Labile pool of cadmium in sludge-treated soils

S.B. Pandeya, A.K. Singh and P. Jha
*Department of Soil Science, Rajendra Agricultural University, Bihar, Pusa-848 125, India**

Abstract

The labile pool of cadmium in sludge-treated soils was determined by application of isotopic dilution principle under laboratory and green house conditions using moong (*Phaseolus aureus* L.) as test crop. The laboratory indices like isotopic distribution coefficient (Kd) of ^{115}Cd in 0.1 M CaCl$_2$, labile pool (LP) of Cd in DTPA–CaCl$_2$– Na acetate (adjusted to pH 5.0, 6.0 and 7.0) and supply parameter (SP) using Kd as intensity and LP as capacity factor of Cd in soils, were computed to compare these values with actual uptake of Cd by the crop to test them as indices of Cd availability. The path-ways of transfer of soil Cd from the discrete chemical pools to plants were also computed. The LP (pH 7.0) and the SP were significantly correlated with the concentration of Cd in plants and its uptake by the crop. They are, therefore, good indices of Cd availability in sludge treated vertisol soils and can be used as reference indices for standarization of chemical extractants. The water soluble + exchangeable Cd and the 0.05 M EDTA extractable Cd were observed to be the two major chemical pools of Cd in soils responsible for supply of this element to plants. As substantial part of 0.1 M Na$_4$P$_2$O$_7$ extractable Cd applied to the soils remains in same form, they are not transfered into the food chain. The amounts of soil Cd extracted by DTPA–CaCl$_2$–TEA (pH 7.3), EDTA–NH$_4$OAC (pH 7.3) and Mg(NO$_3$)$_2$ (pH 6.0) were significantly correlated with concentration of Cd in plants and with uptake of Cd by moong crop.

Introduction

Application of sewage effluents in agricultural land may influence crops by serving as a source of nutrients and, indirectly by altering the chemistry of metal cations in soils (Lutrick et al., 1982; Singh and Nayyar, 1991; Sreeramulu, 1994; Valdares et al., 1983). Land application of municipal sewage sludge is an excellent example of direct addition of trace metals to the soils which may subsequently be taken up by plants. The heavy metals, especially Cd, may cause hazards to human and animal health, if their disposal in a soil is uncontrolled. The reaction mechanisms of humic matter complexed Cd in soil are also very complex and there is no simple relation between plant uptake and amounts present in the soil. Principles of isotopic dilution technique have been widely used for the determination of nutrient supply power of soils. The L value proposed by Tiller et al. (1972), wherein

uptake of nutrient by plants is considered as the index of nutrient supply power, is the best index of nutrient availability. As the method is time consuming, need for alternate quick laboratory indices of nutrient availability for determining nutrient supply power of soils was needed. Tiller et al. (1972) computed isotopic distribution coefficient (E value) in CaCl$_2$ as supporting electrolyte for its comparison with 'L' value of Zn in soils. These two values were very poorly correlated, probably because of the fact that there was irreversible fixation of ^{65}Zn which can be utilized by plants. Use of chelating agents as extractant in place of neutral supporting electrolyte for determination of labile pool of metal cations in soils was tried by Ganai et al. (1982); Lindsay (1973); Lopez and Graham (1972); Sinha et al. (1977); Viets and Lindsay (1973) with varied success. They used isotopic exchange of trace metal cations in DTPA–CaCl$_2$-sodium acetate solutions buffered at different pH values for determination of labile pool (LP) of nutrients. Their results indi-

* FAX No.: +91 -6274 -74226. E-mail: rau@bih.nic.in

cated good agreement between LP and L values. An unified index of nutrient availability, termed as supply parameter (SP) which simultaneously incorporates the intensity, capacity and buffering, was proposed by Khaswaneh (1971) which was found to be good index of availability of nutrients like P_2O_5, and Zn in soils by workers like Khaswaneh and Copeland (1973) and Vig et al. (1978). The laboratory indices of Cd availability is, probably, non-existant in literature.

The determination of size of chemical pools of trace metals with the use of sequential extractions of soils and sludge have been attempted by various workers (Emmerich et al., 1982; Keefer et al., 1979; Soon and Bates, 1982; Sposito et al., 1982; Stover et al., 1976). They tried different versions of sequential extraction procedures making the comparison of different results difficult. There is evident lack of information on the path-ways of sludge borne cadmium by which they are transferred into the food and feed chain.

A number of chemicals in a variety of combinations and concentrations has been tried to evaluate the amount of available Cd in soils and sludges (Binghum et al., 1975; Comb and Dowdy, 1982; Ganai et al., 1982; Gaynor and Halstead, 1976; Latterell et al., 1978; Sommers et al., 1987). The criteria of suitability of extractants put forward by Mitchell et al. (1978), however, could be partially satisfied by these results. Moreover, the soil extracting procedure by reagents for determination of availability of a particular element needs to be tested for specific soil and plant situations before it is adopted by the soil testing laboratories. The objectives of the present investigation were: (i) to determine the labile pool of Cd in sludge treated vertisols, by estimating actual uptake by the crop and to compare these values with those obtained by isotopic exchange in neutral salt and in buffered DTPA; (ii) to evaluate the values of supply parameter, an unified index involving quantity-intensity-buffering parameters, as index of Cd availability by comparing it with actual Cd uptake by the crop; (iii) to evaluate the path-way of transformation of added sludge borne cadmium in discrete chemical pools and their influence on uptake of Cd by plants; and (iv) to test the suitability of chemical extractants as index of available Cd in soils.

Materials and methods

Ten surface soil samples (0–15 cm) of old alluvium non-calcareous, non-saline (vertisol) soils of Patna, Bihar (India) which received sewage effluents for more than a hundred years, were collected for the studies. The treatment of sewage over a period of years in these soils increased the organic carbon content to a large extent; the greater impact being on soils near the point of discharge of sewage sludge. The air dried and sieved (2 mm) soils were analysed for their physical and chemical properties (Table 1). The mechanical analysis of the soils was done according to International pipette method (Piper, 1966) and pH and EC were measured in 1:2 (soil:water) suspension. Walkely and Black's rapid titration method as documented by Jackson (1967) was followed for the estimation of carbon contents of soils.

Sludge

Sludge was collected from Patna sewage sludge treatment plant. The processed sludge contained 45.5% organic carbon, 1.5% nitrogen, 0.56% phosphorus, 1.0 mg kg^{-1} total Cd and 0.10 mg kg^{-1} DTPA extractable cadmium.

Fractionation and purification of fulvic acid from sludge

A modified procedure of Kononova (1966) for fractionation of fulvic acid from sludge and modified Forsyth's (1947) procedure for purification of the fulvic acid, as described in detail by Pandeya (1992) was followed. The fulvic acid had 30.03% carbon, 6.18% hydrogen, 5.58% nitrogen and 58.21% oxygen. Total acidity of the fulvic acid, estimated by potentiometric titration was 548 cmol kg^{-1}. The approximation of molarity of fulvic acid in the aqueous system was made by the procedure of Pandeya (1991). Equimolecular quantities of $CdCl_2$ and fulvic acid (pH adjusted to 8.0) were mixed to prepare stock solution of Cd-FA complex for further use.

Pot experiment

Pots lined with polythene sheets were filled with 1 kg of each of the ten air dried soils in six replications. To each pot, 50 mg kg^{-1} N, 40 mg kg^{-1} P_2O_5 and 40 mg kg^{-1} K_2O in the form of urea, KH_2PO_4 and KCl solutions, respectively, were added. Three pots of each soil received carrier free ^{115}Cd in aqueous solution at rate of 50 μCi kg^{-1} soil. In remaining three pots of each soil sample, $CdSO_4$ at the rate of 89.0×10^{-2} mmol kg^{-1} soil in the form of (1:1) Cd–FA complex and tagged with ^{115}Cd at the rate of 50 μCi kg^{-1}

Table 1. Properties of the soils used for studies

Soil no.	Sand (%)	Silt (%)	Clay (%)	Textural class	pH	EC (dSm^{-1})	Org. C (%)
1	48.20	34.00	7.20	Silt loam	6.71	1.00	1.45
2	50.80	32.40	6.50	Sandy loam	6.91	1.30	1.13
3	41.90	39.20	9.20	Silt loam	6.93	1.30	1.44
4	41.50	38.20	11.40	Silt loam	7.07	1.25	1.22
5	41.70	37.40	10.20	Silt loam	7.02	1.00	1.33
6	45.80	35.60	8.90	Silt loam	6.91	1.10	1.11
7	41.10	38.80	10.60	Silt loam	6.71	1.55	1.32
8	37.00	37.40	16.00	Silt loam	6.60	1.75	1.04
9	41.70	36.60	13.20	Silt loam	6.78	1.60	1.16
10	40.70	35.00	14.00	Silt loam	6.21	2.25	1.32

soil was added. The fertilizers and Cd source applied to the soils were mixed well in the shielded hood to ensure uniform distribution of ^{115}Cd isotope in the soils. Water was applied to the soils to keep the moisture content at field capacity. The pots were then left for equilibration with alternate wetting and drying for two weeks. Five plants of moong (*Phaseolus aureus* L.) were allowed to grow after germination. Irrigation with deionized water was performed to the test crop as per need. The plants were harvested after 60 days growth of the crop. The whole plants were washed thoroughly first in 0.1 *M* HCl and then in distilled water. Two grams of dried shoot of plant samples were weighed and digested in tri-acid (Piper, 1966). The final volume of the digest was made to 10 mL with distilled water. A two mL of aliquot of solution containing the digested plant material was used for counting ^{115}Cd on gamma-ray spectrometer and concentration of Cd in the plant digest was determined on an atomic absorption spectrophotometer. The per cent activity of Cd in plant parts and in fertilizer together with per cent Cd derived from fertilizer were computed with the help of following relationships:

% derived from fertilizer =

$$\frac{\text{specific activity in plant}}{\text{specific activity in fertilizer}} \times 100$$

where

specific activity in plant =

$$\frac{\text{counts of } ^{115}\text{Cd (count per s)}}{\text{total amount of Cd in plant (mmol)}},$$

specific activity in fertilizer =

$$\frac{\text{counts of } ^{115}\text{Cd (count per s)}}{\text{total amount of Cd in fertilizer (mmol)}}.$$

Distribution coefficient (Kd) of Cd in soils

Distribution coefficient in presence of a constant concentration of an electrolyte is the measure of the distribution of Cd isotope in solid and solution phase. It approximates the quantity/intensity relationship provided the ^{115}Cd is only involved in isotopic dilution with the specifically bonded surface Cd and that in the equilibrium soil solution. The distribution coefficient of Cd in sludge treated soils was determined by equilibrating 5 g soil samples in triplicate in 10 mL of 0.1 *M* CaCl$_2$ solution containing carrier free ^{115}Cd isotope at the rate of 0.10 μCi per mL extractant in centrifuge tubes. The tubes were placed on a horizontal shaker and equilibrated for a period of 24 h at room temperature. The suspensions were then centrifuged and 2 mL aliquot was taken for counting the activity of ^{115}Cd on a well type scientillation head of gamma-ray spectrometer. The actvity of Cd in solid phase was calculated from the difference between the activity of Cd before and after equilibration with soil. The values of distribution coefficient of Cd (Kd$_{cd}$) of soil samples were calculated from the equation (Lopez and Graham, 1972):

$$\text{Kd}_{cd} = \frac{\text{Activity of } ^{115}\text{Cd in solid phase (cps)}}{\text{Weight of soil (g)}} \times \frac{\text{Volume of solution added (mL)}}{\text{Activity in solution (cps/mL)}}. \quad (1)$$

Labile pool of cadmium in soils

Stock solutions containing 0.005 *M* DTPA, 0.01 *M* CaCl$_2$ and 0.1 *M* Na-acetate buffered at pH values

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of 5.0, 6.0 and 7.0 were separately prepared. The extractant contained 0.1 μCi of ^{115}Cd per mL. Isotopic exchange was carried out in triplicate by equilibrating 5 g soil with 10 mL extractant. The soil suspension was shaken on a shaker for 48 h at room temperature and a 2 mL aliquot was removed from the centrifuge tube for estimation of activity of ^{115}Cd on gamma-ray spectrometer.

The amounts of stable Cd in the soil extracts were determined by the extraction procedure as described above, but separately, in extracting solution without radio active isotope. The concentration of Cd in the extract was determined by atomic absorption spectrophotometer. The labile pool (LP) of Cd was calculated from the values of Cd concentration and isotopic measurements with the help of following equation (Graham, 1973):

$$LP_{cd} = \frac{\text{Concentration of Cd in soil extract (mg/kg)}}{\text{Radioactivity of } ^{115}\text{Cd in soil extract (\%)}} \times 200$$

Per cent ratio activity $=$

$$\frac{\text{Average count of } ^{115}\text{Cd in extract (cps/mL)}}{\text{Average count of } ^{115}\text{Cd in original solution added in soil (cps/mL)}} \times 100.$$

Supply parameter (SP)

The supply parameter was calculated by taking Kd as the index of intensity and LP as the index of capacity together with the data on adsorption isotherm for the computation of constants K_1 and K_2. It was observed in a pilot trial that the isotherm with data on Kd as an index of equilibrium concentration and LP as index of reserve of Cd in soil solids followed Langmuir adsorption isotherm. The plot of 1/Kd vs. 1/LP also followed straight line relationship. The quantity-intensity relationships of Cd were worked out, using the equation:

$$q = (K_1 C)/(K_2 + C) \tag{2}$$

where q = capacity of nutrient (LP$_{cd}$); C = intensity (Cd remaining in solution or Kd); K_1 and K_2 are constants, which were determined by plotting $1/q$ vs. $1/C$; K_1 = reciprocal of intercept of $1/q$ - $1/C$ plot, and K_2 = slope of the plot $\times K_1$.

The values for the expression called supply meter (SP) which describes the interaction of quanity, intensity and buffering capacity of soil Cd were calculated

by using the relationship (Khaswaneh, 1971)

$$SP = \sqrt{qc/\sqrt{K_1 K_2}}. \tag{3}$$

Fractionation of Cd in soils

A modified procedure of Stover et al. (1976) was used for the fractionation of Cd in post harvest soils sampled from treated and control pots. The composition of the chemical extractants sequentially used, the soil solution ratio, shaking time and other details are presented in Table 2. The procedure of Stover et al. (1976) was modified in the present investigation for fractionation of Cd in sludge-treated soils as follows:

(i) The strength of KNO$_3$ solution as first extractant was kept to 0.5 M in place of 0.1 M. Stover et al. (1976) extracted water soluble + exchangeable Cd with 0.1 M KNO$_3$, while it was 0.5 M KNO$_3$ by Sposito et al. (1982) and Mahler and Ryan (1988).

(ii) Extraction with water as above by Mahler and Ryan (1988) and with KF by Stover et al. (1976) to extract adsorbed Cd in soils was omitted in the present investigation. KF and water as extractant at step two failed to extract measurable amounts of Cd from soils in a pilot study.

Two g of each soil sample was taken in 40 mL centrifuge tubes with stoppers. The first extractant was added to it and was placed on an end to end shaker. After equilibration period, the contents of the tubes were centrifuged and clear supernatant solution was decanted carefully so that the soil settled at the bottom is not disturbed. Approximately one mg of solid DTPA was added to the aliquot to prevent precipitation of Cd and a drop of toluene was added to prevent microbial growth.

The same sample was used in the same centrifuge tube for extraction by other extractants as per procedure described for the first extractant. The content of Cd in the extracts were measured by atomic absorption spectrophotometer. A correction for the amount of Cd extracted by extractants 2, 3 and 4 was done by determining the water retained by the soil after centrifugation and decantation of extracts and concentration of Cd in the extract.

The processed post harvest soil samples of the pot experiment were used for extraction of Cd by different chemical reagents. The nature of the chemical reagent, soil solution ratio, shaking time and other details are reported in Table 2.

Table 2. Experimental details for fractionation of cadmium in soils and its extraction by chemical reagents

Composition of extractant	Soil extrac-tant ratio	Shaking time (h)	Chemical pool extracted	References
Fractionation				
0.5 M KNO$_3$	1:10	16	Water soluble + exchangeable	Emmerich et al. (1982); Sposito et al. (1982); Stover et al. (1976)
0.1 M Na$_4$P$_2$O$_7$	1:20	16	Organically bound or organically complexed	
0.05 M EDTA (pH 7.3)	1:20	8	Inorganically complexed Cd	
4.0 M HNO$_3$	1:10	16	Residual form	
Extraction				
(0.005 M DTPA + 0.01 M CaCl$_2$ + 0.1 M TEA) at pH 7.3	1:2	2	–	Lindsay and Norvell (1978)
(1 M NH$_4$OAC + 0.1 M EDTA) at pH 7.3	1:5	1/2	–	Ganai et al. (1982)
0.1 M Mg(NO$_3$)$_2$ at pH 6.0	1:5	1	–	Ganai et al. (1982)

Table 3. Dry matter yield (g/pot), concentration of Cd in plant (mg/kg) and its uptake (μg/pot) by moong grown in sludge-treated soils

Soil no.	Dry matter yield			Concentration			Uptake		
	Control	Treated	Mean	Control	Treated	Mean	Control	Treated	Mean
1	7.20	7.31	7.26	0.58	0.61	0.59	4.19	4.48	4.33
2	3.76	3.41	3.59	0.65	0.90	0.77	2.46	3.09	2.78
3	5.77	5.68	5.72	0.85	1.34	1.09	4.92	7.59	6.26
4	3.73	3.62	3.67	1.55	1.76	1.65	5.78	6.36	6.07
5	4.83	5.06	4.95	1.02	1.11	1.07	4.92	5.63	5.27
6	3.35	3.48	3.42	0.65	0.77	0.71	2.18	2.71	2.44
7	4.62	4.94	4.78	0.90	0.96	0.93	4.17	4.77	4.47
8	2.79	2.65	2.72	2.24	2.16	2.20	6.24	5.74	5.99
9	3.80	3.47	3.64	1.59	1.82	1.71	6.01	6.33	6.17
10	4.00	4.05	4.03	1.15	1.45	1.30	4.60	5.86	5.23
Mean	4.38	4.37	–	1.12	1.29	–	4.55	5.26	–
Cd (0.05)									
Soils			0.250			0.111			0.596
Levels			0.122			0.050			0.266
Soil×levels			0.161			0.114			0.585

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Results and Discussion

Dry matter yield of moong grown in sludge treated soil

The dry matter yield of moong crop grown in pots with ten sewage-sludge treated soils without or with treatment of Cd–FA, is reported in Table 3. The dry matter yields obtained in pots with ten different soils were observed to be significantly influenced by soil properties. Significant positive correlation coefficient between organic carbon content and dry matter yield of the crop (r = 0.898**) indicates that humic substances in soils help in producing high yield, probably by providing essential nutrients in balanced amounts and in readily available forms. The contribution of organic matter in influencing yield of the crop by way of providing essential nutrients to plants has been reported by several workers (Narwal et al., 1983; Sreeramulu, 1994; Valdares et al., 1983).

The organically complexed Cd was applied to the soils to simulate a system where soils are supplied with organically bound Cd due to continued application of sewage-sludge over a period of years. The application of Cd–FA at the rate of 89.0×10^{-3} mmol kg^{-1} soil did not significantly influence the yield. This indicates that the level of applied Cd is in a range where Cd toxicity is not observed.

Concentration of Cd in plant and its uptake

The observed concentration of Cd in plants (Table 3) were found to be well within the range reported in literature for normal crops by Sommers el. (1987). The clay content of the soils had a significant positive influence (r = 0.886**) on concentration of Cd in plants grown in soil without Cd–FA treatment. It seems possible that the pool of exchangeable Cd on soil clays are in bioavailable forms.

Application of Cd–FA increased the uptake significantly over the corresponding controls (Table 3). Different soils provided statistically different amounts of Cd to plants. The interaction effect of soils and application of Cd was also found to be statistically significant, meaning thereby, that different soils behave differently with respect to determining the uptake of Cd by the crop. Regression equation showing relationship of uptake of Cd and the soil properties

Table 4. Fraction of Cd (per cent) in plant parts derived from applied Cd–FA in sludge-treated soils

Soil no.	Root	Shoot	Total
1	3.04	1.71	2.08
2	4.76	3.18	4.08
3	4.68	1.77	3.32
4	24.60	4.84	14.35
5	9.08	4.31	6.59
6	24.90	4.16	8.75
7	23.80	5.70	11.68
8	29.00	8.23	18.10
9	18.30	5.49	11.90
10	19.00	4.85	11.85
Cd (0.05)	3.51	0.91	1.59

is:

$$\text{Uptake} = -26.80 + 2.88 pH + 0.54 \text{ clay} + 4.85 \text{ organic carbon}$$
$$(0.52) \qquad (1.18) \qquad (0.50)$$
$$R^2 = 0.824^{**}, \qquad Adj. \ R^2 = 0.736^*$$

The figures in the parentheses are the standard regression coefficient. The positive contribution of soil organic carbon on uptake of Cd may be indirect effect because high carbon contents are probably the result of high levels of sludge (and thus Cd) applications.

The data on fractions of Cd in the plants which were derived from the applied source Cd–FA indicates that the percent use-efficiency of Cd–FA by plants differed from soil to soils (Table 4). The values of percent use-efficiency of Cd applied were significantly correlated with clay contents (r = 0.875**) of the soils. The soil properties rather than contents and forms of Cd applied to soils have been found to determine the uptake of Cd by plants by Corey et al. (1987).

Labile pool (LP)

The soils were equilibrated in DTPA–CaCl$_2$–Na acetate solutions of pH 5.0, 6.0 and 7.0 to determine the labile pool of Cd in sludge-treated soils. The pH of the equilibrium solution in three cases were not mantained at all. The soil and the extracting solutions were two different buffered systems and thus, behaved in different ways. Measured mean pH values of 7.1, 7.1 and 7.3 correspond with preset levels of pH 5.0,

Table 5. Laboratory determination of labile pool (LP) of Cd in DTPA–CaCl$_2$–Na acatate solution buffered at pH 5.0, 6.0 and 7.0 and calculated values of distribution coefficient (Kd) of Cd in sludge-treated soils

Soil no.	pH of equilibrium solution				Labile pool (mg/kg)				Cd distribution coefficient (Kd)
	5.0	6.0	7.0	Mean	pH 5.0	pH 6.0	pH 7.0	Mean	
1	7.0	7.1	7.2	7.1	0.24	0.21	0.15	0.20	48.20
2	5.9	7.1	6.7	6.6	0.02	0.15	0.08	0.08	20.90
3	7.2	7.3	7.2	7.2	0.36	0.14	0.26	0.22	40.90
4	7.3	7.3	7.5	7.4	0.56	0.65	0.56	0.59	24.30
5	7.1	7.2	7.4	7.3	0.39	0.20	0.29	0.29	32.40
6	6.7	7.4	7.2	7.1	0.17	0.45	0.16	0.26	20.90
7	7.1	7.4	7.4	7.4	0.39	0.29	0.29	0.32	32.40
8	7.3	7.5	7.5	7.5	0.85	0.78	0.58	0.74	29.90
9	7.2	7.4	7.4	7.3	0.64	0.62	0.51	0.58	24.30
10	7.3	7.3	7.3	7.3	0.64	0.64	0.40	0.56	30.39
Mean	7.1	7.1	7.3	–	0.43	0.41	0.38	–	–
Cd (0.05)									
Soil				0.089				0.029	3.790
pH				0.049				0.016	
Soil×pH				0.182				0.085	

6.0 and 7.0 (Table 5). Lopez and Graham (1972) have suggested that the values of labile pool do not differ much due to change in the pH of the equilibrating solution while, Sinha et al. (1977) demonstrated that the pH of the equilibrating solution provides different values of labile pool. The aspect of varying pH of the equilibrating solution on the values of labile pool however, could not be resolved in the present investigation. Keeping in view the pH of the soils under study falling in neutral range, the extractant adjusted to pH 7.0 may provide extracted amount of Cd near to those available to growing plants. The calculated values of labile pool with extractant of pH 7.0 were therefore, considered for further investigations.

Distribution coefficient (Kd)

A simultaneous determination of distribution coefficient of [115]Cd between solid and solution phase in 0.1 M CaCl$_2$ (Table 5), indicated variation in its values in different soils. The distribution coefficients of Cd in presence of constant concentration of CaCl$_2$ may approximate the *q/I* relationships provided the [115]Cd is only involved in the isotopic dilution with specifically bound Cd and that, in equilibrium solution. It

indirectly provides the measure of the intensity term of Cd in soils which otherwise, is not possible to be measured directly. At relatively low levels of the labile pool of Cd in soils, the distribution coefficient may assume greater significance in controlling its availability to plants. Based on these considerations, a simultaneous determination of the labile pool and distribution coefficients has the premise of delineating soils into different categories of Cd availability.

Supply parameters (SP)

The supply parameter, an unified index of availability of Cd to plants involves the intensity, capacity and buffering capacity of soil, with respect to Cd (Khasawneh and Copeland, 1973). It has generally been assumed that the integrated impact of intensity, capacity and buffering capacity of soils influences the flux of the ions to the plants roots. Consequently, the unified index consisting of these three parameters was computed to test its validity as an index of availability of Cd to plants. The calculated values of constants K_1, and K_2 with the coefficient of determination for straight line fit of relationships between $1/q$ vs. $1/C$ are reported in Table 6. The data in the

Table 6. Constant K$_1$ and K$_2$, the coefficients of determination and the supply parameter (SP) of Cd in sludge-treated soils

Soil no.	K_1	K_2	Coefficient of determination (R^2)	Supply parameter (SP)
1	−1567.6	−3817.0	0.999	0.054
2	2737.5	2337.8	0.999	0.026
3	401.9	442.9	0.996	0.180
4	1152.5	2455.9	0.999	0.079
5	282.2	742.3	0.993	0.144
6	2024.3	4830.0	0.999	0.033
7	−175.6	−149.4	0.706	0.241
8	−188.5	−164.4	0.978	0.304
9	−163.1	−395.9	0.952	0.223
10	−392.5	−576.1	0.996	0.145

Cd (0.05) = 0.020

Table 7. Relationship of laboratory indices of availability in sludge-treated soils with the soil properties

Parameters	Kd	Labile pool (LP) (pH 7.0)	Supply parameter (SP)
pH	−0.192	−0.186	−0.316
EC	−0.163	0.502	0.547
Clay	−0.183	0.888**	0.867
Org. carbon	0.820**	−0.321	−0.292
Kd		−0.196	−0.090
LP at pH 7.0			0.844**

*significant at 5% level, ** significant at 1% level.

present investigation fitted into the straight line equation with high values of coefficient of determination. The supply parameter calculated by taking Kd as the index of intensity together with the data on adsorption isotherm for the computation of constants K_1 and K_2. The values of supply parameter in different soils were significantly different from each other with the maximum value observed in soil no. 8 and minimum in soil no. 2.

The relationship of LP, Kd and SP with soil properties and Cd uptake

The correlation matrices between LP, Kd and SP and soil properties are presented in Table 7. Significant positive correlations between labile pools of Cd in soils for equilibrating solutions having pH 7.0 and the clay contents of the soils were observed. Interestingly, the values of labile pool did not bear any relationship with the organic matter content of soils. The positive significant relationship between organic carbon contents of the soils and the values of distribution coefficients of Cd, however, indicate that the added soluble Cd may first be transferred to organic pools and the ionic activity of Cd in soils is mainly controlled by 0.1 M Na$_4$P$_2$O$_7$ extractable Cd. The calculated values of supply parameter were significantly and positively correlated with clay contents and the values of labile pools of Cd in soils. This was, however, not correlated with values of Kd and other soil properties.

Step down multiple regression equations, showing the relationship of LP, Kd and SP with soil properties are presented in Appendix 1. It is apparent that the values of LP at pH 7.0 could be described on the basis of pH and clay contents of the soils with nearly 89% confidence. Inclusion of some other soil properties improves the description very marginally. The values of Kd could be accounted for with a confidence of 67% on the basis of organic carbon alone, though inclusion of other soil properties improves the confidence considerably (84%). Similarly, the supply parameter could be described with a confidence of 75% on the basis of clay contents of the soils alone.

The values of Kd did not exhibit significant relationship with Cd concentration in plants and uptake of Cd by the crop. This is obvious because of the fact that ambient concentration of Cd in any type of soil is

extremely low. The observations are not in conformity with the reports by Sinha et al. (1977). The nature of soil such as organic matter and clay content which provide adsorption sites on the solid surfaces, may be the reason for such deviation. The relationship of labile pool (LP) of Cd determined at pH 7.0, however, was significantly correlated with concentration of Cd in plants ($r = 0.934^{**}$) and with Cd uptake by the crop ($r = 0.879^{**}$). The labile pool of Cd calculated at pH 7.0 may, therefore, be taken as good index of availability of Cd in sludge-treated soils. The values of supply parameter were found to be positively correlated with the concentration of Cd in plants and its uptake by the crop ($r = 0.855^{**}$ and 0.722^{**}, respectively). This also, therefore, seems to be a valid index of availability of Cd in these soils.

The results as above, indicate that isotopic dilution of radioactive ^{115}Cd as the base indices of labile pool can serve as a reliable reference method for measurement of total labile pool of Cd in sludge-treated soils. This could be used as reference method for calibrating soil chemical testing procedures that removes amounts of Cd equal to or directly proportional to the amount of available Cd to plants.

Chemical pools of Cd in soils

Water soluble and exchangeable cadmium

Water soluble + exchangeable Cd extracted by 0.5 M KNO$_3$ ranged between 1.05×10^3 and 1.89×10^3 mmol Kg^{-1} soil. This constituted 4.2–7.2% of the total Cd present in different soils. Enrichment of soils with respect to water soluble and exchangeable Cd due to application of Cd–FA was substantial, but the percentage of Cd present in this form in treated soils were found to be lower to those in untreated soils. The Cd content in this fraction of soil was significantly correlated with EC ($r = 0.640^*$) and clay content of soils ($r = 0.934^{**}$). This indicates that clay content of the soils by and large, holds Cd in exchangeable forms. The role of organic carbon in determining this form of Cd in soils was non-significant, possibly because Cd is retained by humic substances in non-exchangeable forms and humic matter has high bounding energy for Cd.

0.1 M Na$_4$P$_2$O$_7$ extractable Cd

The pool of cadmium extracted by 0.1 M Na$_4$P$_2$O$_7$ may be designated as organically bound cadmium (Emmerich et al., 1982; Sposito et al., 1982; Stover et al., 1976) or organically complexed Cd. Organic

pool of Cd extracted by 0.1 M Na$_4$P$_2$O$_7$ ranged between 12.1×10^3 to 14.3×10^3 mmol Kg^{-1} in control soils and forms the bulk of the total Cd present. The application of Cd–FA in soils resulted in appreciable increase in the values of this pool of cadmium. The percentage of total cadmium present in 0.1 M Na$_4$P$_2$O$_7$, extractable Cd form, however, increased marginally in the Cd–FA treated soils in comparison to those in controls. The Cd content in this form was observed to be significantly correlated with organic carbon contents of the soils ($r = 0.860^*$). The significant negative correlation of 0.1 M Na$_4$P$_2$O$_7$, extractable Cd with water soluble + exchangeable Cd ($r = -0.701^*$) indicate that Cd^{2+} has higher affinity with the humic matter than with clays. The application of 0.1 M Na$_4$P$_2$O$_7$, extractable Cd in the form of Cd–FA in these soils enriched the pyrophosphate extractable Cd, the extent of which were dependent upon clay content of the soils ($r = 0.671^*$). Protection of humic matter applied to soil by soil clays from biological and chemical decomposition has been postulated by Greenland (1965, 1971) and Pandeya (1992). This may occur through process of clay-humus complex formation by way of ion bridging between positively charged multivalent cation and the negatively charged clay and humus molecules. The role of clay in protecting the 0.1 M Na$_4$P$_2$O$_7$ extractable metals in soils by formation of clay-humus-complexes thus, need not be emphasised.

0.05 M EDTA extractable Cd

Inorganic pool of Cd extracted by 0.05 M EDTA ranged between 4.61×10^3 to 8.4×10^3 mmol Kg^{-1} soil. This constituted 19.8–31.8% of the total Cd present in different sludge treated soils. Enrichment of soils with respect to this chemical pool of Cd by application of Cd–FA at the rate of 89×10^{-3} mmol Kg^{-1} soils was substantial and the percentage of Cd present in this form in treated soils was also a bit higher in comparison to those in control soils. The Cd content in this fraction was significantly correlated with clay ($r = 0.897^*$). Specific site adsorption of Cd in the lattice structure of clay seems to form this pool of Cd in soils. Alternately, Cd of this pool may remain non-exchangeable as they might be occluded by carbonate concretion. A significant positive relationship between EDTA extractable Cd in Cd–FA treated soils and the clay content ($r = 0.641^*$) further substantiates the above observation.

Residual cadmium

Residual Cd extracted by 4 *M* HNO$_3$ ranged between 4.0×10^3 to 4.63×10^3 mmol Kg^{-1} soil. This constituted 15.2–18.5% of the total Cd present in different soils. Application of Cd–FA enriches the soil with respect to residual Cd substantially, but the percentage of Cd present in this form in treated soil was found to be lower than those in control soils. The Cd content in this fraction in control soils was found to be significantly correlated with organic carbon (r = 0.728*). In treated soils, however, the 4 *M* HNO$_3$ extractable Cd was found to bear highly significant positive correlation with clay content of the soils (r = 0.805**). The applied soluble form of complexed Cd seems to get precipitated to enrich this pool.

On the basis of correlation studies it may be postulated that water soluble + exchangeable Cd is in reversible equilibrium with 0.05 *M* EDTA extractable Cd. Similarly, organic pool of Cd is in reversible equilibrium with residual pool of Cd. Transformation of water soluble + exchangeable to organic pool of Cd is unidirectional. Similarly, the 0.1 *M* Na$_4$P$_2$O$_7$ extractable Cd gets converted into 0.05 *M* EDTA extractable Cd in only one direction. 0.1 *M* Na$_4$P$_2$O$_7$ extractable Cd may form part of water soluble + exchangeable pool only after getting converted into 0.05 *M* EDTA extractable Cd form while, the residual Cd will have to follow the path via 0.1 *M* Na$_4$P$_2$O$_7$, extractable and then to 0.05 *M* EDTA extractable Cd form before it may enter into water soluble + exchangeable pool.

Chemical pools of Cd in soils responsible for supply of Cd to plants

A significant positive correlation between Cd-concentration in plants and KNO$_3$ extractable Cd (r = 0.881**) and EDTA extractable Cd (r = 0.889**) was observed. The uptake of Cd by plants was found to be significantly correlated only with the 0.05 *M* EDTA extractable Cd (r = 0.688*). This indicates that exchangeable + water soluble and 0.05 *M* EDTA extractable Cd contribute major portion of Cd to plants. The contribution of different chemical pools of Cd in soils in determining concentration of Cd in plants and its uptake by the crop was analysed by the path analysis. The concentration of Cd in plants are directly influenced by size of the chemical pool, viz., water soluble + exchangeable, 0.05 *M* EDTA extractable and 0.1 *M* Na$_4$P$_2$O$_7$ extractable cadmium. The direct contribution of residual Cd in determining the concentra-

tion was negative meaning, thereby, that this chemical pool has got very little significance in determining the concentration of Cd in plants. The contribution of KNO$_3$ extractable Cd indirectly via 0.1 *M* Na$_4$P$_2$O$_7$ extractable Cd was also a evident. The major pathway through which the 0.05 *M* EDTA extractable Cd become available to plants was through its transformation into water soluble + exchangeable form. The uptake of Cd as function of the size of chemical pools of Cd present in soils indicate that 0.1 *M* Na$_4$P$_2$O$_7$ extractable Cd, water soluble + exchangeable Cd and residual form Cd are important pools determining uptake. The KNO$_3$ extractable Cd seems to have played a dominant role in determining the uptake of Cd by the crop directly. The contribution of 0.05 *M* EDTA extractable Cd directly was of lesser significance in comparison to its contribution after its transformation into water soluble + exchangeable forms.

Suitability of extractants

The amount of Cd extracted by extractants like DTPA, EDTA–NH$_4$OAC and Mg(NO$_3$)$_2$ are reported in Appendix 2. The amount of Cd extracted by DTPA in Cd–FA treated soil was higher in comparison to control. This may be probably due to the fact that a sizeable amount of applied Cd–FA remains in labile form. The average amounts of Cd extracted from soils by these extractants were in the order: EDTA–NH$_4$OAC > DTPA > Mg(NO$_3$)$_2$. The variations in the values of the concentration of Cd in different extractants were significantly different in soils. The application of Cd–FA in soils increased the amounts of Cd extracted by different extractants. A significant positive relationships (r = 0.826**, r = 0.833** and r = 0.811**) of the amounts of Cd extracted from control soils by reagents DTPA, EDTA–NH$_4$OAc and Mg(NO$_3$)$_2$ and the clay content of the soils was observed. Organic carbon content, pH of soils and electrical conductivity did not bear any significant relationship. The results, therefore, indicate that Cd held by the clay forms the major pool of labile cadmium even in sludge-treated organic matter rich soils.

The use of chemical extractants is the most common and accepted practice for determination of available Cd content in soils. Three chemical extractants were tested for their suitability as valid indices of availability of Cd in sludge-treated soils. The correlation matrices drawn indicating relationship between the concentration of Cd in plants or uptake of Cd by the crop and the amount of Cd extracted by DTPA,

EDTA–NH$_4$OAC and Mg(NO$_3$)$_2$ are presented in Appendix 3. The results indicate highly significant and positive relationship of Cd concentration in plants and the amount of Cd extracted from soils by these three extractants. Similar observations were also found to be true for uptake of Cd by plants. The results, therefore, indicate that these three extractants are more or less equally good extractants which may be used for predicting availability of Cd in soils. One of the drawbacks of Mg(NO$_3$)$_2$ as an extractant is that it extracts very small amount of Cd from soils, which may pose problems in its detection in the extracts. Its suitability due to this reason may be limited. Among the extrac-

tants, DTPA may be preferred over EDTA–NH$_4$OAC, as correlation coefficients of the DTPA with concentration and uptake of Cd are higher in comparison to those with EDTA–NH$_4$OAC. The two laboratory indices named labile pool at pH 7.0 and the supply parameter calculated for the sludge-treated soils bear positive and significant correlation with the amounts of Cd extracted by the three extractants. The labile pool and supply parameter, therefore, are two valid indices of availability of Cd in soils which may be used for standardization of extractants for availability of Cd in similar natured sludge-treated soils.

Appendices

Appendix 1. Step down multiple regression equations determining various laboratory indices of Cd availability in sludge-treated soils on the basis of the soil properties

Dependent variables	Multiple regression equations	R^2	R^2 adj.	
LP at pH 7.0	1. $-3.17 + 0.37\,X_1 + 0.08\,X_2 + 0.06\,X_3 + 0.17\,X_4$	0.904**	0.827**	
	2. $-2.56 + 0.29\,X_1 + 0.07\,X_3 + 0.15\,X_4$	0.900**	0.849**	
	$\quad\quad (0.41)\quad\ (1.14)\quad (0.12)$			
	3. $-2.13 + 0.26\,X_1 + 0.06\,X_3$	0.888**	0.856**	
Kd	1. $163.0 - 25.7\,X_1 - 21.4\,X_2 + 1.21\,X_2 + 46.9\,X_4$	0.839**	0.710*	
	$\quad\quad (-0.73)\quad (-0.95)\quad (0.42)\quad (0.76)$			
SP	1. $-0.75 + 0.07\,X_1 - 0.01\,X_2 + 0.09\,X_3 + 0.06\,X_4$	0.778**	0.601	
	$\quad\quad (0.19)\quad\ (-0.03)\quad (1.01)\quad\ (0.08)$			
	2. $-0.81 + 0.08\,X_1 + 0.03\,X_2 + 0.06\,X_3$	0.778**	0.667*	
	$\quad\quad (0.20)\quad\ (1.00)\quad\ (0.09)$			
	3. $-0.64 + 0.07\,X_1 + 0.04\,X_3$	0.772**	0.707*	
	$\quad\quad (0.18)\quad\ (0.96)$			
	4. $-0.15 + 0.03\,X_3$	0.750*	0.719*	
	$\quad\quad (0.87)$			

Values in the parentheses indicates standard regression coefficient. X_1 – pH; X_2 – EC; X_3 – clay; X_4 – organic carbon %.
* significant at 5% level. ** significant at 1% level.

Appendix 2. The concentration of cadmium in the equilibrium soil solution (mg/kg) in post harvest soil samples equilibrated with different chemical reagents

Soil no.	DTPA			EDTA-NH$_4$OAC			Mg(NO$_3$)$_2$		
	Control	Treated	Mean	Control	Treated	Mean	Control	Treated	Mean
1	0.90	4.98	2.94	1.08	6.22	3.65	0.23	0.32	0.27
2	0.83	4.22	2.52	0.92	6.07	3.49	0.23	0.33	0.28
3	1.17	5.64	3.41	1.52	9.43	5.47	0.26	0.34	0.30
4	1.37	6.40	3.89	1.52	9.77	5.64	0.31	0.71	0.51
5	1.20	5.70	3.45	1.42	7.46	4.44	0.29	0.51	0.40
6	0.90	4.45	2.67	1.22	6.16	3.69	0.24	0.37	0.30
7	1.19	5.78	3.48	1.41	6.76	4.09	0.29	0.37	0.33
8	1.58	6.48	4.03	2.00	10.54	6.27	0.38	0.78	0.58
9	1.26	5.75	3.50	1.51	9.13	5.32	0.31	0.65	0.48
10	1.10	5.48	3.29	1.33	8.21	4.77	0.26	0.38	0.32
Mean	1.15	5.49	–	1.39	7.79	–	0.28	0.47	–
Cd (0.05)									
Soil			0.279			0.603			0.042
Level			0.125			0.270			0.019
Soil×Level			0.378			1.020			0.095

Appendix 3. Relationship of concentration of Cd in soil solutions equilibrated with different chemical reagents and concentration of Cd in plants, its uptake by crop and availability indices of Cd in soils

Parameters	EDTA-NH$_4$OAC extractable Cd	Mg(NO$_3$)$_2$ extract-able Cd	Cd concen-tration in plant	Cd uptake	Kd	LP at pH 7.0	SP
DTPA extractable Cd	0.957**	0.961**	0.917**	0.882**	−0.048	0.913**	0.892**
EDTA–NH$_4$OAC extractable Cd		0.920**	0.875**	0.803**	0.002	0.835**	0.835**
Mg(NO$_3$)$_2$ extractable Cd				0.794**	−0.205	0.878**	0.907*
Cd concentration in plant				0.816**	−0.245	0.834**	0.855**
Cd uptake					0.205	0.879**	0.772**
Kd						0.196	−0.090
LP							0.844**

*significant at 5% level. **significant at 1% level.

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