Interactions of Some Heavy Metals in Soil and Effects on their Uptake by Spinach (*Spinacia Oleracea*)

Nlemadim, A. B[1]., Jacob, J. O[1]., Ajai, A. I[1]. and Adeboye, M. K. A.[2]

[1] *Department of Chemistry, Federal University of Technology, Minna, Nigeria*
[2] *Department of Soil Science, Federal University of Technology, Minna, Nigeria*

E-mail: bennlemadim@yahoo.com

ABSTRACT

Physico-chemical analysis, total metal concentration and chemical speciation of soil have been studied using standard analytical methods. Determination of heavy metals in soil and spinach were also carried out. The seeds of spinach were planted in pots and nurtured in a glass house for 30 days, after which the plant was harvested. The soil used for the planting was taken from the research farm of the Federal University of Technology, (FUT) Minna, and treated with the metals Pb, Cd, Cu and Zn in single and combined forms. The initial levels of these metals in soil decreased in the order Zn> Cu> Cd while Pb was not detected. Sequential extraction procedure was used to separate the heavy metals in soil into six geochemical forms: Water soluble, exchangeable, carbonate, Fe/Mn Oxide, organic and residual. Among the four heavy metals studied, the mean of the concentration of Zn in the water soluble fraction was the highest (33.00 mg/kg) while the lowest (0.50 mg/kg) was Pb bound to organic and exchangeable fractions. The mean of the heavy metal concentration in soil for single metal treatment was highest (51.33 mg/kg) in Zn treated soil and lowest (0.33 mg/kg) in Pb treated soil. Similarly, the mean of the heavy metal concentration in soil for combined metal treatment was highest (60.00 mg/kg) in Cu/Zn treated soil and lowest (0.55 mg/kg) in Pb/Zn treated soil. The mean of the heavy metal concentration in spinach for single metal treatment was highest at (57.67 mg/kg) and lowest at (0.33 mg/kg). Similarly, the mean of the heavy metal concentration in spinach for combined metal treatment was highest at (56.67 mg/kg) and lowest at (1.00 mg/kg). It was observed that the concentration of Zn both in soil and spinach fell below the maximum permissible level. The result of the transfer factor showed that Cd is the most mobile metal in spinach while Zn is the least. The order of decreased mobility is Cd> Pb > Cu> Zn.

Key words: Interactions, Sequential Extraction, Antagonistic, Synergistic, Transfer Factor

INTRODUCTION

Heavy metals are non-biodegradable and persistent environmental contaminants which may be deposited on the surfaces and then absorbed into the tissues of vegetables. Plants take up heavy metals by absorbing them from deposits on the parts of the plants exposed to the air from polluted environments as well as from contaminated soils[1].Trace elements play an important role in the metabolic regulations of human body[2]. Human exposure to lead has received a great attention because of its negative effects on the central nervous system[3].

Ingestion of large amounts of Pb may cause anaemia, kidney damage, colic, muscle weakness and brain damage[4]. Low blood Pb levels may cause fatigue, insomnia, irritability, hyperactivity and effects on hearing, vision and motor functions[3]. Long term effects of low doses of Pb exposure also include effects on physical and mental growth and intellectual functioning[5]. The World Health Organization (WHO) recommended values for lead in soil and fresh vegetableshave been set at 20.00 mg/kg and 0.30 mg/kg respectively[6].In soil, cadmium is usually found in the form of Cd^{2+}. This chemical form is the most common form of Cd. Cadmium, when in excess, can cause brown margins on leaves, curled leaves and brown stunted shoots[7].Cadmium exposures are also associated with kidney and bone damage. It has also been identified as a potential human carcinogen, causing lung cancer, gastro intestinal cancer, cancer of the pancreas, urinary bladder or prostate[7].

The maximum permissible levels for cadmium in soil and vegetable have been set at 1.00 mg/kg and 0.20 mg/kg respectively[6].Copper is essential in all plants and animals and is widely distributed in the body. It occurs in the liver, muscle and bone. Copper is transported in the blood stream on a plasma protein called ceruloplasmin[8]. The World Health Organization (WHO) permissible levels of Cu in soil and vegetable are 20.00mg/kg and 73.30 mg/kg respectively[6].

Copper is necessary for normal biological activities of tyrosinase enzyme[9]. It is required as co-factor in different oxidative and reductive enzymes[10]. A low uptake of Cu in human consumption can cause a number of symptoms such as growth retardation, skin ailments and gastro intestinal disorders. Copper deprivation in animals contributes to instability of heart rhythm, hyperlipidemia, increased thrombosis, breakdown of vascular tissue, cardiac hypertrophy and altered corterial function[11]. Copper deficiency is associated with increased oxidative stress, which in turn, leads to low density lipoproteins susceptibility to oxidation[12].Copper toxicity which is linked to genetic disorders can also be a serious health concern[13].

Zinc is well known to be essential for somatic growth of children[14]. It is essential as a constituent of many enzymes involved in several physiological functions, such as protein synthesis and energy metabolism[15,16]. For instance, zinc is required for the proper function of 1,5-deiodinase, the enzyme required for the conversion of thyroxine to the more active form, triiodothyroxine[17]. Zinc deficiency is a major overlapping public health concern in developing countries. It causes not only growth retardation, but also delays in sexual maturation, hypogonadism and thyroid dysfunction[15].

As a result of their essentiality, vegetables contain some concentrations of elements such as Cu and Zn that they acquire from cultivated soils. Unfortunately, much of the soil in which food is grown has been depleted of these essential minerals. A supplementation of Cu and Zn in vegetables is a convenient and easy method in agriculture to improve trace elements nutrition for humans. The maximum permissible levels for zinc in soil and in vegetables have been set as 100.00 mg/kg and 99.40 mg/kg respectively[6].

MATERIALS AND METHODS

Study Site

The soil was collected from the Teaching and Research Farms of Federal University of Technology, Gidan Kwanu, Minna ($9^0$31.860'N; $6^0$27.244' E; 254m). The soil was classified as typic plinthustalf with loamy sand surface soil texture, slightly acidic, low organic carbon, nitrogen and medium phosphorous[18].

Sample Collection and Pre-treatment

Surface soil (0-15cm) samples were collected with an auger, along four diagonal transects, from ten points each, thoroughly mixed and bulked to give four composite samples. Samples were collected from the four composite samples mixed thoroughly and bulked to give one composite sample. The sample was air-dried, crushed gently and passed through a 2 mm sieve.

Physico-chemical Properties of Soil

The physico-chemical properties of soil samples which include pH, organic carbon, soil particle size distribution, electrical conductivity and cation exchange capacity were determined according to the method reported by Adeboye *et al*.[19].

Preparation of Experimental Pots

Composite samples of soil were weighed into experimental pots. Each pot contained 20 kg of composite soil. The pots were divided into two sections. The first section contained 15 pots while the second section contained 33 pots.

Addition of Metals to the Experimental Pots

The nitrates of Pb and Cd and the sulphates of Cu and Zn were added to all experimental pots, comprising single and combined metal treatments in concentrations below their maximum permissible levels. This is illustrated in Tables 1 and 2

Table 1: Single Metal Treatment to Spinach Experimental Pots

Treatment	Number of Spinach pots
Pb	03
Cd	03
Cu	03
Zn	03
Control	03
Total	**15**

Table 2: Combined Metal Treatment to Spinach Experimental Pots.

Treatment	Number of Spinach Pots
Pb/Cd	03
Pb/Cu	03
Pb/Zn	03
Cd/Cu	03
Cd/Zn	03
Cu/Zn	03
Pb/Cd/Cu	03
Pb/Cd/Zn	03
Cd/Cu/Zn	03
Pb/Cd/Cu/Zn	03
Control	03
Total	**33**

Determination of Metal Speciation in Soil

The chemical fractionation of heavy metals in soil samples which included water soluble fraction, exchangeable fraction, acid extractable carbonate bound fraction, reducible Fe/Mn oxides and hydroxide fraction, oxidizable organic matter bound fraction and residue and inert fraction were determined according to the method reported by Finzgaret *et al.*[20].

Determination of Metal Concentrations in Soil

Heavy metal concentrations in soil samples before and after addition of standard amounts of metals were determined according to the method reported by Hooda[21].

Planting of Spinach

The vegetable seeds were sown in 96 pots at the rate of 2-4 seeds per pot. They were allowed to germinate, emerge and grow for 30 days in an ambient environment within which they produced good leaves. The pots were watered regularly with de-ionized water to keep them close to field capacity before harvesting using a clean pair of scissors.

Soil Collection and Preparation

Samples of soil collected from experimental pots were air-dried, crushed gently and passed through a 2 mm sieve for analysis of heavy metals.

Spinach Collection and Preparation

The leaves of the harvested spinach were cut with a clean and pre-washed pair of stainless scissors. They were washed with distilled water followed by de-ionized water. The leaf sample was dried in an oven at 60°C for 3 days and then milled to pass through a sieve with a 0.15 mm mesh.

Determination of Metal Concentration in Spinach.

Heavy metal concentrations in Spinach samples were determined according to the method reported by Hooda[21].

Statistical Analysis

Statistical analysis including mean and standard deviation were computed using. Analysis of Variance (ANOVA) to see if there is significant difference between the means.

RESULTS AND DISCUSSION

Physico-chemical Properties of Soil

The Result of the Physico-Chemical Properties of the Soil from the study area is presented in Table 3. The soil is classified as Loamy sandy with a pH in water of 6.80. It contains sand, Silt and clay with sand having the highest (830.00 g/kg) value while silt has the lowest (70.00g/kg) value. It has an electrical conductivity of 45.39 Ms/m and an organic carbon of 4.30 g/kg. The exchangeable acidity (cmol/kg) is 0.13, while the cation exchange capacity (cmol/kg) which is the summation of exchangeable bases is 7.13.

Table 3: Physico-chemical Properties of Untreated Soil

Parameter	Values
Sand (g/kg)	830.00
Silt (g/kg)	70.00
Clay (g/kg)	100.00
Textural class	Loamy Sandy
pH (H_2O)	6.80
Electrical conductivity (Ms/m)	45.39
Organic carbon (g/kg)	4.30
Exchangeable acidity (cmol/kg)	0.13
Exchangeable bases (cmol/kg)	
Ca^{2+}	4.00
Mg^{2+}	2.60
K^+	0.23
Na^+	0.17
Cation exchange capacity (cmol/kg)	7.13

Initial Concentration of Heavy Metals in Untreated Soil

The result of the initial concentrations of heavy metals in untreated soil is shown in Table 4. Pb was not detected, while the mean concentration of Cd, Cu and Zn are 11.00 ±0.32 mg/kg, 16.00±0.46 mg/kg and 51.00 ± 0.62 mg/kg respectively. This shows that the concentration of the metals decreased in the order Zn > Cu > Cd. The concentrations of Cu and Zn were below the maximum permissible level for agricultural soil while that of Cd was above[22].

Table 4: Initial Concentrations of Heavy Metals in UntreatedSoil

Treatment	Mean Concentration ± S.D. (mg/kg)
Pb	ND
Cd	11.00 ± 0.32
Cu	16.00 ± 0.46
Zn	51.00 ± 0.62

SD = Standard deviation ND = Not detected

Heavy Metal Speciation in Soil

The result of the mean concentration of heavy metals in sequentially extractable soil fractions is shown in Table 5.

Sequential extraction Procedure was used to fragment the metals into six geochemical forms: water soluble, exchangeable, carbonate, Fe/MnO, organic and residual. The mean total concentration of the metals in the six fractions decreased in the order Zn > Cu > Cd > Pb. The highest (4.00 mg.kg) concentration of Pb was observed in the carbonate phase while the lowest (0.50 mg/kg) was observed in the organic and exchangeable phases. The concentration of Cd was highest (8.00mg/kg) in the exchangeable and the residual phases and lowest (5.50 mg/kg) in the Fe/Mno phase. Cu was highest (10. 00 mg/kg) in the carbonate phase and lowest (7.00 mg/kg) in the organic phase. Some workers have attributed low Cu with organic phase to the formation of stability constant of organic copper complexes[23]. The concentration of Zn was highest (33.00 mg/kg) in the water soluble phase and lowest (24.00 mg/kg) in the organic phase. It is generally observed that the sum of the five fractions obtained from sequential extraction procedure was higher than the mean total content of acid digestion. This had also been observed by pare *et al.*[24,25, 26]. This may be due to incomplete digestion with the mixed acid compared to the slow and increasingly stronger attack by the reagents of the sequential extractions. For similar reasons, Pb that was not detected in the determination of the initial concentration of metal was detected in the sequential extraction.

Table 5: Mean Concentrations (mg/kg) of Heavy Metal in Sequentially Extractable Soil Fractions

Metals	Water Soluble	Exchangeable	Carbonate	Fe/MnO	Organic	Residual	Total
Pb	3.00 ±1.16	0.50 ± 0.12	4.00 ± 1.18	1.50 ±0.17	0.50 ± 0.14	1.00 ±1.16	10.50
Cd	6.50 ± 0.23	8.00 ± 1.16	6.50 ± 0.23	5.50 ± 0.20	6.50 ± 0.25	8.00 ± 1.20	41.00
Cu	9.00 ± 1.20	8.00 ± 1.16	10.00 ± 1.30	8.00 ± 1.17	7.00 ± 1.14	9.00 ± 1.22	51.00
Zn	33.00 ± 1.20	29.00 ± 1.80	28.00 ± 1.75	29.50 ± 1.15	24.00 ± 1.65	28.50 ± 1.60	172.00

Mobility Factor of Heavy Metals

The mobility factor (MF) of heavy metals is the percentage of soluble phases relative to the sum of all phases. The six geochemical form of the phases can be represented as water soluble (FI), exchangeable (FII), Carbonate (FIII), Fe/Mno (FIV), Organic (FV) and residual (FVI),

Mobility Factor = $\frac{F1+F11}{SumofP\ hases}$ x 100

The result of the mobility factors of heavy metals is presented in Table 6

Studies have shown that heavy metals are potentially available for plant uptake if the mobility factor is above 10%[27]. The high mobility factors are indications that all the metals are potentially available for plant uptake and this was actually so in this study.

Table 6: Mobility Factors of Metals

Metals	FI + FII	Sum of Fractions	MF (%)
Pb	3.50	10.50	33.02
Cd	14.50	41.00	35.37
Cu	17.00	51.00	33.33
Zn	62.00	172.00	36.05

Heavy Metal Concentrations in Single Metal Treated Soil for Spinach

The result of the heavy metal concentrations in single metal treated soil for spinach is shown in Table 7. In the Pb treated soil, the concentration of Pb in soil was 0.33 mg/kg, Cd was 13.00 mg/kg, Cu was 13.00 mg/kg while Zn was 36.00 mg/kg. However, the initial levels of Cd, Cu and Zn in the untreated soil was 11.00 mg/kg, 16.00 mg/kg and 51.00 mg/kg respectively. Pb was not detected in the untreated soil. It can be concluded that, soil treated with Pb had a synergistic effect with respect to Pb and Cd in soil and an antagonistic effect with Cu and Zn in the soil.

In the Cd treated soil, the concentration of Pb was 1.67 mg/kg, Cd was 19.67 mg/kg, Cu was 16.00 mg/kg and Zn was 50.00 mg/kg. When compared with the initial levels of these metals in the untreated soil, Pb and Cd had a synergistic effect, Zn was antagonistic while Cu showed no change in concentration. In the Cu treated soil, the concentration of Pb was 1.00 mg/kg, Cd was 13.33 mg/kg, Cu was 16,67 mg/kg while Zn was 41.00 mg/kg. In this case, Pb, Cd and Cu had synergistic effects while Zn was antagonistic. In the Zn treated soil, the concentration of Pb was 1.00 mg.kg, Cd was 16.67 mg/kg, Cu was 20.67 mg/kg while Zn was 51.33 mg/kg. In this situation, all the four metals showed a synergistic interaction. The result of the control was almost the same with the initial concentration of these metals in the untreated soil.

Table 7: Heavy Metal Concentrations in Single Metal Treated Soil for Spinach

Treatment	Mean Concentrations of metals ± SD (mg/kg)			
	Pb	Cd	Cu	Zn
Pb	0.33 ± 0.33^{a}	13.00 ± 1.73^{ab}	13.00 ± 2.08^{a}	36.00 ± 1.53^{ab}
Cd	1.67 ± 0.88^{bc}	19.67 ± 0.88^{c}	16.00 ± 1.16^{ab}	50.00 ± 4.36^{bc}
Cu	1.00 ± 0.00^{b}	13.33 ± 1.20^{bc}	16.67 ± 0.88^{ab}	41.00 ± 3.79^{abc}
Zn	1.00 ± 0.58^{b}	16.67 ± 1.86^{a}	20.67 ± 1.20^{b}	51.33 ± 2.60^{a}
Control	ND	10.33 ± 1.20^{d}	16.67 ± 1.76^{ab}	50.67 ± 3.18^{c}

SD = Standard Deviation

ND = Not Detected

Mean ± SD Values followed by different small letters (superscript) within the same column are significantly different ($P>0.05$)

Heavy Metal Concentrations in Combined Metal Treated Soil for Spinach

The result of heavy metal concentrations for combined metal treated soil for spinach is shown in Table 8. In the Pb/Cd treated soil, the concentration of Pb was 0.67 mg/kg, Cd was 11.67 mg/kg, Cu was 14.33 mg/kg while Zn was 46.67 mg/kg. The result shows that Pb and Cd had a synergistic effect while Cu and Zn had an antagonistic effect when compared with the initial concentration of these metals in the untreated soil. In the Pb/Cu treated soil, the concentration of Pb was 1.33 mg/kg, Cd was 14.00 mg/kg, Cu was 19.67 mg/kg, while Zn was 37.00 mg/kg. The result shows that Pb, Cd and Cu had synergistic effects while Zn showed an antagonistic effect when compared with initial concentration of theses metals in the untreated soil.

In the Pb/Zn treated soil, Pb and Cd had synergistic interaction while Cu and Zn were antagonistic when compared with the initial concentration of these metals in the untreated soil. In the Cd/Cu treatment, the concentration of Pb was 1.67 mg/kg, that of Cd was 11.33 mg/kg, Cu was 15.67 mg/kg and Zn was 40.67 mg/kg. This shows that Pb and Cd had a synergistic effect while Cu and Zn were antagonistic when compared with the initial levels of these metals in the untreated soil.In the Cd/Zn treated soil, the result shows a similar trend when compared with the Cd/Cu treatment. Pb and Cd showed a synergistic interaction while Cu and Zn were antagonistic when compared with the initial levels of these metals in the soil.In the Cu/Zn treatment, the result showed that Pb, Cd and Cu showed a synergistic interaction while Zn was antagonistic. In the Pb/Cd./Cu treatment, the results showed a synergistic interaction among the four metals. However, in the Pb/Cd/Zn treatment Pb and Cd were synergistic while Cu and Zn were antagonistic when compared with initial levels of these metals in the untreated soil.

In the Cd/Cu/Zn treatment, Pb, Cd and Zn had synergistic interactions while Cu was unchanged. The last was the Pb/Cd/Cu/Zn treatment, in which Pb and Cd had synergistic interaction while Cu and Zn had an antagonistic effect. In the control, Pb was not detected, Cd was 12. 33 mg/kg, Cu was 13.67 mg/kg while Zn was 46.33 mg/kg.

Table 8: Heavy Metal Concentrations in Combined Metal Treated Soil for Spinach

Treatment	Mean Concentrations of Metals ± SD (mg/kg)			
	Pb	**Cd**	**Cu**	**Zn**
Pb/Cd	0.67 ± 0.33^{a}	11.67 ± 1.86^{a}	14.33 ± 1.86^{ab}	46.67 ± 4.10^{a}
Pb/Cu	1.33 ± 0.88^{c}	14.00 ± 1.00^{b}	19.67 ± 0.88^{b}	37.00 ± 6.11^{b}
Pb/Zn	0.55 ± 0.42^{a}	13.00 ± 1.16^{c}	12.33 ± 1.86^{c}	48.33 ± 2.60^{c}
Cd/Cu	2.00 ± 2.00^{b}	14.33 ± 2.03^{b}	11.33 ± 1.20^{d}	44.67 ± 2.85^{d}
Cd/Zn	ND	15.67 ± 3.38^{ab}	16.33 ± 2.03^{e}	49.33 ± 7.25^{e}
Cu/Zn	1.67 ± 0.67^{c}	15.67 ± 1.45^{ab}	14.33 ± 2.33^{ab}	60.00 ± 1.53^{ab}
Pb/Cd/Cu	0.67 ± 0.33^{a}	11.33 ± 0.88^{a}	15.67 ± 1.20^{bc}	45.67 ± 4.26^{abc}
Pb/Cd/Zn	1.00 ± 0.01^{c}	15.00 ± 2.08^{ab}	12.67 ± 0.88^{c}	42.33 ± 7.34^{bc}
Cd/Cu/Zn	1.33 ± 0.88^{c}	14.33 ± 1.86^{b}	16.00 ± 1.73^{e}	51.33 ± 4.41^{f}
Pb/Cd/Cu/Zn	2.00 ± 0.58^{b}	15.00 ± 2.65^{ab}	12.33 ± 0.67^{c}	36.67 ± 5.70^{g}
Control	**ND**	$\mathbf{15.00 \pm 2.08^{ab}}$	$\mathbf{14.33 \pm 0.88^{ab}}$	$\mathbf{47.33 \pm 1.76^{h}}$

SD = Standard Deviation ND = Not Detected

Mean ± SD Values followed by different small letters (superscript) within the same Column are significantly different (P>0.05).

Heavy Metal Concentrations in Spinach from Single Metal Treated Soil.

The result of heavy metal concentrations in spinach from single metal treated soil is shown in Table 9. In the Pb treatment, the uptake of Pb, Cd, Cu and Zn ws synergistic when compared with the initial levels of these metals in the soil. However, in the Cd treatment, Pb and Cd uptake were synergistic while Cu and Zn were antagonistic. In the Cu treated soil, the uptake of Pb, Cd, and Cu was synergistic while the uptake of Zn was antagonistic. In the Zn treated soil, Pb, Cd. Zn uptake were synergistic while Cu uptake was antagonistic.

Table 9: Heavy Metal Concentrations in Spinach from Single Metal Treated Soil

Treatment	Mean Concentrationsof metals ± SD (mg/kg)			
	Pb	Cd	Cu	Zn
Pb	0.33±0.17^{a}	13.00±1.73^{a}	23.33±2.03^{a}	57.67±4.91^{a}
Cd	1.67±0.33^{a}	15.67±2.19^{b}	13.00±1.73^{b}	50.67±1.20^{b}
Cu	1.33±0.88^{a}	17.33±2.03^{c}	19.00±1.16^{c}	46.33±1.20^{c}
Zn	1.33±0.88^{a}	13.00±2.08^{a}	13.33±1.45^{b}	53.67±2.91^{d}
Control	**ND**	**11.33±1.20^{d}**	**24.33±3.38^{d}**	**45.67±1.86^{e}**

SD = Standard Deviation ND = Not Detected

Mean± SD Values followed by different small letters (superscript) within the same Column are significantly different (P>0.05)

Soil – plant Transfer Factor (TF)

Transfer Factor (TF) is given by the following equation: TF = $\frac{[M]plant}{[M]Soil}$

Where [M] plant is the concentration of a metal in the test plant tissue and [M] soil is the total concentration of the same metal in the soil where the plant is grown. The TF value gives an indication of the mobility of the metal in the soil. A high TF value indicate high rate of transfers of the metal into the plant tissues. This may also involves a risk of human food chain accumulation. Table 10 shows Transfer Factors of Cd, Cu and Zn.

The result shows that Cd is the most mobile heavy metal among the group. This was also reported by Klokee*t al.*[28]. The order of decreased mobility is Cd>Pb>Cu>Zn.

Table 10: Soil-Spinach Transfer Factors for Single Metal Treated Soil

Treatment	Transfer Factors (TF)			
	Pb	Cd	Cu	Zn
Pb	1.00	1.00	1.80	1.60
Cd	1.00	1.42	0.81	0.99
Cu	1.33	1.58	1.19	0.90
Zn	1.30	1.18	0.83	1.05
Mean (TF)	1.17	1.30	1.16	1.14

Heavy Metal Concentration in Spinach from Combined Metal Treated Soil.

The result of heavy metal concentrations in spinach from combined metal treated soil is shown in Table 11. In the Pb/Cd treatment, the interaction of Pb, Cd and Cu was synergistic while Zn was antagonistic. In the Pb/Cu treatment, the interaction Pb, Cd and Cu was synergistic while that of Zn was antagonistic. In the Pb/Zn treatment, all the four metals showed a synergistic interaction. In the Cd/Cu treatment, Pb and Cd showed a synergistic interaction while the interaction of Cu and Zn was antagonistic relative to the initial concentration of these metals in the untreated soil. In the Cd/Zn treatment, Pb and Cd showed a synergistic interaction while Cu and Zn showed an antagonistic interaction. In the Cu/Zn treated soil, Pb, Cd and Cu showed a synergistic interaction while Zn was antagonistic. In the Pb/Cd/Cu treatment, all the four metals showed a synergistic interaction. In the Pb/Cd/Zn treatment, Pb and Cd showed a synergistic interaction. While Cu and Zn showed an antagonistic interaction. In the Cd/Cu/Zn treatment, Pb and Cd showed a synergistic interaction while Cu and Zn showed an antagonistic interaction. In the control, Pb was not detected, Cd was synergistic while Cu and Zn were antagonistic when compared to the initial levels of these metals in the untreated soil. In the Pb/ Cd/ Cu/ Zn treatment, Pb and Cd showed a synergistic interaction while Cu and Zn showed antagonistic interaction. The result are consisted with the results reported by Sarma*et al.*[29], who observed a reduction in the uptake of Zn in the presence of Cd.

Table 11: Heavy Metal Concentration in Spinach for Combined Metal Treated Soil

Treatment	Mean Concentrations of metals ±SD(mg/kg)			
	Pb	**Cd**	**Cu**	**Zn**
Pb/Cd	1.67±0.67^{a}	13.67±1.86^{a}	24.33±1.86^{c}	44.00±2.52^{a}
Pb/Cu	1.67±1.20^{a}	17.33±1.67^{b}	18.33±0.88^{ab}	36.33±0.67^{b}
Pb/Zn	2.33±1.33^{b}	16.00±1.16c	20.33±2.96^{be}	56.67±4.10^{c}
Cd/Cu	1.67±0.67^{a}	11.33±0.88^{d}	15.67±1.45^{a}	40.67±8.69^{d}
Cd/Zn	2.00±2.00^{a}	12.33±1.45^{e}	15.67±1.46^{a}	40.67±1.76^{d}
C u/Zn	1.33±0.33^{a}	13.67±1.20^{f}	16.33±1.76^{a}	44.67±1.45^{a}
Pb/Cd/Cu	1.67±0.67^{a}	15.67±2.60^{g}	17.00±0.58^{e}	55.00±4.16^{e}
Pb/Cd/Zn	1.33±0.33^{a}	14.33±2.33^{h}	15.00±1.53^{a}	41.33±1.33^{f}
Cd/Cu/Zn	1.33±0.88^{a}	13.33±1.45^{f}	14.00±0.58^{f}	44.33±2.33^{a}
Pb/Cd/Cu/Zn	1.00±0.00a	14.00±1.16^{h}	15.33±1.20^{a}	43.67±2.33^{ab}
Control	**ND**	**12.33±1.86^{e}**	**13.67±2.08^{h}**	**46.33±1.86^{bc}**

SD = Standard Deviation ND = Not Detected

Mean± SD Values followed by different small letters (superscript) within the same Column are significantly different (P>0.05)

Soil-Spinach Transfer Factors for Combined Metal Treated Soil

The Soil-spinach transfer factors for combined metal treated soil is shown in Table 12. This result further confirms that Cd is the most mobile metal among the group. This was also reported by Kloke*et al.*,[28]. The order of decreased mobility is Cd>Pb>Cu>Zn.

Table 12: Soil-Spinach Transfer Factorsfor Combined Metal Treated Soil

Treatment	Transfer Factors (TF)			
	Pb	Cd	Cu	Zn
Pb/Cd	2.50	1.24	1.52	0.86
Pb/Cu	1.26	1.50	1.15	0.71
Pb/Zn	1.50	1.46	1.27	1.11
Cd/Cu	0.84	1.50	0.98	0.79
Cd/Zn		1.30	0.98	0.80
Cu/Zn	0.80	1.50	1.02	0.88
Pb/Cd/Zn	2.50	1.43	1.06	1.08
Pb/Cd/Zn	1.33	1.30	0.94	0.81
Cd/Cu/Zn	1.00	1.50	0.88	0.87
Pb/Cd/Cu/Zn	0.50	1.72	0.96	0.86
Mean (TF)	1.47	1.50	1.40	0.88

CONCLUSION

The work carried out within the scope of this research provided information on the much needed single and combined metal treatment in soil. The research showed clearly the effect of the presence of a metal in soil in the presence of another metal. These effects can be antagonistic or synergistic. The distribution patterns in the six geochemical phases showed the various forms in which the metals exist in the soil. The mobility factors of these metals showed that all the metals had mobility factors above 10% indicating that they were all potentially available for plant uptake. The soil-spinach transfer factors showed Cd as the most mobile metal and Zn as the least.

ACKNOWLEDGEMENT

The authors express gratitude to the Tertiary Education Trust Fund (Tetfund) for the funding and permission to publish the paper.

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