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Use of Low Cost Soil Amendments Reduces Uptake of Cadmium and Lead by Tobacco (Nicotiana tabacum) Grown in Medially Polluted Soils

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Abstract:

The study explored the use of low cost soil amendments in reducing lead and cadmium uptake in tobacco. High levels of these metals in tobacco are of concern because of their toxicity and cumulative nature in tobacco consumers. Tobacco was planted in soils amended with cow manure and hydroxyapatite (HA), and non-amended sandy-loamy soil containing 60.57±3.74 μg/g lead and 33.95±0.49 μg/g cadmium in field and pot experiments. Tobacco leaves harvested after 75 days were dried, acid digested and their metal contents determined using atomic absorption spectroscopy (AAS). Cow manure reduced cadmium uptake by 67.20% and 71.75% in field and pot experiments while 1.5% HA reduced lead uptake by 39.69% in field experiments. Pot experiments had higher metals uptake than field experiments while fertilizer treatments recorded highest uptake. However, the levels were within WHO/FAO limits, indicating that amending medially polluted soils with cow manure and HA results in safe harvests of tobacco.

Keywords:

Cadmium and Lead Uptake; Cow Manure; Hydroxyapatite; Soil Amendment; Tobacco Leaves

1. INTRODUCTION

Environmental safety of soil has become a major issue in many developing countries as a result of accumulation of heavy metals in the soil from different kinds of industrial wastewater, exhaust gas, sewage irrigation and sludge farm application through growth in industrialization and urbanization. This has caused great concern in agricultural food production because heavy metals have adverse effects on food quality, crop growth and environmental health [1–3]. Growing of crops such as cabbage and spinach in soils with high levels of heavy metals, especially in lead contaminated area has reported high contamination in vegetables [4, 5]. Due to such contamination, crops grown in soils with high levels of heavy metals, especially those grown using sewage sludge are getting uprooted in many countries as a result of public protests [6–8]. Some crops such as tobacco accumulate heavy metals, which find their way to animal and human food chain and, and thus, causing many diseases, including cancer [4]. Uptake of heavy metals by plants is greatly influenced by soil type, soil pH and organic matter content [9, 10].

Lead and cadmium are heavy metals of greatest concern due to their toxicity and cumulative nature in animals and humans through consumption of contaminated foods. Toxic heavy metal contaminated soils are common in developing countries due to lack of stringent waste disposal regulation, and such soils are used for agricultural purposes.

Common remediation techniques of soils contaminated with heavy metals are expensive, and potential remediation methods need to be environment-friendly and used in-situ [1, 11]. The most convenient and low cost method is the use of soil ameliorates that reduces toxic heavy metal uptake within plants. Soil ameliorates, as by-products and waste materials, when added to contaminated soil immobilize heavy metals and lower their uptake by plants, and thus, represent an inexpensive in-situ remediation method. Some of the soil ameliorates used to immobilize Pb, Cd, and zinc (Zn) in contaminated soil includes biosolids, sawdust, composts, manures, and phosphatic materials [9, 11–16]. Phosphatic ameliorates such as apatite and hydroxyapatite (HA) have been successfully used to reduce the bioavailability and increase the geochemical stability of heavy metals in polluted soil [9, 10, 17]. Jinjun et al. [10] and Gao et al. [18] have reported that apatite effectively removes Pb from aqueous solution, exchange resins, and Pb contaminated soil material, and concluded that apatite has the ability to immobilize Pb in contaminated soils and wastes.

Tobacco growing, especially in developing countries where waste disposal procedures are not well developed and followed is done in contaminated soils. This results in tobacco products containing high levels of heavy metals such cadmium and lead. Use of cow manure in farming results in lower uptake of cadmium and lead due to its dilution effect as well as chelation, complexation, coprecipitation and adsorption between organic matter and cow manure [14]. However, hydroxyapatite (HA) has better reduction effieciency at high levels of about 5% HA than cow manure [19, 20]. The success of soil amendments immobilization is evaluated by their ability to reduce bioavailability of contaminants and their exposure to humans [21, 22]. The present study assessed the ability of soil amendments consisting of cow manure and hydroxyapatite, respectively, to reduce availability of lead and cadmium uptake by tobacco from contaminated soils.

2. MATERIALS AND METHODS

2.1 Preparation of field and pots experiments

The study was carried out in Migori County, Kenya using both field and pot experiments. In field experiment a farm was divided into plots of 1 m by 1 m, soil dug up to 20 cm deep, thoroughly loosened and dried for one day. One litre of water contaminated with cadmium and lead salts was evenly sprinkled to the soil which was then mixed thoroughly. The soil was allowed to dry for a one day, mixed and watered. The same process was repeated for two weeks to ensure that the cadmium and lead were homogenously mixed in the experimental soil. The soil was then amended to give six treatment plots, each in triplicates, as follows: (i) 2% cow manure (w/w), (ii) 1% cow manure (w/w), (iii) 1.5% HA, (iv) 0.75% HA, (v) fertilizer and (i) non-amended treatment (control). In the pot experiment, soil dug from the same farm was prepared in the same way and filled in 2-kg perforated plastic containers (200 cm diameter) to provide the six treatments in triplicates.

The field experiments were carried out in the open natural conditions and in each plot 9 tobacco seedlings were planted. Pot experiments were carried out in green house conditions and one tobacco seedling was planted in each pot. The tobacco seedlings, purchased from British American Tobacco (BAT) Company, Kenya, were regularly watered until maturity. Plant samples were harvested after 75 days of growth. Both, the field and pot samples, were analyzed in a randomized complete block design.

2.2 Laboratory procedures

Soil and tobacco leaf samples were obtained from field and pot experiments in triplicates. Soils samples were taken from a uniform depth of up to 20 cm in the field experiment and longitudinal in pot experiment based on the transient method [23]. Mature leaves ready for harvesting were sampled from the same place where soil samples were picked. The samples were then packed in labeled plastic bags and taken to the laboratory for analyses. The leaves were washed with tap water and distilled water before drying at 70°C for 2 weeks in the oven and then crushing into fine powder. The soil samples were also air dried for seven days and crushed into fine powder.

The soil and leave samples were digested in accordance with the procedure described by L'vov [24]. Specifically, a sample of 0.5 g of soil sample was digested with 5ml concentrated nitric acid at 100°C until brown fumes were observed, the concentrated hydrogen peroxide added to complete the digestion. After cooling, the samples were filtered into 100 ml volumetric flasks, made to the mark with distilled de-ionized water and stored at 4°C awaiting analysis by AAS (Perkin Elmer, model 560).

Soil organic matter content was determined using the ignition method where a sample was heated at 360°C for three hours in the muffle furnace, while electrical conductivity was determined based on Rhoades (1996) procedure. The soil pH was determined with a 1:10 soil-water suspension, after stirring and allowing it to stand for one hour [25]. Student's t-test was used to compare soil characteristics before and after contamination. Analysis of variance (ANOVA) was used to compare levels of Cd and Pb in tobacco leaves grown on differently amended soils.

3. RESULTS

3.1 Soil physicochemical properties

The levels of physicochemical characteristics, as well as cadmium and lead levels of before and after contamination of the soil used in field and pot experiments were determined and the results presented in **Table 1**.

The pH values of the contaminated and uncontaminated soils were slightly acidic and were not significantly different (P=0.59), implying that the contamination process did not change the mobility of H^+. Similarly, there was no significant difference between electrical conductivity of contaminated and uncontaminated soil (p=0.69), though contaminated soil had slightly higher value (1.02±0.14 dSm^{-1}) than uncontaminated soil (0.99±0.08 dSm^{-1}) due to increased salinity from added salts. The soil texture was sandy loamy with moderate organic matter content (14.93±1.72%), which was not significantly different from contaminated soil was (p=0.87). The soil contained cadmium levels of 4.87±0.14 μg/g and lead levels of 11.77±4.79 μg/g before contamination, but the levels increased to 33.95±0.49 μg/g for cadmium and 60.57±3.74 μg/g for lead after contamination. The levels were above the recommended concentration in soils for growing tobacco [20, 26].

Table 1. Mean levels (range) of physicochemical characteristics and metals of soils used (n=27)

Characteristic	Levels (Range) Before	After	P-value
Organic matter	14.93±1.72 (7.59-18.67)	13.89±1.85 (7.49-18.69)	0.87
Soil Ph	6.81±0.21 (6.65-6.92)	6.68±0.03 (6.69-6.90)	0.59
Electrical conductivity (dSm^{-1})	0.99±0.08 (0.90-1.15)	1.02±0.14 (0.88-1.14)	0.89
Concentration of cadmium (μg/g)	4.87±0.14 (2.6- 5.8)	33.95±0.49 (32.20-36.07)	<0.01
Concentration of lead (μg/g)	11.77±4.79 (8.1- 16.4)	60.57±3.74 (45.87-72.47	<0.01

Table 2. Cadmium and lead levels in tobacco leaves grown in field and pot experiments under various soil treatments (n=54)

Treatment	Mean Concentration (μg/g) Lead		P-value	Mean Concentration (μg/g) Cadmium		P-value
	Field	Pot		Field	Pot	
2% Cow manure	20.50 ± 0.20^{a} (10.60-22.40)	39.44 ± 0.82^{b} (23.60-44.40)	$1.33x10^{-24}$	5.98 ± 1.66^{a} (0.20-19.80)	6.81 ± 0.34^{a} (4.40-8.30)	0.584
1% Cow manure	24.38 ± 0.94^{b} (15.80-28.80)	40.61 ± 0.91^{b} (35.40-48.20)	$7.81x10^{-12}$	13.76 ± 2.52^{bc} (4.20-27.60)	8.62 ± 0.11^{b} (4.80-9.30)	0.026
1.5% HA	18.78 ± 1.20^{a} (8.40-31.20)	28.21 ± 1.04^{a} (23.60-36.60)	$1.02x10^{-4}$	9.92 ± 1.33^{ab} (0.60-24.00)	11.95 ± 0.57^{c} (7.4-14.30)	0.175
0.75% HA	26.84 ± 0.83^{bc} (20.80-32.20)	40.03 ± 0.11^{b} (39.00-41.00)	$2.57x10^{-19}$	7.48 ± 0.35^{a} (6.40-11.80)	14.07 ± 1.99^{c} (10.2-22.00)	$1.15x10^{-3}$
Non-amended	31.14 ± 1.23^{c} (25.20-40.80)	45.33 ± 0.27^{c} (42.80-47.80)	$6.82x10^{-13}$	18.24 ± 0.35^{c} (16.8-24.6)	24.14 ± 0.39^{d} (16.0-31.80)	$1.04x10^{-13}$
Fertilizer	38.28 ± 0.66^{d} (33.20-42.40)	48.85 ± 0.26^{d} (46.60-51.00)	$4.23x10^{-16}$	19.74 ± 0.14^{d} (17.0-25.00)	26.41 ± 0.19^{e} (20.6-33.60)	$6.35x10^{-31}$
p-value	<0.05	<0.05		<0.05	<0.05	

Mean values followed with same small letters within the same column are not significantly different at p =0.05 (SNK test)

3.2 Levels of cadmium and lead in tobacco leaves

The levels of lead and cadmium in tobacco leaves harvested from the field and pot experiments are shown in **Table 2**. The results indicate that although 1.5% hydroxyapatite (HA) treatment recorded the lowest uptake of lead in the field experiment it was not significantly different from levels of 2% cow manure 2 treatment ($p<0.05$), just as the levels in 1.5% and 0.75% (HA) treatments in pot experiments were not significantly different ($p>0.05$). There was no significance difference in lead uptake between 2% cow manure and 1.5% hydroxyapatite treatments in field experiments and between 2% and 1% cow manure in pot experiments, but significant difference was found between 2% and 1% cow manure, and between 1.5% and 0.75% HA treatments in field experiments. Fertilizer added treatment recorded the highest level of lead uptake in tobacco leaves that was significantly different ($p<0.05$) with all amendments in both field and pot experiments.

The same trend was seen with cadmium levels, where tobacco leaves harvested from 2% cow manure amendment in field experiment recorded the lowest mean levels followed by 1.5% HA amendment in both field and pot experiments, while leaves from fertilizer added contaminated soils had the highest mean levels. However, while cadmium levels were significantly different in 1% and 2% cow manure amendments in both field and pot experiment, it was not in 1.5% and 0.75% HA amendments, indicating that ameliorative effect for cadmium is more felt in cow manure than in HA, through adsorption sites, surface complexation and coprecipitation [27–29].

The results further show that the levels of metals were significantly different ($p<0.05$) in field and pot experiments, except for cadmium levels in 2% cow manure and 1.5% HA amendments. Generally, all lead and cadmium levels from pot amendment experiments were higher than those in field amendment experiments, except for cadmium in 1% cow manure amendment. The controlled experimental conditions in the pot studies may have contributed to the less ameliorative effect on cadmium [30].

Generally, cadmium had higher uptake reduction in all treatments as compared to lead. HA (1.5%) and cow manure (2%) amendments recorded the highest reduction of 39.67% and 34.16%, respectively, for lead in field experiments compared with the non-amended soil. Cadmium uptake was the most efficiently reduced, at 67.2% and 71.8% by 2% cow manure in field and pot experiments respectively, and followed by 1% manure at 64.3% and 0.75% HA at 59%. Lead showed higher reduction in field experiments, while cadmium showed higher reduction in pot experiments.

3.3 Percentage reduction of cadmium and lead uptake

Figure 1 shows lead and cadmium percentage reduction uptake for tobacco leaves grown in soil amendments in pot and field experiments relative to that in the non-amended soil levels. Generally, cadmium had higher uptake reduction in all treatments as compared to lead. HA (1.5%) and cow manure (2%) amendments recorded the highest reduction of 39.67% and 34.16%, respectively, for lead in field experiments compared with the non-amended soil. Cadmium uptake was the most efficiently reduced, at 67.2% and 71.8% by 2% cow manure in field and pot experiments respectively, and followed by 1% manure at 64.3% and 0.75% HA at 59%. Lead showed higher reduction in field experiments, while cadmium showed higher reduction in pot experiments. Fertilizer treatments had higher levels and thus gave negative reduction.

4. DISCUSSION

The results indicate that the amount of HA amendment in the soil affects the level of uptake of lead in tobacco leaves more than cow manure [29], while for cadmium it was more effective when HA concentration in the soil is low [27, 31]. Higher amounts of HA such as 5% would have produced the same effect as 2% cow manure in situations when cadmium levels are high [20, 32]. Reduced uptake of heavy metals such as lead and cadmium has been reported from various other similar studies [20, 29, 31, 33–35]. High levels of cow manure or HA reduce the uptake of metals to levels below maximum allowed levels in tobacco leaves and thus its products [29, 30, 36, 37].

The high amounts of amendments immobilize cadmium and lead in soil by shifting their available forms in the soil to forms that are associated with organic matter, carbonates or metal oxides, therefore rendering

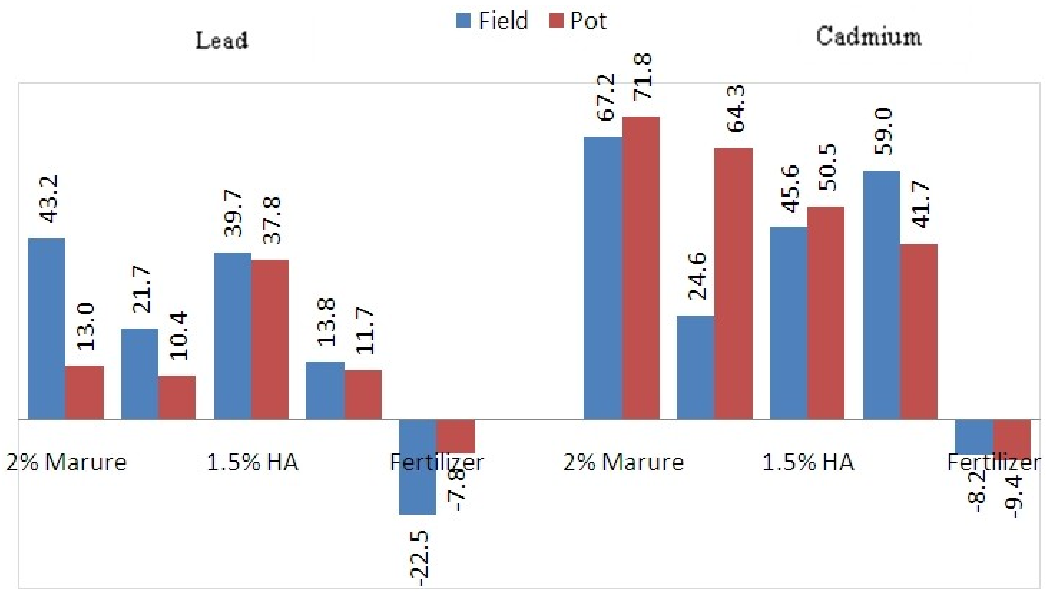

Figure 1. percentage reduction in lead and cadmium uptake in various field and pot soil treatments (n=162)

them unavailable for tobacco uptake [31]. Immobilization comes as a result of chelation, complexation, coprecipitation and adsorption between the the metal ions and the amendments. Hydroxyapatite and cow manure amendments not only create

an ameliorative effect due to increase in surface area and number of specific adsorption sites, but also a dilution effect to cadmium and lead contaminated soil [27, 29, 31, 35].

The levels of cadmium and lead in tobacco leaves harvested from fertilizer added contaminated soils was significantly higher compared to amended and non-amended soils ($p<0.05$). This may be explained by the fact that phosphatic fertilizers lower the soil pH, increase their electrical conductivity and reduce their organic matter content, thereby providing few binding sites for chelation, complexation, adsorption and sorption processes; resulting in cadmium and lead mobilization and availability for uptake [27, 29, 31, 38, 39]. Moreover, fertilizer contains heavy metals that may increase their levels in soil [7]. Cadmium has lower solubility and ability to leach especially at soil pH between 6-8 with light textured soils compared to lead and zinc [39], explaining why field experiments had reduced uptakes as compared to the pot experiments, while easily leached lead had higher reduction in field than pot experiments. These results also indicate that cadmium is more effectively reduced by the amendments than lead because of its high adsorption ability. However, fertilizer amendment increased cadmium uptake through lowering soil pH and thus mobilizing its uptake by tobacco [39, 40].

The results conclude that the cow manure and HA soil amendments reduced uptake of lead and cadmium in tobacco leaves to allowable limits. However, the amount of amendments added and the levels of metals in the soil determine the extent of uptake by tobacco. The 2% cow manure was most effective in reducing uptake of the metals in field and pot experiments, thereby guaranteeing safe tobacco products [41]. HA was most effective in reducing uptake of lead metal, though higher amounts would have produced similar effects as 2% cow manure. The study has shown that use of high amounts of cow manure and HA amendments are efficient in reducing cadmium and lead in tobacco leaves without compromising on productivity of tobacco plants. The amendments can be used in medially contaminated soils to diversify tobacco farming even to peri-urban areas to meet ever growing demand of tobacco that earns tobacco growing countries huge foreign exchange.

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