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"Bioavailable" Lead Concentration in Vegetable Plants grown in Soil from a reclaimed industrial site: health implications.

Anyanwu, EC.[1*], Ijeoma Kanu, Ehiri, J.E., [2]Saleh, M.A.[1]

[1]Department of Chemistry, Environmental Toxicology Program, Texas Southern University, 3100 Cleburne Street, Houston, Texas, USA.

[2]Department of Microbiology, Abia State University, PMB 2000 Uturu, Abia State Nigeria.

*** Correspondence: ebereanyanwu@msn.com**

Abstract
Reclaimed lands are known to contain significant amount of metals and are being used for growing food crops and vegetables. Although, the total elemental contents of a given soil sample can be determined using modern analytical techniques such as AAS and ICP-AES, however there is no established quantitative method for determining directly the exact or fractional amount of metals that are bioavailable to plants. This is because of many factors such as variations in pH, temperature, chemical composition and redox conditions. In this research, the bioavailability of Pb concentration in a given soil sample is determined indirectly by using nitric acid (10 cm3, 25% v/v), disodium EDTA (0.05 M at pH 7.0) and water. The aim was to find out the effectiveness of EDTA in extracting 'bioavailable' Pb in the soil sample relative to other indirect methods. The results showed that the 'total' Pb concentration of the unknown soil sample concentration was 5593.4 mg/l, while the concentration of the reference soil sample was 274 mg/l. The Pb concentration of EDTA and Water extraction methods were 7103.05 mg/l and 135.5 mg/l respectively. Overall, the 'bioavailable' Pb concentration was 1509.65 mg/l while the % of extractable Pb concentration was 27% by calculation. These results showed that EDTA is more effective in extracting 'bioavailable' Pb concentration in the soil.

Introduction
Soils are made up of a mixture of inorganic and organic particles, gaseous and liquid constituents. They therefore act as a sink for pollutant through adsorption processes which bind inorganic and organic pollutants with varying strengths to the surface of soil colloids (McLaren and Crawford, 1973; Evans, 1989; Schnitt and Sticher, 1991; Alloway, 1995). The determination of bioavailability of metals remains an unsolved problem in environmental pollution analysis. Although, the total elemental contents of a given soil sample can be determined using modern analytical techniques such as AAS and ICP-AES, however there is no established quantitative method for determining directly the exact or fractional amount of metals that are available to plants. The problem is further compounded by the fact that the mobility, activity and bioavailability of chemical elements and their compounds are influenced by many factors such as pH, temperature, and chemical composition and redox conditions. However, many indirect methods have been suggested (Elsokkary and Lag, 1978; Sposito et al., 1982; Shuman, 1982; Forstner, 1984; Leschber, 1984; Eriksson et al., 1990). The most indirect methods used by many researchers (Forstner, 1990; Eriksson et al., 1990; Farago and Mehra, 1992), in recent times include the application of chemical ligands such as ethylenediaminetetracetic acid (EDTA), diethlenetriamine pentacetic acid (DTPA) and ammonium acetate or water and hydrochloric acid (Fargo and Mehra, 1992). In this experiment, the bioavailability of Pb concentration in a given soil sample is determined using nitric acid (10 cm3, 25% v/v), disodium EDTA (0.05 M at pH 7.0) and water.

Materials and Methods
Preparation of Standard Solutions (Lead)
Aliquot parts of standard solution 250, 500, 750 and 1000 microlitres of standard solution were pipetted into four 100cm3 volumetric flask respectively and carefully made up to the mark with demonized water. These solutions contain 2.5, 5.0, 7.5 and 10 parts per million (ppm) of lead.

A) Preparation of Vegetable Soil Sample for 'total' metal analysis
i) About 0.2g of the soil sample was added in a 100cm3 beaker and moistened with demonized water. Nitric acid (10 cm^3, 25% v/v) was added and brought to boil on a hotplate to simmer for 15 minutes. The solution was allowed to cool down after which it was filtered through a Whatman 541 filter paper into a 25 cm^3 volumetric flask. The residue on paper was then washed into flask with demonized water and made up

to the mark with demonized water. All the steps were carried in duplicate. The above procedure was carried out in duplicate on 'reference materials' and blank (deionized water) samples. The solutions were then analyzed by AAS for lead.

B) Preparation of vegetable soil for 'bioavailable' Pb analysis

Soil Sample for 'bioavailable' for Pb analysis was prepared according to the method used by the United kingdom Ministry of Agriculture, Fisheries and Food, 1986:

i) EDTA extraction of vegetable soils

About 0.2g of the soil sample weighed and put in a 100 cm^3 conical flask. Fifty cubic centimeters 50 cm^3 of disodium EDTA (0.05M at pH 7.0) was added and shaken for one hour on the shaker. The solutions were filtered and the filtrates were analyzed for lead by AAS.

ii) Water Extraction of vegetable Soil:

The above procedure was carried out, replacing EDTA with demonized water. From the data generated, the unknown Pb concentration, 'total' Pb concentration, 'bioavailable' concentration, % of extractable lead were calculated and tabulated. A calibration curve was plotted using the data from the standard concentration.

Quality Control

Maximum care was taken to minimize random and systematic errors. Calibration curve was obtained and all the tests were carried out in duplicates to establish confidence in the accuracy, reproducibility and reliability of data generated. Health and safety regulations were observed.

Results

The overall results of the analyses are presented in Tables 1-4. From the data generated, the 'total' Pb concentration of the reclaimed soil sample was 5593.4 mg/l, while the concentration of the reference soil sample was 274 mg/l (Table 1). The curve for the standard concentration was based on the data as given in Fig.1.

Table 1: 'Total' Pb analysis using AAS

Samples	Conc. mg/l	X100/0.2 mg/l	Mean conc. mg/l	Total (mean-10-6 blank)	SD	RSD %
S1	11.30	5650	5604	5593.4	0.0896	0.785
S2	11.36	5658	-	-	-	-
Ref 1	0.0384	19.2	284.6	274	0.0206	31.995
Ref 2	1.100	550				
Blank	0.0212	10.6	10.6	10.6	0.0124	58.67

The Pb concentration of EDTA and Water extractions were 7103.05 mg/l and 135.5 mg/l respectively (Tables 1-4). The results showed that the 'total' Pb concentration in the reclaimed soil sample in which the vegetables were planted was 5593.4 mg/l, while the concentration of the reference soil sample was 274 mg/l.

Table 2: Standard Solution analysis

Std soln.ppm/kg-1	Pb conc. mg/l	SD	RSD %
2.5	2.33	0.0118	0.50
5,0	3.122	0.0450	1.44
7.5	3.162	0.0144	0.45
10.0	3.876	0.0111	0.29

The Pb concentrations using EDTA and Water extraction methods were 7103.05 mg/l and 135.5 mg/l respectively. Overall, the 'bioavailable' Pb concentration in the soil sample from the reclaimed land was 1509.65 mg/l while the % of extractable Pb concentration was 27% by calculation (Note/Table 4). In addition, the results showed that EDTA is more effective indirect method for determining 'bioavailable' Pb concentration in the vegetables planted in a reclaimed contaminated soil.

'Bioavailable' Pb extraction from EDTA and Water

Table 3: First treatment of data (HRM1 & HRM2)

Samples	Pb conc./mg/l	SD	RSD %
EDTA 1	13.82	0.2087	1.51
EDTA 2	14.62	0.5566	3.81
Dist-W 1	0.1183	0.0188	15.93
Dist-W 2	0.4367	0.0209	4.80
EDTA blank	0.0139	0.0081	58.05
Dist-W blank	0.0068	0.0076	112.02

Table 4: Second treatment of data

Samples	Mean conc. (mg/l)	SD	RSD %
EDTA 1&2	7110	0.3826	2.66
EDTA-blank	6.95	0.0081	58.05
Dist-W 1&2	138.75	0.01985	10.365
Dist-W blank	3.4	0.0076	112.02

Note: Overall Calculations:

Total EDTA extractable Pb conc. = (7110 - 6.95) = 7103.05 mg/l
Total water extractable Pb conc. = (138.75 - 3.4) = 135.5 mg/l
But the 'total' Pb conc. from Table 1 = 5593.4 mg/l
Therefore, 'bioavailable' Pb conc. = (7103.05 - 5593.4) = 1509.65 ppm/kg-1
% Extractable Pb = 1509.65/5593.4 x 100 = 26.9898 = 27%

Discussion

The experiment has shown some positive results however, there is a huge discrepancy between the Pb values provided for the HRM1 (13.0 mg/kg-1) and HRM2 (510 mg/kg-1) on the one hand and the soil sample Pb concentrations generated from EDTA extraction method on the other. Experimentally, EDTA has an effective capacity in extracting more lead than either water or acid as shown in the 'bioavailable' Pb concentration of 1509.65 mg/l and the percentage (27%) of extractable Pb concentration. However, it is probably arguable to establish the use of EDTA on soil analysis as an ideal method for quantifying the empirical relationships between plant uptake and soil metal contents. Besides the limitations of random and systematic errors and other factors such as pH, temperature, chemical composition and redox conditions, there are many other factors that must be put into consideration for example, soil pH, soil organic matter content and plant genotype which can have marked effects on metal uptake. Other researchers (Kloke et al., 1984; Kabata-Pendias, and Pendias, 1984; O'Neill, 1995) have used 'transfer coefficient' (concentration of metal in the aerial portion of the plant relative to total concentration in the soil) as a convenient way of quantifying the relative differences in bioavailability of metals to plants. This method has its own problems too. Kloke et al. (1984) gave generalized transfer coefficients for soils and plants based on root uptake of metals but it should be realized that plants can accumulate relatively large amounts of metals by foliar absorption of atmospheric deposits on plant leaves. If metabolic processes (including redox activities) and transportation systems in plants were put into consideration, then doubts would be cast upon the validity of using the present indirect methods (including EDTA) for measuring bioavailability of metals to plants. This is more so because of the differential ligand and mineral salt absorptivity, soil difference, plant genotype and the selective membrane permeability of plant tissues.

Health risk and hazards of exposure

The possibility and the danger of lead getting into human food chain through eating contaminated vegetables should not be underestimated. In children for example, such an exposure would lead to adverse health consequences against the developing brain, which may result in long-term cognitive deficits as evidenced in literatures. For example, Kuhlmann, et al. (1997) tested groups of male rats exposed to lead during different developmental periods as adults in a water maze. A highly significant ($P < 0.01$) impairment in water maze performance was measured in rats exposed to Pb only during gestation and lactation (maternal exposure). At the time of testing (100-106 days old), blood and brain Pb concentrations were at control levels. Significant impairments ($P < 0.05$) were also present in rats continuously exposed to Pb from conception through adulthood. Post-weaning Pb exposure alone did not result in impaired performance despite significantly elevated blood and brain Pb levels at the time of testing.

Conclusions

Although, the determination of 'pseudototal' concentrations of metal content of the soil as a representative of bioavailability to plant by boiling concentrated acids (e.g. nitric/perchloric) still remains acceptable in this circumstance, it is envisaged that the application of EDTA could be more viable if new ways of minimizing its limitations could be found. More research work is recommended.

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