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Research Article

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Seasonal Study of Cu, Zn and Pb Accumulation and Distribution in Sediments and PhragmitesAustralisplanted in A Constructed Wetland "Lake Manzala Engineered Wetland Project", (LMEWP), Egypt

Hind A.H.Y.Ali[1*], Ahmed A. Rashed[2], Fawzy G. Kheder[3]

[1]Drainage Research Institute, National Water Research Center, Cairo, Egypt,
[2]National water Research Center. Cairo, Egypt,
[3] Botany Department, Faculty of Science, Zagazig University, Zagazig, Egypt.

***Email:** hendali09 @ gmail.com*

ABSTRACT

Lake Manzala Engineered Wetland project (LMEWP) is a constructed wetland that has been designed for treating drainage water coming from Bahr El-Baqar drain. In this study, the concentration of heavy metals (Cu, Zn and Pb) in water, sediment and different organs of Phragmitesaustralis collected from LMEWP seasonally. The aim of this study was to determine the dynamics of distribution of metals in different parts of phragmitesaustralis, and to determined the bioaccumulation factor (BCF) in different organs of plant phragmitesaustralis and Translocation Factor (TA), particularly to determine the time of maximum accumulation in the above-ground tissues and to assess removal capacity of phragmitesaustralis in LMEWP. Results revealed a high significant correlation between heavy metals concentrations in phragmitesaustralis with its location in LMEWP vegetation cell; start cell> end cell. The highest concentration of metals is mostly found in phragmitesaustralis root. All metals were accumulated in root over time until the end of the growing season after eight months. On the other side, metal concentrations in stem and leaves increased even after the growing season of the plant. Overall, the results suggest to harvestphragmitesaustralis before six months IN the growing season in order to improve the accumulative efficiency of phragmitesaustralis in LMEWP project.

Key words: *Water Quality, Bahr EL-Baqar Drain, Constructed Wetland, PhragmitesAustralis, Heavy Metals, Bioaccumulation and Translocation Ability.*

INTRODUCTION

Constructed wetlands are special engineering systems designed to imitate natural processes in wetland soils, vegetation and associated microorganisms to meet human needs. Wetland improves water quality through removing suspended sediments and pollutants [1]. Constructed wetlands save electricity and labor and they are totally cheaper than conventional treatment methods [2,3]. Wetland plants have been used to reduce the nutrient content of domestic, industrial, and agricultural wastewater [4].

The wetland planted vegetation plays an important role in removing contaminants. The filter bed, consisting usually of sand and gravel, has an equally significant role [5]. Constructed wetlands (CWs) are economical and environmentally friendly systems that need low energy inputs, making them widely appealing for the treatment of different types of wastewater [6].

Heavy metals, such as Cd, Cu, Fe, Pb and Zn cannot be degraded by microbial or chemical processes; therefore, they tend to accumulate in aquatic sediments. The problem is not limited to soils with high metal levels, such as those of mining areas, but also consistes of those with moderate to low metal contamination (Ali et al., 2004) [7]. The levels of heavy metals are associated with dysfunctions in different organisms [8]. Phytoremediation is defined as application of plants to immobilize metals and store them in their below-ground organs. In contrast, during phytoextraction, hyperaccumulators may be used to eliminate metals from the soil and accumulate them in above-ground organs [9,10].

Wetland plants are used for removal of pollutants such as metals. Wetland sediments are generally considered as a sink for metals and, in the anoxic zone, may have very high concentrations of metals in a decreased state. As such, the bioavailability of the metals is low in comparison with terrestrial systems with oxidized soils. Different types of metals have different availability: water-soluble metals and exchangeable metals are the most available formes, metals precipitated as inorganic compounds, metals complexes with large molecular weight humid materials and metals adsorbed to hydrous oxides are potentially available, and metals precipitated as insoluble sulfide and metals bound within the crystalline lattice of minerals are essentially unavailable [11]. Active uptake of elements into plant tissue may enhance immobilization in plant tissues, as seen in constructed wetlands for wastewater treatment and in the application of wetland plants in phytoremediation [12].

Phragmitesaustralis (reed plant) is an indigenous and wild plant species that was found as a pioneer plant growing in various places. This plant has the ability to uptake heavy metals from soil so it is a good biological control method in contaminated soils [13]. *Phragmitesaustrali* is one of the most widely distributed species in the world. It grows fast and able to withstand extreme environmental conditions. The aim of this study is to determine the accumulation and the distribution of metals in different parts of reed plant and the concentration of heavy metals (Cu, Zn, and Pb) in sediment, water and different parts of reed plant in LMEWP during the different seasons to determine the time of maximum accumulation in the different organs of plant.

MATERIAL AND METHODS

Study Area:

Lake Manzala Engineered Wetland project (LMEWP) is located in the north eastern edge of the Nile Delta, 170 km away from Cairo and 15 km from the Port Said. The total area allocated for LMEWP is almost 245 acres (about 100 hectare). The daily hydraulic load of 25,000 m^3 is pumped into LMEWP from Bahr El-Baqar drain. Polluted drainage water come from Bahr EL-Baqar drain starting with pumping station after that retained in the sedimentation basin and stayed there for two days. Wastewater flows down from sloped sedimentation basin to ten surface flow engineered wetland treatment cells each cell is 250m long, 50m wide, and0.3- 0.5mdeep Figure. (1). The wetland cells were designed in parallel mode to investigate the treatment of hydraulic loading rates with a detention time of two days. Wetland cells were planted with a common reed (*Phragmitesaustralis)* for phytoremediation. Currently, treated wastewater is used in fish farms.

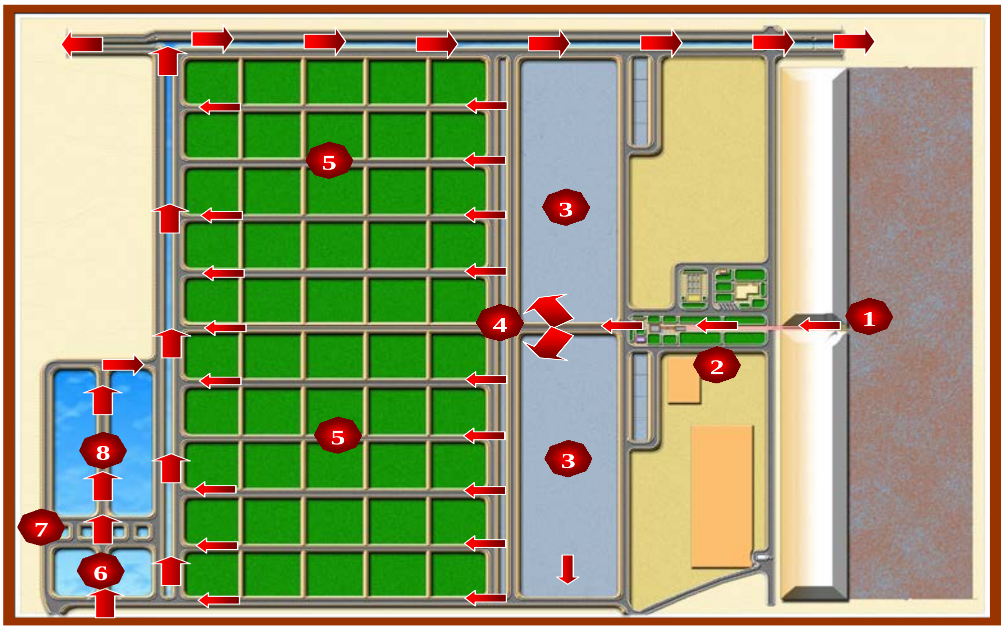


Figure 1: Major components of Lake Manzala Engineered wetland Project (LMEWP). 1, Intake; 2, pump station; 3, sedimentation pond; 4, distribution channel; 5, surface flow cells; 6, reciprocating cells; 7, hatchery ponds; 8, fingerling ponds.

Experimental Design and Field sampling:

All Water samples was collected from three treatment cells in Lake Manzala Engineered wetland Project (LMEWP) inlet and LMEWP outlet, and Bahr El-Baqar drain Water samples were collected every two months starting from February 2015 to March 2016. Three treated cells in LMEWP were chosen. Each cell was divided into two locations (start cell and end cell), and six 5×5 m plot was established in the chosen cells. All aboveground parts of *p. australis* were harvested. After two months from harvesting plant samples were collected every two months starting from February 2015 to October 2015 seasonally in the first year and March 2016 - November 2016 in the second year. sediment samples were collected from 1-1.5 cm from the same places where plant sample were taken in Bahr El-Baqar drain. All Samples were stored in polyethylene bags and then transported to the laboratory before sediment analyses.

Analytical determinations and calculations:

All samplings were carried out according to Standard Methods for Examination of wastewater [14]. Water samples were collected from the subsurface layer (at depth 50 cm) adding several drops of concentrated nitric acid ($pH<2$) to protect against microbial reactions in stopper polyethylene plastic bottles. The collected water samples were stored in an iced cooler box and delivered immediately to the laboratory for analyses. The whole plant was cut from root at 50 cm from the surface of the sediment. Then placed in the bags and delivered to the laboratory for analysis. Plant samples were washed with tap water, 10–4 M HCL solution and demonized water, then oven dried at 65 ^{o}C for 48 hours. Plant materials were grounded and mixed well. Each part of plant was digested in concentrated H_2SO_4 and H_2O_2 at 400 ^{o}C.heavy metals were determined in the digested materials using inductively coupled plasma- Optical Emission spectroscopy (ICP-OES). The samples were homogenized, spread out on plastic trays and allowed to air-dry, then grounded in porcelain mortar to pass a 2 mm sieve and kept for total heavy metal determination. Samples were digested using microwave digestion techniques as reported by Little john et al. [15]. Heavy metals (Cu, Pb and Zn) were measured using the Inductively Coupled Plasma- Emission Spectrometry (ICP-OES). All water, plant and sediment analyses were done in the central laboratory for environmental quality monitoring (CLEQM), El-Kanater EL-Khirya, Egypt.

The Bioconcentration factor (BCF):

The ability of plants to absorb and accumulate metals from the growth media was studied by the Bioconcentration factor (BCF). The BCF value was measured as the ratio of the concentrations of metals in plants and sediments:

$$BCF = [Metal]plant/[Metal]sediment.$$

A higher BCF implies a greater phytoaccumulation ability of the plant.

The translocation ability (TA):

The possibility of plants to transport metals from the roots to the above-ground organs was determined using the translocation ability (TA). The value of the translocation ability was measured as the ratio of the concentrations of metals in roots and a part of the plant: TA = [Metal] root/ [Metal] part of the plant.

A higher TA means a smaller translocation ability.

Statistical Analysis:

The average, one-way ANOVA with the value of $p< 0.05$ was performed using the SPSS (version 11.5) software package (SPPS Inc., Chicago, USA).

RESULTS:

Heavy Metals in Water:

Table (1) shows mean values, average concentration ranges, and removal efficiencies removing efficiencies of metals measured in water. High pH and EC were determined in the inlet flow and decreased in outlet flow, the metals Cu and Zn were significantly correlated with time at LSD ($P<0.05$), but Pb did not show any significant correlation with time. Trend of the heavy metals concentration in water samples were Cu>Pb> Zn as shown in Figure (2), The concentration of heavy metals (Cu, Zn and Pb) in LMEWP outlet were lower than that were in inlet flow, indicating the removal efficiency of LMEWP. This agree with Allam [16] that used wetland project (LMEWP) to remove the pollutants on Bahr EL-Baqar drain water. El-Hawary [17] reported that using project wetland LMEWP decreased heavy metals concentrations coming from Bahr EL-Baqar drain and it can be used safely in fish ponds.

Table 1: parameters (average, minimum, and maximum), and average of removal efficiency (%)

parameters	unit	Inlet flow	Outlet flow	Average removal (%)
PH	-	7.7 (7.2-8.8)	7.4(7.2-7.7)	-
Conductivity	ds/m	5 (3.9-7.3)	4.5(4.1-3.5)	-
Cu	Mgl^{-1}	0.3(0.1-0.5)	0.08(0.1-0.06)	64
Zn	Mgl^{-1}	0.13(0.37-0.06)	0.01(0.01-0.002)	95
pb	Mgl^{-1}	0.35(0.3-0.5)	0.22(0.29-0.1)	87

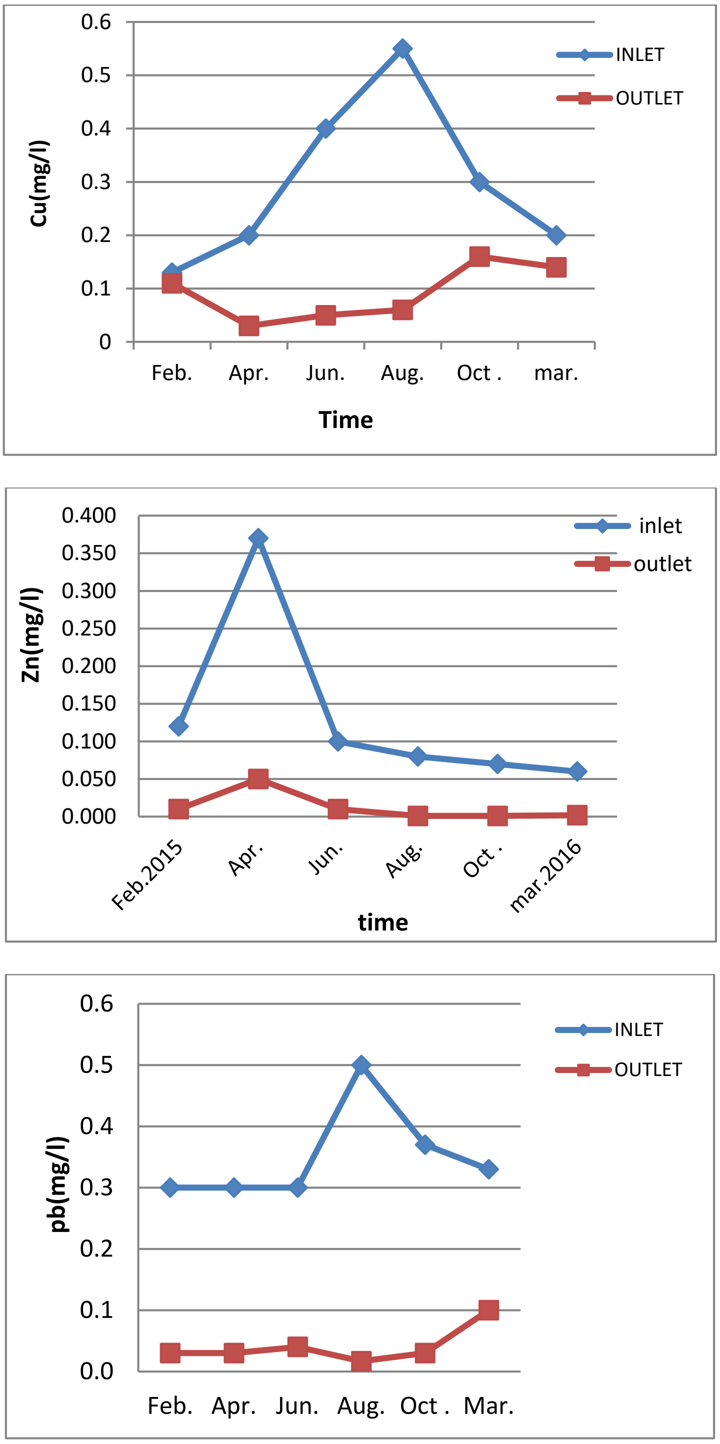


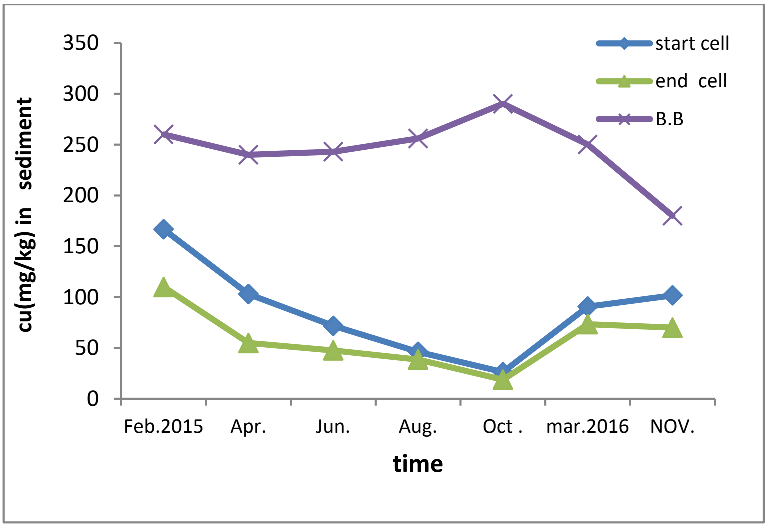
Figure 2: Concentrations of Cu, Zn and Pb in water inlet and outlet flow in wetland project (LMEWP).

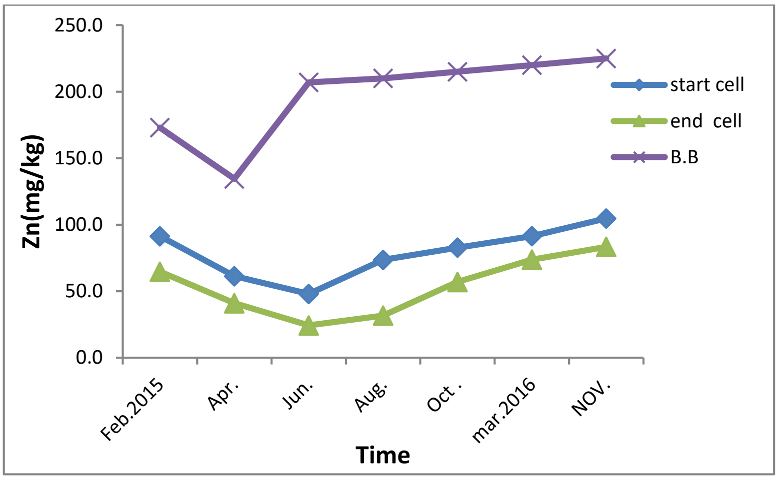
The sediment:

The declining trend of metal concentration in the sediment is as following: Cu>Zn>Pb. This trend is the same in different location sediments but the concentration was higher in sediment B.B; B.B drain>start cell>end cell. Figure (3) shows concentration of Cu were decreased until October in first year while Zn and pb decreased until August in first year, and all metals increased in second year. There is significant correlation between the concentration of metals and the location of cell treatment as there is significant correlation between the concentration of metals and the time. The decreasing of metal concentrations in sediment in first year may be due to the fact that the plant in the growth period while decrease in second year is due to the ability of plant to accumulate metals. Aquatic plants can alter the biogeochemistry of the sediment by modifying redox conditions, pH, and organic matter content, influencing contaminant accumulation in this compartment [18].

Table 2: parameters (average, minimum, and maximum) in sediment.

parameters	unit	B.B	Wetland project	
			Start cell	End cell
PH	--	8(7.5-8.3)	7.7(7.3-8.2)	7.3(6.9-7.6)
Conductivity	ds/m	10(12.2-6.5)	6.9(4.9-8.8)	4(4.7-3.4)
Cu	Mgl^{-1}	245.6(180-290.2)	86.5(46-167)	59(19-110)
Zn	Mgl^{-1}	356.4(388-325)	79.01(48-104)	53.6(24.3-83.3)
pb	Mgl^{-1}	60.8(72.5-44.2)	36.7(25-50.6)	24.5(19.6 -30.2)





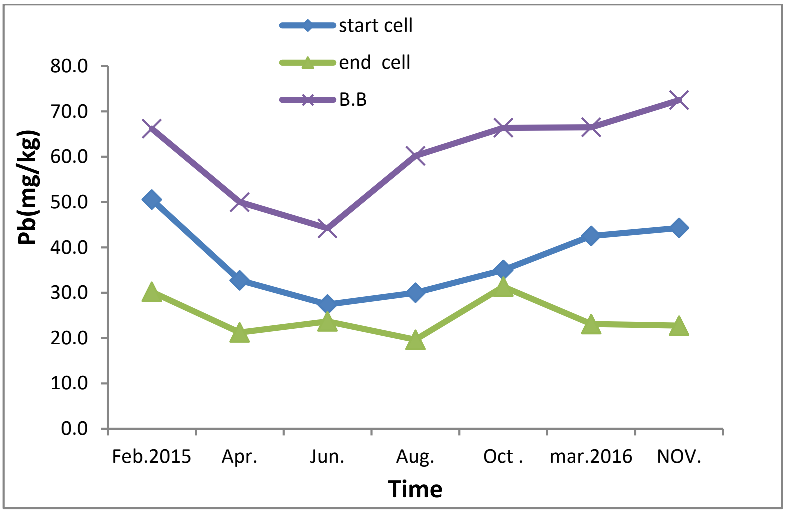
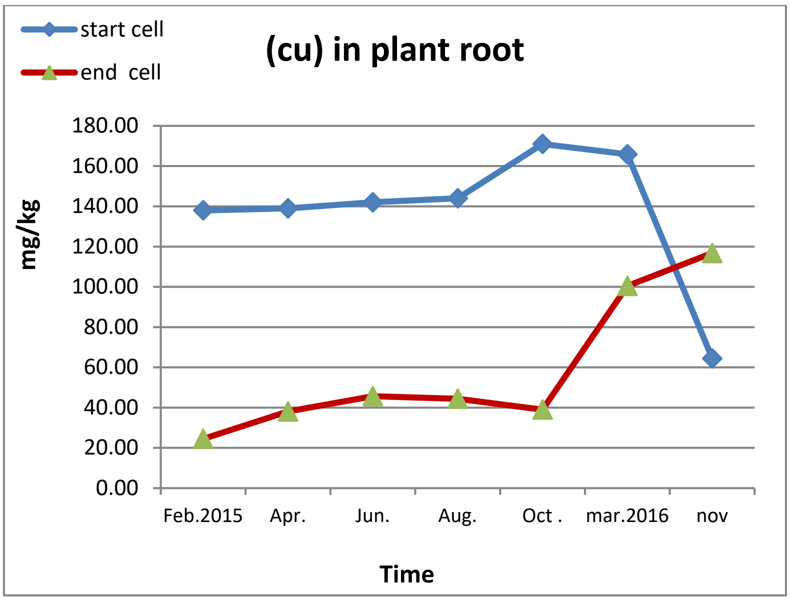
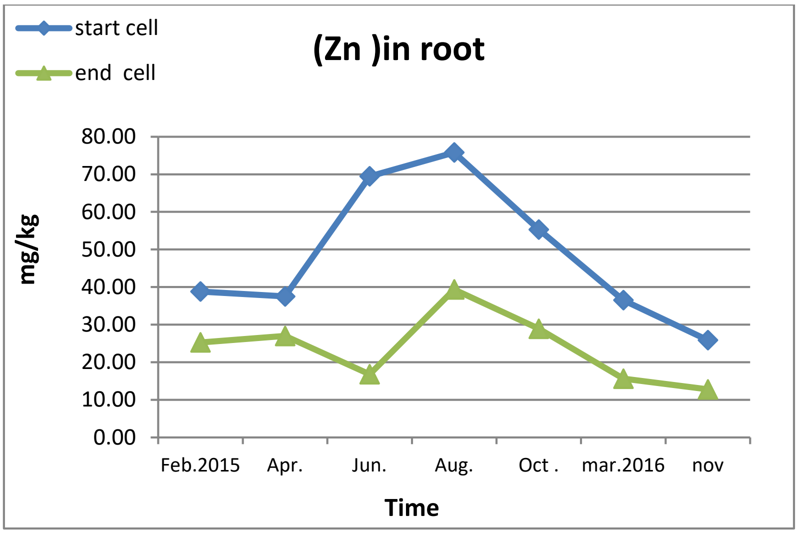


Figure3: Concenteration of metals (Cu, Zn and Pb) in sediment of B.B location and cell treatment of wetland (LMEWP) (stare cell and end cell).

Accumulation of heavy metals in the body of *Phragmitesausturalis*:

Figure (4) illustrates the concentration of metals (mg/kg) in root, stem and leaf of the *P. austuralis*collected from different locations in LMEWP. The trend of concentration of metals in plant organs is similar in all study locations as LMEWP start cell>end cell. Heavy meals accumulation was trended as Cu>Pb> Zn in root; Cu>Zn>Pb in stem; and Cu>Zn>Pb in leaves. The capacity of metal accumulation in root, stem and leaves was positively correlated with time and the age of *P. austuralis* in the first year. On the other hand, metal concentrations were decreased in the second year particularly in November. In second year, metal accumulations were higher in leaves than in roots. There is high significant correlation between the concentration of metal in all plant organs and plant location and time. Cu and Zn accumulation in the first six months (in August) was as root>stem>leaf, and decreased in second year (in November) and the trend was leaf >stem> root; while concentration of pb was as follows: root >stem>leaf in the first six months, after that the concentration of pb decreased in root, stem and increased in leaf but with the same trend.




start cell
end cell
(Zn)in root
mg/kg
80.00
70.00
60.00
50.00
40.00
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20.00
10.00
0.00
Feb.2015
Apr.
Jun.
Aug.
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mar.2016
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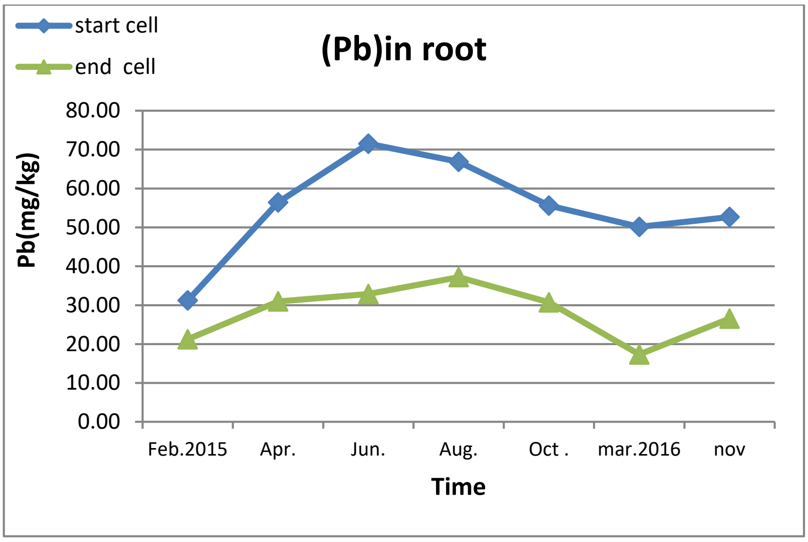
Time


start cell
end cell
(Pb)in root
Pb(mg/kg)
80.00
70.00
60.00
50.00
40.00
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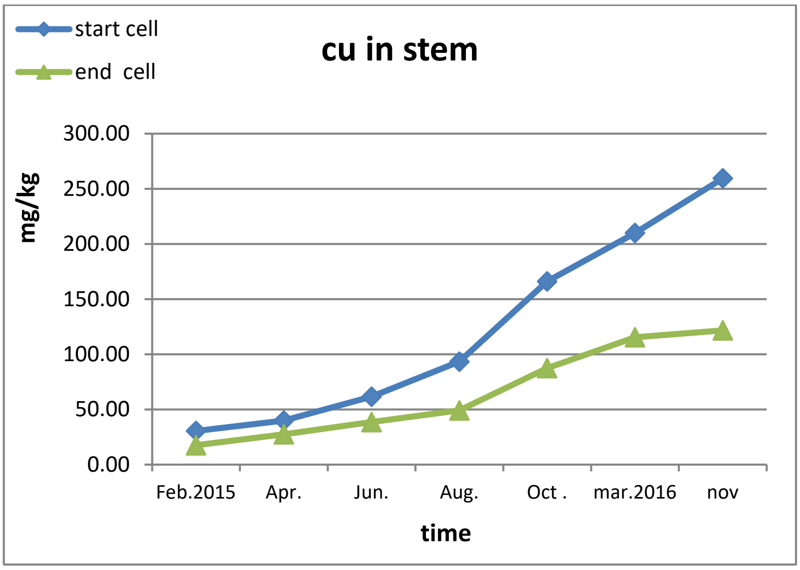
start cell
end cell
cu in stem
mg/kg
300.00
250.00
200.00
150.00
100.00
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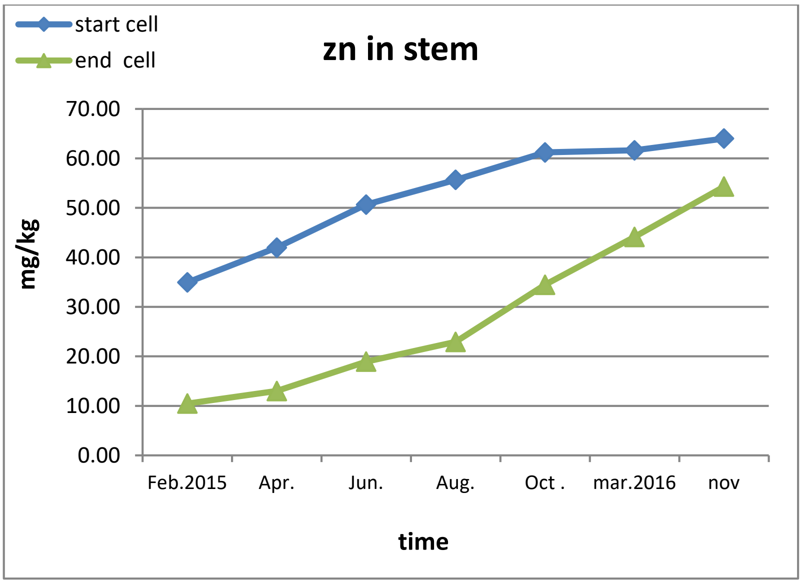
start cell
end cell
zn in stem
70.00
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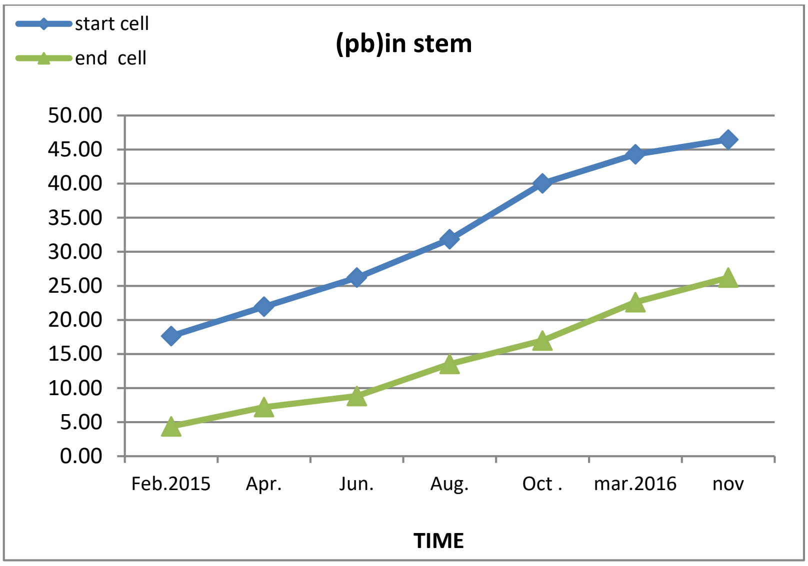
start cell
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(pb)in stem
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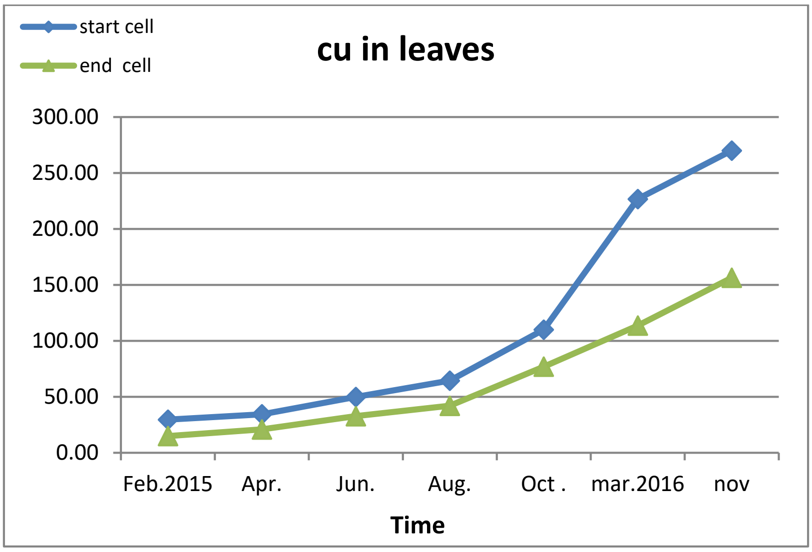
TIME


start cell
end cell
cu in leaves
300.00
250.00
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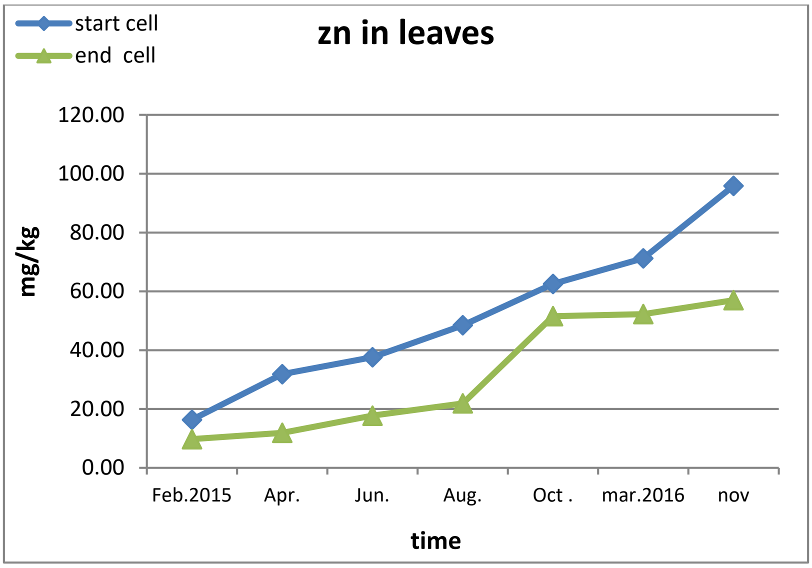

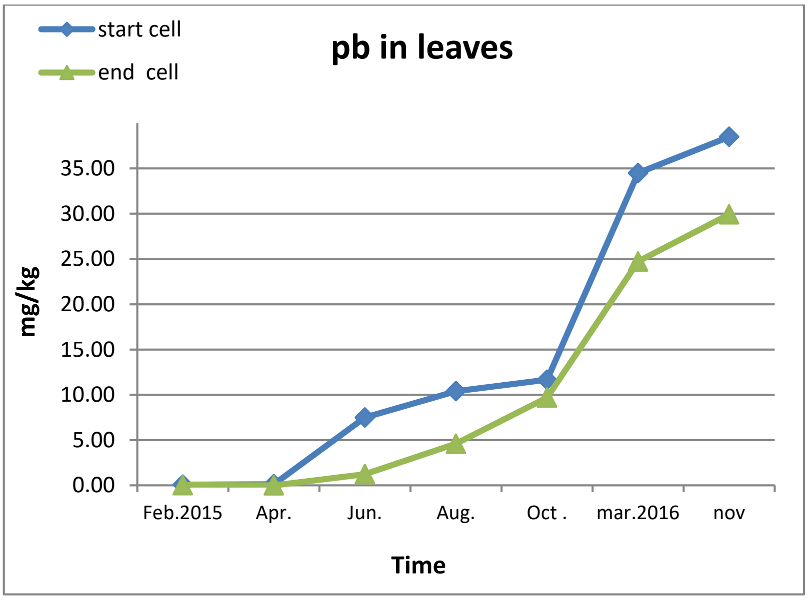

Figure 4: Concenteration of metals (Cu, Zn and Pb) in plant parts (root, stem and leaves) in cell treatment wetland (LMEWP; stare cell and end cell).

Bioconcentration Factor:

Figure (5) shows the seasonal changes in bioconcenteration factor (BCF) for the roots, stems and leaves. In LMEWP, values of BCF in start cell> end cell. BCF_{ROOT} was Cu>pb>Zn metal. BCF_{ROOT} of Cu increased in first year until October and decreased in second year. BCF_{ROOT} for Zn and Pb increased until June and August, respectively (the end of growing season) and then decreased. theBCF_{stem} for Cu increased in October of first year and decreased in second year. BCF_{stem} for Zn and Pb) increased until Augusts then decreased in second year. The result shows that BCF_{leaf}constantly increased in all months in first and second years during and after vegetation period, and all metals (Cu, Zn and Pb) increased with the time in location.

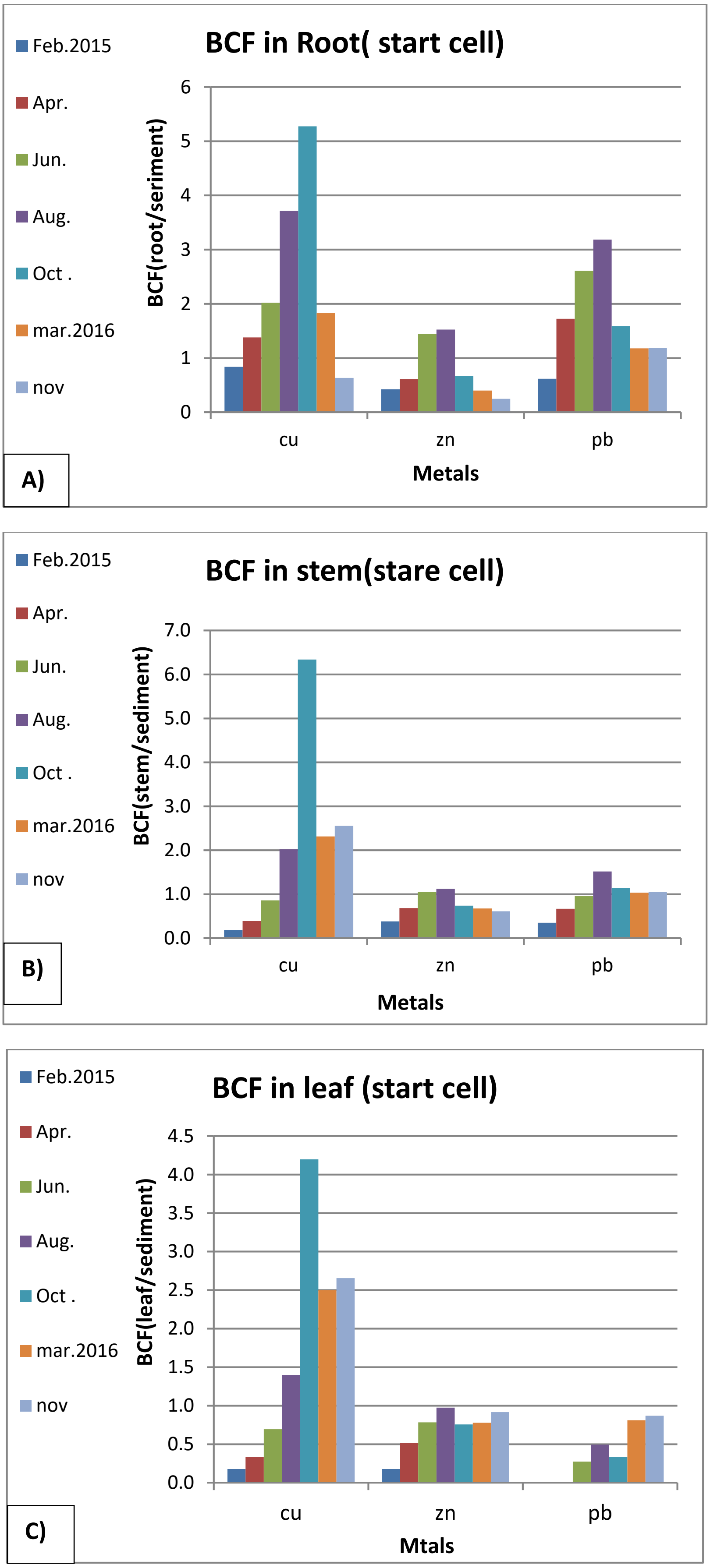

Figure 5: Bbioconecenteration factor (BCF): A): root, B): stem c) leaf in start cell in wetland project.

Table 3: Translocation ability (TA) in wetland project (LMEWP) cell treatment (start cell, end cell).

Metals	parts	Month													
		Feb.2015		April		June		August		October		March2016		November	
		Start cell	End cell	Start cell	End cell	Start cell	End cell	Start cell	End cell	Start cell	End cell	Start cell	End cell	Start cell	End cell
cu	Root/stem	4.6	1.4	3.6	1.4	2.3	1.2	1.8	0.9	0.8	0.5	0.8	0.9	0.2	1
	Root/leaf	4.7	1.6	4.1	1.8	2.9	1.4	2.7	1.1	1.3	0.5	0.7	0.9	0.2	0.7
	Stem/leaf	1.032	1.1	1.16	1.3	1.3	1.1	1.4	1.1	1.5	1.1	0.9	1.02	0.9	0.7
zn	Root/stem	1.1	2.4	0.9	2.1	1.4	0.9	1.4	0.9	1.7	0.8	0.6	0.4	0.4	0.2
	Root/leaf	2.4	2.6	1.2	2.3	1.8	0.9	1.6	0.9	1.8	0.6	0.5	0.3	0.3	0.2
	Stem/leaf	2.1	1.1	1.3	1.1	1.3	1	1.1	1.1	1	0.7	0.9	0.8	0.7	1
	Root/stem	1.8	4.9	2.6	4.3	2.7	3.7	2.1	2.7	1.4	1.8	1.1	0.8	1.1	1
pb	Root/leaf	445	708	470	10322	9.5	27	6.4	8	4.8	3	1.5	1	1.4	1
	stem/leaf	251	146	182	2400	3.5	7	3.1	3	3.4	2	1.3	1	1.2	1

Translocation ability:

The possibility of plants to transport metals from the roots to the above-ground organs was estimated using the translocation ability (TA). A higher TA means a smaller translocation ability. The values of metal translocation ability are given in Table (3). Translocation between different parts of reed plant depended on the type of metal and the sampling period and location. The translocation of metal Cu is as follows: start cell>end cell; while TA of Zn and Pb in trend of end cell> start cell. The translocation of metal Pb from root to stem increased after vegetation period in August to the end of the second year but the translocation of metals Cu and Zn from root to stem increased over time. The translocation of metal from root to leaves increased after vegetation months. The translocation of Cu and Pb from root to leaves increased from October to the end of experiment. The translocation of Zn from root to leaves was increased with time in second year. Translocation of metal Zn from stem to leaves was increased with time while translocations in metal Cu and Pb from stem to leaves were increased in June to the November in second year.

DISCUSSION:

Plant efficiency decreases in the second year of plant life. Accumulation of metals Cu, Zn, and pb in *P. austurales*increased in root in growing season Cu was in Octobers but , Zn and Pb in August in frits year but decreased after that which agree with Nikolaidis *et al*. [19] reported that heavy metals showed increased accumulation in *P. australis* during the growing season, with the maximum values in August and September, and then continuously decrease.Accumulation metals in *p. austurales*arenot distributed evenly, but there are especial organs for bioaccumulation. The underground organ (roots) shows a higher storage than above ground parts (stems and leaves).as many research show of bioaccumulation in plant organs which established that metal concentrations decreased according to the following order rhizome> leaf> stem [20]. The concentrations of metals accumulated in the organs of the common reed may vary during the growing season, as a result of a change in the levels of metal concentration in the surrounding medium, and, on the other hand, the changes in their bioavailability [21]. Cu is an important essential microelement for plants but it can be toxic at higher concentration. In the samples of *P. australis*, the bioconcenteration factor (BCF) of Cu was recorded figure (5). The values of copper accumulation in roots is increased by time than aboveground parts of *P. austratis* in February to October in first year, as the accumulation in stems and leaves were increased. In November, the concentration of Cu in leaves was higher than in the root and stems which was in agreement with Kastratovic et al. [22] that found the following Cu trend: sediment>above-ground part>underground part in *P. australis*. Fitzgerald *et al*. [23] found that *P. australis*translocated Cu more to the shoots. Kastratovic et al. [22] found that the accumulation of Cu in the leaves of *P. australis*was comparable to those measured in the stems and rhizome from July to October.

Zinc (Zn) is an essential and useful element for plants, mainly as a part of various metallo-enzymes. In most aquatic ecosystems, Zn^{2+} can be toxic for the organisms. According to the present study, the roots of *P. australis* actively adsorbed Zn, which contained the most Zn during all the sampling periods. The bioconcentration factors for Zn was low compared with other elements; highest BCF in root was 1.5 in August which decreased after that to lowest BCF in root (0.2) in November of second year. The Distribution of metals in some parts of the plant is the result of differences in the amount and rate of metals input, primarily by root pressure and their release into the environment, mainly through the transpiration of the leaves [24, 25]. Some metals are accumulated in roots, probably due to some physiological barriers for the transport of toxic elements in traces. The metals nessecary for metabolic needs are easily transported to the above-ground parts of the plant.

Lead (Pb) is a potentially hazardous and toxic metal for most of organisms, and it is relatively accessible to aquatic organisms [22]. Pb is particularly present in aquatic environments. In this study, bioaccumulation ability of three investigated organs of *P. australis* for Pb enhanced during the whole research period. Most of the absorbed Pb was in the roots (figure 5). The concentration in the stems was enhanced significantly in August and October. The concentration of Pb in the leaves was increased significantly from April to October, and increased in second year. The absorbed Pb, mainly from sediment, was mostly translocation to the stems during the investigation period with the highest translocationin in March of second year from root to stem. The translocation from roots to leaves was low during April and June, while in August and October (Table 3). The translocation ability of root/leaf increased from March to November of second year, which mainly caused by the increased mobility in the plant. Underground organs, especially the roots, are main organs for the storage of excess trace metals but it is known that various trace metals are deposited even in the leaves [26].

CONCLUSIONS

1. The concentration of all studied metals were decreased in water after treated in LMEWP.
2. The concentration of metals in different organs of *P. australis* varies according to the location and time of sampling.
3. The concentration of all studied metals were increased in roots and stems over time until the end of the growing season and then decreased, while the concentrations in leaves increased even after the period of plant growth.
4. The results of this study showed that the concentrations of metals (Cu, Zn and Pb) were higher in the sediment than in the plant during and after the growing season. At the same time, the concentrations of metals in the plants were much higher than those in the water, which indicates sediment as the major source of the metals absorbed by the plant roots.
5. Bioconcenteration factor (BCF) of metals in start cell was higher than end cell in all studied metals (Cu, Zn and Pb); higher (BCF) in root was found after growing seasons (after eight months).
6. highest Bioconcenteration factor (BCF) of Cu was found in stem in October, while highest Bioconcenteration factor (BCF) of Zn and pb was found in root in August
7. The translocation ability (TA) of Cu in start cell> end cell, while TA of metal Zn and pb in end cell> start cell. This means that Cu have high translocation from plant root to stem and leaf in end cell treatment than start cell; while plants have higher translocation of Zn and Pb from root to stem and leaves in start cell than end cell.

Generally, metal mobility through the plant, from roots to leaves, is generally higher than from sediment to the plant. Then it should be harvested after the growing season because the concentration of metals in the above-ground parts is maximal. There is an inverse relationship between the concentration of elements in the soil and their concentration in the plant throughout the experiment.

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