# *Cyperus longus* L. as a biological purifier of wastewater for irrigation purposes: removal efficiency and Zn, Cd, Cu, Fe and Mn

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# Abstract

The common and cosmopolitan distributed wastewater macrophyte, namely, Cyperus longus L. was tested as a biological wastewater purifier. Indoor experiments were mainly based on conventional wastewater treatment processes, besides the specified design of sand filtration pots, implanted by C. longus L. Untreated and treated wastewater samples were analyzed for their key physicochemical properties and some heavy metals (only the removal efficiency of the heavy metals Zn, Cd, Cu, Fe and Mn by C. longus L. is highlighted in this paper). After 129 days (including 45 days, the time needed for the growth/stabilization of C. longus L. in experiment pots) the C. longus L. was harvested and heavy metals were analyzed in root and shoot systems. The removal efficiency (i.e. uptake/bioaccumulation rate) was then followed up. Results showed that the accumulation rate in the plant roots was much higher than the shoots. Higher metal bioaccumulation per cent was noted in roots; Zn (0.522%), Cu (0.821%), Fe (80.480%), Mn (1.886%) and Cd (0.659%) compared with control (irrigated with clean water); Zn (0.147%), Cu (0.167%), Fe (12.590%), Mn (0.331%) and Cd (0.124%). On the other hand, metal bioaccumulation per cent in shoot system was: Zn (0.412%), Cu (0.458%), Fe (4.540%), Mn (1.719%) and Cd (0.567%) compared with control. Always, the more replicated sand filtration pots the highest removal efficiency of heavy metals was achieved.

Keywords: phytoremediation, Cyperus longus l., removal efficiency, heavy metals.



# 1 Introduction

Nowadays, in many parts of the world, people lack enough water to stay healthy. Meanwhile, the amount of water for drinking is becoming dangerously low (Conant [1]). Heavy metals spread by industrial wastewater to the surrounding area cause serious problems and make water doubtful for irrigation (Orhan et al. [2]). Phytoremediation using aquatic macrophyte has developed a new sorption process for removing pollutants and toxic metal ions from wastewater (Darnall and Gabel [3]). This process is based upon the natural, strong metabolic affinity of shoot and root systems of aquatic macrophyte for different pollutants in wastewater (Ganjo et al. [4]). The main sewage canal of Erbil City/northern Iraq (where this work was carried out), with a population estimated as more than 2 million, is very polluted, assessed as causing irrigation problems and other health risks (Ganjo [5]). Farmers here are using sewage water without any pretreatment for irrigation of uncooked vegetables. The present study was designed to examine the ability of reclamation of wastewater for irrigation, using the Phytoremediation process (the low cost and less technology) via a common and cosmopolitan distributed wastewater macrophyte, namely, Cyperus longus L., cultivated in specifically designed sand filtration pots, and then its removal efficiency for some toxic heavy metals including; Zn, Cd, Cu, Fe and Mn was followed up.

# 2 Methodology

## 2.1 Study area and project design

Wastewater from the main sewage canal of Erbil city was collected using a large water tanker. Immediately, wastewater was brought back to the botanical garden, College of Science, Salahaddin University-Erbil, where the present proposed treatment project was designed (fig. 1A). Wastewater from the tanker was then poured into the storage unit (a polyethylene tank of one cubic meter capacity) and the wastewater was left to drain into an aeration/sedimentation unit and then to experiment pots. For elimination of interferences due to dust, wind, rain, etc., the treatment units were placed inside a well ventilated glasshouse. The flow rate was set at 4 litres per hour. Semi treated water from the sedimentation unit was draining into a series of sand filtration pots through 1/2 inch plastic pipes with separate valves to control the equal flow of wastewater. Seven pots (each with 20 litre capacity) were used as sand filtration units (fig. 1B). Quartz sand, only the resistant sand which does not lose more than 5% of its weight in 40% HCl for 24 hours (WHO [6]) was used as the filtration medium.

## 2.2 The study period and sampling frequency

After the construction phase and plantation, *C. longus* L. (5 healthy and young plants in each pot) were left in their pots for 45 days (the period needed for adaptation), and then water samples at different stages of treatment; Sts. 1 to 5



were analyzed according to a regular schedule at weekly intervals. Always daily appropriate corrections were monitored for the constant flow rate. At the end of the experiment period, estimated as 129 days, the plant tissues, i.e. roots and shoots were analyzed for their metal bioaccumulation content.



Figure 1: (A) and (B): design of the proposed wastewater treatment project; the pot design is also indicated.

#### 2.3 Plant tissue analysis for heavy metal determination

Plant tissues were digested according to Ryan *et al.* [7]. After homogenization of the bulk sample from each pot, a powdered vegetable sample was transferred into porcelain crucibles and/or Pyrex glass beakers, then placed into a cool muffle furnace, and the temperature was increased gradually to 550°C for 5 hours. After cooling, the cooled ash was dissolved in 5ml 2N HCl and then the volume was completed to 50ml using distilled water. Atomic Absorption Spectrophotometer, model (WFX-120. BRAIC/China) was used for trace metal determination. The equation described by Xuerui *et al.* [8] for detection of removal efficiency was followed up, as given below:

#### *Removal efficiency* (%) = [(inlet pollutants - outlet pollutants)/ inlet pollutants] x 100

#### 2.4 Statistical analysis

The observed data during the whole study period was analyzed statistically using the available software programs (SPSS version 13.0 and Microsoft Excel Professional Edition 2003/Data Analysis). Two way classification analysis of variance "ANOVA" combining with LSD test "Least Significant Difference", was used to determine the significant variations between different treatment stages as a spatial variation and between the sampling date intervals as a temporal variation.



## 3 Results and discussion

Zinc (Zn) concentration was ranging from a maximum value of 0.930 to a minimum value of 0.305 ppm, with a total mean of 0.825 ppm having a fluctuation of  $\pm$  0.073 as an average standard deviation around the grand mean during this investigation (table 1). The highest value was recorded at St.1 during April, while the lowest value was observed at St. 5 during June. Statistical analysis indicated significant differences (*P*<0.01) between the studied sites and sampling dates.

Zn concentration of the raw wastewater was ranging from 0.710 to 0.930 ppm with the mean value of 0.825 ppm, but in Sts. 4 and 5 the zinc concentration decreased to very low quantities. This may be attributed to the efficiency of *C. Longus L.* for decreasing zinc in wastewater; however the greater portion of zinc may be sequestered in the cell wall tissue of root through internal completion and detoxification with subsequent translocation of a relatively small amount of the metal to the shoot branches. The findings of Hinchman *et al.* [9] may confirm the present results, however they demonstrated that a considerable amount of zinc can be sequestered into the root rather than other parts of plant. On the other hand, Abdulbary [10] and LeCoultre [11] explained that the bioavailability of the heavy metals (including zinc) depends on the interrelationships of a number of factors, such as rate and frequency of application, soil characteristics, plant species, uptake efficiency, transpiration rate, and the original concentration. This may be true for the present results.

The cadmium (Cd) value posed a grand mean of 0.160 ppm with an average standard deviation of  $\pm 0.023$  during this investigation. The lowest value of 0.054 ppm was recorded at St. 5 during July, while the highest value of 0.186 ppm was observed at St. 1 during April (table 2). The statistical analysis revealed significant differences (P < 0.01) between the studied sites and sampling dates.

Cd content in the storage unit showed the total mean of 0.160 ppm. This may be due to the multi-source of cadmium from industries and pesticide uses in farmlands nearby. While the mean value of Cd throughout the three sand filtration pots (St. 5) was severely decreased, aquatic plants can accumulate high amounts of Cd in such a way that they reflect the toxicity of the water environment, and may serve as a tool for the bio-monitoring of contaminated waters (Cardwell *et al.* [12]). Meanwhile, Lokeshwari and Chndrappa [13] showed that some plant species accumulate Cd depending on environmental conditions and the available Cd form.

The overall mean value of 0.578 ppm was recorded for copper (Cu) concentration with a standard deviation of  $\pm 0.071$  ppm during the entire period of this investigation. However, it was ranging from a maximum value of 0.682 to minimum of 0.347 ppm (table 3). The lowest value was recorded at St. 5 during June, while the highest value was observed at St.1 during April. Statistical analysis revealed clear significant differences (*P*<0.01) between the study sites and sampling dates.



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2.	0.154	0.126	0.112	0.096	0.094	0.096	0.091	0.085	0.092	0.080	0.090	0.086	$0.100^{\circ}$	0.021
3.	0.126	0.110	0.099	0.077	0.083	0.081	0.081	0.081	0.083	0.078	0.069	0.061	$0.086^{b}$	0.018
4.	0.091	0.091	0.080	0.070	0.078	0.074	0.070	0.078	0.073	0.072	0.064	0.054	$0.075^{a}$	0.010
5.	0.087	0.086	0.071	0.066	0.065	0.061	0.068	0.071	0.070	0.068	0.061	0.054	$0.069^{a}$	0.010
Mean	$0.129^{f}$	$0.120^{ef}$	$0.108^{de}$	$0.097^{cd}$	$0.096^{6d}$	0.096 <sup>cd</sup>	0.095 <sup>cd</sup>	$0.096^{cd}$	$0.095^{cd}$	$0.089^{bc}$	$0.082^{ab}$	$0.073^{a}$	0.098	
±SD	0.042	0.039	0.042	0.045	0.039	0.042	0.039	0.037	0.035	0.032	0.026	0.025	0.037	
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Cu value in raw wastewater decreased from a total mean value of 0.578 to 0.378 ppm at St. 5 (i.e. three sand filtration pots) respectively. It seems that *C. longus* L was more efficient in Cu removal from the wastewater. This may be correlated to the capacity of *C. longus* L. to uptake Cu into its tissues (root and leaf) *via* adsorption and/or binding to sand particles. A similar finding was observed by Aganga *et al.* [14].

Iron (Fe) concentration showed an overall average of 1.046 with a standard deviation of  $\pm 0.168$  in all study sites. However, its concentration ranged from 0.329 to 1.298 ppm (table 4). The minimum value was noted at St. 5 during July, while the maximum value was observed at St. 1 during the first week of April. The result of iron content in samples revealed clear significant differences (P < 0.01) between the study sites while, there were no significant variations between sampling dates.

Fe is present in a wide variety of industrial wastewater including mining operations, or milling chemical industrial wastewater, dye manufacture, metal processing, textile mills, petroleum, refining, and others (Goldman and Horn [15]). The iron values showed gradual reduction until April. Results came in accordance with the findings of Ayers and Westcott [16]. However, they demonstrated that iron precipitation is primarily dependent upon two factors; the concentration and the pH of the water. Iron is usually present in wastewater in dilute quantities 1–100 ppm at neutral or acidic pH values <7.0, while with caustic substances, the iron reacts with hydroxide ions to form metal hydroxide solids. The same correlation between pH values and iron was noted (the data is not given here).

Manganese (Mn) values showed a grand mean of 0.686 ppm with an SD value of  $\pm 0.199$  throughout the duration of this study. A maximum value of 1.112 ppm was detected during April at St.1, while a minimum value of 0.268 ppm was noted during July at St. 5 (table 5). Conversely, statistical analysis indicated obvious significant differences (*P*<0.01) between the study sites and sampling dates.

A high amount of manganese accumulated in the root tissues followed by the shoots. Ittana [17] stated that the differences in Mn uptake in plants are attributed to their tolerance range. While Ghaly *et al.* [18] demonstrated that the Mn uptake rate and the maximum amount which can be accumulated in each plant species will be affected by the initial Mn concentration in the wastewater.

The per cent build up of studied metallic cations in the root and shoot of *C. longus* L is given in table 6; a comparatively higher build up of metal was in the roots rather than the shoots, namely, Fe (80.480%), Mn (1.886%), Cu (0.821%), Zn (0.522%), and Cd (0.659%). Always the highest values were observed in St. 3 compared with the control site, which irrigated with clean water, with the following values; Fe (12.590%), Mn (0.331%), Cu (0.167%), Zn (0.147%) and Cd (0.124%). Generally, it seemed that the more replicated the sand filtration pots were, the highest removal efficiency of heavy metals was achieved.



Monitoring Period (Months/Weeks)         June         June         June         June $\pm D$ ite         April         June         June         June $\pm D$ I         June         June         June $\pm D$ I         June         June         June $\pm D$ June $\pm D$ June $\pm D$ <th col<="" th=""><th>Table</th><th>3:</th><th>Copper</th><th>(Cu) val</th><th>ues (ppm</th><th>ı) measu</th><th>red for tl</th><th>he experi</th><th>imental <sub>I</sub></th><th>pots duri</th><th>ng the st</th><th>udied pe</th><th>riod, wit</th><th>h mean ±</th><th>SD.</th></th>	<th>Table</th> <th>3:</th> <th>Copper</th> <th>(Cu) val</th> <th>ues (ppm</th> <th>ı) measu</th> <th>red for tl</th> <th>he experi</th> <th>imental <sub>I</sub></th> <th>pots duri</th> <th>ng the st</th> <th>udied pe</th> <th>riod, wit</th> <th>h mean ±</th> <th>SD.</th>	Table	3:	Copper	(Cu) val	ues (ppm	ı) measu	red for tl	he experi	imental <sub>I</sub>	pots duri	ng the st	udied pe	riod, wit	h mean ±	SD.
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$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		.298	1.215	1.210	1.185	1.145	1.095	0.995	0.986	0.898	0.897	0.812	0.812	$1.046$ $^{\circ}$	0.168
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	[	(.137	1.076	1.064	0.994	0.965	0.901	0.849	0.748	0.735	0.682	0.664	0.596	$0.868^{d}$	0.182
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	_	0.075	0.962	0.942	0.898	0.864	0.856	0.741	0.655	0.602	0.594	0.525	0.424	$0.762^{\circ}$	0.201
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$		766.(	0.895	0.862	0.783	0.765	0.783	0.601	0.596	0.498	0.463	0.432	0.365	$0.670^{b}$	0.205
1.092 <sup>i</sup> 0.987 <sup>h</sup> 0.965 <sup>h</sup> 0.866 <sup>i</sup> 0.756 <sup>e</sup> 0.600 <sup>i</sup> 0.609 <sup>e</sup> 0.565 <sup>b</sup> 0.505 <sup>a</sup> 0.789       -         0.135       0.165       0.179       0.170       0.170       0.194       0.173       0.200       0.173       -		.952	0.786	0.748	0.765	0.686	0.694	0.596	0.466	0.466	0.409	0.393	0.329	$0.608^{a}$	0.194
0.135 0.165 0.179 0.172 0.170 0.150 0.150 0.170 0.194 0.179 0.194 0.173 0.200 0.173 -	-	.092 <sup>i</sup>	$0.987^{h}$	$0.965^{h}$	$0.925^{g}$	$0.866^{f}$	$0.866^{f}$	$0.756^{\circ}$	$0.690^{d}$	$0.640^{\circ}$	$0.609^{\circ}$	$0.565^{\mathrm{b}}$	$0.505^{a}$	0.789	ı
	)	).135	0.165	0.179	0.172	0.170	0.150	0.170	0.194	0.179	0.194	0.173	0.200	0.173	

Different letters means there is a significant difference.



Table	: 5: I	Mangane	se (Mn)	values (p <sub>j</sub>	pm) mea	sured for	the expe	erimental	l pots du	ring the s	studied p	eriod, wi	th mean ∃	- SD.
					Monito	ring Period	l (Months/	Weeks)						
Site	Al	pril		W	ay			Ju	ne		ŋſ	dy	Mean	±SD
	1	2	3	4	5	9	L	8	6	10	11	12		
Γ.	1.112	1.086	0.965	0.952	0.934	0.942	0.926	0.901	0.880	0.872	0.810	0.810	$0.933^{\circ}$	0.093
2.	1.097	0.989	0.904	0.898	0.875	0.823	0.816	0.794	0.765	0.723	0.714	0.703	$0.842^{d}$	0.118
3.	0.875	0.786	0.782	0.714	0.645	0.665	809.0	0.521	0.670	0.489	0.420	0.596	$0.648^{\circ}$	0.132
4.	0.764	0.645	0.652	0.598	0.534	0.584	0.514	0.465	0.502	0.394	0.310	0.460	$0.535^{b}$	0.123
5.	0.642	0.560	0.604	0.542	0.412	0.495	0.448	0.387	0.452	0.340	0.268	0.390	$0.462^{a}$	0.111
Mean	$0.898^{i}$	$0.813^{h}$	$0.781^{gh}$	$0.741^{\mathrm{fg}}$	$0.702^{\circ}$	$0.702^{\rm ef}$	$0.662^{\circ}$	$0.614^{cd}$	$0.654^{d}$	$0.564^{b}$	$0.504^{a}$	$0.592^{\mathrm{b}}$	0.686	1
±SD	0.206	0.223	0.156	0.180	0.203	0.181	0.203	0.222	0.179	0.226	0.244	0.172	0.199	
Same lett	ers means t	there is no	significant	difference.										
Different	letters mea	ans there is	a significaı	nt differenc	e.									

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Table 6:

t. 4 St. 5 900 25 310	460 0.870	504 0.359	410 0.345	414 0.326	004 1.113	300 0.970	240 0.215	388 0.217	476 0.289
St. 3 St. 4 0.480 43.900	1.886 1.460	0.504 0.504	0.522 0.410	0.659 0.414	4.540 3.004	1.719 1.300	0.458 0.240	0.412 0.388	0.567 0.476
1 Metal Fe 8	Wn	Cu	Zn (	Cd	Fe ,	Mn	Cu (	Zn (	Cd

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Generally, removal of metals in sand filtration pots may occur through a number of processes, including sedimentation/coagulation, filtration, plant uptake/removal efficiency, adsorption (i.e. binding to sand particles and root), formation of solid compounds, cation exchange, and microbial-mediated reaction (Watson et al. [19]). Malla et al. [20] concluded that the extent of build-up of metals in wastewater irrigated lands often depends on the period of its application. On the other hand, Zhang et al. [21] outlined that many factors affect the accumulation concentration of heavy metals in plant tissues, among them; stem and root, source of the contamination, dosed concentration, plant type, method of detection, soil properties, and relative accumulation level of heavy metals in edible plant parts and transfer factors. In general, the application of a triple pot caused an increase in dry matter compared with single and double pots. This may be due to the positive effect of the root system in a triple pot on nutrient balance when electrical conductivity EC and other parameters decreased (the data is not given here). These results were in agreement with those found by Zhang et al. [21].

# 4 Conclusion

Results showed that levels of Fe, Mn, Cu, Cd and Zn in the wastewater of Erbil City were much higher than the permissible norms, considered fit for irrigation. Sand filtration pots implanted by *Cyprus longus* L, seem to be a good possibility for removal of Fe, Mn, Cu, Cd and Zn from the wastewater. The treated wastewater *via* the present proposed design may improve the water quality for irrigation purposes. The accumulation rate in the plant root was higher than plant shoots.

# 5 Recommendation

Phytoremediation is a technology with great potential, using a combination of high-biomass with hyper accumulator mechanisms will successfully remove heavy metal contaminants from the environment. Long term field investigations, taking into account the role of other microorganisms (i.e. bacteria and aquatic fungi), are recommended for confirmation of the present results.

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