Phytoremediation of Heavy Metals by Four Hyper Accumulation Timber Tree Species Irrigated With Treated Wastewater. 1) Concentrations of Heavy Metals in Tree Parts and Soil Depths

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***Received:** 9/11/2011*

Abstract: This study was carried out in two seasons 2009 and 2010 at Serabium Forest at Ismailia, Egypt. Four tree species accumulating Fe, Mn, Zn, Cu, Pb, Ni, Cd and Cr were studied. These species are *Eucalyptus citriodora, E. camaldulensis, Cupressus sempervirens and Pinus halepensis* and they were irrigated by drip irrigation system of primary treated wastewater. Two ages (four and eight years) were analyzed. Also heavy metals DTPA under trees in two soil depths (0-25 cm) and (25-50 cm) were analyzed. Significant differences occurred among the tree species, tree ages and soil depths. Older trees had hyper accumulation capacity than the younger trees. In the first season, *Eucalyptus camaldulensis* trees had highest Mn and Pb in the total parts of tree (222.95 and 7.6166 mg kg^{-1}, respectively) as compared with other tree species. While, *Cupressus sempervirens* trees had highest Cr conc. in the stem (0.307 mg kg^{-1}) and it gave the highest Fe conc. in the roots (402.66 mg kg^{-1}). Also, *Eucalyptus citriodora* trees gave the highest Cd conc. in the stem (0.180 mg kg^{-1}) and it gave the highest Zn and Cu conc. in the roots (102.83 and17.20 mg kg^{-1}, respectively). As for the available concentrations of heavy metals in the soil, data revealed that Zn, Cu, Ni and Cd concentrations were found in low concentrations at soil supporting *E. citriodora* trees in the two soil depths. However, in the second season, *Eucalyptus camaldulensis* trees had highest Fe, Zn, Cu and Pb in the total parts of tree (398.58, 72.0, 8.475 and 7.533 mg kg^{-1}, respectively), and it gave the highest Fe, Mn and Cu in the stem (398.16, 190.33 and 9.033 mg kg^{-1}, respectively). While, *Cupressus sempervirens* trees had highest Ni, Cd and Cr in the total parts of tree (1.218, 0.198 and 0.211 mg kg^{-1}, respectively). On the other hand, *Pinus halepensis* trees gave the lowest concentrations of the available Mn, Cd and Cr in the two soil depths. Results recommend that planting *Eucalyptus camaldulensis* trees for phytoremediation of Fe, Mn, Zn, Cu and Pd, *Cupressus sempervirens* trees for phytoremediation of Fe, Ni, Cd and Cr, *Eucalyptus citriodora* trees for phytoremediation of Zn, Cu, Ni and Cd, and *Pinus halepensis* trees for phytoremediation of Mn, Cd and Cr.

Keywords: Heavy metals, hyper accumulation capacity, phytoremediation

INTRODUCTION

With increasing heavy metal contamination, due to various human and natural activities, ecosystems have and are being contaminated. The recycling of wastewater and sewage sludge are examples of events that contribute to contamination of our environment.

Industrial wastes, heavy metals, dust and SO_2 emission as the main pollutants from mining and smelting activities in many countries of the world. These imposed diverse effect on plant communities that led to complete degradation of plant cover and severe soil erosion (Regvar and Vogel, 2000). Approximately 400 metal hyperaccumulators have been identified so far (Baker *et al.*, 1994). Hyperaccumulator plants are grown on soils high in metals to determine their potential for management of polluted soils and especially for metal extraction (Shallari *et al.*, 1998).

Phytoremediation is using tree to extract the heavy metals from the contaminated ground, and accumulate them in root, stem and branches. Logically, repeating cycles of planting and harvesting of trees accumulated with heavy metals will eventually reduce the concentration of potentially toxic elements of heavy metals in soils to an acceptable level for other uses (Ang *et al.*, 2010). Using timber species for phytoremediation approach has additional benefit as it also yields timber at the end of its rotation (Ang *et al.* 2000). All tree species have the capacity to uptake heavy metals but each species may differ in its bioaccumulation potential. It also depends on the species preference and availability of heavy metals in ionic forms. Many studies in the temperate show that tree species have been used as bioaccumulation to clean heavy metals such as: Ni, Pb, Zn, Cu and Cd (Pulford and Watson, 2003 and Li, 2006).The present study aimed to evaluating four timber tree species for removing heavy metals from soil.

MATERILS AND METHODS

The experiment was carried out in Serabium forest at Ismailia, Egypt, for 24 months in two seasons (July 2009 and July 2010) to study species accumulating exceptionally large concentrations of Fe, Mn, Zn, Cu, Pb, Ni, Cd and Cr of four timber trees species of two ages and the heavy metals concentrations of soils in two depths. The design was a factorial randomized complete block (RCBD) with three replicates. Factors were (1) trees (4 species) and (2) age of trees (two ages for each species i.e. 4 and 8 years). The forest covers about 1000 Faddan and it has been successfully greened with many timber tree species. Each species planted in plot, about 4 Faddan (200 × 75 m) and the space between trees were 3 × 3 m.

Trees:

Four timber tree species were used in this study i.e. *Eucalyptus citriodora, E. camaldulensis, Cupressus sempervirens, and Pinus halepensis*. These species have been introduced in Egypt more than a hundred years ago.

They are fast growing species, grow well in different soils and are used as windbreaks. The timber is used for pulp production, polywood manufacture, charcoaling and furniture.

Soil:

The soil used in the experiment was a virgin soil in Serabium. Soil physical and chemical properties were determined according to Black *et al.* (1965). Available Fe, Mn, Zn, Cu, Pb, Ni, Cd and Cr were extracted by DTPA- reagent and measured by atomic absorption spectrophotometer, these data are shown in table (1).

Irrigation water:

The water used for irrigation was primary treated waste water collected from the oxidation ponds of Serabium and it consists of a mixture of domestic and industrial water. Each tree species was irrigated three times weekly by drip irrigation system since the time of planting trees from (4-8) years till the end of experiment.

Water analysis:

The concentration of total heavy metals (Fe, Mn, Zn, Cu, Pb, Ni, Cd and Cr) was measured by atomic absorption spectrophotometer after acidifying with nitric acid (APHA (2000), table (2) shows analysis of irrigation water.

Three homogenous trees of species were randomly selected for analysis of leaves, stems, roots, and fruits. Samples were dried at 70°C for 48 hours to constant weight, and then analyzed for heavy metals (Chapman and Pratt, 1961). Also, soil samples around each tree were taken at two depths namely, 0 - 25 and 25 – 50 cm.

Regarding to the effect of metal ions in water, the maximum concentration of the heavy metals especially in the second season (2010) may be due to the increase industrial activities during this periods and natural process of chemical wreathing of sedimentary rocks.

Table (1): The main chemical and physical properties of Serabium virgin soil of the current study

Soil Depth (cm)	PH	EC (ds m^{-1})	Water soluble cations (mmole L^{-1})				Water soluble anions (mmole L^{-1})				Available heavy metals ($mg\ Kg^{-1}$) DTPA-extract							
			Ca^{++}	Mg^{+}	Na^{+}	K^{+}	$Co_3^{=}$	Hco_3^{-}	Cl^{-}	$So_4^{=}$	Fe	Mn	Zn	Cu	Pb	Ni	Cr	Cd
0 - 50	7.88	1.1	2.5	3.5	4.2	0.7	—	0.5	7.5	2.9	3.34	0.57	0.38	0.33	0.14	0.08	.006	0.01

Table (2): Analysis of primary treated wastewater used for irrigation of the trees

Parameter / Years	pH	Ec ds m^{-1}	SAR	Elements mg L^{-1}							
				Fe	Mn	Zn	Cu	Pb	Ni	Cd	Cr
2009	6.6	1.55	5.38	0.55	0.25	0.14	0.70	0.09	0.06	0.04	0.08
2010	6.6	1.77	4.36	7.50	3.15	1.05	0.35	1.15	0.38	0.23	0.26
Recommended by FAO 1992	6.5- 6	0.7-3.0	0.7-0.2	5.00	0.20	2.00	0.20	5.00	0.20	0.01	0.10

RESULTS AND DISCUSSION

Contents of heavy metals in trees: analysis was done in two seasons, 2009 and 2010. Parts of tree analyzed were leaves stems, roots, fruits and total (mixed of all parts of tree).Tables 3 to 7 showed, heavy metals contents in trees. Tables 8 and 9 showed heavy metals in soil.

The leaves:

In the first season, the results in table (3) revealed that the zinc and nickel concentrations in leaves differed significantly between species, and *P. halepensis* trees gave the lowest Zn and Ni conc. in the needles (47.83 and 0.583 mg kg^{-1}, respectively).

In the second season, the data showed that the iron and nickel conc. in leaves differed significantly between species. *Pinus halepensis* trees gave the highest Fe conc. in needles (398.16 mg kg^{-1}). While, *Eucalyptus citriodora* trees gave the lowest Fe and Ni conc. in leaves (363.33 and 0.867 mg kg^{-1}, respectively).

Generally, the oldest trees (8 years) gave the highest mean values of Fe, Mn, Ni and Cr (406.83, 186.75, 1.242 and 0.228 mg kg^{-1}, respectively) as compared with the younger trees (4 years). Also, the interaction between species and ages was highly significant for iron conc. in leaves as compared with other metals.

The stem:

In the first season, cadmium and chromium concentrations in stem were significantly differences between species as shown in table (4).Also, the interaction between species and ages was highly significant for Mn, Zn, Ni, Cd, and Cr. *Eucalyptus citriodora* trees gave the highest Cd conc. (0.180 mg kg^{-1}).While, *Cupressus sempervirens* trees gave the highest Cr conc. in the stem (0.307 mg kg^{-1}) as compared with other species.

In the second season, the data in table (4) indicated that the mean value of Fe, Mn, and Cu conc. in stem differed significantly between species. And, *Eucalyptus camaldulensis* trees gave the highest Fe, Mn and Cu conc. (398.16, 190.33 and 9.033 mg kg^{-1}, respectively). As well as, the significance between different ages indicates that Fe, Mn and Zn conc. of each species increased with the older trees as compared with the younger trees (401.25, 191.33 and 73.33 mg kg^{-1}, respectively).

The roots:

In the first season, the results of the analysis in table (5) revealed that the Fe, Zn, Cu, Ni and Cd conc. in the roots differed significantly between species. *Cupressus sempervirens* trees gave the highest Fe conc. (402.66 mg kg^{-1}). And, *Eucalyptus citriodora* trees gave the highest Zn and Cu conc. (102.83 and 17.20 mg kg^{-1}, respectively).While, *Pinus halepensis* trees gave the highest Ni and Cd conc. in the roots (2.142 and 0.173 mg kg^{-1}, respectively). On the other hand, the interaction between species and ages was highly significant differences of Mn, Zn, Ni and Cd concentrations.

In the second season, the data indicated that the Fe, Mn, Zn, Pb and Ni conc. in the roots differed significantly between the two ages, and the older trees gave the highest level conc. of Fe, Mn, Zn, Pd and Ni (413.75, 186.41, 71.58, 7.45 and 1.263 mg kg^{-1}, respectively) as compared with the younger trees, as shown in table (5).

The fruits:

Data presented in table (6) indicated that Mn, Zn, Cu, Ni and Cd conc. in fruits was significantly different between species, in the first and second seasons. On the other hand, *Eucalyptus citriodora* trees gave the lowest Mn conc. in the first and second seasons (137.33 and 180.33 mg kg^{-1}, respectively). Also, it gave the lowest Pd conc. in the first and second seasons (5.33 and 7.00 mg kg^{-1}, respectively). So, *E. camaldulensis* trees gave the lowest Ni conc. in the first and second seasons (0.683 and 0.683 mg kg^{-1}, respectively). However, *P. halepensis* trees gave the highest Cd conc. in cones in the first and second seasons (0.160 and 0.252 mg kg^{-1}, respectively).

The ages and species – ages interaction effect was generally evident throughout the first season, especially with Mn, Zn, Pd, Ni, Cd and Cr concentrations in fruits.

The total:

As shown in table (7), Fe, Cu, Pd, Ni and Cr conc. in the total part of tree were highly significant affected by the species in the first and second seasons. As well as, the trees ages and the interaction between the species – ages indicted highly significant differences of Fe, Pb, and Cd conc. in the total parts of tree in the first and second seasons.

In the second season, *E. camaldulensis* trees gave the highest Fe, Mn, Zn, Cu and Pb conc. (398.58, 189.08, 72.00, 8.47 and 7.53 mg kg^{-1}, respectively). While, *C. sempervirens* trees gave the highest Ni, Cd, and Cr conc. in the total part of tree (1.218, 0.198 and 0.211 mg kg^{-1}, respectively).

The available heavy metals in different depths of the soil:

A-At the first depth (0-25 cm):

It is clear from the results in table (8) that the available Fe, Mn, Zn, Cu, Ni and Cd (DTPA-extractable) differed significantly between species in the first and second season. And, *Eucalyptus citriodora* trees gave the lowest available Fe, Mn, Zn, Ni, and Cd (DTPA- extractable) (2.237, 1.94, 1.005, 0.263, 0.215 and 0.138 mg kg^{-1}, respectively), in the first season. However, *C. sempervirens* trees gave the lowest available Pb and Cr (DTPA- extractable) (2.247 and 0.185 mg kg^{-1}, respectively), in the first season.

On the other hand, the trees ages and the interaction between species- ages indicated highly significant different of Fe, Zn, Pb and Cd concentrations in the first and second seasons.

B- At the second depth (25-50 cm):

The mean values of the different species, ages and interaction between species- ages in table (9) indicated that they significantly respond to decrease the concentration of the available Fe, Zn and Ni (DTPA-extractable) through the soil in the first and second season.

Pinus halepensis trees gave the lowest concentration of the available Fe, Mn and Cr (DTPA-extractable) (2.380, 1.983 and 0.135 mg kg^{-1}, respectively). While, *Eucalyptus citriodora* trees gave the lowest conc. of the available Zn, Cu, Ni and Cd (DTPA- extractable) (1.148, 0.278, 0.198 and 0.132 mg kg^{-1}, respectively), in the first season.

On the other hand, in the second season, the significance between different ages indicated that the available Fe, Zn and Ni (DTPA- extractable) in the soil of each species decreased with older trees(10. 04, 3.267 and 0.710 mg kg^{-1}, respectively) as compared with the younger trees (13.22, 3.792 and 0.808 mg kg^{-1}, respectively).

Generally, in the first and second seasons, the result indicated that *Eucalypus citriodora, Pinus halepensis* and *Cupressus sempervirens* trees gave the lowest heavy metals concentration in the two soil depths. So, the roots of this species were more diffusion, deepen and high absorption of heavy metals than other trees species.

The obtained results are in agreement with the results of Mireles *et al.* (2004), they presented the range of variation that was observed for metals such as, Fe, Cr, Co, Mn, Ni, Cu, Zn and Pb, when irrigation with wastewater for long periods of time. The results of Singh and Singh (2000) showed that the contents of each metal in leaves, bark, wood and roots were correlated with levels occurring in the environment.

El Gendi *et al.* (1999) they found that the amounts of total Cd, Pb, Co and Ni were higher in soil irrigation for seven years by sewage effluent. So, Selem *et al.* (2000) found that the amounts of DTPA-extractable elements increase in the upper soil layer (0-30 cm). Also, Hassan *et al.* (2003) showed that extractable metals (Cu, Cd, Mn, Pb, Ni and Zn) increased in the soil as a result of sewage water irrigation, with higher accumulation at surface layers. Assareh *et al.* (2008) indicated that *Eucalyptus* is able to grow areas where some metals in soil are accumulated.

In general, different interaction may be related with the amount of each metal added to soil, soil properties, physicochemical properties of heavy metals determine, plant species, plant factors and structural properties of plant tissues (Nogales *et al.*, 1997, Grytsyuk *et al.*, 2006 and Ataabadi *et al.*, 2010).

Table (3): Mean values of the concentrations of iron, manganese, zinc, copper, lead, nickel, cadmium and chromium (mg kg^{-1} of dry weight) in the leaves of the four tree species irrigated by primary treated wastewater at the first season (2009) and the second season (2010)

Treatments	Fe		Mn		Zn		Cu		Pb		Ni		Cd		Cr	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
E. citriodora	349.83	363.33	173.33	168.33	75.00	62.00	10.500	6.050	6.500	6.183	1.100	0.867	0.120	0.183	0.238	0.183
C. sempervirens	381.83	384.16	175.16	168.16	71.50	69.83	9.250	8.317	6.433	7.167	0.867	1.433	0.128	0.232	0.223	0.217
E. camaldulensis	364.33	395.50	186.00	184.16	72.00	73.00	8.050	8.683	6.983	7.250	1.267	0.970	0.128	0.188	0.213	0.195
P. halepensis	393.16	398.16	178.00	181.16	47.83	66.50	5.183	7.467	6.150	7.317	0.583	0.933	0.105	0.162	0.158	0.197
Sig.	NS	**	NS	NS	*	NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS
LSD	-	19.02	-	-	12.30	-	-	-	-	-	0.398	0.314	-	-	-	-
Age1	386.25	406.83	174.66	186.75	69.91	74.41	8.200	8.367	6.525	7.233	1.058	1.242	0.118	0.206	0.217	0.228
Age2	358.33	363.75	181.58	164.16	63.25	61.25	8.292	6.892	6.508	6.725	0.850	0.860	0.122	0.177	0.200	0.168
Sig.	NS	*	NS	*	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	*
Sp1 x Age 1	367.33	368.66	180.00	178.33	74.66	62.66	8.667	6.767	6.000	5.900	1.100	0.933	0.110	0.187	0.207	0.150
Sp1 x Age 2	383.33	427.00	170.66	180.66	72.33	78.00	9.067	8.633	7.000	7.300	0.767	1.867	0.110	0.243	0.243	0.260
Sp2 x Age 1	362.00	410.33	192.00	184.00	74.00	82.33	8.200	9.133	7.200	7.833	1.600	1.100	0.153	0.217	0.247	0.260
Sp2 x Age 2	432.33	421.33	156.00	204.00	58.66	74.66	6.867	8.933	5.900	7.900	0.767	1.067	0.100	0.177	0.170	0.243
Sp3 x Age 1	332.33	358.00	166.66	158.33	75.33	61.33	12.333	5.333	7.000	6.467	1.100	0.800	0.130	0.180	0.270	0.217
Sp3 x Age 2	380.33	341.33	179.66	155.66	70.66	61.66	9.433	8.000	5.867	7.033	0.967	1.000	0.147	0.220	0.203	0.173
Sp4 x Age 1	366.66	380.66	180.00	184.33	70.00	63.66	7.900	8.233	6.767	6.667	0.933	0.840	0.103	0.160	0.180	0.130
Sp4 x Age 2	354.00	375.00	200.00	158.33	37.00	58.33	3.500	6.000	6.400	6.733	0.400	0.800	0.110	0.147	0.147	0.150
Sig.	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LSD.	-	26.96	-	-	-	-	-	-	-	-	-	-	-	-	-	-

** Highly significant   * Significant   NS- non significant   Sig.: Significant

Species: Sp1: *Eucalyptus citriodora*   Sp2: *Cupressus sempervirens*   Sp3: *Eucalyptus camaldulensis*   Sp4: *Pinus halepensis*

Age: Age 1: 8 years   Age 2: 4 years

Table (4): Mean values of the concentrations of iron, manganese, zinc, copper, lead, nickel, cadmium and chromium (mg kg^{-1} of dry weight) in the stem of the four tree species irrigated by primary treated wastewater at the first season (2009) and the second season (2010)

Treatments	Fe		Mn		Zn		Cu		Pb		Ni		Cd		Cr	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
E. citriodora	388.83	372.66	182.33	172.50	87.33	62.83	13.25	5.900	7.150	6.633	1.393	0.867	0.180	0.158	0.280	0.187
C. sempervirens	381.83	363.66	188.33	164.16	87.83	60.66	13.46	6.617	7.150	6.883	1.490	0.992	0.167	0.202	0.307	0.190
E. camaldulensis	349.66	398.16	221.83	190.33	75.83	77.66	12.35	9.033	7.775	7.233	1.703	0.975	0.133	0.187	0.255	0.158
P. halepensis	385.50	390.66	213.16	186.83	108.16	66.16	14.68	7.767	6.783	7.350	1.775	1.167	0.133	0.170	0.227	0.238
Sig.	NS	*	NS	*	NS	NS	NS	*	NS	NS	NS	NS	*	NS	*	NS
LSD	-	25.56	-	16.47	-	-	-	1.89	-	-	-	-	0.0279	-	0.05	-
Age1	384.75	401.25	172.50	191.33	79.58	73.33	14.16	7.883	7.700	7.233	1.355	1.029	0.153	0.178	0.258	0.214
Age 2	368.16	361.33	230.33	165.58	100.00	60.33	12.70	6.775	6.729	6.817	1.826	0.971	0.154	0.180	0.277	0.173
Sig.	NS	**	**	**	*	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sp1 x Age 1	377.66	382.66	216.00	181.33	99.00	66.66	13.86	6.267	7.833	6.533	1.420	0.900	0.153	0.170	0.223	0.160
Sp1 x Age 2	411.33	385.33	163.66	173.33	85.00	65.00	13.53	6.233	7.933	6.800	1.200	0.850	0.190	0.203	0.273	0.210
Sp2 x Age 1	351.00	416.00	180.00	201.66	56.00	88.33	15.20	9.367	7.200	7.567	1.933	1.000	0.120	0.170	0.260	0.173
Sp2 x Age 2	399.00	421.00	130.33	209.00	78.33	73.33	14.06	9.667	7.833	8.033	0.867	1.367	0.147	0.170	0.273	0.313
Sp3 x Age 1	400.00	362.66	148.66	163.66	75.66	59.00	12.63	5.533	6.467	6.733	1.367	0.833	0.207	0.147	0.337	0.213
Sp3 x Age 2	352.33	342.00	213.00	155.00	90.66	56.33	13.40	7.000	6.367	6.967	1.780	1.133	0.143	0.200	0.340	0.170
Sp4 x Age 1	348.33	380.33	263.66	179.00	95.66	67.00	9.50	8.700	8.350	6.900	1.473	0.950	0.147	0.203	0.250	0.143
Sp4 x Age 2	372.00	360.33	296.00	164.66	138.00	59.00	15.30	5.867	5.733	6.667	2.683	0.967	0.120	0.170	0.180	0.163
Sig.	NS	NS	**	NS	*	NS	NS	NS	NS	NS	*	NS	*	NS	**	*
LSD.	-	-	74.17	-	31.70	-	-	-	-	-	1.08	-	0.039	-	0.07	0.087

** Highly significant * Significant NS- non significant Sig.: Significant

Species: Sp1: *Eucalyptus citriodora* Sp2: *Cupressus sempervirens* Sp3: *Eucalyptus camaldulensis* Sp4: *Pinus halepensis*

Age: Age 1: 8 years Age 2: 4 years

Table (5): Mean values of the concentrations of iron, manganese, zinc, copper, lead, nickel, cadmium and chromium (mg kg^{-1} of dry weight) in the roots of the four tree species irrigated by primary treated wastewater at the first season (2009) and the second season (2010)

Treatments	Fe		Mn		Zn		Cu		Pb		Ni		Cd		Cr	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
E. citriodora	359.16	378.83	258.83	178.66	102.83	59.16	17.20	5.817	7.750	6.783	1.745	0.877	0.153	0.185	0.302	0.148
C. sempervirens	402.66	367.83	177.83	157.33	56.16	66.66	9.850	7.100	7.233	7.117	0.817	1.300	0.130	0.202	0.255	0.205
E. camaldulensis	392.83	406.16	214.83	185.83	85.50	69.33	13.23	8.150	7.417	7.400	1.302	1.117	0.135	0.205	0.235	0.188
P. halepensis	394.00	389.33	225.66	178.66	99.66	59.33	14.28	7.250	7.867	7.200	2.142	1.117	0.173	0.182	0.283	0.190
Sig.	*	NS	NS	NS	**	NS	**	NS	NS	NS	**	NS	*	NS	NS	NS
LSD	26.22	-	-	-	14.46	-	3.32	-	-	-	0.54	-	0.027	-	-	-
Age1	396.91	413.75	188.16	186.41	81.66	71.58	13.33	7.400	7.525	7.458	1.373	1.263	0.148	0.187	0.257	0.201
Age 2	377.41	357.33	250.41	163.83	90.41	55.66	13.95	6.758	7.608	6.792	1.630	0.942	0.148	0.199	0.280	0.165
Sig.	NS	**	*	*	NS	*	NS	NS	NS	*	NS	*	NS	NS	NS	NS
Sp1 x Age 1	359.00	389.00	292.00	173.33	114.66	67.66	18.23	5.633	7.600	6.500	2.490	0.853	0.177	0.160	0.303	0.137
Sp1 x Age 2	417.33	415.66	150.66	172.00	65.66	70.66	10.56	6.700	7.367	6.967	0.833	1.333	0.107	0.197	0.220	0.240
Sp2 x Age 1	417.00	433.00	147.00	196.00	62.00	78.66	10.80	7.833	7.433	7.767	0.800	1.333	0.140	0.187	0.240	0.217
Sp2 x Age 2	394.33	417.33	163.00	204.33	84.33	69.33	13.73	9.433	7.700	8.600	1.367	1.533	0.170	0.207	0.267	0.210
Sp3 x Age 1	359.33	368.66	225.66	184.00	91.00	50.66	16.16	6.000	7.900	7.067	1.000	0.900	0.130	0.210	0.300	0.160
Sp3 x Age 2	388.00	320.00	205.00	142.66	46.66	62.66	9.133	7.500	7.100	7.267	0.800	1.267	0.153	0.207	0.290	0.170
Sp4 x Age 1	368.66	379.33	282.66	175.66	109.00	60.00	15.66	8.467	7.400	7.033	1.803	0.900	0.130	0.223	0.230	0.160
Sp4 x Age 2	393.66	361.33	288.33	153.00	115.00	49.33	14.83	5.067	8.033	5.800	2.917	0.700	0.177	0.157	0.300	0.170
Sig.	NS	NS	*	NS	**	NS	NS	*	NS	**	**	NS	*	NS	NS	NS
LSD.	-	-	84.11	-	20.43	-	-	2.41	-	1.068	0.765	-	0.039	-	-	-

** Highly significant * Significant NS- non significant Sig.: Significant

Species: Sp1: *Eucalyptus citriodora* Sp2: *Cupressus sempervirens* Sp3: *Eucalyptus camaldulensis* Sp4: *Pinus halepensis*

Age: Age 1: 8 years Age 2: 4years

Table (6): Mean values of the concentrations of iron, manganese, zinc, copper, lead, nickel, cadmium and chromium (mg kg^{-1} of dry weight) in the fruits of the four tree species irrigated by primary treated wastewater at the first season (2009) and the second season (2010)

Treatments	Fe		Mn		Zn		Cu		Pb		Ni		Cd		Cr	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
E. citriodora	337.66	394.16	137.33	180.33	87.66	62.83	7.700	5.900	5.333	7.000	1.000	1.500	0.100	0.180	0.230	0.198
C. sempervirens	346.66	372.83	188.66	186.83	93.83	73.83	10.850	7.483	6.733	7.567	1.617	0.967	0.150	0.152	0.225	0.235
E. camaldulensis	350.00	398.50	269.16	198.50	68.33	69.66	5.500	9.467	8.283	8.267	0.683	0.683	0.147	0.157	0.138	0.200
P. halepensis	369.00	432.66	212.00	227.38	92.00	84.11	11.067	8.067	6.667	9.394	3.067	1.117	0.160	0.252	0.167	0.282
Sig.	NS	NS	**	*	**	*	**	**	**	NS	**	*	**	*	**	NS
LSD	-	-	25.7	18.47	5.14	8.51	1.59	0.937	0.74	-	0.115	0.409	0.008	0.038	0.031	-
Age 1	352.66	405.41	187.08	198.68	83.50	72.61	8.875	7.508	7.117	8.165	1.342	1.154	0.140	0.185	0.171	0.234
Age 2	344.66	380.66	258.33	188.00	85.00	68.77	7.983	7.911	6.783	7.467	1.650	0.933	0.147	0.162	0.220	0.204
Sig.	NS	NS	**	NS	*	NS	NS	NS	**	NS	**	NS	NS	NS	**	NS
Sp1 x Age1	337.66	392.33	137.33	179.66	87.66	61.33	7.700	5.333	5.333	7.167	1.000	1.567	0.100	0.197	0.230	0.150
Sp1 x Age 2	349.00	398.00	130.66	187.00	82.66	75.33	10.567	7.067	8.100	7.533	0.567	1.333	0.157	0.163	0.150	0.260
Sp2 x Age 1	355.00	398.66	268.33	200.66	71.66	69.66	6.167	9.567	8.367	8.567	0.733	0.600	0.143	0.130	0.137	0.243
Sp2 x Age 2	369.00	432.66	212.00	227.38	92.00	84.11	11.067	8.067	6.667	9.394	3.067	1.117	0.160	0.252	0.167	0.282
Sp3 x Age 1	.	396.00	.	181.00	.	64.33	.	6.467	.	6.833	.	1.433	.	0.163	.	0.247
Sp3 x Age 2	344.33	347.66	246.66	186.66	105.00	72.33	11.133	7.900	5.367	7.600	2.667	0.600	0.143	0.140	0.300	0.210
Sp4 x Age 1	345.00	398.33	270.00	196.33	65.00	69.66	4.833	9.367	8.200	7.967	0.633	0.767	0.150	0.183	0.140	0.157
Sp4 x Age 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Sig.	NS	NS	**	NS	**	NS	NS	NS	**	NS	**	NS	*	NS	**	NS
LSD.	-	-	36.31	-	7.29	-	-	-	1.048	-	0.161	-	0.012	0.054	0.043	-

** Highly significant * Significant NS- non significant Sig.: Significant

Species: Sp1: *Eucalyptus citriodora* Sp2: *Cupressus sempervirens* Sp3: *Eucalyptus camaldulensis* Sp4: *Pinus halepensis*

Age: Age 1: 8 years Age 2: 4 years

Table (7): Mean values of the total concentrations of iron, manganese, zinc, copper, lead, nickel, cadmium and chromium (mg kg^{-1} of dry weight) in the all parts of the four tree species irrigated by primary treated wastewater at the first season(2009)and the second season(2010)

Treatments	Fe		Mn		Zn		Cu		Pb		Ni		Cd		Cr	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
E. citriodora	316.66	375.70	180.457	149.5	77.25	61.125	11.2	5.827	6.015	6.637	1.182	1.027	0.125	0.178	0.233	0.171
C. sempervirens	378.25	373.41	182.5	168.41	77.332	67.915	10.852	7.282	6.887	7.129	1.197	1.218	0.143	0.198	0.252	0.211
E. camaldulensis	364.20	398.58	222.95	189.08	75.415	72.00	10.232	8.475	7.612	7.533	1.3	0.915	0.135	0.178	0.210	0.188
P. halepensis	339.04	348.41	180.70	164.79	75.415	58.375	8.912	6.607	6.032	6.629	1.507	0.945	0.123	0.158	0.188	0.189
Sig.	**	**	*	NS	NS	*	*	**	**	**	*	*	*	NS	**	*
LSD	19.08	14.22	30.86	-	-	8.987	1.42	0.945	0.435	0.46	0.23	0.207	0.011	-	0.020	0.013
Age1	380.14	410.27	180.60	179.25	78.665	73.332	10.57	7.522	7.215	7.606	1.312	1.204	0.139	0.189	0.225	0.223
Age 2	318.93	337.79	202.70	156.64	74.04	56.375	10.02	6.575	6.057	6.358	1.282	0.849	0.124	0.167	0.216	0.156
Sig.	**	**	NS	NS	NS	**	NS	*	**	**	NS	**	**	*	NS	*
Sp1 x Age 1	360.41	385.33	206.33	128.75	94	65	12.11	5.89	6.69	6.633	1.502	1.096	0.135	0.182	0.240	0.141
Sp1 x Age 2	390.25	416.5	153.91	179.75	76.415	72.665	10.93	7.14	7.6	7.241	0.84	1.437	0.140	0.203	0.221	0.255
Sp2 x Age 1	371.25	416.58	196.83	197.91	65.915	80.582	9.84	8.075	7.55	8.091	1.39	1.008	0.139	0.173	0.220	0.24
Sp2 x Age 2	398.66	422.66	165.33	210.58	78.33	75.08	9.41	8.982	7.025	8.458	1.515	1.275	0.144	0.198	0.219	0.258
Sp3 x Age 1	272.91	366.08	154.58	170.25	60.5	57.25	10.28	5.765	5.34	6.641	0.865	0.958	0.116	0.174	0.226	0.2007
Sp3 x Age 2	366.25	330.33	211.08	157.08	78.25	63.16	10.77	7.42	6.17	7.016	1.55	1.0	0.146	0.194	0.283	0.168
Sp4 x Age 1	357.16	380.58	249.08	180.25	84.91	63.41	10.62	8.87	7.67	6.97	1.21	0.822	0.132	0.184	0.2	0.135
Sp4 x Age 2	279.41	274.16	196.08	119.0	72.5	41.66	8.40	4.23	5.04	4.8	1.5	0.616	0.101	0.118	0.156	0.120
Sig.	**	**	*	*	**	NS	NS	**	**	**	**	*	**	*	**	**
LSD.	26.78	19.96	43.31	51.39	11.03	12.61	-	1.32	0.61	0.645	0.32	0.292	0.016	0.038	0.028	0.018

** Highly significant   * Significant   NS- non significant   Sig.: Significant

Species: Sp1: *Eucalyptus citriodora*   Sp2: *Cupressus sempervirens*   Sp3: *Eucalyptus camaldulensis*   Sp4: *Pinus halepensis*

Age: Age 1: 8 years   Age 2: 4 years

Table (8): Mean values of the available of iron, manganese, zinc, copper, lead, nickel, cadmium and chromium (mg kg^{-1}) in the soil depth at (0-2°cm) of the four tree species irrigated by primary treated wastewater at the first season (2009) and the second season (2010)

Treatments	Fe		Mn		Zn		Cu		Pb		Ni		Cd		Cr	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
E. citriodora	2.237	12.50	1.940	10.50	1.005	3.425	0.263	1.185	2.885	3.700	0.215	0.690	0.138	0.720	0.187	0.642
C. sempervirens	6.077	9.475	2.718	9.100	1.140	3.100	0.580	1.470	2.247	3.525	0.397	0.667	0.222	0.507	0.185	0.625
E. camaldulensis	6.327	13.31	3.917	8.933	1.358	3.450	0.635	1.598	2.595	3.750	0.267	0.815	0.210	0.710	0.200	0.625
P. halepensis	2.825	9.925	2.530	7.833	1.275	4.000	0.330	1.578	2.915	3.500	0.235	0.712	0.160	0.492	0.225	0.460
Sig.	**	**	**	**	**	**	**	**	**	NS	**	NS	**	**	*	**
LSD	0.244	1.632	0.938	1.08	0.031	0.207	0.019	0.106	0.078	-	0.021	-	0.02	0.079	0.028	0.05
Age1	2.912	9.783	2.805	8.942	1.425	3.238	0.337	1.451	2.973	3.838	0.353	6.862	0.155	0.544	0.196	0.581
Age 2	5.821	12.82	2.747	9.242	0.964	3.750	0.568	1.465	2.348	3.400	0.203	0.787	0.210	0.670	0.203	0.595
Sig.	**	**	NS	NS	**	**	**	NS	**	**	**	NS	**	**	NS	NS
Sp1 x Age 1	3.463	13.70	3.450	11.70	1.840	3.750	0.407	1.160	3.620	4.300	0.270	0.563	0.127	0.630	0.193	0.573
Sp1 x Age 2	2.873	8.250	2.480	7.900	1.270	2.700	0.310	1.590	2.743	3.850	0.613	0.573	0.173	0.383	0.170	0.630
Sp2 x Age 1	3.580	10.23	3.620	10.10	1.740	3.100	0.440	2.027	3.540	3.800	0.320	0.810	0.170	0.710	0.240	0.710
Sp2 x Age 2	1.730	6.950	1.670	6.067	0.850	3.400	0.190	1.027	1.990	3.400	0.210	25.50	0.150	0.453	0.180	0.410
Sp3 x Age 1	1.010	11.30	.430	9.300	0.170	3.100	0.120	1.210	2.150	3.100	0.160	0.817	0.150	0.810	0.180	0.710
Sp3 x Age 2	9.280	10.70	2.957	10.30	1.010	3.500	0.850	1.350	1.750	3.200	0.180	0.760	0.270	0.630	0.200	0.620
Sp4 x Age 1	9.073	16.40	4.213	7.767	0.977	3.800	0.830	1.170	1.650	3.700	0.213	0.820	0.250	0.710	0.160	0.540
Sp4 x Age 2	3.920	12.90	3.390	9.600	1.700	4.600	0.470	2.130	3.840	3.600	0.260	0.750	0.170	0.530	0.270	0.510
Sig.	**	**	**	**	**	**	**	**	**	**	**	NS	**	*	**	**
LSD.	0.343	2.290	1.31	1.52	0.043	0.290	0.026	0.150	0.109	0.311	0.030	-	0.028	0.112	0.040	0.071

** Highly significant   * Significant   NS- non significant   Sig.: Significant

Species: Sp1: *Eucalyptus citriodora*   Sp2: *Cupressus sempervirens*   Sp3: *Eucalyptus camaldulensis*   Sp4: *Pinus halepensis*

Age: Age 1: 8 years   Age 2: 4 years

Table (9): Mean values of the available of iron, manganese, zinc, copper, lead, nickel, cadmium and chromium (mg kg^{-1}) in the soil depth at (2°-50cm) of the four tree species irrigated by primary treated wastewater at the first season (2009) and the second season (2010)

Treatments	Fe		Mn		Zn		Cu		Pb		Ni		Cd		Cr	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
E. citriodora	5.460	13.28	3.305	10.26	1.148	3.750	0.278	1.263	3.003	3.800	0.198	0.710	0.132	0.750	0.205	0.650
C. sempervirens	6.938	9.750	3.735	9.675	1.332	2.983	0.663	1.338	2.388	3.700	0.413	0.660	0.262	0.607	0.202	0.657
E. camaldulensis	6.627	13.20	4.025	9.183	1.485	3.400	0.660	1.692	2.305	3.750	0.367	0.865	0.215	0.780	0.160	0.635
P. halepensis	2.380	10.30	1.983	8.100	1.400	3.983	0.290	1.612	2.305	3.525	0.247	0.800	0.153	0.580	0.135	0.533
Sig.	**	**	**	**	**	**	**	**	NS	NS	**	**	**	**	**	**
LSD	0.249	0.958	0.210	1.009	0.110	0.209	0.037	0.152	-	-	0.059	0.039	0.020	0.094	0.018	0.050
Age1	3.030	10.04	2.747	9.246	1.485	3.267	0.333	1.480	3.001	3.837	0.372	0.710	0.146	0.646	0.173	0.628
Age 2	7.673	13.22	3.778	9.367	1.198	3.792	0.612	1.472	2.000	3.550	0.241	0.808	0.235	0.713	0.177	0.610
Sig.	**	**	**	NS	**	**	**	NS	**	*	**	**	**	NS	NS	NS
Sp1 x Age 1	3.570	14.36	3.460	10.93	1.917	3.900	0.437	1.217	3.837	4.000	0.207	0.570	0.133	0.670	0.220	0.570
Sp1 x Age 2	3.227	8.700	2.530	9.150	1.253	2.800	0.317	1.507	2.917	4.000	0.617	0.610	0.163	0.563	0.183	0.663
Sp2 x Age 1	3.013	10.10	3.180	10.50	1.620	3.200	0.350	2.133	3.150	3.700	0.420	0.820	0.160	0.810	0.150	0.760
Sp2 x Age 2	2.310	7.000	1.817	6.400	1.150	3.167	0.230	1.063	2.100	3.650	0.243	0.840	0.127	0.540	0.140	0.517
Sp3 x Age 1	7.350	12.20	3.150	9.600	0.380	3.600	0.120	1.310	2.170	3.600	0.190	0.850	0.130	0.830	0.190	0.730
Sp3 x Age 2	10.65	10.80	4.940	10.20	1.410	3.167	1.010	1.170	1.860	3.400	0.210	0.710	0.360	0.650	0.220	0.650
Sp4 x Age 1	10.24	16.30	4.870	7.867	1.350	3.600	0.970	1.250	1.460	3.800	0.313	0.910	0.270	0.750	0.170	0.510
Sp4 x Age 2	2.450	13.60	2.150	9.800	1.650	4.800	0.350	2.160	2.510	3.400	0.250	0.760	0.180	0.620	0.130	0.550
Sig.	**	**	**	**	**	**	**	**	*	NS	**	**	**	NS	*	**
LSD.	0.350	1.345	0.295	1.416	0.155	0.293	0.053	0.214	1.014	-	0.083	0.055	0.028	-	0.026	0.070

** Highly significant * Significant NS- non significant Sig.: Significant

Species: Sp1: *Eucalyptus citriodora* Sp2: *Cupressus sempervirens* Sp3: *Eucalyptus camaldulensis* Sp4: *Pinus halepensis*

Age: Age1: 8 years Age 2: 4 years

CONCLUSION

Results showed that the concentration of heavy metals in trees and two soil depths varied according to species.

Generally, concentrations of Fe, Mn, Zn and Pb in trees and soil depths were higher than that of Cu, Ni, Cd and Cr. Also, significant difference was observed among the trees species, trees aged and soil depths for heavy metals concentrations. Hence, the older trees had high accumulation capacity than the younger trees, may be the huge of vegetative growth of the old trees.

Eucalyptus camaldulensis trees had higher hyper accumulating capacity to the concentrations of Fe, Zn, Cu and Pb, followed by other tree species, and it gave the highest Fe, Mn and Cu in the stem, especially in the second season. While, *Cupressus sempervirens* trees had hyper accumulating capacity to the concentrations of Ni, Cd and Cr, in the second season. As well as, it gave the highest Cr conc. in the stem, in the first season. However, *Eucalyptus citriodora* trees gave the highest Cd conc. in the stem and it gave the highest Zn and Cu conc. in the roots, especially in the first season as compared with other tree species.

It is pointed out that roots contained more heavy metals than leaves, stems and fruits. Plant roots are reported to contribute in mobilization of nutrients (Huang, 1997). Also, the species which had high concentration of heavy metals in the stem and root recommended for phytoremediation because, it has a difficult analysis of heavy metals.

On the other hand, in the first season, *Eucalyptus citriodora* trees gave the lowest available conc. of Zn, Cu, Ni and Cd in the two soil depths. However, in the second season, *Pinus halepensis* trees gave the lowest available conc. of Mn, Cd and Cr in the two soil depths, followed by the other tree species. So, the concentrations of available heavy metals DTPA metal as shown by the study are contents lower than level recommended by the National Academy of Sciences and National Academy of Engineering, (1972).

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المعالجة النباتية للمعادن الثقيلة بواسطة أربعة أنواع شجرية عالية التراكم تروى بمياه الصرف الصحى والصناعى المعالج، ١) تركيز المعادن الثقيلة فى أجزاء الأشجار وأعماق التربة

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أجريت هذه التجربة خلال عامين٢٠٠٩ ،٢٠١٠ بغابة سرابيوم – الأسماعيلية – مصر. لدراسة الأنواع الشجرية عالية التراكم للحديد والمنجنيز والزنك والنحاس والرصاص والنيكل والكادميوم والكروم وهى :-

الكافور الليمونى والكافور البلدى والسرو والصنوبر الحلبى. وذلك عند عمرى (٤ و٨ سنوات) وعند عمقين للتربة (٠- ٢٥ سم) ،(٢٥- ٥٠ سم). والتى تروى بطريقة الرى بالتنقيط بمياه الصرف المعالجة أوليا منذ بداية زراعة الأشجار من ٤و٨ سنوات بالغابة.

وقد أوضحت النتائج الأتى :-

لوحظ أن تركيز المعادن الثقيلة فى أجزاء الأشجار وفى عمقى التربة يختلف تبعا للأنواع الشجرية. كما لوحظ وجود أختلافات معنوية بين الأنواع الشجرية وأعمار الأشجار وأعماق التربة. وقد كانت الأشجار الأكبر عمرا أكثر قدرة على التراكم عن الأشجار الأصغر عمرا.

فى السنة الأولى أظهرت النتائج أن أشجار الكافور البلدى ذات سعة تراكم عالية للتركيز الكلى من المنجنيز والرصاص(٢٢٢,٩٥ و٧,٦١ ملليجرام/كيلوجرام على التوالى) بينما أشجار السرو أعطت أعلى تركيز من الكروم فى الساق(٠,٣٠٧ ملليجرام /كيلوجرام) وأعلى تركيز للحديد فى الجذور(٤٠٢,٦٦ مللىجرام/كيلو جرام) بالمقارنة مع باقى الأنواع. أما أشجار الكافور الليمونى فقد اعطت أعلى تركيزللكادميوم فى الساق(٠,١٨ ملليجرام/كيلو جرام) واعلى تركيز للزنك والنحاس فى الجذور (١٠٢,٨٣و١٧,٢٠ ملليجرام/كيلو جرام على التوالى) هذا بالأضافة الى أن أشجار الكافورالليمونى أعطت أقل تركيز متاح من الزنك والنحاس والنيكل والكادميوم خلال عمقى التربة.

بينما فى السنة الثانية فقد وجد أن أشجار الكافور البلدى ذات سعة تراكم عالية للتركيز الكلى من الحديد والزنك والنحاس والرصاص (٣٩٨,٥٨، ٧٢,٠٠، ٨,٤٧٥، ٧,٥٣٣ ملليجرام /كيلوجرام ،على التوالى) وقد أعطى هذا النوع أيضا أعلى تركيز للحديد والمنجنيز والنحاس فى الساق(٣٩٨,١٦، ١٩٠,٣٣ و٩,٠٣٣ ملليجرام /كيلو جرام ، على التوالى). بينما أشجار السرو كانت لها سعة تراكم عالية للتركيز الكلى من النيكل والكادميوم والكروم (١,٢١٨، ٠,١٩٨، ٠,٢١١ ملليجرام / كيلوجرام ،على التوالى) ومن ناحية أخرى فقد أعطت أشجار الصنوبر الحلبى أقل تركيز متاح للمنجنيز والكادميوم والكروم خلال عمقى التربة.

ومن النتائج السابقة يوصى بزراعة كل من الكافور البلدى فى المعالجة النباتية للتخلص من التركيزات العالية من الحديد والمنجنيز والزنك والنحاس والرصاص.،والسرو للتخلص من التركيزات العالية من الحديد والنيكل والكادميوم والكروم،والكافور الليمونى للتخلص من التركيزات العالية من الزنك والنحاس والنيكل والكادميوم ،والصنوبر الحلبى للتخلص من التركيزات العالية من المنجنيز والكادميوم والكروم فى التربة التى تروى بمياه الصرف المعالجة أوليا.