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Original Article

Experimental study on removal of toxic metals and nutrient salts from secondary treated wastewater using *Echinochloa pyramidalis* and *Ludwigia stolonifera*

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ABSTRACT

The reuse of treated wastewater, in particular for irrigation, is an increasingly common practice, encouraged by governments and official entities worldwide. Constructed wetlands have become known systems that use many plants varieties to withdraw pollutants from the nature. The present research goal is to examine the using of *Echinochloa pyramidalis* and *Ludwigia stolonifera* to extract toxic metals and nutrients from wastewater. The plants were used each individually and together for 10 days to remove toxic metals and nutrients from wastewater with different concentrations. Higher removal efficiency of Lead (95.77 %) at concentration of 10 mg/l was obtained when they planted together than that of planted individually, while a removal efficiency of Nickel (49.40 %) at treatment of 20 mg/l was obtained by planted *L. stolonifera* alone and removal efficiency of Cadmium (75.47 %) at 20 mg/l. was obtained by planted *E. pyramidalis* alone. A positive correlation was noticed between the time of detention and removal of toxic metals, and negative and significant correlation factors for toxic metals corresponding to the detention period were confirmed. Excepting Lead, *L. stolonifera* showed greater absorption capacity than *E. pyramidalis* for all studied toxic metals and *L. stolonifera* achieved the maximum removal efficiency of 56.52% and 43.93%, for (NO2-N) and (NO3-N) respectively while, *E. pyramidalis* scored the maximum removal efficiency of 49.84% and 41.26%, for (PO4-P) and (TP) respectively. It is concluded that both studied plants can be used to remove toxic metals and nutrients from wastewater.

KEYWORDS

contaminants,

removal efficiency,

aquatic plants,

wastewater.

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INTRODUCTION

The sustainable use of the water resources is a crucial issue that has been worldwide discussed over the last decades. Due to some factors such as climate change and resulting drought, population growth, and increased pollution, water shortage will be also increasing (**Pintilie *et al*. 2016**; **Aboelkassem *et al*. 2020**).

The re-using of wastewater in agricultural is very visible in urban areas of developing countries and it is estimated that the total area irrigated by wastewater is about 20 million hectares worldwide (**Varkey *et al*. 2015**; **Jiménez & Asano 2008**).

The availability of plant nutrients such as N, P or K is essential for plant growth (**Haygarth *et al*. 2013**;

White & Greenwood 2013). One advantage of wastewater irrigation is to provide nutrients that may be suitable for replacing, or at least limiting, the use of artificial fertilizers in agriculture. (**Adrover *et al*. 2012**). Toxic metals pose a significant risk to the environment and all life forms because they can be easily transported through media and can lead to poisoning of tissues and organs (**Adesiyan *et al*. 2018**; **Chinedu *et al*. 2018**). They could be accumulated in the vital organs of the human body, causing numerous serious health disorders, such as destroying immune system, Alzheimer's diseases, and disabilities associated with malnutrition (**Bhargava *et al*. 2017**).

The traditional methods of removing toxic metals such as precipitation, coagulation, ….. etc are very expensive (**Kumari & Tripathi 2015**). Therefore, it was important to propose an economical and environmentally friendly technique for removing these toxic metals. Removal of toxic metals and other contaminants from wastewater using aquatic plants has been informed as a low-cost and effective technique (**Rai 2008**).

Aquatic plants have been widely used in the last few decades to remove toxic metals and nutrients from wastewater (**Fawzy *et al*. 2012**; **Hua *et al*. 2013**). One of the well-known and important functions that aquatic plants perform is to absorb dissolved nutrients such as nitrogen, phosphorous, ….. etc. from highly polluted waters and are widely used in built-up wetlands around the world to remove excess nutrients and toxic metals. (**Zhang *et al*. 2018**; **Nandakumar *et al*. 2019**). Constructed Wetlands are man-made under similar conditions to the natural wetlands and contains aquatic plants that are applicable to treat contaminated water. (**Zhang *et al*. 2014**). Constructed Wetlands are mainly used to efficiently remove organic matter, suspended solids, nutrients, and some metals from wastewater, and also to remove organic pollutants, such as pesticides (**Matamoros & Salvado 2012**) and hydrocarbons (**Guittonny-Philippe *et al*. 2015**). The aim of this study is to test the possibility of using Echinochloa pyramidalis and Ludwigia stolonifera to remove toxic metals and nutrients from wastewater.

MATERIALS AND METHODS

Experimental Setup

The study experiments were performed at the Faculty of Agriculture, Sohag University, Egypt, using two plants (*Echinochloa pyramidalis* and *Ludwigia stolonifera*). The plants were fixed in a wooden frame on the top of four plastic tanks (500 mm length ×500 mm width × 960 mm height) as shown in figure 1. The tanks were filled with wastewater, which was brought from wastewater treatment plant outflow, at Gerga - Sohag governorate, Egypt. Processing concentrations of 10 and 20 mg/L of the studied metals [Lead (Pb), Cadmium (Cd) and Nickel (Ni)] were mixed together in this study. Cd $(NO_3)^{-2}$, Ni (SO^4), and Pb $(NO_3)^{-2}$ Sigma, St. Louis, MO were used to make toxic metal solutions. Experiments were carried out in four categories. The first group used *E. pyramidalis* plants, the second group used *L.* stolonifera plants, and the third group used the two plants together (*E. pyramidalis* + *L. stolonifera*). The fourth group, which served as a monitor for evaluating natural toxic metal removal, did not have any plants. The studied plants were cleaned with fresh water before used in each run and with distilled water after being acclimated for 7 days. 220 liters of wastewater were used in each experimental group.

Analytical procedures

A 15 mL water samples was taken from each compartment after detention time 1, 3, 5, 7, and 10 days for toxic metals analysis. At the start and end of the experiment, plant samples of (5 g) were collected. The plant samples were washed and dried in a convection oven at 80°C for 48 hours before being ground to a fine powder to digest according to **Campbell and plank** (**1998**) method. An atomic absorption spectrophotometer (PerkinElmer Analyst 400, USA) was used to assess the concentrations of toxic metals in wastewater and tissue samples.

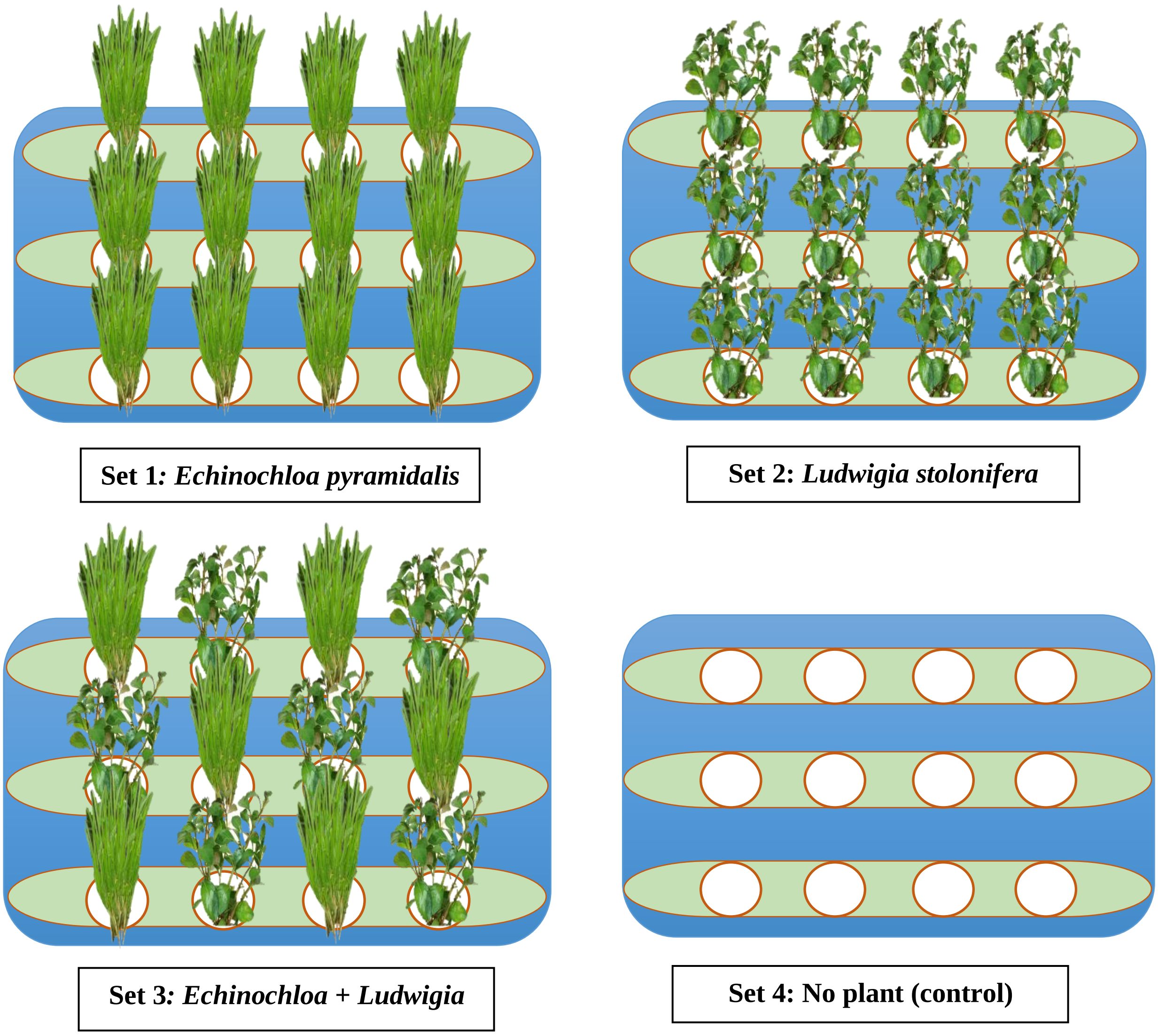

Figure (1) Diagram of the experimental set up.

Statistical Analysis

Triplicate controls and tests were carried out. Using the SAS computer software framework, the impact of plant use, toxic metal concentrations at different retention periods, and removal efficiency of different toxic metals were investigated (SAS Institute, 2002). Tukey's multiple comparison tests were used to find statistically significant variations between mean values in Tukey (P <0.05). The Spearman rank order correlation was used to assess the relationship between toxic metal levels and detention period.

RESULTS AND DISCUSSION

Removal of nutrients

The maximum removal efficiencies of (NO_2-N) were 47.83% (extracted by using *E. pyramidalis*) and 56.52% (extracted by using *L. stolonifera*), while the maximum removal efficiencies of (NO3-N) were 40.96% (extracted by using *E. pyramidalis*) and 43.93% (extracted by using *L. stolonifera*) (Table 1).

Table (1) Nutrient salts content of the wastewater enriched with concentration (10 and 20) mg/l of mixture of Cd, Ni, and Pb by *E. pyramidalis* and *L. stolonifera* during the experiment duration. All the values are mean of triplicates ±SD.

Plants	Time	Nutrient salts			
		NO_2-N (mg/L)	**NO_3-N (mg/L)**	**PO_4-P(mg/L)**	**TP (mg/L)**
E. py.	**Initial**	0.23±0.02	11.45±1.95	3.09±0.47	7.44±0.26
	Day-10	0.12±0.01	6.76±1.05	1.55±0.21	4.37±0.33
L. st.	**Initial**	0.23±0.02	11.45±1.95	3.09±0.47	7.44±0.26
	Day-10	0.10±0.03	8.42±0.91	1.59±0.18	4.57±0.21

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

The main biological processes responsible for the elimination of nitrogen in the wetland systems include ammonification (mineralization), nitrification, and denitrification (**Jamieson *et al*. 2003**). Vegetation is known to play an important role in nitrogen removal in Constructed Wetlands due to plant uptake and microbial activity around the rhizome. Nutrient uptake by plants is dependent on their productivity and the concentration of nutrients in the plant tissues (**Fountoulakis *et al*. 2017**).

The maximum removal efficiencies of (PO_4–P) were 49.84% (extracted by using *E. pyramidalis*) and 48.54% (extracted by using *L. stolonifera*), while the maximum removal efficiencies of (TP) were 41.26% (extracted by using E. pyramidalis) and 38.58% (extracted by using *L. stolonifera*) (Table 1). The reason of high phosphorous removal in the mentioned Constructed Wetlands can be due to the plant absorption process and more stable temperature during wetland operation and long contact time inside the wetland units (**Valipour & Ahn 2016**). Phosphorous removal is usually low and only 40 to 60% quantities are monitored during domestic wastewater treatment (**Vymazal 2004**). More than 60% of the phosphorus removal was accumulated by plant uptake in bio-rack wetlands (**Wang *et al*. 2012**).

Removal of toxic metals

Aquatic plants are chosen over other terrestrial plants due to their improved ability to accumulate nearly 1,450-fold the heavy metals in the water (**Rezania *et al*. 2016**). Another advantage of using aquatic plants is their rapid growth rate and increased biomass production, high ability to absorb pollutants, and clearer purification due to their direct contact with water. (**Wani *et al*. 2017**). The efficiency of wastewater treatment in Constructed Wetlands depends on the characteristics of the effluent wastewater (**Hargreaves *et al*. 2018**). Lead, Cadmium and Nickel removal by natural procedure (degradation and precipitation) were determined from reference experiments as shown in the table 2. The results showed low removal concentration of toxic metals by natural procedure. In order to effectively remove toxic metals, Lead, Cadmium and Nickel removal were studied by using *E. pyramidalis* and *L. stolonifera* individually and in combination.

In combination cases, significant improvement in Lead, Cadmium and Nickel removal as shown in table 2 regarding to that of natural procedure. Possible techniques involved in removing heavy metals are the consumption, immobilization and absorbing of metals by plants, as insoluble salts and substrate-bound metals while natural precipitation is caused by the oxidation of the compounds by the dissolved oxygen. (**Ranieri *et al*. 2011**; **Mulkeen *et al*. 2017**; **Sekomo *et al*. 2012**). Different mechanisms were used by plants for detoxifying contaminated areas; these include phytoextraction, phytostabilization, rhizofiltration, and phytovolatilization (**Danh *et al*. 2014**).

Table (2) Removal efficiency of toxic metals by *E. pyramidalis*, *L. stolonifera*, and their combination.

Conc (mg/l)	Heavy metals (%)	*E.py + L.st*	*E.py*	*L.st*
10 mg/l	**Cd**	53.33 ± 17.64[a]	50.88 ± 6.14[a]	55.07 ± 14.12[a]
	Ni	28.40 ± 4.91[ab]	24.47 ± 2.53[b]	37.91 ± 5.40[a]
	Pb	88.45 ± 6.23[a]	65.41 ± 11.65[b]	64.81 ± 14.48[b]
20 mg/l	**Cd**	53.57 ± 12.23[a]	55.30 ± 15.78[a]	54.74 ± 9.67[a]
	Ni	36.19 ± 8.04[b]	35.13 ± 2.44[b]	46.02 ± 3.48[a]
	Pb	86.64 ± 6.14[a]	69.03 ± 9.33[b]	68.63 ± 10.25[b]

E.py = Echinochloa pyramidalis and *L.st = Ludwigia stolonifera*. The values followed by same letter are not significantly different at the 0.05 probability level.

When both plants were combined, the removal of Lead (Pb) is improved from individually grown plants. Pb removal improvement may be due to a change in the behavior of plants as they grow together (the synergistic effects) (**Kumari & Tripathi 2015**). The roots of these plants have been increased, which will increase the plant ability to absorb metals and the liquidation and movement of Phyto-siderophores that contains free metal ions increase the absorption performance of the plant (**Tsednee *et al*. 2012**; **Hu *et al*. 2014**). Cadmium and Nickel removal was higher by using *L. stolonifera* compared of that by using *E. pyramidalis*, while the removal of Lead by using *E. pyramidalis* was higher compared of that by using *L. stolonifera*. That because the plant types may have different efficiencies in absorbing the studied metals, similar results were found in **Mishra & Tripathi** (**2008**). In general, ANOVA result (Figure 2) showed that there is a significant variation in the concentrations of toxic metals in wastewater in relation to plant species and retention time.

The preferential order for removing toxic metals in this study by each plant individually and by both plants in combination was Lead > Cadmium > Nickel as it is clear from Table 2. The plant defense system may block increased metal uptake as a survival mechanism and rather increase the uptake of essential nutrients.

This could be an indication that plants selectively take up substances from the environment based on their usefulness and capacity for uptake (**Ekperusi *et al*. 2018**). In this study, the removal of Cd and Pb using aquatic plants was higher than that found in algal and duckweed pond (**Elkhatib *et al*. 2014**; **Sekomo *et al*. 2012**). The current process removes more Cd from wastewater (55.30%). The greater removal of Cd may be due to the plants' greater uptake efficiency of metals when compared with the other advanced technologies. **Rai *et al*. (2015)**

reported an 86% decrease using *T. latifolia*, *P. australis* and *C. esculenta* for urban wastewater treatment in the summer season (April to May) in India. **Syukor *et al*.** (**2016**) introduced a study on the withdraw of toxic metals by two plants and obtained removal of 49.3% for Cadmium (Cd) and 54.7% for Lead (Pb), while the present research obtained a higher removal of 53.57% and 88.45% for Cadmium (Cd) and Lead (Pb) respectively. Also in this study, a higher removal of Cd and Pb was observed compared to what **Kumar & Chopra** (**2018**) reported using *Trapa natans* L. for municipal wastewater treatment. The higher removal of metals may be due to the fact that emerging plants accumulate metals in higher ranges of floating plants due to their root structure (**Vymazal 2013**). The relationship between pH and removal of toxic metals through precipitation and absorption, were examined. The pH of the wastewater was slightly alkaline and through the study runs, pH value was (6.86±0.01–7.82±0.01). This can be attributed to the addition of oxygen through the photosynthesis activity of plants. The neutral state to the slightly alkaline state indicates that the possible mechanisms for removing metals are the immobilization in plant rhizosphere and their absorption through the plant roots. Similar results have been reported by **Barakat** (**2011**) and **Abid *et al*.** (**2015**).

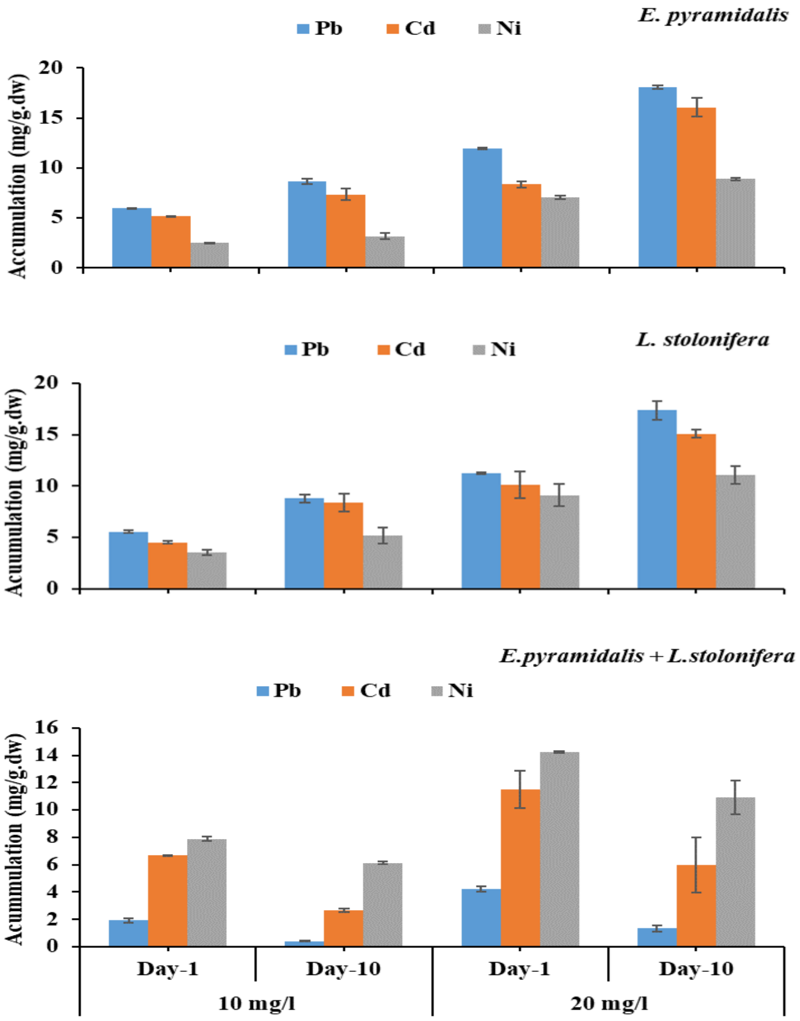

Figure (2) Accumulation of toxic metals in *E. pyramidalis* and *L. stolonifera* (mg/g.dw) were grown individually and co-grown in different concentrations of toxic metals during the experiment duration.

Relation of detention time and toxic metals removal

Spearman rank order correlation was used, and correlation analysis was done between the toxic metal levels and detention time for all experiments results. A significant and negative correlation coefficients of Cadmium (Cd), Nickel (Ni), and Lead (Pb) levels with retention period were determined that ensured the positive relation of retention period and removal percentages at P< 0.001 as shown in table 3. Similar results were obtained by **Bello *et al*. (2018)**.

Table (3) Simple linear correlation coefficient (r) between detention time and toxic metals removed at $P < 0.001$.

Species	Conc. of Heavy metals (mg/L)	Heavy metals		
		Cd	Ni	Pb
E.py	**10**	-0.809	-0.796	-0.864
	20	-0.941	-0.74	-0.84
L.st	**10**	-0.943	-0.827	-0.914
	20	-0.865	-0.755	-0.851
E.py+ L.st	**10**	-0.972	-0.855	-0.76
	20	-0.892	-0.878	-0.761

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

Accumulation of toxic metals

The accumulation of studies metals in both plants at different retention times are shown in Figure 3, A comparison between the accumulated amounts of Cadmium (Cd), Nickel (Ni), and Lead (Pb) in *E. pyramidalis* and *L. stolonifera* had shown that Ni amounts were lower than that of Cd and Cd amounts were lower than that of Pb for the ten-day exposure. This may be due to the differential plant growth rate and the absorption of heavy metals. Similar results were reported by **Kumari & Tripathi 2015**. Nickel is required at least in the metabolism of the plant, **Rana & Maiti** (**2018**) reported the lowest accumulation of Ni by plants compared to other metals.

Also, the absorption of Nickel (Ni) was higher in L. stolonifera than that of *E. pyramidalis*, while the absorption of Lead (Pb) was higher when *L. stolonifera* and *E. pyramidalis* are combined compared to that of individually planted for all concentrations (10 and 20) mg / L.

CONCLUSION

Constructed Wetlands are economical and environmentally friendly techniques that use many plants varieties to withdraw pollutants from the nature. The present research goal is to examine the using of *Echinochloa pyramidalis* and *Ludwigia stolonifera* to extract toxic metals and nutrients from wastewater. According to aforementioned results, it was found that, for all toxic metals except Lead (Pb), *L. stolonifera* had higher absorption ability than *E. pyramidalis* and higher removal efficiency of Lead (Pb) at concentration of 10 mg/l was obtained when both species were planted together than that of planted individually. Also *L. stolonifera* showed the highest removal efficiency of Nickel (Ni) at concentration of 20 mg/l, and *E. pyramidalis* showed the highest removal efficiency of Cadmium (Cd) at concentration of 20 mg/l. For removal efficiency of nutrients, *L. stolonifera* had the highest removal efficiency for (NO_2-N) and (NO_3-N), and *E. pyramidalis* had the highest removal efficiency for (PO_4-P) and (TP).

It was concluded from this study that both *E. pyramidalis* and *L. stolonifera* could be used to remove toxic metals and nutrients and improve wastewater treatment process.

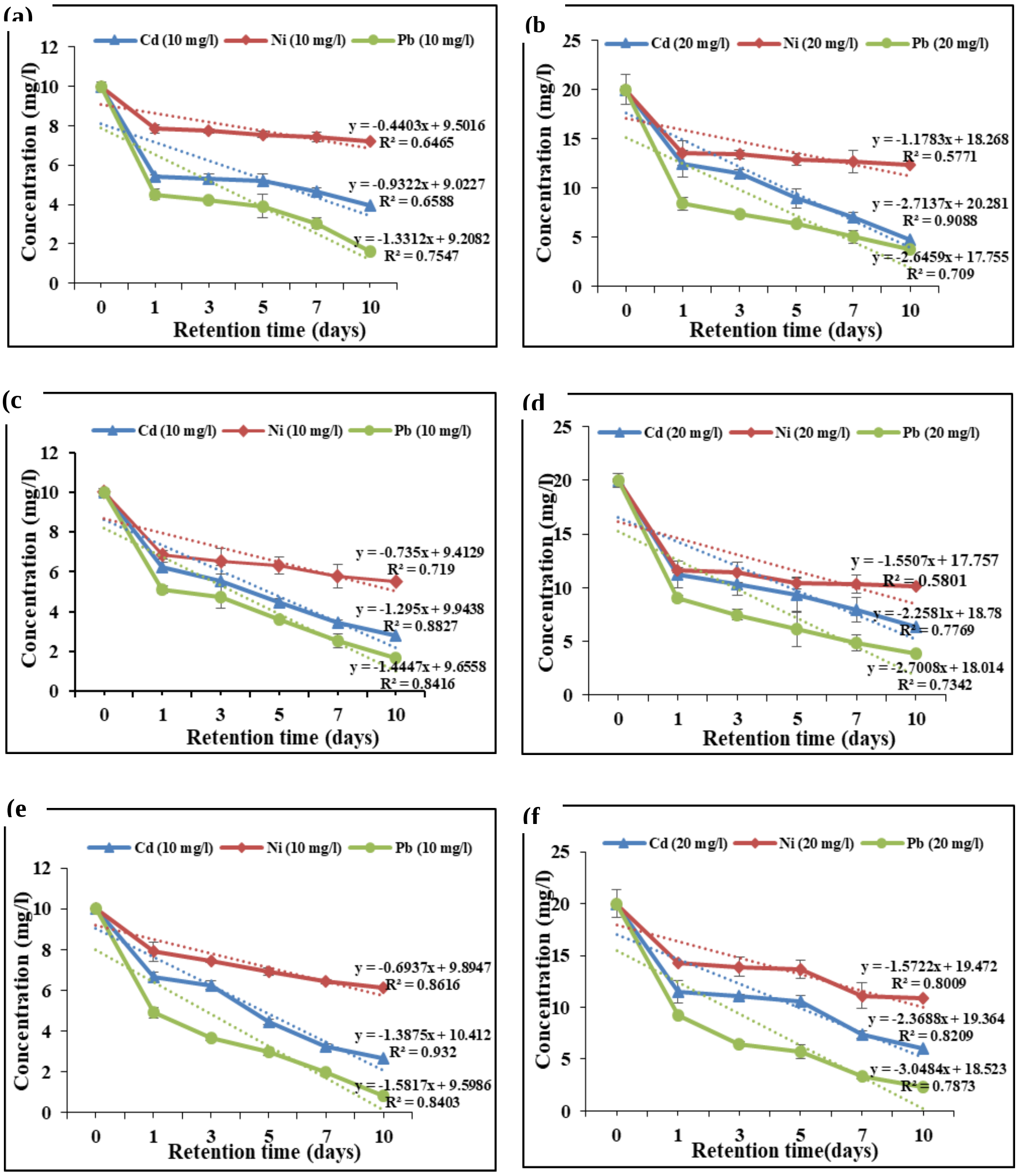

Figure (3) Effect of *E. pyramidalis* (a-b), *L. stolonifera* (c-d) and their combination (e-f) on Cd, Ni, and Pb concentrations of wastewater at different retention times.

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