.3, with an efficiency of 4.28% (Table 3).

Table 3

Quality parameters and metal analysis of water collected from tailings dumps at Rosia Montana and Salistei Valley in the bioremediation experiment with *Pistia stratiotes* (14.03.2016-28.03.2016)

Variants		E.C ( $\mu\text{S/cm}$ )	TDS (mg/L)	Salinity (‰)	Eh (mV)	pH
Drinking plain water as blank	A	373	236	0.1	-18.9	6.9
	B	343	219	0.1	-48	7.4
Water from Abrudel river downstream GuraRosiei tailings dump	A	258	166	0	-24.5	7
	B	239	152	0	-41	7.3
Water from Salistei Valley downstream Salistei tailings dump	A	3570	2258.77	1.8	209.2	2.9
	B	4410	2815.71	2.3	231.7	2.6

A = before bioremediation (14.03.2016); B = after bioremediation (28.03.2016).

During the experiments the quality of water parameters was improved when *V. spiralis* was used. EC ( $\mu\text{S/cm}$ ) of the water from Abrudel river downstream tailings dump Gura Rosiei and Water from Salistei Valley downstream tailings dump had increased after two weeks with *V. spiralis* (with the Efficiency of E = 284.1% for Abrudel water and 3.36% for Salistei Valley water). *V. spiralis* had TDS increasing of the water from Abrudel river downstream tailings dump Gura Rosiei and from the water of Salistei Valley downstream tailings dump. In water from Abrudel river downstream tailing dump Gura Rosiei with *V. spiralis* had efficiency on the decreasing of REDOX potential (E = 94.28%). In the water from Abrudel river downstream tailings dump Gura Rosiei, *V. spiralis* had an effect in reducing acidity from pH 7 to 8.2 with an efficiency of 17.14% (Table 4).

Table 4

Quality parameters and metal analysis of water collected from tailings dumps at Rosia Montana and Salistei Valley and in the bioremediation experiment with *Vallisneria spiralis* (28.03.2016-08.04.2016)

Variants		E.C ( $\mu\text{S/cm}$ )	TDS (mg/L)	Salinity (‰)	Eh (mV)	pH
Drinking plain water as blank	A	373	236	0.1	-18.9	6.9
	B	1070	685	0.5	-103.6	8.5
Water from Abrudel river downstream GuraRosiei tailings dump	A	258	166	0	-24.5	7
	B	992	632	0.4	-90	8.2
Water from Salistei Valley downstream Salistei tailings dump	A	3570	2258.77	1.8	209.2	2.9
	B	3690	2368.28	1.9	190.0	3.6

A = before bioremediation (14.03.2016); B = after bioremediation (28.03.2016).

The phytoextraction in water from Abrudel river downstream tailings dump Gura Rosiei with *L. minor* species has different efficacy during the experiment: for Na 25.44%; K 74.19%; Ba 66.90%; Al 66.90%; Ni 62.27%; Zn 48.37%, and Mn 62.57%. Phytoextraction in water from Abrudel river downstream tailings dump Gura Rosiei with *P. stratiotes* in the same sample has efficacy of phytoextraction after two weeks: for Cd 100%; Ni 78.63%; Zn 32.91%, and for Mn 28.07%. *V. spiralis* species has different efficacy during the experiment: for Pb 25.80%. The phytoextraction in the water from Salistei Valley downstream tailings dump with *L. minor* species has different efficacy during the experiment: for Ba 6.38%; Al 30.48%; Co 33.33%; Ni 21.10%, and Pb 60.86%. The phytoextraction in the water from Salistei Valley downstream tailings dump with *P. stratiotes* species has the efficacy for Co 33.33%. Phytoextraction in water from Salistei Valley downstream tailings dump with *V. spiralis* species has different efficacy during the experiment: for Mn 9.82% and for Fe 60.86% (Table 5).

After the experiment of bioaccumulation of pollutants in the green tissue of aquatic species (mg/kg dry mater) (Table 6), in the water from Abrudel river downstream tailings dump Gura Rosiei, *L. minor* species has accumulated most Fe, Al, Zn, Cu, and *P. stratiotes* accumulated most Pb. In the water from Salistei Valley downstream tailings dump Salistei Valley, *L. minor* has accumulated most Fe, and *V. spiralis* has accumulated most Al, and most Cr. *L. minor* species in drinking plain waters blank accumulated most Cr (Table 6).

Table 5

Tests on pollutant concentration (µg/L) of acid water from mining area Rosia Montana after phytoextraction with aquatic plants (*Lemna minor*, *Pistia stratiotes*, *Vallisneria spiralis*) increased for 14 days on different types of water

	Before phytoremediation			After phytoremediation								
	V1	V2	V3	<i>Lemna minor</i> + V1	<i>Lemna minor</i> + V2	<i>Lemna minor</i> + V3	<i>Pistia stratiotes</i> + V1	<i>Pistia stratiotes</i> + V2	<i>Pistia stratiotes</i> + V3	<i>Vallisneria spiralis</i> + V1	<i>Vallisneria spiralis</i> + V2	<i>Vallisneria spiralis</i> + V3
µg/L	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Li	64.4	2.3	7.0	54.7	1.2	7.1	70.0	2.4	8.0	59.1	10.2	24.9
Na	25566.2	2226.6	2227.2	22744.4	1659.1	3767.0	25361.0	3043.7	4563.9	41563.4	19890.9	20059.5
Mg	1736.6	1827.9	23442.7	954.5	1220.1	26416.4	1760.6	2645.1	28950.5	5744.4	7962.7	37642.4
Al	52.9	108.6	83.3	28.1	36.6	58.0	85.5	187.6	170.3	67.1	97.9	844.1
K	1915.1	757.2	10017.3	274.2	195.4	17481.8	2732.4	878.7	23936.8	45048.8	47362.1	67465.1
Ca	10484.8	18209.5	79270.0	6662.1	17240.5	87033.2	8685.8	23805.5	89773.2	21386.6	29889.2	178238.3
Ti	20.6	36.5	390.8	14.8	32.8	409.4	19.3	47.6	448.6	44.5	70.3	476.7
V	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.3	0.4
Cr	0.2	1.0	2.4	0.0	0.9	4.4	0.0	0.0	3.1	0.0	0.0	6.2
Mn	10.2	17.1	43329.4	34.5	6.4	45046.0	37.8	12.3	47594.2	37.9	83.9	39074.0
Fe	204.2	418.8	163309.2	304.7	403.6	166971.2	490.0	461.5	167396.5	395.6	499.5	18435.5
Co	0.6	0.3	36.6	0.2	0.2	40.3	0.2	0.2	42.7	0.5	0.6	55.5
Ni	10.3	22.0	54.5	3.8	8.3	43.0	4.4	4.7	90.0	7.4	8.9	79.3
Cu	9.2	15.8	13.2	14.6	16.0	75.9	12.1	20.4	108.8	28.3	20.1	45.4
Zn	21.3	40.1	166.4	18.3	20.7	219.8	20.8	22.7	319.6	18.8	26.9	303.6
Cd	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.3	0.1	0.1	0.9
Ba	15.9	27.8	9.4	6.2	9.2	8.8	13.9	21.4	21.4	42.6	26.7	102.9
Pb	2.1	3.1	4.6	4.3	5.5	1.8	1.4	2.9	4.6	2.7	2.3	13.3

V1 = drinking plain water as blank; V2 = water from Abrudel river downstream Gura Rosie tailings dump; V3 = water from Salistei Valley downstream Salistei tailings dump.

Table 6

Tests concerning the bioaccumulation of pollutants (mg/kg d.m.) in aquatic plants (*Lemna minor*, *Pistia stratiotes*, *Vallisneria spiralis*), increased for 14 days in different types of water

	<i>Lemna minor</i> + V1	<i>Lemna minor</i> + V2	<i>Lemna minor</i> + V3	<i>Pistia stratiotes</i> + V1	<i>Pistia stratiotes</i> + V2	<i>Pistia stratiotes</i> + V3	<i>Vallisneria spiralis</i> + V1	<i>Vallisneria spiralis</i> + V2	<i>Vallisneria spiralis</i> + V3
mg/kg d.m.	P1	P2	P3	P4	P5	P6	P7	P8	P9
Li	76.4	3.7	0.4	12.1	2.4	0.2	13.1	4.6	1.4
Na	27330.2	12099.8	297.4	6772.0	2280.2	155.3	6416.7	4235.3	1436.9
Mg	6246.9	5217.3	804.5	3393.1	3023.1	614.8	3093.2	2350.6	1254.8
Al	203.3	739.5	67.3	179.6	603.7	33.5	266.8	180.8	236.6
K	38441.2	36145.4	1015.0	S	S	2348.9	32466.0	31443.4	4105.1
Ca	21333.7	19718.6	0.0	17954.9	15900.4	6224.0	5222.7	5621.0	0.0
Ti	77.2	75.5	9.7	43.5	41.6	3.4	14.0	18.4	15.0
V	296.1	161.5	48.5	34.1	19.6	5.5	91.2	78.0	41.0
Cr	21.4	13.3	5.2	7.8	4.2	1.5	8.0	4.4	8.0
Mn	200.0	671.9	1579.4	58.7	169.6	744.4	311.8	223.5	767.2
Fe	2993.5	6167.2	144849.1	544.4	1502.6	58284.7	2011.5	879.6	33574.1
Co	1.2	3.2	1.5	0.5	1.2	0.6	2.1	1.1	2.3
Ni	10.8	11.4	1.7	17.7	16.4	2.5	9.1	6.0	7.0
Cu	198.8	236.3	10.5	100.6	81.4	4.1	54.4	41.9	49.2
Zn	157.2	199.2	9.3	172.9	148.8	9.3	126.2	78.5	49.5
Cd	0.4	1.0	0	0.2	0.4	0	0.5	0.5	0.3
Ba	35.6	65.7	4.8	87.1	83.1	10.4	26.4	16.8	16.7
Pb	1.4	4.3	0.9	2.2	6.2	0.7	2.4	1.5	5.4

V1 = drinking plain water as blank; V2 = water from Abrudel river downstream Gura Rosie tailings dump; V3 = water from Salistei Valley downstream Salistei tailings dump.

In water from Abrudel river downstream tailings dump Gura Rosie *L. minor* has shown a phytoremediation efficiency on increasing pH 7.14 % and EC ( $\mu\text{S}/\text{cm}$ ) (14.72%); on decreasing in trend negative of Eh (mV) (94.2%) and increasing TDS (mg/L) (10.84%). *P. stratiotes* has shown a phytoremediation efficiency on increasing pH 4.28 %; on decreasing of trend negative Eh (mV) (67.34.2%) and decreasing TDS (mg/L) (8.43%). *V. spiralis* has shown a phytoremediation efficiency on increasing pH 17.14 % and EC ( $\mu\text{S}/\text{cm}$ ) (284.49%); on decreasing of Eh (mV) (267.34%) and decreasing TDS (mg/L) (280%). The phytoextraction in the water from Abrudel river downstream tailings dump Gura Rosie have different efficacy. *L. minor* plants have extracted with an efficiency of 74.18% for K; Na 25.54%; Ba 66.90%; Al 66.29%; Co 33.33%; Cr 10%; Ni 62.27%; Mn 62.57%; Zn 48.37%; Fe 3.62%. *P. stratiotes* has different efficacy in the pollutants phytoextraction: Ba 23%; Co 33.33%; Ni 78.63%; Mn 28%; Zn 43.39%; Pb 6.45%; Cd 100%. *V. spiralis* plant has extracted with an efficiency of 3.95% for Ba ; Al 9.85%; Ni 59.54%; Zn 32.91%, Pb 25.80%. From the water of Abrudel river, downstream tailings dump Gura Rosie, the bioaccumulation of pollutants in the green tissue (mg/kg dry mater) to *L. minor* was highest for Al, Co, Cu, Zn, Pb; *P. stratiotes* plants accumulated the most Al, Co, Pb and *V. spiralis* plants did not have higher accumulations compared to the control variant.

In water from Salistei Valley downstream tailings dump *L. minor* has shown a phytoremediation efficiency on increasing acidity pH 2.9 to 2.6 and increasing EC ( $\mu\text{S}/\text{cm}$ ) (18.20%); on decreasing in trend positive of Eh (mV) (10.32%) and increasing TDS (mg/L) (19.41%). *P. stratiotes* has shown a phytoremediation efficiency on increasing acidity pH 2.9 to 2.6; on increasing in trend positive Eh (mV) (10.75%) and increased TDS (mg/L) (24.69%). *V. spiralis* has shown a phytoremediation efficiency on increasing pH 24.13 % and EC ( $\mu\text{S}/\text{cm}$ ) (3.36%); on decreasing of Eh (mV) (9.18%) and increasing TDS (mg/L) (4.84%). The phytoextraction in the water from Salistei Valley downstream tailings dump the aquatic species have different efficacy: *L. minor* plants have extracted: Ba 6.35%; Al 30.48%; Ni 21.04%; Pb 60.86%; *V. spiralis* plant has extracted Mn 9.82% and Fe 88.71%; *P. stratiotes* had the effect of increasing: Na, K , Ba, Al, Co, Cr, Ni, Mn, Zn, Cu, Pb, Cd, Fe (Figure 4).

In the water from Salistei Valley downstream tailings dump, the bioaccumulation in the green tissue sample (mg/kg dry mater) of *L. minor* accumulated most Mn, Fe; *P. stratiotes* accumulated most K, Mn, Fe; *V. spiralis* accumulated most Co, Cr, Mn, Pb, Fe (Figure 5).



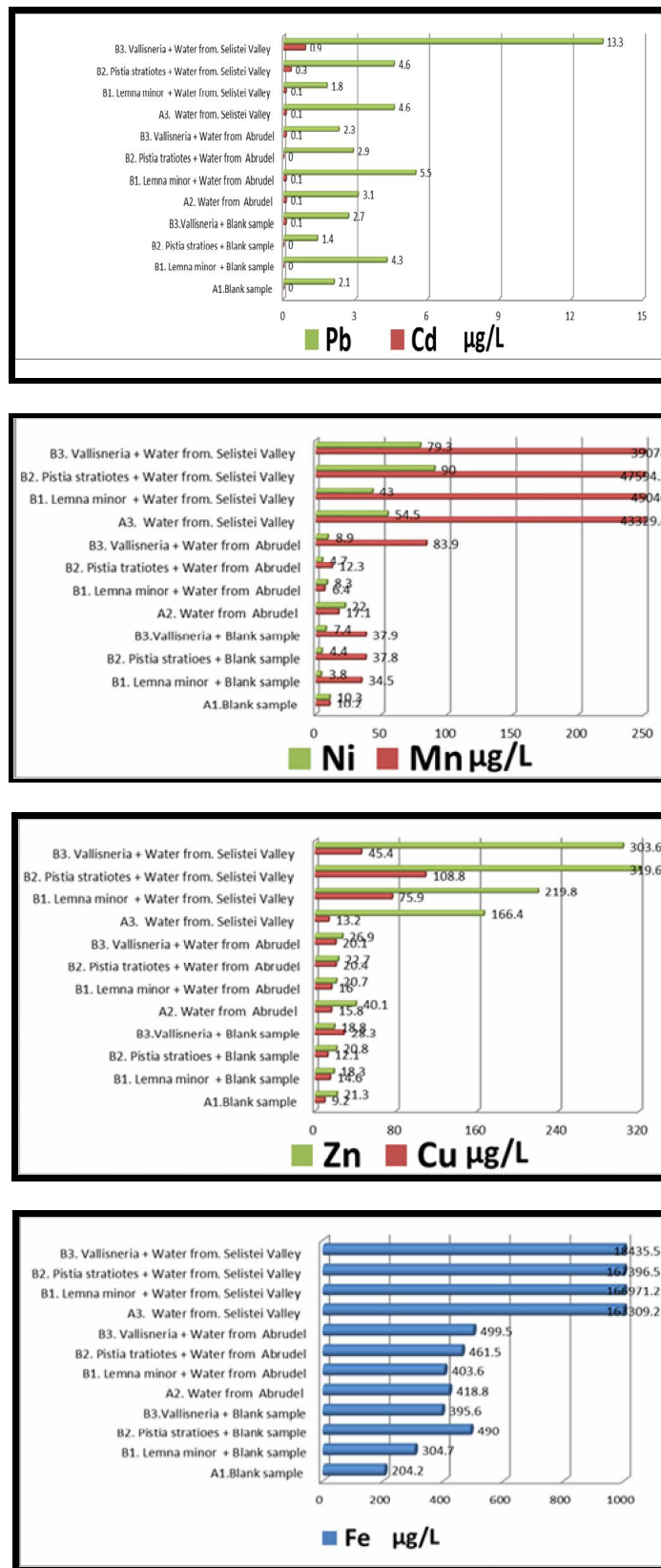


Figure 4. Heavy metal concentrations, phytoextraction efficiency (28.03.2016-08.04.2016).

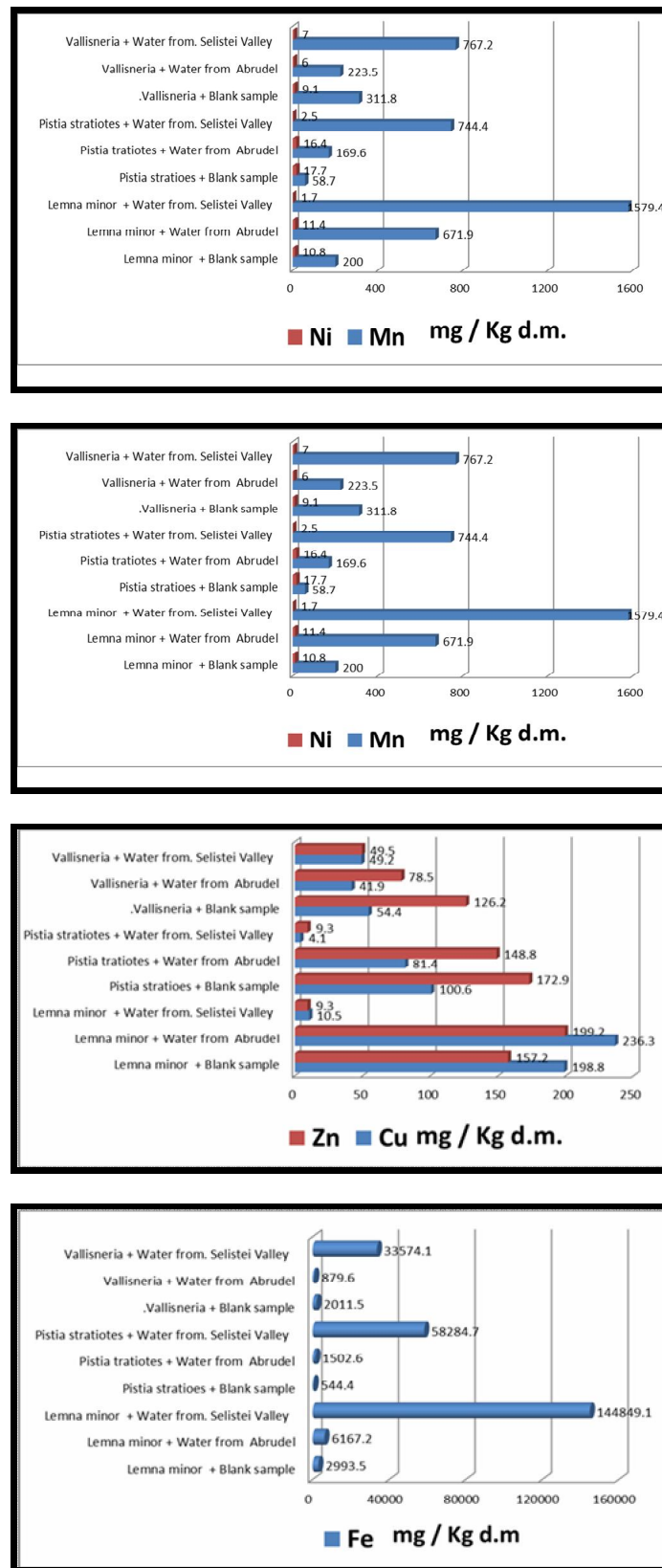


Figure. 5. Heavy metal Bioaccumulation (28.03.2016-08.04.2016).

**Conclusions.** The analysis of physico-chemical parameters showed an improvement after the fitoremediation with aquatic species. The results from the laboratory experiment confirmed that associating plants as *Lemna minor*, *Pistia stratiotes* and *Vallisneria spiralis* is a viable method for decontamination and improvement of the physical and chemical parameters of polluted water. These aquatic species are important for the phytoremediation effects produced by phytoextraction and bioaccumulation of heavy metals. These can be used in biotechnological systems of constructed wetlands.

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Authors:

Alexandru Dan Petaca, Babeş-Bolyai University, Faculty of Environmental Sciences and Engineering, 30 Fântânele Str., 400294 Cluj-Napoca, Romania, e-mail: dan\_petaca@yahoo.com

Dana Malschi, Babeş-Bolyai University, Faculty of Environmental Sciences and Engineering, 30 Fântânele Str., 400294 Cluj-Napoca, Romania, e-mail: danamalschi@yahoo.com

Erika Levei, INCDO INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath Str., 400293, Cluj-Napoca, e-mail: erika.levai@icia.ro

Claudiu Tanaselia, INCDO INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath Str., 400293, Cluj-Napoca, e-mail: claudiu@tanaselia.ro

Carla Nicoară, Babeş-Bolyai University, Faculty of Environmental Sciences and Engineering, 30 Fântânele Str., 400294 Cluj-Napoca, Romania, e-mail: carlanicoara@yahoo.com

Elena Rinba, Babeş-Bolyai University, Botanical Garden, 42 Republicii Str., Cluj-Napoca, Romania, e-mail: rinba.elena@yahoo.com

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