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REMOVAL OF SOME HEAVY METALS FROM POLLUTED WATER USING PHYTOREMEDITION WITH PHRAGMITES PHRAGMTES AUSTRALIS PLANT

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ABSTRACT

A pot experiment was made to study the use of *Phragmites australis* to absorb and accumulate of Ni, Cd and Pb from enriched water with a formenied heavy metals, where *Phragmites australis* (common reed) plant was growing. The soil used was calcareous in nature which irrigated with such waters up to 120 days. Plant samples (shoots and roots) were taken after 40, 80 and 120 days from transplanting which put to heavy metals content. Obtained results showed that Eh values of the soil-water system affected the accumulation of the three studied metals in plant (shoots and roots), in turn, mainly due to the change of soil pH under such redox conditions. The overall results showed that all studied heavy metal uptakes increased with increasing metal concentrations of artificial heavy metal polluted water comparing to the control treatment. It is worth to mention that, roots tend to accumulate higher amounts of metal content than those of shoots. ANOVA results showed that the studied heavy metal concentrations in artificial polluted water and their interactions with both (roots and shoots) of the plant affected only Ni uptake by common reed plant after 120 days. In most cases metal translocation into common reed upper plant (shoots) showed no affect by increasing heavy metal concentrations in polluted water except for Cd and Ni after 40 and 120 days from transplanting, respectively where the metal concentration affect the translocation processes as it increases with increasing metal concentrations.

Key words: Phytoremediation, *Phragmites australis*, Heavy metal and Artificial heavy metal polluted water.

INTRODUCTION

The pollution of soil and water with heavy metals is an environmental concern today. The high cost of existing clean up technologies led to the search for new clean-up strategies that have the potential to be low-cost, low-impact, visually benign, and environmentally sound.

Phytoremediation describes the treatment of environmental problems through the use of plants that mitigate the aforementioned problem without the need to excavate the polluted material and dispose of it elsewhere.

Phytoremediation consists in mitigating high metal concentrations in polluted soils, water with some plants. It refers to the natural ability of certain plants called hyper-accumulators to bio-accumulate, degrade, or render harmless pollutants in soils and water.

Research related to this relatively new technology needs to be promoted and emphasized and expanded in developing countries since it is low cost.

In situ, solar driven technology makes use of vascular plants to accumulate and trans-locate metals from roots to shoots.

Harvesting the plant shoots can permanently remove these polluted from the soil. Phytoremediation does not have the destructive impact on soil fertility and structure that some more vigorous conventional technologies have such as acid extraction and soil washing. This technology can be applied "in situ" to remediate shallow soil, ground water and surface water bodies.

Also, phytoremediation has been perceived to be a more environmentally-friendly "green" and low-tech alternative to more active and intrusive remedial methods. *Phragmites australis* (common reed) which is widely spread in Egypt is one of the most widely distributed species in the world **(Hocking *et al.* 1983; Graneli 1984; Van der Werff, 1991).** This plant can withstand extreme environmental conditions, including the presence of toxic heavy metal contaminants, such as Zn, Pb and Cd **(Kufel and Kufel 1980; Schierup and Larsen 1981; Van der Werff, 1991).**

In the last two decades, *P. australis* has been widely used in constructed wetlands for treatment of industrial heavy metal enriched waters containing metals **(Dunbabin and Bowmer, 1992).**

However, it is not clear whether the distribution of this species is related to its capacity to evolve metal tolerance when growing in metal-contaminated soil and sediments or whether it has an innate metal tolerance throughout its range, even when growing on relatively uncontaminated sites.

It may be important to study the phytoremediation potential of common reed and correlate different soil characteristics and plant organs to its ability for metal uptakes. In this concern, the present work studied the applicability of common reed to remove metal pollutants from soil treated by heavy metal enriched water. The aim of the current study are to; (i) compare the degree of metal removal (Ni, Cd and Pb) from artificial heavy metal enriched water by common reed, (ii) study the metal accumulation rate in roots and shoots as a result of plant exposure to different heavy metal concentrations in water and (iii) develop predictive algorithms for metal uptake by common reed from some studied soil properties.

MATERIALS AND METHOD

The main target of the current study was to evaluate the common reed marsh plant as a phytoremediation tool to remove some heavy metals from enriched heavy metal synthetic polluted water.

Soil used:

Soil sample was collected from Experimental Farm of Faculty of Agricultural Environmental Sciences, El-Arish, Suez Canal University.

The sample was air dried, crushed 2 mm sieved and then analysed for main physical and chemical properties according to standard methods **(Jackson, 1967).** The main physical and chemical properties of the soil used are presented in Table (1).

Plant used:

Common reed marsh plant was used as an indicator plant which collected from Grada Sewage Station, El-Arish. The initial concentrations of studied heavy metals in the studied plant are presented in Table (2).

Enriched heavy metal synthetic polluted water used:

Enriched heavy metals synthetic polluted water was prepared using metal lead, cadmium sulphate and nickel nitrate as a source of lead, cadmium and nickel, respectively, which dissolved in tap water to obtain the required concentrations. The chemical properties of the tap water used are presented in Table (3).

Table (1): Some physical and chemical characteristics of the soil used

Soil parameter	value
Soil particle distribution (%)	
Sand	97.80
Silt	1.00
Clay	1.20
Soil texture	Sand
$CaCO_3$ (g kg^{-1})	160
EC (dSm^{-1})	1.81
pH	7.93
OM (g kg^{-1})	16.0
Cations and anions (mg kg^{-1})	
Ca^{2+}	132
Mg^{2+}	60.2
Na^{+}	122
K^{+}	42.9
CO_3^{2-}	n.d.*
HCO_3^{-}	19.2
Cl^{-}	249
SO_4^{2-}	451
Total metal concentrations (mg kg^{-1})	
Pb	0.330
Ni	0.135
Cd	3.82

***n.d. no detectable**

Table (2): Initial heavy metal concentrations in common reed plant shoots and roots, mg kg^{-1}

Part	Pb	Cd	Ni
	Heavy metals concentration, mg kg^{-1}		
Shoots	6.925	2.200	5.425
Roots	8.925	2.700	7.700

Table (3): Correlation coefficients (r) between the metal uptake (Pb, Cd and Ni (mg plant^{-1}) by *C. papyrus* and pH and Eh (mV).

	pH		Eh	
	shoots	**roots**	**shoots**	**roots**
Pb	-0.47**	-0.53**	0.26	0.42*
Cd	-0.62**	-0.49**	0.44*	0.29
Ni	-0.66**	-0.41*	0.51**	0.30

Significant correlation at 1% (**) or 5% (*).

A pot experiment was carried out in a greenhouse to investigate the potential use of common reed plant to extract Pb, Cd and Ni from artificially heavy metal polluted water Twenty kgs of the air-dried surface soil sample (0–20 cm) were packed in plastic containers (45 cm internal diameter and 36 cm height). Common reed was transplanted which collected Garada Swage Station, North Sinai, Egypt. On each soil that irrigated with synthetic water enriched with three different concentrations of Pb, Cd and Ni. Three level of metal concentration water were prepared from the salt metal form; ML_1: 10, 1.0 and 0.1 mgl^{-1}, ML_2: 20, 2.0, 0.2 mgl^{-1} while ML_0 (tap water) was used as control treatment. The pot experiment was arranged in a complete randomized block design.

The prepared solutions were added to each pot treatment to stand 5 cm over the soil surface in order to mimic the natural plant conditions. A supplementary of deionized water was added if necessary to recover the water lost by evaporation process from each pot. Plant samples (shoots and roots) were taken after 40, 80 and 120 days from transplanting by cutting the stems approximately 2 cm above the soil surface. While root samples were collected, sieved to get rid of soil particles and washed with running water and then distilled water. Eh and pH were measured in situ immediately before each harvest course. Plant samples (shoots and roots) were dried at 60 °C to a constant weight, grounded into fine powder, sieved with a 2 mm plastic mesh.

One half g of the powdered samples was digested with 4 ml of concentrated sulphuric acid (~7 min) and subsequently with 10 ml of a H_2O_2 solution and analyzed for Ni, Cd and Pb concentrations using an inductively coupled plasma (ICP) technique.

1. Translocation ratio (TR):

This parameter is necessary for environmental transfer models which are useful in prediction of the pollutant concentrations in agricultural crops for estimating dose intake by man. TR is calculated by the relation: the ratio of concentration of heavy metal in the shoots to the concentration of metal in the roots **(Cui *et al.* 2007).**

$$TR = \left(\frac{M_{Sh}}{M_{Ro}}\right)$$

Where TR is a translocation ratio, M_{Sh} and M_{Ro} are the metal concentration in shoot and root systems respectively (mgkg^{-1}). This factor was used to evaluate the Ni, Cd and Pb phyto-extraction capacity of each plant species.

2. Statistical analysis:

Data were statistically analysed to test the ANOVA and Pearson correlation coefficient using IBM SPSS Statistics v. 20 and Microsoft Excel 2010.

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RESULTS AND DISCUSSION

1. Changes of pH and Eh in the soils:

Data presented in Fig. (1) show the effect of elapsed experimental periods on pH and Eh in the soil cultivated with *c.papyrus* plant under different studied heavy metal concentrations of irrigation water (ML1 and ML2 of Pb, Cd and Ni). Soil flooding resulted in changes in pH and Eh.

All treatments exhibited decreasing in Eh following soil flooding by metal enriched irrigated water (Fig. 1).

As general, the Eh values of the soil cultivated with *C. papyrus* was +288 mV. Over a growing period of 40 days, Eh value fell from +540 mV at zero time to approximately +190 mV. The redox potential then remained fairly constant over 80 days so that the soil-solution systems appeared to be strongly poised around this region. In this concern, **Ponnamperuma (1972**) referred to that plant is hard hit by anaerobic conditions and some plants are specially adapted to survive water logged conditions, e.g. rice and marsh plants and here O_2 is transported through the plants from shoot to roots.

The decrease in Eh with time suggests the development of anaerobic conditions in the soil cultivated with studied plant. Such anaerobic conditions result from microbial respiration during which readily available organic carbon is metabolized and soil oxygen is exhausted.

Flooding drastically limits exchange of gases between soil and the atmosphere and an oxygen-deprived environment is established.

The oxygen diffusion in water is lower than that of air by 10,000 times **(Ponnamperuma, 1972).** Moreover, soil pH (Fig.1) was initially lowered by about 0.5 – 1.0 units after 40 days in the soil but then tend to fluctuate within a narrow range of pH changing (pH between 7.0 to 6.4) after 80 days till the end of growing period with the exemption of soils treated with ML2, which tend to continuous decreasing in pH values. Overall, pH values under ML1 and ML2 concentrations fall to lower values compared to the control treatment which rose back to the pH 7 (Fig. 1).

There was a general increase in soil pH values with decreasing soil redox potential (Eh) (Fig. 2) with the soils subject to limiting aeration conditions. The correlation coefficients (r values) showed a significant relationship at P value less than 0.01 ($r = -0.62$).

This is in agreement with expected trends **(Ponnamperuma 1972, Hesterberg 1998, Miao et al. 2006, Fiedler *et al,* 2007).**

Under reduced conditions, the pH of soils tends with elapsing time towards neutral values (around pH 7 as shown in Fig. 1) and such effect could be due mainly to the consumption of H^+ ions during reduction processes, balanced by a build-up of CO_2 from soil organic matter decomposition and plant roots respiration **(Ponnamperuma 1972).**

2. Factor Affecting Metal Uptake by C. Papyrus:

a. Soil pH and Eh

Table (3) shows correlation coefficient between shoot and root metal uptakes by common reed and both pH and Eh (mV) of the soil.

The results showed that a negative significant relationship has been found between all metals taken up by both roots and shoots of common reed plant and pH.

Moreover, a positive significant relationship has been found between the same values and redox potential. However, a non-significant correlation was observed in case of Cd and Ni with root only (r = 0.39) and shoot (r = 0.19) and root(r = 0.33) respectively with Eh.

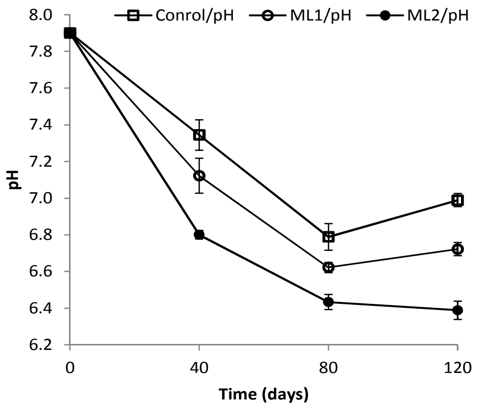
The results indicated that the metal uptake pool increased with decreasing soil pH. The increasing of metal uptake under relatively high Eh values may be due mainly to decreasing in soil pH. Metal uptake is significantly correlated to the solubility forms of metals in the soil solution which, in turn, related to their available pool in soil solid phase. **Miao *et al.* (2006)** studied Zn solubility under both oxic and anoxic conditions by controlling soil pH (from 7.1 to 5.8) and Eh (from 500 to -200 mV). They found that Zn concentrations were mainly dependent on pH and were at a maximum under oxic conditions (at pH = 5.8) which increase the proportional of metal that could be taken up by plants.

This pattern of dependence of metal uptake by plants on soil pH is fairly well recognized in previous studies **(Mench *et al.* 1994, Mench *et al.* 2000, Lombi *et al.* 2002, Cappuyns and Swennen 2005).** In general, metal cations are more mobile under lower pH values of the soil-water system.

b. Metal concentrations and elapsing time:

Metal uptakes with elapsing time (i.e. 40, 80 and 120 days from transplantation) under different metal concentrations of enriched heavy metal polluted water (i.e. Control; 0.00 mgl^{-1}, ML_1 and ML_2, of Pb, Cd and Ni; 10 and 20 mgl^{-1}, Cd; 1.0 and 2.0 mgl^{-1} and Ni; 0.1 and 0.2 mgl^{-1}, respectively) are shown in Figs 3-5. The overall results show that all metal uptake increased with increasing metal concentration comparing to the control treatment.

The highest uptake values were corresponded with high metal concentrations after 80 days in both shoots and roots. This might suggest that lowering of metal concentrations from soil solution is primarily a result of process associated with the presence of plants. In the other word, the highest uptake values should be associated with the depletion process from the soil-solution system. Generally speaking and as expected, the uptake process was enhanced by increasing metal concentrations in all treatments with some exceptions. However, these variations from the expected results were almost recovered when metal uptake calculated using the whole plant as seen in right hand site graphs (Figs 3, 4 and 5).



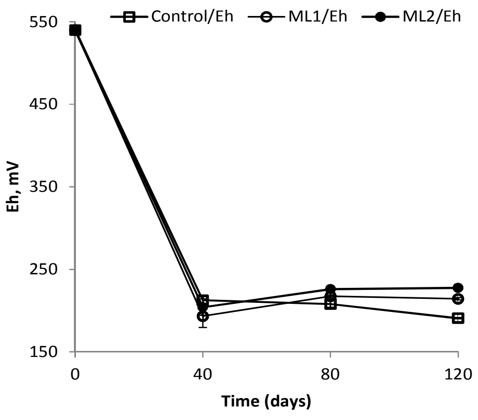


Fig. (1): Changes in soil pH and Eh (mV) as a function of elapsing time.

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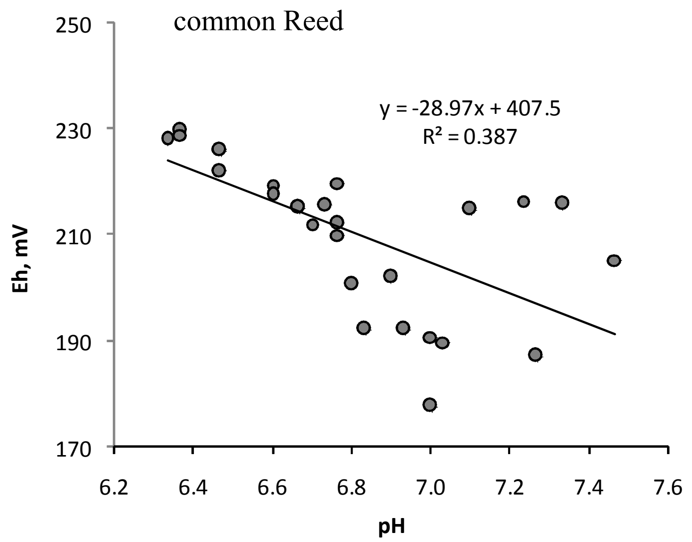

Fig. (2): Soil pH versus soil Eh (mV) values soils cultivated with Common reed conditions.

Therefore, Analysis of variance (ANOVA) test has been undertaken to show the effect of heavy metal concentrations in synthetic polluted water and plant parts (roots and shoots) on metal uptake by C. papyrus plant.

Obtained results given in Table (4) showed that the different metal concentrations and their interactions with plant parts affected only Ni uptake by common reed plant after 120 days form transplantation. In addition, Pb uptake has been affected by neither metal concentrations nor plant parts. Plant parts and metal concentrations showed more effect on Cd uptake by plant comparing to the other metals (Ni and Pb).

Such effect may be due to the competing effect of Cd ions over other heavy metals.

Some heavy metals may effectively compete for the same transmembrane carriers used by other heavy metals. Toxic heavy metals such as cadmium may effectively compete for the same transmembranic carrier as used by micronutrient heavy metal.

This relative lack of selectivity in transmembrane ion transport may partially explain why toxic heavy metals can enter cells, even against a concentration gradient. Kinetic data demonstrate that essential elements Cu2+ and Zn2+ and nonessential Ni2+ and Cd2+ compete for the same transmembrane carrier **(Crowley *et al.* 1991).** Moreover, Pb and Ni may show low solubility in the current study where increasing Pb and Ni solubility is likely to be affected significantly by higher decomposable organic carbon (DOC) concentrations under anoxic conditions as it is well recognised that Pb and Ni is more strongly bound with soluble humus ligands **(Sauve *et al.* 1997, Cances *et al.* 2003, Grybos *et al.* 2007)**. Metal selectivity is the preferential uptake of one metal over another and can be defined in terms of total uptake or concentration in either the entire plant or plant parts.

Translocation of metals into upper plant:

Translocation ratio was determined by dividing the total shoot concentration by the concentration in the plant roots **(Cui *et al.,* 2007).**

Important differences in Pb, Zn, Cu and Cd in endurantplants distributed in an old smeltery. Environmental Geology 51 (6), 1043–1048. translocation of metals within the plant were noted. In most cases metal translocation into common reed upper plant parts did not affect by increasing metal concentration Table (4).

This pattern was not appear with Cd and Ni after 40 and 120 day, respectively, where the metal concentration affect the translocation processes as it increases with increasing in the metal concentrations Table (4).

Metals taken up from metal concentration treatments were found in higher amounts in the roots when compared with the shoots in case of Pb (Figs. 3, 4 and 5).

This trend suggests that translocation of metal into upper vegetative plant part is driven by the concentration of that metal and by the presence of other metals (competing effect). However, the data shown in table 5 suggested that the efficiency of this translocation mechanism is plant species dependent.

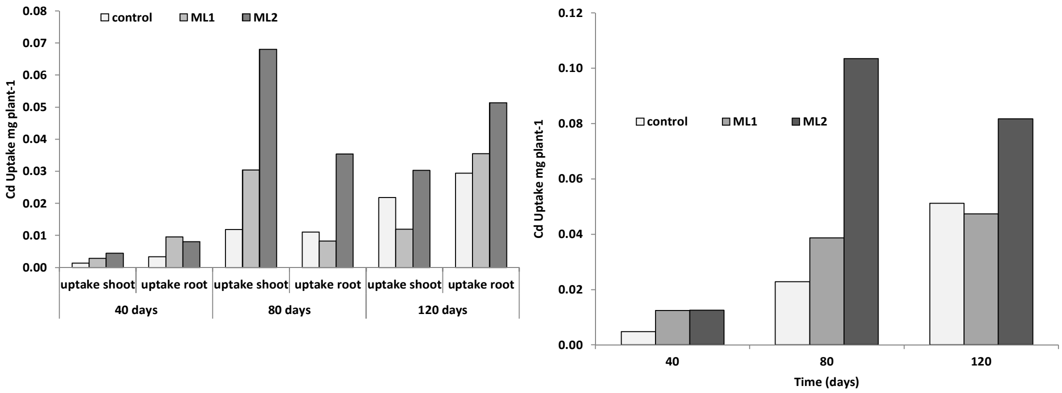

Fig. (3): **Cadmium taken up (mg plant^{-1}) by common reed roots and shoots (left side) and whole plant (right side) irrigated by enriched water (Control; 0.00 mgl^{-1}, ML1 and ML2, of Ni; 0.1 and 0.2 mgl^{-1}, respectively).**

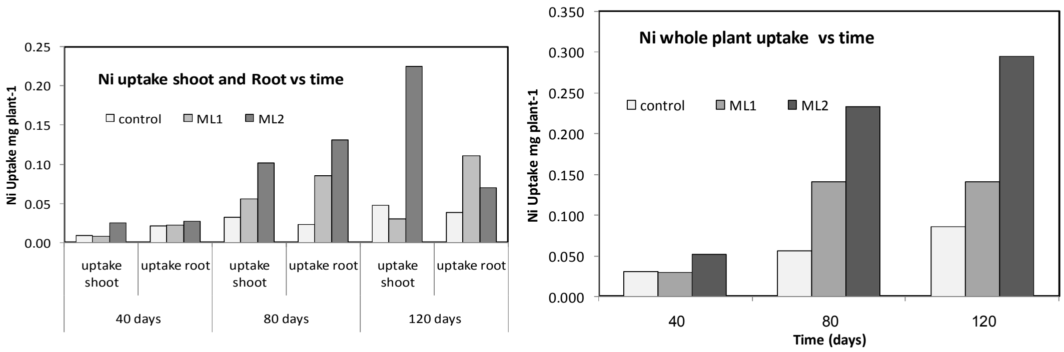

Fig.(4): Nickel taken up (mg plant^{-1}) by common reed plant roots and shoots and whole plant irrigated by heavy metal enriched polluted water (Control; 0.00 mgl^{-1}, ML1 and ML2, of Pb; 10 and 20 mgl^{-1}, respectively).

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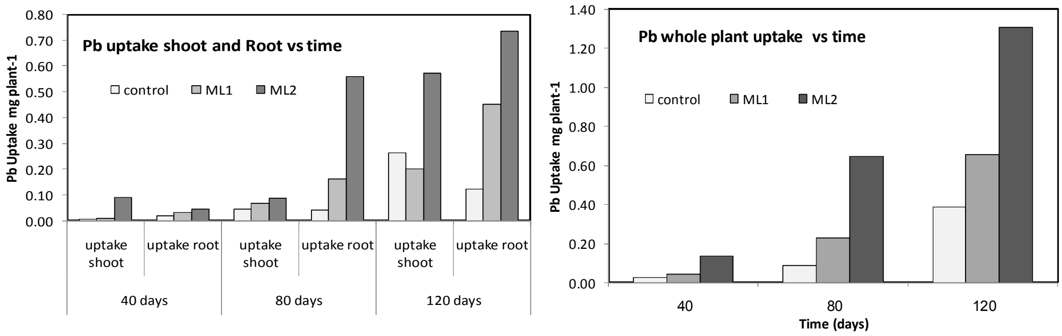

Fig. (5): Lead taken up (mg plant^{-1}) by Common reed plant roots and shoots and whole plant irrigated by heavy metal enriched polluted water (Control; 0.00 mgl^{-1}, ML1 and ML2, of Pb; 10 and 20 mgl^{-1}, respectively).

Table (4): Analysis of variance (ANOVA) performed for Cd, Ni and Pb uptake as a function of different metal concentrations and common reed plant parts.

	Cd uptake		Ni uptake		Pb Uptake	
	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
			40 days*			
concentrations	1.86	0.20	2.59	0.12	5.81	0.02
Plant parts	4.69	0.05	4.17	0.06	0.06	0.80
interaction	0.52	0.60	0.66	0.53	2.20	0.15
			80 days			
concentration	7.97	0.01	2.44	0.13	1.32	0.30
Plant parts	4.52	0.05	0.25	0.62	1.63	0.23
Iinteraction	1.15	0.35	0.15	0.86	0.97	0.41
			120 days			
concentration	1.43	0.28	4.23	0.04	3.57	0.06
Plant parts	3.71	0.08	0.85	0.38	0.39	0.55
Interaction	0.30	0.75	5.12	0.02	0.67	0.53

*., Bold figures represent significant values.

Table (5): Percentage of total metals transported into the shoots of common reed under different metal concentration treatments and over different growing periods.

	Heavy metal								
	Cd			Ni			Pb		
days	40	80	120	40	80	120	40	80	120
Control	51.1	80.7	10.8	16.2	94.3	45.2	12.8	88.9	84.7
ML1	65.5	6.36	2.05	12.5	14.3	69.5	15.7	2.9	3.56
ML2	55.8	13.1	21.0	24.9	75.7	56.2	6.3	10.5	24.8

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الملخص العربي

إزالة بعض العناصر الثقيلة من المياه الملوثة بواسطة المعالجة النباتية باستخدام نبات البوص

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أجريت تجربة أصص لدراسة أثر استخدام نبات البوص لامتصاص وتراكم عناصر الرصاص والكاديميوم والنيكل من الماء الملوث بتلك العناصر الثقيلة. وتم تنمية النبات المستخدم في الدراسة في تجربة أصص باستخدام ماء مدعم بثلاث مستويات هي:

١- معاملة الكنترول (ماء صنبور).

٢- ماء مدعم بثلاث مستويات من العناصر الثقيلة باستخدام عنصر الرصاص النقي وكبريتات الكاديميوم ونترات النيكل كمصدر لتلك العناصر وكان المستوى الأول يمثل ضعف المستوى المسموح به لتلك العناصر في المياه.

٣- يمثل المستوى الثالث من هذه العناصر ضعفي المستوى المسموح به بين تلك العناصر في ماء الري. أقيمت التجربة في أصص احتوت على ٢٠ كجم تربة وأضيف الماء لارتفاع ٥سم أعلى سطح التربة طول التجربة. استمرت التجربة لمدة ١٢٠ يوم وقد قدرت تركيزات العناصر الثقيلة الثلاثة تحت الدراسة في النبات عند بداية التجربة (زمن صفر) وبعد ٤٠، ٨٠، ١٢٠ يوم من شتل النبات وتم قياس رقم pH النظام تربة – ماء وكذلك جهد الأكسدة والاختزال (Eh) لذلك النظام عند الفترات السابقة.

وكانت أهم النتائج المتحصل عليها ما يلي:

١- أن قيم جهد الاكسدة والاختزال Eh للنظام (تربة – ماء) قد أثر على تراكم العناصر الثلاثة المدروسة في كلا من الجذور والمجموع الخضري وربما يرجع ذلك إلى انخفاض رقم pH ذلك النظام مما أدى إلى زيادة ذوبان العناصر الثلاثة وبالتالي زيادة امتصاصها وتراكمها في النبات.

٢- أن امتصاص العناصر الثلاثة المدروسة قد زاد بواسطة النبات بزيادة تركيزات تلك العناصر في ماء الري.

٣- أن تراكم العناصر الثلاثة المذكورة قد زادت في المجموع الجذري للنبات أكثر من المجموع الخضري.

٤- أنه يمكن استخدام نبات البوص في عملية (المعالجة النباتية) لإزالة العناصر الثقيلة من المياه الملوثة بها.

الكلمات الاسترشادية: العناصر الثقيلة، المعالجة النباتية، نبات البوص، المياه الملوثة.
