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ORIGINAL PAPER

Heavy metals from soil and domestic sewage sludge and their transfer to Sorghum plants

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Abstract We studied the mobility and transport of heavy metals such as Cu, Zn, As, Cd, Cr, Ni, and Pb, from soil and soil amended with sewage sludge to sorghum plants. The total and ethylenediaminetetraacetic acid (EDTA) extractable heavy metals in agricultural soil and untreated domestic sewage sludge (DWS) samples were determined. The correlation between the total and extractable metals in soil and sewage sludge was investigated. The total and extractable heavy metals in soil, sewage sludge and sorghum grain were analysed by flame and electro thermal atomic absorption spectrometer (FAAS/ETAAS), after digestion in microwave oven. Statistically good correlations were obtained between the total contents of all heavy metals and their respective extractable fractions in soil and domestic wastewater sludge. Transfer factors of all heavy metals from domestic sewage sludge to sorghum grains were determined.

Keywords Sewage sludge · Soil · Sorghum grains · Exchangeable heavy metals · Transfer factor

Introduction

The application of sewage sludge (biosolid) to agricultural land has become a common practice over the past several decades. Recycling of sewage sludge to agricultural land is generally considered to be the best practicable environmental option because this practice is inexpensive, logical and easy to carry out. Since sewage sludge (SS) contains plant nutrients and organic matter, it may be used to supplement or replace commercial fertilizers for crop production (Albadejo et al. 1994; Spinosa and Vesilind 2001). The beneficial effect of using sewage sludge on agriculture has been proven by numerous researchers (Korentajer 1991). It has been shown that SS application improves the physical, chemical and biological properties of soil (Aggelides and Londra 2000). Nutrients contained in sewage sludge increase plant biomass and yield. Reed et al. (1991) and Cogger et al. (2001) reported that sludge and nitrogen fertilizer applications as the source of applied N did not affect the grain and silage yield of corn, indicating that the fertilizer value of sludge was comparable to that of commercial fertilizers. Pedreno et al. (1996) found that tomato yield was clearly favored by SS fertilization and no difference was observed from other organic fertilizer treatments.

The characterization of heavy metals (HMs) in sewage sludge is an important requirement for sludge disposal or application to farmland because there is a risk of toxic elements accumulating in the soil (Alvarez et al. 2002). Metal transfer from biosolids to soil and subsequently to plants pose potential health risks since they can enter the food chain and the environment. It is common conception nowadays that the total concentrations of metals in soil are not a good indicator of bioavailability, or a good tool for potential risk assessment either, due to the different and complex distribution patterns of metals among various chemical species or solid phases (Chen et al. 1996). The total metal content of soil will usually include fractions that are not immediately available to plants, microorganisms and soil fauna (Henning et al. 2001) although many studies have demonstrated good correlation between total metal content and uptake by plants (Hooda et al. 1997). The toxic

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effects of HMs have also been related to some operationally defined extractable fractions (Shrivastava et al. 2004; McLaughlin et al. 2000). There were a variety of reports on the single extraction procedures with different chemical agents, for available metals in the literatures (Pascual et al. 2004; Qiao et al. 2003; Maiz et al. 1997). The mobility of trace metals, their bioavailability and related eco-toxicity to plants, depend strongly on their specific chemical forms or ways of binding (Fernandez et al. 2000; Pueyo et al. 2001). The lixiviation of HMs from soils and sewage sludge using selective extractants give valuable information, especially for agricultural purposes. The two reagents validated by a group of European researchers coordinated by the Measurements and Testing Program of the Commission of the European Community, in single extraction procedures (Sahuquillo et al. 1995), are EDTA 0.05 M, in either the di-sodium or di-ammonium salt form, has been used extensively as an extractant of potentially plant-available HMs. In some trials, EDTA was found to give a very good indication of the HMs pollution hazard in soils as well as being a reliable test for predicting plant-available metals (Cajuste and Laird 2000). Neutral salt extractants are generally weaker extractants than EDTA and give an indication of the immediately exchangeable (therefore immediately plant-available) metals.

In the present study, the total and extractable HMs were determined in ten batches of soil and untreated domestic sewage sludge collected from different areas of Hyderabad city, Pakistan. In our country there are not many planned sewage treatment plants, and so generally the untreated SS is used as agricultural fertilizer for growing vegetables and grain crops near the cities. The aim of this study was to evaluate the biocycling of HMs from sewage sludge to grain crops (sorghum). The extractable HMs by 0.05 M EDTA pH 7 from all soils and DWS were determined. The uptake of HMs by edible part of sorghum plants grown in soil and soil amended with DWS are discussed. The analytical methodology for total HMs in all three samples were based on microwave assisted digestion method, it is less time consuming as well as reliable, and accurate. The relationships between the total concentrations and extractable fractions of these HMs in soil and soil amended with DWS and their total contents in sorghum grains were examined.

Experimental

Instrumentation

Centrifugation was carried out using a WIROWKA Laboratoryjna type WE-1, nr-6933 centrifuge (speed range 0–6,000 rpm, timer 0–60 min, 220/50 H_Z, Mechanika Phecyzyjna, Poland). A WTW pH meter was employed for pH adjustments of the reagents and the pH determination of the SS. A Milestone microwave oven was used (Rotar MPR-300/12S, pressure 35Bar, with maximum temperature 300°C). The determination of metals in extracts and digests was carried out by means of a double beam Perkin-Elmer atomic absorption spectrometer model 700 (Norwalk, CT, USA) equipped with a graphite furnace HGA-400, pyrocoated graphite tube with integrated platform, an auto sampler AS-800. Hollow cathode lamps were used as radiation sources. Lamp intensity and bandpass width were used according to the manufacturer's recommendations. The Cu and Zn were measured under optimized operating conditions by flame atomic absorption spectrometer (FAAS) with air-acetylene flame, while the As, Cd, Cr, Ni and Pb were determined by electro thermal atomic absorption spectrometer (ETAAS). Conditions for all elements are shown in (Table 1).

Reagents

Ultra pure water obtained from a Milli-Q purifier system (Millipore Corp., Bedford, MA, USA) was used throughout the work. Nitric acid and hydrogen peroxide were analytical reagent, grade from E. Merck (Darmstadt, Germany) used. Concentrated nitric acid and hydrogen peroxide were used for the wet acid digestion method. Nitric acid was purified by sub-boiling distillation in quartz still using ultrapure grade HNO_3 as feed. Standard solutions of As, Cd, Cu, Cr, Ni, Pb, Zn of 1,000 ppm were prepared from certified standards (Fluka kamika, Switzerland).

Extractant solution 0.05 M EDTA pH 7 was prepared by dissolving di-sodium dihydrogen ethylene diamine tetra-acetate salt dihydrate (Na_2 EDTA $\times$ $2H_20$ Merck). The pH solution was adjusted to 7.0 adding NH_4OH solution (trace element quality, Fisher).

Certified reference material BCR 483 and BCR 189 was purchased from the Bureau of references of European communities. All glassware and plastic material used was previously treated for 24 h in 2 N suprapur nitric acid and rinsed with ultra-pure water. The moisture content was determined separately by drying a 1 g sample of soil, sewage sludge and Sorghum grains at 110°C to constant weight. From this, a mean correction 'to dry mass' was obtained and applied to all analytical values reported.

Sampling

Soil samples were collected from five sites in Hyderabad division (South Western Pakistan). The sites are located in the small town. All soils were taken from the surface layer (0–25 cm) of cultivated soils placed in plastic containers

Table 1 Measurement conditions for AAS 700

Parameters	As	Cd	Cr	Ni	Pb
Lamp current (mA)	18	4	30	30	10
Wave length (nm)	193.7	228.8	357.9	232.0	283.3
Slit-width (nm)	0.7L	0.7L	0.7L	0.2L	0.7L
Background correction	D_2 lamp	D_2 lamp	–	D_2 lamp	D_2 lamp
Cuvette	Tubes	Tubes	Tubes	Tubes	Tubes
Dry temperature (°C)	140	140	140	140	140
Dry time (ramp/hold) (s)	15/5	15/5	15/5	15/5	15/5
Ashing temperature (°C)	1,300	850	1,650	1,400	700
Ashing time (ramp/hold) (s)	10/20	10/20	10/20	10/20	10/20
Atomization temperature (°C)	2,300	1,650	2,500	2,500	1,800
Atomization time (ramp/hold) (s)	0/5.0	0/5.0	0/5.0	0/5.0	0/5.0
Cleaning temperature (°C)	2,600	2,000	2,600	2,600	2,200
Cleaning (ramp/hold) (s)	1/3	1/3	1/3	1/3	1/3
Carrier gas (ml/min)	200	200	200	200	200
Sample volume (μl)	10	10	10	10	10

Measurement conditions for FAAS

Elements	Wave length (nm)	Slit width (nm)	Lamp current (mA)	Oxidant (air) L/min	Fuel (acetylene) L/min
Zn	213.9	0.7H	30	17.0	2.0
Cu	324.8	0.7H	30	17.0	2.0

and transported to the laboratory for preparation. The soils were air-dried, ground and sieved to pass through a 1 mm plastic mesh for further use. Precautions were taken to avoid contamination during sampling, drying, grinding and storage. Soil properties, pH, organic matter (OM), organic carbon (OC) and cation exchange capacity (CEC) were determined using standard methods (Lao 1988). These results are given in Table 2.

Sampling of sewage sludge and its preparations

The sewage sludge or biosolid samples were collected from five sites of urban areas of Hyderabad city, Pakistan, at sites where the wastewater was separated from solid wastes. This solid waste is mostly used for the agricultural land near the city, where vegetables and grain crops are grown. Sampling was performed randomly after every

Table 2 Physico-chemical characteristics of the sampled soils and domestic sewage sludge from different areas of Hyderabad city

Site	pH	Organic matter (%)	Organic carbon (%)	Available phosphorus mg kg^{-1}	Silica (%)	Total nitrogen (mg/kg)	Total sulfur (mg/kg)	CEC (meq/100 gm)
S1	7.33 ± 0.2	22.6 ± 1.6	13.2 ± 1.2	35.3 ± 1.5	47.7	2850.2 ± 47.5	850.2 ± 23.2	14.4 ± 1.1
S2	7.88 ± 0.2	26.5 ± 1.9	15.5 ± 1.3	37.3 ± 2.3	47.7	2920.7 ± 62.3	902.5 ± 52.3	15.6 ± 0.8
S3	7.73 ± 0.2	25.9 ± 1.5	15.1 ± 1.5	40.5 ± 2.5	47.8	2945.3 ± 65.5	968.1 ± 56.8	13.9 ± 1.5
S4	7.66 ± 0.3	26.7 ± 2.1	15.6 ± 1.2	41.6 ± 3.5	48.1	2895.6 ± 81.9	935.6 ± 54.2	16.2 ± 1.3
S5	7.53 ± 0.4	26.9 ± 2.2	15.7 ± 1.3	39.8 ± 4.0	47.9	2880.5 ± 53.6	1043.2 ± 87.2	14.8 ± 1.1
DWS1	6.92 ± 0.3	35.6 ± 2.6	20.8 ± 1.8	29.8 ± 2.2	54.6	7750.2 ± 340.5	770.3 ± 35.2	
DWS2	7.66 ± 0.5	33.9 ± 2.9	19.8 ± 1.7	28.1 ± 2.8	52.5	8050.6 ± 290.5	850.6 ± 33.5	
DWS3	8.23 ± 0.4	35.8 ± 3.5	20.9 ± 1.8	31.8 ± 2.1	55.2	10686.7 ± 402.6	910.2 ± 65.4	
DWS4	8.4 ± 0.4	36.1 ± 3.0	21.1 ± 1.7	32.6 ± 3.2	53.2	9365.5 ± 410.2	802.6 ± 54.7	
DWS5	8.1 ± 0.2	36.8 ± 3.2	21.5 ± 1.9	30.8 ± 3.5	52.1	11686.5 ± 462.5	790.5 ± 41.3	

All values presented as mean ± standard deviation, $n = 6$

15 days from January to December in 2005 (so 24 individual samples of biosolid were collected). To ensure that the samples were as representative as possible, each biosolid was collected by taking subsamples (between 2 and 5 kg boisolid). Samples were collected using a polypropylene shovel, and subsequently transferred to clean polypropylene bags. In the laboratory, all of the sewage sludge samples were spread on plastic trays in fume cupboards and allowed to dry at ambient temperature. The samples were homogenized to make a representative sample, after air-drying for 8 days; each representative sample was ground with a mortar and pestle, and after this initial grinding, the samples was passed through a 1 mm nylon fiber sieve. It was then further ground to pass <90 μm sieve, and these final samples were kept in polypropylene containers labeled with the collection date at ambient temperature before analysis. All analyses were performed in triplicate. Blanks were simultaneously run. The analytical accuracy of the procedure utilized to determine the total and extractable HMs in soil and sewage sludge, were confirmed by analysis of a certified reference material of soil amended with sewage sludge (BCR 483) (Kazi et al. 2005) and whole meal flour BCR 189 (Jamali et al. 2007).

Measurement of pH, organic matter, organic carbon, cation exchange capacity

The pH, OM, OC, and CEC were assessed by official methods of soil analysis of the Italian legislation issued in 1992, as described below. A 1:2.5 soil–water suspension (8.0 g of soil for 20 ml of highly purified water (HPW)) was prepared and left standing overnight for pH measurement. The samples of soil and DWS of each batch were burned in muffle furnace at 550 °C for 4 h to determine the OM content, which then was calculated gravimetrically based on the weight difference. The OC was determined by Walkley–Black method (Soltner 1988). It was oxidized to carbon dioxide with potassium dichromate in the presence of sulphuric acid. The unreacted potassium dichromate was titrated with iron (II) sulphate. CEC of soil was determined by the ammonium acetate procedure pH 7. All analyses were performed in triplicate. Blanks were run simultaneously.

In the green house of research centre (National centre of excellence in analytical chemistry, Sindh University Jamshoro, Pakistan), ten plots of $45 \times 35 \times 35$ cm (length × width × height) were prepared. Five plots were filled with agricultural soil as control, while five plots filled with soil amended with SS (3:1) ratio were used for planting sorghum. The seeds of Sorghum variety (PARC SV-1) were obtained from seed certification department; the collected seed samples were thoroughly rinsed with deionized water, and germinated on a filter paper. After the sorghum seeds were germinated for 36 h at 20°C in the dark, uniformly germinated seeds with radical emerged were sown in the control and test plots. The relative humidity of all plots was kept by adding deionized water daily. After 12 weeks, sown sorghum plants grown in control and test samples were harvested and separated into edible and nonedible parts. The edible part of grains was washed thoroughly with tap water and then with deionized water (Hargrove et al. 1985; Wong et al. 2001). The sorghum grain samples were oven-dried for 72 h at 70°C and then ground to pass a 1.0 mm sieve for analyzing HMs concentration.

Extraction procedure

EDTA 0.05 M extract. 0.5 g of soil and DWS sample separately was transferred to an extraction bottle (250 ml polypropylene bottles) and 50 ml of EDTA 0.05 M was added. The mixture was shaken in an end-over-end operating at 30 rpm for 1 h at room temperature. The extract was immediately separated by centrifuging at 3,000 g and the supernatant liquid was filtered and stored in polyethylene bottles at 4°C until analysis (Jamali et al. 2006).

Digestion method for soil, DWS and Sorghum grains

The total metal contents of the agricultural soil and DWS samples were determined via digestion with nitric acid and hydrogen peroxide. In this work, a microwave-assisted digestion procedure was applied to achieve a shorter digestion time (Florian et al. 1998). About 200 mg of duplicate air-dried samples of soil, sorghum grain, and all 24 batches of sewage sludge and five samples of BCR 483 and BCR 189 were weighed and placed in a PTFE reactor, and 65% Suprapur HNO_3 (4.0 ml) and 2.0 ml of 30 % H_2O_2 were added and kept for 2 h at room temperature. Then the reactor was sealed and placed in an oven; it was then heated following a one-stage digestion programmed (250 W, 30 min). After cooling, sample digests were filtered through a Whatman 42 filter paper, transferred into a 25 ml flask and brought to volume with MilliQ water. Analytical blanks were prepared in the same way without addition of any sample.

Statistical analysis

All experimental data shown in the tables and figures were examined statistically by analysis of variance. Means of three replicates was subject to Duncan's New Multiple Range Test at 0.05 probability level using SPSS software.

Result and discussion

Physicochemical parameters of soil and sewage sludge

In Table 2 are presented the physico-chemical parameters of agricultural soils and DWS considered in this study. The results indicate the OM contents range: from 22.6 to 26.9 and 33.9 to 36.8%, while OC found in the range of 13.2–15.7 and 19.8–21.5% in soil and DWS, respectively. The pH of all five samples of agricultural soil was in the range of 7.33–7.88, while the pH values of 24 batches of DWS were found in the range of 6.92–8.4. At low pH (at 6.5 for instance), the mobility and leaching of toxic metals increases, and their mobility and availability decreases as the pH approaches neutral or rises above seven. The considerable amount of total nitrogen, sulfur and phosphate highlights the beneficial use of DWS as agricultural fertilizer.

Bioavailable metals in soil and DWS

To know the potential risk of the bioavailability of HMs from soil and amended soil with DWS to plants, animals, and human beings, it is necessary to evaluate their mobile and/or available fraction of HMs. Many researchers have tried to find a way of measuring the plant available fraction of metals in soils using different extraction procedures. A variety of complexing agents have been proposed to evaluate the pool of elements that is mobile or available to plants (Rauret 1998).

These studies have mostly been validated in field experiments by correlating plant contents with extractable soil contents; e.g. the analysis of EDTA soil extracts are widely used in agriculture; their role is the prediction and assessment of trace element deficiency or toxicity in crops or in animals eating them. In this work 0.05 M EDTA at pH 7 was chosen as extractant solution because this reagent has been recommended by the MAT of the European Community BCR to determine the extractable or mobile fraction of HMs from soils and sediments (Ure et al. 1993). EDTA solution is assumed to extract principally organically bound and carbonate bound fractions of metals by forming strong soluble complexes. However, this reagent can also extract metals included in Fe amorphous oxides (Das et al. 1995). Quevauviller et al. (1998) have proposed the extraction of trace metal contents in calcareous soils with EDTA solutions, relating the extracted fraction bioavailable to plants.

The extractable concentrations of HMs in EDTA agricultural soil and DWS extracts including the percentage of extracted elements relative to total content are listed in Table 3. Result shows that the available fractions of these elements were high in DWS samples as compared to obtain from the soils. In fact, statistically significant correlations of total concentrations of HMs in soils and DWS samples with those in the EDTA extracts were found (Table 4). The low mobility of Cr, Zn, and Ni has been observed in soils and/or to a low efficiency of the EDTA solution to extract these fractions. The extractable concentration of As, Cu, Cr, Cd, Ni, Pb and Zn in soils, ranged from, 0.038–0.05, 0.35–0.37, 0.07–0.078, 0.046–0.056, 0.49–0.54, 0.43–0.48 and 2.28–2.32 mg/kg (3.15–3.4, 3.1–3.23, 3.3–3.41, 3.1–3.3, 1.79–1.85, 2.1–2.2 and 2.19–2.2% of the total content). However, in DWS samples of five regions of Hyderabad city, significant high extractable fractions of all HMs (two–ten fold) were observed. These values are higher than the proposal for maximal acceptable concentrations that can cause phytotoxicity in agriculture (Chanatachon et al. 2002). In general, copper in soils from different agricultural lands presented lower mobility than in untreated DWS samples. The extractable and total concentrations of As, Cd and Cr were lower in agricultural soil as compared to those obtained from different samples of DWS. The high content of toxic metals in DWS are due to small domestic industries (welding, battery and tanneries) working in domestic areas.

EDTA extractable HMs in soil and sewage sludge samples were lower as compared to the total content of these elements in the range of (1.79–3.45) and (1.9–16.8%), respectively, because the EDTA practically cannot extract these metals from Fe-oxides and OM at pH 7 (Barona et al. 2001). Since there is no regulation in Pakistan about mobile or available content of HMs in agricultural soil and sewage sludge used frequently as untreated manure, we have observed that the extractable fraction of all HMs were lower in both soil and DWS samples than the mean range of this element in unpolluted soils (Helgensen and Larsen 1998).

Total heavy metals in sorghum grains

To understand the mobility and transport of HMs to edible parts of sorghum plants, these elements were determined in sorghum grains grown in unamended and amended soil DWS. The accumulation of HMs forms in unamended and amended soil DWS and its subsequent uptake by sorghum grains represent a direct pathway of these elements into the human food chain, which is a major concern. Determination of trace elements in environmental samples requires quality control of the analytical methodology employed. For this purpose, certified reference material, wholemeal flour BCR 189 those matches as closely as possible the sorghum grains matrix were analyzed. Recoveries of HMs from these CRMs are listed in Table 5. The experimental values are in agreement with those (Table 5). The concentrations of all seven metals in sorghum grains, on the

Table 3 Metals concentrations (mg/kg) in 0.05M EDTA at pH 7 of soil and DWS extracts

	Cu	Zn	As	Cd	Cr	Ni	Pb
S1	0.337 ± 0.02 (3.2)[a]	2.30 ± 0.2(2.2)	0.043 ± 0.003(3.23)	0.049 ± 0.006(3.16)	0.07 ± 0.01(3.23)	0.52 ± 0.04(1.85)	0.45 ± 0.035(2.14)
S2	0.342 ± 0.022(3.14)	2.32 ± 0.2(2.2)	0.038 ± 0.004(3.2)	0.046 ± 0.005(3.2)	0.079 ± 0.01(3.45)	0.49 ± 0.036(1.79)	0.43 ± 0.39(2.1)
S3	0.35 ± 0.018(3.15)	2.3 ± 0.24(2.2)	0.046 ± 0.005(3.25)	0.052 ± 0.003(3.25)	0.072 ± 0.012(3.3)	0.55 ± 0.04(1.86)	0.45 ± 0.04(2.15)
S4	0.37 ± 0.03(3.2)	2.28 ± 0.2(2.2)	0.048 ± 0.004(3.3)	0.053 ± 0.004(3.24)	0.074 ± 0.01(3.35)	0.5 ± 0.04(1.8)	0.46 ± 0.05(2.18)
S5	0.37 ± 0.035(3.23)	2.3 ± 0.2(2.2)	0.049 ± 0.005(3.3)	0.056 ± 0.005(3.2)	0.076 ± 0.012(3.4)	0.51 ± 0.045(1.83)	0.49 ± 0.052(2.25)
DWS1	0.70 ± 0.06(1.9)	4.9 ± 0.35(2.09)	0.212 ± 0.02(11.26)	0.35 ± 0.03(15.6)	0.16 ± 0.011(6.2)	2.70 ± 0.2(8.0)	2.31 ± 0.2(3.18)
DWS 2	0.74 ± 0.06(1.96)	5.2 ± 0.39(2.11)	0.208 ± 0.02(11.3)	0.33 ± 0.035(15.27)	0.16 ± 0.015(6.11)	2.83 ± 0.24(8.04)	2.36 ± 0.22(3.03)
DWS 3	0.81 ± 0.07(2.0)	4.9 ± 0.4(2.11)	0.216 ± 0.02(11.1)	0.40 ± 0.039(16.2)	0.18 ± 0.017(6.4)	2.90 ± 0.26(8.1)	2.38 ± 0.25(2.94)
DWS 4	0.76 ± 0.1(1.93)	4.8 ± 0.35(2.1)	0.22 ± 0.02(11.0)	0.41 ± 0.035(16.7)	0.22 ± 0.015(7.0)	3.0 ± 0.25(8.2)	2.34 ± 0.22(3.07)
DWS 5	0.82 ± 0.08(1.98)	4.8 ± 0.4(2.08)	0.214 ± 0.02(11.24)	0.44 ± 0.04(16.8)	0.26 ± 0.02(7.8)	3.2 ± 0.3(8.3)	2.30 ± 0.25(3.29)

[a] ( ) Percentage of the extracted element relative to total concentration in soils and DWS

Values presented as mean ± standard deviation, $n = 6$

Table 4 Relationship between the extractable fractions of heavy metals extracted by 0.05M EDTA pH 7 solution and their total concentration in soil and DWS samples

Element	Soil	Domestic sewage sludge
Relationships Y = extractable fraction in soil and DWS, X = Concentration in soil and DWS (mg/kg)		
Cu	$y = 0.039 - 0.082$	$y = 0.026x - 0.238$
	$R^2 = 0.902$	$R^2 = 0.955$
Zn	$y = 0.025x - 0.287$	$y = 0.022x - 0.246$
	$R^2 = 0.962$	$R^2 = 0.967$
As	$y = 0.038x - 0.008$	$y = 0.073x + 0.075$
	$R^2 = 0.993$	$R^2 = 0.988$
Cd	$y = 0.033x - 0.002$	$y = 0.242x - 0.192$
	$R^2 = 0.981$	$R^2 = 0.989$
Cr	$y = 0.071x - 0.083$	$y = 0.13x - 0.181$
	$R^2 = 0.979$	$R^2 = 0.978$
Ni	$y = 0.026x - 0.225$	$y = 0.106x - 0.879$
	$R^2 = 0.982$	$R^2 = 0.997$
Pb	$y = 0.051x - 0.624$	$y = 0.008x + 1.759$
	$R^2 = 0.967$	$R^2 = 0.983$

basis of dry weight, are shown in Table 6. The concentration of different HMs in sorghum grains obtained from control samples is lower as compared to those obtained from test samples. The level of Cu and Zn was 3.6 and 1.5 fold higher, respectively, in sorghum grains grown in soil amended with DWS, fitting within the normal range reported for herbaceous plants (5–20 mg/kg) (Alloway 1995), whereas Zn concentrations in sorghum grains grown as test sample contain 68.0 mg/kg and control samples contain 47.0 mg/kg. The accumulation of As, Cr, Ni, Pb and Cd was higher in sorghum grain obtained from test samples as compared to those obtained from control, but the difference was not significant ($P > 0.05$).

Inter-elemental relationship provides interesting information on HMs sources and pathways. As can be seen in (Fig.1a) copper in sorghum grains grown in agricultural soil shows a non-significant correlation with Pb, Ni and Cr (R^2 0.3754, 0.0016 and 0.0005), whereas greater extent was observed in correlation of Zn with Pb, Cd and As in sorghum grains grown in agricultural soil (R^2 0.0996, 0.3676 and 0.5934) (Fig. 1b). The inter-elemental relationship in sorghum grains obtained from test samples, between Cu and Ni, Pb and Cr, only Ni and Cr shows significant correlation (R^2 0.8484 and 0.7004) and to a lesser extent with Pb as shown in (Fig. 2a), the results confirmed the similar anthropogenic contamination source for Cu and Ni in the untreated DWS and show a similar behavior of sorghum grains to uptake and to metabolize both metals. The correlation of Zn with As and Cd in sorghum grains grown on soil amended with DWS showing higher extent (R^2 0.704 and 0.752) respectively, while lesser in the case of

Pb (R^2 0.0475) as shown in (Fig. 2b). These results indicate common anthropogenic contamination sources for these elements. It is important to remark that the Zn/ Cu, Cr, Cd, Ni, Pb and as concentration ratio in test samples of sorghum grains was in the range of (8.6, 243, 80, 28, 18 and 340), respectively. The concentration ratios of EDTA extractable Zn with other HMs in DWS samples have the same trends. The results of some researches indicated that the Zn, Cu, Cd and Cr concentrations of plant tissues increased with sludge application (Frost and Ketchum 2000). However, in the present work, metal concentrations were in the normal range and did not reach the phytotoxic levels reported by Lopez-Mosquera et al. (2000). This can be explained by the low heavy metal concentrations in the sludge and the slightly high pH than 7. Peles et al. (1998) found that heavy metal concentrations (Cu, Zn, Cd and Pb) were significantly lower in plants collected from soils with high pH. Although the sewage sludge used in this study is suitable for agricultural use, but if it is applied in high amounts to soil over long periods, Zn and other HMs can reach a dangerous level for public health. The soil in agricultural lands near by the Hyderabad city has a pH value above 7 and this is an advantage for preventing the toxic effects of HMs.

Table 5 Certified and obtained heavy metals in BCR 483 and BCR 189 (n = 6)

Element	Certified value (μg/g)	Obtained value (μg/g)	Recovery (%)
Cu	215.0 ± 11.0	217.5 ± 14.6	101.2
Zn	612.0 ± 19.0	610.2 ± 17.9	99.7
Ni	28.7 ± 1.7	28.9 ± 2.3	100.7
Cr	28.6 ± 2.6	28.4 ± 2.2	99.3
Pb	229.0 ± 8.0	235.6 ± 13.6	102.9
Cd	24.3 ± 1.3	24.2 ± 2.1	99.6
Whole meal flour BCR 189			
As	0.018 ± 0.0005[a]	0.019 ± 0.0004	99.6
Cu	6.4 ± 0.2	6.2 ± 0.18	105.6
Zn	56.5 ± 1.7	57.4 ± 1.9	96.9
Ni	0.35 ± 0.04[a]	0.342 ± 0.04	101.6
Cr	0.065 ± 0.001[a]	0.068 ± 0.002	98.3
Pb	0.38 ± 0.012	0.36 ± 0.014	104.6
Cd	0.072 ± 0.003	0.071 ± 0.002	98.6

[a] (indicative values)

Values presented as means ± standard deviation

Relationships between total and extractable heavy metals in soil and DWS samples and its content in sorghum grains

Concentrations of elements in plants can be influenced by various factors including total or extractable contents of the elements in soils and sewage sludge, pH, organic matter content of soils and sewage sludge samples, plant species, plant parts and the metabolism pathway that present some plant species for toxic elements. To demonstrate the total contents of HMs in soils from different agricultural lands and DWS from different domestic sites of Hyderabad city or the fractions extracted by EDTA presented different behaviors on their transfer to sorghum grains, the relationship between these concentrations and their total content in sorghum grains was studied with reference to soil and soil amended with sewage sludge.

The statistical correlations were determined between the total concentration of HMs in the edible part of sorghum crop and total as well as extractable concentrations of these

Table 6 Heavy metal concentrations in Sorghum grain grown in unamended and amended soil with DWS (mg/kg)

Elements	Sorghum grain grown in soil	Sorghum grain grown in DWS
Cu	2.2 ± 0.13	7.9 ± 0.5
Zn	47.0 ± 2.12	68.0 ± 4.9
As	0.14 ± 0.02	0.2 ± 0.02
Cd	0.38 ± 0.03	0.85 ± 0.08
Cr	0.21 ± 0.01	0.28 ± 0.02
Ni	1.5 ± 0.1	2.45 ± 0.17
Pb	2.0 ± 0.11	3.78 ± 0.17

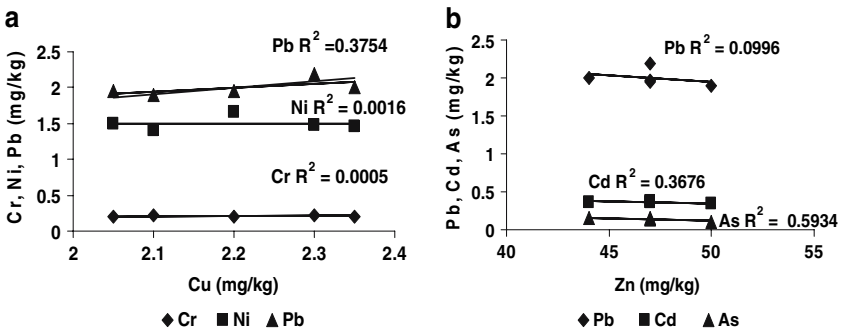

Fig. 1 **a** Inter relationship of Cu with Cr, Ni and Pb in Sorghum grain grown in soil. **b** Inter relationship of Zn with Pb, Cd and As in Sorghum grain grown in soil

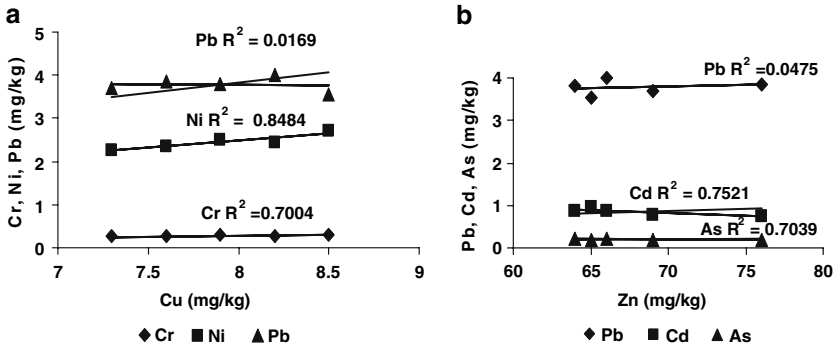

Fig. 2 **a** Inter relationship of Cu with Cr, Ni and Pb in Sorghum grain grown in DWS. **b** Inter relationship of Zn with Pb, Cd and As in Sorghum grain grown in DWS

metals in soils (Table 7). It was observed that all total as well as extractable elements showed significant correlation ($P < 0.05$), except in the case of EDTA extractable Cu which showed lower correlation (R^2 0.96) with those values obtained in sorghum grains grown as control. While in the case of test samples of sorghum grains grown in soil amended medium with DWS samples, higher correlation coefficients were found between total and extractable contents of all metals under study in sorghum grains except in the case of EDTA extractable Zn and Pb showed lower correlation (R^2 0.947) and (R^2 0.955) respectively, with those values obtained in sorghum grains grown as test samples. This fact showed that Zn and Pb compounds in the DWS are not effectively absorbed by sorghum grains. On the contrary, the absorption of As was lesser effective in both cases.

On the basis of these results, individual transfer factors (T_f) of the extractable fraction of HMs in soil and DWS samples, to the respective sorghum grains, defined as the ratio between the concentrations of HMs in sorghum grains and the respective concentration in the EDTA extracts of soil and DWS were evaluated (Table 8). As can be observed, T_f of Zn in soils was higher (20.45) and the lower for Ni (2.83). Although the T_f is high in control sorghum grain samples, but the level EDTA extractable metals was lower in soil samples. While in the case of test samples of sorghum grains, the highest T_f was observed for Zn and Cu (13.81 and 10.32) and the lowest for Ni (0.84). This fact showed that the uptake of Zn and Cu by sorghum crops is more efficient. This fact showed that the uptakes of Cu and Zn by sorghum grains from the DWS are efficient, probably due to the particular climate of this extreme arid zone under study. Since both temperature and light influence plant growth, one can explain the effect of these two parameters on the Cu and Zn uptake as a combination of effects caused by favorable growth and direct effect of high temperature and light, is consistent with other studies (Gregori et al. 2003). With respect to the percentage of other EDTA extractable toxic metals such as As, Cd and Ni in DWS were found to be, 11.26, 16.8 and 8.3%, but both toxic metals have low transfer factors. This fact shows that, although As and Cd have high mobility in DWS samples,

Table 7 Relationship between the total concentrations of heavy metals in Sorghum grain (mg/kg) and the respective total and extractable concentration in unamended and amended soil with DWS (mg/kg)

Elements	Correlation with total metals in soil	Correlation with extractable metals in soil	Correlation with total metals in DWS	Correlation with extractable metals in DWS
Cu	$y = 0.339x - 1.580$ $R^2 = 0.993$	$y = 8.101x - 0.667$ $R^2 = 0.961$	$y = 0.248x - 1.838$ $R^2 = 0.991$	$y = 9.38x + 0.719$ $R^2 = 0.969$
Zn	$y = 3.321x - 300.3$ $R^2 = 0.994$	$y = 131.1x - 254.2$ $R^2 = 0.983$	$y = 0.658x - 86.43$ $R^2 = 0.993$	$y = 28.7x - 73.2$ $R^2 = 0.947$
As	$y = 0.177x - 0.103$ $R^2 = 0.989$	$y = 4.599x - 0.066$ $R^2 = 0.998$	$y = 0.344x - 0.458$ $R^2 = 0.999$	$y = 4.68x - 0.801$ $R^2 = 0.983$
Cd	$y = 0.128x + 0.156$ $R^2 = 0.998$	$y = 3.767x + 0.1671$ $R^2 = 0.968$	$y = 0.430x - 0.178$ $R^2 = 0.999$	$y = 1.761x + 0.170$ $R^2 = 0.992$
Cr	$y = 0.187x - 0.204$ $R^2 = 0.99$	$y = 2.58x + 0.018$ $R^2 = 0.974$	$y = 0.069x + 0.081$ $R^2 = 0.998$	$y = 0.516x + 0.179$ $R^2 = 0.974$
Ni	$y = 0.111x - 1.614$ $R^2 = 0.997$	$y = 4.169x - 0.638$ $R^2 = 0.991$	$y = 0.094x - 0.945$ $R^2 = 0.996$	$y = 0.891x - 0.157$ $R^2 = 0.996$
Pb	$y = 0.266x - 3.604$ $R^2 = 0.996$	$y = 5.39x - 0.464$ $R^2 = 0.981$	$y = 0.039x + 0.822$ $R^2 = 0.992$	$y = 4.978x - 7.86$ $R^2 = 0.955$

Table 8 Transfer factors (concentration in Sorghum grains/concentration in 0.05M EDTA soil and DWS Extract) of heavy metals in soil and DWS to Sorghum grains

	Cu	Zn	As	Cd	Cr	Ni	Pb
S1	6.08	20.43	3.07	7.24	2.86	2.91	4.36
S2	6.14	21.55	2.86	7.38	2.84	2.86	4.37
S3	6.29	20.48	3.15	6.92	2.85	3.02	4.33
S4	6.35	19.34	3.19	6.89	2.81	2.92	4.35
S5	6.22	20.43	3.27	6.79	2.80	2.90	4.47
Mean ± SD	6.21 ± 0.11	20.45 ± 0.78	3.11 ± 0.15	7.04 ± 0.25	2.83 ± 0.02	2.92 ± 0.06	4.38 ± 0.05
DWS1	10.43	14.08	0.89	2.26	1.61	0.83	1.60
DWS 2	10.27	14.62	0.84	2.27	1.63	0.83	1.64
DWS 3	10.12	13.47	0.97	2.20	1.53	0.84	1.68
DWS 4	10.39	13.33	1.05	2.15	1.35	0.83	1.62
DWS 5	10.37	13.54	0.92	2.16	1.19	0.84	1.54
Mean ± SD	10.32 ± 0.12	13.81 ± 0.53	0.93 ± 0.08	2.21 ± 0.06	1.46 ± 0.19	0.84 ± 0.01	1.62 ± 0.05

only a limited fraction is transferred to edible part of the sorghum crop. This last phenomenon is favorable when taking into account that the untreated sewage sludge used as fertilizer near the cities of Pakistan for growing grain crops that are consumed by humans and animals, decreasing the possibility of toxic metals intake by this route. Cadmium (Cd) in the soils, for example, derived mainly from industrial processes, mining activities, repeated agricultural use of sewage sludge and phosphate fertilizers, is extremely toxic to living cells even at low concentrations (Sandalio et al. 2001).

Conclusion

The studied EDTA extractable procedure presented good precision and the quality of the data was evaluated by comparing the obtained extractable Cd, Cu, Pb and Zn concentrations with the indicative values reported for the BCR CRM 483 soil. These results demonstrate the importance of measuring extractable as well as total heavy metal concentrations in untreated sewage sludge, when assessing HMs uptakes, and setting sewage sludge quality criteria. The results from this study indicate that total soil and sewage sludge metal concentration measurements do not provide the best indicator of metal availability to plants, with better correlations observed between metals in edible part of sorghum and EDTA extractable metal concentrations. It is therefore recommended that assessments of the impact of HMs in untreated sewage sludge of domestic origin, required a measure of extractable as well as total soil–metal concentrations. Nevertheless, toxic elements, such as Cd and As, accumulated in the plants, Moreno et al. (2003) and Bahour et al. (2001) due to the phytoremediation of agricultural soil amended with sewage sludge, cause the plant material to be considered harmful for human consumption and use as animal fodder.

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