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Effects of Physico-Chemical Properties on Uptake of Lead and Cadmium in Tobacco Grown in Medially Polluted Soils

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Abstract:
Background and Aims
Tobacco is a heavy metal accumulator that uptakes Cd and Pb in large amounts depending on soil type and soil physico-chemical properties. Cadmium and lead are heavy metals of greatest concern because of their toxicity and cumulative nature. Tobacco bio-accumulates Cd and Pb incorporated in the soil as a result of excessive use of fertilizers and pesticides, irrigation with polluted water, contaminated mulches and polluted run-off that interacts with the soil. This study used six sets of experimental soils with different pH, EC, % OM to investigated influence of soil physico-chemical properties on uptake of Cd and Pb in tobacco.
Methods
Tobacco seedlings were transplanted to fields for growth till 75, 90 and 105 days for first, second and third harvest respectively. Harvested leaves were cleaned, dried, ground and acid digested before analyzed for Cd and Pb by AAS.
Results
The soil with lowest pH (6.63+0.01) recorded highest uptake of Cd (23.53 + 0.14) and Pb (38.28+0.17), while soil with highest % OM (soil 1, 17.17%, EC 1.38ds/m) had the lowest Cd and Pb concentration levels of 7.3 and 20.30 ugg^{-1} *respectively.*
Conclusions
Physico-chemical properties affects levels of Cd and Pb uptake in tobacco. The tobacco grown in amended soils is within WHO/FAO limits. The results suggest that heavy metals uptake in tobacco is reduced by soil amendments that alters soil physico-chemical properties indicating that tobacco can be grown in heavy metal polluted areas using soil amendments.

Keywords*: physico-chemical properties, bio-accumulates, Cadmium, lead, Tobacco soil amendments*

1. Introduction

Accumulation of heavy metals in soil is a matter of greatest concern in agricultural production of food in terms of its quality and safety (Zueng-sang et al. 2010). The industrialization has highly contributed to ever increasing levels of heavy metal load of soils worldwide (Simmons et al. 2005; Anita, 2011). Tobacco is among such crops that accumulates Cd and Pb in large amounts. Many factors influences absorption and accumulation of heavy metals in plants, some of which include soil physico-chemical properties, amount of heavy metals in soil, composition and intensity of atmospheric deposition like precipitations and phase of plant vegetation (NJF, 2005; Nouri et al. 2009). Other sources are the generation by agricultural technologies such as irrigation with wastewater, the administration of organic manure, mineral fertilizers and application of pesticides that contains Cd and Pb in their structure increasing heavy metal load in the soil (Hart et al. 2005; Prabu, 2009; Nwachukwu, 2008;Singh &, Agrawal, 2010; Dhulap and Chavan, 2013). The soil type is considered one of the most important factors that determine heavy metal content of food crops, probably because this is the binding and retention site for the toxicants (Nouri et al. 2009; Anita, 2011).
Tobacco and its products are known to contain heavy metals incorporated in the growing tobacco plant from such sources as the soil and soil mulches, fertilizers, pesticides and polluted rainfall (Rotkittikhun et al. 2007; Anita, 2011). The heavy metals are in turn inhaled by tobacco product users who end up having higher levels in blood streams than non-users (Keller et al. 2005). Since

there is increase in use of tobacco products in Kenya and globally, the demand of tobacco is increasing indicating optimism result for foreign exchange (Levy, 2006). But anti-tobacco crusaders are using the presence of heavy metals in tobacco products in threatening the promotion of tobacco growing due to not only the health effects from tobacco but also the effects of heavy metals such as lead and cadmium. The uptake of heavy metal by the tobacco plant minimization through soil physico-chemical properties by amending soil is a sure way of guaranteeing sustainable development of tobacco industry. A number of soil amendments both inorganic and organic, change soil physico-chemical properties of the soil such as soil pH, soil % OM and soil EC that influence immobilizing of heavy metals in contaminated soils. Some of the soil physico-chemical properties that have been variedly tested with success to reduce Cd and Pb availability to plants include soil pH, soil % OM and soil EC and CEC (Keller et al. 2005). Application of calcium carbonate–containing amendment increases soil pH, thereby significantly decreasing the solubility of heavy metals in contaminated soils (Liu et al. 2007; Zvinowanda et al. 2009) and increased soil EC, and all these combined to decrease the metal uptake by rice, wheat, tobacco and cabbage (Fawzy, 2008). Many reports indicated that sulfur-containing amendments and organic-waste application to contaminated soils lowered the concentration of soluble Cd and Pb in soils (Maria et al. 2008 Zarei &Savaghebi ,2011).

The influence of soil pH on heavy metal availability is related to its effect on the controlling reactions of heavy metal concentrations in the soil solution. Under acid conditions, sorption of heavy metal cations by soil colloids is at a minimum, and the solution concentrations are relatively high (Zarei andSavaghebi, 2011; Hao et al. 2013). As soil pH rises, sorption of heavy metal cations increases and the solubility of oxides decreases. The sorption of heavy metals that occur in anionic forms decreases with increasing soil pH, and hence solution concentrations and availability increase heavy metal concentrations soluble in soils are likely to be influenced to some extent by the total concentrations of heavy metals present in soils. The soils pH is the main factor affecting the mobility and bioavailability of heavy metals (Ismail et al. 2005). Most of the heavy metals are rendered less mobile and less available for plants uptake if the soil pH is kept near neutral or above by amending with cow manure. At these pH levels the heavy metals are generally bound to soil elements and structure hence they are not easily available for plant uptake. The lower the pH of the soil the smaller the adsorption of heavy metals is, making them more soluble. This is however not true for hexavalent chromium which is soluble in a wide range of pH conditions (Brady and Weil, 2002). Soils with generally high pH values are soils with high lime content. Low pH values are generally connected to large granulated soils, high amounts of precipitation and high contents of humus (Liqiang *et al*. 2012).

Organic matter makes strong complexes with heavy metals. Solid organic matter is for example able to hold metals in the solid phase of the soil. As organic materials influence the binding of heavy metals in soil, it may also affect plant uptake (Jinjun et al. 2013). Active solid organic matter is also quite effective in reducing the soluble and toxic hexavalent chromium (Cr^{6+}) to the more stable and less toxic form of trivalent chromium (Cr^{3+}) (Nwachukwu et al. 2010). However amendment with organic matter may change the soil pH to more acid and thereby indirect influence the bioavailability of metals to be more prone (Hao et al. 2013). In a study made by Hao et al. (1998) they found that metals that bound to the organic matter the first year it was applied to soil were probably released as the organic matter decomposed in the following years. They also found that for some metals such as copper and zinc, application of organic matter may even have increased their plant availability. In some soils such as peat soil the application of organic matter does not seem to reduce the plant availability of heavy metals.

The cow manure treatments had the highest EC as compared to other treatment which enabled it to be more efficient in reducing the uptake of cadmium and lead in tobacco leaves. On the other hand, non-amended control and fertilizer added had low EC that highly contributed to their lowest efficiency of these treatments in reducing uptake of cadmium and lead in tobacco leaves as they have few adsorption sites for exchange of heavy metals which are eventually immobilized. The 2% cow manure and 1% cow manure EC are significantly different indicating the higher amount of of the amendment added the higher the electrical conductivity of the soil as more binding sites are added to reduce uptake of cadmium and lead in tobacco leaaves. Simillar findings were found by Rattanawat et al. 2010 who reported that the addition of 10% and 20% cow manure resulted in much higher values of EC (3.2 and 4.1 dS m^{-1}, respectively) when compared with non-amended soil (EC 0.5 dS m^{-1}).

The soil electrical conductivity in the pot experimental contaminated amended and non-amended soil were determined potentiometrically and the average EC values recorded in Table 4.3. The table shows in the pot experiment the cow manure treatments had the highest EC as compared to other treatment which enabled it to be most effective in reducing the uptake of cadmium and lead in tobacco leaves. The 2% cow manure had the highest electrical conductivity that's reason it has the highest cadmium uptake reduction potential in tobacco leaves. On the other hand, non-amended control and fertilizer added had low EC that highly contributed to ineffective of these treatments in reducing uptake of cadmium and lead in tobacco leaves as they have few adsorption sites. The 2% cow manure and 1% cow manure EC are significantly different indicating the higher amount of of the amendment added the higher the EC of the soil as more binding sites are added to reduce uptake of Cd and Pb in tobacco leaves (Rattanawat et al. 2010).

This study sought to investigate effect of physico-chemical properties on uptake of Cd and Pb in tobacco.

2. Methodologies

2.1. Field Procedures

The study was conducted in Isebania, Migori county, Kenya with highly contaminated soils. A fieid of 1m by 1m was marked, soil dug upto 20 cm deep which was thoroughly loosened sprinkled with Cd/Pb contaminated water and dried for one day. Six such fields were made before triplicating the experiment. To give varied soil physico-chemical properties, experimental soil was treated as follows; soil1: soil + 1% cow manure, soil2; soil + 2% cow manure, soil3; soil + 0.75% HA, soil 4; soil + 1.5% HA, soil 5; soil without additive, soil 6; soil + 1kg fertilizer. In each soil thorough mixing, watering and drying every two days for a period of one month to obtain homogenous experimental soil. Tobacco seedlings one month old were transplanted to 1m by 1m fields. A total of 9 seedlings per field which was allowed to grow for 75, 90 and 105days before first, second and third harvests were made respectively. Harvested leaves were washed with distilled deionized water, oven dried, ground and acid digested before analyzed by AAS for Cd and Pb concentration in tobacco leaves.

2.2. Determination of Organic Matter Content

Soil was analyzed for organic matter content (OM) using loss on ignition (LOI) method at 360^oC for 3 hours in the muffle furnace (Maria et al. 2008). The equation used was:

$W_V = (m_b – m_c)$ x 100

$(m_b – m_c)$

Where

- W_v is the loss of ignition of the dry mass of the solid
- M_a is the mass of the empty crucible, in grams
- M_b is the mass of the crucible containing the dry mass, in grams
- M_c is the mass of the crucible containing the ignited dry mass, in grams

2.3. Determination of Soil pH and Electrical Conductivity

Soil pH was measured potentiometrically by an AB 15 Accumet [TM] basic pH meter in a suspension of a 1:10 soil: water mixture. About 10 g of air-dried soil (<2 mm) was weighed and transferred in to 100 ml beaker and 100 ml of water was added. The sample was stirred by a magnetic stirrer and the pH was measured after allowing the suspension to stand for 1 hour at room temperature (Zarei &Savaghebi, 2011). Each determination was made in triplicates. Similarly EC values (solid: distilled water = 1:10) of soil samples were measured using same procedure by an EC meter (Ekwumemgbo et al. 2013).

2.4. Laboratory Procedures

Soil and tobacco leaf samples were obtained from field and pot experiments in triplicates. For the field experiments, the soils samples were taken from a uniform depth of up to 20 cm based on the transient method (Mitei, 1992; Prabu, 2009). For the pot experiment soil samples were longitudinal taken on basis of transient method (Prabu, 2009), where 3 pots were picked for each treatment. The samples were collected in labeled plastic bags and then the above procedure was repeated. Mature leaves ready for harvesting were sampled from the same place where soil samples were picked. The leaves were washed with tap water and distilled water before drying at 70^0C for 2 weeks in the oven. The dry leaves were then being crushed in a Wiley mill into fine powder.

The soil and leave samples were digested in accordance with the procedure described by L'vov (2005). A sample of 0.5 g of soil sample was digested with 5ml concentrated nitric acid added first at 100^0C until brown fumes are observed before addition of hydrogen peroxide to complete the digestion. Digestion was done at 100^oC. After cooling, the samples were filtered into 100 ml volumetric flasks; filtrate was made to the mark with distilled water and then stored at 4^oC in freezer to await analysis by Perkin Elmer model 560 AAS at Mines and Geology department, Nairobi.Data was analyzed by ANOVA.

2.5. Data Analysis

Student's t-test was used to compare levels of Cd and Pb in soil samples. Analysis of variance (ANOVA) was used to compare levels of Cd and Pb in tobacco leaves grown on cow manure and hydroxyapatite (HA) amended soils as well as those grown without soil amendments. Correlation analysis was used to compare levels of Cd and Pb in the pot and field experiment as well as levels in soil and tobacco leaves.

3. Results and Discussions

3.1. Soil Physicochemical Properties

The main physicochemical characteristics such as soil texture, soil organic matter, soil pH and electrical conductivity of Cd/Pb contaminated soil in the field experiment were assessed to determine the physico-chemical factors that influenced the uptake of cadmium and lead in tobacco leaves. The soil texture was sandy loamy with moderate organic matter content. The soil contained high levels of Cd and Pb. The soil pH in the field and pot experimental contaminated amended and non-amended soil were determined potentiometrically the average soil pH values obtained are given in Table I.

Treatment	**Physicochemical property**		
	Soil pH	**EC (dS/m)**	**OM (%)**
2% cow manure	7.39±0.05[e] (7.19-7.55)	1.23±0.02[bc] (1.21-1.24)	17.17±1.02[a] (13.14-19.75)
1% cow manure	7.26±0.02[d] (7.17-7.37)	1.41±0.39[c] (1.14-1.91)	15.53±0.81[a] (13.48-18.74)
1.5% Hyroxyapatite	7.34±0.02[de] (7.27-7.43)	1.10±0.13[ab] (0.93-1.19)	15.74±1.86[a] (9.05-21.90)
0.75%	7.08±0.06[c]	0.86±0.40[a]	17.84±1.43[a]

Hydroxyapatite	(6.8-7.23)	(0.46-1.14)	(12.15-21.24)
Non-amended	6.81±0.03[b] (6.69-6.9)	0.89±0.14[a] (0.88-1.14)	14.89±1.85[a] (7.49-18.69)
Fertilizer added	6.43±0.02[a] (6.35-6.53)	0.88±0.02[a] (0.86 -0.90)	12.69±0.91[a] (10.42-18.69)
p-value	$2.41x10^{-24}$	2.793×10^{-07}	0.147

Table 1: Physicochemical properties of soil treated with various amendments (n=54).
Mean values followed with same small letters within the same column are not significantly different at p =0.05. (SNK test)

The soil pH is significantly different in all treatments because of the effect various amendments had on the soil (Table I). Soil amended with 2% cow manure had the highest pH of 7.19-7.55, this is because manure immobilized some of the H^+ ions making bioavailability of cadmium and lead by plants at high pH values reduce. This agrees with results obtained by Rattanawat et al. 2010 that showed that addition of 10% and 20% cow manure resulted in lowering pH values from 7.3 to 7.0–7.1.

The soil added with commercial fertilizer had the lowest pH values of 6.35-6.53 probably due to excessive of commercial fertiliser on the soil where tobacco was grown. Fertilizers have acidic effect on soils mobilising heavy metals like cadmium and lead. This is the reason why we have highest uptake levels of cadmium and lead in this soil treatment although, non-amended soils had low pH value, addition of fertiliser to contaminated soil had an effect on lowering soil pH further. The hydoxyapatite treatment had high pH because of liming effect of burned bones that acted as hydroxyapatites, 1.5% HA had a pH range of 7.27-7.43 that probably reduces uptake of cadmium and lead in tobacco leaves. They are usually effective in reducing uptake of lead (Keller et al. 2005; Francisco et al. 2006).

The 2% cow manure and 1% cow manure treatments had the highest EC as compared to other treatment which enabled it to be more efficient in reducing the uptake of cadmium and lead in tobacco leaves. On the other hand, non-amended soil (control) and fertilizer added soil had low EC that highly contributed to their lowest efficiency of these soils in reducing uptake of cadmium and lead in tobacco leaves as they have few adsorption sites for exchange of heavy metals which are eventually immobilized (Francisco et al. 2006; Khan & Jones, 2009). The 2% cow manure and 1% cow manure treatments' EC are significantly different indicating the higher amount of of the amendment added the higher the electrical conductivity of the soil as more binding sites are added to reduce uptake of cadmium and lead in tobacco leaaves. Simillar findings were found by Rattanawat et al. 2010 who reported that the addition of 10% and 20% cow manure amendments resulted in much higher values of EC (3.2 and 4.1 dS m^{-1}, respectively) when compared with non-amended soil (EC 0.5 dS m^{-1}).

In the field experiment the organic content between treatments were not significantly differently (p=0.147). However, organic matter was different in various treatments, with the 0.75% HA amended soil having the highest organic matter content, followed 1% cow manure amended soil. The fertilizer added and non-amended (control) had the lowest organic matter. Low organic matter influences high uptake of cadmium and lead in tobacco leaves since there were less adsorption sites for the heavy metals (Khan & Jones, 2008). Related results have been realized by several studies on low uptake of cadmium amended with 2% cow manure due to the fact that organic amendments can decrease heavy metal bioavailability, shifting them from plant available forms to fractions associated with organic matter, carbonates, or metal oxides (Mahmood, 2010). Additionally, organic matter amendments to metal contaminated soils can have an ameliorative effect due to increased surface area and an increase in the number of specific adsorption sites (Singh & Agrawal, 2010) and also the dilution effect when they are mixed with metal contaminated soil (Chiu et al. 2006; Khan & Jones, 2009; Ekwumemgbo et al. 2013).

3.2. Levels of Cd and Pb in tobacco leaves from Field and Pot experiment

The concentration of cadmium and lead in tobacco leaves harvested from experimental contaminated soil in amended and non-amended soil were determined using atomic absorption spectroscopy technique and the mean concentration are recorded in Table II.

Treatment	**Mean Concentration (µg/g)**			
	Lead		**Cadmium**	
	Field	**Pot**	**Field**	**Pot**
2% cow manure	20.79±0.43[b] (15.20--27.20)	21.79±1.45[b] (6.00-44.40)	8.30±0.62[a] (0.20-19.80)	5.37±0.31[a] (1.40-10.30)
1% cow manure	23.55±0.49[c] (16.90-30.80)	21.79±1.57[b] (7.20-48.20)	12.33±0.79[b] (5.20-27.60)	8.20±0.27[a] (4.30-11.90)
1.5% Hyroxyapatite	14.15±0.69[a] (3.60-31.20)	13.03±1.27[a] (1.20-36.60)	11.92±0.61[b] (0.60-24.00)	11.29±0.46[b] (6.20-18.60)
0.75% Hydroxyapatite	19.88±0.94[b] (1.00-32.20)	20.83±1.5[b] (7.30-41.00)	8.47±0.33[a] (1.20-12.80)	22.05±1.23[c] (9.40-42.00)
Non-amended	31.53±0.50[d] (25.20-40.80)	33.92±1.11[c] (9.80-4780)	18.19±0.46[c] (9.40-24.60)	30.52±1.57[d] (14.30-51.80)
Fertilizer added	38.20±0.27[e] (33.20-42.40)	36.43±1.14[c] (22.60-51.00)	20.52±0.39[d] (15.10-25.00)	32.38±1.65[d] (15.10-53.60)

p-value	<0.001	<0.001	<0.001	<0.001

Table 2: Cd and Pb levels in tobacco leaves grown in soils under various amendments (n=162).

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

As can be seen in Table II, levels of Cd for pot experiment were significantly different with $p<0.05$) for different treatments, 2% cow manure tobacco leaves recorded the lowest mean concentration levels of 8.23 ± 0.34 $\mu g g^{-1}$, while leaves from fertilizer added Cd/Pb soils had the highest mean concentration levels of cadmium of 31.89 ± 1.19 $\mu g g^{-1}$ as shown by Table II. There was no significance difference in cadmium concentration in pot experiment between 1% cow manure and 2% cow manure amended soils, although 2% cow manure treatment had lower mean concentration of cadmium. This was because the 2% cow manure amendment was not enough to immobilize 70.86 $\mu g g^{-1}$ cadmium in the soil to a significantly different concentration level as compared to 1% cow manure which had only 42.11 $\mu g g^{-1}$ cadmium. However there was a significant difference ($p<0.05$) for 1.5% HA and 0.75% HA treatment, indicating that hydroxyapatite amending affects the uptake of cadmium in tobacco leaves. There was significant difference between contaminated non-amended and fertilizer added Cd/Pb amended soil ($p=1.69x10^{-5}$). However fertilizer added treatment resulted in higher concentration of cadmium because the added fertilizer was source of Cd and Pb and it also lowered soil pH leading to higher uptake in tobacco. Experimental and environmental conditions in the pot experiment are controlled unlike the field experiment. Hence the uptake of cadmium in tobacco leaves is not significantly different as fertiliser added did not affects much the factors that mobilizes the cadmium as mobility increases between soil pH 6-7 (Pidwirny, 2006; Francisco et al. 2006).

The 2% cow manure treatment produced tobacco leaves that was within limit reported in Kenyan tobacco by Mitei (1996) and Wangoli (2007). The low uptake of cadmium in soil treated with 2% cow manure is due to the fact that the amendment provides more adsorption sites for cadmium and lead, surface complexation and coprecipitation (Chiu et al. 2006; Mohammad, 2012). The highest mean concentration levels of cadmium and lead in tobacco were recorded in the soil 6 that was fertilizer amended soils because fertilizer reduces soil pH, increases electrical conductivity and reduces the % organic matter content thereby providing few binding sites for chelation, complexation, adsorption and sorption of cadmium (Tomov & Kalinka, 2006). The use of cow manure amendment in relatively higher amounts reduces the uptake of cadmium to levels allowed by WHO/FAO in tobacco products (Clemante et al. 2005; Keller et al. 2005; Chiu et al. 2006; Pitchel and Bradway, 2008; Singh & Agrawal, 2010; Zarei andSavaghebi, 2011; Hao et al. 2013). This is because when higher amounts of cow manure are applied to cadmium/lead contaminated soil its sufficient to immobilize cadmium and lead by providing more adsorption sites and increases soil pH thereby reducing uptake by tobacco (keller et al. 2005).

Cadmium levels between field and pot experiment in all soils except 2% cow manure and 1.5% HA were significantly different with field tobacco having lower levels as compared with potted plants grown in respective treatments. This is because field tobacco immobilized cadmium more effectively than pot reducing uptake. However cadmium levels between field and pot tobacco for soil 2% cow manure treatment ($p=0.584$) and 1.5% HA ($p=0.175$) treatment was not significantly different. This is because the cadmium was efficiently adsorbed by the amendment to minimize uptake in field tobacco. Comparison between pot and field experiments using ANOVA indicates that concentration levels of cadmium showed a significant difference ($p<0.05$) as shown by Table II. This is because has been observed to be more mobile heavy metal especially at soil pH between 6-8 with light textured soils, when compared to lead and zinc. Therefore, if experimental conditions are not well controlled like in the field experiment leaching, erosion and high mobility of cadmium at low soil pH is likely to cause a significant difference when compared to pot experiment (Pidwirny, 2006; Chiu et al. 2006; Hao et al. 2013).

In the field experiment, the lowest cadmium concentration in tobacco leaves for soil treated with 2% cow manure, 1% cow manure and 1.5% HA treatments were 0.20, 0.60 and 1.20 $\mu g\ g^{-1}$ respectively which are within recommended concentration levels of cadmium in tobacco. Similary, in the pot experiment, the lowest cadmium concentration in tobacco leaves for 2% cow manure and 1% cow manure treaments were 4.40 and 4.80 $\mu g\ g^{-1}$ in respective amendments which are within allowed concentration levels of cadmium in tobacco leaves of 0.5-5 $\mu g\ g^{-1}$ (Wangoli, 2007; Lucien et al. 2012).

Comparison between pot and field experiments using ANOVA test indicated that there was no significant difference for the concentration levels of lead in tobacco leaves as $p<0.05$ (Table II). This is because lead metal is as mobile as cadmium when the soil pH is slightly lowered to 6-7 although cadmium is more mobile. Research done by Pidwirny (2006) pointed out that cadmium was observed to be the most mobile element and was leached to a depth of 30-40 mm in a soil pH of 7.5, light textured soil when compared with lead and zinc. There was significance difference between pot and field experiments, indicating that concentrations levels of lead were affected by environmental variable factors predominant in the field experiment like lead leaching, soil erosion by water and wind, aeration, temperatures, moisture content, competing with other tobacco plants for nutrients and light (Pidwirny, 2006).

The cow manure could have been better in reducing uptake of lead in tobacco leaves in both pot and field experiment if it could have been applied in relatively higher amounts than 2% cow manure such as 5% cow manure, 10% cow manure and 20% cow manure treatments. Several studies have demonstrated the efficiency of this amounts of cow manure in reducing heavy metals like lead accumulation in plants (Clemente et al. 2005; keller et al. 2005; Pitchel and Bradway, 2008; Liqiang et al. 2012). However if higher application rates of amendments is done some of the elements like copper and zinc are immobilized which are essential micronutrients in cellular metabolism and serve as structural and catalytic components of proteins and enzymes which eventually affects productivity of tobacco (Lasat, 2002 and Nwachukwu et al. 2010).

3.3. Effect of amendments on percentage metal uptake in tobacco

Based on mean concentration of cadmium and lead in the tobacco leaves harvested after 75 days, percentage reduction on metal uptake in the field and pot experiment were calculated as given in Table III.

Treatment	% Reduction in metal uptake			
	Lead		Cadmium	
	Field	Pot	Field	Pot
2% manure	34.16	12.99	67.20	71.75
1% manure	21.71	10.41	24.56	64.34
1.5% Hydroxyapatatite	39.69	37.77	45.63	50.48
0.75% Hydroxyapatatite	13.81	11.69	58.97	41.72
Non-amended	0.00	0.00	0.00	0.00
Fertilizer added	-22.48	-7.77	-8.23	-9.43

Table 3: Percentage reduction in metal uptake in various amendments (n=162)

Field lead concentration levels in tobacco harvested from soil 1.5% HA treated soil recorded the highest reduction of 39.67% as compared with the non-amended control followed by 2% cow manure amended soil with 34.16% lead uptake reduction. These results indicate that soil 1.5% HA amendment was most effective in reducing lead uptake in tobacco by providing many binding sites for lead adsorption. However tobacco harvested in fertilizer added soil had increased lead levels of 22.48% as compared to the control. Similarly 1.5% HA reduced lead uptake in the pot experiment by 37.77% while tobacco harvested from fertilizer added soils increased lead uptake by 7.77% as compared to non-amended soil (control).

Cadmium uptake in field was most efficiently reduced by 2% cow manure by 67.20% followed by 0.75% cow manure with 58.97% cadmium uptake reduction. However fertilizer amendment increased cadmium uptake by 8.23% as soil pH was lowered mobilizing cadmium to be readily taken by tobacco (Pidwirny, 2006). In pot experiment 2% cow manure reduced cadmium uptake by 71.75% followed by 1% cow manure that reduced cadmium uptake by 64.34%. However fertilizer added increased cadmium uptake by 9.43% as it lowers soil pH mobilizing cadmium to increase its uptake in tobacco.

The percentage reduction of Pb in 2% cow manure treatment in the field experiment was 34.16% while reduction by same treatment in pot experiment was 12.99%. But beyond expectations field Cd percentage reduction was lower (67.20%) than percentage Cd reduction in pot experiment (71.75%) as shown in Table III. This is because cadmium has lower solubility and leaching at soil pH of 7-8. This is because Cd been observed to be more mobile heavy metal especially at soil pH between 6-8 with light textured soils, when compared to lead and zinc. Research done by Pidwirny (2006) pointed out that cadmium was observed to be the most mobile element and was leached to a depth of 30-40 mm in a soil pH of 7.5, in light textured soil when compared with lead and zinc Hence no much Cd was leached accounting for field experiment having lower percentage Cd reduction in field as compared to the pot experiment which in case of easily leached Pb to account for its higher % reduction in field lead than potted plants (Pidwirny, 2006; Freitas et al. 2007).

4. Conclussion

The results of the study affirm the need to grow tobacco in medially polluted soils for tobacco diversification to meet its ever growing demand. The study focused to determine whether the soil physicochemical characteristics like soil pH, electrical conductivity and percentage organic matter of the experimental soil correlates with uptake of cadmium and lead in tobacco leaves. The results indicate that this soil physicochemical characteristic correlates with the uptake of cadmium and lead in tobacco leaves. Soil pH had negative correlation in the uptake of cadmium and lead in tobacco leaves that's the lower soil pH the more Cd^{2+} and Pb^{2+} are mobilized hence higher uptake in tobacco leaves. However, addition of soil amendments increases soil pH that immobilizes cadmium and lead to reduce their uptake in tobacco leaves. The results indicates the higher the electrical conductivity the lower the uptake of cadmium and lead in tobacco leaves because at higher EC there are more adsorptions sites for exchange of Cd and Pb which are eventually immobilized leading to low uptake by tobacco leaves. Cow manure and hydroxyapatite amended soil increases EC which influences low uptake of cadmium and lead in tobacco leaves. Similarly, the results indicate higher percentage organic matter decreases uptake of Cd and Pb in tobacco leaves because of increase in adsorption sites of Cd^{2+} and Pb^{2+} ions. It may also be due to surface complexation and coprecipitation of these ions to reduce their uptake in tobacco (Shah et al. 2008).

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