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Fractionation Of Heavy Metals In Soil Samples From Rice Fields In New Bussa, Nigeria

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Abstract : This study was conducted to assess the effects of long term use of fertilizers, herbicides and pesticides on heavy metal (Cd, Cr, Cu, Pb and Zn) contents in soil used for rice cultivation. Five rice farming areas were chosen for the study and soil samples were collected in the dry season from rice fields (1 ha each) within these areas. Physico-chemical parameters of soil (pH, electrical conductivity, organic matter, total nitrogen, available phosphorus, total phosphorus and cation exchange capacity), total heavy metal contents and metal contents in the different soil fractions were determined. The analytical results reveal that mean values of pH, electrical conductivity, organic matter and cation exchange capacity were significantly higher (P<0.05) in samples from site 3 (S3) compared to others. Comparison of mean total metal concentrations with UNEP/ BMFT recommended limits for heavy metals in cultivated soil, showed that while the limits for Cr and Cu were not exceeded, mean Pb and Zn concentrations at all sites exceeded the limits. However, metal concentrations were below the Canadian Quality Guidelines values for soil meant for agricultural use. The distribution of the metals in the soil fractions shows that Cr, Cu and Zn were mostly associated with the Fe and Mn oxide-hydroxide bound or reducible fraction while Cd and Pb were mostly associated with the organic matter bound or oxidisable fraction. At all sites, the water soluble fraction generally had the lowest mean values. **Keywords :** Fertilizers, herbicides, pesticides, heavy metals, soil fractions.

Introduction

The three basic processes through which soil quality can be degraded are: physical – arising from erosion; chemical – due to nutrient deficiency, heavy metal contamination etc. and biological – resulting from loss of organic matter ¹. Heavy metal accumulation is a principal factor in soil quality degradation and reduction of the capacity of the soil to grow healthy plants ^{2,3}. Soils may become contaminated from the accumulation of heavy metals through application of fertilizer, animal manures, biosolids (sewage sludge), compost, pesticides, etc⁴. Heavy metals exist in various forms in the soil but the forms that are available to plants are of greatest importance and include the exchangeable, oxides and hydroxides, carbonate and organic matter bonded metals ⁵. The presence of heavy metals pollutants in the soil is of great concern because their absorption by plants could put human and animal consumers of plants at great risk. In addition, it has adverse effects on microbial activities, soil porosity, cation exchange capacity (CEC), mineral composition, seed germination etc. ^{6,7}. Traditionally, total metal contents was used to determine concentrations of metals in soil samples but it is a poor indicator of the mobility, bioavailability and risk of remobilization of metals which depend on the chemical form and type of binding the metals undergo. Fractionation of metals into operationally defined forms using sequential extraction provides information on the distribution, mobility and bioavailability of metals and is also important in determining whether metals are of anthropogenic or lithogenic origin ^{8,9}. Metals associated with the exchangeable, carbonatic, reducible and organic matter fractions are considered to be more mobile and are mostly from anthropogenic sources ¹⁰. They give information regarding the type and severity of the pollution to which a particular environment has been exposed. Metals in the residual (inert fraction) may be regarded as having originated from geochemical or natural sources (i.e., of lithogenic origin).

Niger State is one of the key rice producing states in Nigeria and New Bussa is an important rice producing area in the state. Rice farms in New Bussa are usually treated with herbicides and pesticides to kill grasses and eliminate rice pests. In addition the soil is amended with organic (compost, chicken and cow manure) and inorganic fertilizers (NPK – nitrogen-phosphorus-potassium and urea). This study was designed to determine the effects of long term use of organic fertilizers, herbicides and pesticides on soil physico-chemical parameters, total heavy metal content and metal contents in the different soil fractions. The results were used to determine whether metal concentrations have accumulated to dangerous levels in the soil.

Materials and Methods

Study Area



Figure 1. Borgu Local Government Area and New Bussa., (Insert – map of Nigeria showing Niger State)

New Bussa is in Borgu Local Government Area of Niger State and is situated in the semi-arid zone of Nigeria (Figure 1) and is located between latitude 9° 51'N – 10° 55' N and longitude 4° 23'E – 4° 45'E. It has a tropical continental climate characterized by a shorter wet season (May to September) and a longer dry season (October to April) with a temperature range of 15 °C to nearly 40 °C. The months, February, March and April, are usually the hottest months of the year and the hot period reaches its peak in April. Annual rainfall is about 1000 mm but there's considerable variation in amount and distribution of rainfall among years. The vegetation is guinea savanna which is characterized by undistributed woodland with trees 15 – 18 m tall. The soil is generally alluvium but is highly variable in physical composition, generally low in phosphorus and nitrate but

rich in potassium. The rice fields (rain-fed lowland systems) have been under long term (at least 10 years in each case) amendment with cow and chicken manure, NPK and urea fertilizers, herbicides and pesticides. Organic fertilizers are usually applied during soil preparation. After tilling of the soil, the rice seeds are planted by direct seeding followed by the application of herbicides before the rice germinates. NPK is usually applied to the rice seedlings after some weeks while urea is applied during the flowering stage to boost grain production. Urea is a nitrogen based fertilizer and the rice plant requires a lot of nitrogen for growth. Inorganic fertilizers are usually applied at the rate of 56 kgha⁻¹ (urea) and 200 kgha⁻¹ (NPK). Manual weeding of the rice is done when the need arises. Rice varieties with three to four (FARO 47, FARO 52, FARO 57 etc.) month maturity periods are planted in these areas.

Sampling

Soil samples were collected from the rice fields in Dogon Gari (Site 1 or S1), Kuruwasa (S2), Tamanai (S3), Karabonde (S4) and National Institute for Freshwater Fisheries Research (NIFFR) rice area (S5). Five samples were collected from each rice field with an acid washed polyethylene corer from the surface layer (0 - 30 cm). The samples were then mixed to give a composite sample. Extraneous materials were removed from the composite soil samples. All samples for organic matter and heavy metal analyses were oven dried at 110 °C ¹¹ while those for other analyses were air dried. Dry soil samples from each treatment were ground into fine powder with acid washed plastic mortar and pestle. Samples from each treatment were divided into two and passed separately, through 2 mm and 0.2 mm sieves. They were then stored in dry acid washed polyethylene bottles with screw caps, at room temperature, prior to analyses. The 2 mm samples were used for the determination of pH, electrical conductivity (EC), total phosphorus, available phosphorus and nitrogen, while the 0.2 mm samples were used for organic matter, CEC and metal analyses.

Quality Assurance

Analytical grade reagents and chemicals were used for this study. Distilled-deionised water was also used in all relevant analyses. All digestion and analyses were done in triplicate. Procedural and reagent blanks were used and a clean laboratory environment was ensured during the analysis and preparation of solutions. Glassware, plastic containers, crucibles, pestle and mortar were washed with liquid soap, rinsed with distilled-deionised water and then soaked in 10 % HNO₃ solution for 24 h. They were washed with distilled-deionised water again and dried in a Gallenkamp drying oven at 80 $^{\circ}$ C for 5 h.

Buck Scientific (model 2.0 VGP) Flame Atomic Absorption Spectrophotometer (FAAS) was used for metal analyses. It was calibrated with Multi-element standard Solution (MESS) and the calibration standards were analysed after 10 sample runs to ensure that the instrument remained calibrated. The following salts of the heavy metals of interest were used to prepare a Multi-Element Standard Solution (MESS): $Cd(NO_3)_2.4H_2O$; $Cu(NO_3)_2.3H_2O$; K_2CrO_4 ; $Pb(NO_3)_2$ and ZnO. The concentrations of the metals in the MESS were: Cd, 2; Cr, 2; Cu, 5; Pb, 3 and Zn, 5 mgL⁻¹. Aliquots of the MESS were used to spike 2 g each of triplicate soil samples for the spiking/recovery experiment in lieu of standard reference materials. The samples were digested together with blanks and run on FAAS. Concentrations of metals in spiked and unspiked samples were used to calculate percent recovery in order to validate the method.

Physico-chemical Analyses of Soil Samples

Soil pH was determined by inserting the probe of a pre-calibrated pH meter into 2:1 (soil/water) slurry made with 2 mm air dried soil sample in water and taking the reading. Electrical Conductivity (EC) was determined with the 0.1 % sodium metaphosphate method ¹². Organic Matter (OM) was determined by oxidation of 1 g of oven-dried sample with 50 % H_2O_2 solution and ignition in a muffle furnace. Total nitrogen (TN) was analysed using the Kjeldahl method while total phosphorus was determined using the colorimetric method after acid digestion of the sample and extraction of neutralized digest with deionized waster ¹². Available Phosphorus was analysed using the Bray-1 method ¹³. Particle size analysis was done using the Bouyoucos hydrometer method which utilizes 50 % Calgon (sodium hexametaphosphate) as dispersing agent ¹⁴. Texture of the soil samples were classified according to International System of Textural Classification. Cation exchange capacity (CEC) was analyzed using the ammonium acetate method ¹².

Total Heavy Metal Analyses

Soil samples were digested by placing 2 g of sediment sample into a beaker followed by the addition of 9 ml of concentrated HNO₃ and 3 ml of concentrated HCl respectively ¹⁵. The beaker was placed in a microwave oven and digested at 700 W for 5 min in order to reach 180 $^{\circ}$ C. It was then digested at 500 W for 10 min to maintain a temperature of 180 $^{\circ}$ C. The sample was allowed to cool followed by centrifugation at 3500 rpm for 5 min. The extract was removed with pipette and filtered through Whatman No. 42 filter paper into a 50 ml volumetric flask and made up to mark with distilled-deionised water prior to FAAS analysis.

Heavy Metal Fractionation in Soil Samples

Soil samples were subjected to a modified six step sequential extraction (SE) ¹⁶ as follows:

SE1 – Water soluble fraction: 1 g of soil sample was placed in a centrifuge tube and shaken at room temperature with 20 ml of distilled-deionised water for 1 h followed by centrifugation. The resulting supernatant was made up to 40 ml with distilled/deionised water prior to analysis.

SE2 – Exchangeable fraction: Residue from SE1 was shaken at room temperature with 16 ml of 1 mol dm⁻³ Mg(NO₃)₂ at pH 7.0 for 1 hr, centrifuged and the supernatant was made up to 40 ml with distilled-deionised water prior to analysis.

SE3 – Oxidizable fraction (Bound to organic matter): Residue from SE2 + 10 ml of 8.8 mol dm⁻³ H₂O₂ + 6 ml HNO₃ 0.02 mol dm⁻³, were shaken for 5 +1 hr at 98°C. 10 ml of 3.5 mol dm⁻³ CH₃COONH₄ was added as an extracting agent, centrifuged and the supernatant made up to 40 ml with distilled-deionised water prior to analysis.

SE4 – Acid soluble fraction (Bound to carbonates): 25 ml of 0.05 mol dm⁻³ Na₂EDTA was added to the residue from SE3, shaken for 6 hr and centrifuged. The supernatant was made up to 40 ml with distilled-deionised water prior to analysis.

SE5 – Reducible fraction (Bound to Fe/Mn oxides and hydroxides): Residue from SE4 + 17.5 ml of 0.1 mol dm⁻³ NH₂OH·HCl + 17.5 cm³ CH₃COONH₄ 3.5 mol dm⁻³, was shaken for 4 + 1hr at 98 °C. It was extracted with 10 ml of 3.5 mol dm⁻³ CH₃COONH₄, shaken for 1hr, centrifuged and the supernatant was made up to 40 ml with distilled-deionised water prior to analysis.

SE6 – Residual fraction (Bound to silicates and detrital materials): Residue from SE5 was digested using aqua regia/hydrofluoric acid (HCl-HNO₃/HF) (0.35:12 w/v sediment/solution ratio) in an acid digestion Teflon cup. It was evaporated to dryness for 2h. The residue was diluted to 40 ml with distilled-deionised water prior to analysis.

After each successive extraction, the samples were centrifuged at 4500 rpm for 15 min ¹⁷. The supernatants were removed with pipette and filtered with Whatman No. 42 filter paper. The residue was washed with distilled-deionized water followed by vigorous hand shaking and 15 minutes of centrifugation before the next extraction. The volume of rinse water was kept to a minimum to avoid excessive solubilization of solid materials. Finally, the extracts collected were analyzed using FAAS to determine the concentrations of Cd, Cr, Cu, Pb and Zn.

Statistical analysis

Statistical analysis was done using the SPSS (version 13.0) for Windows software package. Mean concentrations and standard deviations were calculated for each parameter. The results were also subjected to analysis of variance (ANOVA) and means were compared using Duncan Multiple Range Test. Pearson correlation analysis was also performed to analyse and confirm the relationship between parameters.

Results and Discussion

Table 1 shows the percent recoveries calculated for each metal in soil samples from the spiking experiment. Zn had the highest recovery (97.30 %) from the soil sample while Cu had the lowest. Mean percentage recovery values were all above 80 % and so the digestion method and the FAAS machine are reliable. Table 1 also shows the settings and conditions for the FAAS analysis and these are standard conditions which were maintained throughout the analysis.

Metal	Percentage Recovery	Conditions for AAS					
	(Mean ±SD)	Wavelength (nm)	Slit width (nm)	Current (mA)	Flow (L/min)		
Cadmium	90.16 ±2.25	228.9	0.7	8	1.8		
Chromium	94.30 ± 1.80	357.9	0.7	10	2.8		
Copper	80.20 ± 2.18	324.7	0.7	6	1.8		
Lead	92.75 ±1.95	283.2	0.7	12	2.0		
Zinc	97.30 ± 3.42	213.9	0.7	8	2.0		

 Table 1. Percentage Recovery of Heavy Metals from Spiking Experiment Characteristic

 Conditions for AAS Analysis

Percentage Recovery values represent the mean and standard deviation for three replicates

The physico-chemical parameters of soil samples are shown in Table 2. These results show

Sampling	Physico-chemical Parameters of Soil Samples						
Sites	pН	EC	OM (%)	TN (%)	ТР	AP	CEC
		(µScm ⁻¹)			(mg kg ⁻¹)	$(mg kg^{-1})$	(mmol cm ⁻¹)
S 1	7.90	105.18a	1.08	0.56	28.33	9.17	10.25 ±2.13a
	±0.25a	±7.24	±0.15a	±0.19a	±2.16a	±1.39a	
S2	8.13	146.59b	1.37	0.30	44.08	15.20	13.64 ±2.59b
	±0.26a	± 13.20	±0.24b	±0.06b	±6.51b	±4.85b	
S 3	8.92	170.62c	3.21a	1.05	78.12	23.97	15.80 ±1.96b
	±0.30b	± 14.20	±0.92c	±0.40c	±10.44c	±4.04c	
S 4	7.50	160.51c	2.05	0.44	31.60	10.22	$12.07 \pm 3.44a$
	±0.79a	± 8.33	±0.84d	±0.12a	±7.29a	±2.45a	
S5	6.92	89.82d ±3.50	0.87	0.27	17.21	5.40	7.90 ±2.29c
	±0.30c		±0.10a	±0.08b	d±4.60	±1.88d	

Table 2. Physico-chemical Parameters (Mean ±SD) of Soil Samples

EC = Electrical conductivity, OM = Organic matter content, TN = Total nitrogen, TP = Total phosphorus, AV = Available phosphorus, CEC = Cation exchange capacity

Values represent the mean and standard deviation for three replicates

Means in the same column with different letters are significantly different (P<0.05), that mean pH was significantly higher (P<0.05) in soil sample from S3 compared to others. This may be as a result of higher fertilizer application at S3 ^{18,19}. However, the pH of NPK fertilizers are usually in the acidic region ²⁰. Mean EC values of the samples is also shown in Table 2. Mean EC was significantly lower (P<0.05) in S5 compared to the other sites and this may be as a result of lower application of manure in S5 since the application of animal manure may lead to increase of soil EC ^{21,22}. OM in S3 was significantly higher (P<0.05) than in the other sites. This probably means that more organic amendment is used in S3 compared to other sites. Likewise, mean total nitrogen content was significantly higher (P<0.05) in soil samples from S3 compared to the other sites. There was positive correlation between TN and OM (S1 r = 0.325, S2 r = 0.084, S4 r = 0.562 and S5 r = 0.489 at p<0.01). Significant positive correlation (r = 0.898, P<0.01) was observed for S3.

Total phosphorus (TP), as the name implies, refers to the total concentration of P species in the soil while available phosphorus (AP) refers to the labile pool of soil phosphorus from which plants and animals draw to meet their phosphorus requirements. Mean value for TP in S3 was significantly higher (P<0.05) than those of other sites and this points to higher use of organic soil amendments in S3 compared to other sites. Nitrogen content in soil samples had positive correlation with TP (S1 r = 0.439, S2 r = 0.158, S3 r = 0.998, S4 r = 0.782 and S5 r =0.377 at P<0.01). Mean AP value for S3 was significantly higher (P<0.05) than values for other sites. AP values were positively correlated to TN (S1 r = 0.258, S2 r = 0.098, S3 r = 0.681, S4 r = 0.460, S5 r = 0.069 at P<0.05).

Table 2 also shows the CEC of soil samples. Mean CEC was significantly lower (P<0.05) in S5 compared to others. Cations are held in the soil by the negatively charged clay and organic matter particles through electrostatic forces and these cations are easily exchanged with other cations making them available for use by plants. The cation exchange capacity (CEC) of a soil represents the total amount of exchangeable cations that the soil can adsorb ²³. CEC increases as the amount of clay and organic matter of soil increases. The factors that are most likely to influence metal sorption in the soil are OM and CEC and increasing the soil OM content

may lead to increase in soil CEC ¹⁸. CEC was positively correlated with OM and correlation coefficients were: S1, 0.336; S2, 0.051; S3, 0.695; S4, 0.449 and S5, 0.251 at P<0.05. Profile for cation exchange capacity (CEC) in the experiment followed that of OM and TP with the highest value in S3 which also had the highest mean values for OM and TP.

Table 3 shows the particle size distribution and texture classes of soil samples from the sites. Soil samples from S1, S2, S3 and S4 were in the texture class, loam. Their mean silt contents were significantly higher (P<0.05) than that of S5. However, mean sand content in S5 was significantly higher (P<0.05) than that in soil samples from the other sites and it was of the texture class, sandy loam.

Sampling	Par	Texture		
Sites	Clay	Silt	Sand	Class
S1	25 ±1.0a	34 ±0.0a	41 ±1.0a	Loam
S2	21 ±1.0b	34.5 ±0.5a	44.5 ±1.5a	Loam
S3	27 ±1.0a	36.5 ±1.5a	36.5 ±0.5a	Loam
<u>S</u> 4	23 ±1.0b	36 ±0.0a	41 ±1.0a	Loam
S 5	19.5 ±0.5b	17 ±1b	63.5 ±1.5b	Sandy loam

Table 3. Mean (+SD) Particle size and Texture of Soil Samples (dry basis)

Values represent the mean and standard deviation for three replicates

Means in the same column with different letters are significantly different (P<0.05)

Total Metal Concentrations in Soil Samples

Table 4. Mean	Total Metal	concentrations in	Soil Sample	s (mgkg ⁻	¹ , Dry	Weight)
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Metals	Sites								
	S1	S2	S3	S4	S5				
Cd	$0.341\pm0.082a$	0.603 ±0.071b	0.714±0.098c	0.402±0.049a	0.324±0.063a				
Cr	$2.168 \pm 0.504a$	3.192 ±0.740b	2.499 ±0.856a	2.329±0.736a	3.741 ±0.142a				
Cu	11.397 ±4.374a	15.309 ±2.821b	14.014 ±1.621c	12.630 ±2.427a	7.692 ±0.381d				
Pb	4.047±1.317a	7.047±1.317b	5.127±2.224c	4.589±1.302a	2.312 ±0.216d				
Zn	61.725 ±10.689a	$83.560 \pm 5.300b$	106.159 ±4.261c	57.126 ±6.269a	$80.469 \pm 10.368b$				
Manualin									

Means in the same row with different letters are significantly different (P<0.05)

Mean total metal concentrations in soil samples under the different treatments are shown in Table 4. Mean Cd, Cr, Cu and Pb were highest in S3 (Oli) compared to other rice farming sites. Mean Zn was highest in S3 and lowest in S4. Zn concentration was significantly higher (P < 0.05) than the concentrations of other metals at all the sites while Cd had the lowest concentrations (Figure 2). The general profile for mean total metal concentrations in soil samples from all the sites was Zn>Cu>Pb>Cr>Cd with the exception of S5 which had the profile Zn> Cu>Cr>Pb>Cd. Cr and Zn concentrations were negatively correlated to EC concentrations at the sites while Cd, Cu and Pb concentration were positively correlated to EC. Cd, Cr and Zn had negative correlations with organic matter (OM) contents of the soil samples while Cu and Pb concentrations were positively correlated with OM. Only Zn was positively correlated with CEC at all the sites. Concentration and bioavailability of heavy metals in soils and sediments increase with decreasing grain size of sediment (is a function of particle size)²⁴. Metal concentrations tend to be higher in soils with higher organic mater content and clav content ²⁵. This may be the reason for lower mean total metals in S5 compared to other sites. S3 had the highest clay and OM contents but only mean total Cd and Zn contents were higher in S3 compared to the other sites. It is not exactly clear the levels of soil heavy metals that are safe or unsafe since there are no universally accepted soil quality guideline values. The recommended tolerance contents of elements in cultivated soils with regards to the health of vegetation, livestock and man are Cr 10-50, Cu 0-20, Pb 0.1-2 and Zn 10-50 mg kg^{-1 26}. While the limits for Cr and Cu in all the sites were not exceeded, mean Pb and Zn concentration exceeded these limits at all the sites. However, the Canadian Quality Guidelines values for soil meant for agricultural use ²⁷ have the following limits for the metals analyzed in this study: Cd 1.4, Total Cr 64, Cu 63, Pb 70 and Zn 200 mg kg⁻¹. On this basis, it can be concluded that the soils should be under constant monitoring so that proactive remedial measures can be taken appropriately.



Metal Concentrations in Soil Fractions

Soil samples were subjected to a six step sequential extraction to determine the concentrations of the metals in soil fractions. Table 5 shows mean metal concentrations in the six soil fractions (water soluble, WS; exchangable fraction, Ex; Fe and Mn oxide-hydroxide bound or reducible fraction, OB; organic matter bound or oxidisable fraction, OMB; carbonate bound or acid soluble fraction, C and residual or silicate bound fraction, R. In S1, mean Cd and Pb were significantly higher (P<0.05) in the OMB fraction than in other fractions and had the lowest value in the WS. However, Cr, Cu and Zn were significantly higher (P<0.05) in the OB fraction compared to the other fractions.

In S2, the lowest values occurred in the WS fraction except for Cd which had the lowest value in the Ex fraction. Cd concentrations in OB and OMB were similar (P>0.05) but were significantly higher (P<0.05) than concentrations in the other fractions. For Cr and Zn, the highest mean concentration was in the OB fraction, but for Cu and Pb, the OMB fraction contained higher amounts of the metals compared to other fractions.

Cd Cu and Pb had significantly higher (P<0.05) concentrations in OMB fraction in S3 compared to other fractions. However, Cr and Zn concentrations were highest in the OB fraction. WS fraction has the lowest concentrations of the metals except for Cu where concentration in the Ex fraction was lower than WS.

In S4, mean concentrations Cr and Pb were significantly higher (P<0.05) in the OMB fraction while Cr, Cu and Zn had significantly higher (P<0.05) mean values in the OB fraction. The WS fraction contained the lowest mean metal concentrations. Zn concentrations in all the fractions were higher than the corresponding fraction for all the other metals.

For S5, the OMB fraction contained significantly higher (P<0.05) mean concentrations of Cd, Cr and Pb compared to other fractions while mean values were significantly higher (P<0.05) in the OB for Cu and Zn. Cr values were rather high in S5 compared to the other sites and this may point to the use of substances containing higher Cr concentration at the site.

The distribution of the metals in the soil fractions shows that Cr, Cu and Zn are mostly associated with the OB fraction while Cd and Pb are mostly associated with the OMB fraction. This result is similar to that obtained in a study of metal fractionation in soils near a waste site where it was found that Cu, Pb and Zn were mostly associated with the OB fraction²⁸. At all sites, the WS fraction generally had the lowest mean values. The WS fraction is very mobile and can easily leach to ground water making metal contents in it low compared to other fractions²⁹.

Sites	Fractions	Metal Concentrations				
		Cd	Cr	Cu	Pb	Zn
S 1	WS	0.019 ± 0.006	0.188 ± 0.027	0.420 ± 0.153	0.084 ± 0.023	1.986 ±0.205
	Ex	0.029 ± 0.011	0.175 ± 0.055	0.396 ±0.131	0.120 ± 0.055	4.375 ± 0.824
	OB	0.107 ± 0.035	0.811 ± 0.106	3.275 ± 1.015	0.981 ± 0.105	22.330 ± 4.687
	OMB	0.141 ± 0.058	0.440 ± 0.082	2.402 ± 0.811	1.470 ± 0.399	11.051 ± 1.900
	С	0.049 ± 0.024	0.316 ± 0.096	1.369 ± 0.405	0.940 ± 0.129	14.226 ± 3.117
	R	0.034 ± 0.014	0.265 ± 0.060	3.105 ± 1.075	0.826 ± 0.133	8.167 ±1.215
S2	WS	0.049 ± 0.022	0.298 ± 0.086	0.570 ± 0.108	0.203 ± 0.074	1.890 ± 0.242
	Ex	0.040 ± 0.019	0.307 ± 0.073	0.805 ± 0.177	0.622 ± 0.109	5.750 ± 1.762
	OB	0.219 ± 0.048	0.930 ± 0.199	3.190 ± 0.987	2.449 ± 0.851	32.353 ±9.119
	OMB	0.195 ± 0.045	0.724 ± 0.157	4.130 ± 0.781	3.075 ± 0.960	19.504 ± 3.347
	С	0.107 ± 0.039	0.477 ± 0.101	1.611 ± 0.420	0.820 ± 0.330	10.593 ± 6.068
	R	0.061 ± 0.012	0.390 ± 0.080	2.429 ± 0.978	0.871 ± 0.256	13.780 ± 4.586
S 3	WS	0.081 ± 0.022	0.190 ± 0.053	0.541 ± 0.122	0.149 ± 0.014	3.113 ± 0.550
	Ex	0.090 ± 0.019	0.251 ± 0.079	0.397 ± 0.097	0.287 ± 0.038	8.570 ±1.12 5
	OB	0.186 ± 0.038	0.833 ± 0.270	3.115 ±0.244	1.380 ± 0.345	40.149 ± 7.234
	OMB	0.224 ± 0.095	0.548 ± 0.113	5.004 ± 0.984	1.820 ± 0.091	22.198 ±3.191
	С	0.128 ± 0.026	0.411 ± 0.097	2.196 ±0.294	0.788 ± 0.105	15.099 ±2.217
	R	0.095 ± 0.018	0.320 ± 0.115	3.037 ± 0.898	0.833 ±0.139	11.276 ± 1.200
S4	WS	0.037 ± 0.010	0.180 ± 0.023	0.743 ±0.121	0.163 ± 0.041	1.320 ± 0.930
	Ex	0.060 ± 0.021	0.215 ± 0.101	0.948 ± 0.208	0.280 ± 0.085	5.941 ±0.977
	OB	0.075 ± 0.014	0.711 ±0.153	4.451 ± 1.009	1.508 ± 0.160	18.978 ± 2.413
	OMB	0.126 ± 0.033	0.514 ± 0.158	2.170 ± 0.740	1.941 ± 0.5308	10.530 ± 1.008
	С	0.059 ± 0.017	0.431 ± 0.110	1.502 ± 0.581	0.864 ± 0.139	13.207 ±2.410
	R	0.040 ± 0.015	0.317 ± 0.103	2.396 ± 0.745	0.661 ±0.113	9.033 ±2.190
S5	WS	0.023 ± 0.009	0.087 ± 0.022	0.355 ± 0.125	0.170 ± 0.065	2.630 ± 0.222
	Ex	0.035 ± 0.015	0.105 ± 0.047	0.487 ± 0.201	0.165 ± 0.032	7.941 ±0.691
	OB	0.076 ± 0.019	0.925 ± 0.130	2.480 ± 0.920	0.481 ± 0.099	27.285 ± 4.120
	OMB	0.130 ± 0.034	1.338 ±0.425	1.633 ±0.371	0.916 ± 0.218	23.180 ± 3.708
	С	0.044 ± 0.012	0.814 ± 0.305	1.855 ± 0.444	0.200 ± 0.066	15.722 ± 1.875
	R	0.038 ± 0.010	0.550 ± 0.086	0.910 ± 0.206	0.372 ± 0.104	5.680 ± 0.968

Table 5. Metal Concentrations in Soil Fractions (mg kg⁻¹)

WS = water soluble fraction; Ex = Exchangable fraction; OB = Fe and Mn oxide-hydroxide bound or reducible fraction; OMB = organic matter bound or oxidisable fraction; C = carbonatic or carbonate bound fraction; R = silicate bound residual fraction

Conclusion and Recommendations

The study shows the presence of five heavy metals analyzed at various concentrations in the soils collected from rice fields. It is believed that in addition to background levels, the soils were enriched with these metals as a result of fertilizer, herbicide and pesticide application since heavy metals are contained in these substances. Indeed, the application of organic and inorganic fertilizers on soils is known to be a source of heavy metals in soils. However, only mean total Pb and Zn concentration exceeded the UNEP/BMFT recommended levels in samples from all the sites and this is of great importance due to the deleterious effects of Pb on biological systems. Constant monitoring of cultivated soil is recommended and future work on the incidence of heavy metals in the rice plants grown in these areas should be carried out to confirm the possible health effects on human consumers of rice grown in such fields. It is also important that international soil quality guidelines are harmonized since different and sometimes conflicting guidelines exist.

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