Carpathian Journal of Earth and Environmental Sciences, February 2014, Vol. 9, No. 1, p. 107 - 116

PHYTO-EXTRACTION OF MUNICIPAL WASTEWATER'S AND APPLIED SOLUTION OF COPPER, LEAD AND ZINC, USING HIGH BIO-MASS CROPS, *ZEA MAYS* AND *BRASSICA NAPUS*

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Abstract: Heavy metal pollution is one of the major global concerns which require urgent attention. Considering this, pot experiments were designed to assess the phyto-extraction ability of *Zea mays* and *Brassica napus* plants grown in soils irrigated with municipal wastewater and amended with varying levels of copper, lead and zinc at Faculty of agriculture, Gomal University, Dera Ismail Khan, Pakistan. In this study, four treatments such as tap water, municipal wastewater, 300 mg kg^{-1} soil each of Cu, Pb and Zn (medium toxic), and 600 mg kg^{-1} soil each of Cu, Pb and Zn (high toxic) were used in completely randomized design with three replications. The salt solutions of Cu (copper sulphate), Pb (lead acetate trihydrate) and Zn (zinc sulphate) were added as post-emergence application to the respective pots. The maize and brassica plants were harvested at 42 days of life, and uptake of heavy metals by shoot and root tissues were analyzed. In addition, the dry matter yield as affected by the various treatments was determined. Maize and brassica plants receiving wastewater accumulated maximum heavy metals and excelled the medium and high toxic solutions of 300 and 600 mg kg^{-1} soil of each metal, respectively. Among plant organs, shoots of both *Z. mays* and *B. napus* in general, accumulated two to four times more heavy metals compared to roots. Maize due to its vigorous growth accumulated two times more heavy metals than brassica. Apart from control, the dry matter yields were maximum in both maize and brassica plants treated with municipal wastewater followed by medium toxic metal solutions. However, high toxic metal solution revealed minimum dry matter yield in both crops. Results suggested that *Zea mays* and *Brassica napus* could be used as viable phyto-extractors for heavy metals.

Keywords: Phytoremediation, heavy metals, Cu, Pb & Zn, wastewater, pollution, high biomass crops, *Z. mays, B. napus*

1. INTRODUCTION

The valuable land and water resources are continuously under stress, exploited, degraded and over polluted due to anthropogenic activities. Pollution especially heavy metal poisoning results from emissions, effluents and solid discharge from industries and municipalities, vehicular exhaust, mining and smelting or excessive use of fertilizers and pesticides (McGrath et al., 2001; Gisbert et al., 2003; Knezevic et al., 2009; Ahmad et al., 2011; Khan et al., 2012). Heavy metals on one hand are important constituents for plants and humans when present only in small amounts; while such elements on the contrary may also be toxic to both animals and humans at higher concentrations. However, there are few trace elements such as arsenic, cadmium, mercury and lead which are toxic even at small concentrations (Reeves, 2003; Ragnarsdottir & Hawkins, 2005; Divrikli et al., 2006). Among metals, the concentrations of copper and lead is constantly increasing in the environment due to human activities (Lăcătușu et al., 2007; Mihali et al., 2013), and at high concentration, these metals can cause severe damage to physiological and biochemical activities of plants (Nicholls & Mal, 2003; Marchiol et al., 2004; Perveen et al., 2011; Wani et al., 2012; Truta et al., 2013).

Heavy metals viz., Cu, Pb, Zn, Cr and Hg have been identified in the polluted environments, and the negative effects of the atmospheric emissions on soils, vegetation, water and animals have been observed (Lăcătușu & Lăcătușu, 2008; Secu et al., 2008). Soils and vegetables were heavily tainted, and in soil, the heavy metals ranging between 9.1-2593 (Cu), 1.1-27 (Cd), 83-8040 (Pb) and 60-11445 mg kg^{-1} (Zn), while in vegetables the said ranges were 5.8-196, 0.3-5.2, 0.5-15 and 21-126 mg kg^{-1}, respectively and the plants showed retention of heavy metals in the soil (Big et al., 2012). The clinical course of the copper sulphate intoxicated patient is often complex involving intravascular hemolysis, jaundice and renal failure (Kavitha et al., 2007). Acute gastrointestinal and respiratory damages in human being are associated with Zn toxicity. Lead is a toxic metal with a cumulative effect which competes with the essential metals in the human body (Ca, Fe, Cu, and Zn) during physiological processes of bone tissues remodeling. Part of the Pb (II) ions by migration through oral and other biological fluids; reach other remote organs, like brain, kidneys, and the liver (Pocock et al., 1994; Vig & Hu, 2000). Exceeded amounts of heavy metals disturb metabolic mechanism in plants i.e. the bio-physico-chemical processes (Garbisu & Alkorta, 2001; Schmidt, 2003; Khan et al., 2012). Heavy metals may also cause changes in the soil reactions by inhibiting soil microbial community, and adversely affect the soil characteristics (Giller et al., 1998; Kozdroj & Elsas, 2001; Kurek & Bollag, 2004).

Heavy metals in general, are non-destructible and therefore, persist in the environment (Tandi et al., 2004). The remediation of metal-contaminated environment including soils thus becomes extremely important since polluted soils around the globe cover large areas that have become unsuitable for agronomic production. In the three neighbouring countries viz., Pakistan, India, and Bangladesh, the soil and water pollution ascended and on risk where untreated industrial and sewage water reaches the farm areas in covered/un-covered drains irrigating the crops (Lone et al., 2008). In this context, physicochemical methods such as immobilizations or extraction have been attempted but have been found expensive and are often recommended only for small areas where the rapid and complete decontamination is required (Martin & Bardos, 1996; Gadd, 2008).

Physicochemical measures are the excavations and burial of soil materials at a hazardous waste site, tying up and immobilizing the metals, leaching down and return of clean soil back to the site (Salt et al., 1995). Physicochemical remediation of heavy metals polluted soils; especially the larger areas are beyond the access of poor community due to huge expenses and the reclamation side effects (Martin & Bardos 1996; Raskin et al., 1997; Nriagu & Pacyna, 1988; Schalscha & Ahmuda, 1998; McGrath et al., 2001). Biological remediation is the use of special type of green plants that inactivate the metals, translocate them to the above ground portion and decontaminate the soil (Chaney et al., 1997; Lombi et al., 2001; Chen et al., 2004). The physicochemical remediation approach is though quick but costly, i.e. the cleaning of 0.4 ha of lead polluted soil by excavation and landfill, the estimated cost is US$ 500, while through phytoremediation (extraction, harvest and disposal) which is time consuming, however, costs US$150 with 50-65% saving with same piece of land (Blaylock et al., 1997; Garbisu & Alkorta, 2001; Gisbert et al., 2003).

Phytoremediation is alternative approach used to clean up the contaminated soils using plants and has gained considerable attention around the world (Pilon-Smits, 2005; Turan & Esringu, 2007; Zhixin et al., 2009). Phytoremediation i.e. *phytofiltration*- removal of metals by roots or seedling's from aqueous wastes; *phytostabilization*- pollutants absorption by plant roots and to keep them in the rhizosphere; *phytovolatization*- to volatilize pollutants by plants such as Se and Hg; *phytodegradation*- plants and associated microorganisms to degrade and *phytoextraction*- the accumulation of metals in the plant's upper ground portion and the plant parts removed, disposed off or burnt, recovering metals, is the use of green plants depending upon the mechanisms of remediation (Schnoor, 1997; Salt et al., 1998; Schmidt, 2003; Nascimento & Xing, 2006). These techniques are considered newer and highly promising for reclaiming polluted sites and are cheaper than physicochemical ones (Raskin et al., 1997; Garbisu & Alkorta, 2001; McGrath et al., 2001; Marchiol et al., 2004).

More than 400 species are metal hyper-accumulating plant (capability>1000 mg kg^{-1} dw) (Reeves, 2003) contributing less than 0.2% of all the angiosperms (Baker & Walker, 1990). The species i.e. *Malva aegyptiaca* for Cd, *Frankenia thymifolia* for Zn, *Peganum harmala* for Cu and *Cittrulus* sp. for Sr were found most effective in the translocation of these heavy metals, however, the absorption of Cr and Ni was very low (Galfati et al., 2011). The species *Rumex acetosela, Solanum nigrum, Chenopodium album, Aster pannonicum* and *Phragmytes australis* in descending order accumulated the heavy metals in their aerial parts i.e. Cu (140 mg kg^{-1}), Zn (5213), Cd (270 mg kg^{-1}) and Zn (10128 mg kg^{-1}) (Lăcătușu et al., 2012).

Brassica napus L. and other species of brassica are moderately higher accumulators of Cu and Zn (Ebbs et al., 1997). *Brassica napus* is moderately tolerant, grown on multi-metal contaminated soil (Marchiol et al., 2004). *Zea mays* L. has high tolerance capacity to heavy metals and can be used as remediator (Schmidt, 2003; Pilon-Smits, 2005). Maize is a quick, vigorous and tall (2-3 m) plant growing with broad (5-10 cm) and long (50-100 cm) leaves, has the capability of phytoextraction. In maize, the roots translocate the metals to shoots (Nascimento & Xing, 2006) and grouped as an accumulator and a metal tolerant plant especially for Cd and Zn (Mathe-Gaspar & Anton, 2005), and that is why the accumulation of Pb above certain limits by maize plants has defined maize as hyper-accumulator.

In order to maintain sustainable, economically feasible and contamination free good quality soil and water environment, the present study was planned to determine the phyto-extraction ability of *Zea mays* and *Brassica napus* irrigated with metal containing municipal wastewater and soils amended differently with varying levels of Cu, Pb and Zn.

2. MATERIALS AND METHODS

2.1. Experimental design and procedure

Pot experiments were carried out in a complete randomized design (CRD) with three replications at Faculty of agriculture, Gomal University, Dera Ismail Khan, Pakistan. Dera Ismail Khan lies between 31°, 49′ North latitude and 70°, 55′ East longitude. A bulk soil (sandy loam textured alluvial soil) was collected from the *Indus River* bank and was passed through 2 mm sieve, then washed with distilled water and dried after removing the weeds/crops fibrous roots and residues. Poly vinyl chloride (PVC) made pots were used having volume of 1050 cm^3 and were filled with three kg air dried and sterilized soil. Four treatments included were - (i) T_1 (tap water) (ii) T_2 (municipal wastewater) (iii) 300 mg kg^{-1} soil each of Cu, Pb and Zn (T_3) and (iv) 600 mg kg^{-1} soil each of Cu, Pb and Zn (T_4). The heavy metals were applied in the form of copper sulphate ($CuSO_4$), lead acetate trihydrate ($PbCH_3OO$) and zinc sulphate ($ZnSO_4$) (with 99% purity) by dissolving the calculated amount in one liter of water and that solution applied as post-emergence of seedling in the PVC pots. The ammonium sulphate, orthophosphoric acid and potassium sulphate were used as basal dose of Nitrogen (N), Phosphorus (P) and Potassium (K) i.e. NPK @ 200:300:400 mg kg^{-1} of soil, respectively.

2.2. Physico-chemical properties and heavy metals of wastewater, tap water and soil

Physico-chemical properties and heavy metals of wastewater, tap water, and NEQS (National Environmental Quality Standards) are provided in table 1. The characteristics of wastewater i.e. pH, electrical conductivity (EC_e) and sodium adsorption ratio (SAR) were in the range of 10.31, 3.87 and 36.23, respectively showing high salinity status.

Table 1. Physico-chemical properties of municipal wastewater, tap water and NEQS

Parameters	Measure unit	Municipal wastewater	Tap water	NEQS
pH	-	10.31	8.01	6 to 9
EC	dSm^{-1}	3.87	2.07	Nil
SAR	-	36.23	9.33	NIL
COD	mgL^{-1}	1589	89	400
BOD	mgL^{-1}	850	57	250
Grease + oil	-	14.30	Nil	10
CO_3	mgL^{-1}	2.53	0.51	NGVS
HCO_3	mgL^{-1}	11.19	2.80	NGVS
Cl^{-1}	mgL^{-1}	2631	213	1000
Cu	mgL^{-1}	1.70	Nil	1
Fe	mgL^{-1}	15.35	1.73	8
Zn	mgL^{-1}	5.83	0.09	5
Mn	mgL^{-1}	2.21	0.06	1.50
Ni	mgL^{-1}	2.55	Nil	1
Cd	mgL^{-1}	0.41	Nil	0.10
Pb	mgL^{-1}	2.19	Nil	0.50
Cr	mgL-1	1.69	Nil	1
Sulphate	mgL^{-1}	1435	135	1000
Phosphorus	mgL^{-1}	26	1.12	15
NO_3-N	mgL^{-1}	87.20	5.27	Nil

NEQS-National environmental quality standards; NVGS-no guidelines value standards; EC-electrical conductivity; SAR-sodium adsorption; COD-chemical oxygen demand; BOD-biochemical oxygen demand

Table 2. Physico-chemical properties and heavy metal contents of the soil used in pots

Sand (%)	Silt (%)	Clay (%)	pH	EC_e (dS m^{-1})	OM (%)	$CaCO_3$ (mg kg^{-1})
24.13	59.42	16.45	7.47	1.31	0.003	17.00
Cu (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Pb (mg kg^{-1})	Ni (mg kg^{-1})	Cr (mg kg^{-1})
0.67	4.00	0.87	3.40	1.87	1.52	2.30
OM-organic matter						

The heavy metals found in wastewater were Cu, 1.70; Fe, 15.53; Zn, 5.83; Mn, 2.21; Ni, 2.55; Cd, 0.41; Pb, 2.19 and Cr, 1.69 mg L^{-1} of wastewater (Table 1). The physico-chemical properties and heavy metals of the soil used in pots are given in table 2. The soil contained 24.13% sand, 59.42% silt and 16.45% clay particles defining the soil texture as sandy silt loam, while the soil pH and EC values were 7.47 and 1.31, respectively (Table 2). The heavy metals contained in the soil were Cu, 0.67; Fe, 4.00; Mn, 0.87; Zn, 3.40; Pb, 1.87; Ni, 1.52 and Cr, 2.30 mg kg^{-1} of soil.

Five healthy seeds of maize and brassica were sown in pots. After two weeks of germination, the plants were thinned to one plant per pot and maintained. The pots having treatments T_1 (control) and T_2 were irrigated with tap water and wastewater, respectively on regular basis whereas, the pots with medium (T_3) and high toxicity (T_4) solutions were irrigated with distilled water regularly and the moisture level was maintained at field capacity i.e. water content held at potential -33 J/kg throughout the growth period. The experiment was conducted in complete randomized design (CRD) in fenced research area and in an open prevailing temperature and there were three replications and four treatments. The experiment was repeated three times.

At 42 days of life, the maize and brassica plants were harvested and moved to the laboratory. Plants were separated into shoots and roots, gently washed three times with distilled water to remove the dust and air-dried. After air-drying, the plant organs were oven dried (70°C) for 48 h for complete removal of moisture. After drying, the dry matter yields in both crops were determined for all the treatments. The shoot and root of both crops were ground manually using pestle and mortar.

2.3. Metal concentration

Wet digestion procedure (AOAC, 1990) was followed using 0.5 gram oven dried tissues samples of maize and brassica shoot and root treated with 10 mL concentrated HNO_3 and kept over night. The digested solution was mixed with 4 mL $HClO_4$ and the mixture was digested in block digester using hot plate. In the digested samples of shoot and root tissues of maize and brassica after dilution, the heavy metals were determined with the help of Atomic Absorption Spectrophotometer (Buck Scientific Accusys-211, Germany) using their respective standards for quality control.

2.4. Traits measurement and statistical analysis

The accumulation of Cu, Pb and Zn in maize and brassica shoot and root tissues (mg kg^{-1}), and dry matter yield (g $plant^{-1}$) were recorded. The data were subjected to analysis of variance (ANOVA) appropriate for CRD to compare the mean differences as outlined by Steel & Torrie (1980). LSD test with 1% level of probability was applied for means separation and further comparison.

3. RESULTS

3.1. Heavy metals uptake by *Zea mays*

Heavy metals uptake by maize shoot and root tissues varied significantly ($p \leq 0.01$) among different irrigation water and metals added toxic solutions (Table 3). Results showed that uptake of heavy metals by shoot and root tissues were highest in plants receiving municipal wastewater as compared to the other treatments (Table 4, Fig. 1). Maize irrigated with municipal wastewater accumulated 164.2 259.6 and 271.3 mg kg^{-1} Cu, Pb and Zn, respectively in shoots which was 10, 55 and 14 times higher than control (tap water) followed by medium (T_3) and high toxic (T_4) solutions (Table 4, Fig. 1). Due to harmful and inhibiting effect, the ratio of heavy metals accumulation in maize shoots was much reduced in presence of high toxic than medium toxic solutions i.e. Cu (151 and 175.2 mg kg^{-1}), Pb (107.20 and 119.7 mg kg^{-1}) and Zn (221.2 and 247.5 mg kg^{-1}), respectively. The analysis of maize roots showed that Cu was eight times more in municipal wastewater (26.8 mg kg^{-1}) treated plants relative to control (3.5 mg kg^{-1}), followed by medium toxic solution (22.6 mg kg^{-1}) (Table 4, Fig. 1).

Table 3. Mean squares for heavy metals in *Zea mays* and *Brassica napus* shoot and root tissues

Crop	Plant organs	Heavy metals	Mean Squares			CV %
			Reps.	Treatments	Error	
		D.F.	2	3	6	
Zea mays L.	Shoot	Cu	553.39	16607.27**	554.51	18.60
		Pb	2.606	32916.02**	1.356	0.95
		Zn	0.518	40052.33**	0.013	0.06
	Root	Cu	0.294	301.38**	0.368	3.41
		Pb	11.005	8144.59**	21.203	9.26
		Zn	0.069	1448.32**	0.555	2.03
Brassica napus L.	Shoot	Cu	0.519	3938.62**	0.748	1.18
		Pb	0.194	18546.44**	0.205	0.60
		Zn	1.49	15527.64**	0.282	0.53
	Root	Cu	0.106	681.47**	0.699	3.67
		Pb	1.01	3664.99**	0.143	1.21
		Zn	1.27	1115.31**	0.056	0.84
Dry matter (Maize)	-	-	22.099	417.28**	32.262	7.21
Dry matter (Brassica)	-	-	0.328	691.86**	0.869	1.99

** Significant at $p \leq 0.01$

Table 4. Uptake of heavy metals by shoot and root tissues of *Zea mays*

Treatments	Shoot (mg kg^{-1})			Root (mg kg^{-1})		
	Cu	Pb	Zn	Cu	Pb	Zn
Tap water (control)	16.0	4.7	19.2	3.5	2.3	4.1
Municipal wastewater	164.2	259.6	271.3	26.8	130.5	61.1
Medium toxic solution	175.2	119.7	247.5	22.6	69.6	73.3
High toxic solution	151.0	107.2	221.2	18.0	46.6	66.6
$LSD_{0.01}$	61.73	3.05	0.298	1.59	5.095	0.87

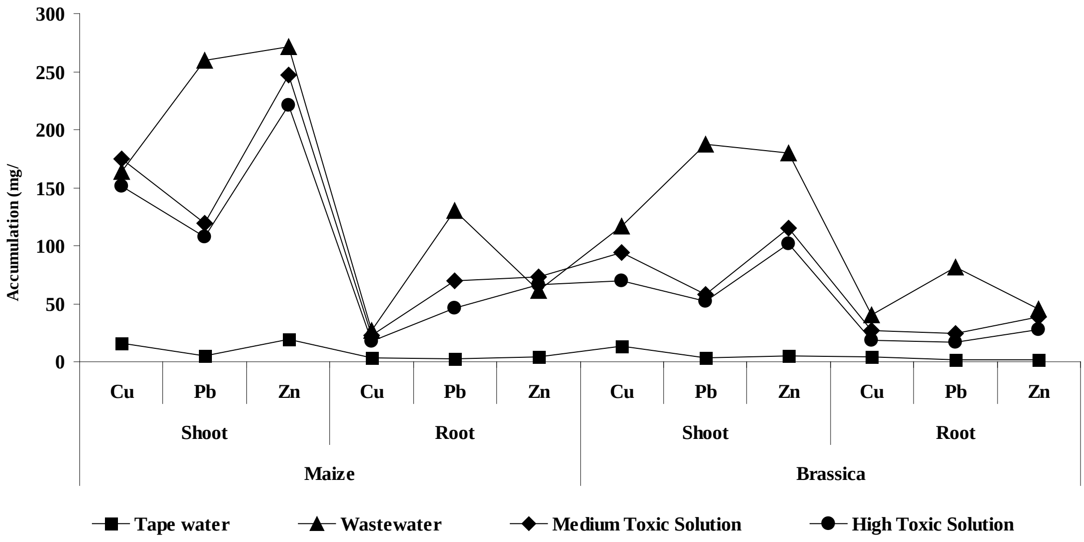

Figure 1. Heavy metal accumulation in *Zea mays* and *Brassica napus* shoot and root tissues

Table 5. Uptake of heavy metals by shoot and root tissues of *Brassica napus*

Treatments	Shoot (mg kg^{-1})			Root (mg kg^{-1})		
	Cu	Pb	Zn	Cu	Pb	Zn
Tap water (control)	13.1	3.5	5.1	4.5	1.7	1.6
Municipal wastewater	116.6	187.3	179.9	40.3	81.7	45.4
Medium toxic solution	93.9	57.7	115.5	27.2	24.6	38.9
High toxic solution	69.4	52.1	101.7	18.5	17.2	27.9
$LSD_{0.01}$	2.67	1.187	1.392	2.176	0.99	0.62

Table 6. Effects of tap water, municipal wastewater and medium and high toxic solutions on dry matter yields of *Zea mays* and *Brassica napus*

Treatments	*Zea mays* (g $plant^{-1}$)	*Brassica napus* (g $plant^{-1}$)
Tap water (control)	80.4	50.6
Municipal wastewater	94.6	66.3
Medium toxic solution	74.2	38.7
High toxic solution	66.3	31.6
$LSD_{0.01}$	14.60	2.44

Whereas, the Pb concentration in maize roots irrigated with wastewater reached to the level of 130.5 mg kg^{-1} and was 57 times greater than those accumulated in tap water irrigated plants (2.3 mg kg^{-1}). However, in maize roots with high (69.6 mg kg^{-1}) and medium toxic solutions (46.6 mg kg^{-1}), the Pb accumulation was 30 and 20 times more respectively as compared to control (2.3 mg kg^{-1}). The maize roots efficiently absorbed Zn to the extent of 61.1, 73.3 and 66.6 mg kg^{-1} in wastewater, medium and high toxic solutions against control (4.1 mg kg^{-1}), and showed 15, 18 and 16 times more buildup of Zn as compared to tap water. The maize roots showed higher uptake of Zn in medium (73.3 mg kg^{-1}) followed by high toxic solution (66.6 mg kg^{-1}) and wastewater (61.1 mg kg^{-1}).

4. DISCUSSIONS

Generally, the heavy metals accumulation was maximum in both plant species receiving municipal wastewater than rest of solutions in shoots followed by roots, which might be due to maximum ratio of heavy metals in municipal wastewater. In maize, the roots formed above the ground level, translocate the metals from roots to shoots (Nascimento & Xing, 2006). Mathe-Gaspar & Anton (2005) also grouped the maize an accumulator and a heavy metal tolerant plant especially for Cd and Zn. Several maize inbred lines have been identified, which accumulated high levels of Cd, however, these lines were susceptible to Zn toxicity and, therefore, could not be used to cleanup soils at the normal Zn:Cd ratio of 100:1 (Hinesly et al., 1978; Chaney et al., 1999). Brewer et al., (1997) generated somatic hybrids between *T. caerulescens* (a Zn hyperaccumulator) and *B. napus* (canola), followed by hybrid selection for Zn tolerance, and high biomass hybrids with superior Zn tolerance were recovered.

The irrigation water with medium toxic solution and municipal wastewater provide more chances to uptake enhanced heavy metals by both species, however, in extreme high toxic solution, the metals uptake was less which might be due to adverse toxic effects of heavy metals on physiological processes. In soil, and *B. napus* foliage and seed, the Pb, Cd, and Cr concentrations enhanced consistently with increased level of sewage water (Ahmad et al., 2011). Heavy metals cause changes in the soil reactions by inhibiting soil microbial community, which adversely affect the soil properties and plant health (Giller et al., 1998; Kozdroj & Elsas, 2001; Kurek & Bollag, 2004). In the present study, the enhanced deposition of Zn was observed in maize roots with medium and high toxic solutions followed by municipal wastewater. However, the maize plants irrigated with wastewater were leading in uptake of Cu and Pb in shoot and root tissues while high toxic solution was on the bottom. Corn inbred lines differ in accumulation of Zn and Cd in leaves and grain, while the uptake of Zn was relatively high in leaf. However, grain tissue did not always accumulate high concentrations of Cd, and absorption of Zn and Cd appeared to be independent each other (Hinesly et al., 1978). In past studies, the uptake ability of plants varies with toxicity ratio of heavy metals with different solutions in different crop species (Knezevic et al., 2009; Zhixin et al., 2009; Galfati et al., 2011; Big et al., 2012; Lăcătușu et al., 2012). However, Lombi et al., (2001) reported that EDTA (ethylene-diamine-tetraacetic acid) increased metal mobility in soil and uptake by roots, but did not substantially increase the transfer of metals (Cd, Zn, Pb, Cu) to corn shoots. The high-biomass crop plants work as hyper-accumulators, such as corn, barley, peas, oats, rice, and Indian mustard having natural metal-accumulating capacity, and revealed enhanced phyto-extraction (Lombi et al., 2001; Chen et al., 2004).

In case of *Brassica napus*, the plant shoot and root tissues treated with waste water, revealed highest uptake of heavy metals, followed by medium toxic solution. However, the plants with high toxic solution exhibited least values for all the three heavy metals in *B. napus* shoot and root tissues. Indian mustard exposed to Pb and EDTA in nutrient solution and accumulated 11,000 mg kg^{-1} Pb in dry shoot tissue (Vassil et al., 1998). Another synthetic chelator, HEDTA (hydroxyethyl-ethylenediamine-triacetic acid) applied at 2.0 g kg^{-1} soil contaminated with 2,500 ppm Pb, increased Pb accumulation in shoots of Indian mustard from 40 ppm to 10,600 ppm (Huang & Cunningham, 1996). Present studies revealed that by comparing the both specie crops, the maize due to its vigorous growth accumulated two times more heavy metals than brassica. Under chelate-induced conditions, Indian mustard (Blaylock et al., 1997) and maize (Huang & Cunningham, 1996) have been successfully used to remove Pb from solution culture and contaminated soil, respectively. However, adding 10 mmol of EDTA kg^{-1} soil, showed increased Pb accumulation in maize shoots (1.6 wt% of dry biomass) (Blaylock et al., 1997). Present findings further revealed that the logic behind low uptake of heavy metals in maize and brassica in presence of high toxic solutions might be attributed to the adverse toxic effects of these metals. Accumulation of heavy metals (Fe, Ni, Cr, Cu, Mn, Pb and Cd) increased in roots and leaves of vegetables (Kozanecka et al., 2002; Knezevic et al., 2009; Perveen et al., 2011; Big et al., 2012), and present observations were in parallel analogy with these findings.

The dry matter yields of *Zea mays* and *Brassica napus* were significantly affected by wastewater, medium and high toxic solutions in comparison with tap water. In both species, the dry matter yield was highest in wastewater and least in high toxic solution. The reason might be of contribution of organic matter and other plant food material found in rich nature of the municipal wastewater. However, in high toxic solution, the uptake ability of the nutrients disturbed and reduced in both species due to some disturbance in physiological processes, which eventually decreased the dry matter yield. In heavy metals, the Pb causes retardation of plant growth and inhibition of seed germination (Iqbal & Shazia, 2004), with significant negative impacts on seedling biomass, root and shoot lengths (Uveges et al., 2002). The Zn significantly changes mitotic activity (Rout & Das, 2003), disturbs membrane integrity and permeability (Stoyanova & Doncheva, 2002) and finally kills the plant cell (Change et al., 2005). Furthermore, the Pb, Zn and Cd also reduced the plant uptake rate of essential elements like Mn, Fe, K, Mg and Ca (Wu et al., 2007), which further accelerating toxicity in plants in term of reduction in plant height and weight. The Pb and Cd also adversely affect the process of photosynthesis, respiration and metabolism of plants (Paolacci et al., 1997). The Canola and Indian mustard biomass affected by the contaminated soil, and on average, the heavy metals caused a reduction of about 75% in root and shoot dry matter yield (Turan & Esringu, 2007). However, the zinc is one of the essential elements for many physiological processes in plants, however, in higher concentration it become toxic (Baccio et al., 2005).

Municipal wastewater application reduced plant vegetative as well as reproductive growth in addition to adverse effects on soil health and environment and it was probably due to heavy metals, high pH, sodium adsorption ratio (SAR) and salinity (Khan et al., 2012). Wastewater with higher concentration of heavy metals is potent to retard plant growth and development and adversely affect the yield in *B. napus* (Ahmad et al., 2011). In Indian mustard, the dry matter yield was significantly affected through application of the heavy metals in untreated water; however, the 0.1 mmol kg^{-1} of Pb treated soil produced nearly twice biomass than plants receiving 10 mmol kg^{-1} chelate application (Blaylock et al., 1997). Municipal wastewater was leading for dry matter yield in maize and brassica, which might be due to organic matter and some nutrients found in wastewater, followed by tap water due to least toxic effects. However, the medium toxic solution revealed medium values of dry matter, while high toxic solution revealed least values of dry matter yield in both species.

Overall, the heavy metals accumulation was less in *Zea mays* and *Brassica napus* shoot and root tissues of the plants irrigated with tap water. However, the said uptake gradually increased through enhanced concentration of heavy metals found in municipal wastewater and medium toxic solutions. Nevertheless, in extreme adding of heavy metals in high toxic solution, the accumulation decreased which may be due to the adverse toxic effects of the heavy metals. However, in both crops the shoots accumulated two to four times more heavy metals than roots. In comparison of both crops, the maize due to its vigorous growth accumulated two times more heavy metals as compared to brassica.

5. CONCLUSIONS

Of the two different plant species, maize due to its vigorous growth accumulated two times more heavy metals than brassica. Among plant organs, shoots of both maize and brassica in general, accumulated two to four times more heavy metals compared to roots. Conclusively, since *Zea mays* and *Brassica napus* are considered high biomass producing crops, these could therefore, be successfully used to clean up metal contaminated environment. However, more trials are required to substantiate these metal removing/extracting abilities of the two crops.

ACKNOWLEDGMENTS

The authors are greatly thankful to “Prof. Dr. Md.

Saghir Khan, Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India" for his critical review.

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Received at: 28. 08. 2013
Revised at: 02. 12. 2013
Accepted for publication at: 09. 12. 2013
Published online at: 16. 12. 2013