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Review Article

Testing Bioaccumulation of Cd, Pb, and Ni in Plants Grown in Soil Amended with Municipal Sewage Sludge at Three Kentucky Locations

George F. Antonious[1]*, Eric T. Turley[1], and Tejinder S. Kochhar[2]

[1]Division of Environmental Studies, Kentucky State University, USA

[2]Division of of Applied Health and Natural Science, Blue Grass Community and Technical College, USA

*Corresponding author

George F. Antonious, Division of Environmental Studies, College of Agriculture, Food Science, and Sustainable Systems, Kentucky State University, 107 Chappell Building, Frankfort, KY 40601-2355; Tel: 502/597-6005; Fax: 502/597-6763; Email: george.antonious@kysu.edu

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Abstract

Current trends in crop production permit the utilization of municipal sewage sludge (SS) as alternative fertilizer. The impact of SS on Cd, Pb, and Ni accumulation in plants grown under this practice was examined at three locations in Kentucky (Adair, Meade, and Franklin Counties). Soil and soil amended with SS were examined for total metals using nitric acid solution and metal ions available to plants using $CaCl_2$ solution. Inductively coupled plasma mass spectrometer (ICP-MS) was used to assess the transport of metals (Cd, Pb, and Ni) from soil mixed with SS into plants and potential bioaccumulation of these metals in edible plants at harvest. Tobacco leaves, potato tubers, green beans, pepper fruits, squash fruits, tomato fruits, broccoli buds, onion leaves and bulbs, sweet potato, beet, and okra fruits grown under this practice were collected at harvest for metal analyses. In Meade county, results indicated that Cd bioaccumulation factor (BAF) of pepper, onion leaves, onion bulbs, tomato fruits, and okra fruits was >1 whereas, the BAF values of bean pods, bean seeds, green squash, yellow squash, and beet heads were <1. BAF values of Pb in all of the above mentioned crops were <1. Other than tomato fruits and beet heads, Ni BAF values were all >1. In Franklin County, Ni BAF values were < 1 in potato, pepper fruits, onion bulbs and all other plants tested. Only Ni BAF values in tobacco leaves and onion bulbs were >1 in Adair County. Accordingly, our results revealed that low Cd-, Pb-, and Ni-accumulator plants could be grown in soil contaminated with such metals.

INTRODUCTION

Plant nutrients exist in soil in many different forms, soluble forms that are readily available to plants (weakly bound forms that are in rapid equilibrium with soluble forms), and strongly bound or precipitated forms that are insoluble and become available to plants only over long time periods. Soil organic matter provides slow release nutrients as it decomposes. However, recycling waste such as municipal sewage sludge (SS) for use as alternative to synthetic fertilizers, has been a matter of frequent concern. SS used for land farming typically retain metals bearing crop potential hazard to animal life. However, recycling wastes for use in agricultural production systems provides a useful source of nutrients needed to restore the nutritional composition and fertility of deteriorated soil [1,2]. The use of organic amendments in vegetable production has been clearly proven to be beneficial [3-5]. The use of SS in crop production is approved by the U.S. Environmental Protection Agency (USEPA) due in part to the decreased dependency on synthetic fertilizers and increased growers' financial rewards. The presence of substantial concentration of organic matter, and macro- and micro-nutrients in SS that enhance soil physical, chemical, and microbial conditions also improve soil bulck density, drainage, root penetration and pesticide bioremediation in contaminated soil [6,7].

SS used in growing field crops such as maize and sorghum, and vegetables such as cucumbers, beans, potatoes, and lettuce enlarged soil physical properties due to the increased soil organic matter content [8]. Studies have shown that SS composts alleviate the physical properties of soil and soil water holding capacity and drop soil density [9]. Results have shown that the addition of lime (calcium carbonate) to SS compost enhanced the soil chemical properties due to the increased pH and reduced mobility of toxic metals to growing plants [10]. Buildup of toxic metals (Cd and Pb) in soil of plants fertilized with SS [10-12] represents a potential problem that needs a nonstop soil and plant analyses. Dangers of soil contamination when recycled wastes are incorporated with soil as fertilizer have been studied [13]. Metals such as Cd and Pb are harmful to human, plant or animal life if present in plant beyond permitted limits. As indicated by Thuy et al. [14], metals are the pollutants of most fear worldwide. Metals that accumulate in edible plants are unsafe chemicals [15]. The bioaccumulation factor (BAF) is characterized by the ratio of the metal content in plant and total metal content in the soil. Cd can cause kidney and skinny damage as well as reproductive deficiencies. [16] Cd concentration in soil amended with SS [17,18] could be taken by publics consuming plants matured is such soils. Pb and Ni also are potential pollutants in SS. Dangerous levels of Pb have been described by the US EPA [19]. Pb is a carcinogenic agent that can also cause liver, brain, and central nervous system damage [20] and the existence of Ni above certain levels can cause respiratory difficulties [21,22].

Soil microorganisms need certain metals for their existence and survivals, however, metals are deadly to soil microorganisms when existing above certain concentrations [23]. Metals in soil have shown harmful impact on helpful soil microorganisms as described by diminished activities of the enzymes they release. Phytotoxicity is caused by uptake of metals by plants. Plant and animal cells absorb and accumulate certain metals and molecules that could be harmful substances such as metals. The objectives of this investigation were to: 1) assess the impact of soil incorporated with municipal SS on Cd, Pb, and Ni content of soil and potential mobility of these metals into field-grown plants at three locations in Kentucky (Adair, Meade, and Franklin Counties), 2) determine the bioaccumulation factor (BAF) of Cd, Pb, and Ni in plants grown under this practice, and 3) compare metal concentrations detected in plants to their standard limits.

MATERIALS AND METHODS

The study was established at three locations (Adair, Meade, and Franklin Counties; Figure 1) by collecting plants leaves and fruits at harvest time from areas where small farmers use SS as an organic fertilizer for vegetable production. Before planting, the native soil was incorporated with municipal SS purchased from the Metropolitan Sewer District in Louisville, KY and used at 15 t acre^{-1} (on dry weight basis). SS was added to the top 15 cm soil. Thirty-cm extra space was allowed between planting rows, and the plants were watered, irrigated, and weeded during the growing season according to Kentucky Vegetable Growers Guide [24], but no mineral fertilizer was applied. At harvest, four plants (tobacco, *Nicotiana tabacum* cv. Burley; red potato, *Ipomoea batatas* cv. Norland Red; onion, *Allium cepa* cv. Super Star- F1; and sweet potato, *Ipomoea batatas* cv. Beauregard) from Adair County; seven plants (white potato, *Ipomoea batatas* cv. Kennebec; green pepper, *Capsicum annuum* cv. Aristotle X3R; tomato, *Lycopersicon esculentum* cv. Mountain Spring; onion, *Allium cepa* cv. Super Star- F1; broccoli, *Brassica oleracea* cv. Packman; yellow squash, *Curcurbita pepo* cv. Conqueror III; and sweet potato, *Ipomoea batatas* cv. Beauregard) from Franklin County; and eight plants (green beans, *Phaseolus vulgaris* cv. Blue Lake; green pepper, *Capsicum annuum* cv. Aristotle X3R; green squash, *Cucurbita pepo* cv. Costata Romanesco; yellow squash, *Curcurbita pepo* (Conqueror III); onion, *Allium cepa* cv. Super Star- F1; tomato, *Lycopersicon esculentum* cv. Mountain Spring; okra, *Abelmoschus esculentus* cv. Clemson Spineless; and beets, *Beta vulgaris* cv. Red Ace –F1) from Meade County were sampled at harvest. Randomly selected fruits, pods, bulbs, or leaves (n=3) from each location were collected at full maturity from throughout the growing plants to reduce the effect of fruits, pods, bulbs, and leaves position on the concentration of metals monitored.

Samples of soil (three replicates per location) also were collected from the rhizosphere (the zone where soil and root make contact) that has an increased microbial and enzyme activity to a depth of 15 cm using a soil core sampler equipped with a plastic liner (Clements Associates, Newton, IA, USA) of 2.5 cm i.d. Soil samples were air-dried, passed through a 2 mm sieve, and kept in plastic bags at 4 °C up to 48 h before use. A glass electrode was used to determine soil pH in a soil: distilled water slurry (1:5, w/v). Soil organic matter was calculated as dry weight minus ash content [25]. Nitrogen (N) was determined by the Kjeldahl method [26]. An Inductively Coupled Plasma Spectrometer (ICP) was used to determine all metals after digestion and extraction with HNO_3 [27].

Metal analysis

For quantification of metals in harvested plants, ten fruits, pods, bulbs, or leaves of appropriate size were randomly collected from each of the three field locations in Adair, Meade, and Franklin Counties (three replicates from each plant). Soils were carefully removed from plants and washed with deionized water in order to remove any attached soil particles. Plants were separated into leaves, roots, and fruits and dried in an oven at 65 °C for 48 h. Using ceramic mortar and pestle, the dried samples were manually ground to pass 2 mm non-metal sieve. Samples were re-dried using an oven to obtain constant weight. Ten mL of concentrated nitric acid (HNO_3) trace metal grade was added to 1 g of each dry sample powder and the mixture was allowed to stand overnight. The mixture was then heated for 4 h at 125 °C on a hot plate and diluted to 50 mL with double distilled water and filtered through Whatman filter paper No.1. Metal concentration was determined using ICP-mass spectrometer (ICP-MS) in standard mode following the U.S. EPA method 6020a [27] and using an octopole collision cell ICP-MS (7500cx, Agilent, Santa Clara, CA, USA). All metal standards were NIST traceable. Spike recovery ranged from 85-100%. Instrument detection limits (IDL) were 0.0755, 0.0113, and 0.0454 ppb (ng mL^{-1}) for Ni, Cd, and Pb, respectively. The final m/z values used for quantification were 60, 112 and 208 for Ni, Cd and Pb, respectively.

Soluble concentrations of metals in soil were also determined using calcium chloride $CaCl_2$ [28] to quantify soluble and extractable metals in soil. Soil (10 g) samples were suspended in 25 mL of 0.01 $CaCl_2$ and heated at 90 °C on a hot plate for 30 min. The resulting supernatants were filtered hot through Whatman filter paper #42, and 2 drops of 1 M HNO_3 trace metal grade were added to prevent metal precipitation and to inhibit microbial growth in sample extracts [29]. Extractable Cd, Ni, and Pb were also determined in soil extracts using ICP spectrometer [13]. Metal concentrations in soil, SS incorporated with soil, and plants grown under this practice were statistically analyzed using ANOVA procedure [30]. Means were compared using Duncan's multiple range test.

RESULTS AND DISCUSSION

The soil texture and chemical characteristics of soils and soils amended with SS at the three locations in Kentucky are shown in Figure (1) and Table (1), whereas, the concentrations of total Cd, Pb, and Ni extracted using nitric acid and soluble metals extracted using $CaCl_2$ from native soil and SS mixed soil are presented in Table (2). Carbon, N, organic matter (OM), and pH were higher in each of the three locations after the additions of SS to native soil (Table 1). Other than Ni in samples collected from Meade County (Table 2), metal concentrations in soil extracted using nitric acid (an aggressive solvent) were greater than their soluble forms (metal ions) extracted using $CaCl_2$ solution (a mild solvent). These findings indicated that metal extracted using nitric acid solutions might be in a conjugated form that is not soluble in the mild $CaCl_2$ solution that extracts only metal ions available to plants.

Bioavailability is the amount of the metals in soil that are available for the incorporation into biota [31]. The bioaccumulation factor (BAF) [32] is useful in screening plants for bioaccumulation and potential phytoremediation. Phytoremediation (phytoextraction) is used to increase the uptake of metals from contaminated sites through translocation to aboveground plant parts and accumulation of metals for soil cleanup [33-35]. Whereas, in biofortification, the aim is to increase nutrient uptake mainly from poor soils and translocation to the edible plants [36-38]. The common purpose for both biotechnological applications is to raise metal transfer from the roots to the shoots. Results in Figure (2) (upper graph) revealed that Cd BAF of tobacco (BAF =0.95) is < 1 and significantly greater than red potato tubers, onion bulbs, and sweet potato (BAF values of =0.17, 0.1, 0.05, respectively. This could be due to the high surface area of tobacco leaves g^{-1} dry tissues compared to edible tissue of other vegetables tested in Adair County. Tobacco, *Nicotiana tabacum* has been characterized as a leaf and root Cd accumulation [39]. Cd in tobacco leaves is moveable in tobacco smoke [40]. John and Laerhoven [41] have shown that variability in Cd accumulation occurs even among commercial varieties in lettuce. Researchers have found that varietal type and plant species varied in Cd accumulation in a variety of plant species [42,43] and even among genotypes of the same species [44]. It was also found that vegetable foods contribute to about 75% of daily intake of the nonessential toxic Cd to the U.S. population [45,46].

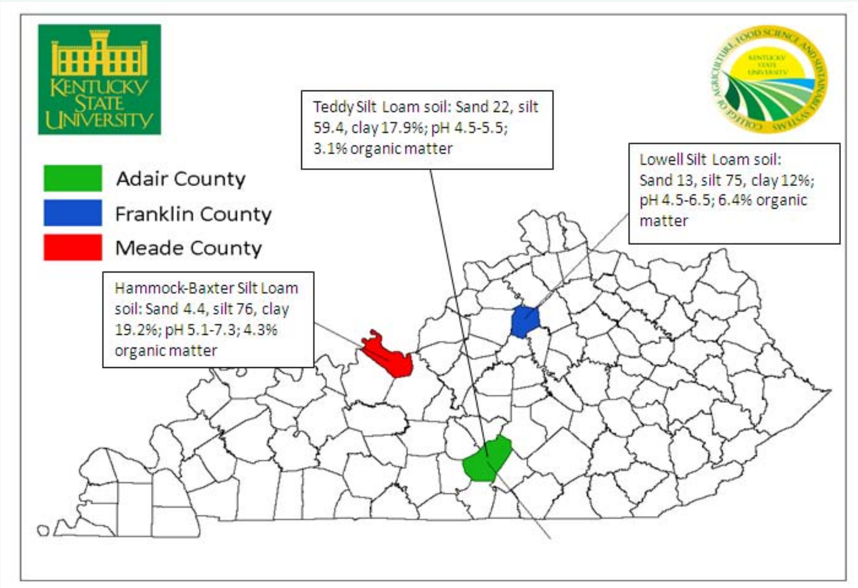

Figure 1 Soil texture, pH, and % organic matter of soils used for growing vegetables at three locations in Kentucky.

Table 1: Chemical characteristics of native soil and soil amended with municipal sewage sludge (SS) at three locations in Kentucky.

Location	Soil	Soil Type	% C	% N	% OM	pH
Adair	Native Soil	Teddy Silt Loam	0.77 ± 0.30	0.07 ± 0.01	1.2 ± 0.4	4.5 ± 0.15
	SS Soil Mix	Teddy Silt Loam	1.89 ± 0.25	0.18 ± 0.03	3.1 ± 0.07	5.5 ± 0.18
Meade	Native Soil	Hammack-Baxter Silt Loam	1.72 ± 0.15	0.21 ± 0.05	1.75 ± 0.0	5.1 ± 0.12
	SS Soil Mix	Hammack-Baxter Silt Loam	2.52 ± 0.55	0.31 ± 0.06	4.3 ± 0.19	7.3 ± 0.33
Franklin	Native Soil	Lowell Silt Loam	1.60 ± 0.23	0.15 ± 0.01	2.1 ± 0.06	4.5 ± 0.61
	SS Soil Mix	Lowell Silt Loam	3.70 ± 0.75	0.39 ± 0.08	4.3 ± 0.11	7.6 ± 1.22

Each value in the table is an average ± std. error of the mean.

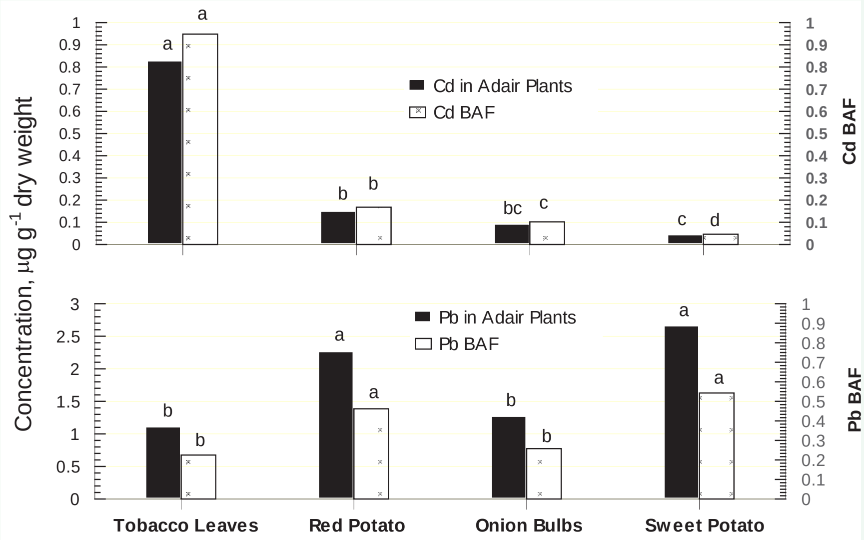

Figure 2 Concentration (expressed as µg g-1 dry plant tissue) and bioaccumulation factor (BAF) of Cd (upper graph) and Pb (lower graph) in Adair County plants grown in soil amended with sewage sludge. Statistical comparisons were carried out among plants for each parameter. Bars accompanied by the same letter(s) for each parameter are not significantly different (P> 0.05) using Duncan's multiple range test (SAS Institute 2003).

Concentrations of Pb in sweet potato and red potato (belowground crops) grown in soil amended with SS (2.65 and 2.25 µg g^{-1} dry tissue, respectively) in Adair plants were significantly greater than Pb concentration in onion bulbs and tobacco leaves (Figure 2, lower graph). BAF of Pb in red potato and sweet potato (0.46 and 0.54, respectively) were also significantly greater compared to onion bulbs and tobacco leaves (0.26 and 0.22, respectively). According to Thornton et al. [47], substantial proportions of Pb in contaminated soils were relatively insoluble and the low solubility of Pb in soil did not necessarily present a risk to exposed population groups. Antonious et al. [28], also found that elevated concentrations of trace elements in soil do not necessarily reflect metals available to plants. Compounds of Pb-phosphates, for example, are significantly less soluble than Pb (II) sulfide (PbS) and Pb (II) sulfate ($PbSO_4$) commonly reported in contaminated soils. According to Zia et al. [48], Pb bioavailability in soils is largely controlled by phosphate, iron oxides, organic matter and pH. Iron (oxy) hydroxides such as goethite and amorphous Fe $(OH)_3$ (ferrihydrite) and organic matter create surface sorption or chelation sites for binding Pb^{2+}, whereas, dissolved phosphate causes Pb precipitation [49-51]. In a similar way, an increase in pH also decreases Pb mobility and bioavailability as fewer H^+ ions are available to compete with Pb^{2+} ions for binding sites [52]. Lead is defined by USEPA as potentially toxic to most forms of life. According to the Codex Alimentarius Commission of the Joint FAO/WHO Food Standards [53], the maximum Pb level in most vegetables is 0.1 mg kg^{-1} on fresh weight basis. Pb is a toxic metal in the environment [54] and has no biological role in animals, plants, and microorganisms; however, it forms a bond with the sulfhydryl group of proteins, and hence can disrupt the metabolism and biological activities of many proteins and has caused cancer in rodents [54].

Table 2: Average concentrations of heavy metals expressed as µg g^{-1} dry soil amended with municipal sewage sludge (SS) at three locations in Kentucky using two extraction procedures.

Location	Heavy Metal	Extraction with HNO_3	Extraction with $CaCl_2$
Adair	Cd	0.87 ± 0.11	0.030 ± 0.00
	Pb	4.87 ± 0.98	0.998 ± 0.20
	Ni	1.05 ± 0.16	0.580 ± 0.10
Meade	Cd	0.23 ± 0.07	0.113 ± 0.03
	Pb	8.05 ± 0.95	0.921 ± 0.08
	Ni	0.88 ± 0.08	0.87 ± 0.05
Franklin	Cd	0.17 ± 0.03	0.002 ± 0.00
	Pb	29.3 ± 2.01	0.093 ± 0.02
	Ni	17.33 ± 2.05	0.073 ± 0.02

Each value in the table is an average ± std. error of the mean of three replicates.

Ni concentrations in tobacco leaves and onion bulbs (1.82 and 1.59 µg g^{-1} dry tissue, respectively) were significantly greater than Ni concentrations in red potato and sweet potato (Figure 3). Ni BAF values were 1.74, 0.35, 1.51, and 0.72 in tobacco leaves, red potato, onion bulbs, and sweet potato, respectively. As mentioned earlier, plants can transfer and concentrate metals from soil into their roots and shoots. Ni can cause respiratory problems and is carcinogenic [21,22]. In terms of solubility of elements in soil and water, two groups can be classified: readily soluble compounds (salts of mineral acids) and weakly soluble compounds (mineral oxides). Metal oxides and salts entering the soil present different potential dangers for environment and living organisms. For example, Ni (II) chloride ($NiCl_2$), Ni (II) sulfate ($NiSO_4.6H_2O$), and Nickel (II) nitrate Ni $(NO_3)_2$ solubility in water are 66.8, 44.4, and 94.2 g per 100 g of water at 20°C, respectively. While, Nickel hydroxide Ni $(OH)_2$ and nickel oxide (Ni O) solubility in water is 0.01 g per 100 g of water at 20 °C [55]. This means that element oxides getting into the soil have lower ecological danger because of their low water solubility compared

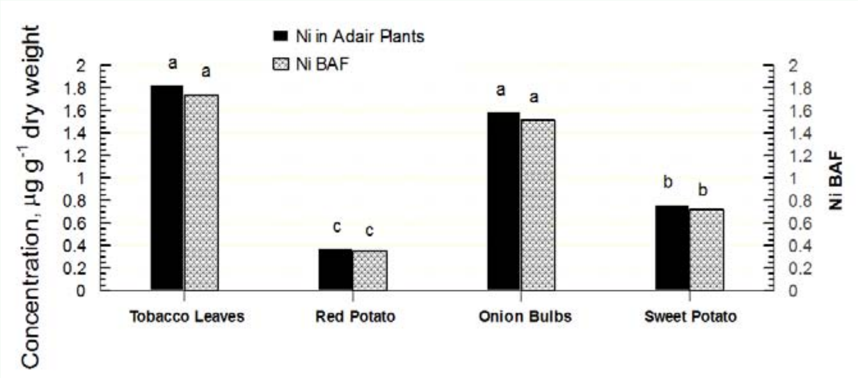

Figure 3 Concentration (expressed as µg g^{-1} dry plant tissue) and bioaccumulation factor (BAF) of Ni in Adair County plants grown in soil amended with sewage sludge. Statistical comparisons were carried out among plants for each parameter. Bars accompanied by the same letter(s) for each parameter are not significantly different (P> 0.05) using Duncan's multiple range test.

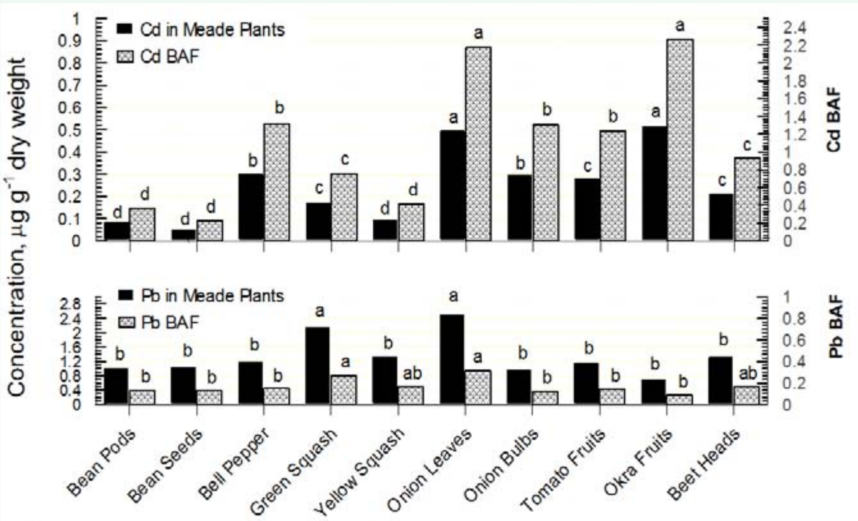

Figure 4 Concentration (expressed as µg g^{-1} dry plant tissue) and bioaccumulation factor (BAF) of Cd (upper graph) and Pb (lower graph) in Meade County plants grown in soil amended with sewage sludge. Statistical comparisons were carried out among plants for each parameter. Bars accompanied by the same letter(s) for each parameter are not significantly different (P> 0.05) using Duncan's multiple range test.

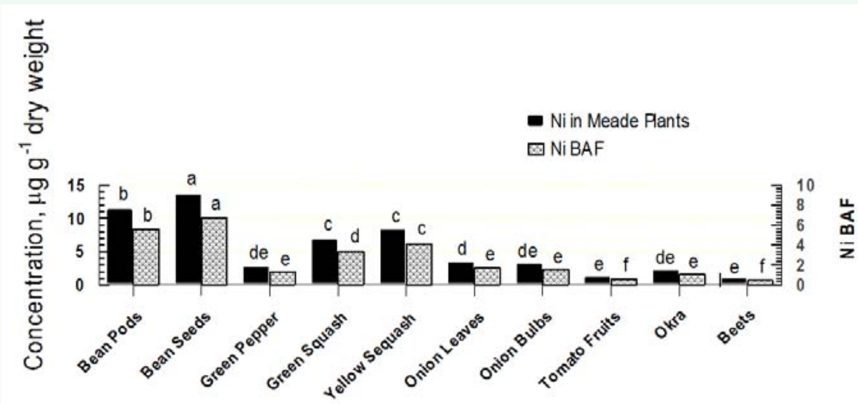

Figure 5 Concentration (expressed as µg g^{-1} dry plant tissue) and bioaccumulation factor (BAF) of Ni in Meade County plants grown in soil amended with sewage sludge. Statistical comparisons were carried out among plants for each parameter. Bars accompanied by the same letter(s) for each parameter are not significantly different (P> 0.05) using Duncan's multiple range test.

to metal salts. Soil often contains between 4 and 80 ppm of Ni [22]. The highest soil Ni concentrations (up to 9,000 ppm) are found near industries that extract Ni from ore. Great levels of Ni rise as dust released into air from stacks during metal processing and settle on the ground. Humans may be exposed to Ni in soil by skin contact. Children may also be exposed to nickel by eating soil. Food contains Ni and is the major source of Ni exposure for the general population. A person eats about 170 μg of Ni in his/her food every day.

Regarding Cd, the majority of Cd compounds are more soluble than other heavy metals, so it is more available to plants that accumulate it in different parts through which Cd enters the food chain [56]. There is a duality in plant tolerance to metals, [57] some elements like Fe, Cu, Zn and Mn at low concentration are valuable for plants through improving plant growth, biofortification, or both and in this way they are beneficial for all living organisms in the food chain. Metals also can damage plants at low or high concentration depending on the plant specific/tolerance mechanisms. High levels of metals in soils, including Cd, could damage biomemebranes and cause uncontrolled uptake and translocation of Cd in plants [56]. In addition to this toxicity-induced Cd accumulation, plants may accumulate significant amounts of Cd in different parts without any apparent toxicity or yield loss [58].

Results of this investigation also revealed that okra fruits and onion leaves of plants grown in soil mixed with SS (Meade County) accumulated greatest concentrations of Cd (0.51 and 0.49 μg g^{-1} dry tissue, respectively) compared to other vegetables tested, i.e. bean pods, bean seeds, pepper, and squash (Figure 4, upper graph), in which onion leaves, pepper, onion bulbs, tomato, and okra revealed Cd BAF values >1 compared to other plants grown under the same environmental conditions. Pb BAF values in plants tested in Meade County were all below 1 (Figure 4, lower graph). Ni concentrations in bean seeds and pods and their BAF were significantly greater compared to other vegetables tested in Meade County (Figure 5). Analyses of Cd in plants collected from Franklin County revealed that potato tubers, pepper fruits, and onion bulbs BAF vales >1 compared

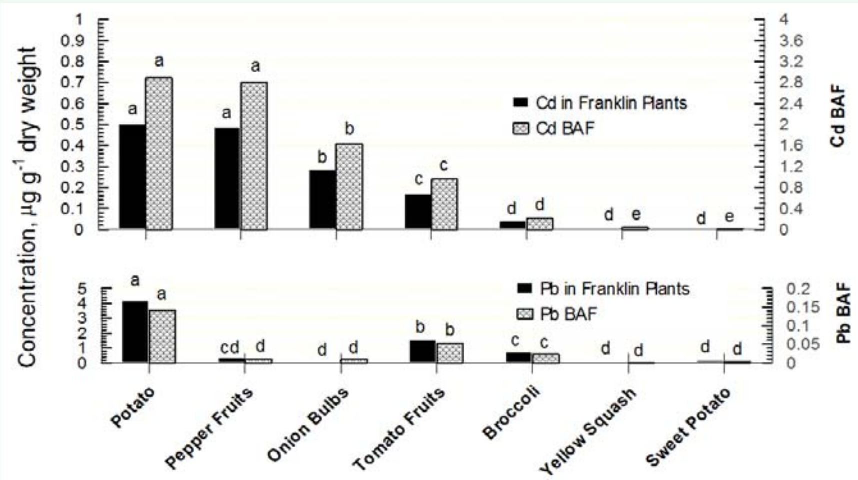

Figure 6 Concentration (expressed as μg g^{-1} dry plant tissue) and bioaccumulation factor (BAF) of Cd (upper graph) and Pb (lower graph) in Franklin County plants grown in soil amended with sewage sludge. Statistical comparisons were carried out among plants for each parameter. Bars accompanied by the same letter(s) for each parameter are not significantly different (P> 0.05) using Duncan's multiple range test.

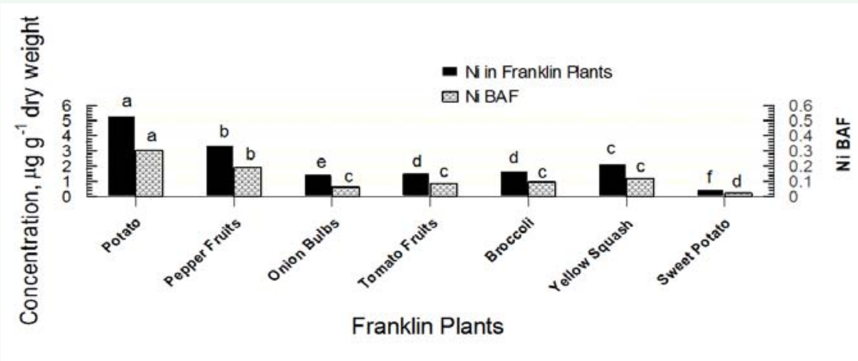

Figure 7 Concentration (expressed as μg g-1 dry plant tissue) and bioaccumulation factor (BAF) of Ni in Franklin County plants grown in soil amended with sewage sludge. Statistical comparisons were carried out among plants for each parameter. Bars accompanied by the same letter(s) for each parameter are not significantly different (P> 0.05) using Duncan's multiple range test.

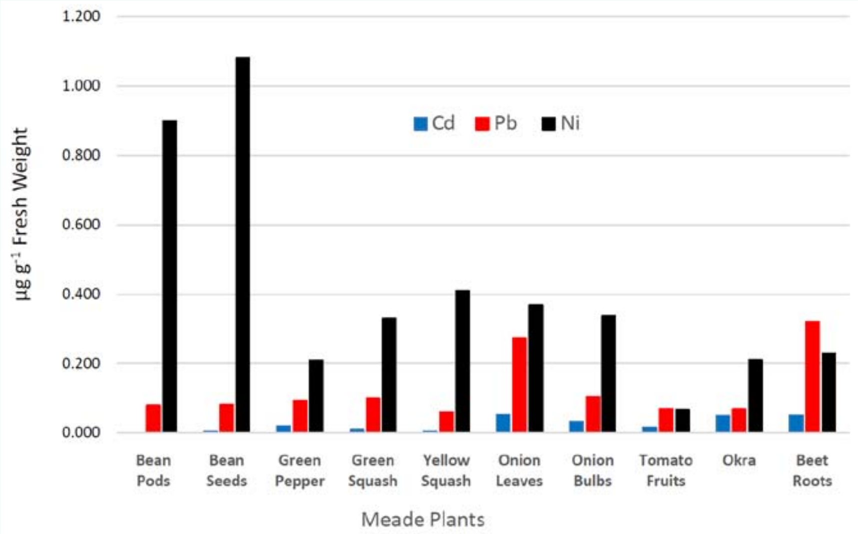

Figure 8 Concentration of Cd, Pb, and Ni expressed as µg g-1 fresh plant tissue in Meade County plants grown in soil amended with sewage sludge.

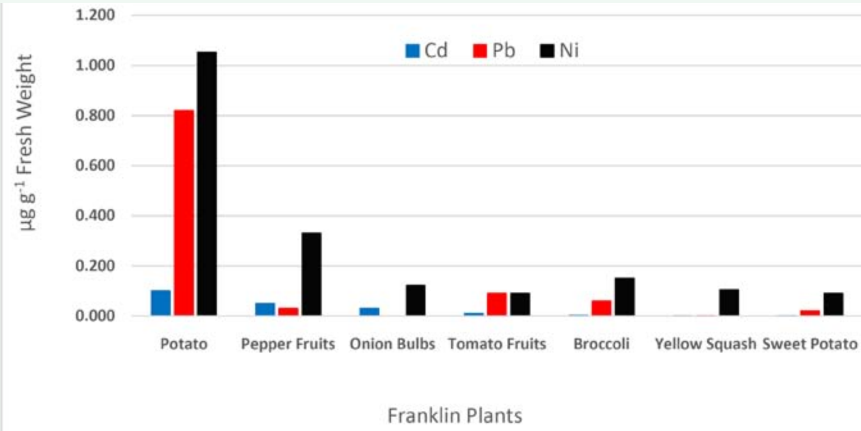

Figure 9 Concentration of Cd, Pb, and Ni expressed as µg g-1 fresh plant tissue in Franklin County plants grown in soil amended with sewage sludge.

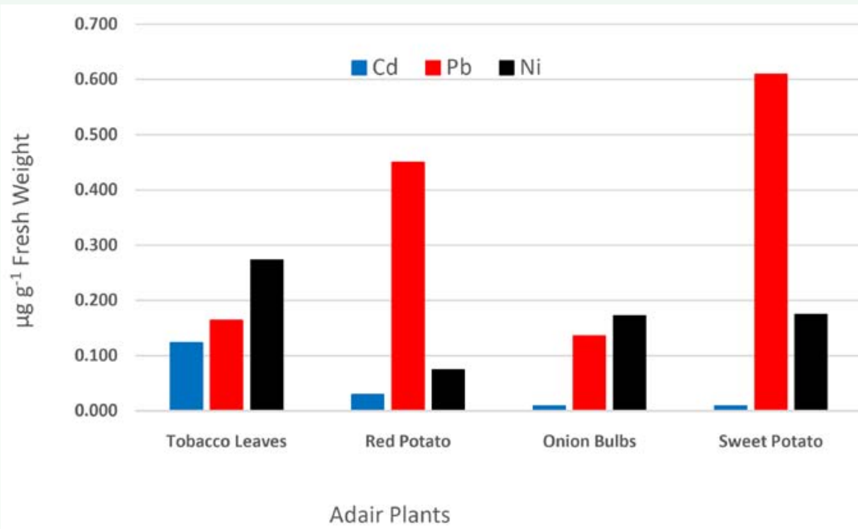

Figure 10 Concentration of Cd, Pb, and Ni expressed as µg g-1 fresh plant tissue in Adair County plants grown in soil amended with sewage sludge.

to other plants (tomato, broccoli, yellow squash, and sweet potato) collected from the same location (Figure 6, upper graph). Although the concentration of Pb was significantly greater in potato (Figure 6, lower graph) compared to other vegetables, its BAF is < 1. Ni concentration and its BAF were also greater in potato (Figure 7) compared to other vegetables tested in Franklin County. Therefore, assessing metal levels in soil amended with SS and screening plants into high- and low-metal accumulators could be explored in phytoremediation of contaminated soils. Whereas, low Cd-accumulator plants, low Pb-accumulator plants, and low Ni-accumulator plants might be appropriate selection for growing in Cd-, Pb-, and Ni-contaminated soils. In addition to metal solubility in soil, soil sorption affinity for metals due to the presence of organic matter in municipal SS compost could effectively reduce metals availability to plants.

Metals detected in plants (Figures 2-7) investigated in this study are all expressed as μg g^{-1} dry weight. In order to compare these results with metal allowable limits, which are listed on fresh weight basis (μg g^{-1} fresh plant tissues), we determined the moisture content in each plant part and used this information to transfer metal content in plants from μg g^{-1} dry weight into μg g^{-1} fresh plant tissue as shown in Table (3). Considering that the maximum allowable limit (MAL) of Cd is 0.1 μg g^{-1} [59] of plant tissue on fresh weight basis and MAL of Pb is also 0.1 μg g^{-1} [59] on fresh weight basis, Figure (8) shows that Cd concentration in plants collected from Meade County was below the permissible limits, while Pb in onion leaves and beet heads was above the allowable limit of Pb. There is a lack of information about MAL of Ni in vegetables. Figure (8) also shows that the concentrations of Ni in plants collected from Meade County ranged from 0.07 – 1.08 μg g^{-1} fresh plant tissue.

Figure (9) shows that Cd concentration in plants collected from Franklin County was below the permissible limits, whereas Pb in potato (belowground tubers) was above the allowable limit of 0.1 μg g^{-1} fresh tissue. Pb levels in all other vegetables tested in Franklin County ranged from 0.0-0.8 μg g^{-1} fresh tissue and were all below the MAL. These results indicated that potato tubers are high Pb accumulators. Baghour et al. [60], found that their potato plants accumulated far higher quantities of Pb and Cd and most of the Cd taken up in these potato plants was retained in the roots (Grant et al.) [61], and small concentrations were translocated towards the aerial plant parts. Cherfi et al. [62], also showed that within the many selected fruits and vegetables, the highest concentrations of Pb were found in the root vegetables (potato, onions, and carrots). Their data are for plants grown in native un-amended soils, whereas the results obtained from the present investigation (our data) are collected from plants grown in SS amended soils, which are often contain greater concentrations of metals [15]. Figure (10) shows the concentrations of Cd, Pb, and Ni in plants collected from Adair County. Cd in tobacco leaves was 0.12, whereas Pb in tobacco was about 0.16 μg g^{-1} fresh leaves. Pb in red potato, onion bulbs, and sweet potato exceeded the MAL of Pb in these three edible plants.

CONCLUSION

The addition of SS to agricultural soils is a management practice that can be utilized to replace synthetic inorganic fertilizers. This constitutes a significant saving especially for limited resource farmers. However, potential bioaccumulation and mobility of metals from SS into growing plants could boost the transfer of metals through crops to animals (feed crops) and humans (food crops) [59]. Metals are not biodegradable and therefore can accumulate in the vital organs of human bodies [60] and cause toxic effects if consumed with food that has concentrations of metals above the recommended level. Metals are toxic to soil microorganisms [23]. Excessive metals in soil can be detrimental to the plants themselves. The wilted look of Ni treated plants, in which water was not a limiting factor, proposed impediment in facilitated movement of water from roots to the leaves. Cd^{2+} is the main Cd form in soil and tends to be very mobile in soil systems and very available to plants. Plants vary in their tendency to accumulate Cd. Pb tends to accumulate in surface horizon of soil because of its low water solubility and mobility. Total metal content in soil is not a predictor of metal accumulation in plants since only soluble of metals (metal ions) are accessible for plant uptake. Plants accumulate Cd, Pb, and Ni from soils at different levels depending on plant species, genotypes within the same species, soil pH, and organic matter content. To ensure that Cd and Pb maximum allowable levels are not exceeded, routine

Table 3: Concentrations of Cd, Pb, and Ni in plants grown in soil mixed with sewage sludge at three locations in Kentucky (Meade, Franklin, and Adair Counties) expressed as μg g^{-1} fresh plant tissue.

Meade	**Bean Pods**	**Bean Seeds**	**Green Pepper**	**Green Squash**	**Yellow Squash**	**Onion Leaves**	**Onion Bulbs**	**Tomato Fruits**	**Okra**	**Beet Roots**
Cd	0.001	0.004	0.020	0.008	0.004	0.053	0.032	0.016	0.050	0.052
Pb	0.079	0.080	0.094	0.100	0.060	0.272	0.104	0.069	0.068	0.320
Ni	0.900	1.080	0.210	0.330	0.410	0.369	0.337	0.067	0.211	0.229
Franklin	**Potato**	**Pepper Fruits**	**Onion Bulbs**	**Tomato Fruits**	**Broccoli**	**Yellow Squash**	**Sweet Potato**			
Cd	0.100	0.050	0.030	0.010	0.003	0.001	0.001			
Pb	0.820	0.030	0.000	0.090	0.060	0.0001	0.020			
Ni	1.052	0.330	0.121	0.090	0.150	0.105	0.090			
Adair	**Tobacco Leaves**	**Red Potato**	**Onion Bulbs**	**Sweet Potato**						
Cd	0.123	0.029	0.009	0.009						
Pb	0.164	0.450	0.136	0.609						
Ni	0.273	0.074	0.172	0.174						

Each value is an average of three replicates of samples collected at harvest.

surveillance of vegetables grown in SS amended soils should be regularly monitored in the produce.

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