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Removal of heavy metals from wastewater by natural growing plants on River Nile banks in Egypt

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Abstract

Green remediation is a known technology that uses different types of plants to extract contaminants from the environment. This study aims to remove heavy metals from treated wastewater by using natural growing plants on River Nile banks in Egypt. Secondary treated effluent was collected from West Gerga wastewater treatment plant, located in Sohag city, Egypt. Experiments using two types of aquatic plants were carried out. They were planted individually and in combination with different densities on the secondary treated wastewater surface for 10 days' retention time to remove cadmium (Cd), nickel (Ni) and lead (Pb). It was concluded that both plants have high capabilities to remove heavy metals directly from treated wastewater. The removal efficiency of Cd and Pb was higher when they were planted together than when individually planted. A positive relationship was observed between detention time and heavy metals removal. The removal efficiency of heavy metals increased with the increase of plant density for both plant types. Also, the availability of aquatic plants and their free cost makes their use an economically attractive alternative. In addition, the removal of these plants from River Nile improves the performance of water distribution networks in Egypt.

Key words: aquatic plants, cadmium, lead, nickel, River Nile, wastewater

Highlights

- Removal of heavy metals from treated wastewater using aquatic plants named *Echinochloa pyramidalis* and *Ludwigia stolonifera*.
- Both *E. pyramidalis* and *L. stolonifera* have high capabilities to remove heavy metals directly from treated wastewater.
- The removal efficiency of heavy metals increases with the increase of plant density for both plant types.
- The availability of aquatic plants and free cost make their use in the wastewater treatment process to remove heavy metals an economically attractive alternative.
- The removal of these plants from the River Nile improves the performance of water distribution networks in Egypt.

INTRODUCTION

Increasing population growth, unexpected climate change and poor management of water resources are the main reasons for water scarcity, so efficient management of available water resources is needed, such as designing water harvesting structures, reusing wastewater, and improving the desalination plants especially in developing countries (Asthana & Shukla 2014). Recently, in non-urban areas of developing countries, the reuse of wastewater is presented as a perennial source of nutrients for agricultural production (Varkey *et al.* 2015).

Many types of industries, such as automobile and metal processing industries, are responsible for heavy metals contamination of our environment (Matouq *et al.* 2015). Heavy metals are toxic materials and harmful to the environment because they are non-biodegradable and toxic at trace concentrations (Ayyappan *et al.* 2005; Gautam *et al.* 2013). Metal toxicity causes many human diseases, such as cancer and nervous disorders (Nagjyoti *et al.* 2010; Lee *et al.* 2012). Removal of heavy metals from treated wastewater is required before its reuse in agriculture. Removal of heavy metals by traditional treatment methods such as precipitation, coagulation–flocculation, adsorption, and other advanced oxidation processes comprises a big cost (Lara *et al.* 2014). Therefore, it has become mandatory to induce a cost-effective green technology to remove these heavy metals and improve the effluent standards (Gonzalez *et al.* 2014).

Constructed wetlands (CWs) are designed and constructed to simulate the biological, chemical, and physical processes that happen in natural wetlands (Zhang *et al.* 2014). Wetlands are one of the most productive life support systems in the world and they are very important to mankind in socio-economic and ecological fields (Sanyal *et al.* 2015). Wetlands with macrophytes are natural systems that can be used for the treatment of domestic wastewater. Using macrophytes to treat wastewater is a cost-effective process due to a low cost of construction, operation, and maintenance. Phytoremediation is a known process that uses different types of plants to reduce and remove contaminants from soil and water (Akinbile & Yusoff 2012; Gupta *et al.* 2012; Kumar *et al.* 2012; Ugya *et al.* 2015; Lu et al. 2016; Prasad & Maiti 2016). Phytoremediation is also called green remediation, botano-remediation, agro-remediation, and vegetative remediation (Kumar & Chopra 2016).

Wetlands are capable of providing removal rates ranging from 60% to over 95% for many pollutants; they provide important functions such as habitat enhancement and they are less intrusive and provide a more environmentally sensitive approach to pollution abatement. One of the disadvantages of wetlands is that the reliability of their treatment systems can often be less consistent than that of traditional treatment systems, as nature can be unpredictable and external factors such as weather and pests often cause sites to display inconsistent contaminant removal rates (Davis 1995; Tousignant *et al.* 1999).

Ajibade & Adewumi (2017) evaluated the phytoremediation potential of three aquatic macrophytes (*Commelina cyanea*, *Phragmites australis* and Water Hyacinth (*Eichhornia crassipes*)) for treatment of municipal wastewater. *Phragmites australis* gave the highest removal efficiency for phosphate (85.8%).

This study aims to remove heavy metals from treated domestic wastewater using two types of plants that grow naturally on the River Nile banks in Egypt, namely *Echinochloa pyramidalis* and *Ludwigia stolonifera*, which were planted individually and in combination with different densities on the secondary treated wastewater from West Gerga wastewater treatment plant, Sohag, Egypt. These plants are undesirable, since they retard and increase the roughness of water flow. Egypt's government has already removed and disposed of these plants, which comprises a big cost without any benefits from these removed plants, to improve the performance of irrigation water distribution networks and enhance the maintenance working environment of irrigation structures.

METHODS AND MATERIALS

Studied plant species

Studies performed in previous works, the laboratory, and the field have provided encouraging insights into the suitability of aquatic plants to act as bio-indicators of water quality, especially in relation to the monitoring of trace metals.

Fawzy *et al.* (2012) investigated the concentrations of Cd, Cu, Pb, and Zn in sediments, water, and different plant organs of six aquatic vascular plant species: *Ceratophyllum demersum*; *Echinochloa pyramidalis*; *Eichhornia crassipes*; *Myriophyllum spicatum*; *Phragmites australis* and *Typha domingensis* growing naturally in the Nile system (Sohag Governorate), and reported that the concentrations of heavy metals in water, sediments, and plants possess the same trend: Zn > Cu > Pb > Cd, which reflects the bio-monitoring potentialities of the investigated plant species (Fawzy *et al.* 2012).

Due to their availability on the River Nile banks in Egypt, and their free cost, *Echinochloa pyramidalis* and *Ludwigia stolonifera* were used in the present study.

Batch experiments were configured at the same environmental conditions (i.e. weather, temperature, sunlight, wind speed, etc.), to avoid their influences on experimental results, from October 2017 to December 2017, using *Echinochloa pyramidalis* and *Ludwigia stolonifera* aquatic plants. Secondary treated effluent was collected from west Gerga wastewater treatment plant, located in Sohag city, Egypt. Batch tests were carried out with similar samples obtained during similar phases in the Chemical Department laboratories, Faculty of Science, Sohag University. Characteristics of the wastewater samples, obtained by using laboratory devices, are shown in Table 1.

Table 1 | Characteristics of the secondary treated wastewater effluent from West Gerga plant

Parameters	Values[a]	Laboratory device
Temperature (°C)	23.74 ± 0.730	Dry mercury thermometer.
pH	7.63 ± 0.125	pH meter PC 300 (Eutech Instruments)
Electrical conductivity (μS cm $^{-1}$)	1,313 ± 10.81	Conductivity meter PC 300 (Eutech Instruments).
Dissolved oxygen (mg L^{-1})	1.48 ± 0.043	Schott handy lab OX 1/SET portable meter.
Biochemical oxygen demand (mg L^{-1})	59.33 ± 2.32	Schott handy lab OX 1/SET portable meter.
Nitrite (mg L^{-1})	0.23 ± 0.02	WPA CO 7500 colorimeter.
Nitrate (mg L^{-1})	11.45 ± 1.95	WPA CO 7500 colorimeter.
Orthophosphate (mg L^{-1})	3.09 ± 0.0.47	WPA CO 7500 colorimeter.
Total phosphate (mg L^{-1})	7.44 ± 0.26	WPA CO 7500 colorimeter.

[a]Values are mean of three replicates + standard error.

Reparation of heavy metal concentrations

In this study, constant concentrations of 5 mg/l of the tested metals (Cd, Ni, and Pb) were used as a mixture. Lead, cadmium and nickel solutions were prepared using $Pb(NO_3)_2$ (Sigma, St. Louis, MO A.R., 99.9%), $Cd(NO_3)_2$ (Aldrich Chemical Co., 98%), and $Ni(NO_3)_2$. The concentrations were calculated based on the individual element versus their compound form. The reagents were dissolved in distilled water to achieve the appropriate concentration level.

Experimental setup

The experiments were executed at the Experimental Farm of the Faculty of Agriculture, Sohag University, Sohag, Egypt. In this study, an effluent from West Gerga wastewater treatment plant, with a constant artificial concentration of 5 mg/l of the tested metals (Cd, Ni, and Pb) was used as a mixture. The experimental setup consists of seven plastic tanks (50 cm length × 50 cm width × 96 cm height) with a working depth of 88 cm. Each tank has a wooden frame at the top to hold the plants up (Figure 1). *Echinochloa pyramidalis* and *Ludwigia stolonifera* plants were collected from the mainstream of the River Nile's east bank (N: 26° 33′ 29″; E: 31° 42′ 33″). The dimensions of

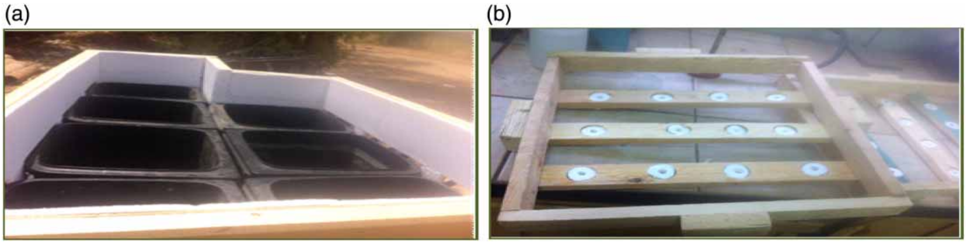


Figure 1 | Photograph of the experimental tanks (a) and wooden frame (b).

these plants were not the same; the average dimensions, with a standard error of a single plant, were 43 ± 3.1 cm height and 8.5 ± 0.5 g fresh weights of *Echinochloa pyramidalis* and 56 ± 3.4 cm height and 38.6 ± 0.8 g fresh weight of *Ludwigia stolonifera*.

Experimental process

Acclimatization of the selected plants was done using distilled water for 7 days; the selected plants were thoroughly washed with freshwater before being placed in each experimental set. Then, 220 litres of secondary treated effluent was poured into each experimental set and subjected to 10 hours of sunlight daily throughout the length of the experiment. Distilled water was added at fixed intervals to compensate for the evaporation losses and keep the pH of the wastewater at a constant value.

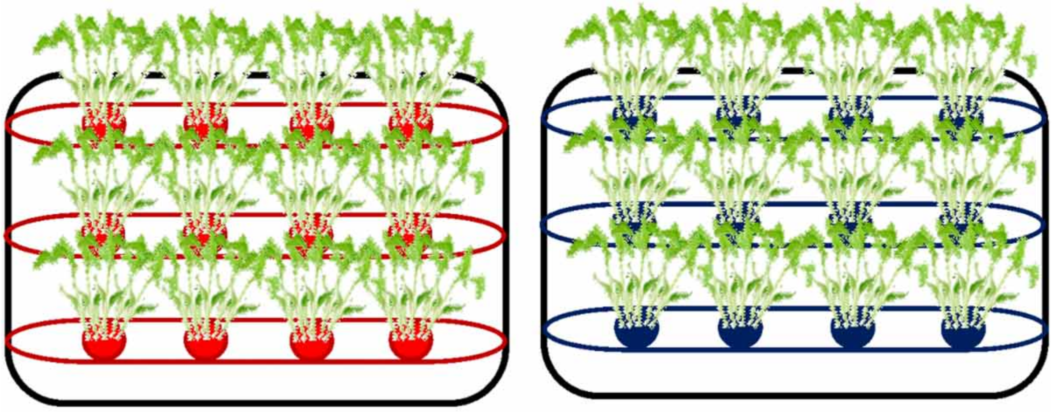
The removal of Cd, Ni, and Pb was studied by planting the two plants each individually and both in combination, with different densities.

Four sets of experiments, as shown in Figure 2, were executed; the first set consisted of only *E. pyramidalis* plants with a maximum density, which could be fixed in the wooden frame, of 80 plants per square metre of wastewater surface. The second set consisted of only *L. stolonifera* plants, which were kept at a density of 80 plants per square metre. In the third set, three different experiments were carried out, each having a different density of combined plants (*E. pyramidalis* + *L. stolonifera*); the first density was 100% (i.e. 40 *E. pyramidalis* plants/m^2 and 40 *L. stolonifera* plants/m^2), the second was 60% (i.e. 24 *E. pyramidalis* plants/m^2 and 24 *L. stolonifera* plants/m^2), and the third was 20% (i.e. 8 *E. pyramidalis* plants/m^2 and 8 *L. stolonifera* plants/m^2). In the fourth set, no plants were planted and it was used as an experimental control case for the determination of natural heavy metals removal.

For each experiment, three wastewater samples (triplicate) each of 15 ml volume were collected, at the same time of day from each compartment at different places within the root depth, at intervals of 1, 3, 5, 7 and 10 days (i.e. number of samples of each experiment, $n = 15$) for heavy metals analysis. Also complete plant samples, each weighing 5 grams, were collected at the beginning and at the end of each experiment.

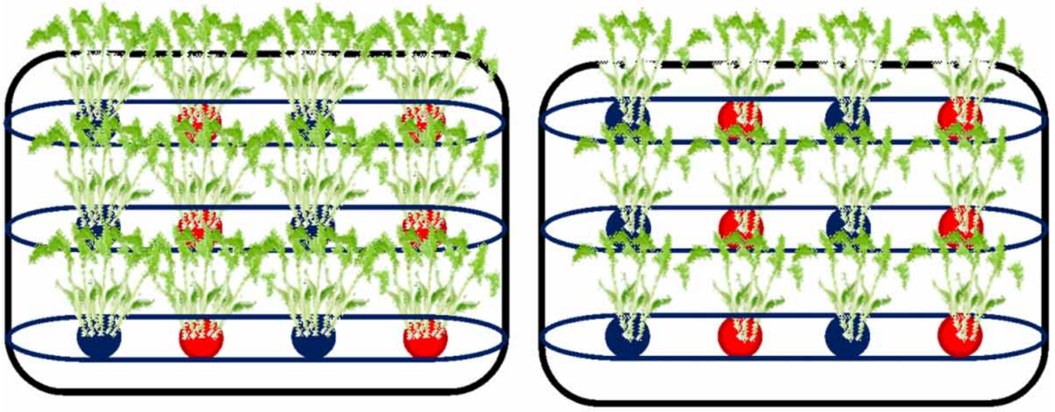
Analytical procedures

After washing, the plant samples were dried in a convection oven for 24 hours at 48 °C, and then ground and powdered using a mortar to ensure homogeneity for facilitating organic matter digestion. Dry plant materials were digested according to the wet digestion procedure involving concentrated nitric acid (Campbell & Plank 1998). Cd, Ni, and Pb concentrations in both wastewater and plant samples were measured using an Atomic Absorption spectrophotometer (PerkinElmer Analyst 400, USA). Measurements were repeated three times. Heavy metal concentrations were expressed as mg/l (ppm), for wastewater samples and mg/g of dry weight for plant samples.



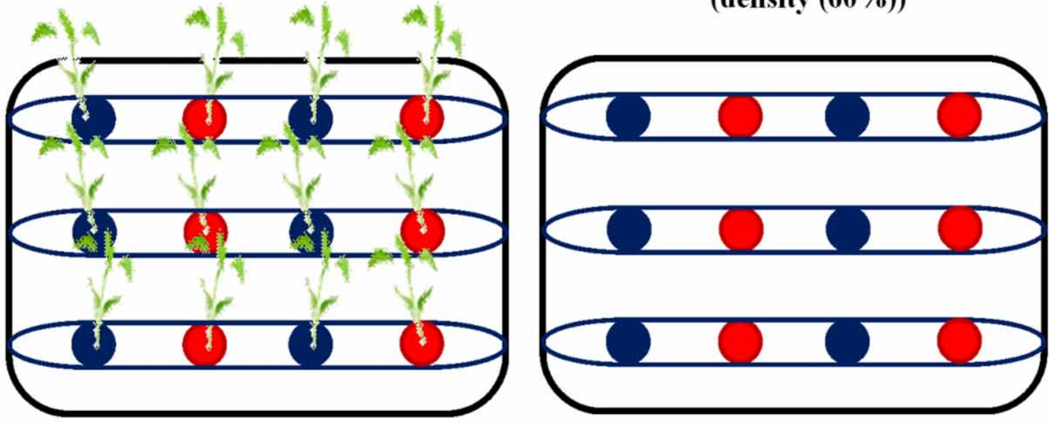
Set 1*: Echinochloa pyramidalis*

Set 2: *Ludwigia stolonifera*



Set 3(a): *Echinochloa* + *Ludwigia* (density (100%))

Set 3(b): *Echinochloa* + *Ludwigia* (density (60%))



Set 3(c): *Echinochloa* + *Ludwigia* (density (20%))

Set 4: No plants (control set)

Figure 2 | Schematic description of the experimental sets.

Statistical analysis

Complete randomized design (CRD) was used with three replications for each treatment. Analysis of variance (ANOVA) was carried out, using Proc Mixed of SAS package version 9.2 (SAS 2008) and means were compared by Duncan test at 5% level of significance, to determine the effect of using aquatic plants, with different planted cases at various detention times, on the removal efficiency of heavy metals (Steel & Torrie 1981; SAS Institute 2008).

Finally, correlation analysis was performed to examine the relationship between the variables according to Spearman rank order correlation as heavy metal levels were the intendant variable and detention time was the dependent one.

The heavy metal removal percentage was obtained using the following equation:

$$\% \text{ Removal efficiency} = \frac{(C_i - C_f)}{C_i} * 100 \tag{1}$$

where C_i and C_f are the initial and final heavy metal concentrations, respectively.

RESULTS AND DISCUSSION

Role of plants in the removal of heavy metals

Removal of Cd, Ni, and Pb due to natural processes such as degradation and precipitation was determined from control experimental sets without using plants, and results are shown in Table 2, which shows clearly the removal of a very little amount of heavy metals through the natural precipitation process. Keeping this result as a reference, two aquatic plant types; that is, *Echinochloa pyramidalis* and *Ludwigia stolonifera*, were used for the efficient removal of heavy metals. A significant enhancement in the removal of Cd, Ni, and Pb (Table 2) over the values of the control experimental set was observed when both plants were planted individually. The removal efficiency of Cd increases by about 34% and 44% for *E. pyramidalis* and *L. stolonifera* respectively; the removal efficiency of Ni increases by about 16% and 26% for *E. pyramidalis* and *L. stolonifera* respectively; and the removal efficiency of Pb increases by about 78% and 74% for *E. pyramidalis* and *L. stolonifera* respectively with respect to control experiments.

Table 2 | Removal of Cd, Ni, and Pb by *E. pyramidalis*, *L. stolonifera*, their combination, and reference set

Heavy metal removal (%)	Combination of *E. pyramidalis* and *L.stolonifera*	*E. pyramidalis*	*L. stolonifera*	Control set
Cd	61.52 ± 14.01	37.99 ± 12.37	48.04 ± 16.48	4.16 ± 0.79
Ni	27.8 ± 6.46	22.25 ± 2.89	32.3 ± 6.68	6.36 ± 1.59
Pb	93.22 ± 4.87	88.74 ± 10.71	84.29 ± 14.84	10.47 ± 1.58

Values are the mean ± standard deviation for $n = 15$.

The possible processes concerned in heavy metals removal are metal consumption by these wetland plants, precipitation, and co-precipitation as insoluble salts and metal bound to the substrate (Brix 1994; Ranieri *et al.* 2011). Rhizosphere microbes play an important role in phytoremediation. Since the rhizosphere is the immediate vicinity of the root, the chemical and physical changes in that environment can easily affect heavy metal uptake by the plant. The rhizosphere of *P. australis* and *T. latifolia* provided the supporting media for the growth of microorganisms, which are the main places for heavy metals immobilization and uptake by plants (Jacob & Otte 2004).

When *E. pyramidalis* and *L. stolonifera* were grown in combination, there was a further increase in the removal of Cd and Pb compared to the plants grown individually. As is clear from the ANOVA analysis results shown in Table 2, the removal efficiency of Cd and Pb by a combination of the two plants increases by about 13% and 5% respectively with respect to that of the individual plants.

Perhaps the reason for the improved removal of Cd and Pb is due to the change of the plants' behavior when grown in combination, which is known as synergistic effects of the plant species (Tripathi & Upadhyay 2003). The increase in the rhizosphere of plants increases the surface area of the rhizomes, which would further increase the rhizomes' cell wall ability to uptake metals through immobilization (Tsednee *et al.* 2012).

The solubilization and movement of phytosiderophores by plants result in a complex with free heavy metal ions that may be taken as a secondary cause for the increase in absorption performance (Hu *et al.* 2014). Removal of Cd and Ni was significantly higher in *L. stolonifera* culture than that of *E. pyramidalis* culture, whereas in *E. pyramidalis* culture removal of Pb was higher. The possible reason would be that the selected plant species may have different uptake efficiency for the heavy metals used. Similar findings were introduced by Mishra & Tripathi (2008), where significantly different removal efficiencies for Fe, Cu, Cd, Cr, and Zn using *Pistia stratioties*, *Siprodela polyrrhiza* and *Eichhornia crassipes* support the present observation (Mishra & Tripathi 2008). Generally, the variation of heavy metal concentrations in wastewater, through each experiment day, in relation to plant species (P) and detention time (T), as analyzed by the two way repeated measures ANOVA, was significant as is clear from the ANOVA – F values are shown in Table 3.

Table 3 | ANOVA – F values of heavy metals concentrations in wastewater for different plant species at various detention times

Variables	Cd	Ni	Pb
Plant species (P)	81.38*	45.33*	88.54*
Retention times (T)	236.66*	190.63*	4,453.0*
P*T	4.41*	3.72*	27.87*

*$p < 0.01$.

The preferential order of heavy metal removal in the present study was Pb > Cd > Ni in *E. pyramidalis, L. stolonifera* and their combination (Table 2). The possible reason for higher removal of Cd and Pb might be that emergent plants with a long root system have higher metal absorption capacity than that of free-floating plants. Sargın *et al.* (2015), using pollenechitosan microcapsules, observed lower removal of Cd (26.6%) and Ni (16.2%) compared with findings of the present work (Sargın *et al.* 2015). The present process performs greater Cd removal from wastewater (61.52%) than that observed by Ahmadi *et al.* (2014) while treating aqueous solutions with maghemite nanoparticles (20%) (Ahmadi *et al.* 2014). The greater removal of Cd may be due to the plants' greater uptake efficiency for metals when compared with the other advanced technologies. Abdul Syukor *et al.* (2016) carried out an experiment on the uptake of heavy metals by *Typha angustifolia* and *Limnocharis flava* plants and achieved 49.3% Cd and 54.7% Pb removal, while the present work achieved 61.52% and 93.22% higher removal for Cd and Pb when two plants (*E. pyramidalis* and *L. stolonifera*) were applied together (Abdul Syukor *et al.* 2016). While treating heavy metal contaminated wastewater with *Phragmites australis* and *Typha latifolia*, Kumari & Tripathi (2015) found a removal efficiency of 48.1% for Cd and 53.6% for Pb, which are 13.42% and 39.62% less than our findings in the present study, respectively (Kumari & Tripathi 2015).

Bello *et al.* (2018) investigated the phytoremediation ability of *Phragmites australis* to remove cadmium (Cd), lead (Pb), and nickel (Ni) from contaminated water, The results of the study showed that *P. australis* had a removal efficiency of 93% for cadmium, 95% for lead and 84% for nickel over a 6-week period, which was a higher removal efficiency than that by *E. pyramidalis* and *L. stolonifera* in the present study.

To study the effect of wastewater pH on the removal efficiency of heavy metals through precipitation and absorption, heavy metal concentrations and pH were measured during the experiments.

Initially, pH of the secondary treated effluent wastewater was slightly alkaline (Table 1), and during the experiment varied from neutral to slightly alkaline (6.86 ± 0.01–7.82 ± 0.01). This change in pH may be due to the addition of oxygen (O_2) via the diffusion of the atmospheric O_2 and photosynthetic activity of plants. That variation of pH from neutral to slightly alkaline during the experiments implies that the heavy metals removal from the wastewater was achieved by metals solubilization in the rhizosphere and absorption through the plant roots.

Aquaculture with high pH and high concentration of heavy metal ions has more chances of precipitation as a dominant process of metal immobilization, where the presence of anions such as SO_4^{2-}, CO_3^{2-}, OH-, and HPO_4^{2-} facilitates the process (Hong *et al.* 2007; Ok *et al.* 2010).

Khan *et al.* (2009) reported a pH value equal to and greater than 7 was best for removing heavy metals from storm wastewater (Khan *et al.* 2009). Abid *et al.* (2015) reported that the optimum heavy metals removal efficiency using the phytoremediation process occurs in the alkaline pH range (Abid *et al.* 2015).

Relation of detention time and heavy metals removal

The relation of detention time and heavy metals removal was studied using Spearman rank order correlation. In all the planted experimental sets of wastewater, significant negative correlation coefficients of Cd, Ni, and Pb levels with retention period were determined, which ensured the positive relation of retention period and Cd, Ni, and Pb removal percentages at $p < 0.001$ (Table 4). Similar findings were observed by Mishra *et al.* (2009) for the removal of heavy metals from coal mine effluent (Mishra *et al.* 2009).

Table 4 | The simple linear correlation coefficient (r) between detention time and heavy metals removal at $p < 0.001$

	Correlation coefficient value of heavy metals removal		
Species	**Cd**	**Ni**	**Pb**
E.py	− 0.942	− 0.791	− 0.802
L.st	− 0.947	− 0.889	− 0.851
E.py + L.st	− 0.913	− 0.908	− 0.734

E.py = *Echinochloa pyramidalis* and L.st = *Ludwigia stolonifera*.

The removal of Cd, Ni, and Pb from wastewater by *E. pyramidalis* and *L. stolonifera* at different retention times is shown in Figure 3, which illustrates the decrease in heavy metal concentration, for all the three metals, with detention time during 10 days of exposure. The possible reason for enhancement in heavy metals removal might be the continuous absorption of metals by plants. Similar findings by Kumari & Tripathi (2014) support the present observation (Kumari & Tripathi 2014).

Sorption of heavy metals in plants

Mass balance calculations of heavy metals at the end of the experiment for a contaminated sample are shown in Table 5. The values in Table 5 indicate that the loss of Cd, Ni, and Pb from the wastewater equals their net sorption in plants (mg), which was measured from the dry plant samples using an Atomic Absorption spectrophotometer, and loss due to natural precipitation (mg). Metal sorption for Cd and Ni was greater in *L. stolonifera* compared with *E. pyramidalis*, although this was not true for Pb. Natural metals precipitation (mg) occurs through the oxidation of materials in the

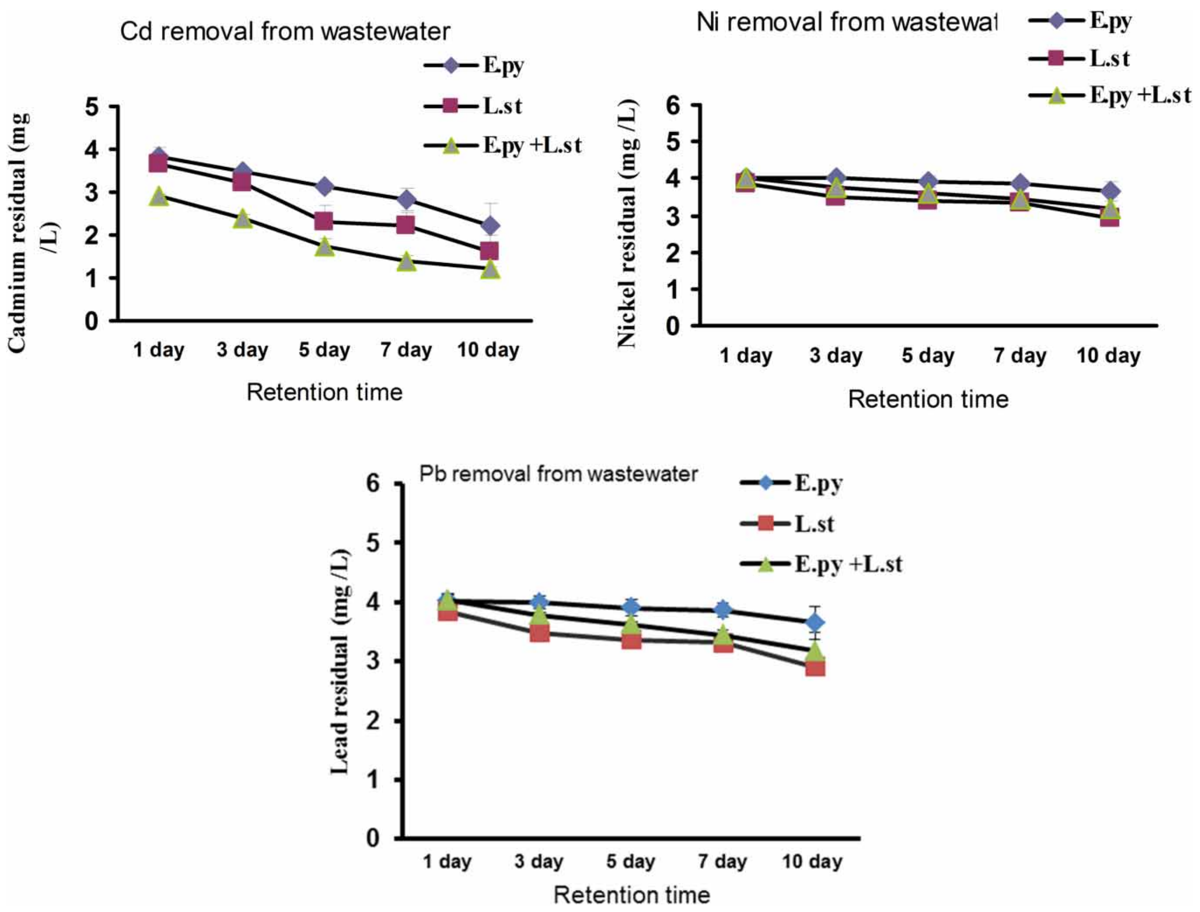


Figure 3 | The variations of metal residuals in wastewater with retention time for *E. pyramidalis, L. stolonifera* and their combination, the first for Cd, the second for Ni and the third for Pb.

Table 5 | Mass balance calculations of heavy metals in *E. pyramidalis*, *L. stolonifera* and their combination (retention period 10 days)

Heavy metals	Plant sp.	Initial concentration (mg)	Concentration after treatment (mg)	Measured net metals sorption in plants (mg)	Natural metals precipitation R (mg)
Cd	E.py	5.00 ± 0.04	2.23 ± 0.51	2.56 ± 0.59	0.21 ± 0.04
	L.st	5.00 ± 0.04	1.60 ± 0.41	3.19 ± 0.42	0.21 ± 0.04
	E.py + L.st	5.00 ± 0.04	1.22 ± 0.04	3.57 ± 0.07	0.21 ± 0.04
Ni	E.py	5.00 ± 0.03	3.65 ± 0.27	1.03 ± 0.27	0.32 ± 0.08
	L.st	5.00 ± 0.03	2.91 ± 0.13	1.77 ± 0.11	0.32 ± 0.08
	E.py + L.st	5.00 ± 0.03	3.17 ± 0.31	1.51 ± 0.27	0.32 ± 0.08
Pb	E.py	5.00 ± 0.06	0.16 ± 0.09	4.31 ± 0.12	0.53 ± 0.08
	L.st	5.00 ± 0.06	0.24 ± 0.06	4.23 ± 0.17	0.53 ± 0.08
	E.py + L.st	5.00 ± 0.06	0.08 ± 0.003	4.39 ± 0.22	0.53 ± 0.08

Values are the mean of three replicates ± standard error. E.py = *Echinochloa pyramidalis* and L.st = *Ludwigia stolonifera*. R = Concentration in the reference set (without plant).

presence of dissolved oxygen. It indicates the deposition of heavy metals in the form of oxidized salts on the bottom of the experimental set without any influence of experimental plants.

Accumulation of heavy metals

The accumulated heavy metals in *E. pyramidalis* and *L. stolonifera* plants were measured from the dry plant samples using an Atomic Absorption spectrophotometer at the initial time, after 1 day and 10 days, and the results are presented in Table 6. The table illustrates that the accumulation of Cd and Ni was higher in *L. stolonifera* than *E. pyramidalis*, while *E. pyramidalis* showed higher accumulation for Pb.

Table 6 | Accumulation of heavy metals in *E. pyramidalis* and *L. stolonifera* (mg/g.dw)

	E. pyramidalis			**L. stolonifera**		
Time	**Cd**	**Ni**	**Pb**	**Cd**	**Ni**	**Pb**
Initial	0.013 ± 0.001	0.038 ± 0.002	0.026 ± 0.003	0.037 ± 0.003	0.056 ± 0.004	0.045 ± 0.002
Day-1	2.13 ± 0.0.65	1.33 ± 0.21	3.87 ± 0.22	3.01 ± 0.87	2.01 ± 0.17	3.41 ± 0.27
Day-10	3.77 ± 0.32	1.84 ± 0.17	5.09 ± 0.14	4.61 ± 0.51	2.89 ± 0.41	4.89 ± 0.14

Values are mean of three replicates ± standard error.

A comparison between the accumulated amounts of Cd, Ni and Pb within *E. pyramidalis* and *L. stolonifera* showed that Pb amounts were higher than that of cadmium, and Cd amounts were higher than that of Ni for the ten-day exposure. This may be a result of different response or tolerance of species to kinds of metals, and reflects the complexity of plant metal uptake under field conditions (Amany 2012).

Effect of the density of aquatic plants on heavy metals removal

In this study, the maximum removal efficiency of heavy metals by different intensities of the mixture of *E. pyramidalis* and *L. stolonifera* was at a density of 100%, while the minimum was at a density of 20%, and results are shown in Figure 4. The preferential order of the residual of heavy metals removal by the mixture of *E. pyramidalis* and *L. stolonifera* was Ni > Cd > Pb. The results showed a rapid reduction in metals residual since the first day exposure, with a different value for each metal type, since the results show the ability of both plants to absorb a larger amount of Pb in the first day than of Cd and Ni, while the metals removal continued over the remaining days of the experiment and decreased gradually by the end of the experiment for the three metals.

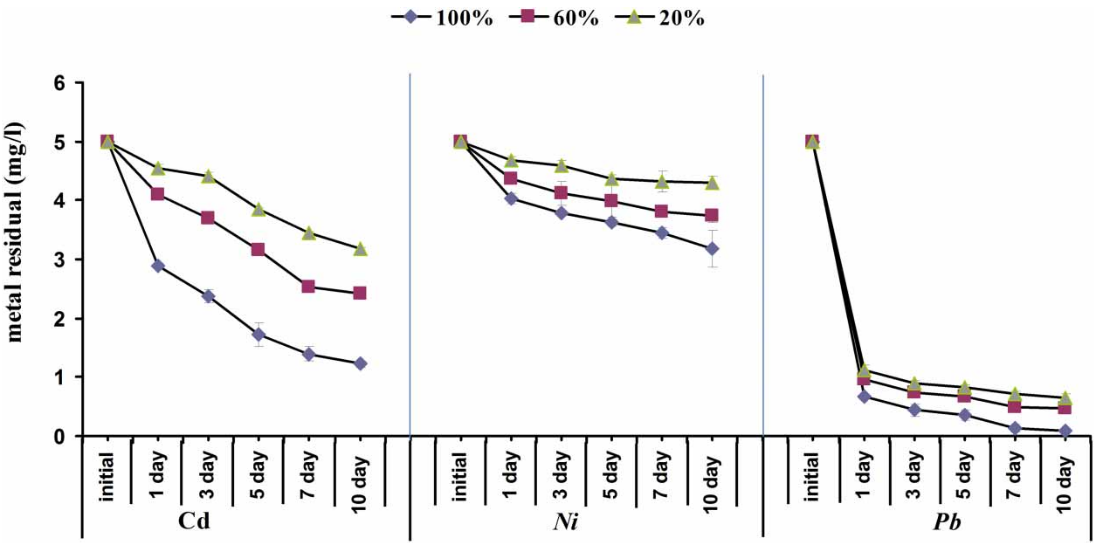


Figure 4 | Metals residual in the wastewater enriched with concentration (5 mg/l) of a mixture of Cd, Ni, and Pb by the different intensities of the mixture of *E. pyramidalis* and *L. stolonifera* during the experiment's duration.

The possible reason for enhanced removal of Cd, Ni, and Pb might be the increase in the rhizosphere of these plants due to the increase of plant density, which results in more surface area of the rhizomes, which would further augment the rhizomes' cell wall capability to absorb metals through immobilization. Similar findings were observed by Saleh *et al.* (2017) for removal of cesium and cobalt radionuclides from simulated radioactive wastewater by *Ludwigia stolonifera* aquatic plants (Saleh *et al.* 2017).

CONCLUSIONS

In the present study, the heavy metals removal from secondary treated wastewater using two types of natural growing plants on the River Nile banks in Egypt, namely *Echinochloa pyramidalis* and *Ludwigia stolonifera*, was tested. Plantings these two plants in combination showed the highest removal of Cd and Pb, while *L.stolonifera* showed the highest removal of Ni. There was a positive relationship between detention time and removal of Cd, Ni, and Pb in the wastewater used for the experiment, which was confirmed by negative and significant correlation coefficients of Cd, Ni, and Pb concentrations with detention time at $p < 0.001$. Preferential order of heavy metals removal indicates higher affinities of Pb towards plants followed by Cd > Ni. Mass balance calculations showed that the loss of Cd, Ni, and Pb from wastewater was equal to their net sorption in plants and loss due to natural precipitation. The sorption ability of *L.stolonifera* is greater than that of *E. pyramidalis* for the tested heavy metals, except for Pb. The removal efficiency of heavy metals increases with the increase of plant density for both plant types.

The availability of aquatic plants and their free cost make their use in the wastewater treatment process to remove heavy metals an economically attractive alternative.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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