

Research article

# SEQUENTIAL EXTRACTION PROCEDURE FOR PARTITIONING OF LEAD, COPPER, CADMIUM, CHROMIUM AND ZINC IN CONTAMINATED ARABLE SOILS OF NIGERIA

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## Abstract

The accumulation of heavy metals in soil arising from anthropogenic impacts are serious environmental problem. The arable soils were sequentially extracted to fractionate heavy metals into water soluble fraction (WSF), exchangeable fractions (EF), bound to carbonate fraction (BCF), bound to metal oxide fraction (BMO), organically bound fraction (OBF) and residual fraction (RF). The average mobility of Pb, Cu, Cd, Cr and Zn levels in all the six fractions were in the order: Cd>Cu>Zn>Pb>Cr. Speciation of heavy metals in soils determines the availability of metals for plant uptake and ecotoxicity. The risk associated with the presence of metals in soils is the ability of their transfer in water or plants. Chemical properties such as pH, CEC, organic matter, texture and textural classification of the concerned soils were also analyzed. The bioavailability of heavy metals, their biological uptake and ecotoxicological impacts on soils biota can better be understood in terms of their geochemical and speciation properties. **Copyright © AJEPR, all rights reserved.**

**Key words:** Arable Soil, Sequential Extraction, Mobility Factor, Heavy Metals, Speciation, Nigeria.

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## Introduction

Sequential extraction is an analytical process that chemically and selectively leaches metal out of soil, sediment and sludge samples. The purpose of sequential or “selective” extraction is to simulate the release of the selective metals into solution under various environmental conditions. The theory behind sequential extraction is that the most mobile metals are removed in the first fraction and continue in order of decreasing of mobility <sup>[1, 2, 3, 4, 5]</sup>.

It is well known that metals are present in soil in different chemical forms, which influence their reactivity and hence their mobility and bioavailability. The list of sites contaminated with metals grow larger every year due to antropogenic impacts, presenting a serious health problem and a formidable danger to the environment <sup>[6]</sup>. Most of the previous works in the early eighties have been limited to the determination of total concentration of the metals only. Although the total concentration of trace elements in soil gives some indication of the level of enrichment, depletion or pollution, it provides no insight into element bioavailability of potentially toxic metals or mobility <sup>[7]</sup>. Total metal content of soils is useful for many geochemical applications, but often the speciation that is bioavailability and mobility of these metals is more of an interest agriculturally in terms of what is biologically extractable <sup>[8]</sup>. Speciation is defined as the identification and quantification of the different, defined species, forms or phases in which an element occurs, and is essentially a function of the mineralogy and chemistry of the soil sample examined <sup>[9]</sup>.

Metal in soils can be divided into two fractions <sup>[10]</sup>: (i) inert fraction assumed as the non – toxic fraction and (ii) the labile fraction, assumed to be potentially toxic. To assess the availability of heavy metals, only the soil labile fraction is taken into account because this fraction is often called by extension, the bioavailable fraction<sup>[11]</sup>. However, the bioavailable fraction can differ from one metal to another and from one receptor to another. The mobility, bioavailability and toxicity of heavy metals for plants uptake depend upon their specific chemical forms and their interactions with the different soil and sediment constituents. Consequently, detailed information about the interactions between the toxic heavy metals and the soil matrix is required to judge their environmental impacts. The impact of these metals in soils, is their possible transfer into plants or water, which is defined by the term of bioavailability.

## Bioavailability Factor of Heavy Metals

The bioavailability factor is expressed as the ratio of the available concentration of a metal in soil to its total concentration. It shows the potentials of a particular metal from the soil matrix to enter the soil solution from which it can be absorbed by plants <sup>[12]</sup>.

This equation is used to calculate the bioavailability factor as follows:

$$BF = \frac{A_1 + A_2}{A_1 + A_2 + \dots + A_n}$$

Where BF is the bioavailability factor,  $A_1$  is concentration of the first fraction,  $A_2$  is the concentration of the second fraction and  $A_n$  is the concentration of the nth fraction.

### **Mobility factor of Heavy Metals**

The mobility factor of metals in soil samples may be assessed on the basis of percentage absolute and relative content of fractions weakly bound to soil components, and it is calculated on the basis of the following equation <sup>[12, 13, 14]</sup>.

$$MF = \frac{F_1 + F_2 + \dots + F_n}{F_1 + F_2 + \dots + F_n} \times \frac{100}{1}$$

Where MF is the mobility factors,  $F_1$ ,  $F_2$ ,  $F_n$  are the first, second and nth fractions respectively.

Cd and Pb are considered as the most important environmental pollutants in agricultural soils because of the potential harmful effects they may have on food quality and health of soil <sup>[15]</sup>. Copper which is an active ingredient of fungicides is reported as one of the most toxic metals to soil microorganism and soil health <sup>[16]</sup>. Some heavy metals e.g. Zn are essential for the healthy development of plants, animals and humans, but they can be toxic and harmful if available in certain concentrations.

One commonly used sequential extraction procedure is designed to partition different trace metal based on their chemical nature. Of the many procedures in publication, some are designed to operate within specific parameters while others are designed for more broad applications <sup>[17]</sup>. The modified <sup>[18]</sup>, procedure is the most commonly used to determine both the actual and potential mobility of trace elements in soils and sediments. This consists of five steps in which heavy metals are partitioned among different phases.

In each of the steps, calculated concentrations of chemicals and buffers are added and the sample is shaken on an end – over – end shaker. The leachate from each step is then analyzed depending on the project requirement <sup>[3]</sup>. This multi – step procedure assures that all the metals of concern are completely extracted and quantified from the sample. Factors such as pH of the acid use for adjustment, temperature and duration of extraction are the critical factors that control the concentration of metal extracted from the sample <sup>[19]</sup>.

Various sequential chemical extraction schemes have been adopted for farming soils. These schemes vary in reagent types, volume, strength and extraction time. In spite of the variability in reagents and modification of approaches, they all aimed at correlating each fraction with plant bioavailability. Much research on speciation

have been conducted on heavy metals contamination in soils from various anthropogenic sources such as industrial wastes<sup>[17,20,21,23]</sup>, automobile emission<sup>[24,25]</sup>, mining activity<sup>[12,26,27]</sup> and agricultural practice<sup>[28,29]</sup>.

This study highlights the behavior of Pb, Cu, Cd, Cr and Zn in selected arable soils in the vicinity of mining and dumping sites in Nigeria, by identifying the farming soil compartments in which they are most concentrated and their implication on agro – ecosystems. Six different extraction procedures were used to establish the extractability and to predict the phyto availability of heavy metals in the farming soils investigated.

## **MATERIALS AND METHODS**

Location of the Research: The study areas are within latitude 4<sup>0</sup> and 14<sup>0</sup> North and longitude 3<sup>0</sup> and 15<sup>0</sup>, East, Nigeria. These include:

- (a) Yargalma farming soil, near local gold ore, mining site in Bukkuyun, Zamfara State, North West Nigeria.
- (b). Dareta arable soil, near gold / lead ores mining site in Anka, Zamfara State, North West Nigeria.
- (c). Itakpe farming soil, near iron ore mining site in Okene, Kogi State North Central Nigeria.
- (d) Ray Field Resort farming soil near tin ore mining site in Jos South, Plateau State, North, Central, Nigeria.
- (e) Udi farmin soil, near Coal mining site in Enugu East, Enugu State, South East, Nigeria.
- (f) Chalawa arable soil in the vicinity of tannery waste dumping site in Kumbotso, Kano State, North West, Nigeria.
- (g) Court Road farming soil, near general dumping site in Kumbotso, Kano State North West, Nigeria.

The dominant formations in the area are carboniferous deposits with layers from Tertiary and Quaternary Period. The predominant types of soils in the study areas are arenosols and ultisols. In small areas, histosols occur.

The study areas are agricultural, industrial and mining communities. The farmland covers over 65% of the studied areas. The farming activity in the studied areas deals mainly with crop growing such as yam, cassava, maize, pepper, okra, leafy vegetables, cocoyam, rice, millet, guinea corn, tomatoes, carrot, lettuce and onions.

**Sample collection and Analytical procedure:** The surface soil layer to a dept of 0 – 20cm (ploughing layer) was sampled with a sharp edged plastic spatula and directly transferred the soil sample into

labeled polyethylene homogenization container and mixed thoroughly to obtain a homogenous sample representative of the entire sampling interval. Each sample from equi-spaced sample station was a composite of 30 – sub – samples from a distance of 20m per a sub – sample. When compositing was completed, the labeled homogenization polyethylene bags were closed tightly and returned same to the laboratory for pre treatment and analysis<sup>[30,31]</sup>.

The farming soil samples were air dried under laboratory conditions for two weeks, ground, sieved through a 2 mm polyethylene sieve and dried to constant mass in an oven at 75<sup>0</sup>C and kept in a desiccators for further analysis. 0.25g of the oven dried soil samples were weighed into platinum crucibles. The digestions were conducted with a mixture of 3cm<sup>3</sup> of conc. HNO<sub>3</sub>, 2 cm<sup>3</sup> of conc. HF and 1cm<sup>3</sup> of 40% H<sub>2</sub>O<sub>2</sub> solution on a sand bath at a temperature of 200 - 230<sup>0</sup>C, for the determination of the total metal contents of the arable soil sample.

The six sequential extraction procedure used in this study was designed to separate metals into:

- a. Water Soluble Fraction
- b. Exchangeable Fraction
- c. Bound to Carbonates Fraction
- d. Bound to Metal Oxide Fraction
- e. Organically Bound Fraction
- f. Residual Fraction

Typically, metals of anthropogenic inputs tend to reside in the first two fractions and metals found in the residual fraction are of natural occurrence in the parent rock<sup>[32]</sup>.

## **1. Water Soluble Fraction**

50cm<sup>3</sup> of de-ionized water was added to 50g soil samples in 100cm<sup>3</sup> polypropylene centrifuge tubes with screw caps. The extraction was performed by shaking in a mechanical, end-over-end shaker (Model New Brunswick Scientific, Innova 4000 Incubator Shaker) at a speed of 300rpm and at a room temperature of 28<sup>0</sup>C for 5 hours, and left overnight. The solution was centrifuged at 3000rpm gravity for 15 minutes and the supernatant was filtered into plastic container through No.1 Whatman filter paper and stored in a refrigerator at 4<sup>0</sup>C for analysis.

## **2. Exchangeable Fraction**

50cm<sup>3</sup> of 1M Ammonium Oxalate was added to the residue from the water soluble fraction in 100cm<sup>3</sup> polypropylene centrifuge tube with screw caps, and the extraction conducted according to the above extraction procedure.

### **3. Bound to Carbonates Fraction**

The residue from step 2 above, was extracted with 50cm<sup>3</sup> of 2.5% Acetic acid solution according to the above extraction procedure.

### **4. Bound to Metal Oxide Fraction**

The residue from step 3 was extracted with 50cm<sup>3</sup> of 0.1M oxalic acid, 0.17M Ammonium oxalate at pH 3.25, according to the above extraction procedure.

### **5. Organically Bound Fraction**

The residue from step 4 above was extracted with 50cm<sup>3</sup> acid mixture, 2% HNO<sub>3</sub>, 30% H<sub>2</sub>O<sub>2</sub> in the ratio of 3cm<sup>3</sup> : 7cm<sup>3</sup>; pH 2, according to the above extraction procedure, except that the temperature was controlled at 85°C.

### **6. Residual Fraction (Silicates)**

The residue from step 5 above was evaporated to dryness. 0.25g sub-sample was taken and this was analysed by tri-acid mixture digestion with 3cm<sup>3</sup> conc. HNO<sub>3</sub>, 2cm<sup>3</sup> conc. HF and 1cm<sup>3</sup> (40%) H<sub>2</sub>O<sub>2</sub> solution in a platinum crucible. After the acids had been digested and evaporated, 40cm<sup>3</sup> of 0.25M HNO<sub>3</sub> was added and warmed on a sand bath and filtered into 50cm<sup>3</sup> labeled plastic containers and filled to volume with the 0.25M HNO<sub>3</sub> solution. Total metal content of the original soil samples were similarly determined earlier using 0.25g of soil samples, to estimate the total amount of Pb, Cu, Cr, Cd, Zn, recovered from all the sequential extraction steps.

The digested residue samples were stored in the refrigerator at 4°C for analysis. Reagent blanks were similarly conducted for every step. Samples from the sequential extraction the (extracts), the reagent blanks and standard solutions were analysed using Atomic Absorption Spectrometer, Model – Buck Scientific UPG 210.

Soil pH was measured potentiometrically in 1m KCl with a soil / extratant ratio of 1:5 in three replicates per sample. The organic carbon was determined by Tiurin method. It was oxidized to carbon dioxide with potassium dichromate in the presence of conc. Sulphuric acid. The unreacted potassium dichromate was titrated with

ammonium iron (II) Sulphate. Considering that the average content of carbon in soil organic matter was equal to 58%, the conversion factor 1.724 was used to calculate the percentage of organic matter from the content of organic carbon.

The cation exchange capacity (CEC) is the maximum amount of cations that 100g of dry soil can absorb. The CEC was determined using the Direct saturation method. The particle size composition (sand, silt and clay) of the soil samples were determined by the hydrometer method. The texture of the soil samples was established by charting the percentages of sand, silt and clay fractions with a textural triangle.

## **Results**

The studied areas include Fig. 1 to 6. Fig 7 shows the flow diagram for the partitioning of metals in farmland soils into six steps. The pH of the soils (Fig 8) tested ranged from 4.1 – 8.1, indicating acidic, neutral and slightly alkaline. Humus content (Fig. 9) ranged from 0.6 – 4.6%. Fig. 10 shows the general frequency distribution pattern of the CEC for the soil samples and is skewed towards low frequency of high concentrations. A direct consequence of low soil CEC is that cations added in the form of fertilizers would be loosely held and therefore easily lost by leaching. Table 1 shows the soil texture and textural classification. The textural analysis of the farming soils shows that sand > silt > clay. The textural classification shows dominantly, sandy loam. Tables 2 – 6 show the concentrations of soil Pb, Cu, Cd, Cr and Zn in the individual sequential extraction fractions.



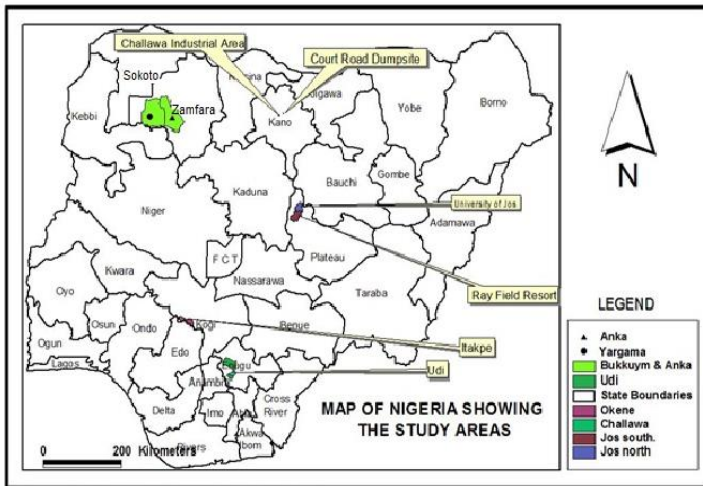


Fig. 1: Map of Nigeria Showing the Study Areas

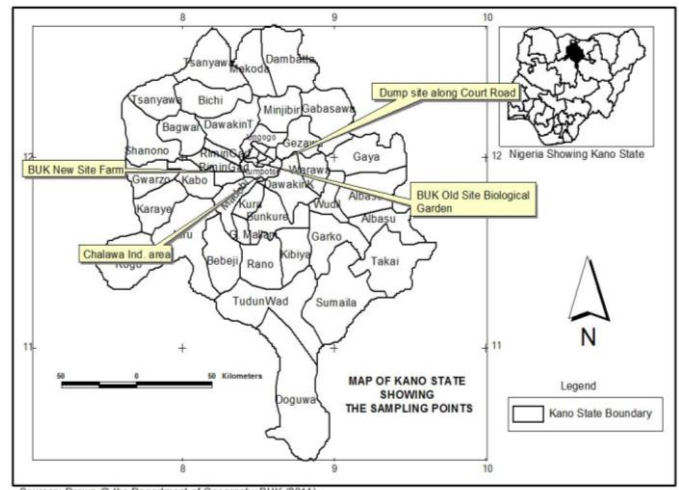


Fig.4: Map of Kano State Showing the Sampling Points

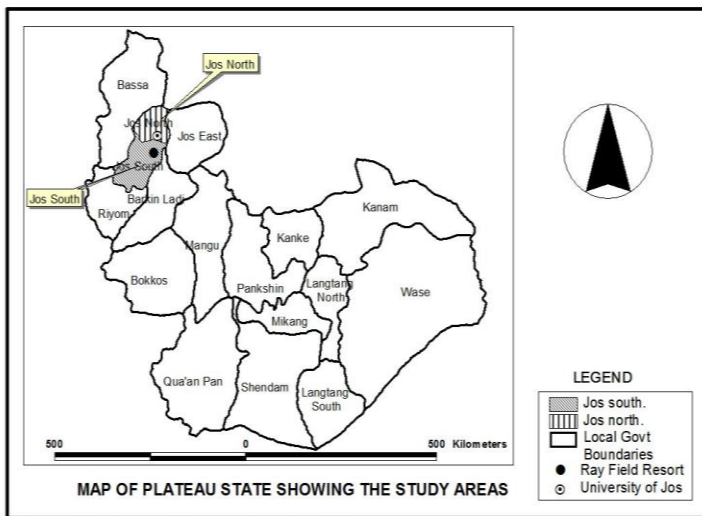


Fig.2: Map of Plateau State Showing the Study Areas

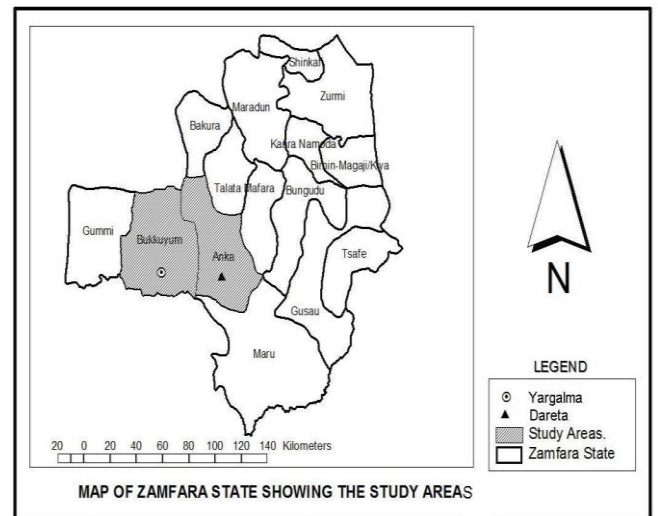


Fig.5: Map of Zamfara State Showing the Study Areas

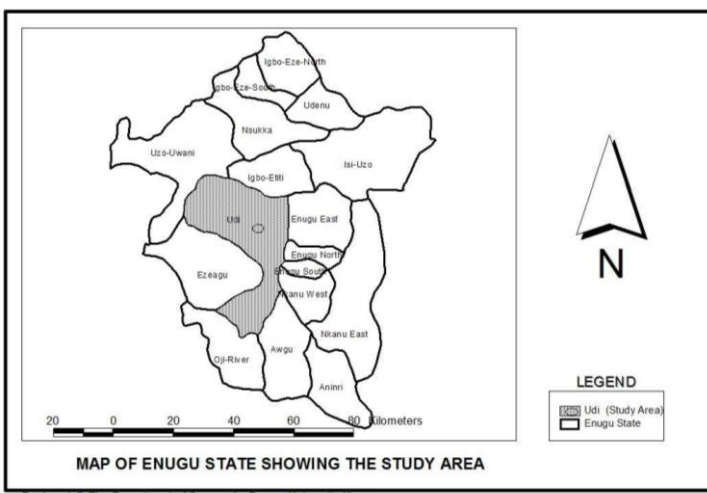


Fig.3: Map of Enugu State Showing the Study Area

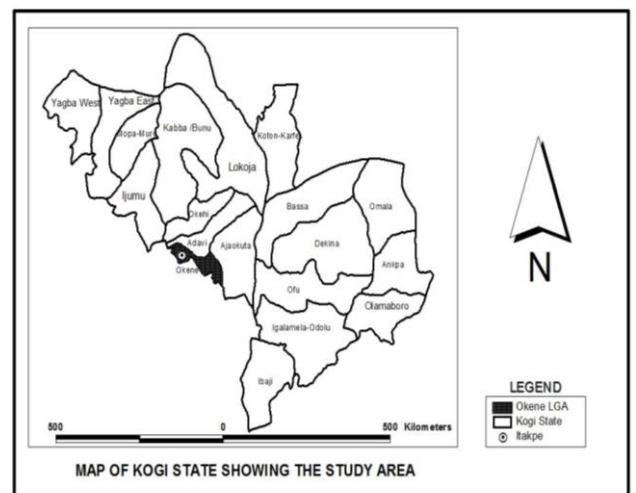


Fig. 6: Map of Kogi State Showing the Study Area



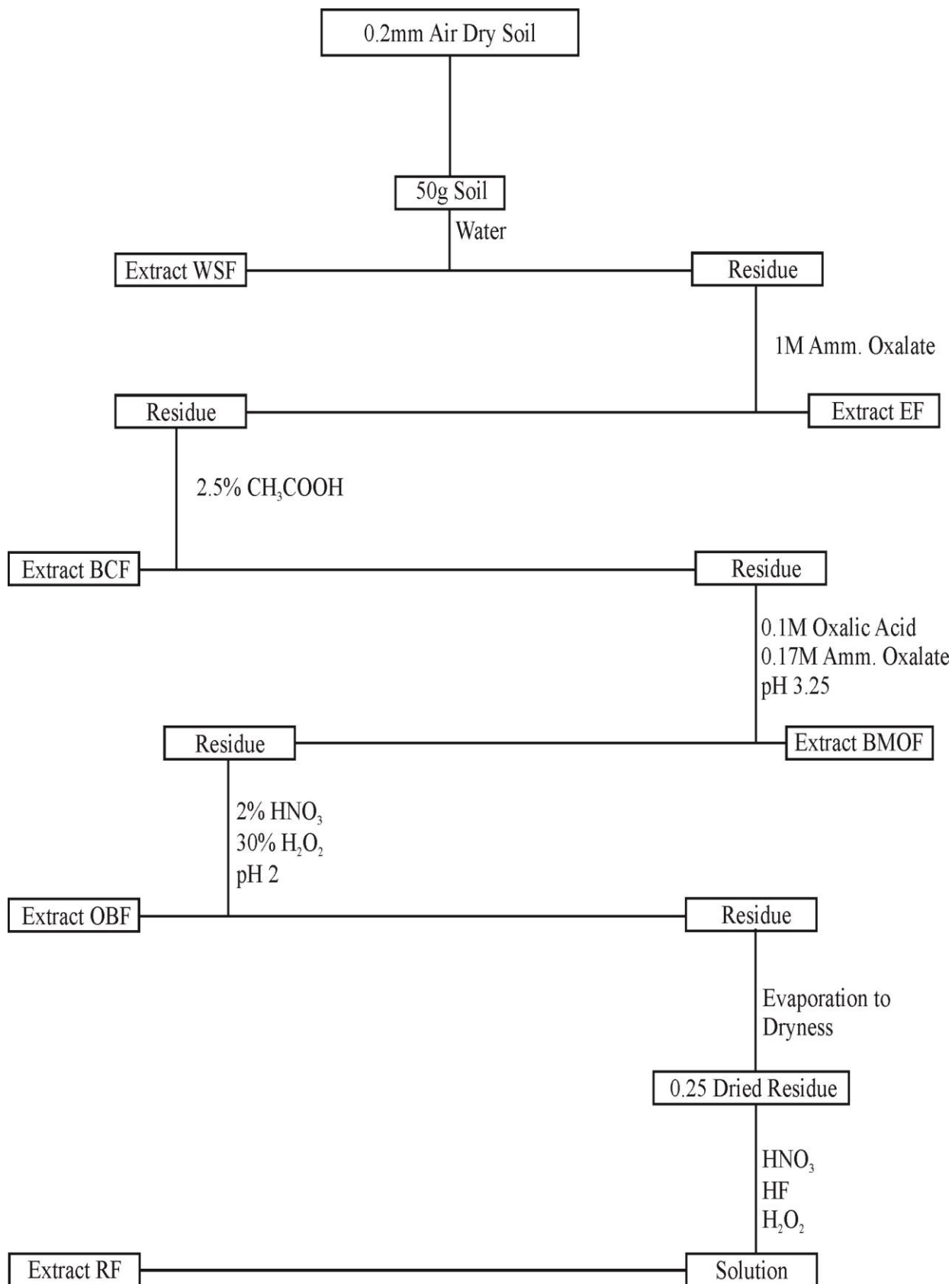
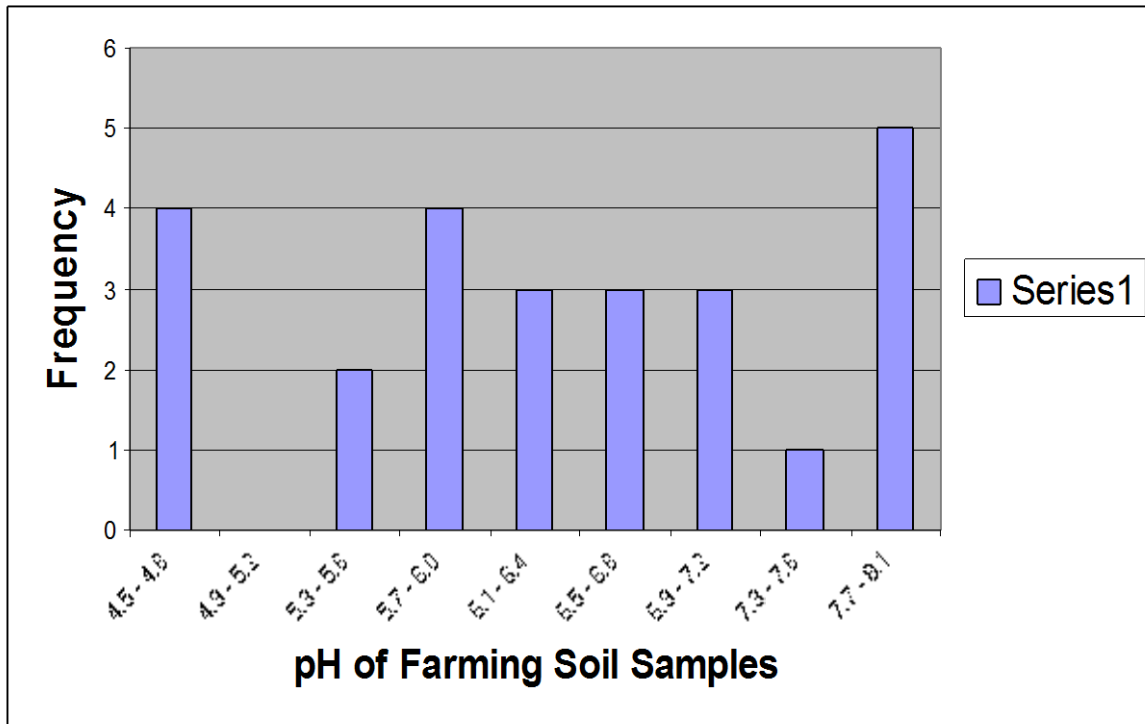
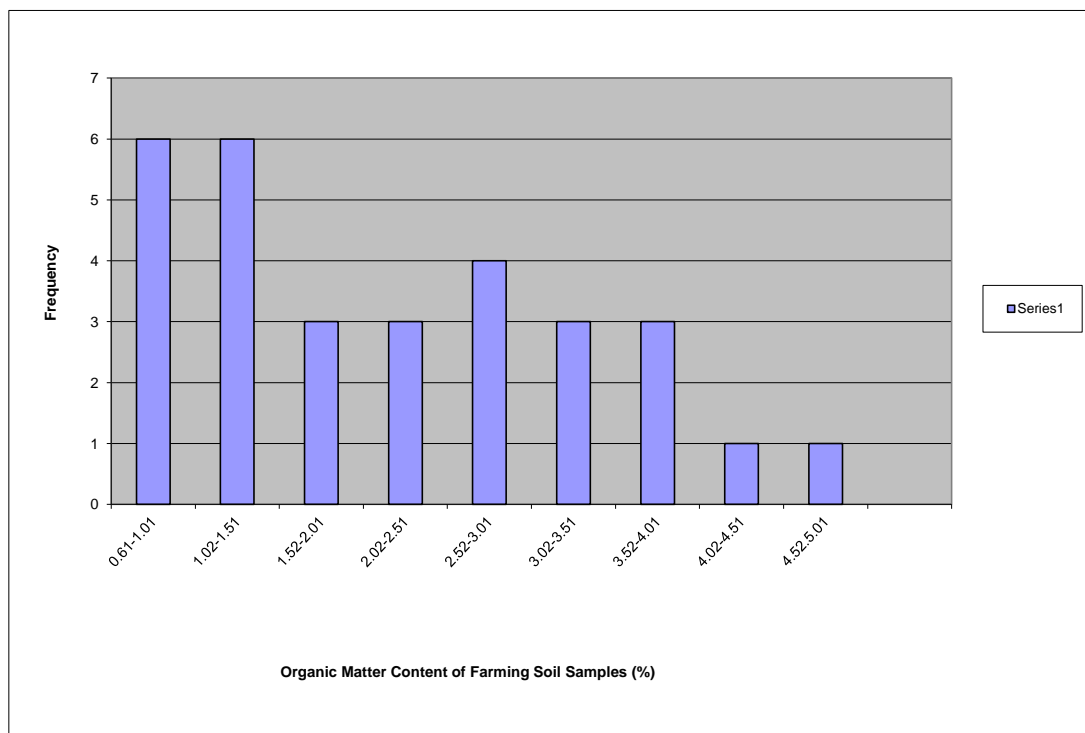


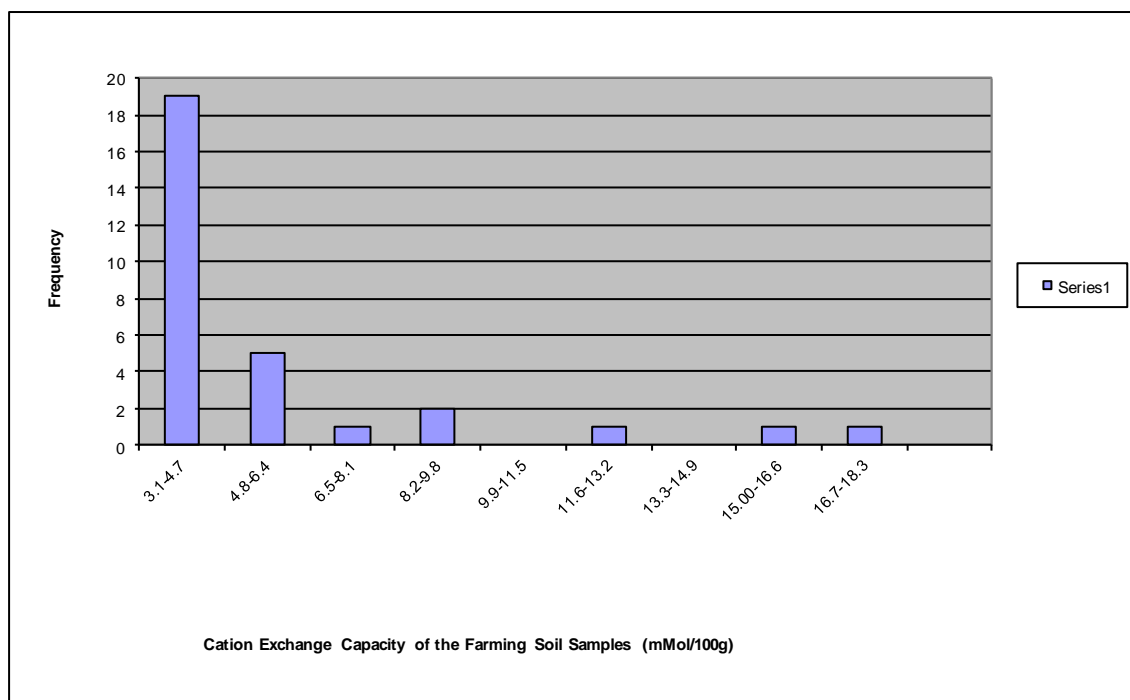
Fig. 7 Flow Diagram for the Partitioning of Metals in Farming Soils



**Fig.8** Distribution Pattern for pH in Farming Soil Samples



**Fig.9** Frequency Distribution Pattern for Organic Matter Content of Farming Soil Samples



**Fig.10** Frequency Distribution Pattern for Cation Exchange Capacity of Farming Soil Samples

**Table 1** Soil Texture and Textural Classification

S/ N	Hydrometer Reading			2 hour Hydrometer Reading			Percentage sand, silt and clay				Textural class
	Temp. 20 <sup>0</sup> C	HR <sub>1</sub>	CHR <sub>1</sub>	Temp. 21 <sup>0</sup> C	HR <sub>2</sub>	CHR <sub>2</sub>	Silt + Clay	Clay	Silt	Sand	
1.	20 <sup>0</sup> C	31	31	21 <sup>0</sup> C	9+.3	9.3	62	19	43	38	Silt loam
2.	20 <sup>0</sup> C	30	30	21 <sup>0</sup> C	9+.3	9.3	60	19	41	40	Silt loam
3.	20 <sup>0</sup> C	27	27	21 <sup>0</sup> C	8+.3	8.3	54	17	37	46	Silt loam
4.	20 <sup>0</sup> C	23	23	21 <sup>0</sup> C	12+.3	12.3	46	25	21	54	Loam
5.	20 <sup>0</sup> C	21	21	21 <sup>0</sup> C	4+.3	4.3	42	9	33	58	Sandy loam
6.	20 <sup>0</sup> C	20	20	21 <sup>0</sup> C	4+.3	4.3	40	9	31	60	Sandy loam
7.	20 <sup>0</sup> C	17	17	21 <sup>0</sup> C	3+.3	3.3	34	7	27	66	Sandy loam
8.	20 <sup>0</sup> C	11	11	21 <sup>0</sup> C	2+.3	2.3	22	5	17	78	Loamy sand
9.	20 <sup>0</sup> C	20	20	21 <sup>0</sup> C	3+.3	3.3	40	7	33	60	Sandy loam
10.	20 <sup>0</sup> C	16	16	21 <sup>0</sup> C	2+.3	2.3	32	5	27	68	Loamy sand
11.	20 <sup>0</sup> C	15	15	21 <sup>0</sup> C	3+.3	3.3	30	7	23	70	Loamy sand
12.	20 <sup>0</sup> C	12	12	21 <sup>0</sup> C	2+.3	2.3	24	5	19	76	Loamy sand
13.	20 <sup>0</sup> C	13	13	21 <sup>0</sup> C	3+.3	3.3	26	7	19	74	Loamy sand
14.	20 <sup>0</sup> C	13	13	21 <sup>0</sup> C	2+.3	2.3	26	5	21	74	Loamy sand
15.	20 <sup>0</sup> C	14	14	21 <sup>0</sup> C	3+.3	3.3	28	7	21	72	Loamy sand
16.	20 <sup>0</sup> C	10	10	21 <sup>0</sup> C	2+.3	2.3	20	5	15	80	Loamy sand
17.	20 <sup>0</sup> C	13	13	21 <sup>0</sup> C	2+.3	2.3	26	5	21	74	Loamy sand
18.	20 <sup>0</sup> C	15	15	21 <sup>0</sup> C	3+.3	3.3	30	7	23	70	Loamy sand
19.	20 <sup>0</sup> C	15	15	21 <sup>0</sup> C	3+.3	3.3	30	7	23	70	Loamy sand
20.	20 <sup>0</sup> C	21	21	21 <sup>0</sup> C	3+.3	3.3	42	7	35	58	Sandy loam
21.	20 <sup>0</sup> C	23	23	21 <sup>0</sup> C	6+.3	6.3	46	13	33	54	Sandy loam
22.	20 <sup>0</sup> C	29	29	21 <sup>0</sup> C	7+.3	7.3	58	15	43	42	Silt loam
23.	20 <sup>0</sup> C	24	24	21 <sup>0</sup> C	4+.3	4.3	48	9	39	52	Sandy soil
24.	20 <sup>0</sup> C	23	23	21 <sup>0</sup> C	4+.3	4.3	46	9	37	54	Sandy loam
25.	20 <sup>0</sup> C	13	13	21 <sup>0</sup> C	3+.3	3.3	26	7	19	74	Loamy sand

26.	20 <sup>0</sup> C	22	22	21 <sup>0</sup> C	3+.3	3.3	44	7	37	56	Sandy loam
27.	20 <sup>0</sup> C	24	24	21 <sup>0</sup> C	2+.3	2.3	48	5	43	52	Sandy loam
28.	20 <sup>0</sup> C	21	21	21 <sup>0</sup> C	3+.3	3.3	42	7	35	58	Sandy loam
29.	20 <sup>0</sup> C	22	22	21 <sup>0</sup> C	5+.3	5.3	44	11	33	56	Sandy loam
30.	20 <sup>0</sup> C	18	18	21 <sup>0</sup> C	2+.3	2.3	36	5	31	64	Sandy loam

S/No.	WSF	EF	BCF	BMO	OBF	RF	Sum of Pb Fraction	Pb Conc. in the Original Sample	% Recovery	Mobility Factor	Bioavailability
1	1.41	2.28	3.73	2.42	3.32	6.71	19.87	20.5	96.93	19	0.19
2	0.67	1.68	2.38	3.86	4.40	ND	12.99	13.7	94.82	18	0.18
3	1.91	2.65	3.43	5.51	6.31	ND	19.81	20.5	96.63	23	0.23
4	1.07	1.41	2.49	3.86	4.23	ND	13.06	13.7	95.33	19	0.19
5	2.02	2.66	3.40	5.68	6.05	ND	19.81	20.5	96.63	24	0.24
6	1.35	1.89	2.29	3.67	3.88	ND	13.02	13.7	95.04	25	0.25
7	1.19	2.86	5.11	5.95	5.01	6.74	27.65	27.4	100.91	18	0.18
8	2.35	3.49	4.70	4.47	4.84	6.74	26.59	27.3	97.40	22	0.22
9	3.03	5.62	7.16	8.17	9.05	6.72	39.75	40.9	97.19	22	0.22
10	1.68	2.86	3.71	5.80	5.66	ND	19.71	20.5	96.15	23	0.23
11	1.82	2.60	3.31	5.78	6.15	ND	19.66	20.5	95.90	22	0.22
12	4.10	5.10	7.15	8.16	8.36	6.72	39.59	41.0	96.56	23	0.23
13	1.75	2.43	3.84	5.90	5.90	ND	19.82	20.5	96.68	21	0.21
14	2.35	4.06	5.84	5.23	8.19	6.72	32.39	34.2	94.71	20	0.20
15	0.50	0.64	1.38	1.65	1.98	ND	6.15	6.8	90.44	19	0.19
16	2.39	4.67	5.97	6.18	7.28	6.75	34.14	34.4	99.53	23	0.23
17	3.91	5.56	7.18	7.79	8.56	6.72	39.72	40.9	97.11	24	0.24
18	2.49	3.29	4.94	4.07	4.87	6.75	26.41	27.3	96.74	22	0.22
19	1.66	2.60	3.59	5.99	5.61	ND	19.45	20.5	94.88	22	0.22
20	1.01	1.32	1.65	1.99	2.33	ND	8.30	13.7	60.58	28	0.28
21	2.22	2.92	3.43	5.27	5.51	ND	19.35	20.5	94.39	27	0.27
22	1.14	1.71	2.28	3.36	4.06	ND	12.55	13.7	91.61	23	0.23
23	0.50	0.84	1.18	1.78	1.95	ND	6.25	6.8	91.91	21	0.21
24	2.85	3.79	3.72	3.86	4.36	6.72	25.30	27.3	92.67	26	0.26
25	2.83	3.50	8.44	8.48	9.85	6.74	36.84	41.0	89.85	17	0.17
26	2.92	3.93	4.94	4.13	5.10	6.72	27.74	27.3	101.61	25	0.25
27	1.17	1.85	2.35	3.26	4.06	ND	12.69	13.6	93.31	24	0.24
28	2.25	3.73	4.87	3.89	4.66	6.71	26.11	27.3	95.64	23	0.23
29	2.62	3.93	6.14	3.66	7.89	6.74	30.98	34.2	90.58	21	0.21

30	4.66	6.38	8.15	9.66	10.84	6.71	46.40	47.8	97.07	24	0.24
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**Table 2:** Concentration of Soil Pb in the Individual Fractions (mg/kg)

**Table 3:** Concentration of Soil Cu in the Individual Fractions (mg/kg)

S/No.	WSF	EF	BCF	BMO	OBF	RF	Sum of Cu Fraction	Cu Conc. in the Original Sample	% Recovery	Mobility Factor	Bioavailability
1	2.75	3.16	4.05	4.87	3.89	6.33	25.05	25.3	99.01	24	0.24
2	1.90	2.37	2.82	5.89	4.91	ND	17.89	19.0	94.16	24	0.24
3	3.32	4.24	5.16	6.62	5.32	6.34	31.00	31.7	97.79	24	0.24
4	1.33	1.23	1.71	4.34	3.10	ND	11.71	12.7	92.20	25	0.25
5	2.09	2.47	2.89	2.60	1.71	6.34	18.10	19.0	95.26	25	0.25
6	2.70	3.12	3.97	4.86	3.82	6.34	24.81	25.3	98.06	23	0.23
7	3.49	4.34	5.17	6.53	5.33	6.36	31.22	31.8	98.18	25	0.25
8	6.08	7.25	7.82	9.15	6.84	12.72	49.86	50.7	98.34	27	0.27
9	5.23	6.12	6.53	7.49	6.91	6.33	38.61	37.9	101.87	29	0.29
10	2.22	2.54	2.89	2.45	2.00	6.34	18.44	19.0	97.05	26	0.26
11	5.10	6.66	8.22	9.52	7.58	12.67	49.75	50.6	98.32	24	0.24
12	1.74	2.15	2.98	3.07	2.15	6.34	18.43	19.0	97.00	21	0.21
13	3.15	3.53	3.88	4.42	3.65	6.32	24.95	25.3	98.62	27	0.27
14	2.06	2.18	2.72	3.13	1.99	6.34	18.42	19.0	96.95	23	0.23
15	1.27	1.30	1.71	4.38	3.14	ND	11.80	12.7	92.91	22	0.22
16	5.19	6.46	7.06	10.00	8.67	6.36	43.74	44.5	98.29	27	0.27
17	3.34	4.42	4.93	6.64	5.34	6.33	31.00	31.6	98.10	25	0.25
18	2.60	2.82	3.17	6.12	3.74	6.36	24.81	25.3	98.06	22	0.22
19	0.64	0.87	1.02	1.82	1.56	ND	5.87	6.3	93.17	25	0.25
20	4.90	6.27	7.07	12.03	8.63	6.33	45.23	44.4	101.87	25	0.25
21	3.07	3.67	4.88	7.38	5.48	6.34	30.82	31.7	97.22	22	0.22
22	2.41	2.75	3.04	5.25	4.62	ND	18.07	19.0	98.42	29	0.29
23	0.63	0.66	1.11	1.96	1.46	ND	5.82	6.3	92.38	22	0.22
24	3.42	4.37	4.84	6.84	5.32	6.34	31.13	31.7	98.20	25	0.25
25	2.47	3.33	4.16	4.47	3.93	6.36	24.72	25.3	97.71	23	0.23
26	3.99	5.45	5.92	8.27	7.22	6.34	37.19	38.0	97.87	25	0.25
27	0.95	1.65	1.87	4.46	3.48	ND	12.41	12.6	98.49	21	0.21
28	3.67	4.56	4.97	5.98	3.23	6.32	28.73	31.7	90.63	29	0.29
29	2.03	2.37	2.78	3.13	2.53	6.36	19.20	31.7	60.57	23	0.23
30	6.74	8.54	9.75	14.08	10.98	12.66	62.75	63.3	99.13	24	0.24

S/No.	WSF	EF	BCF	BMO	OBF	RF	Sum of Cd Fraction	Cd Conc. in the Original Sample	% Recovery	Mobility Factor	Bioavailability
1	1.17	1.43	2.59	1.29	0.86	2.48	9.82	9.9	99.19	26	0.26
2	1.00	1.15	1.66	2.18	1.28	ND	7.36	7.4	99.46	29	0.29
3	1.58	1.91	2.79	2.33	1.09	2.48	12.18	12.4	98.23	29	0.29
4	0.34	0.36	0.55	0.73	0.35	ND	2.33	2.5	93.20	30	0.30
5	1.90	2.26	3.41	3.05	1.63	2.49	14.74	14.8	99.59	28	0.28
6	1.30	1.67	2.46	1.72	0.98	2.49	10.62	9.9	107.27	28	0.28
7	1.59	1.75	2.64	2.11	0.86	2.49	11.44	12.4	92.26	29	0.29
8	1.46	2.69	3.87	3.75	2.11	2.49	16.37	17.3	94.62	25	0.25
9	0.81	1.07	1.90	2.16	1.18	ND	7.12	7.4	96.23	26	0.26
10	1.57	1.89	2.94	2.62	0.78	2.49	12.29	12.4	99.11	28	0.28
11	1.94	2.30	3.46	3.10	1.46	2.48	14.24	14.8	96.23	30	0.30
12	2.11	2.43	4.20	4.12	1.76	2.49	17.11	17.3	98.90	27	0.27
13	0.70	0.82	1.12	1.28	0.98	ND	4.90	7.4	66.23	31	0.31
14	1.19	1.50	2.47	1.29	0.82	2.49	9.76	9.9	98.59	28	0.28
15	0.61	0.76	1.19	1.42	0.80	ND	4.78	4.9	97.55	29	0.29
16	1.60	2.07	2.83	2.21	1.43	2.49	12.63	12.4	101.85	29	0.29
17	1.83	2.14	3.64	3.03	1.53	2.48	14.65	14.8	98.99	27	0.27
18	2.18	2.66	4.00	3.94	2.04	2.50	17.32	17.3	100.12	28	0.28
19	0.96	1.15	1.69	1.50	0.74	ND	6.04	7.4	81.62	35	0.35
20	1.39	1.52	2.13	1.85	1.22	ND	8.11	9.9	81.92	36	0.36
21	1.07	1.14	1.68	2.11	1.12	ND	7.12	7.4	96.22	31	0.31
22	1.97	2.28	3.28	2.97	1.76	2.48	14.74	14.8	99.59	29	0.29
23	0.32	0.35	0.53	0.72	0.35	ND	2.27	2.5	90.80	30	0.30
24	2.13	2.53	4.24	4.08	2.34	2.48	17.80	17.3	102.89	26	0.26
25	1.58	1.74	2.80	2.09	1.39	2.49	12.01	12.4	96.85	28	0.28
26	1.95	2.31	3.48	3.24	1.15	2.49	14.62	14.8	98.78	29	0.29
27	0.81	1.05	1.86	2.23	1.28	ND	7.23	7.4	97.70	26	0.26
28	2.22	2.89	4.29	3.87	2.07	2.48	18.64	17.3	107.75	27	0.27
29	1.58	1.86	2.80	2.52	2.11	2.49	13.36	12.4	107.74	26	0.26
30	1.86	2.18	3.37	3.18	1.51	2.48	14.58	14.8	98.51	28	0.28



**Table 4:** Concentration of soil Cd in the Individual Fractions (mg/kg)

S/No.	WSF	EF	BCF	BMO	OBF	RF	Sum of Cr Fraction	Cr Conc. in the Original Sample	% Recovery	Mobility Factor	Bioavailability
1	6.96	3.55	5.22	31.67	23.77	28.97	100.14	104.6	95.74	10	0.10
2	7.61	5.07	6.45	26.59	25.80	14.50	86.02	89.6	96.00	15	0.15
3	7.76	5.66	3.77	36.40	31.76	29.02	114.37	119.5	95.71	12	0.12
4	1.38	2.61	1.88	11.38	10.51	ND	27.76	29.9	92.84	14	0.14
5	5.23	7.70	2.47	30.87	24.55	29.03	99.85	104.4	95.64	13	0.13
6	7.72	7.06	4.59	26.35	24.68	14.52	84.92	89.6	94.78	17	0.17
7	11.47	7.62	8.35	33.18	27.88	29.11	117.61	119.9	98.09	16	0.16
8	5.0	2.61	3.70	15.81	15.16	14.56	56.84	59.8	95.05	13	0.13
9	7.12	3.56	5.23	31.74	23.82	29.01	100.48	104.4	96.25	11	0.11
10	6.26	5.97	10.11	39.37	37.99	29.03	128.73	134.5	95.71	10	0.10
11	3.43	5.03	6.71	29.25	26.55	14.51	85.48	89.5	95.51	10	0.10
12	8.12	4.06	6.02	36.47	31.61	29.04	115.32	119.5	96.50	11	0.11
13	5.97	7.93	3.86	31.65	21.83	28.96	100.20	104.6	95.79	14	0.14
14	1.45	1.74	4.06	7.46	8.55	14.52	37.78	59.8	63.18	8	0.8
15	2.18	2.03	2.69	10.24	9.15	ND	26.29	29.8	88.22	16	0.16
16	3.41	6.81	10.15	26.67	24.86	14.57	86.47	89.9	96.18	12	0.12
17	6.26	8.45	12.60	29.05	29.63	29.01	115.00	119.4	96.31	13	0.13
18	3.56	5.37	7.04	25.70	28.53	29.15	100.35	104.6	95.94	9	0.9
19	3.73	2.48	5.04	17.68	14.17	14.56	57.66	59.7	96.58	11	0.11
20	6.49	4.37	10.86	34.91	29.74	14.49	100.86	119.5	84.40	11	0.11
21	2.47	5.00	3.70	26.32	21.03	14.52	73.04	74.7	97.78	10	0.10
22	4.20	8.41	6.23	29.35	22.98	28.97	100.14	104.6	95.74	13	0.13
23	2.54	1.89	1.16	11.82	10.37	ND	27.78	29.8	93.22	16	0.16
24	4.13	6.59	7.54	28.70	26.96	14.51	88.43	89.6	98.69	12	0.12
25	8.13	6.03	3.99	30.87	21.64	29.11	99.77	104.6	95.38	14	0.14
26	5.80	5.66	8.63	37.63	40.46	43.55	141.73	149.3	94.93	8	0.8
27	2.61	2.90	4.28	21.67	20.15	14.50	66.11	74.6	88.62	8	0.8
28	4.57	6.45	6.74	31.38	33.55	28.96	111.65	119.5	93.43	10	0.10
29	7.46	9.28	11.09	20.65	21.59	29.13	99.20	104.6	94.84	17	0.17
30	6.96	13.91	18.48	39.20	35.80	29.00	143.35	149.3	96.01	15	0.15

**Table 5:** Concentration of Soil Cr in the Individual Fractions (mg/kg)

S/No.	WSF	EF	BCF	BMO	OBF	RF	Sum of Zn Fraction	Zn Conc. in the Original Sample	% Recovery	Mobility Factor	Bioavailability
1	11.98	15.32	20.44	21.69	16.52	19.32	105.27	106.4	98.94	26	0.26
2	8.45	11.21	19.71	20.68	16.18	19.33	95.56	96.7	98.82	20	0.20
3	11.60	12.91	19.38	23.64	18.32	9.67	95.52	87.1	109.67	25	0.25
4	6.52	7.25	11.30	13.00	8.89	9.67	56.63	58.0	97.64	24	0.24
5	10.80	11.67	17.09	14.96	12.83	19.35	86.70	86.9	99.77	26	0.26
6	9.32	11.89	19.90	17.76	15.63	19.36	93.86	96.7	97.06	23	0.23
7	11.71	14.81	23.33	26.48	19.12	19.41	114.86	116.5	98.59	23	0.23
8	12.76	16.92	25.43	28.86	20.55	19.42	123.94	125.8	98.52	24	0.24
9	11.38	15.54	23.73	25.90	19.03	19.34	144.83	115.8	99.16	23	0.23
10	16.64	23.00	35.80	38.47	30.08	29.03	173.02	174.1	99.38	23	0.23
11	13.76	15.95	25.14	27.47	22.90	19.35	124.57	125.6	99.18	24	0.24
12	20.69	26.49	47.17	47.56	40.07	38.72	220.70	222.3	99.28	21	0.21
13	38.27	43.36	47.68	48.26	47.82	38.62	264.01	280.4	94.15	31	0.31
14	19.47	24.98	36.33	39.57	25.36	38.71	184.42	183.8	100.34	24	0.24
15	7.80	10.27	15.49	19.22	13.99	9.70	76.47	77.3	98.93	24	0.24
16	8.60	10.20	18.80	15.41	11.98	19.42	84.41	87.3	96.69	22	0.22
17	12.04	13.83	23.93	27.91	17.91	19.34	114.96	115.9	99.19	23	0.23
18	13.07	18.00	27.05	30.25	25.89	19.43	133.69	135.4	98.74	23	0.23
19	6.82	9.06	13.63	16.21	11.59	9.70	67.01	67.7	98.98	24	0.24
20	15.36	19.49	33.48	36.30	28.91	19.32	152.86	154.8	98.75	23	0.23
21	15.57	20.69	33.64	36.45	26.44	29.03	161.82	164.4	98.43	22	0.22
22	14.40	18.60	27.14	30.49	24.02	19.32	133.99	135.4	98.96	25	0.25
23	11.94	14.50	25.14	26.54	17.21	19.33	114.66	115.9	98.93	23	0.23
24	9.08	11.45	18.07	14.83	13.14	19.35	85.92	87.0	98.76	24	0.24
25	8.67	13.17	19.56	21.01	15.64	19.41	97.46	96.7	100.79	22	0.22
26	10.01	8.80	15.47	18.27	13.49	9.68	75.72	77.3	97.96	25	0.25
27	3.48	3.00	3.77	5.07	4.49	9.67	29.48	48.3	61.04	22	0.22
28	9.66	12.17	20.10	20.00	14.01	19.31	95.25	96.7	98.50	23	0.23
29	9.52	10.48	14.69	16.86	14.15	9.71	75.41	77.4	97.43	27	0.27
30	13.57	12.37	16.67	18.50	15.46	9.67	86.24	87.0	99.13	30	0.30

**Table 6:** Concentration of Soil Zn in the Individual Fractions (mg/kg)

## DISCUSSION

### 23 Element Recoveries

Validation of the analytical results was tested by recovery experiments because there was no standard reference material (SRM), which is more preferential or needed to control the accuracy of the method studied, in our laboratory. An important consideration in the reliability of a sequential extraction data is the percentage recovery relative to a single digestion using a mixture of strong mineral acids or generally a mixture of strong acids at the digestion of the residual phase of the sequential extraction protocol employed <sup>[38]</sup>. Recovery is defined as follows:

$$\% \text{ Recovery} = \left( \frac{\sum n \text{ Sequential Extraction Procedure}}{\text{Single Digestion With Strong Acids}} \right) \times 100$$

Where n is the concentration of a given element, and the single digestion with strong acids used for reference was a mixture of strong acids used in the residual fraction digestion <sup>[38]</sup>. The analytical results acquired are depicted in tables 2 – 6. Recovery values of the tested elements for the scheme were calculated according to the equation above, and generally agreed with each other although some recoveries deviated from acceptable values. For example, low recoveries were obtained for Pb (60.58%), for Cu (60.57%), for Cr (63.18%), for Cd (66.23%), for Zn (61.04%), respectively. Some recoveries, however, were higher than 100% for Pb, Cu, Cd, Cr, Zn,. Already, in the use of sequential extraction procedures for partitioning/speciation of metals, sample contamination or loss could occur during the extraction steps. This phenomenon may cause the experimental errors, i.e., obtaining low or high recovery.

The difference in pH of the soils (Fig 8) highlight the displacement of the ions H<sup>+</sup> adsorbed on the exchange sites of the absorbing complex from soil towards the soil solution. pH is one of the factors which influence the bioavailability and the transport of heavy metal in the soil and according to <sup>[34]</sup>, heavy metal mobility decreases with increasing soil pH due to precipitation of hydroxides, carbonates or formation of insoluble organic complexes. Heavy metals are generally more mobile at pH <7 than at pH >7. The amount of heavy metals mobilized in soil environment is a function of pH, properties of metals, redox conditions, soil chemistry, organic matter content, clay content, cation exchange capacity and other soil properties<sup>[35,36,37]</sup>.

The sequential extraction procedures results provided information on the potential mobility and bioavailability of the studied elements in this research. The distribution of heavy metals in the sample allows us to predict their mobility and bioavailability. The bioavailability factor was expressed as the ratio of the available concentration of a metal in soil to its total concentration. It shows the potentials of a particular metal from the soil matrix to enter the soil solution from which it can be absorbed by plants. Mobility factor was expressed as percentage of the Bioavailability factor. The metal concentrations, percentage recovery, mobility and bioavailability factors of all the sequential extraction steps including the residual phase fractions, determined at each extraction step in Tables 2 -6, indicate that each metal has a characteristic distribution pattern.

Table 2, shows the mobility, and bioavailability factors and percentage recovery of pb for all the sequential extractions steps. The MF of pb gave average value of 22% while average Bf of pb was 0.22. The percentage recovery of Pb ranged from 60.58 -101.61%. The high MF and BF values of soil pb may be interpreted as symptoms of relatively high liability and biological availability of the metals in soil<sup>[25,29]</sup>. Similar characteristics distribution patterns were observed for Cu, Cd, Cr and Zn (Tables 3 -6). The average mobility of Pb, Cu, Cd, Cr and Zn levels in all the six fractions were in the order: Cd > Cu > Zu > Pb > Cr.

The water soluble fraction (i.e Cd, Cu, Zu and Pb) is certainly the most biologically active. The water soluble fraction has highest potential of contamination of food chain, surface water and ground water<sup>[40,41]</sup>.

The high amount of Cd, Pb and other heavy metals associated with the non – residual fractions show that they may be easily transferred into food chain through water reservoirs, uptake by plants growing in the soils or any other mechanism, may have a potentially negative impact on environmental quality and human health. Generally, Cd seemed to be easily mobilized element in this study. Cd and Pb metals have toxic effects on living organisms because they are not required nutrient elements. Elemental speciation information is crucial today because the toxicity and biological activity of many elements depend not only on their quantities but also on their chemical forms<sup>[42]</sup>.

## **Conclusion**

The increasing of the total metals (Cd, Cu, Zn, and Cr) content in the soil is due to anthropogenic activities. Once in the soil, Cd binds preferentially to carbonates which explains its greater mobility and its availability to plants. This may present a real threat as cadmium is transferred into the food chain from soil contaminated by

this metal. The kidney, central nervous systems and the liver are the major target organs of Cd accumulation and exposure to Cd leads to renal tubular dysfunction, poor bone mineralization and testicular necrosis.

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