
ABSTRACT

Wastewater is a major receptacle for contaminants as it possesses an ability to bind various toxicity substances. These chemicals can exist in various forms in wastewater and growing crops different forces. It is essential to study these interactions because the toxicity of chemicals may strongly depend on the form in which they exist in the environment. Another thing is that crops variability in some environmental properties may change in soil and cause leaching of trace toxic elements. Metals associated with urban wastewater and crop species are great concern because of their direct and indirect effects on human health. The main purposes of this phytoremediation study undertaken in the Mysuru city Municipal wastewater irrigated zones were to identify heavy metals with dangerous environmental load and to find out of their environmental impact (Cd, Cu, Fe, Ni, and Zn). The purpose of this work was to provide information on heavy metals concentration in selected crops and heavy metal speciation in crops carried out were shows that all metals were mainly associated with the oxidizable and residual fraction, which allows us to predict their mobility in the crop.

KEYWORDS: Phytoremediation, Wastewater, Toxicity, Fractions, Chemicals and Municipal

INTRODUCTION

Phytoremediation is defined as the use of plants to remove pollutants from the environment from less harmless (Salt et al., 1998). Some methods, such as soil washing, have an adverse effect on biological activity, soil structure and fertility, and some require significant engineering costs. Consequently, the low-technology, in-situ come close to of phytoremediation is smart as it offers site reinstatement, partial decontamination, maintenance of the biological activity and physical structure of soils, and is potentially cheap, visually self-effacing, and there is the possibility of recovery of Heavy metals (Baker et al., 1991, 1994). The development of phytoremediation is being driven primarily by the high cost of many other soil remediation methods, as well as a desire to use a 'green', sustainable process. Initially, much interest focused on hyperaccumulator plants capable of accumulating potentially phytotoxic elements to concentrations more than 100 times than those found in nonaccumulators (Salt et al., 1998; Chaney et al., 1997; Raskin and Ensley, 2000). These plants have strongly expressed metal sequestration mechanisms and, sometimes, greater internal requirements for specific metals (Shen et al., 1997). Some species may be capable of mobilizing metals from less-soluble soil fractions in comparison to non hyperaccumulating species (Mc Grath et al., 1997). Metal concentrations in the shoots of hyperaccumulators normally exceed those in the roots. Phytoremediation has many advantageous features that make it an appropriate and successful technology, giving practitioners a valuable option for remediation. Its major advantage is the low cost, estimated by the USEPA to be 50 – 80% lower than the alternatives for some applications of phytoremediation (EPA, 2000). It can be used for removal of heavy metals as well as for organic. It is a novel, cost-effective, efficient, environment- and eco-friendly, in situ applicable, and solar-driven remediation strategy (Clemens, 2001; Suresh and Ravishankar, 2004; Le Duc and Terry, 2005; Chehregani and Malayeri, 2007; Odjegba and Fasidi, 2007; Turan and Estringu, 2007; Lone et al., 2008; Kawahigashi, 2009; Vithanage et al., 2012). Plants generally handle the contaminants without affecting topsoil, thus conserving its

utility and fertility. They may improve soil fertility with inputs of organic matter (Mench *et al.*, 2009). The determination of the total concentration of the metals gives no information on their various forms. Therefore, the determination and monitoring of specific chemical forms of the heavy metals in environmental samples such as airborne particulates, waters, biological materials, soils or sediments are extremely important.

The development of analytical techniques to reliably measure the concentrations of the various chemical forms (speciation) of trace metals in an environmental sample such as sediment is one of the most challenging problems presently facing environmental chemists. Speciation techniques have been widely used for the determination of heavy metals in sediments in the past two decades (T.M. Florence, 1982); (G.E. Batley, 1989). Determination of total metal in crops may provide information concerning possible pollution Levels, but generally there are insufficient details for estimating their biological effects, because it is the chemical form of the metal in the crops that determine its mobilization capacity and behavior in the environment. An experimental approach commonly used for studying the mobility, transport and bioavailability of metals in crops is the use of selective sequential extraction procedures (Mc Laren and Clucas, 2001). Heavy metal speciation in soil indicated that metals were mainly associated with the oxidizable and residual fraction, which allows us to

Predict their mobility in the soil sample. The residual fraction has the maximum percentage of heavy metals, whereas only a small fraction of heavy metals (Fe, Cu, Cr, Zn and Ni) is extracted in soluble fractions, exchangeable and carbonate fractions. It indicates that the bioavailability index is low. Mobility and availability of heavy metals by sequential extraction has been proved to be of great significance in provide a valuable information (Doddaiiah Shivakumar, 2012). In operationally defined speciation, the physical or chemical fractionation process applied to the sample defines the fraction obtained. In sequential multiple extraction techniques, different chemical extractants of various types are sequentially applied to the sample of sediment, soil and crops, each follow-up treatment being more drastic in chemical action or different in nature from the previous one. Applications of these methods have been used for atmospheric particulates by Walter *et al.* (1989); street dust and roadside soils by (Harrison *et al.* 1981); biosolids by (Pérez-Cid *et al.* 1996), (Rudd *et al.* 1986), (Salveti *et al.* 1994) and (Ure and Davidson 1995); incinerated sludge ash by (Fraser and Lum 1983) and (Vela *et al.* (1993); sediments by (Gonzalez *et al.* 1995), (Rauret *et al.* 1989b) and (Quevauviller *et al.* 1993) and soils by (Singh *et al.* 1996), (Cañadas *et al.* 1986), (Lindsay, 1979) and (Li *et al.* (1995). Several speciation methods have been reviewed in the literature (Forstner 1993; Forstner *et al.* 1989; Lum *et al.* 1982; Tack and Verloo 1995; Ure and Davidson 1995). Many of these schemes are based on the five-stage schemes developed by (Tessier *et al.* 1979), method or its modifications, by (Rauret *et al.* 1989 a, b). These sequences are designed to differentiate between the exchangeable, carbonate, reducible (hydrated Fe/Mn oxides), oxidizable (sulfides and organic phases) and residual (mineral) fractions. These sequences are designed to differentiate between the exchangeable, carbonate, reducible (hydrated Fe/Mn oxides), oxidizable (sulfides and organic phases) and residual (mineral) fractions. Zinc, copper, nickel and chromium are the principal elements which restrict the application of crops on agricultural land. Their potential accumulation in human tissues and bio-magnification through the food-chain cause both human health and environmental concerns. These considerations are usually based on total heavy metal content of biosolid. However, it is now widely accepted that the determination of total elements does not give an accurate estimation of the potential environmental impact. This is the case because it is becoming more and more apparent that both bioavailability and toxicity are critically dependent on the chemical form of heavy metals (Alvarez *et al.* 2002). The main aim of this present study is to convert the metals bound in the sediment phases into soluble forms with the extractant used at the each step. A series of single reagents is used to extract defined phases from the sediment in a defined sequence and investigate the potential pollution of heavy metals which are present in crops followed by the study of speciation of cadmium, copper, iron, nickel, lead and zinc in crops grown by using municipal wastewater by using sequential extraction procedure proposed by Tessier method. The crop species for the present study have been collected from crops irrigated areas of Mysore, Karnataka, India.

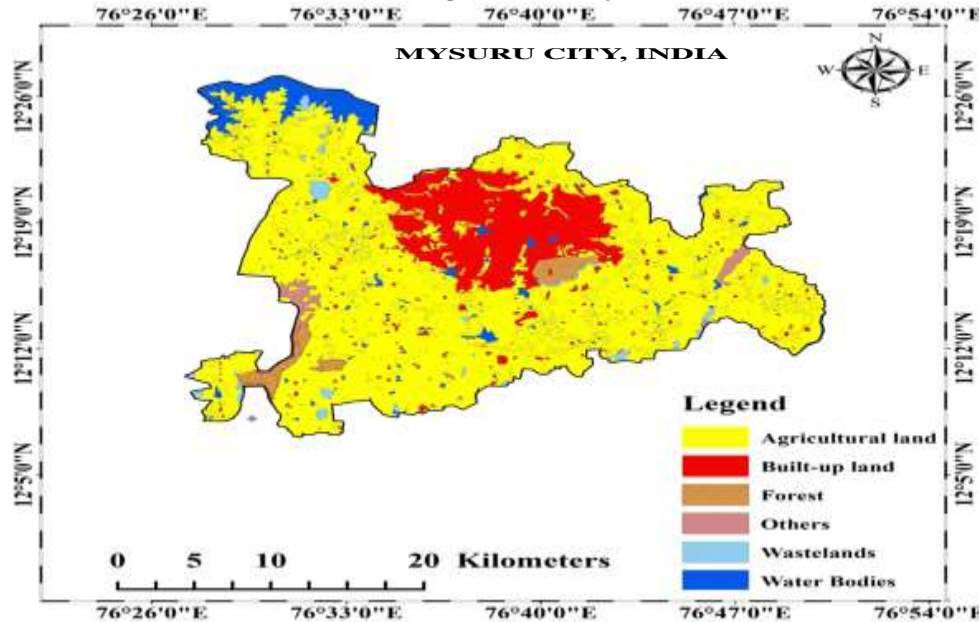
MATERIALS AND METHODS

Study area

Mysuru is a city in the Indian state of Karnataka, and the administrative seat of Mysuru District, one of the largest districts in Karnataka. Mysuru was the former capital of the Kingdom of Mysuru. Mysuru is located at 770 m above sea level at 12.18° N 76.42° E and is 135 km from Bangalore, the state capital. Mysuru being one of the growing cities of Karnataka, largely due to the presence of industrial resources and a well developed communication network. In recent decades industrialization is the main cause of city's growth. A large number

of small, medium and large scale industries exists in and around the Mysuru city, including engineering, chemical, pharmaceutical, food, brewery, distillery, textile, rubber, steel and metal smelting. Over the past decade Mysore has transformed itself into a destination for modern industries in the manufacturing service and Information and Technology sector.

Figure: 01. Study area



Multi-step sequential extraction of crop bio-mass

The sequential extraction procedure used in this study is Tessier *et al.* (1979) method. The extraction was carried out increasingly on an initial mass of 1.00 g of sample of crop biomass including the composition of (root + shoot + leaf). The samples for sequential extraction were dried in an oven at 60°C for 24 h in order to avoid, as far as possible, the transformation of some chemical forms. This procedure is having five steps fractionization. The extractants and operationally defined chemical fractions were as follows: **Fraction 1 (F1): Exchangeable metal fraction.** The sample was extracted with 8 ml of 1 M MgCl₂ for 1 h with continuous agitation, at room temperature. **Fraction 2 (F2): Carbonate bound metal fractions.** The residue from F1 was leached for 5 h with 8 ml of 1 M sodium acetate adjusted to pH 5.0 with acetic acid, at room temperature and with agitation. **Fraction 3 (F3): Elements associated with Fe– Mn oxide metal fraction.** The residue from F2 was extracted with 20 ml of 0.04 M hydroxylamine hydrochloride in 25% v/v acetic acid for 5 h in boiling water bath and with periodic agitation. **Fraction 4 (F4): Organic and sulfide metal fractions.** The residue from F3 was extracted with 3 ml of 0.02 M HNO₃ and 8 ml of 30% H₂O₂ adjusted to pH 2.0 with HNO₃ for 5 h at 85°C with occasional agitation and then at room temperature with 5 ml of 3.2 M ammonium acetate in 20% HNO₃. **Fraction 5 (F5): Residual metal fraction.** This fraction was calculated as the difference between the total metals and the sum of extracted metals. The selective extractions were conducted in 50 ml capacity centrifuge tubes. After each extraction step, the sample was subjected to 30 min of centrifugation at 4,000 rpm; the supernatant was separated from the residue with a pipette and transferred into a 25-ml calibrated flask. The residue was centrifugation and later washed thoroughly, the obtained second supernatant was added to the flask, which was diluted to the desired volume. The extracts obtained were acidified using aqua regia and stored in stopper polyethylene vessels until their analysis by using inductively coupled plasma atomic emission spectroscopy techniques (ICP-OES). The total content of metals was determined after digesting 0.4 g of sample with aqua regia. The concentration of particular heavy metals was expressed per 1 kg of air dry sample. The content of heavy metals in the obtained solution was determined by using the Perkin-Elmer Optima 8000, ICP-OES. Speciation and geochemical behavior of Heavy metals evaluated results were tabulated in Table 4 to Table 9.

Table.1: Crop species grown around Mysore city using Municipal Wastewater (MWW)

Name of plant species irrigated using (MWW)	Family of the species
<i>Amaranthus trititis</i> L	Amaranthaceae
<i>Anethum graveolens</i> L	Apiaceae
<i>Coriandrum sativum</i> L	Apiaceae
<i>Mentha longifolia</i> L	Lamiaceae
<i>Oryza sativa</i> L	Poaceae
<i>Pennisetum purpureum</i> Schumach	Poaceae
<i>Spiracia oleracea</i> L	Amaranthaceae
<i>Trigonella foenum graecum</i> L	Fabaceae
<i>Zea mays</i> L	Poaceae

RESULTS AND DISCUSSION

In the exchangeable fraction (F1) the metal bonding is very weak in its ionic form which shows a higher mobility from one tropic level to another tropic level and it can be drained by water. The fraction related to the carbonates (F2) is extractable and can be accumulated in the living organisms and it's also shows a moderate mobility in the soil and water environment. The fraction related to the oxides of iron and manganese (F3) and that related to organic matter (F4) contain metals enclosed in the matrix. The fraction (F5) contains the inert metals like mobility is comparatively very less and the metals which is present in the residual fraction can't move in the further levels. which may hold metals within their arrangement. These metals are not probable to be released in resolution over a reasonable time span under the circumstances normally encountered in nature (Stylianou *et al.* 2007). In the analyzed metals in the crops species grown in the agriculture field by using MWW showed a highest mobility of metals in the *P.purpureum Schumach* species for all the analyzed metals. Cadmium is trace for all the crop species, indicates its lesser concentration and non availability to the plants. In the uptake of Copper, the mobility in the F1 and F2 indicates its higher mobile nature in the plants. Whereas F5 showed its total accumulation in the plant body and that tends towards its residual nature. Iron is the major element for the plant growth. Major source of iron is corrosion of pipes and garbage and its associated with the soil as nutrient and MWW and plays a vital role as well as uptake. Hence the uptake of iron from the plant species is usually high. Nickel was bound to F1 in the *P.purpureum Schumach*, *Oryza sativa* L, *Trigonella foenum graecum* L, *Zeamays* L and *Amaranthus trititis* L species. It shown that its mobility and it can be further moved in to next tropic level by accumulating higher concentration. Lead concentration was once again BDL except *P.purpureum Schumach* in all the fraction. The concentration of Zinc was recorded for all the fractions and observed its concentration. *Coriandrum sativum* L and *Mentha langifolia* L species have shown BDL. The speciation analysis show that cadmium, in the crops of different crop species, has been found mainly in the fraction bound to carbonates It is below detection limit in exchangeable fractions, thus cadmium in the study samples would not be mobile. Copper is bound much more tightly by organic matter than Ni and is much less likely to be displaced by the hydroxylamine hydrochloride reagent. Similar results are reported by McLaren and Clucas (2001). Copper in the crops is not present in the ionic forms which reduce its toxicity. In exchangeable fraction, except iron, the concentration of all metals in this fraction is lower than the detection limit. The amounts of metals released in the carbonate fraction represent a low proportion of the total metal concentration. Copper, chromium and zinc are below the detection range and iron has low concentrations in this fraction. In the fractions bound to hydrated iron and manganese oxides, the amounts of nickel, iron and zinc associated are relatively high. These amounts of metals would be released under reducing conditions. The metal levels in fraction associated with organic matter are relatively high and they represent a large fraction of the total metal concentrations. These amounts of metals are released in soil under strong oxidizing conditions and consequently this fraction constitutes an important source of potentially available trace metals. It can be observed that the greatest part of the metals studied, especially iron, and was associated with the residual fraction.

Table 2: Total content of Cadmium and its distribution among particular fractions in crops

Cadmium (Cd)						
Crops	Plants grown by using MWW (mg/L)					
	F1	F2	F3	F4	F5	Total
<i>Amaranthus tritris</i> L	BDL	BDL	BDL	BDL	0.1	0.1
<i>Anethum graveolens</i> L	BDL	0.2	BDL	BDL	0.1	0.3
<i>Coriandrum sativum</i> L	BDL	BDL	BDL	BDL	BDL	BDL
<i>Mentha langifolia</i> L	BDL	0.1	BDL	BDL	BDL	0.1
<i>Oryza sativa</i> L	BDL	BDL	BDL	BDL	BDL	BDL
<i>P.purpureum Schumach</i>	0.2	BDL	0.3	BDL	0.1	0.6
<i>Spiracia oleracea</i> L	BDL	BDL	BDL	BDL	BDL	BDL
<i>Trigonella foenum graecum</i> L	BDL	BDL	BDL	0.2	BDL	0.2
<i>Zeamays</i> L	BDL	0.1	BDL	BDL	0.2	0.3

Table 3: Total content of Copper and its distribution among particular fractions in crops

Copper (Cu)						
Crops	Plants grown by using MWW(mg/L)					
	F1	F2	F3	F4	F5	Total
<i>Amaranthus tritris</i> L	0.1	BDL	0.2	BDL	0.1	0.4
<i>Anethum graveolens</i> L	0.1	0.1	BDL	0.1	0.1	0.4
<i>Coriandrum sativum</i> L	BDL	BDL	BDL	BDL	0.1	0.1
<i>Mentha langifolia</i> L	BDL	BDL	BDL	BDL	0.1	0.1
<i>Oryza sativa</i> L	BDL	BDL	BDL	BDL	0.2	0.2
<i>P.purpureum Schumach</i>	0.3	0.8	1.0	0.2	0.2	1.6
<i>Spiracia oleracea</i> L	0.3	0.2	BDL	BDL	0.4	0.9
<i>Trigonella foenum graecum</i> L	BDL	BDL	BDL	BDL	0.2	0.2
<i>Zeamays</i> L	0.1	0.3	0.2	BDL	0.8	1.5

Table 6: Total content of Iron and its distribution among particular fractions in crops

Iron (Fe)						
Crops	Plants grown by using MWW (mg/L)					
	F1	F2	F3	F4	F5	Total
<i>Amaranthus tritris</i> L	8.9	28.1	34.6	48.2	36.1	155.9
<i>Anethum graveolens</i> L	7.3	14.6	28.9	36.8	80.6	168.2
<i>Coriandrum sativum</i> L	4.0	14.8	16.3	45.2	43.0	123.3
<i>Mentha langifolia</i> L	6.9	17.4	36.2	29.3	61.6	151.4
<i>Oryza sativa</i> L	12.3	38.6	78.1	48.2	24.1	201.3
<i>P.purpureum Schumach</i>	31.0	68.2	148.9	269.1	165.9	683.1
<i>Spiracia oleracea</i> L	4.9	21.3	48.9	18.9	60.5	154.5
<i>Trigonella foenum graecum</i> L	3.2	18.6	34.2	31.7	68.1	155.8
<i>Zeamays</i> L	8.7	24.3	54.1	87.6	76.6	251.3

Table 7: Total content of Nickel and its distribution among particular fractions in crops

Nickel (Ni)						
Crops	Plants grown by using MWW (mg/L)					
	F1	F2	F3	F4	F5	Total
<i>Amaranthus tritris</i> L	0.2	0.3	0.1	BDL	0.23	0.83
<i>Anethum graveolens</i> L	BDL	BDL	0.2	0.2	0.3	0.70
<i>Coriandrum sativum</i> L	BDL	BDL	BDL	0.25	0.31	0.56
<i>Mentha langifolia</i> L	BDL	BDL	BDL	0.12	0.2	0.32
<i>Oryza sativa</i> L	0.12	0.1	0.2	0.3	0.15	0.87

<i>P.purpureum Schumach</i>	1.1	2.5	1.8	1.3	1.5	8.2
<i>Spiracia oleracea L</i>	BDL	BDL	0.12	0.24	0.28	0.64
<i>Trigonella foenum graecum L</i>	0.1	0.12	0.24	0.12	0.31	0.89
<i>Zeamays L</i>	0.4	0.6	0.6	0.5	1.1	3.2

Table 8: Total content of Lead and its distribution among particular fractions in crops

Lead (Pb)						
Crops	Plants grown by using MWW (mg/L)					
	F1	F2	F3	F4	F5	Total
<i>Amaranthus trititis L</i>	BDL	BDL	0.1	BDL	BDL	0.1
<i>Anethum graveolens L</i>	0.1	BDL	BDL	BDL	BDL	0.1
<i>Coriandrum sativum L</i>	BDL	BDL	BDL	BDL	BDL	BDL
<i>Mentha langifolia L</i>	BDL	BDL	0.2	BDL	BDL	0.2
<i>Oryza sativa L</i>	BDL	BDL	BDL	BDL	BDL	BDL
<i>P.purpureum Schumach</i>	0.8	0.8	1.2	0.9	0.9	4.6
<i>Spiracia oleracea L</i>	0.2	BDL	BDL	BDL	BDL	0.2
<i>Trigonella foenum graecum L</i>	BDL	BDL	BDL	BDL	BDL	BDL
<i>Zeamays L</i>	BDL	0.2	BDL	BDL	0.2	0.4

Table 9: Total content of Zinc and its distribution among particular fractions in crops

Zinc (Zn)						
Crops	Plants grown by using MWW (mg/L)					
	F1	F2	F3	F4	F5	Total
<i>Amaranthus trititis L</i>	0.26	0.3	0.39	0.4	1.32	2.67
<i>Anethum graveolens L</i>	0.1	0.1	0.2	0.2	0.73	1.33
<i>Coriandrum sativum L</i>	BDL	BDL	BDL	0.2	0.46	0.66
<i>Mentha langifolia L</i>	BDL	BDL	0.2	0.3	0.28	0.78
<i>Oryza sativa L</i>	0.3	0.4	0.2	0.3	0.9	2.1
<i>P.purpureum Schumach</i>	2.9	4.2	14.1	12.9	12.4	46.5
<i>Spiracia oleracea L</i>	0.1	0.1	0.17	0.24	0.22	0.83
<i>Trigonella foenum graecum L</i>	0.5	0.8	0.4	0.8	0.7	3.2
<i>Zeamays L</i>	0.4	0.7	0.9	0.4	1.1	3.5

CONCLUSION

The results obtained in the present research, heavy metal speciation in crop sample indicated that all metals were generally allied with the oxidizable and residual fraction, which allows us to predict their mobility in the crops sample. The residual fraction has the maximum percentage of heavy metals, whereas only a small fraction of heavy metals is extracted in soluble fractions, exchangeable and carbonate fractions. It indicates that the bioavailability index is low. The study clearly state that, mobility and availability of heavy metals by sequential extraction has been proved to be of great significance in providing valuable information, since the toxicity of heavy metal in selected crops in Mysuru city. Further it may have the chances of increasing toxicity of heavy metals by regular availability and regular adoption of MWW to irrigation and cause the serious problems of bio accumulation and bio magnification in crops as well as environment.. These research works is novel and enlighten the application of sequential extraction procedures for determination of speciation of heavy metals in crop grown by using Municipal Wastewater and provides an evaluation of the real mobility of these elements.

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