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"Gheorghe Asachi" Technical University of Iasi, Romania

BIOLOGICAL TREATMENT OF WASTEWATER CONTAMINATED WITH Cu(II), Fe(II) AND Mn(II) USING *Ludwigia stolonifera* AQUATIC PLANT

Hosam M. Saleh[1*], Hazem H. Mahmoud[1, 2], Refat F. Aglan[3], Talat A. Bayoumi[1]

[1]*Radioisotope Department, Nuclear Research Center, Egyptian Atomic Energy Authority, Dokki 12311, Giza, Egypt*
[2]*Central Laboratory for Elemental and Isotopic Analysis, Nuclear Research Center, Egyptian Atomic Energy Authority, Egypt*
[3]*Department of Analytical Chemistry, Hot Laboratories Center, Egyptian Atomic Energy Authority, 13759, Egypt*

Abstract

The release of metals from many industrial processes into aquatic ecosystems is currently a major environmental threat, especially in developing and emerging countries. Most traditional treatment approaches are not efficient in terms of removal yield and cost. Plants have the ability to stabilize metals in a fast and cost-efficient way. In this context, aquatic plants are successfully applied for remediation of hazardous elements from industrial wastewater. This study demonstrates the capability of the wetland macrophyte, *Ludwigia stolonifera,* in uptake and accumulation of three metal species (Cu, Fe and Mn) dissolved in artificial industrial wastewater. Four days treatment of artificial wastewater containing 100 ppm of Cu, Fe and Mn remove about 86%, 74%, 93%, respectively, of the individual metals. *L. stolonifera* displayed a considerable metal recovery potential after exposure to different environmental conditions regarding acidity, illumination and metal concentration. The data obtained in this study underline the high capability of *L. stolonifera* for phyto-mediated purification of wastewater contaminated by metals, which is widely independent from the cultivation conditions.

Keywords: phytoremediation, *Ludwigia stolonifera*, metals, wastewater

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1. Introduction

Rapid urbanization, use of pesticides, hormones and fertilizers have resulted in ongoing pollution of both terrestrial and aquatic environments especially in developing and emerging countries. Despite the undisputed economic and social benefits of industry, its harmful impact on the environment and water resources must be considered, especially regarding contaminants discharged into water bodies and the steadily increasing water demand (Akinbile and Yusoff, 2012; Rathner et al., 2017).

Environmental metal toxicity became a worldwide concern, causing the occurrence of various health threats (Mishra and Tripathi, 2009). Contamination of aquatic ecosystems resulting from the discharge of industrial contaminants into water bodies significantly contributes to prevailing environmental worries. The liberation of metals resulting from rapid industrialization and urbanization may damage both natural and artificial ecosystems (Burada et al., 2017; Tyler et al., 1989).

However, metals have essential tasks in the life functions of microorganisms. Fe, Cu and Mn serve as micronutrients and play an important role to regulate osmotic pressure and redox processes to immobilize molecules during the electrostatic interactions and act as essential agents of various enzymes (Terry, 1981). Nutrients essential to plant growth are classified into macronutrients, secondary nutrients and micronutrients (Benton Jones, 2012). Plants are generally able to accumulate both "essential" metals

*Author to whom all correspondence should be addressed: e-mail: hosamsaleh70@yahoo.com; Phone: +2 01005191018; Fax: +202 37493042

(i.e., Ca, Co, Cu, Na, Fe, K, Mg, Mo, Mn, Ni, Se, V and Zn) as micronutrients in different concentrations for growth and development, and other "non-essential" metals (i.e., Al, As, Au, Cd, Cr, Hg, Pb, Pd, Pt, Sb, Te, Tl and U) of undefined biological effect (Djingova and Kuleff, 2000). Cu, Fe and Mn, in low concentrations, are essential as micronutrients for plant metabolism; however, when present in excess, they could negatively impact the plant´s metabolism (Vincze et al., 2018; Williams et al., 2000).

Conventional physical or chemical techniques applied for purification of wastewater contaminated with these elements are already available. This encompasses the removal of metals by performing reverse osmosis, ion exchange, precipitation, membrane technologies, solvent extraction, sorption, and electrochemical treatment (Abou El-Nour, 2016; Rai et al., 2002). Developing countries like Egypt have to devote priority of consideration towards economic development; however, more recent technologies applied in the treatment of wastewater cannot be disregarded in order to combine economic progress with environmental sustainability. In this context, metal stabilization by living organisms (Saleh, 2012; Saleh et al., 2017), and biosorption by dried material (Abu-Khadra et al., 2016), are considered eco-friendly and cost-effective techniques for the remediation of metals and radionuclides from wastewater prior to subsequent solidification (Bayoumi and Saleh, 2018; Osmanlioglu, 2016; Saleh, 2014).

Recently, phytoremediation processes have received attention as bioremediation strategies to purify contaminated soil and water. This technique uses aquatic plants for detoxification or immobilization of metals (Barbes and Barbulescu, 2017; Singh et al., 2012). A plant used for bioremediation should efficiently remediate metals at different concentrations, display tolerance to the metal accumulated, exhibit fast growth and formation of high biomass concentration, possess a highly branched root system, and should be easy to harvest (Eapen and D'Souza, 2005). Aquatic plants are beneficial compared to terrestrial plants for the treatment of wastewater; this is due to their rapid growth, high biomass production capacity, and pronounced capacity for uptake of contaminants when immersed in contaminated water (Sood et al., 2012).

In particular, floating plants have considerable ability to uptake, translocate, and stabilize various toxic metals of high concentrations in their harvestable parts (Rahman and Hasegawa, 2011). Most aquatic plants reveal different capability to survive in relatively high-contaminated water and behaved as an efficient natural filter for different metals and hazardous contaminants (Sharma et al., 2015).

Invasive species, so called *Neophytae*, represent significant problems to ecosystem integrity, biodiversity, fisheries, agriculture, and health (Lee, 2002). These unwanted species have a considerable harmful impact on economic, social and ecological welfare. Often, the invasive plant *Ludwigia stolonifera* is found accompanied by the most common indigenous macrophyte, *Eichhornia crassipes* (Adam et al., 2002). These macrophytes display high reproductive abilities, tolerance to wide salinity ranges, metals and pronounced adaptability to varying climatic conditions. *L. stolonifera*, a water primrose, was evaluated as an economic plant nominated to refine water from common agricultural chemicals to improve the quality of pure water for drinking (Abu-Ziada, 2007). Moreover, it was recently demonstrated that about 2 g of *L. stolonifera* are able to take up and translocate more than 95% of radiocobalt and 65% of radio-cesium from radioactive waste solution (Saleh et al., 2017) and eliminates up to 65%, 97% and 99% of Cd, Cr and Pb respectively (Saleh et al., 2019).

Two mechanisms are described to contribute to the uptake of metals dissolved in water by aquatic plants: (i) the fast metabolism-independent surface reaction, which consists of a diffusion process followed by binding or sorption of metals on the cell wall, and, (ii) the slow metabolism-dependent cellular uptake, which involves mass transfer from the exterior cell wall to the interior cell wall (Khosravi et al., 2005).

Generally, metal accumulation by using plants is depending on the time of the process, type of metal, concentrations of metal ions, if the metals occur individually or in the mixture, and on environmental variables such as illumination, pH-value of the medium, and the bulk mass of the plant (Emenike et al., 2016). The present study aims to investigate the phytoremediation performance of *L. stolonifera* as one of the most important invasive plants in order to evaluate the potential of this scarcely studied species for application in phytoremediation technology; its ability to remove metals such as Mn, Fe and Cu individually or as a mixture in water under several conditions was the major objective of the study. This plant was selected according to its fast growth and abundance in many Egyptian freshwater sources. Specific objectives were: (1) determination of the uptake tendency of metals separately or as a mixture using the aquatic plant; (2) comparison of the removal of nominated metals from artificially contaminated wastewater under different conditions; and (3) evaluation of the optimum process conditions to remove high decontamination levels within a reasonable time frame.

2. Materials and method

2.1. Sample collection, processing, and cultivation

Phytoremediation scenarios for wastewater decontaminations were simulated using healthy young floating plants of the aquatic macrophyte, *L. stolonifera*, collected from one branch of the El-Mansourya Canal, a branch of the Nile River, as one of the water bodies contaminated with sewage. Inductively coupled plasma-optical emission spectrometry (ICP-OES) was used to determine the concentration of individual elements in the Nile river water as the natural habitat of the plant (results see Table 1). For the subsequent analyses, the samples were collected at a depth of 2 cm from the water surface during the period of treatment. The plants were rinsed properly by tap water, and samples of approximately 10 to 12 g were put into glass bottles containing 100 mL of artificially contaminated water; the bottles had narrow necks in order to minimize evaporation. The treatment process was performed under typical temperature and humidity conditions prevailing in the laboratory. The experiment was carried out for extended time periods up to more than one week to determine the time needed for optimum metal accumulation/removal. Compositions of the artificially contaminated water, illumination regime, quantity of plant biomass, and pH-value were adapted according to the aims of the individual experiments.

Table 1. Elemental analysis of water from El-Mansourya Canal (without artificial contamination) using ICP-OES

Element	***Concentration, ppm***
Na	27.14
K	41.35
Ca	22.32
Mg	18.01
Fe	0.05
Cu	ND
Mn	ND

ND: not determined/detected

2.2. Artificial contamination of the water samples

Aqueous solutions of different concentrations of metal ions and aqueous media adjusted to various pH-values by HNO_3 or NaOH were prepared before immersing the floating plants for the remediation process. For this purpose, analytical grade Cu, Fe and Mn metal salts solutions were diluted by tap water to predetermined concentrations of 10, 50 and 100 ppm. To compensate the loss of water through evapotranspiration, bottles were continuously refilled with tap water to maintain the original volume of 100 mL.

2.3. Testing the impact of different quantities of plant biomass

Different weights or numbers of plants were utilized to evaluate the influence of mass and number of plants on the remediation process. Exposure of the macrophyte to light and single/multi-metal systems were investigated. The uptake values for Cu, Fe and Mn from the simulated wastewater using *L. stolonifera* were followed and evaluated under the above described conditions during more than four days.

2.4. Identification of remaining metal ions

Prodigy axial high dispersion (ICP-OES) TELEDYNE Leeman Labs, USA, was applied to analyze the remaining concentrations of Cu, Fe and Mn in water for various samples after the treatment process under the different conditions.

2.5. Metal extraction evaluation

Percent removal of metals from artificially contaminated water was calculated according to (Eq. 1):

$$PR = (1 - Cf/Ci) \times 100\% \quad (1)$$

where *Cf* = pollutant concentration after treatment, *Ci* = metal concentration prior the treatment (Kumari and Tripathi, 2014).

2.6. Statistical analysis

Two-way classification by ANOVA using SPSS Statics was applied to verify statistical significance of differences in removal performance among various treatments; differences were considered significant at $p<0.05$.

3. Results and discussions

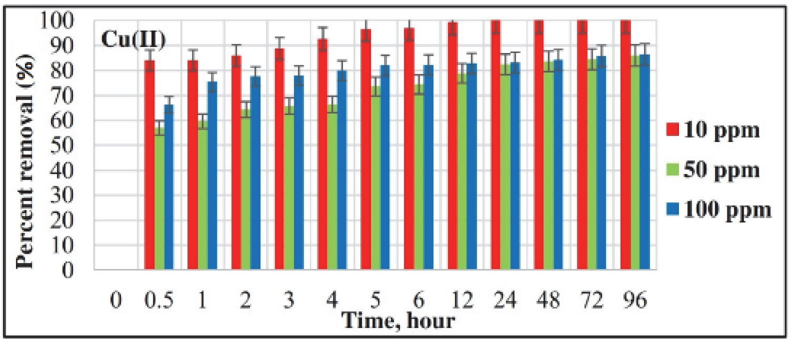
3.1. The uptake potential for metals at different concentration during extended periods

In general, the higher the metal concentrations in aqueous media, the higher the uptake obtained by the plants (Saleh, 2012). This relationship between metal concentrations in the water and the percent removal (PR) is presented in Fig. 1a-c. A significant increase in metal uptake was observed during the first six hours of treatment. The removal of Cu, Fe and Mn (with the highest initial concentration of 100 ppm) at the end of the investigation after four days (96 h) reached 86%, 74%, and 93% (Figs. 1a-c), respectively. About 100% uptake was recorded at the lowest concentration level of 10 ppm for all the three investigated metal ions at the end of the investigation. In the case of Cu and Fe, 100% recovery of metals from setups with the lowest contamination level (10 ppm) was observed after 12 h (Fig. 1a) and 3 h (Fig. 1b), respectively, whereas in the case of Mn, a recovery of 100% was only approached at the end of the process (Fig. 1c). No remarkable difference was observed for setups with 50 ppm and 100 ppm, independent from the type of metal ion.

Foliar symptoms and growth reduction are the direct effect of a metal toxicity on plant metabolism. At high metal content, diverse indicator metabolites can display either increased or decreased production in response to the presence of toxins (Burke et al., 1990). Macroscopically, growth of the accumulator plant during immersion in water polluted with low concentration of the metals (10 ppm) occurred without visible differences between the individual metals; no undesired changes in the plant´s macroscopic appearance were observed. This is in contrast to the outcomes when investigating higher concentrations (50 ppm and 100 ppm) of polluting metals; here, yellowish and dry leaves appear. Obviously, increasing metal concentration in the aqueous medium results in the observed impairment of the plant´s physiological state. Previous studies have stated two principal mechanisms used by the plants to react to the high concentration of metals located in the treatment medium: on the one hand, the "exclusive technique" describes the behavior of plants to absorb and transport metals at excessive quantities, on the other hand, the "accumulation technique" allows plants to collect and transport extremely high metal contents to the rhizosphere (McGrath et al., 1997). After metals have entered the rhizosphere, they are either accumulated in the root, or translocated to the shoots (Ghosh and Singh 2005). In spite of the high-contaminated aqueous medium containing various metals, *L. stolonifera* turned out as a metal hyperaccumulator of remarkable efficiency. The morphological observations (yellowish leaves and leaves loss) occurring during the experiment display the most obvious changes on the macrophyte.

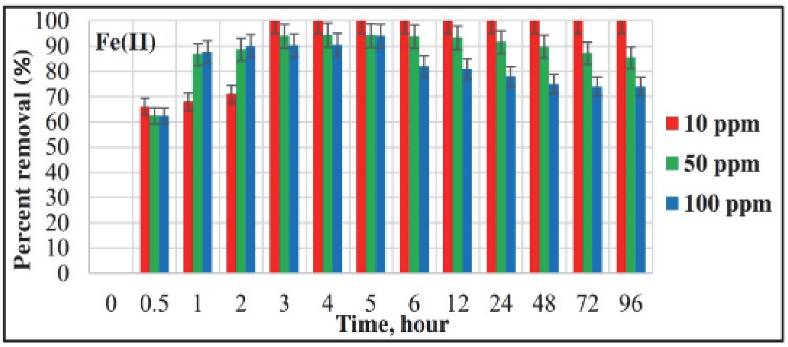
3.2. Stabilization of the metals in the plant in dependence on different pH-values

Wastewater bodies usually are contaminated by different pollutants and therefore the nature of the aqueous medium varies between low alkaline to high acidic. The effect of the pH-value on metal ion recovery is dependent on the composition of the aqueous medium used, particularly on the metals present in this medium.



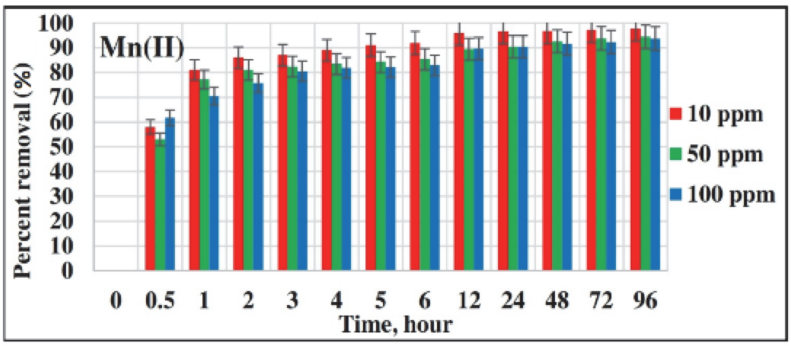
F_{time}=24.78* P<0.05, F_{Ci}=51.60* P<0.05

(a)



F_{time}=30.44* P<0.05, F_{Ci}=4.89* P<0.05

(b)



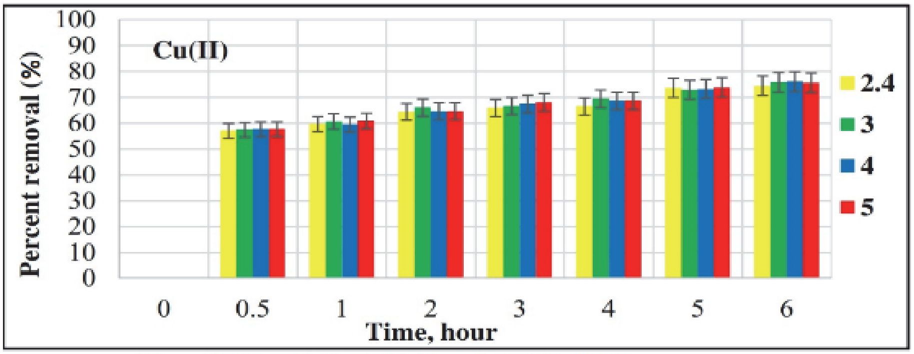
F_{time}=367.74* P<0.05, F_{Ci}=22.29* P<0.05

(c)

Fig. 1. Uptake of bivalent metal ions at different initial concentrations during extended times: (a) Cu(II), (b) Fe(II), (c) Mn(II)

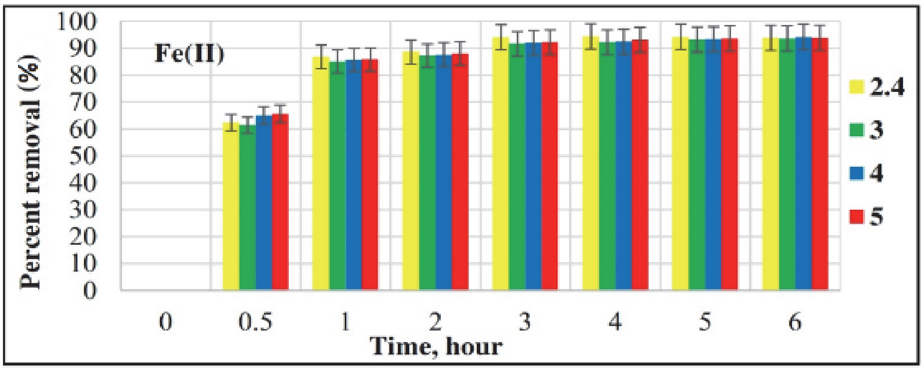
Metal content and the bioavailability of metals for the plants depend on many parameters such as the pH-value of water and sediment. Generally, ascending pH-values are accompanied by descending presence of free metal-ions remaining in wastewater as a consequence of the precipitation of metal hydroxides and diverse metal complexes. Aqueous solutions with fixed metal ions concentration of 50 ppm were adjusted to different pH-values varying between neutral and highly acidic (6.6-1.8). As illustrated in Fig. 2, a slight increase of the accumulation rate is observed for all metal ions, when the pH-value increases from 2.4 to 4; in the case of Cu(II) ions, this accumulation rate slightly decreases when the pH-value further increases to 5 (Fig. 2a).

Mn accumulation generally tends to decrease when the pH-value increases (Fig. 2c). This behavior may be due to the increasing presence of Mn in different oxidation states than Mn(II), such as Mn (III) and Mn (IV): these higher oxidation states of Mn are not bioavailable by plants, and thus cannot be accumulated (Millaleo et al., 2010). In contrast, uptake of Fe(II) ions does not vary with different pH-values. For all three metal ions, the behavior of *L. stolonifera* matches well with previously reported studies, which demonstrate that the solubility and transport of many metals into roots is increased in acidic media (Meagher, 2000). In spite of pH-mediated tolerance related to the metal behavior in the media, pH-value may play a minor role as tolerance mechanism.



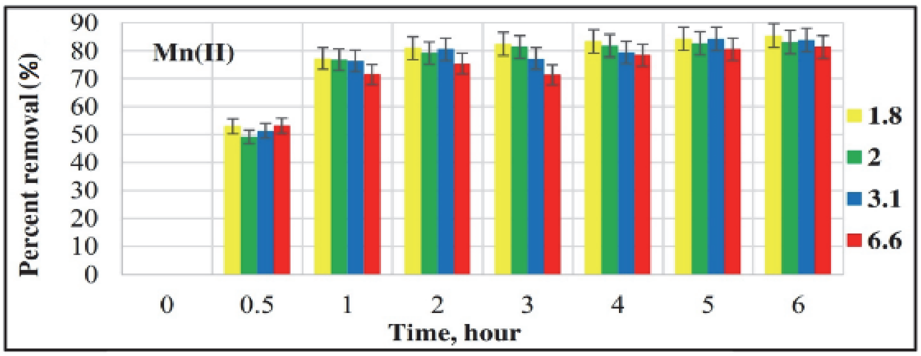
F_{time}=5922.69* P<0.05, F_{pH}=4.04* P<0.05

(a)



F_{time}=5810.75* P<0.05, F_{pH}=3.18* P<0.05

(b)



F_{time}=855.41* P<0.05, F_{pH}=6.66* P<0.05

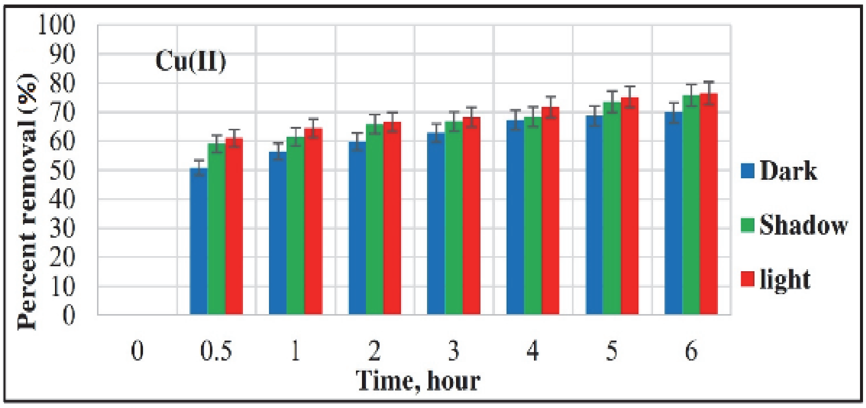
(c)

Fig. 2. Uptake of metals from different pH aqueous media: (a) Cu(II), (b) Fe(II), (c) Mn(II)

Consequently, only insignificant relation between the uptake ratio and the pH-value is observed in Fig. 2, which matches previous reports (Taylor, 1991). It could be concluded that the plant has the potential to survive and uptake high amounts of metal ions both in low or highly acidic media, and that the plant displays a tendency to tolerate different media, which is also in accordance to previous studies (Jafari, 2010).

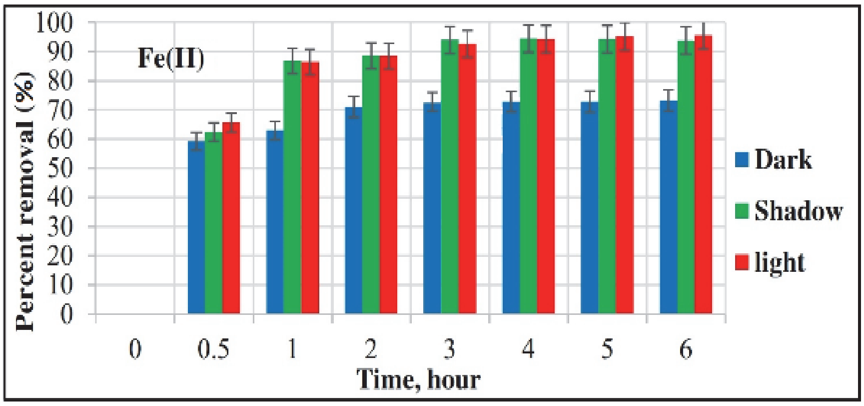
3.3. Remediation of metals in dependence on illumination

The photoperiod, i.e., changing dark and light conditions, can affect the growth performance based on the essential role of light in photosynthesis and consequently the potency of macrophytes to accumulate excess metal ions and retard the toxicity (Fig. 3).



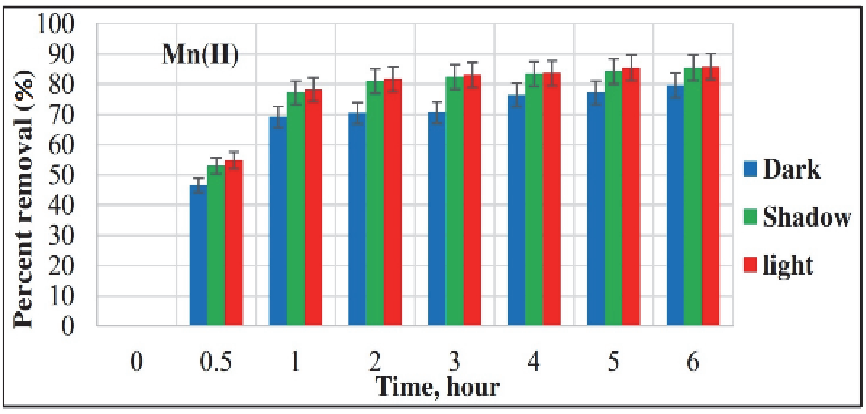
F_{time}=601.93* P<0.05, F_{light}=26.67* P<0.05

(a)



F_{time}=90.5* P<0.05, F_{light}=25.79* P<0.05

(b)



F_{time}=548.95* P<0.05, F_{light}=33.12* P<0.05

(c)

Fig. 3. Efficiency of the aquatic plant to uptake metals due to different lighting conditions: (a) Cu(II), (b) Fe(II), (c) Mn(II)

Stabilization of metal ions by aquatic plants in dependence on different illumination regimes was evaluated at three levels of light exposure (i.e., sunlight, shade and dark). Generally, it could be stated that, when cultured in contaminated water, illumination had no dramatic influence on the overall removal potency of *Ludwigia stolonifera;* even in darkness, removal capacity was not drastically reduced compared to full illumination. Looking into the details, uptake of metals behaves differently among the three metals studied. The illumination effect can be clearly observed in case of iron. Here, using illumination (light or shadow conditions) increased the uptake efficiency by about 25% more if compared to the treatment in dark; this trend is visible starting after the sampling at t = 1 h until the end of the experiment (6 h), as illustrated in Fig. 3b. This outcome could be explained by the correlation between photosynthesis rate driven by sunlight on the one hand, and, on the other hand, rapid growth and highly vegetative reproduction rates due to bioaccumulation performance under ultraviolet radiation (Mkandawire and Dudel, 2005).

For all metal ions, plants cultivated in shadow display a slightly decreased metal removal capacity in comparison to full illumination, but significantly higher capacity than observed for cultivations carried out in the dark (Fig. 3 a-c). However, in the case of Cu(II) and Mn(II), differences observed between setups under dark or illumination (light and shadow) were less pronounced (Fig. 3b and 3c, respectively) than for Fe(II) (Fig. 3a). The different behavior of the macrophyte in uptake of divalent iron and copper or manganese might be related to their different valences; divalent metal cations are structurally very similar (Jayakumar and Abdul Jaleel, 2009). Consequently, iron ions are to a high extent removed during exposure to daylight.

3.4. Identification of optimum mass and number of plants for higher accumulation

Twice duplication of mass or number of individual plants were performed to evaluate the uptake efficiency of three metal ions individually to evaluate the role of mass and number of the accumulator. Fig. 4 shows that the multiplication of number or mass of *L. stolonifera* does not result in any variation of metal ions removal during 6 hours of immersion in simulated wastewater containing 50 ppm of Fe, Cu or Mn as sole contaminants, not in combination.

This experiment confirms the high potential of the aquatic plant for phytoremediation of such contaminated media already by a low quantity of plant biomass; using lower biomass quantities, reduced secondary waste (biomass with accumulated metals) will be released. It is self-evident that a high amount of plants produced as solid waste displays is challenging the subsequent disposal process to a higher extent. Consequently, identifying the optimal mass or number of macrophytes required to uptake defined amounts of metal ions from contaminated waste bodies is an issue of economic and logistic importance. According to our results obtained (Fig. 4), it could be stated that already a low amount of biomass is sufficient to remediate relatively high shares of the metal contamination. For all investigated metal ions, no significant difference was observed for the individual setups; metal ion recovery was independent from number (1, 2, or 3) or mass (10 g, 15 g, or 20 g) of plants. Moreover, in comparison with other species used for phytoremediation, the stabilization performance for the metals by *L. stolonifera* biomass was superior to, e.g., *E. crassipes* in all density combinations studied, thus confirming the high efficiency of this plant (Njambuya and Triest, 2010).

3.5. Accumulation capacity of the plant using water contaminated with combinations of metals

It should be noted that the uptake performance of the plant for an individual metal might differ in dependence on the metal ion being present as sole contaminant or in combination with other metals. This originates from specific factors affecting plant uptake such as the interaction between individual metal ions present in the simulated wastewater.

Cu toxicity has a significant effect on root growth of many species where high concentrations inhibit the production of root hairs, often before any effects on shoots occur (Minnich et al., 1987). This behavior is presented clearly in Fig. 5, showing the lower uptake percentage for Cu than for Fe or Mn, when these metals are present in a combined form. In the case of iron, chemical transformation is essential for its metabolism. Fe(III) is the main form of iron in oxidized medium, while it is relatively toxic and essentially not bioavailable for macrophytes. Plants exposed to excess iron use a reductive mechanism for iron extraction from contaminated medium. In general, ferric iron is reduced to ferrous iron, before being stabilized into plant root cells as required to become available as a nutrient element (Meagher, 2000). Despite the common symptom toxicity of Mn, its uptake behavior is relatively similar to Fe and higher than in the case of Cu (Fig. 5).

Bioaccumulation is a result of the dynamic equilibrium between uptake, excretion, storage and degradation within the macrophyte and the outside medium (Zhou et al., 2008). Accordingly, bioaccumulation is affected by the presence of the metal with others of the same concentration. In agreement with present observations, Ouzounidou detected that uptake of Mn and Fe was decreased by excess Cu due to the Fe deficiency induced by the chlorotic symptoms on plant leaves in the presence of Cu (Ouzounidou, 1995).

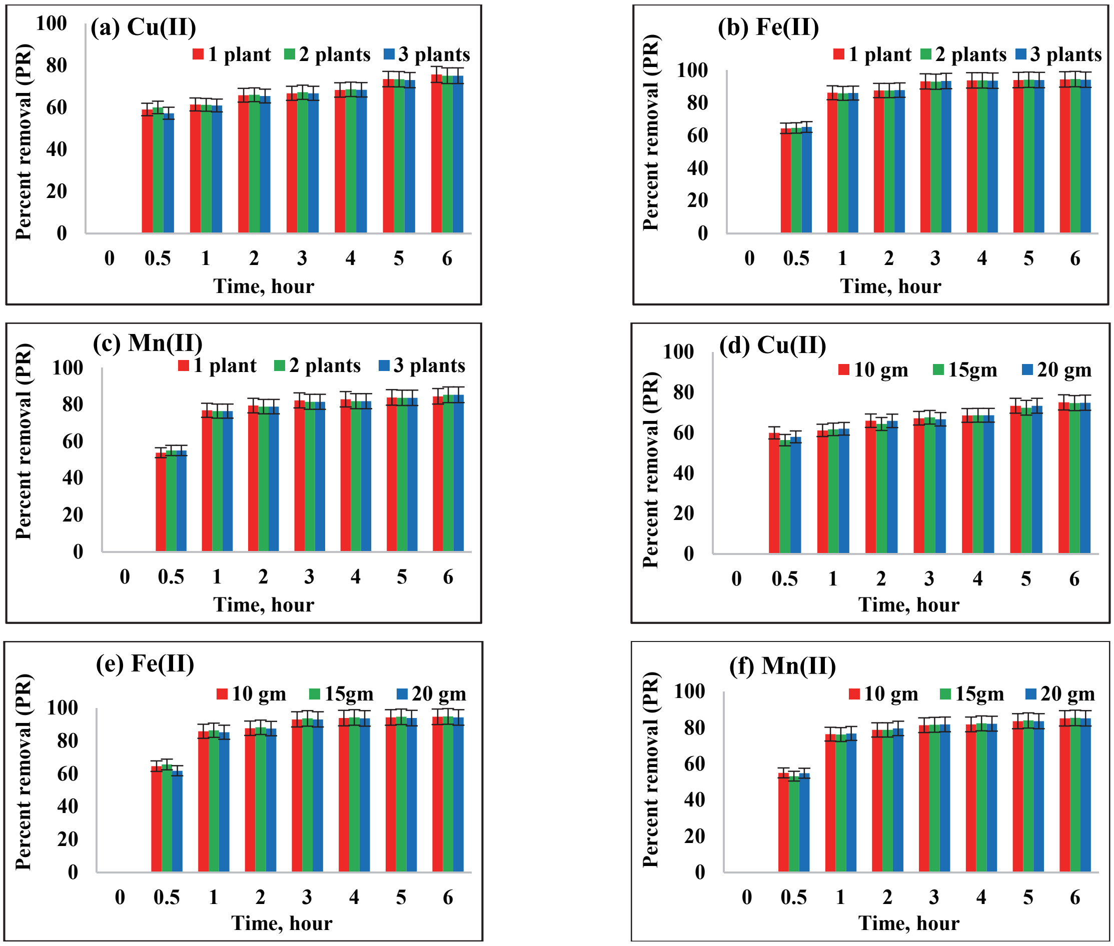


Fig. 4. Bioaccumulation of metal ions by *L. stolonifera* in dependence on number and mass of plants: (a) Cu(II), (b) Fe(II), (c) Mn(II) with different numbers of plants; (d) Cu(II), (e) Fe(II), (f) Mn(II) with different masses of plants

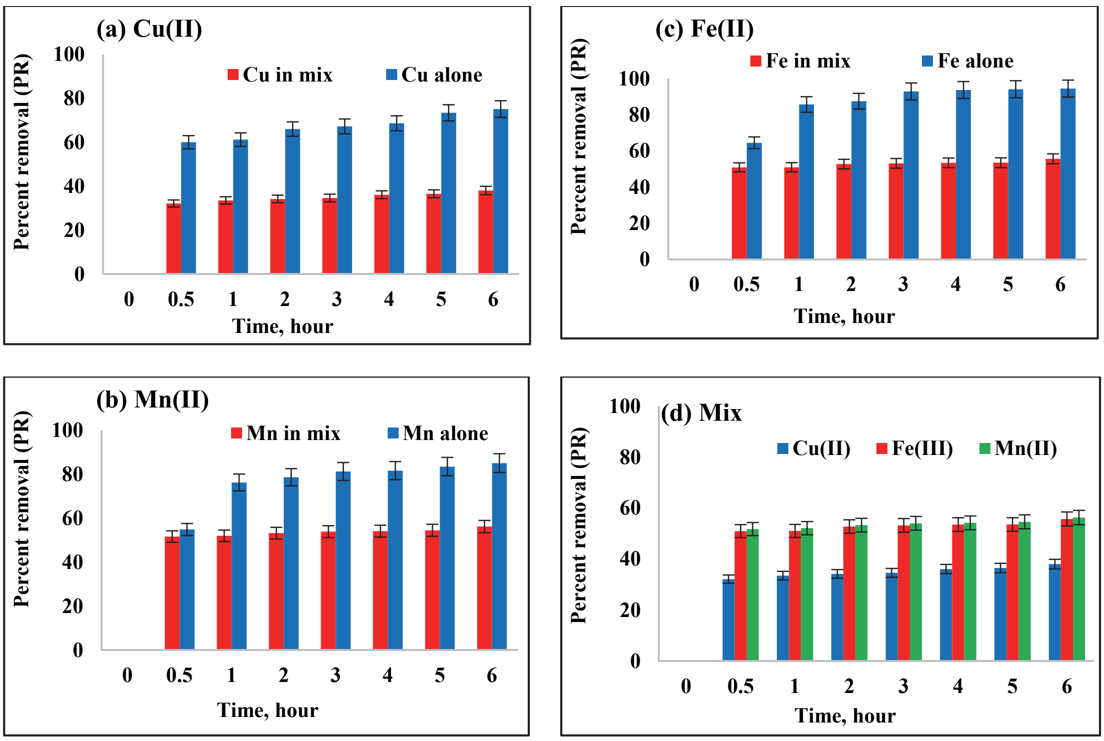


Fig. 5. Accumulation of different metals individually and in a mixture with others: (a) Cu(II), (b) Mn(II), (c) Fe(II) and (d) mixture of three metals

Table 2 and Fig. 5 illustrate the maximum uptake tendency of the three metals individually and in the mixture. The difference between two conditions typically amounts to about 38% for three metals. Meanwhile, it can be concluded from the data obtained that *L. stolonifera* accumulates 38%, 55%, and 56% of copper, iron and manganese, respectively, in a mixture during just six hours (Fig. 5); this further evidences its potential as efficient candidate species for phytoremediation and purification of wastewater**.**

Table 2. Maximum uptake of three metals in presence individually or in mixing

Metal ions	***Cu***	***Fe***	***Mn***
Initial concentration, ppm	50	50	50
Maximum uptake, % (individual metals)	75.1	94.6	94.6
Maximum uptake, % (in mix)	38.04	55.72	56.3
Difference, %	37.06	38.88	38.30

4. Conclusions

Phytoremediation technology as a green technology constitutes an eco-friendly and cheap tool for concentration and stabilization of many pollutants to decontaminate water and soil systems. The accumulation of the metals Cu, Fe and Mn in a simulated wastewater was processed by the native aquatic plant species *L. stolonifera*.

The most significant observation of high uptake potential at increasing concentrations for the three metals under different physical-chemical conditions encourages the use of *L. stolonifera* as a hyper-accumulator of environmentally hazardous compounds.

Many factors affecting the bioaccumulation of metals by aquatic plants, such as ambient metal content, pH-value of the medium, concentration of metal ions, competition between metals for binding sites, were evaluated. As major outcome, it was shown that recovery performance is not drastically influenced by major cultivation conditions such as pH-value or the illumination regime, and is independent from the mass of plant biomass.

A comparison was subsequently made between uptake of metals individually with maximum efficiency of 75%, 94%, 94% and if applied as a mixture, with 38%, 55%, 56% for Cu, Mn, and Fe respectively.

However, extra experiments are required to evaluate the metal stabilization in plant tissue as a hazardous waste before the subsequent disposal. *L. stolonifera* or other plants from this family might be studied to remediate other toxic metals present in contaminated soils or water bodies.

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