



ISSN: 0974 - 0376

*The Ecoscan* : Special issue, Vol. IX: 387-393: 2016  
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES  
www.theecoscan.com

## CHEMICAL FRACTIONATION OF HEAVY METALS IN SOILS UNDER LONG TERM FERTILIZATION OF PADDY (*ORYZA SATIVA* L.) IN CAUVERY DELTA ZONE

E. Parameswari *et al.*,

### KEYWORDS

Fertilizers  
Heavy metal fractions  
Long term experiment  
Paddy soil

Proceedings of National Conference on  
Harmony with Nature in Context of  
Resource Conservation and Climate Change  
(HARMONY - 2016)  
October 22 - 24, 2016, Hazaribag,  
organized by  
Department of Zoology, Botany, Biotechnology & Geology  
Vinoba Bhawe University,  
Hazaribag (Jharkhand) 825301  
in association with  
NATIONAL ENVIRONMENTALISTS ASSOCIATION, INDIA  
www.neaindia.org



E. PARAMESWARI<sup>1\*</sup>, V. DAVAMANI<sup>2</sup> AND S. ARULMANI<sup>2</sup>

<sup>1</sup>Tamilnadu Rice Research Institute, Aduthurai - 612 101

<sup>2</sup>Horticultural College and Research Institute, Periyakulam - 625 604,

Tamil Nadu Agricultural University, Tamil Nadu, INDIA

e-mail: parameswariphd@gmail.com

## ABSTRACT

In the present investigation sequential extraction was used to fractionate heavy metals (Cd, Pb, Cu and Zn) from long term fertilizer experiment into six operationally defined groups: water soluble, exchangeable, carbonate bound, metal organic complex, Fe-Mn oxide bound and residual. The residual fraction was the most abundant pool for all four metals examined. A significant amount (7 to 10 %) of Zn was present in the potentially available fraction *i.e* nonresidual fraction. A portion (1 to 3.0 %) of Cu was associated with Organic complex bound, Exchangeable and water soluble fractions in all the treatments. Contamination of Cd and Pb in these soils was not as severe as Zn and Cu. The apparent mobility and potential bioavailability for these four metals in the soils were: Zn > Cu > Cd > Pb. However, when residual fraction was taken into account, all the metals originally present in soil are relatively immobile and unavailable to plants. Accumulations in higher concentrations of these heavy metals are associated with higher level of single super phosphate addition as well as zinc sulphate.

## INTRODUCTION

In intensive cropping, continuous use of high levels of chemical fertilizers often leads to nutritional imbalance in soil and decline in crop productivity (Nambiar, 1994). Increased environmental concern is frequently expressed now a days about the concentration of heavy metals especially cadmium and lead and their possible effects on human beings after entering into food chain through the food crops raised in such heavy metal contaminated soils. Phosphatic fertilizers like single super phosphate derived from the phosphatic rocks are the chief sources of heavy metals like cadmium and lead. Lead is one of the heavy metals which also find its way to the soil through phosphatic and zinc fertilizers. Phosphatic fertilizers are indispensable in modern agriculture, although a reduction in the heavy metal concentration of them made from high heavy metal containing phosphate rocks is technically possible but it may be difficult to justify economically. Hence, it becomes a necessary evil to apply heavy metal contained fertilizers, which are the sources of pollution. Higher concentration of heavy metals in the soil would increase the potential uptake of metals by plants. The best tool to monitor the heavy metal accumulation in agricultural soils is the long term fertilizer experiments, where in each treatment receives specified quantity of fertilizers every crop season (Parameswari *et al.*, 2014).

Monitoring of heavy metals in the ecosystem is important for safety assessment of environment and human health in particular (Kashyap *et al.*, 2015). Based on primary accumulation mechanisms in sediments, heavy metals can be classified into five categories: (i) adsorptive and exchangeable, (ii) bound to carbonate phases, (iii) bound to reducible phases (Fe and Mn oxides), (iv) bound to organic matter and sulfides and (v) detrital or lattice metals. Heavy metals present in these categories have different remobilization behaviors under changing environmental conditions. Geochemical forms of heavy metals in soil affect their solubilities, which directly influence their bio availability. Therefore, determining total content of heavy metals is insufficient to assess the environmental impact of contaminated soils and sediments, because it is the chemical form that determines metal behavior in the environment and its remobilization ability. Sequential extraction gives at most a qualitative idea about the reactivity and mobility of total contents present in the soil (Saraswathy *et al.*, 2010). Selective sequential extraction procedures have been commonly used for studying metal mobility and availability in soils. Numerous extraction schemes for soils and sediments have been described in the literature. The extraction scheme used here is based on operationally defined fractions: water soluble, exchangeable, carbonate bound, metal organic complex, Fe-Mn oxide bound and residual. These procedures are not entirely specific, and there will be overlap between the fractions. Despite uncertainties as to the selectivity of the various extractants and to possible problems due to readsorption, extraction procedures provide qualitative evidence regarding the forms of association of trace metals and indirectly, of their bioavailability (Ma and Rao, 1997).

The primary objectives of this study were to investigate the chemical partitioning of Cd, Cu, Pb and Zn in long term fertilizer experiment (LTFE) and to evaluate the

\*Corresponding author

effect of total metal concentrations on metal partition into different fractions.

## MATERIALS AND METHODS

The LTFE was initiated in the year 1994 using two varieties in six fertilizer treatments under split plot design. Rice varieties ADT 43 and ADT 45 are main treatments and different levels of N, P and K as sub treatments. Totally twelve treatments were compared in both *Kharif* and *Rabi* seasons.

### Main plot treatments (promising rice varieties)

Kharif: Variety 1: ADT 43, Variety 2: ADT 45

Rabi : Variety 1: ADT 38, Variety 2: ADT 39

### Sub plot treatments (inorganic fertilizers)

A fixed crop rotation of rice-rice- rice fallow pulses in a year is being followed year after year, rice fallow pulses were grown without fertilizers.

### Treatment details

Treatments	Kharif (kg/ha)			Rabi (kg/ha)		
	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
T <sub>1</sub>	Control	0	0	0	0	0
T <sub>2</sub>	+ PK	0	80	120	0	57.3
T <sub>3</sub>	+ N	150	0	0	132	0
T <sub>4</sub>	+ NP	150	80	0	132	57.3
T <sub>5</sub>	+ NK	150	0	120	132	0
T <sub>6</sub>	+ NPK	150	80	120	132	57.3

{N applied as 3 splits at 15, 30 and 45 DAT (Days After Transplanting) in Kharif and 15, 35, 50 and 70 DAT in Rabi, P applied as full basal, K applied in two splits (50% basal, 50% at panicle initiation) and ZnSO<sub>4</sub> @ 25 kg ha<sup>-1</sup> common to all}.

**Table 1: Soil characteristics of long-term fertility experiment during 1994 (Before 16 years)**

Properties	Value
pH (1:2)	6.9
EC (dS m <sup>-1</sup> )	0.49
Clay (%)	46.53
Water holding capacity (%)	38.9
Bulk density (g cm <sup>-3</sup> )	1.5
Organic C (%)	0.57
Free CaCO <sub>3</sub> (%)	0.55
CEC (c mol (p <sup>+</sup> ) kg <sup>-1</sup> )	40.5
Available N (kg/ha)	231
Available P (kg/ha)	30
Available K (kg/ha)	228
Bioavailable Zn (mg/kg)	1.2
Bioavailable Cu (mg/kg)	2.2
Bioavailable Cd (mg/kg)	BDL
Bioavailable Pb (mg/kg)	BDL

BDL: Below Detectable Limit

**Table 2: Physico-chemical properties of soil after harvest of Kharif- 2010 rice crop**

Properties	Fertilizer treatments (Sub plot)						Mean
	Control	+ PK	+ N	+ NP	+ NK	+ NPK	
Ph	7.16	7.17	7.22	7.22	7.23	7.22	7.20
EC (dS/m)	0.53	0.54	0.54	0.54	0.54	0.58	0.55
Org. C (%)	0.62	0.66	0.70	0.74	0.65	0.78	0.69
Avail. N (kg/ha)	175	181	216	246	240	256	219.0
Avail. P (kg/ha)	40.0	61.7	43.6	61.4	46.2	70.9	53.97
Avail. K (kg/ha)	127	267	128	269	279	291	226.8

The soil samples were collected after the harvest of 31<sup>st</sup> crop. The sequential extraction procedure described by Krishnamurthi et al. (1995) was followed (Fig.1).

The heavy metal concentration was calculated by using the following formula, (Noble and Hughes, 1991).

$C \times (E + M) - (C^1 \times M)$ , Where,

C = Concentration of heavy metals in the extraction solution (?g/ml)

E = Mass (g) of the extractant (25cm<sup>3</sup> or ml)

M = Mass (g) of the entrained solution carried over from previous extraction.

C<sup>1</sup> = Concentration of the heavy metal in the extraction solution of preceding step of the sequence (mg/ml).

Initial weight of the centrifuge tube and weight of the centrifuge tube with residue after each extraction was weighed to calculate the mass of the entrained solution. The heavy metal content in the solution was measured by following standard instrument conditions using Varion Spectra AA 100/200 Flame Atomic Absorption Spectrophotometer (USEPA, 1979).

## RESULTS AND DISCUSSION

The experimental soil is fine montmorillonitic, isohy perthermic, *Udorthentic chromusterts* with heavy clay texture which belong to Kalathur soil series. Soil physico-chemical parameters were estimated during initiation of the experiment as well as at the end of 16<sup>th</sup> year and results are presented in Tables 1 and 2. Soil fertility status at the end of sixteenth year (2010) showed marked variations when compared to initial status (1994). The soil pH and Electrical Conductivity (EC) showed only marginal variations over the years as well as among the treatments. Though, there were some fluctuation in pH values in the treatments varied between 7.16 (absolute control) to 7.22 (NPK), these values are within the neutral range of a rice system (Ponnamperuma, 1972).

The soil's ability to immobilize heavy metals increases with rising pH and peaks under mildly alkaline conditions. According to Aydinalp and Marinova (2003), in acid soils (pH 4.2-6.6) the elements viz., Cd, Zn are highly mobile whereas Cu and Pb are practically immobile, in case of neutral to alkaline (pH 6.7-7.8) soils Cd and Zn are moderately mobile, which was in concurrence with the findings of Mousavi (2010). Regarding the Electrical Conductivity (EC), there was a slight significant increase among the treatments at the end of 16<sup>th</sup> year (32<sup>th</sup> crop) as compared initial soil, but the values are well below the normal rice soil (0.4 to 0.62 dS/m). In general the EC in submerged rice system will tend to fluctuate due to

**Table 3: Cadmium concentrations in each fraction of LTFE soils**

Sl.No	Fractions	Cd concentrations (Mg/kg)						Mean
		T <sub>1</sub> Control	T <sub>2</sub> + PK	T <sub>3</sub> + N	T <sub>4</sub> + NP	T <sub>5</sub> + NK	T <sub>6</sub> + NPK	
1	Water soluble	BDL	BDL	BDL	0.001	BDL	0.011	0.002
2	Exchangeable	0.02	0.031	0.022	0.035	0.024	0.045	0.030
3	Carbonate bound	BDL	BDL	BDL	BDL	BDL	BDL	0.000
4	Organic complex bound	BDL	BDL	BDL	BDL	BDL	BDL	0.000
5	Fe-Mn oxide bound	0.020	0.029	0.020	0.034	0.025	0.041	0.028
6	Residual	0.282	3.048	1.492	2.956	1.300	3.781	2.143
Sum of all fractions (ppm)		0.32	3.11	1.53	3.03	1.35	3.88	2.203
Non-residual (%)		12.50	1.93	2.75	2.31	3.63	2.50	4.269
Residual (%)		88.13	98.01	97.49	97.56	96.31	97.44	95.82

BDL: Below Detectable Limit

**Table 4: Lead concentrations in each fraction of LTFE soils**

Sl.No	Fractions	Pb concentrations (Mg/kg)						Mean
		T <sub>1</sub> Control	T <sub>2</sub> + PK	T <sub>3</sub> + N	T <sub>4</sub> + NP	T <sub>5</sub> + NK	T <sub>6</sub> + NPK	
1	Water soluble	BDL	BDL	BDL	BDL	BDL	BDL	-
2	Exchangeable	0.01	0.07	0.00	0.06	0.04	0.08	0.04
3	Carbonate bound	0.00	0.01	0.07	0.11	0.09	0.13	0.07
4	Organic complex bound	0.11	0.13	0.10	0.13	0.11	0.16	0.12
5	Fe-Mn oxide bound	0.12	0.19	0.13	0.21	0.14	0.24	0.17
6	Residual	21.36	79.68	36.58	81.36	41.23	84.37	57.43
Sum of all fractions (ppm)		21.60	80.08	36.88	81.87	41.61	84.98	57.84
Non-residual (%)		1.11	0.50	0.82	0.62	0.91	0.72	0.78
Residual (%)		98.89	99.50	99.18	99.38	99.09	99.28	99.22

BDL: Below Detectable Limit

**Table 5: Copper concentrations in each fraction of LTFE soils**

Sl.No	Fractions	Cu concentrations (Mg/kg)						Mean
		T <sub>1</sub> Control	T <sub>2</sub> + PK	T <sub>3</sub> + N	T <sub>4</sub> + NP	T <sub>5</sub> + NK	T <sub>6</sub> + NPK	
1	Water soluble	0.11	0.15	0.13	0.19	0.18	0.21	0.16
2	Exchangeable	1.16	2.95	2.92	3.09	2.86	3.11	2.68
3	Carbonate bound	BDL	BDL	BDL	BDL	BDL	BDL	-
4	Organic complex bound	2.04	3.08	3.07	3.05	2.85	3.14	2.87
5	Fe-Mn oxide bound	BDL	BDL	BDL	BDL	BDL	BDL	-
6	Residual	25.1	92.8	88.4	106	103	116	88.55
Sum of all fractions (ppm)		28.41	98.98	94.52	112.33	108.89	122.46	94.27
Non-residual (%)		11.65	6.24	6.47	5.64	5.41	5.28	6.78
Residual (%)		88.35	93.76	93.53	94.36	94.59	94.72	93.22

BDL: Below Detectable Limit

the dissolved salts contributed from soil, water and the release of ionic species of reduction process. However this will stabilize after some times.

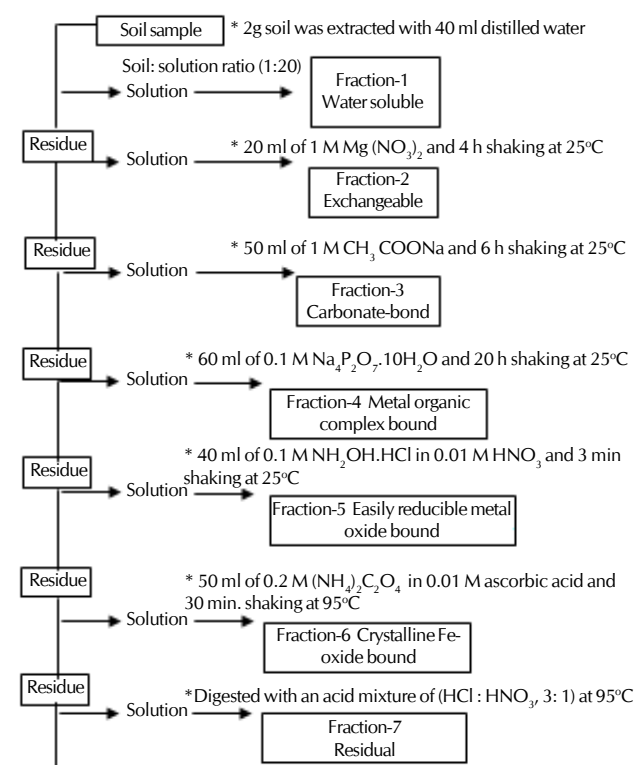
The organic carbon is an important component, because it tends to either soluble form or sub-soluble complexes with the heavy metals, to migrate, or to be retained in the soil (Mousavi, 2010). The percentage of organic carbon (OC) present in the treatments ranged between 0.62 (absolute control) to 0.78 (NPK), a slight significant increase among the treatments was noticed. The increase in the available N, P and K in their respective treatment plots over control was due to the continuous addition of inorganic fertilizers.

### Fractionation of heavy metals

Geochemical forms of heavy metals in soil affect their solubilities which directly influence their bioavailability. Therefore, determining total content of heavy metals is insufficient to assess the environmental impact of contaminated soils and sediments. Selective sequential extraction procedures have been commonly used for studying metal mobility and availability in soils. The extraction scheme used here is based on operationally defined fractions: water soluble, exchangeable, carbonate bound, organic complex bound, Fe-Mn oxides bound and residual fractions. Extraction procedure provides qualitative evidence regarding the forms

**Table 6: Zinc concentrations in each fraction of LTFE soils**

Sl.No	Fractions	Zn concentrations (Mg/kg)						Mean
		T <sub>1</sub> Control	T <sub>2</sub> +PK	T <sub>3</sub> + N	T <sub>4</sub> + NP	T <sub>5</sub> + NK	T <sub>6</sub> + NPK	
1	Water soluble	0.15	0.6	1.65	1.05	0.9	1.95	1.05
2	Exchangeable	1.53	2.4	2.23	2.47	2.2	2.25	2.18
3	Carbonate bound	1.98	3.25	3.31	3.47	3.11	3.29	3.07
4	Organic complex bound	1.64	2.98	3.01	3.15	3.01	3.24	2.84
5	Fe-Mn oxide bound	3.15	6.12	5.64	6.89	6.23	7.14	5.86
6	Residual	85	165	148	202	183	220	167.17
Sum of all fractions (ppm)		93.45	180.35	163.84	219.03	198.45	237.87	182.17
Non-residual (%)		9.04	8.51	9.67	7.78	7.79	7.51	8.38
Residual (%)		90.96	91.49	90.33	92.22	92.21	92.49	91.62



2g of soil was taken and after each treatment, the extract was collected by centrifugation for 10 min. at 12000g (10,000 rpm). The supernatant was filtered through whatman No. 42 filter paper and the filtrate was collected in a polythene bottle and stored at 4°C.

**Figure 1: Fractionation Scheme**

of association of trace metals and indirectly to assess their bioavailability (Ma and Rao, 1997).

Sequential extraction procedures assume that metal bioavailability decreases with each successive extraction step in the procedure. Therefore, metals in water soluble and exchangeable fractions would be readily bio-available to the environment, whereas the metals in the residual fractions are tightly bound and would not be expected to be released under natural conditions.

The water soluble fraction includes highly mobile and potentially available heavy metal species. The exchangeable fraction contains weakly bound (electrostatically) can be

released by ion-exchange with cations such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  or  $\text{NH}_4^+$  also available for plant uptake. The oxidizable fraction released under oxidizing condition metals linked to organic matter within the soil matrix into solution. The reducible fraction provides unstable metal forms connected with amorphous Mn-hydroxides, which are easily discharged and approachable by the surrounding biota under reducing conditions. In the reducible + residual fraction, the metals are bound to amorphous Fe-hydroxides (reducible part) and under reducing conditions they are expected to be released into nature. The residual includes naturally occurring crystalline Mn-hydroxide minerals, which may hold heavy metals within their crystalline matrix. Thus heavy metals are not likely to be discharged under normal environmental conditions (Rogan, 2008). It had been assumed that metals in the non-residual fractions are more bio-available than metals associated with the residual fraction. The non-residual fraction is the sum of all fractions except the residual fraction.

The concentration of Cd varied from 0.32 (absolute control) to 3.88mg/kg (NPK) (Table 3). The limit values for potentially toxic elements (PTE) proposed by the Council of European Economic Committee (Smith, 1996) for Cd concentration in soils is 1-3.0mg/kg. In this present investigation, the Cd level fell above this limit in all the P fertilizer added treatments (3.11mg/kg in +PK, 3.03 in +NP and 3.88 in +NPK). Since, the control plot does not receive any fertilizer, the least concentration of Cd (0.32mg/kg) was recorded. The urea added in the N alone treatment and NK treatment had low Cd concentration in the soil as compared to other treatments. Pendias and Pendias (2001) reported that the maximum allowable limit for Cd in soil was  $0.5\mu\text{g/g}$ (ppm). The increase in the residual and non-residual fraction of Cd in all the treatments was due to the addition of Cd through single superphosphate which contained on an average about 70 mg/kg of Cd. This ensured with the study of Palaniappan et al. (2002).

The mean water soluble fraction of Cd was very low (0.002mg/kg) and the residual fraction was high 2.143mg/kg. The percentage of residual fractions ranged from 88.13 to 97.44. Assuming that bioavailability is related to solubility, then Cd association with different fractions followed in the order: Residual > Exchangeable > Fe-Mn bound > Water soluble. The Carbonate and Organic bound forms are recorded below detectable limit. According to Ma and Rao (1997), the metals

present in the exchangeable fraction are usually thought to be readily available for plant uptake. Cd will be present in all fractions when the total Cd concentration was >50mg/kg. In the present study, Cd concentrations were not present in the organic and carbonate fractions. Cd was found available in exchangeable form and immobile as Fe-Mn oxide bounds.

The total concentration of Pb in all the fractions ranged between 21.60 (absolute control) to 84.98mg/kg (NPK) (Table 4). The Pb concentrations in all the treatments were within the maximum permissible limits proposed by the council of European Economic Committee (5-300mg/kg of dry soil and annual addition of 15kg/ha). Lead in the form of Fe-Mn oxide bound was relatively higher followed by residual fractions. Pb in paddy soils tend to accumulate with oxides of Fe and Mn, as stated by Jamil *et al.* (2011). The mean water soluble fraction of Pb was below detectable limit (BDL), the exchangeable fraction was very low (0.04mg/kg), but the residual fraction (57.43mg/kg) was very high. The sum of all the fractions was recorded as 57.84ppm. The percentage of residual fractions ranged from 98.89 (control) to 99.50(+PK). The Pb association with different fractions followed in the order: Residual>Fe-Mn bound>Organic Complex bound>Carbonate bound>Exchangeable. The Pb in water soluble form was below detectable limit (BDL). The metals present in the exchangeable fraction are usually thought to be readily available for plant uptake. It had been assumed that Pb found in this soil is mostly unavailable form. This study was in accordance with the report of Palaniappan *et al.* (2002).

Continuous incorporation of phosphotic fertilizers resulted in the buildup of Cd and Pb in surface soils. Accumulation of higher concentration of these heavy metals is associated with higher level of single superphosphate application for crop growth. The rate of heavy metal increase necessitates close monitoring in the production system. The increase in the control as well as N alone plots could be due to the input from other sources like irrigation water and atmospheric deposits.

The lowest concentration of total Cu (28.41mg/kg) was recorded in control and the highest concentration was recorded in NPK treatment (122.46 mg / kg) (Table 5). The mean water soluble fraction of Cu was very low (0.16mg/kg) and the residual fraction was high (88.55mg/kg). The percentage of residual fractions ranged from 88.35(control) to 94.72(NPK). The Cu association with different fractions followed in the order of: Residual>Organic Complex bound>Exchangeable >Water soluble. The Cu present in the exchangeable fraction are usually thought to be readily available for plant uptake. The major association of Cu with the organic fraction may be due to the higher formation of constant organic-Cu complexes. Copper was found only below detectable limit (BDL) in Carbonate and Fe-Mn oxide forms. This was in accordance with the findings of Ma and Rao (1997).

The water soluble fraction of mean Zn was very low (1.05mg/kg), and the residual fraction was very high 167.17mg/kg (Table 6). The average sum of all the fractions over treatments was 182.17 ppm. The percentage of residual Zn ranged from 90.33 (N alone treated plot) to 92.49 (NPK treated plot). The percentage of residual fractions in the control was slightly higher than N alone treatment might be due to leaching of Zn

through ionic exchange with N. The Zn association with different fractions followed in the order of: Residual>Fe-Mn bound>Carbonate bound>Organic Complex bound >Exchangeable >Water soluble. Among the non-residual fractions, the Fe-Mn oxide fraction contained the greatest amount of Zn it may be due to the high stability constants of Zn oxides and the concentration of Zn in other fractions were relatively low. This was in accordance with the study of Ma and Rao (1997).

In the present study the exchangeable form of heavy metals were very low indicating that under present conditions, the availability of heavy metals to plants were minimum. This is in line with the study of Aydinalp and Marinova (2003).

Cadmium was found in exchangeable and immobile as Fe-Mn oxide forms but water soluble and other fractions are below detectable limit. Pb was found in least as exchangeable and carbonate bound form, but in appreciable quantity as organic and oxide bound forms. Copper and Zn were present in appreciable quantities as organically bound form. The more possible contamination in all the soil samples was due to Cd, followed by Pb and Zn. Heavy metals Pb and Zn are mainly linked to amorphous Fe-Mn hydroxides in the reducible fraction and thus under reduction conditions are very unstable and mobile, which are not expected to escape under normal environmental conditions. Copper is dominantly bound to organic matter and consequently released under oxidizing conditions into the environment. This is in accordance with the study of Rogan (2008). However, when the residual fraction was taken into account, all the metals originally present in the soil are relatively immobile and unavailable to plants which indicates the added metals through fertilizers tends to become immobilized.

Different geochemical fractions are operationally defined by an extraction sequence that generally follows the order of decreasing solubility. The residual fraction was the most abundant pool for all the metals in the soils studied. Continuous incorporation of phosphatic fertilizers resulted in the buildup of Cd and Pb concentration in surface soils. Overall, the order of contamination was Cu > Zn > Cd > Pb. Accumulations in higher concentrations of these heavy metals are associated with higher level of single super phosphate addition as well as with the addition of zinc sulphate. Though the concentration of these two heavy metals in soil has not reached toxic levels even after sixteen years, the rate of increase necessitates close monitoring the production system involving single superphosphate and zinc application in order to sustain them.

## REFERENCES

- Aydinalp, C. and Marinova, S. 2003. Distribution and forms of heavy metals in some agricultural soils. *Polish J. Env. Studies.* **12(5)**: 629-633.
- Jamil, H., Theng, L. P., Jusoh, K., Razali, A. M., Ali, F. B. and Ismail B. S. 2011. Speciation of heavy metals in paddy soils from selected areas in Kedah and Penang, Malaysia. *African J. Biotechnol.* **10(62)**: 13505-13513.
- Kashyap, R., Verma, K. S. and Chand, H. 2015. Heavy metal contamination and their seasonal variations in rewalsar lake of Himachal Pradesh, India. *The Ecosacr.* **9(1&2)**: 31-36.
- Krishnamurthi, G. S. R., Huang, P. M., Van Rees, K. C. J., Kozak, L.

- M. and Rostad, H. P. W. 1995.** Speciation of particulate-bound cadmium of soils and its bioavailability. *The Analyst*. **120**: 659-665.
- Ma, L. Q. and Rao, G. N. 1997.** Chemical fractionation of cadmium, copper, nickel and zinc in contaminated soils. *J. Environ. Qual.* **26**: 259-264.
- Mousavi, S. M. 2010.** Lead and Cadmium availability and uptake by rice plant in response to different bio-solids and inorganic fertilizers. *Am. J. Agri. Biol. Sci.* **5(1)**: 25-31.
- Nambiar, K. K. M. 1994.** Soil fertility and crop productivity under long term fertilizer use in India. *ICAR publications*, New Delhi, p. 144.
- Palaniappan, M., Shanmugam, K. and Ponnusamy, S. 2002.** Soil degradation due to heavy metal accumulation under long term fertilization. Symposium no.46, paper No:33, 17<sup>th</sup> WCSS, 14-21 Aug 2002, Thailand, pp. 333-1-7.
- Parameswari, E., Davamani, V., Arulmani, S. and Rathinasami, A. 2014.** Soil degradation due to heavy metal accumulation under long term fertilization of paddy (*Oryza sativa L.*). *J. Appl. and Nat. Sci.* **6(1)**: 182-188.
- Pendias, A. and Pendias, H. 2001.** Trace elements in Soils and Plants. 3<sup>rd</sup> edn. Boca Raton: CRC Press.
- Ponnamperuma, F. N. 1972.** The chemistry of submerged soils. *Adv. Agron.* **24**: 32-96.
- Rogan, N. Dolenc, T., Serafimovski, T., Tasev, G. and Dolence, M. 2008.** Determination of heavy metals in paddy soils (Kocani field, Macedonia) by a sequential extraction procedure. *Materials and geoenvironment.* **55(4)**: 444-455.
- Saraswathy, G., Shanmugapriya, A. and Sudha, P. N. 2010.** Speciation and determination of heavy metals in polluted soils of Ranipet industrial area, India. *The Ecoscan.* **4(2&3)**: 221-223
- Smith, S. R. 1996.** Agricultural recycling of sewage sludge and the environment. *CAB international*.
- USEPA (United States Environmental Protection Agency) 1979.** Method 281.4. Chromium, hexavalent. *In: methods for chemical analysis of water and wastes.* EPA-600/4/79-020 (USEPA, Environmental monitoring and support laboratory, Cincinnati, OH).

