Environmental Engineering and Management Journal July 2018, Vol.17, No. 7, 1753-1764
http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu

"Gheorghe Asachi" Technical University of Iasi, Romania

RESEARCH ON WASTEWATERS BIOREMEDIATION WITH AQUATIC SPECIES FOR CONSTRUCTED WETLANDS

Dana Malschi[1]*, Liviu Muntean[1], Ioan Oprea [1], Carmen Roba[1], Gabriela Popiţa[1], Lucrina Ştefănescu[1], Bianca Malschi Florian[2], Elena Rînba[3]

[1]*Babeş-Bolyai University, Faculty of Environmental Sciences and Engineering, 30 Fântânele Street, 400294, Cluj-Napoca, Romania*
[2]*National Environmental Protection Agency, 99-101 Dorobanţilor, Cluj-Napoca, Romania*
[3]*Babeş-Bolyai University, Botanical Garden, 42 Republicii, Cluj-Napoca, Romania*

Abstract

With the aim of contribute to a bioremediation technology drafting, this paper presents a preliminary research on bioremediation of wastewaters polluted with heavy metals and other toxic contaminants. Bioremediation tests were performed with aquatic species *Lemna minor, Vallisneria spiralis, Eichhornia crassipes, Hydrilla verticillata, Pistia stratiotes, Cladophora glomerata,* on samples of contaminated waters resulting from the toxic pond of waste landfill Șomârd-Mediaș, Sibiu County, Romania, during the 2012-2013 period. The studies were conducted in microcontainers with contaminated water using constructed wetlands. In order to check the phytoextraction and bioremediation potential of these species, a comparative analysis of water samples was performed, based on the determination of heavy metals and different pollution parameters. The results on phytoextraction tests have shown that: *L. minor* plants were able to extract Cu, Fe and Pb; *V. spiralis* plants were able to extract Cu, Fe, Zn and Ni; *H. verticillata, E. crassipes, P. stratiotes* and green algae *C. glomerata* were able to extract Cu, Fe and Zn. This research has shown that the aquatic plants grown for two weeks in the toxic pond water have important effects on wastewater bioremediation. All species have shown a significant decrease of NH_4^+ concentration. *L. minor, V. spiralis, H. verticillata, E. crassipes, P. stratiotes* have determined the increase of Eh and the decrease of CCO-Mn, TDS, EC. Stronger effects of bioremediation in the tests with ½ diluted water from the toxic pond were recorded. The results prove the significance of the bioremediation of contaminated waters using aquatic species for constructed wetlands in order to implement this biotechnology.

Key words: bioremediation, *Eichhornia crassipes, Lemna minor, Pistia stratiotes, Vallisneria spiralis*

Received: February, 2014; Revised final: September, 2014; Accepted: October, 2014; Published in final edited form: July 2018

1. Introduction

Studies on the bioremediation of the contaminated sites entail a wide approach due to the opportunities of drafting the adequate technologies for biological depollution (Barbu and Sand, 2004; Buta et al., 2011; Elekes et al., 2010; Gavrilescu, 2010; Glick and Stearns, 2011; Malschi, 2009, Malschi et al., 2013; Oros, 2002, 2011; Pivetz, 2001).

Bioremediation of contaminated aquatic ecosystems may be conducted by using aquatic plant associations and microorganisms involved in biological depollution. Waters polluted with heavy metals may be efficiently treated by artificial constructed wetland ecosystems. The removal of heavy metals by constructed wetland has been the subject of significant researches (Dickinson et al., 2009; Jing et al., 2001; Juang and Chen, 2007; Kuschk et al., 2005; Rai, 2008; Wang et al., 2016).

Numerous experimental results highlighted the effect of the common duckweed *Lemna minor L.*, Araceae family in phytoremediation, by pollutants

* Author to whom all correspondence should be addressed: e-mail: danamalschi@yahoo.com, Phone: +40745 367373, Fax: +40264-30 70 30

bioaccumulation, metals accumulation and wastewater treatment (Dosnon-Olette et al., 2011; Malschi et al., 2013; Rahmani and Sternberg, 1999; Rahman et al., 2007; Willey, 2007). Bioremediation research has revealed the effect of aquatic species such as eel grass V*allisneria spiralis L.* (Hydrocharitaceae) (McCutcheon and Schnoor, 2004; Masi et al., 2002; Núñez et al., 2011; Sim et al., 2007 etc.) on metals hyperaccumulation (Malschi et al., 2013; Oprea et al., 2013); water hyacinth *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae) on biosorption and metals accumulation (Buta et al., 2011); waterthyme *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae), water lettuce *Pistia stratiotes* L. (Araceae) (Stoica et al., 2009) and green algae *Cladophora glomerata* (L.) Kütz. (Chlorophyta) (Yalçına et al., 2008) on metals accumulation and wastewater treatment.

Studies on biological pollution indicators in the aquatic environment reveal an important interest due to the significance of biomonitoring in pollution assessment, determination of ecotoxicity and development of bio- and eco-technologies (Glick and Stearns, 2011; Malschi, 2009; Oros, 2002, 2011; Oros and Drăgici, 2002; Pavel et al., 2012).

Constructed wetlands are used for the decontamination of industrial wastewater and leachate from wastes landfills (Jing et al., 2001; Kuschk et al., 2005; Masi et al., 2002; Sim et al., 2007). The constructed wetlands provide the biological processes determining biological degradation, transformation, biosorption and phytoextraction of pollutants by aquatic species (Dyck et al., 2009; Juang and Chen, 2007). The use of constructed wetlands (composed of water plants, green algae, cyanobacteria and various species with high biological effect of pollutants bioaccumulation) can remove various heavy metals and other pollutants (Jing et al., 2001; Kuschk et al., 2005; Malschi, 2009; Malschi et al., 2013; Oprea et al., 2013; Xu et al., 2016).

This paper presents preliminary research to prove the importance of the contaminated waters bioremediation technology using aquatic species for constructed wetlands. The novelty of the paper consists in controlling the heavy metals phytoextraction potential of the tested species and measuring the bioremediation potential of waste waters quality parameters. In order to check the phytoextraction and bioremediation potential of these species, the experimental tests were performed with *L. minor, V. spiralis, E. crassipes, H. verticillata, P. stratiotes, C. glomerata.*

2. Material and methods

During the 2012-2013 period, researches on bioremediation of polluted waters were conducted in the Biotechnology Laboratory of the Faculty of Environmental Science and Engineering within the Babeş-Bolyai University, Cluj-Napoca. The polluted water samples were collected from the toxic pond of municipal and industrial waste landfill Şomârd-Mediaş, Sibiu County.

Various toxic pollutants and heavy metals have accumulated in this pond causing an important environmental problem, which has been intensely studied (Mihăiescu et al., 2010; Muntean, 2003; Oprea et al., 2010).

Lemna minor, Vallisneria spiralis, Eichhornia crassipes, Hydrilla verticillata, Pistia stratiotes and *Cladophora glomerata* have been used to study the bioremediation and phytoextraction of water pollutants, in micro containers tests such as constructed wetlands. The biologic material of the aquatic species has been grown under greenhouse conditions at the Botanical Garden of the Babeş-Bolyai University, Cluj-Napoca. During the experiment, the best conditions for plant growing in laboratory were provided in transparent plastic pots adequate as volume for the waters as follows: 700 mL for *L. minor* variants, 1 liter for *P. stratiotes* variants, 2 liters for the variants of *E. crassipes, V. spiralis, H. verticillata*, 400 mL for *C. glomerata* variants. The experimentation time was of two weeks for each aquatic species (24.10.2012-07.11.2012) with additional light for 16 hours daily. Three types of experimental waters for each species were placed in micro containers: 1. drinking plain water as blank; 2. water collected from the toxic pond of Şomârd waste landfills; 3. water with the 1/2 dilution from the toxic pond of Şomârd waste landfills. The development of plants phytotoxic effects of water samples subjected to testing and the phytoremediation effects were highlighted by photographing the visible differences between the samples compared to the situation in the unpolluted blank sample.

Before and after phytoremediation and phytoextraction tests, analysis of water quality parameters of the samples were conducted. A multiparameter type WTW Multi 350 was used for the following parameters: the values of O_2, Eh-redox potential, CCO-Mn, EC-electrical conductivity, TDS-total dissolved solids, S–salinity.

The concentrations of anions (F^-, Cl^-, NO_2^-, NO_3^-, SO_4^{2-}) and cations (Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, NH_4^+) have been determined using ICS 1500 Dionex Ion Chromatography (IC) analysis method. Water samples were filtered with qualitative filter paper 125 mm and before analyzing were diluted to an electrical conductivity lower than 100 μS/cm. An analytical column IonPac AS23 for ion exchange was used for the separation of the major anions. The suppressor was an ASRS 300 ULTRA II (4 mm) Anions Self Regenerating Suppressor. As anion mixture standard Seven Anion Standard II (Dionex, USA) was used. The injection volume was 25 μL, and the flow rate 1.0 mL/min, the eluent was an aqueous solution consisting of 4.5 mM Na_2CO_3 and 0.8 mM $NaHCO_3$.

For the separation of the major cations, an Analytical column IonPac CS12A for ion exchange was used and a CSRS 300 ULTRA II (4 mm) Cations Self Regenerating Suppressor. Six Cation II Standard (Fluka, Germany) has been used as cation standard mixture. The eluent was an aqueous solution consisting of 20 mM methanesulfonic acid.

Heavy metal concentrations (Pb, Cu, Zn, Ni, Fe) have been determined with atomic absorption spectrometry (AAS) (ZEEnit 700 Analytik Jena) method for determination of trace elements from solution (flame method, mg/L). Before analyzing, 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.

3. Result and discussions

Previous studies performed in 2009-2010 on the bioaccumulation potential of pollutants recommend the cultivation of *L. minor* plants as a method of bioremediation. Bioaccumulation of heavy metals and metalloids was studied on *L. minor* plants grown in polluted waters. *L. minor* was noticed to be a very good bioaccumulator for Cr, Mn, Zn, As, Ba and a moderate bioaccumulator of Cu, Pb, V from the polluted waters in the waste heaps and tailings dams areas of mining operations, toxic ponds and waste platforms in the chemical industry (Malschi et al., 2013). The study on the parameters of polluted water revealed an extremely high level of heavy metals (Fe, Cd, Zn) pollution in the water samples collected from Şomârd-Mediaş municipal waste landfill, in Sibiu county, also mentioned in previous studies (Mihăiescu et al., 2010; Muntean, 2003; Oprea et al., 2010). The results obtained after applying the Pb, Cd, Zn phytoextraction and bioremediation tests (Oprea et al., 2013) proved the role of the wastewater bioremediation treatment using *L. minor* and *V. spiralis* as component of constructed wetlands in lab. micro containers. After two weeks of bioremediation with *V. spiralis* it was noticed that these plants have a great capacity to biotransform the pollutants and to make them form a viscous oil film on the water surface.

The new tests of bioremediation and phytoextraction with *L. minor* (Fig.1), *P. stratiotes* (Fig. 2), *E. crassipes* (Fig. 3), *V. spiralis* (Fig. 4), *H. verticillata* (Fig. 5), *C. glomerata* (Fig. 6A) were performed on waste water samples collected from historically polluted sites, from the toxic pond of municipal and industrial waste landfill Şomârd, Sibiu county, during 2012-2013. In order to study the phytoextraction and bioremediation potential of aquatic species, a comparative analysis was performed between the samples of polluted water and control unpolluted water, in terms of pollution parameters modifications depending on plants activity.

The experimental data obtained indicate that significant effects on heavy metals phytoextraction and biosorption were noticed in *L. minor, V. spiralis, E. crassipes, H. verticillata, P. stratiotes, C. glomerata*. Biotransformation of some toxic contaminants and their removal from the water in a film of oil on the water surface has been shown for *V. spiralis, H. verticilata, E. crassipes, C. glomerata* (Fig. 6B). Present experimental results have illustrated that *L. minor, V. spiralis, E. crassipes, H. verticillata, P. stratiotes, C. glomerata* have a very important role in the bioremediation of wastewater pollution with various toxic compounds and heavy metals, and are recommended for use in micro ecosystems of constructed wetlands (Fig. 7).

3.1. Phytoextraction/biosorption of heavy metal with aquatic species with plants and green algae

The concentrations of heavy metals in water samples collected from the Şomârd/Mediaş landfill toxic pond in 2012 exceeded the maximum permitted limits for surface waters (Ord. 161, 2006). Toxic pond water has such concentrations of heavy metals that it fitted the 5th water quality class for Cu and Pb, the 3rd water quality class for Ni and the 2nd water quality class for Zn, Fe.

By biosorption and phytoextraction on the experimental tests (during the 24.10.2012-07.11.2012 period), the plants of *L. minor, V. spiralis, H. verticillata, E. crassipes, P. stratiotes* and the green algae *C. glomerata* were able to extract copper. Only *L. minor* plants extracted lead and only *V. spiralis* plants extracted nickel. The plants of *L. minor, V. spiralis, H. verticillata, E. crassipes, P. stratiotes* and green algae *C. glomerata* were able to extract iron. *V. spiralis, H. verticillata, E. crassipes, P. stratiotes* plants and *C. glomerata* were able to extract zinc (Fig. 8).

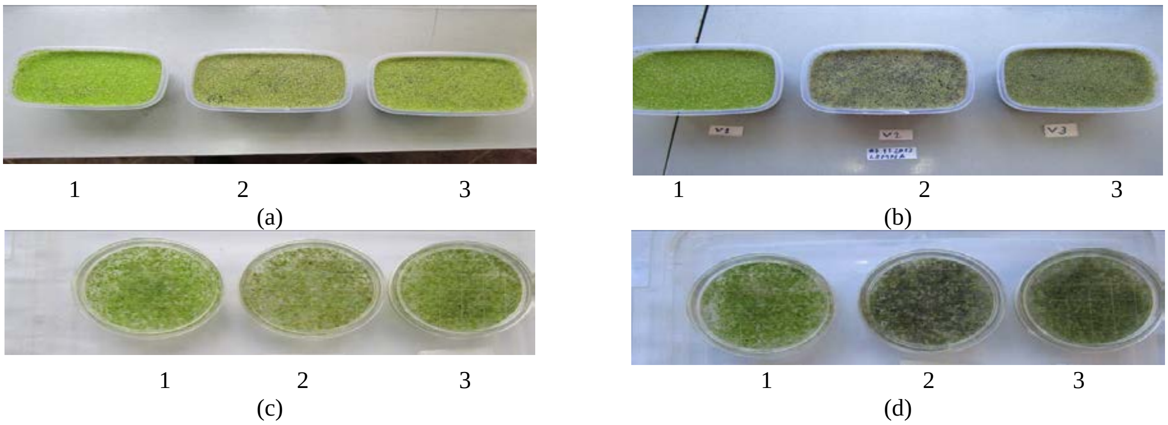
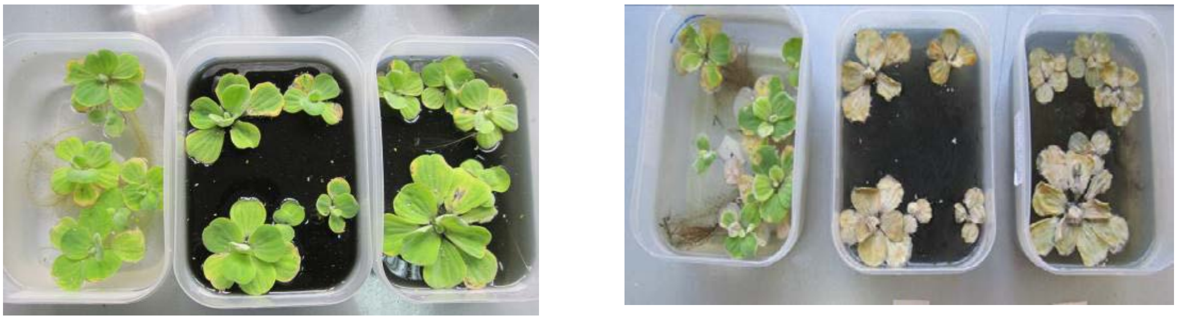


Fig. 1. Phytoextraction and biomonitoring with *Lemna minor* in water from Şomârd waste landfills: (a) Phytoextraction tests during 23-29.10.2012; (b) Phytoextraction tests during 23.10-6.11.2012; (c) Biomonitoring tests/29.10.2012; (d) Biomonitoring tests/6.11.2012. 1=Control, 2=water from toxic pond, 3=the 1/2 dilution of water from the toxic pond



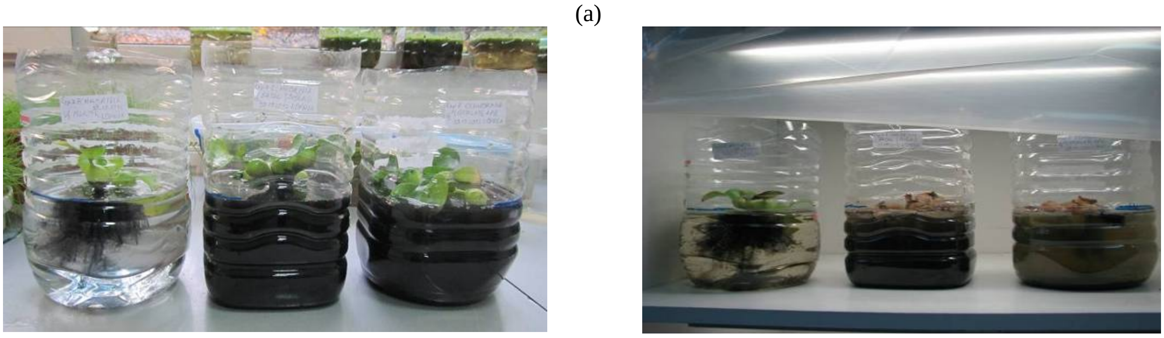
1 2 3 1 2 3

(a) (b)

Fig. 2. Phytoextraction tests with *Pistia stratiotes* in water from Şomârd-Mediaş waste landfills: (a) Before phytoextraction/23.10.2012; (b) After phytoextraction tests/6.11.2012. 1=Control, 2=water from the toxic pond, 3= the 1/2 dilution of water from the toxic pond

1 2 3

(a)



1 2 3 1 2 3

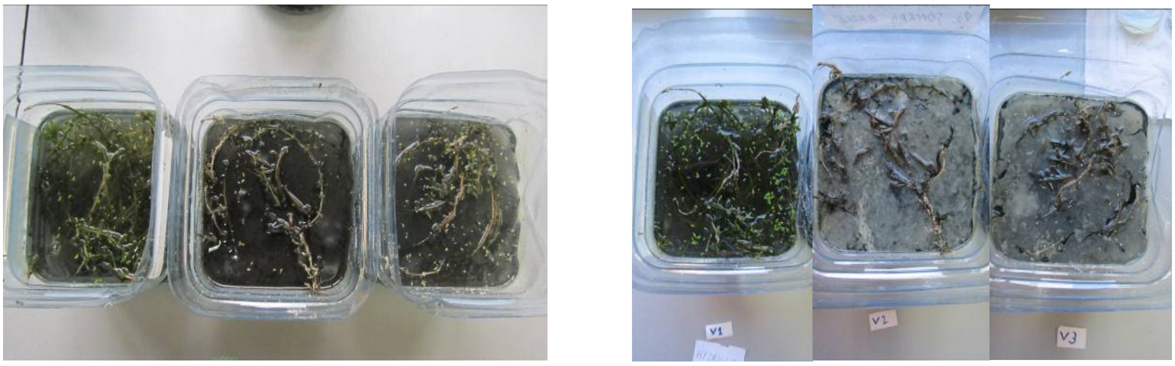
(b) (c)

Fig. 3. Phytoremediation tests with *Echhornia crassipes* in water from Şomârd-Mediaş waste landfills.
a) and b) Phytoextraction tests/29.10.2012; c) After phytoextraction/13.11.2012. 1=Control, 2=water from the toxic pond, 3= the 1/2 dilution of water from the toxic pond

1 2 3 1 2 3

(a) (b)

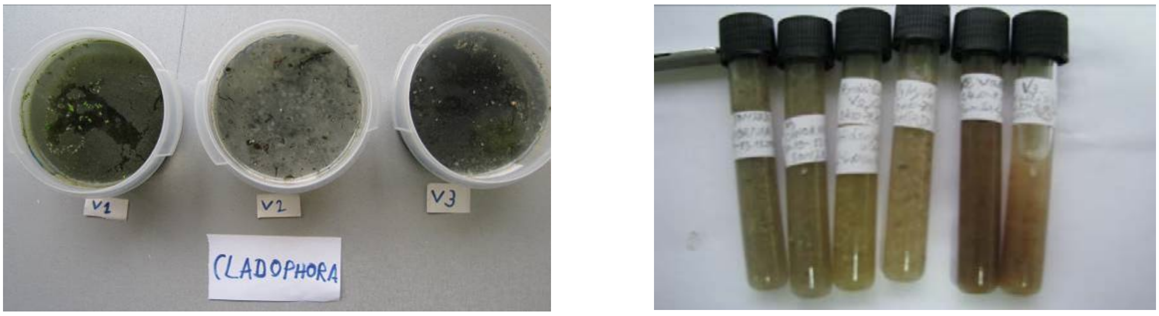
Fig. 4. Phytoremediation tests with *Vallisneria spiralis* in water from Şomârd-Mediaş waste landfills. a) Before phytoextraction/24.10.2012; b) After phytoextraction/6.11.2012. 1=Control, 2=water from the toxic pond, 3=the 1/2 dilution of water from the toxic pond



1 2 3 1 2 3

(a) (b)

Fig. 5. Phytoremediation tests with *Hydrilla verticillata* in water from Şomârd-Mediaş waste landfills.
a) Phytoextraction/29.10.2012; b) After phytoextraction/6.11.2012. 1=Control, 2=water from the toxic pond, 3= the 1/2 dilution of water from the toxic pond



1 2 3 1 2 3

(a) (b)

Fig. 6. Bioremediation with *Cladophora glomerata* in water from Şomârd waste landfills
a) Bioremediation tests with *Cladophora glomerata*, after phytoextraction/15.11.2012. 1- control, 2 - water from the toxic pond, 3 - the 1/2 dilution of water from the toxic pond, b) Biotransformation of pollutants from waste water after phytoremediation. Isolation of pollutants oily film after the treatment with: 1=*Cladophora glomerata*, 2=*Hydrilla verticillata;* 3=*Valisneria spiralis*

1. Bioremediation tests /14-25.11.2012

2. Bioremediation tests /14-25.11.2012

2. Photos of lab tests / 25.11.2012

Fig. 7. Bioremediation in laboratory micro-constructed wetlands consisting in aquatic species associations: *Lemna minor*, *Valisneria spiralis*, *Eichhornia crassipes*, *Hydrilla verticillata*, *Pistia stratiotes*, *Cladophora glomerata* in toxic pond polluted water from Şomârd-Mediaş waste landfills. 1- bioremediation tests /14-25.11.2012 / with species association in 1/2 dilution toxic pond water. 2 - bioremediation tests /14-25.11.2012 / with species association in 1/4 dilution toxic pond water

Preliminary results on phytoextraction tests during 24/10/2012 - 07/11/2012 have shown that: *L. minor* plants have extracted Cu, Fe and Pb, *V. spiralis* plants have extracted Cu, Zn, Fe, Ni, *H. verticillata*, *E. crassipes*, *P. stratiotes* and *C. glomerata* have extracted Cu, Zn and Fe (Fig. 8, Table 1)

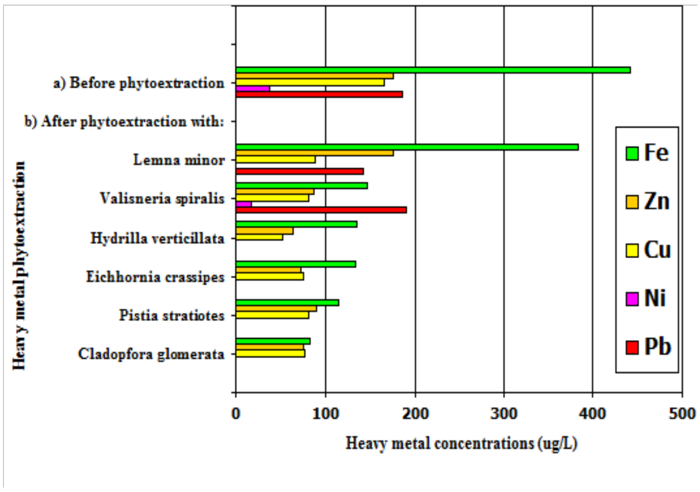


Fig. 8. Laboratory tests with *Lemna minor, Vallisneria spiralis, Eichhornia crassipes, Hydrilla verticillata, Pistia stratiotes, Cladophora glomerata* used in heavy metal biosorption from the Șomârd landfill toxic pond water /24.10.2012-07.11.2012. Heavy metal concentrations:
a) Before phytoextraction / 24.10.2012;
b) After phytoextraction with plants / 07.11.2012

Table 1. Chemical quality standards for some heavy metals to set out the ecological status of surface waters / maximum limits / quality classes (Ord. 161, 2006)

Quality indicator	***U/M***	***Quality classes***				
Heavy metals		1	2	3	4	5
Cu	µg/L	20	30	50	100	> 100
Zn	µg/L	100	200	500	1000	> 1000
Pb	µg/L	5	10	25	50	> 50
Cd	µg/L	0,5	1	2	5	> 5
Ni	µg/L	10	25	50	100	> 100
Fe^{2+} + Fe^{3+}	µg/L	300	500	1000	2000	> 2000

3.2. Bioremediation of wastewater from the toxic pond of Şomârd/Mediaş landfills with aquatic species L. minor, V. spiralis, E. crassipes, H. verticillata, P. stratiotes, C. glomerata

In 2012, the water from Şomârd/Mediaş landfills toxic pond had very high concentrations of anions Cl^-, F^-, SO_4^{2-} fitting the 5th quality class (poor) for surface waters (Ord. 161, 2006). After the phytoremediation experimental tests, in case of the control samples with drinking water, the N-NO_2^- appears in *Lemna*, *Vallisneria*, *Pistia*, *Cladophora*. The presence and activity of these species have led to the accumulation of nitrogen in the form of NO_2^-, in unpolluted waters. The environmental effect of nitrogen fixation does not occur in the toxic pond waters (Tables 2, 3). The presence of *L. minor* plants in drinking water determined the increase of concentrations of Cl^-, F^-, SO_4^{2-} and fixing N to NO_2^-. In the toxic pond water and in the ½ dilution toxic pond water, *L. minor* plants have determined the increase of $SO_4^{2-}$and decrease of Cl^-, F^-. *V. spiralis* plants in drinking water type determined the increase concentrations of F^-, SO_4^{2-}, decrease of Cl^- and fixing N to NO_2^- during testing. In the toxic pond water and in 1/2 dilution toxic pond water, *V. spiralis* determined the increase of SO_4^{2-} and decrease of Cl^-, F^-. *H. verticillata* determined the increase of Cl^-, F^- concentration, decrease of SO_4^{2-}. *H. verticillata* determined the increase of SO_4^{2-} and decrease of Cl^-, F^- in the toxic pond water and the ½ dilution toxic pond water. The *E. crassipes* plants determined the increase of Cl^-, F^-, SO_4^{2-} concentrations in drinking water, while *E. crassipes* plants determined the increase of SO_4^{2-}, Cl^- and a decrease of F^- in the toxic pond water and the 1/2 dilution toxic pond water.

The activity of *P. stratiotes* plants in drinking water determined the increase of Cl^-, F^-, SO_4^{2-} concentrations and fixing of N to NO_2^-. *P. stratiotes* plants have determined the increase of SO_4^{2-} and decrease of F^-, Cl^- in the toxic pond water, while in the 1/2 dilution toxic pond water, the plants have determined the increase of SO_4^{2-}, Cl^- and a decrease of F^-. *C. glomerata* produced the increasing of Cl^-, F^-, SO_4^{2-} concentrations and fixing N to NO_2^-. *C. glomerata* algae have determined the increase of Cl^-, SO_4^{2-} and decrease of F^- in toxic pond water and in ½ dilution toxic pond water (Tables 2, 3).

Very high concentrations of SO_4^{2-}, Cl^- and F^- were in toxic pond waters before the experiment. After phytoremediation experiments, the concentration of SO_4^{2-} increased. The concentration of Cl^-, F^- and salt were variable in different waters types of tests (Tables 2, 3). Preliminary research on phytoremediation of environmental status of waters have shown that: in the toxic pond water and the 1/2 dilution toxic pond water, *L. minor*, *V. spiralis and H. verticillata*, plants have determined the increase of SO_4^{2-} and decrease of Cl^-, F^- anions, while *E.crassipes* and *C. glomerata* have determined the increase of Cl^-, SO_4^{2-} and the decrease of F^-. *P. stratiotes* plantsproduced the increase of SO_4^{2-} and decrease of Cl^-, F^- anions in the toxic pond water.

3.3. Cations bioremediation with aquatic species

In 2012, the water from the toxic pond had a small amount of Mg^{2+}, very high concentrations of Na^+, K^+, NH_4^+ fitting the 5th quality class (poor) and moderate amount of Ca^{2+} (Tables 4, 5), fitting the 3rd quality class of surface waters and concentrations (Ord. 161, 2006). Aquatic plants grown for two weeks in unpolluted water (plain water) caused different changes as compared to the cation concentrations in the control water samples (before bioremediation tests).

L. minor, *E. crassipes*, *P. stratiotes* have caused the decrease of K^+ and Ca^{2+} concentration. *V. spiralis, H. verticillata* and *C. glomerata* caused the appearance of K^+ and the increase of the Ca^{2+} amount. The presence of aquatic plants reared for two weeks in toxic pond water has caused important changes compared to the control water samples.

P. stratiotes has decreased the concentration of K^+, Ca^{2+}, Mg^{2+}, Na^+, Li^+ and more pronounced Mg^{2+}. *L. minor* caused the decrease of Ca^{2+}, Mg^{2+} concentration and the increase of K^+ concentration; *V. spiralis* increased amount of K^+, Ca^{2+}, Mg^{2+}; while *H. verticillata and E. crassipes* decreased Ca^{2+} and increased K^+, Mg^{2+}. The *E. crassipes* increased Na^+ and Li^+. *C. glomerata* was revealed from the increase amount of K^+, Ca^{2+}, Mg^{2+}, Na^+, Li^+ (Tables 4, 5).

Preliminary results on bioremediation have shown that the presence of aquatic plants reared in water from the toxic pond has caused important effects on cations concentration. All species have resulted in significantly decreased amount of NH_4^+. *L. minor, H. verticillata, Eichhornia crassipes* have decreased Ca^2. With important effect of increasing cations concentrations have been noted: *L. minor, V. spiralis, H.verticillata, E. crassipes, C. glomerata* which have increased amount of K^+. The presence of plants grown for two weeks in water from the toxic pond with ½ dilution water determined changes as compared to the control samples.

Table 2. Laboratory tests for phytoremediation with aquatic species *Lemna minor, Vallisneria spiralis, Eichhornia crassipes, Hydrilla verticillata, Pistia stratiotes, Cladophora glomerata* of the anions environmental status of water from the Șomârd waste landfills toxic pond

Anions concentrations	***F^- mg/L***	***Cl^- mg/L***	***NO_2^- mg N/L***	***NO_3^- mg N/L***	***SO_4^{2-} mg /L***
A. Before bioremediation tests / 24.10.2012 / control water samples					
Control-Drinking plain water	1.53	16.65	-	6.02	41.98
Water from the toxic pond	137.05	1662.09	-	-	2785.43
1/2 dilution water from the toxic pond	60.63	810.73	-	-	1307.87
B. After bioremediation tests / 7.11.2012 with:					
Lemna minor					
Control-Drinking water	2.02	27.30	19.64	-	52.48
Water from the toxic pond	41.21	1443.08	-	-	3053.09
1/2 dilution water from the toxic pond	21.49	746.85	-	-	1503.59
Vallisneria spiralis					
Control-Drinking water	1.69	12.91	8.83	-	53.01
Water from the toxic pond	39.98	1525.09	-	-	3031.76
1/2 dilution water from the toxic pond	22.08	736.70	-	-	1417.86
Hydrilla verticillata					
Control-Drinking water	1.84	26.10	-	-	25.94
Water from the toxic pond	38.84	1514.99	-	-	3230.43
1/2 dilution water from the toxic pond	21.77	785.50	-	-	1563.50
Eichhornia crassipes					
Control-Drinking water	4.29	84.58	-	-	321.81
Water from the toxic pond	45.26	1803.99	-	-	3744.97
1/2 dilution water from the toxic pond	26.36	847.15	-	-	1783.26
Pistia stratiotes					
Control-Drinking water	2.07	25.86	44.23	-	66.38
Water from the toxic pond	41.43	1436.59	-	-	3042.64
1/2 dilution water from the toxic pond	23.02	850.47	-	-	1822.10
Cladophora glomerata					
Control-Drinking water	2.09	35.11	30.57	-	96.36
Water from the toxic pond	48.92	2048.04	-	-	4543.16
1/2 dilution water from the toxic pond	25.71	1169.99	-	-	2525.91

Table 3. Chemical and physicochemical quality standards for some nutrients to set out the ecological status of surface waters/maximum limits/quality classes (Ord. 161, 2006)

The quality indicator	***U/M***	***Quality classes***				
Nutrients		**1**	**2**	**3**	**4**	**5**
Nitrites ($N\text{-}NO_2$)	mg N/L	0.01	0.03	0.06	0.3	>0.3
Nitrates ($N\text{-}NO_3$	mg N/L	1	3	5.6	11.2	>11.2
Chlorides (Cl^-)	mg/L	25	50	250	300	>300
Sulfates (SO_4^{2+})	mg/L	60	120	250	300	>300

Table 4. Laboratory tests for phytoremediation with aquatic species *Lemna minor, Vallisneria spiralis, Eichhornia crassipes, Hydrilla verticillata, Pistia stratiotes, Cladophora glomerata* of the cation environmental status of water from the Șomârd waste landfills toxic pond

Cations concentrations	***Li^+***	***Na^+***	***K^+***	***Mg^{2+}***	***Ca^{2+}***	***NH_4^+***
	mg/L	***mg/L***	***mg/L***	***mg/L***	***mg/L***	***mg/L***
Before bioremediation tests / 24.10.2012 / control water samples						
Control-Drinking water	-	119.17	-	3.36	25.66	-
Water from the toxic pond	15.35	2372.78	528.99	5.28	242.29	32.45
1/2 dilution water from the toxic pond	8.32	1387.03	293.00	4.63	136.17	26.67
B. After bioremediation tests /7.11.2012 with:						
Lemna minor						
Control-Drinking water	-	111.03	45.46	3.05	24.09	-
Water from the toxic pond	14.32	2293.52	546.20	4.30	209.37	25.20
1/2 dilution water from the toxic pond	6.02	866.65	268.57	0.06	92.21	17.25
Vallisneria spiralis						
Control-Drinking water	-	104.49	10.32	2.05	38.09	-
Water from the toxic pond	15.41	2447.90	648.09	6.79	276.42	27.91
1/2 dilution water from the toxic pond	6.71	1147.35	328.29	1.73	119.96	20.27
Hydrilla verticillata						
Control-Drinking water	-	120.81	23.66	2.96	32.25	-
Water from the toxic pond	14.06	2353.30	588.14	7.99	209.96	26.48
1/2 dilution water from the toxic pond	7.39	1207.47	305.84	2.39	137.37	-
Eichhornia crassipes						
Control-Drinking water	-	123.97	14.39	2.74	18.03	-
Water from the toxic pond	17.49	2845.40	638.56	11.65	194.34	13.55
1/2 dilution water from the toxic pond	8.19	1292.19	321.34	2.97	147.01	-
Pistia stratiotes						
Control-Drinking water	-	120.54	28.44	2.72	19.55	-
Water from the toxic pond	13.09	2035.26	512.69	0.07	232.71	0.75
1/2 dilution water from the toxic pond	8.01	1333.96	327.10	5.35	117.34	-
Cladophora glomerata						
Control-Drinking water	0.60	35.19	41.18	4.25	60.17	-
Water from the toxic pond	17.69	2854.91	660.62	5.59	250.37	10.47
1/2 dilution water from the toxic pond	10.48	1903.78	417.70	13.60	187.54	-

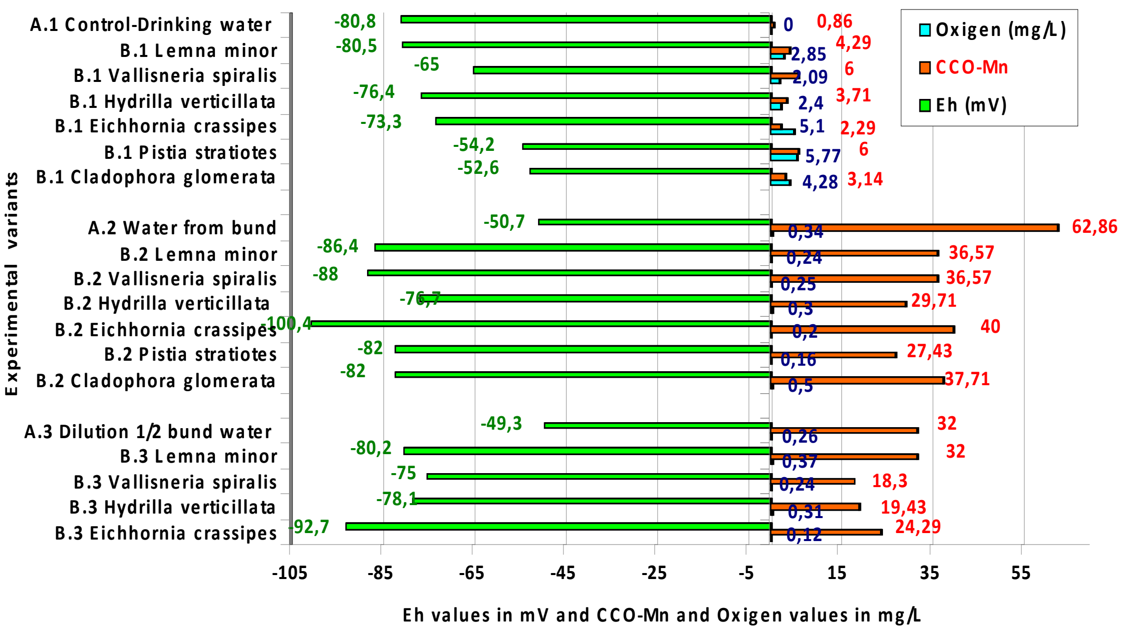
Table 5. Chemical and physicochemical quality standards for some cations to set out the ecological status of surface waters/maximum limits/quality classes (Ord. 161, 2006)

The quality indicator	***U/M***	***Quality classes***				
		1	**2**	**3**	**4**	**5**
Na^+	mg/L	25	50	100	200	>200
Mg^{2+}	mg/L	12	50	100	200	>200
Ca^{2+}	mg/L	50	100	200	300	>300
NH_4^+	mg/L	0.4	0.8	1.2	3.2	>3.2

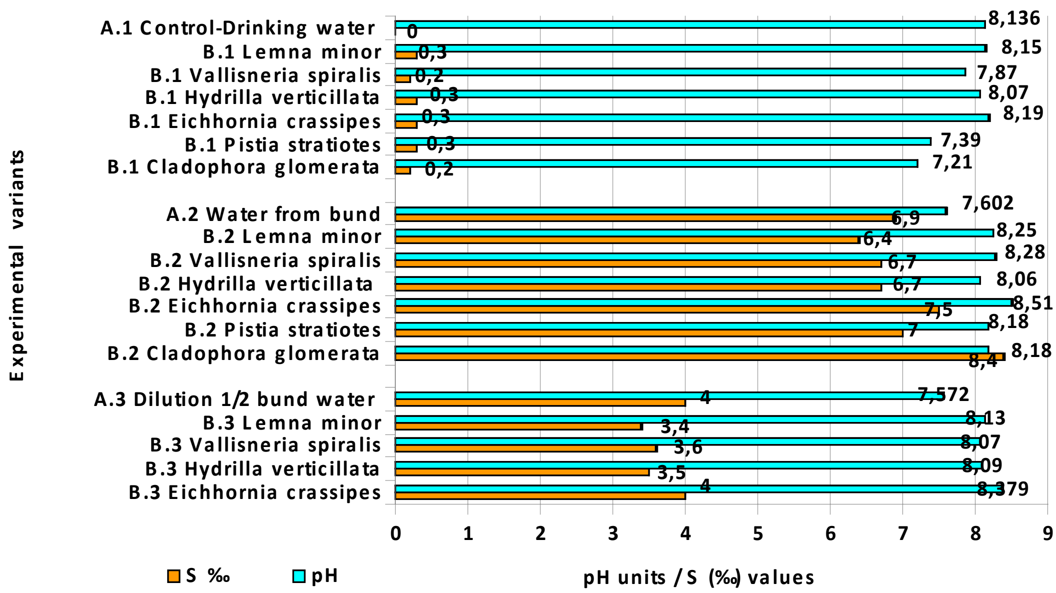
L. minor decreased amount of K^+, Ca^{2+}, Mg^{2+}, Na^+, Li^+; *V. spiralis* and *H. verticillata* caused the decrease of Ca^{2+}, Mg^{2+}, Na^+, Li^+ and the increase of K^+; while *E. crassipes* decreased amount of Mg^{2+}. *P. stratiotes* increased the concentration of Mg^{2+}, K^+ and decreased Ca^{2+} (Tables 4, 5). Stronger effects of bioremediation in the tests with aquatic plants grown for two weeks in ½ dilution water from the toxic pond as compared to the control samples were recorded. *H. verticillata*, *E. crassipes*, *P. stratiotes* and *C. glomerata* have decreased to zero the amount of NH_4^+. *L. minor* and *V. spiralis* significantly decreased amount of NH_4^+. *L. minor* decreased the amount of Ca^{2+}, Na^+, K^+; *V. spiralis* and *H. verticillata* caused the decrease of Ca^{2+} and Na^+. *V. spiralis*, *H. verticillata* and *P. stratiotes* caused the increase of K^+. *C. glomerata* caused the increase the concentration of K^+, Ca^{2+}, Mg^{2+}, Na^+, Li^+.

3.4. Bioremediation of wastewater by means of aquatic species: L. minor, V. spiralis, E. crassipes, H. verticillata, P. stratiotes, C. glomerata

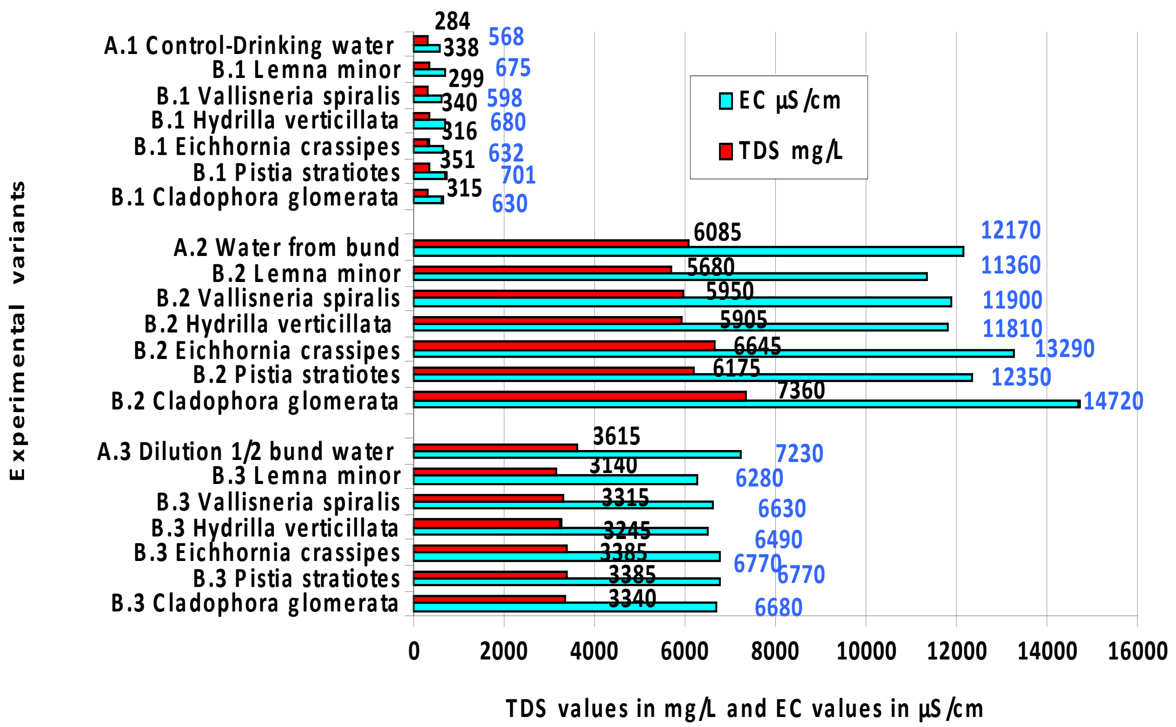
The toxic pond water from the Şomârd/Mediaş waste landfills, as compared to the control unpolluted water samples, shows lower values of O_2 mg/L and of Eh - oxidation-reduction potential and higher values of CCO-Mn, of EC - electrical conductivity, of TDS - total dissolved solids and of S – salinity, in 2012 (Fig. 9).



(a)



(b)



(c)

Fig. 9. Laboratory tests for phytoremediation of the environmental status of waters from the Şomârd Mediaş waste landfills toxic pond, with aquatic species *Lemna minor, Vallisneria spiralis, Eichhornia crassipes, Hydrilla verticillata, Pistia stratiotes, Cladophora glomerata.*
A. Before phytoremediation / 24.10.2012; B. After phytoremediation / 7.11.2012.
1. Control-Drinking water; 2.Water from the pond; 3. 1/2 dilution toxic pond water.
Quality parameters: **9 a** - dissolved O_2 mg/l; CCO-Mn; Eh–redox potential; **9 b** - pH; S-salinity; **9 c** - TDS-total dissolved solids; EC-Electrical conductivity

Plants evolution in control samples with unpolluted water shows that after phytoextraction, *L. minor, V. spiralis, H. verticillata, E. crassipes, P. stratiotes* and *C. glomerata* have determined the increase of the dissolved O_2, CCO-Mn, EC and S and the decrease of Eh - redox potential. *H. verticillata, E. crassipes, P. stratiotes* and *C. glomerata* have also determined the increase of TDS.

Preliminary tests on bioremediation have shown that in the samples with water from the toxic pond and with ½ dilution water from the toxic pond: *L. minor* plants, *V. spiralis, H. verticillata, E. crassipes, P. stratiotes* have determined the increase of Eh, the decrease of CCO-Mn, TDS and EC. In the samples with water from the toxic pond: *P. stratiotes* have determined the decrease of salinity, as well as a slight decrease of O_2; *E. crassipes* and *C. glomerata* have determined the increase of salinity and conductivity, as well as a slight decrease of TDS and O_2.

The present studies results and laboratory experiments on bioremediation as a method of decontaminating polluted areas from the toxic pond of municipal landfill waste Şomârd platform-Mediaş, Sibiu county, recommended the aquatic species *L. minor, V. spiralis, E. crassipes, H. verticillata, P. stratiotes, C. glomerata* for use as constructed wetlands method (Fig. 9).

4. Conclusions

Research results proved the importance of the bioremediation applied for constructed wetlands polluted with heavy metal ions, by using aquatic plants *Lemna minor, Vallisneria spiralis, Eichhornia crassipes, Hydrilla verticillata, Pistia stratiotes* and green algae *Cladophora glomerata*, producing the improvement of environmental status of wastewater.

Strong effects on heavy metals phytoextraction, the increase of Eh, the decrease of CCO-Mn, TDS, EC and significant decrease of NH_4^+ were recorded. These plants and algae can be used in biotechnological systems of constructed wetlands for ecological restoration.

References

Barbu C. H., Sand C., (2004), *Theory and Practice of Modern Remediation of Soils Polluted with Heavy Metals* (in Romanian), Alma Mater Press, Sibiu, Romania.

Buta E., Paulette L., Mihăiescu T., Buta M., Cantor M., (2011), The influence of heavy metals on growth and development of *Eichhornia crassipes* species, cultivated in contaminated water, *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **39**, 135-141.

Dickinson N.M., Baker A.J.M., Doronila A., Laidlaw S., Reeves R.D., (2009), Phytoremediation of inorganics: realism and synergies, *International Journal of Phytoremediation*, **11**, 97-114.

Dosnon-Olette R., Couderchet M., Oturan M.A., Oturan N., Eullaffroy P., (2011), Potential use of lemna minor for the phytoremediation of isoproturon and glyphosate, *International Journal of Phytoremediation*, **13**, 601-612.

Dyck V.M., Rob V.D., Geel K., (2009), On-site wastewater treatment in Flanders, opportunities and threats for constructed wetlands, project TISA, Belgium, On line at: http://www.constructedwetlands.net/vandyck_IWA_nepal_jaar.pdf.

Elekes C.C., Busuioc G., Ionita Gh., (2010), The bioaccumulation of some heavy metals in the fruiting body of wild growing mushrooms, *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **38**, 147-151.

Gavrilescu M., (2010), Environmental biotechnology: achievements, opportunities and challenges, *Dynamic Biochemistry, Process Biotechnology and Molecular Biology, Global Science Books*, **4**, 1-36.

Glick B.R., Stearns J.C., (2011), Making phytoremediation work better: maximizing a plant's growth potential in the midst of adversity, *International Journal of Phytoremediation*, **13**, 4-16.

Jing S.R., Lin Y.F., Lee D.Y., Wang T.W., (2001), Using constructed wetland systems to remove solids from highly polluted river water, *Water Science and Technology*: *Water Supply*, **1**, 89–96.

Juang D.F., Chen P.C., (2007), Treatment of polluted river water by a new constructed wetland, *International Journal of Environmental Science and Technology*, **4**, 481-488.

Kuschk P., Wießner A., Müller R., Kästner M., (2005), *Constructed Wetlands – Treating Wastewater with Cenoses of Plants and Microorganisms,* A Research Association at UFZ Centre for Environmental Research Leipzig-Halle, In the Helmholtz Association, Published by UFZ Centre for Environmental Research Leipzig-Halle. Medien GmbH, On line at: https://www.ufz.de/export/data/2/93454_CW_Booklet.pdf.

Malschi D., (2009), *Biology, Ecophysiology and Microbiology Elements,* Course notes and practical applications (in Romanian), Electronic Manual, Bioflux Publishing House, Cluj-Napoca, Romania, On line at: http://www.editura.bioflux.com.ro/docs/malschi2.pdf.

Malschi D., Roman C., Miclean M., Șenilă M., Ștefănescu L., Malschi-Florian B., Bolonyi A., Ghira G. B., Brăhaița I. D., Crihan Al. B., (2013), Study on phytoextraction bioremediation of heavy metals and metalloids from industrially polluted lands and waters using lab tests based on Lolium perenne and Lemna minor, *Environmental Engineering and Management Journal*, **12**, 1109-1112.

Masi F., Bendoricchio G., Conte G., Garuti G., Innocenti A., Franco D., Pietrelli L., Pineschi G., Pucci B., Romagnolli F., (2002), *Constructed Wetlands for Wastewater Treatment in Italy: State-of-art and Obtained Results*, Proc. of the IWA 7th Int. Conf. on Wetland System for Water Pollution Control, International Water Association, Arusha, Tanzania, 274-283.

McCutcheon S. C., Schnoor J. L., (2004), *Index of Names of Plants*, In: *Phytoremediation: Transformation and Control of Contaminants*, McCutcheon S.C., Schnoor J.L. (Eds.), John Wiley & Sons, Inc., Hoboken, NJ, USA, 925-939.

Mihăiescu R., Muntean O. L., Bodea C., Modoi C., Malos C., Mihăiescu T., Arghius V., Rosian Gh., Baciu N., (2010), Alternative closing municipal landfills. Case study: municipal landfill Medias, Sibiu County (in Romanian), *ProEnvironment*, **3**, 529-533.

Muntean O.L., (2003), A study of environmental reconversion in Copșa Mică Area (Romania), *Wurzburger Geographische Manuskripte*, **63**, 63-71.

Núñez S.E.R., Marrugo Negrete J.L., Arias Rios J.E., (2011), Hg, Cu, Pb, Cd, and Zn accumulation in macrophytes growing in tropical wetlands, *Water, Air and Soil Pollution*, **216**, 361-373.

Oprea I.C., Malschi D., Muntean O.L., (2013), The biomonitoring and the bioremediation of toxic water resulting from municipal waste storage of Somard, Sibiu county, *Advances in Environmental Sciences,* **5**, 189-196.

Oprea I.C., Modoi C., Muntean O.L., (2010), *The Integrated Management of Solid Urban Wastes from the Medias Municipality*, Proc. of the 2010 SIMTEX Conference, Bucharest, 244-253.

Ord. 161, (2006), Order 161/2006 approving the Norms on surface water quality classification in order to determine the ecological status of water bodies, Published in *Romanian Official Monitor* no. 511 of 13 June 2006.

Oros V., (2002), *Biomonitoring,* In: *Environmental Pollution and Monitoring,* Transilvania University of Brașov Publishing House, Brasov, Romania, 60-73.

Oros V., (2011), *Elements of Ecotoxicology and Ecotoxicological Tests*, Risoprint Publishing House, Cluj-Napoca, Romania.

Oros V., Drăgici C., (2002), *Waste Management* (in Romanian), Transilvania University of Brasov, Romania.

Pavel V.L., Diaconu M., Bulgariu D., Statescu F., Gavrilescu M., (2012), Evaluation of heavy metals toxicity on two microbial strains isolated from soil: *Azotobacter sp.* and *Pichia sp*., *Environmental Engineering and Management Journal*, **11**, 165-168.

Pivetz B.E., (2001), *Phytoremediation of Contaminated Soil and Ground Water at Hazardous Waste Sites, Ground Water Issue*, United States Environmental Protection Agency, Washington DC, On line at: https://www.epa.gov/sites/production/files/2015-06/documents/epa_540_s01_500.pdf.

Rahman M.A., Hasegawa H., Ueda K., Maki T., Okumura C., Rahman M.M., (2007), Arsenic accumulation in duckweed (S*pirodela polyrhiza* L.): A good option for phytoremediation, *Chemosphere.* **69**, 493-499.

Rahmani G.N.H., Sternberg S.P.K., (1999), Bioremoval of lead from water using *Lemna minor*, *Bioresource Technology,* **70**, 225-230.

Rai P.K., (2008), Heavy metal pollution in aquatic ecosystems and its phytoremediation using wetland plants: An ecosustainable approach, *International Journal of Phytoremediation*, **10**, 133-160.

Sim C.H., Yusoff K.M., Shutes B., Sim H.C., Mansor M., (2007), Nutrient removal in a pilot and full scale constructed wetland. Putrajaya city, Malaysia, *Journal of Environmental Management,* **88**, 307-317.

Stoica M., Jinescu G., Godeanu M., Nisipeanu S., (2009). *Biosorption of lead on Pistia stratiotes*, Proc. XIII Balkan mineral Processing Congress, Bucharest, 842-849.

Wang W.D., Wang X.G., Zhou L.C., Liu H.B., Ding Z.Z., Liang Y.T., (2016), Wastewater treatment in a constructed wetland followed by an oxidation pond in a rural area of China, *Environmental Engineering and Management Journal*, **15**, 199-205.

Willey N., (2007), *Phytoremediation: Methods and Reviews*, Willey N. (Ed.), Humana Press Inc., New Jersey.

Xu D.F., Li Y.X., Howard A., (2016), Effect of the interaction of earthworms and substrates on photosynthetic characteristics, nitrogen and phosphorus uptake by *Iris pseudacorus* in constructed wetland system, *Environmental Engineering and Management Journal*, **15**, 123-132.

Yalçına E., Çavusoglu K., Maras M.¸ Bıyıkoglud M., (2008), Biosorption of Lead (II) and Copper (II) metal ions on *Cladophora glomerata* (L.) Kütz. (Chlorophyta) algae: effect of algal surface modification, *Acta Chimica Slovenica*, **55**, 228–232.