EXTRACTANTS FOR THE ASSESSMENT OF PHYTO-AVAILABLE CADMIUM TO RICE GROWN IN CADMIUM CONTAMINATED SOILS

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Phyto-available concentration of cadmium (Cd) is considered a good indicator for the assessment of environmental quality of Cd contaminated soils and is more important than total metal contents in soil. A pot study was conducted using two different textured soils, collected from raw sewage irrigated areas of the Faisalabad. The extraction efficiency of extractants for phyto-available Cd from soils was compared. Phyto-available Cd was correlated to Cd concentrations in shoots and grains of rice (cv. Basmati-2000). Cadmium chloride salt was used @ 20 mg Cd kg^{-1} of soil to impregnate these soils and was allowed to equilibrate for 30 days at moisture level of about field capacity. Chemicals viz. AB-DTPA, 0.005 *M* DTPA, 0.05 *M* EDTA, 0.01 *M* $CaCl_2$, 1 *M* NH_4NO_3 and 0.1 *M* $NaNO_3$ were used to extract Cd and its concentration was determined with atomic absorption spectrophotometer. Correlation analysis between the extractable and plant Cd concentration determined by diacid method was performed. The AB-DTPA extractable Cd had more positive and significant correlation with Cd concentration in rice straw and grains harvested from sandy clay loam soil than the other extractants under test. For loamy sand, maximum correlation of Cd concentration in rice straw was recorded with $NaNO_3$-Cd while in grains it was maximum with ABDTPA-Cd. Overall, AB-DTPA gave better and comparable results than those with other extractants and neutral salts gave good results for light textured calcareous soil. It seems important that future research may focus to investigate the effect of soil properties and plant species as variables to assess the efficiency of extractants.
Keywords: Extractants, phytoavailable Cd, rice, contaminated soil

INTRODUCTION

Cadmium exists in various chemical forms in soils and is getting more and more attention owing to one of the most ecotoxic heavy metals in the environment which could exhibit highly adverse effects on soil biological activities, biodiversity, plant metabolism, and health of human beings and animals. This metal is not required for any known biological functions in plants, but due to its higher mobility in soils, it could be easily absorbed by crops and could also contaminate ground water. Toxicity of Cd is more to humans than animals through eating Cd contaminated food, because of its longevity and its accumulation in their essential organs (Tudoreanu and Phillips, 2004). It could result in painful bone demineralization (osteoporosis) because Cd could replace calcium in bones. Other toxic effects of Cd in humans include targeting lungs, kidneys, blood, liver and testes.

In soils, determination of total Cd is a weak indicator of its availability to plants. Different efforts have been made to find out phyto-available fraction of Cd by using different single extraction techniques (Lindsay and Norvel, 1978) or sequential extraction methods (Tessier *et al.*, 1979; Ure *et al.*, 1993). The sequential extraction methods are laborious and time consuming compared to single extraction methods using a single extractant for this purpose. Water soluble cadmium represents readily bio-available proportion of Cd (Seguin *et al.*, 2004). Chelating agents (DTPA, AB-DTPA, EDTA) and strong acids (HCl, CH_3COOH) could extract large fraction of total Cd from the solid phase (Over estimation), and are considered suitable for the prediction of plant uptake only in acidic conditions (Ure *et al.*, 1993). Neutral salts ($NaNO_3$, NH_4NO_3, $CaCl_2$) have been found better reflector of bio-available fraction of Cd from calcareous alkaline soils (Novozamsky *et al.* 1993; Pueyo *et al.*, 2004).

Linear correlation between Cd concentration by different extractants and that in plant parts is often used for the identification of the best-suited assessment method for soils. Keeping this in view, six extractants were used to determine soil bioavailable Cd and these were correlated with Cd in rice grains and straw using diacid method (Ryan *et al.*, 2001). Uptil now, there is no accepted single extractant estimating the bioavailability of Cd from soils (Pueyo *et al.*, 2004). Objectives of this study were to compare different chemicals to extract phyto-available fraction of Cd from texturally different soils and correlate it with plant concentration so that the best extractant can be identified.

MATERIALS AND METHODS

Soil samples were collected from agricultural fields having different texture, irrigated with untreated (raw) city effluent from the suburbs of Faisalabad city. The sandy clay loam soil was collected from village 236/R.B., Kajlianwala and the loamy sand soil was collected from the Land utilization Farm, University of Agriculture, Faisalabad located at village 217/R.B., Uchkera. These samples were collected from the upper 0.20 m soil depth. These samples were air-dried, ground with wooden roller, passed through 2 mm sieve and stored in plastic jars for analyses.

Pre-treatment of soils

Both the soils (Table 1) were artificially contaminated with Cd @ 20 mg kg^{-1} by spraying and mixing $CdCl_2$ solution equal to 75% of the saturation percentage of respective soil. After mixing, soils were placed in large containers lined with polyethylene sheet. These Cd treated soils were kept at a moisture level of about field capacity and allowed to equilibrate with periodic mixing for four weeks. So Cd spiked soil was filled @ 11 kg soil per pot.

Table 1. Characteristics of soils used in the study

Characteristic	**Unit**	**Value**	
Textural class	-	Loamy sand (LS)	Sandy clay loam (SCL)
Sand	%	85.80	49.10
Silt	"	10.36	24.50
Clay	"	3.84	26.40
pH_s	-	7.95	8.16
EC_e	dS m^{-1}	2.05	3.50
SAR	(mmol L^{-1})$^{1/2}$	1.60	16.00
CEC	cmol$_c$ kg^{-1}	4.30	6.15
$CaCO_3$	%	1.10	1.78 %
OM	%	0.58	0.82
Cd	mg kg^{-1}	[a]0.95 (0.081)	[a]1.21(0.099)

[a]Total metals and values in parenthesis represent AB-DTPA extractable Cd.

Experimentation

This experiment was conducted in wire house; where rice (cv. Basmati-2000) was grown during 2006, nursery of which was grown in May 2006 in an uncontaminated soil. Thirty days old five seedlings were transplanted in each pot on July 17, 2006 and finally three plants per hill and four hills per pot were maintained. Fertilizer was applied @ 300-100-100 kg ha^{-1} (150-50-50 mg kg^{-1} soil) of NPK as urea, single super phosphate (SSP) and sulphate of potash (SOP), respectively. Whole of the SSP and SOP and 1/3 urea were added at sowing, while the rest of nitrogen was applied in two equal splits at the plant age 65 days and 100 days. Crop was irrigated with pumped ground water (Table 2) used to keep the pots submerged through out the growth period.

Table 2. Analysis of irrigation water used to raise crops in pots

Parameter	Unit	Value	*Permissible limit
EC	dS m^{-1}	0.59	<1.5
SAR	(mmol L^{-1})$^{1/2}$	0.57	<7.5
RSC	mmol$_c$ L^{-1}	Nil	<2.0
Cd	mg L^{-1}	Nil	0.01
Remarks	-	Suitable	-

* Ayers and Westcot (1985)

Soil and plant analysis

Soils were analyzed for saturation paste pH (pH_s), saturation paste extract EC_e, organic matter (OM), lime ($CaCO_3$), soil texture and cation exchange capacity (CEC) following methods described by the US Salinity Lab. Staff (1954) and Page *et al.* (1982). The AB-DTPA-extractable Cd was determined following the method of Soltanpour (1985) while total Cd was determined following the method of Amacher (1996). The extracted Cd was determined with the help of atomic absorption spectrophotometer (Model Thermo S-Series).

At physiological maturity, rice plants were harvested and biomass was recorded from each pot separately. The crop was threshed manually to separate grains and straw. Soon after harvesting, plants were gently rinsed in 1% HCl and then with distilled water, blotted with tissue paper and were separated into grains and straw. Plant samples were oven-dried at 70 ^{0}C to a constant weight. All the plant samples were ground in Willy mill fitted with stainless steel blades and were digested in di-acid mixture of nitric acid and perchloric acid (3:1) and analysed for Cd (Ryan *et al.*, 2001) with atomic absorption spectrophotometer (Model Thermo S-Series). Soil samples from all the pots were drawn with the help of a stainless steel sampling tube and were analysed for Cd determination in soil extracts.

Preparation of extractants for phyto-available Cd in soils

Different extractants used and procedures of extraction were:

(i) AB-DTPA: 1 *N* NH_4HCO_3+ 0.005 *M* DTPA (Soltanpour, 1985).
Ten gram soil was taken in 20 mL of freshly prepared AB-DTPA solution, shook for 15 minutes, filtered and filtrate was used for Cd concentration.

(ii) 0.005 *M* DTPA: 0.005 *M* DTPA + 0.01 *M* TEA + 0.01*M* $CaCl_2$ (Lindsay and Norvell, 1978).
Ten gram soil was taken in 20 mL freshly prepared DTPA solution, adjusted to pH 7.3, shook for 2 h, filtered and filtrate was used for Cd concentration.

(iii) 0.05 *M* EDTA: 0.05 *M* EDTA (Wear and Evans, 1968).

Two gram soil was taken in 20 mL freshly prepared EDTA solution, adjusted to pH 7.0, shook for 1h, filtered and filtrate was used for Cd concentration.

(iv) 0.01 *M* $CaCl_2$: 0.01 *M* $CaCl_2$ (Novozamsky *et al.*, 1993).
Two gram soil was taken in 20 mL freshly prepared $CaCl_2$ solution, shook for 3 h, filtered and filtrate was used for Cd concentration.

(v) 1 *M* NH_4NO_3: 1 *M* NH_4NO_3 (Symeonides and McRae, 1977).
Ten gram soil was taken in 50 mL of freshly prepared NH_4NO_3 solution, shook for 2 h, filtered and filtrate was used for Cd concentration.

(vi) 0.1 *M* $NaNO_3$: 0.1 *M* $NaNO_3$ (Gupta and Aten, 1993).
Eight gram soil was taken in 20 mL freshly prepared $NaNO_3$ solution, shook for two h, filtered and filtrate was used for Cd concentration.

Statistical analysis of data

Simple correlation between phyto-available Cd concentrations in soils extracted with different extractants and that in rice straw or grains was determined using Minitab software.

RESULTS

Crop yield

Rice straw yield was more from sandy clay loam soil (98.04 g pot^{-1}) compared to that from loamy sand (93.96 g pot^{-1}) soil. Grain yield of rice was more from sandy clay loam soil (27.69 g pot^{-1}) compared to that from loamy sand (23.06 g pot^{-1}) and is shown in Fig. 1. The effect of soil texture on straw and grain yields of rice was statistically non-significant.

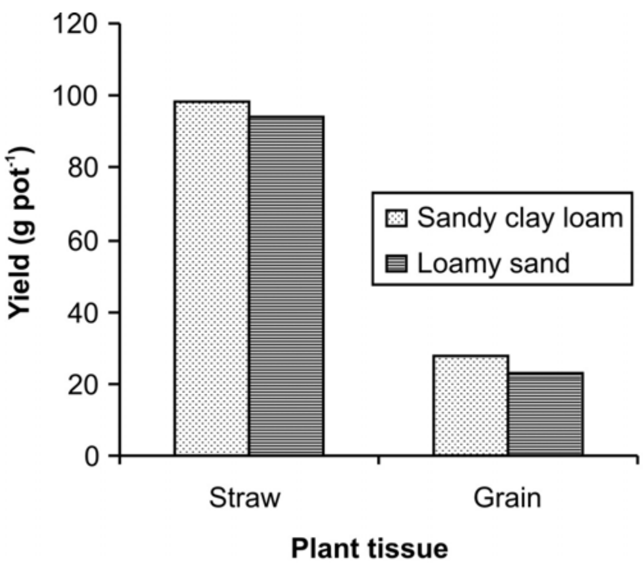

Fig. 1. Growth response of rice in texturally different Cd contaminated soils

Concentration of Cd in plants

More Cd concentration in straw (9.21 mg kg^{-1}) was recorded from loamy sand compared to that in straw (9.07 mg kg^{-1}) from sandy clay loam soil (Fig 2.) In grains, maximum Cd concentration was recorded for sandy clay loam (9.07 mg kg^{-1}) compared to that for loamy sand soil (8.16 mg kg^{-1}). The effect of soil texture on Cd concentration in straw and grains was statistically non-significant.

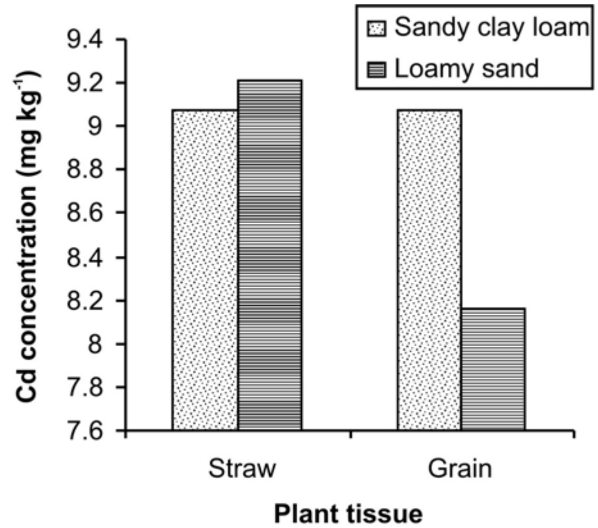

Fig. 2. Concentration of Cd in rice straw and grains from texturally different Cd contaminated soils.

Uptake of Cd by crops

In the present studies, Cd uptake by rice straw was more from sandy clay loam soil (0.89 mg pot^{-1}) compared to that in rice straw (0.87 mg pot^{-1}) from loamy sand soil (Fig. 3). In rice grains Cd uptake was more from sandy clay loam (0.22 mg pot^{-1}) compared to that with Cd uptake by grains from loamy sand soil

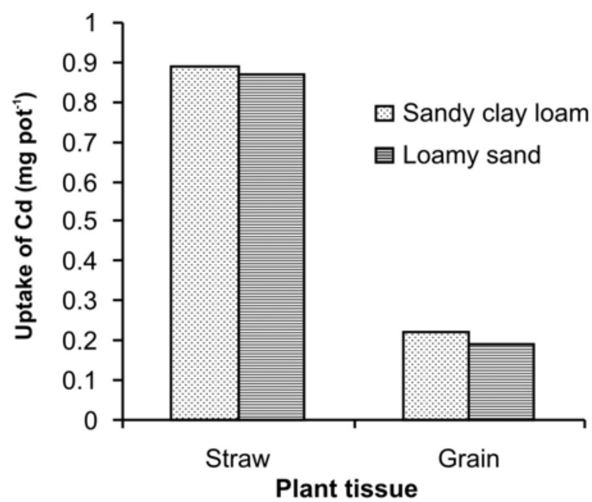

Fig. 3. Uptake of Cd by rice straw and grains grown in Cd contaminated texturally different two soils

(0.19 mg pot^{-1}). The effect of soil texture on Cd uptake by straw and grains was statistically non-significant.

Efficiency of extractants for Cd

Total Cd contents were 20.51 and 20.46 mg kg^{-1} in sandy clay loam and loamy sand soils respectively (Fig. 4). Maximum Cd (mg pot^{-1}) was extracted with EDTA (16.20) followed by DTPA (9.46), AB-DTPA (8.56), $CaCl_2$ (6.53), NH_4NO_3 (5.34) and $NaNO_3$ (4.20) which were 79, 46, 42, 32, 26 and 20 % of the total Cd concentration, respectively in sandy clay loam soil (Fig. 4). For loamy sand soil, maximum Cd (mg pot^{-1}) was extracted with EDTA (16.29) followed by DTPA (10.25), AB-DTPA (8.57), $CaCl_2$ (6.68), NH_4NO_3 (5.32) and $NaNO_3$ (4.22) which were 80, 50, 42, 32, 26 and 20 %, respectively of the total Cd concentration (Fig. 4).

(Table 3) followed by DTPA (r = 0.590). While EDTA-Cd was negatively correlated (r = -0.421) with Cd in rice straw. In grains, AB-DTPA gave maximum positive correlation (r = 0.924) followed by DTPA (r = 0.859) with rice straw Cd obtained from sandy clay loam soil. The EDTA-Cd positively correlated (r = 0.261) but was statistically similar at p = 0.05.

In loamy sand, AB-DTPA and DTPA-Cd had significant positive correlation with Cd concentration in straw and grains (Table 3). In straw, maximum positive correlation was recorded with DTPA-Cd (r = 0.814) compared to that with AB-DTPA (r = 0.623) and EDTA (r = 0.175). While in grains Cd concentration more positively correlated with AB-DTPA (r = 0.978) compared to that with DTPA (r = 0.937) and EDTA (r = -0.264).

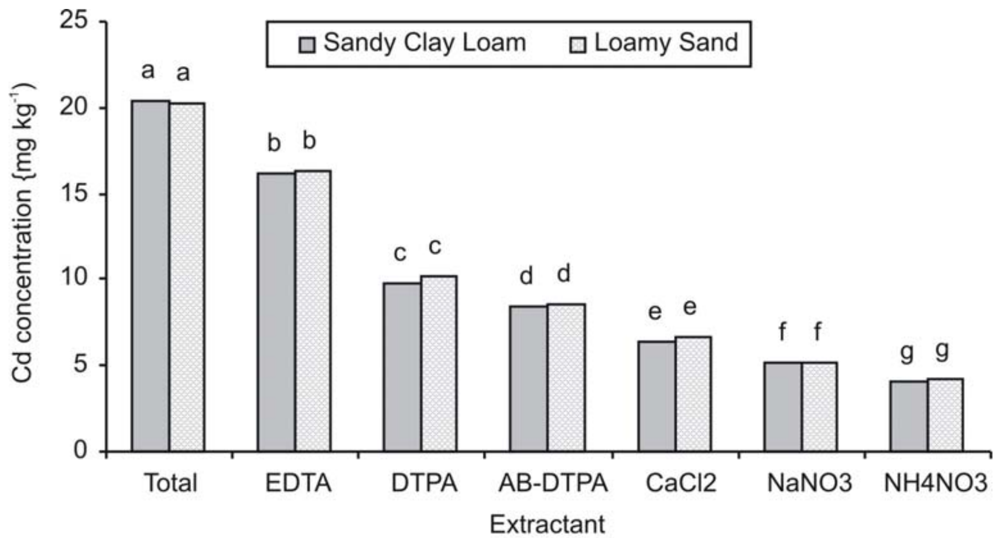

Fig. 4. Concentration of Cd extracted from Cd contaminated soils (Letters on bars indicate statistical status of extractants at P = 0.05)

Correlation between total Cd and plant Cd concentration

For sandy clay loam soil, rice straw showed negative correlation(r = -0.758) with total Cd contents in the soil (Table 3). While Cd in rice grains positively correlated with total soil Cd (r = 0.321) but the correlation was statistically similar. For loamy sand, rice straw showed negative correlation (r = -0.271) with soil Cd (Table 3). While Cd in rice grains positively correlated with total soil Cd (0.220) but the correlation was statistically similar.

Correlation between extracted Cd from soil and plant Cd concentration

For sandy clay loam, AB-DTPA gave maximum positive correlation (r = 0.979) with rice straw Cd

For sandy clay loam, $CaCl_2$, gave highly positive correlation (r = 0.656) with rice straw Cd (Table 3). While $NaNO_3$ and NH_4NO_3 showed negative correlations (r = -0.199 and r = -0.143 respectively) for rice straw Cd. In grains, $CaCl_2$ and $NaNO_3$ gave highly positive correlation (r = 0.668) followed by NH_4NO_3 (r = 0.544) with Cd in rice grains obtained from sandy clay loam soil.

For loamy sand soil, $CaCl_2$- and $NaNO_3$-Cd had significant positive correlation (at p = 0.05) with Cd contents in straw and grains of rice. Cadmium in straw gave maximum positive correlation when extracted with $NaNO_3$ (r = 0.878) compared to that with $CaCl_2$ (r = 0.829) and NH_4NO_3 (r = -0.130). While Cd concentration in grains more positively correlated with $NaNO_3$ (r = 0.926) compared to that with $CaCl_2$ (r = 0.834) and NH_4NO_3 (r = -0.006).

DISCUSSION

Correlation between soil Cd and plant Cd concentrations

Concentration of Cd in rice straw showed a negative correlation with total contents of Cd extracted from both the sandy clay loam and lomy sand soils (Table 3). In rice grain, this correlation was positive but weak and non-significant for both the textured soils. It is common concept now-a-days that total contents of Cd in soils are not a good indicator of phyto-availability, or a good tool for potential risk assessment due to different and complex distribution patterns of metals including Cd among various chemical species or solid phases (Chen *et al.*, 1996). For example, chelated to organic matter, adsorbed onto mineral surfaces or present as recently formed precipitates, which would be relatively less available to plants.

due to their dependance upon the soil characteristics like organic matter contents, soil pH, and the amount, source and form of metal contaminants, and aging of contaminant in soils. Both the DTPA and EDTA consume organic ligands for forming strong complexes with metals as the basis for the extraction process.

In our studies, $CaCl_2$-Cd gave more significant positive correlation with Cd in straw and grain of rice than $NaNO_3$-Cd and NH_4NO_3-Cd for both the soils. For loamy sand, $NaNO_3$-Cd gave more significant positive correlation with Cd in straw and grain of rice than $CaCl_2$-Cd and NH_4NO_3-Cd in soils. The NH_4NO_3-Cd correlated either poorly or negatively with Cd in grains and straw of rice. The Cd extracted with neutral salts is considered reliable technique for the estimation of heavy metals from soils (Meers *et al.*, 2005a, 2006a, b). The $CaCl_2$ extraction offers a good operational prediction for soil solution Cd and Zn (Degryse *et al.*, 2003). For the

Table 3. Correlation between Cd concentration in rice grains or straw Cd extracted from sandy clay loam and loamy sand soils

		Total-Cd	EDTA-Cd	DTPA-Cd	ABDTPA-Cd	$CaCl_2$-Cd	$NaNO_3$-Cd	NH_4NO_3-Cd
Sandy clay loam	Straw	-0.758 (0.004)	-0.421 (0.173)	0.586 (0.045)	0.979 (0.001)	0.656 (0.021)	-0.199 (0.535)	-0.143 (0.658)
	Grain	0.321 (0.310)	0.261 (0.412)	0.859 (0.001)	0.924 (0.001)	0.668 (0.027)	0.668 (0.018)	0.544 (0.067)
Loamy sand	Straw	-0.271 (0.395)	0.175 (0.587)	0.814 (0.001)	0.623 (0.031)	0.829 (0.001)	0.878 (0.001)	-0.130 (0.687)
	Grain	0.220 (0.492)	-0.264 (0.408)	0.937 (0.001)	0.978 (0.001)	0.834 (0.001)	0.926 (0.001)	-0.006 (0.986)

Values in parenthesis show the level of significance, n value for each correlation is 12.

Among different complexing agents, EDTA-Cd showed a poor correlation with Cd in grains and straw of rice obtained from both the soils. Krishnamurti *et al.* (2000) found that the two extractants based on EDTA provided the poorest prediction of Cd availability. The EDTA-Cd resulted in relatively lower correlation coefficient with Cd in *B. juncea* roots (Gupta and Sinha, 2007). Significant positive correlation was recorded with DTPA and AB-DTPA extractable Cd with Cd in straw and grains of rice from both the soils. The DTPA has been widely used to assess the phytoavailability of many trace metals, and concentration of DTPA-extractable trace metals have been reported to correlate well with their plant uptake (Cajuste *et al.*, 2000; Simmons and Pongsakul, 2004). The possible reason could be the fact that the DTPA contains a high concentration of $CaCl_2$, of which Ca^{2+} may affect desorption of other bivalent cations, especially Cd^{2+} and Zn^{2+} from soils (Lindsay and Norvel, 1978). This apparent conflict in the reported effectiveness of complexing extractants may be partially harmonisation of single extraction procedure, $CaCl_2$ procedure is found more protective in terms of risk assessment because of its higher leachability (Sahuquillo *et al.*, 2003). An important advantage is that the ionic strength of this extracting solution is mostly similar to that of the soil solution (Houba *et al.* 1996, 2000). In addition, Ca^{2+} is generally the most abundant cation present in soil solution of calcareous soils.

CONCLUSIONS

Results are consistent where AB-DTPA extractable Cd had more positive significant correlation with Cd concentration both in straw and grains of rice harvested from sandy clay loam soil than other extractants. For loamy sand soil, $NaNO_3$-Cd had more positive correlation with rice straw Cd than other extractants. Overall, AB-DTPA gave comparable results to other extractants but soil properties and plant species could play a great role in the efficiency of any single extraction procedure. Neutral salts especially $CaCl_2$ and $NaNO_3$ gave promising results, especially under alkaline conditions. So future research may focus on soil properties and plant species as a variable of efficiency of extractants and neutral salts should be given importance.

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