Chemosphere 167 (2017) 188–192

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Removal of mercury from gold mine effluents using *Limnocharis flava* in constructed wetlands

José Marrugo-Negrete [a,**], Germán Enamorado-Montes [a], José Durango-Hernández [a], José Pinedo-Hernández [a], Sergi Díez [b,*]

[a] University of Córdoba, Faculty of Basic Sciences, Department of Chemistry, Water, Applied and Environmental Chemistry Group, Laboratory of Toxicology and Environmental Management, Montería, Colombia
[b] Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research, IDÆA-CSIC, Barcelona, Spain

HIGHLIGHTS

- The *Limnocharis flava* showed successful Hg accumulation from polluted water.
- The effectiveness in removing Hg varied according to the exposure time.
- The removal rate of Hg in a constructed wetland was 9 times higher than the control.
- *L. flava* showed a great potential for water remediation in constructed wetlands.

ARTICLE INFO

Article history:
Received 21 March 2016
Received in revised form
23 September 2016
Accepted 26 September 2016

Handling Editor: Martine Leermakers

Keywords:
Phytoremediation
Pilot-scale
Wetland
Mining effluents
Hg
Colombia

ABSTRACT

Phytoremediation has received increased attention over the recent decades, as an emerging and eco-friendly approach that utilizes the natural properties of plants to remediate contaminated water, soils or sediments. The current study provides information about a pilot–scale experiment designed to evaluate the potential of the anchored aquatic plant *Limnocharis flava* for phytoremediation of water contaminated with mercury (Hg), in a constructed wetland (CW) with horizontal subsurface flow (HSSF). Mine effluent used in this experiment was collected from a gold mining area located at the Alacran mine in Colombia (Hg: 0.11 ± 0.03 μg mL^{-1}) and spiked with $HgNO_3$ (1.50 ± 0.09 μg mL^{-1}). Over a 30 day test period, the efficiency of the reduction in the heavy metal concentration in the wetlands, and the relative metal sorption by the *L. flava*, varied according to the exposure time. The continued rate of removal of Hg from the constructed wetland was 9 times higher than the control, demonstrating a better performance and nearly 90% reduction in Hg concentrations in the contaminated water in the presence of *L. flava*. The results in this present study show the great potential of the aquatic macrophyte *L. flava* for phytoremediation of Hg from gold mining effluents in constructed wetlands.

1. Introduction

Heavy metals such as mercury (Hg) pose a growing environmental pollution problem at the global level (Driscoll et al., 2013). Anthropogenic activities have significantly increased the concentrations of mercury in the environment. Because of the high toxicity of Hg, as well as its persistence and ability to bioaccumulate and bioconcentrate, aquatic mercury pollution, generated by industrialization or natural processes, is one of the most critical environmental problems today (Fitzgerald and Lamborg, 2007; Zizek et al., 2007). The main source of Hg pollution at the global level is small–scale gold mining (Cordy et al., 2011). Mining activities are carried out during the recovery of metals from metal ores, in which the rock is disintegrated with water and treated with small amounts of chemicals that facilitate the release of these metals (Trois et al., 2007). One important physicochemical property of mercury is its ability to form amalgams with other metals, including gold. This property has contributed to the extensive use of mercury in mining activities in several countries. Constructed wetlands (CWs), also known as artificial or treatment wetlands, use the same processes that take place in natural wetlands and

* Corresponding author.
** Corresponding author.
E-mail address: sdsqam@cid.csic.es (S. Díez).

http://dx.doi.org/10.1016/j.chemosphere.2016.09.130

represent a feasible option to solve pollution problems due to their low cost of maintenance and operation, which makes them very attractive from an economic and technical point of view, since they can adequately reduce contaminants in polluted waters. Heavy metal removal mechanisms in horizontal subsurface constructed wetlands (HSSF CW) are complex and not fully understood (Kadlec and Wallace, 2008). Major mechanisms include: (i) binding to sediments and soils (through sedimentation, flocculation, adsorption, cation and anion-exchange, complexation, oxidation and reduction); (ii) precipitation and co-precipitation as insoluble salts; and (iii) plant uptake and, to a lesser extent, microbial metabolism (Galletti et al., 2010). Among other plants, *Limnocharis flava* (L.) Buchenau (yellow velvetleaf), which is a common species found in floodplains in tropical and subtropical America, was chosen in this work as the plant for Hg removal, due to its ubiquitous presence in the study area (Abhilash et al., 2008) and its potential to hyper-accumulate Hg, with good removal efficiency (52%) (Anning et al., 2013). These results demonstrate the suitability of the *L. flava* for phytoremediation, and the usefulness of CWs to improve the quality of water. The aim of this study was to evaluate the potential of phytoremediation of Hg–contaminated waters using the macrophyte species, *L. flava*, in HSSF CW on a laboratory scale.

2. Materials and methods

2.1. Constructed wetlands

The constructed wetland with subsurface flow (HSSF CW) was constructed in the greenhouse at the University of Córdoba, Colombia (Fig. 1). Effluents from the mining area El Alacrán, municipality of Puerto Libertador (Córdoba, Colombia) with a concentration of total Hg (T-Hg) of 0.11 ± 0.03 μg mL^{-1} were used with the objective of simulating a polluted environment. The effluent was doped with mercury nitrate ($HgNO_3$) to achieve a final THg concentration of 1.5 ± 0.13 μg mL^{-1}.

For the construction of the artificial wetland (Fig. 1), the following materials were used: four trays of polyethylene fibre with dimensions of 50 × 20 × 20 cm; porous gravel (1–2 cm; D_{60}: 10 mm); one plastic container with a capacity of 80 L to feed the wetland; 20 cm long piezometers to regulate the water flow as well as ensure the subsurface flow, with a depth of 18 cm water level The theoretical hydraulic retention time (HRT) in the wetland was 5 days, and the system was fed daily with a flow of 1 mL min^{-1} over 30 days. Therefore, for T-Hg monitoring, 6 outflow samples were taken at 6, 12, 18, 24 and 30 days. The temperature was maintained in the range of 27.1 °C–39.5 °C with an average of 29.5 °C, whereas the average relative humidity was 73% (49%–87%).

2.2. Transplant and acclimation of the plants

The *L. flava* plants were collected from sites without mining history. Seedbeds were constructed with a density of 40 individuals, ensuring normal conditions, adjustment and stabilization during 1 month. *L. flava* was selected because they usually grow in the study area. As a control, biomass production under natural conditions was determined in a water channel containing wastewater. Abundance was calculated using the density of *L. flava* species in a 1 m^2 area.

2.3. Sampling

Water samples were collected at outer currents in both of systems (constructed wetlands and control) and stored in sterile polyethylene bottles. Vegetal material consisting of the aerial parts of plants ($n = 3$) were collected at about 10 cm above water level and packed in plastic bags (Gomes et al., 2014). Plant materials were oven-dried at 60 °C to constant weight, pulverized and thoroughly homogenized.

2.4. Sample analysis

The analytical method used to determine the total Hg (T-Hg) concentration in the plant samples (30 mg dw) was based on thermal decomposition detected by atomic absorption spectrometry using a milestone DMA-80 (Direct Mercury Analyzer) (US- EPA, 1998). T-Hg in unfiltered water samples was measured using cold vapour atomic absorption spectroscopy (CVAAS) using a Thermo Scientific iCE 3000 series analyzer after digestion with diluted $KMnO_4$-$K_2S_2O_8$ solutions for 2 h at 95 °C (US-EPA, 1994). Analytical quality control of the methods was evaluated in triplicate with certified materials for tomato leaves (CRM 1753a, 34 ± 4 ng g^{-1}), and 'natural water - Mercury' (NIST-1641d, T-Hg 1.557 ± 0.020 μg mL^{-1}), and the percentage recovery was 98% and 97%, respectively. The detection limit for T-Hg was 0.14 μg L^{-1} for water and 0.01 μg g^{-1} dw for plants, calculated as the mean plus three times the standard deviation (SD) (Buccolieri et al., 2006).

2.5. Transfer coefficient (TC)

The water–plant transfer coefficient (TC) was calculated according to Gomes et al. (2014) as the relationship between the Hg concentration in the aerial part of the plant (dry mass) and in the contaminated water. TC was designed to represent a proper way to express the relative metal absorption by the *L. flava*.

2.6. Statistical analysis

Analyses of T-Hg in samples were reported as the mean ± SD of triplicate determinations from three plants and three water samples. The experimental results were statistically evaluated using the software Statgraphics Centurion 15.2.06. The variance of Hg concentrations in water samples between the constructed wetland and the control experiment were compared through ANOVA, by applying Tukey's test. Pearson's linear correlation was used to identify whether any relationships exist between variables. The differences were considered significant with $p < 0.05$.

3. Results and discussion

3.1. Variation of the Hg concentration in the water

Fig. 2 shows that the initial T-Hg concentration in the control experiment decreased from 1.50 ± 0.13 to 1.19 ± 0.10 μg/mL over 30 days. Therefore, the Hg concentration in the system without planting was reduced by 21± 2% at the end of the trial (30 days) mainly due to Hg sorption by particulate material. Other factors, such as the physicochemical conditions of the surrounding environment (e.g. pH, redox conditions of the water, presence of organic substances capable of forming chemical complexes, microorganisms involved in biodegradation processes and/or various climatic factors) are responsible for this decline (Hadad et al., 2006). The most probable pathways of Hg removal are volatilization, biomass uptake, or precipitation as organic and inorganic compounds (Gomes et al., 2014). In the system planted with *L. flava*, the T-Hg concentration was reduced from 1.50 ± 0.13 to 0.15 ± 0.04 μg mL^{-1} over 30 days, and, unlike the system without planting the experiments showed a high (90%) removal efficiency of Hg at the end of the experimental period. Statistically significant differences were found between the two treatments evaluated ($p < 0.05$), demonstrating effect of plants on the Hg removal efficiency, which

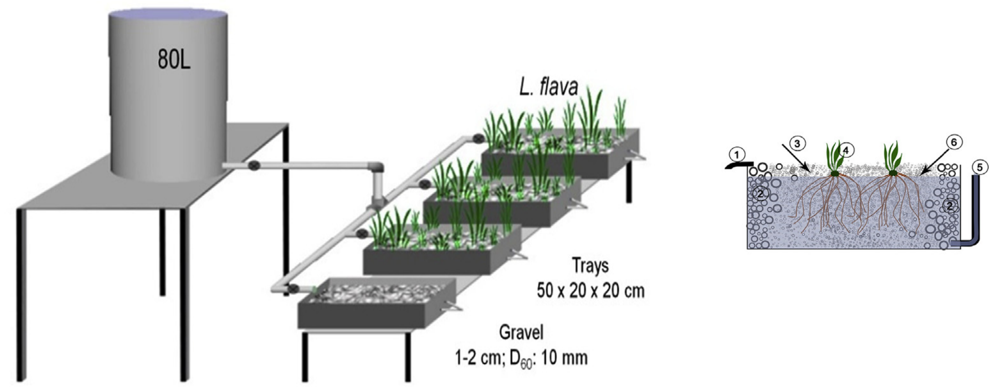

Fig. 1. Schematic view of experimental setup in the pilot-scale constructed wetland system. Lateral section of planted systems *L. flava*: 1. Supply pipe; 2. Distribution area (coarse gravel); 3. Gravel 1–2 cm; D_{60}: 10 mm; 4. *L. flava*; 5. Adjustable output pipe to control water level; 6. Water level.

contributes to the creation of conditions at the microcosm, that have an impact on the effectiveness of the system (Stefanakis et al., 2014). Results are similar to those reported by Gomes et al. (2014) in a HSSF CW using *Typha domingensis* as the plant species, where the reduction of Hg was 99.6± 0.4% in contaminated water. Dorman et al. (2009) reported a 67–99% removal of Hg in a pilot–scale constructed wetland treatment system designed to decrease the concentration and toxicity of metals and metalloids in ash basin water from coal–burning power plants. Kamal et al. (2004) evaluated the capacity of three aquatic plants to remove heavy metals from contaminated waters. Their experiment lasted for 21 days and the initial Hg concentration was 0.5 μg mL^{-1}. The efficiencies obtained with *Myriophylhum aquaticum*, *Ludwigina palustris and Mentha aquatic* were 99.97%, 99.74% and 99.99%, respectively. These efficiencies were higher than those reported in HSSF CW (Anning et al., 2013) at the mesocosm scale (2.1 m^2) used to treat the polluted waters of the Wiwi and Sisa rivers in Ghana using the macrophytes *L. flava* (51.6%), *Thalia geniculata* (45.0%) *and Typha latifolia* (46.6%). Lower efficiencies were reported in other studies, including in a constructed treatment wetland in south Florida, where removal of Hg was 49% (Zheng et al., 2013) or in a constructed wetland used to treat a polluted tributary of the Truckee River, where removal of Hg was reported at 78.2% (Chavan et al., 2007). Finally, removal of Hg varied between 25 and 50% in HSSF CW in the Czech Republic (Kropfelova et al., 2009).

The uptake of heavy metals by aquatic plants is affected by several parameters including pH, temperature, flow, evaporation, solar radiation, chemical constituents (such as chlorides, sulfates, phosphates), water nutrients, dissolved oxygen, biological oxygen demand, total organic carbon, total dissolved solids and total suspended solids (Abhilash et al., 2009). However, in the present study we have considered only two important parameters in detail – exposure time and concentration of Hg to which the *L. flava* are exposed. Fig. 2 shows that, in the presence of *L. flava*, a gradual decrease in the Hg content of the water occurred, leading to a statistically significant correlation between the exposure time and the Hg content ($r = -0.988$, $p < 0.05$). Hg was continuously taken up by *L. flava* over the 30 days of exposure, as demonstrated by the statistically significant correlation between Hg concentration in the plant and exposure time ($r = 0.993$, $p < 0.05$). Thus, the uptake process apparently followed a linear pattern with a linear increase in metal concentration in the plant. The cumulative Hg uptake was highest between 12 and 18 days of exposure. Hg content did not decrease in plants during the exposure period, which indicates that this species may be used for bioremediation over longer time periods and/or using higher initial Hg concentrations. These results also indicate that, at the concentrations and exposure times investigated, no tolerance mechanisms are used by the plants to exclude or restrict metal uptake by the roots. Accordingly, the water Hg concentrations gradually decreased with time.

A similar correlation between the Hg concentration in the water and the Hg content in the aerial part of the plant, was also observed by Mishra et al. (2009) in the treatment of a mining effluent (initial Hg concentration of 10 ng mL^{-1}) using *Pistia stratiotes* and *Azolla pinnata* macrophytes, which are also species commonly grown in mining areas. These results indicate that most of the one of the main Hg uptaken up by routes in the plant is in the form of through the soluble Hg. Furthermore, the fulfilment of the requirements proposed by Wittig (1993), together with the significant correlations presented in this study between Hg concentrations in the aerial part and the contaminated water, suggest that *L. flava* should be considered a useful biomonitor of Hg in wetlands.

3.2. Transfer coefficient (TC)

The Hg concentration in the aerial part during the experiment, was significantly higher after 30 days ($p < 0.05$) compared to shorter exposure times, demonstrating that Hg levels increase with

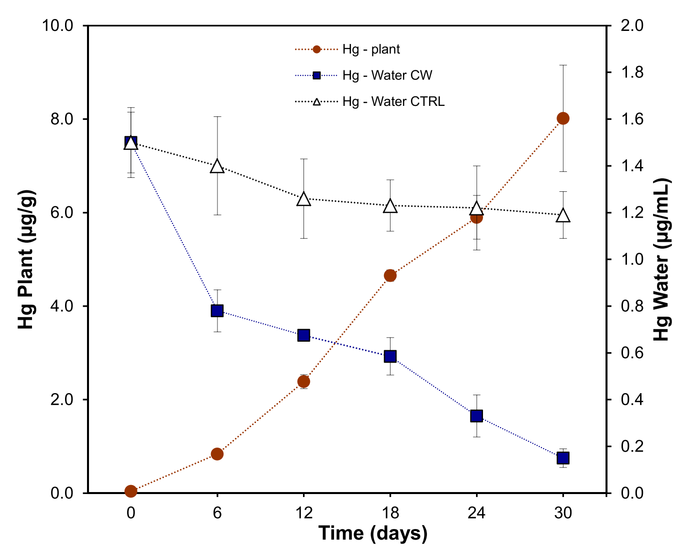

Fig. 2. Variation of the concentration of Hg as a function of exposure time in aerial parts of the plants, to the line of control (Water CTRL) and for the constructed wetland (Water CW).

increasing exposure time. The TC for the background concentration of Hg (0.04 ± 0.01 μg g^{-1}) was 0.02 mL g^{-1} and the TC reached a maximum value of 53.44 mL g^{-1} for an exposure time of 30 days, corresponding to an Hg concentration of 8.02 ± 1.14 μg g^{-1} in the aerial part of the plant. These results differ from those of Gomes et al. (2014), who, using *T. domingensis*, obtained a TC of 7751 ± 570 mL g^{-1} after 27 days, and an initial Hg concentration of 9.0 ± 0.4 g mL^{-1}. Anning et al. (2013) found that, for exposure times of up to 5 months, with initial Hg concentrations of about 0.05 mg mL^{-1} in the water column, accumulation of Hg in the aerial parts of *L. flava* were above 10 μg g^{-1}. Based on a background Hg concentration of 5.14 ± 0.5 μg g^{-1}, the TC reached about 200 mL g^{-1}. The low capacity of a plant to bioaccumulate metal has been associated with high metal retention in the roots, minimizing the possibility of transport to the aerial part (Cobbett, 2007; Vig et al., 2003). The relative lack of selectivity in transmembrane transportation of metals may be the reason for entry of nonessential heavy metals into cells (Soda et al., 2012). In addition, this transport depends on the plant species, type of metal and certain environmental conditions, such as pH, redox potential, temperature, salinity, organic matter content, the concentrations of certain nutrients or even the presence of other metals (Greger, 1999; Fritioff et al., 2005; Yang and Ye, 2009).

Fig. 3 shows an exponential regression plot between the TC and exposure time, providing a significant correlation ($r = 0.842$, $p < 0.05$), indicating that the accumulation of Hg was proportional to time. This relationship between time and accumulation of heavy metals has been previously described (Lai et al., 2010; Gomes et al., 2014). The ratio C/C_0 against time shows the efficiency of the wetland for Hg removal after 30 days (Fig. 3b). The first order kinetic constant, *k*, for the wetland (0.068 d^{-1}) was about 9 times higher compared to the control (0.008 d^{-1}) under similar experimental conditions, indicating that removal of Hg (90%) is due to the presence of *L. flava*.

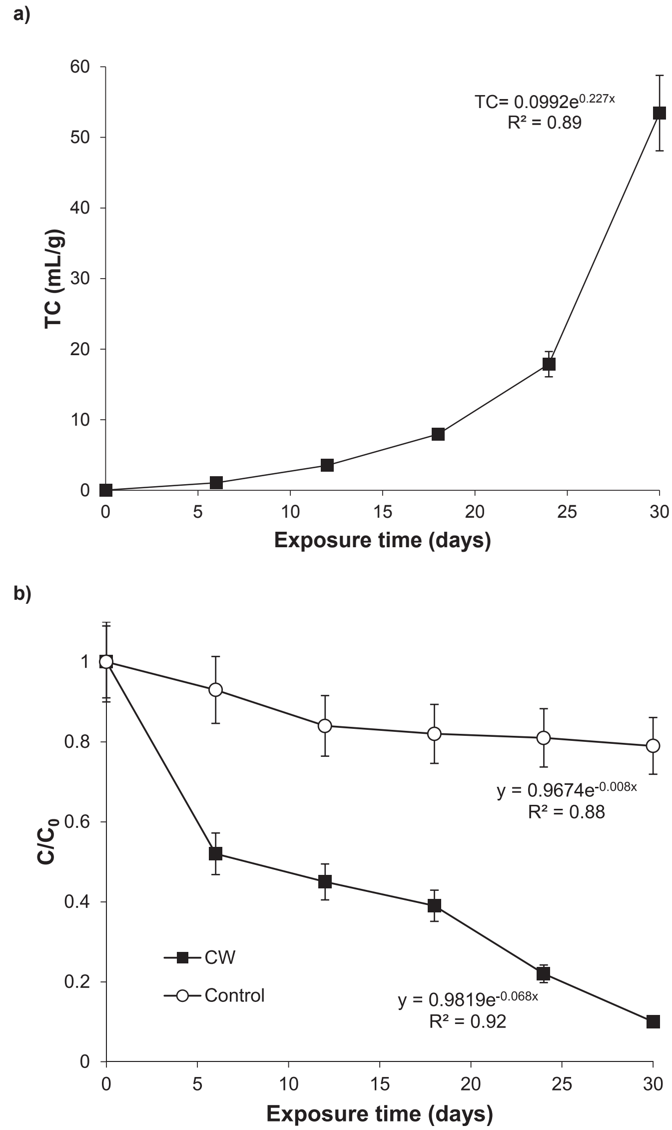

Fig. 3. a) Variation of the water-plant transfer coefficient as a function of exposure time; b) Performance of the constructed wetland on Hg removal.

3.3. Potential Hg removal by L. flava in the field

Under natural conditions, the total biomass production of *L. flava* on a dry basis (root + aerial part), reached values between 41.5 and 93.8 g per plant. The density of plants was approximately 5.6–26.2 plants m^{-2}; therefore, the total amount of biomass (dry weight) per square meter was between 529.23 and 2101.51 g m^{-2}. In the present study, the total accumulation of Hg in the aerial part of *L. flava* was 8.02 ± 1.14 μg g^{-1}. From this estimate, the absorption and accumulation per unit area of Hg can be calculated, and ranged from 4.24 to 16.85 mg m^{-2}. This result is lower than that reported by Abhilash et al. (2009) to remove Cd using this same species. However, our study has not taken into account the absorption of Hg in the roots, a value which is usually much higher than in the aerial part of the plant (Lyubenova et al., 2012; Anning et al., 2013). The reason for not including the roots in the trial is because, in a full-scale wetland the roots cannot be removed from the system, instead, the pruning or removal of the aerial part is an advisable practice in the treatment of metals using constructed wetlands (Vymazal et al., 2010; Sultana et al., 2015). Although the laboratory investigation differs from an on-site investigation in many ways (e.g. the impact of various factors such as microclimate, hydrobiology, hydrochemistry etc.), our study nevertheless provides quantitative information using *L. flava* to remove Hg from low-level Hg–contaminated water and lays the foundation for more detailed field trials. The higher TC values of the tested species enables them to accumulate large amounts of hazardous metals in their harvested parts and, if not disposed properly, the accumulated heavy metals may be returned to the system or can enter into the food chain. Proper disposal of the harvested plant parts is the final and most important step in any kind of plant–based remediation technology. The evaluation of Hg removal by electrodeposition from vegetal biomass samples obtained from mining zones is a technological option (Marrugo et al., 2013). However, the cost benefit analysis of the recovery process should be conducted in order to evaluate the feasibility of this technique.

4. Conclusions

Treatment performance data from this pilot–scale system indicate a reduction in the concentrations of Hg in water from gold mine effluents using the macrophyte *L. flava* in a constructed wetland. These results show that the most important variable governing the rate of Hg removal from the water was exposure time. The removal and relative uptake of Hg from gold mine effluents by *L. flava* in constructed wetlands were studied. Metal absorption by the plant increased with the exposure time according to first order kinetics with a rate constant about 9 times higher than that of the control experiment. The results show a high potential of *L. flava* to perform phytoremediation of Hg contaminated waters in

HSSF CW. These plant species demonstrated excellent remediation, reducing the Hg concentration of polluted water by 90%. In sum, results of this pilot-scale study suggest that HSSF CWs are a viable option for treating Hg from gold mine effluents. In fact, our results will be the basis to construct a full-scale integrated system in El Alacrán gold mining site. Therefore, concerning the future perspectives of this study, an on-site demonstration-scale treatment wetland, at a scale smaller than the full-size system, is planned to be designed and built at the mining site. This phase allows for final site-specific optimization of the design before constructing the full-scale system, including refining the parameters needed for accurate full-scale sizing, and evaluating its performance over several years.

Acknowledgments

The authors would like to thank the University of Córdoba, the Laboratory of Toxicology and Environmental Management; COLCIENCIAS, for financing the project: “Spatial distribution of heavy metals and nutrients in flooded soils of the Mojana region: Environmental implications and recovery strategies”, identified with the code 1112-569-35214 and contract number 0211-2013. We appreciate the anonymous reviewers and the Associate Editor for their valuable comments and suggestions to improve the quality of the manuscript.

References

Abhilash, P.C., Vimal, C.P., Srivastava, P., Rakesh, P.S., Chandran, S., Singh, N., Thomas, A.P., 2009. Phytofiltration of cadmium from water by *Limnocharis flava* (L.) Buchenau grown in free-floating culture system. J. Hazard. Mater 170, 791–797.

Abhilash, P.C., Singh, N., Sylas, V.P., Ajay, B., Kumar, J.C., Mathew, R., Satheesh, Thomas, A.P., 2008. Eco-distribution mapping of invasive weed *Limnocharis flava* (L.) Buchenau using geographical information system: implications for containment and integrated weed management for ecosystem conservation. Taiwania 53, 30–41.

Anning, A.K., Korsah, P.E., Addo-Fordjour, P., 2013. Phytoremediation of wastewater with *Limnocharis flava*, Thalia geniculata and Typha latifolia in constructed wetlands. Int. J. Phytoremediation 15, 452–464.

Buccolieri, A., Buccolieri, G., Cardellicchio, N., Dell'Atti, A., Di Leo, A., Maci, A., 2006. Heavy metals in marine sediments of Taranto Gulf (Ionian Sea, southern Italy). Mar. Chem. 99, 227–235.

Chavan, P.V., Dennett, K.E., Marchand, E.A., Gustin, M.S., 2007. Evaluation of small-scale constructed wetland for water quality and Hg transformation. J. Hazard. Mater 149, 543–547.

Cobbett, C., 2007. Phytochelatins and their roles in heavy metal detoxification. IEEE. Plant Physiol. 123, 825–832.

Cordy, P., Veiga, M.M., Salih, I., Al-Saadi, S., Console, S., Garcia, O., Mesa, L., Velásquez López, P., Roeser, M., 2011. Mercury contamination from artisanal gold mining in Antioquia,Colombia : the world's highest per capita mercury pollution. Sci. Total Environ. 410, 154–160.

Dorman, L., Castle, J.W., Rodgers, J.H., 2009. Performance of a pilot-scale constructed wetland system for treating simulated ash basin water. Chemosphere 75, 939–947.

Driscoll, C.T., Mason, R.P., Chan, H.M., Jacob, D.J., Pirrone, N., 2013. Mercury as a global pollutant: sources, pathways, and effects. Environ. Sci. Technol. 47, 4967–4983.

Fitzgerald, W.F., Lamborg, C.H., 2007. Geochemistry of mercury in the environment. In: Holland, H.D., Turekian, K.K. (Eds.), Treatise on Geochemistry. Pergamon, Oxford, pp. 1–47.

Fritioff, A., Kautsky, L., Greger, M., 2005. Influence of temperature and salinity on heavy metal uptake by submerged plants. Environ. Pollut. 133, 265–274.

Galletti, A., Verlicchi, P., Ranieri, E., 2010. Removal and accumulation of Cu, Ni and Zn in horizontal subsurface flow constructed wetlands: contribution of vegetation and filling medium. Sci. Total Environ. 408, 5097–5105.

Gomes, M.V., Rodrigues de Souza, R., Teles, V., Mendes, É., 2014. Phytoremediation of water contaminated with mercury using Typha domingensis in constructed wetland. Chemosphere 103, 228–233.

Greger, M., 1999. Metal availability and bioconcentration in plants. In: Prasad, M.N.V., Hagemeyer, J. (Eds.), Heavy Metal Stress in Plants: from Molecules to Ecosystems. Springer, Berlin, pp. 1–27.

Hadad, H.R., Maine, M.A., Bonetto, C.A., 2006. Macrophyte growth in a pilot-scale constructed wetland for industrial wastewater treatment. Chemosphere 63, 1744–1753.

Kadlec, R.H., Wallace, S.D., 2008. Treatment Wetlands, second ed. CRC Press, Boca Raton.

Kamal, M., Ghaly, A.E., Mahmoud, N., Côté, R., 2004. Phytoaccumulation of heavy metals by aquatic plants. Environ. Int. 29, 1029–1039.

Kropfelova, L., Vymazal, J., Svehla, J., Stıchova, J., 2009. Removal of trace elements in three horizontal sub-surface flow constructed wetlands in the Czech Republic. Environ. Pollut. 157, 1186–1194.

Lai, H.-Y., Juang, K.-W., Chen, Z.-S., 2010. Large area experiment on uptake of metals by twelve plants growing in soils contaminated with multiple metals. Int. J. Phytoremediation 12, 785–797.

Lyubenova, L., Pongrac, P., Vogel, K., Kukec, G., Vavpetic, P., Grlj, N., Kump, P., Necemer, Marjana M., Peliconc, P., Schrodera, P., 2012. Localization and quantification of Pb and nutrients in *Typha latifolia* by micro-PIXE. Metallomics 4, 333–341.

Marrugo-Negrete, J., Pinedo – Hernández, J., Baeza – Reyes, J., 2013. Optimization of the electrodeposition conditions for mercury removal from vegetal biomass with Response surface methodology. Port. Electrochimic. Acta 31, 107–117.

Mishra, V.K., Tripathi, B.D., Kim, K.H., 2009. Removal and accumulation of mercury by aquatic macrophytes from an open cast coal mine effluent. J. Hazard. Mater 172, 749–754.

Soda, S., Hamada, T., Yamaoka, Y., Ike, M., Nakazato, H., Saeki, Y., Kasamatsu, T., Sakurai, Y., 2012. Constructed wetlands for advanced treatment of wastewater with a complex matrix from a metal-processing plant: bioconcentration and translocation factors of various metals in Acorus gramineus and Cyperus alternifolius. Ecol. Eng. 39, 63–70.

Sultana, M.Y., Chowdhury, A. K. Md.M. B., Michailides, M.K., Akratos, C.S., Tekerlekopoulou, A.G., Vayenas, D.V., 2015. Integrated Cr(VI) removal using constructed wetlands and composting. J. Hazard. Mater 281, 106–113.

Stefanakis, A., Akratos, C.S., Tsihrintzis, V.A, 2014. VFCW components. In: Stefanakis, A., Akratos, C.S., Tsihrintzis, V.A. (Eds.), Vertical Flow Constructed Wetlands. Elsevier, Amsterdam, pp. 39–55.

Trois, C., Marcello, A., Pretti, S., Trois, P., Ross, G., 2007. The environmental risk posed by small dumps of complex arsenic, antimony, nickel and cobalt sulphides. J. Geochem. Explor 92, 83–95.

US-EPA, 1994. Methods 245.1 for Determination of Mercury in Wáter. U.S. Environmental protection Agency, Cincinnati. Ohio.

U.S EPA, 1998. Mercury in Solids and Solutions by Thermal Decomposition. Amalgamation and Atomic Absorption Spectrophotometry (Method 7473).

Vig, K., Megharaj, M., Sethunathan, N., Naidu, R., 2003. Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review. Adv. Environ. Res. 8, 121–135.

Vymazal, J., Kropfelova, L., Svehlac, J., Stichovac, J., 2010. Can multiple harvest of aboveground biomass enhance removal of trace elements in constructed wetlands receiving municipal sewage? Ecol. Eng. 36, 939–945.

Wittig, R., 1993. General aspects of biomonitoring heavy metals by plants. In: Markert, B. (Ed.), Plants as Biomonitors/Indicator for Heavy Metals in the Terestial Environment. VCH Publisher, Weinheim, pp. 3–28.

Yang, J., Ye, Z., 2009. Metal accumulation and tolerance in wetland plant. Front. Biol. 4, 282–288.

Zheng, S., Gu, B., Zhou, Q., Li, Y., 2013. Variations of mercury in the inflow and outflow of a constructed treatment wetland in south Florida, USA. Ecol. Eng. 61, 419–425.

Zizek, S., Horvat, M., Gibicar, D., Fajon, V., Toman, M.J., 2007. Bioaccumulation of mercury in benthic communities of a river ecosystem affected by mercury mining. Sci. Total Environ. 377, 407–415.