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Effects On Nanaofertilizers On Cd AND Pb Uptake In Kales Grown In Medially Polluted Experimental Soil.

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Abstract:
***Background**: Most agriculturalists use excess commercial fertilizers with the notion that some will be eroded and leached eventually causing eutrophication and alters soil pH that increases Cd and Pb uptake in crops .Nanoparticles have numerous adsorption sites that reduce Cd and Pb uptake in crops and it has controlled release of nutrients guaranteeing longer interaction with the crops to ensure safer and higher crop yields to feed sporadic growing population.*

***Materials and Methods**: This study uses nanohydroxyapatite adsorbents from waste bones to synthesis nanofertilizers by encapsulating with DAP and NPK commercial fertilizers. Pots study in green house for contaminated soils were conducted with eight treatments in triplicate; DAP added, NPK added, NPK-DAP added, DAP nanofertilizer, NPK nanofertilizer, NPK-DAP nanofertilizer and non-treated control (ck) treatments, for sukuma wiki (kales) in Kitutu Chache south sub-county, Kisii County, Kenya. Nine pots were set for each treatment with one seedling each planted per pot. Nanofertilizers were applied at normal application rates during planting. Crops were left grown for 30 days to 90 days until they matured and harvested three times on a monthly basis. Harvested leaves were washed with distilled deionized water, oven dried and then acid digested and then analyzed by AAS for Cd and Pb. Data obtained was analyzed by ANOVA and t-test to compare means of various treatment, Cd and Pb in contaminated soils.*

***Results**: The nanofertilizers treatment recorded lowest levels of Cd and Pb in sukuma wiki harvested leaves. The bone ash (nHA added) had the lowest concentration levels of Cd (18.14±0.29) and Pb (15.91±0.57) with NPK nanofertilizers also recording lower concentration levels of Cd (27.29±0.50) and Pb (21.59±0.74). The DAP added fertilizers recorded highest concentration levels of Pb (56.74±5.75) while NPK added fertilizers recorded highest concentration levels of Cd (53.46±2.55). The bone nanofertilizer grown sukuma wiki had higher growth performance and lower levels of Cd and Pb to the permissible levels allowed by WHO/FAO in Kenyan vegetables. The bone ash nano treatment posted the lower heavy metals concentration levels of Cd (18.14±0.29) and Pb (15.91±0.57) while in synthetic experiments leaves harvested from synthetic ash nanofertilizer treated pots had Cd (23.71±1.92) and Pb (14.60±0.30). Cadmium concentration levels in kales harvested from bone ash recorded the highest reduction of 60.69% as compared with the non-treated control followed by bone NPK nanofertilizer with 30.70% cadmium uptake reduction. Lead uptake in bone set-up was most efficiently reduced by bone ash (nHA) by 59.25% while reduction by same treatment in synthetic experiment was 58.71%. The bone NPK nanofertilizer had 44.70% lead uptake reduction. However fertilizer amendment increased lead uptake with DAP added treatment hiking lead uptake in kales by 14.04%.*

***Conclusion:** The result is recommended for nanofertilizers to be used in growing other crops such as maize, beans, spinach potatoes, wheat, rice and other vegetative crops in medially polluted soils, especially in peri-urban farming to boost food productivity to feed ever growing population.*

***Key Word**: Nanofertilizer; Amendment; Uptake; Nanohydroxyapatite; Bone Ash.*

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I. Introduction

Due to an exponential increase in the use of heavy metals such as lead and cadmium in several industrial processes and products, humans' exposure to heavy metals has risen sporadically in the last half of the century. Heavy metals exposure to the human body is through food, water, air and skin absorption (Ngorwe *et al*., 2014; Zhang *et al*., 2011; Sheykhbaglou *et al*., 2010). However, acute exposure has been reported through mercury amalgam dental filling, lead in paint and tap water through lead pipes, chemical residues in processed foods, and personal care beauty products. Consequently, toxic heavy metals damages and reduces functioning of the central nervous system, lower availability of biological energy, and cause damage in lungs, kidneys, liver, blood composition and other essential body organs (Rezvani *et al*., 2012; Zhang *et al*., 2011; Nwachukwu, 2008). But, long-term exposure to heavy metals may result in chronic physical, muscular, and neurological degenerative processes such as mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple selerosis (Ngorwe *et al*., 2014; Song *et al*., 2011; Duruibe *et al*., 2007;). Also, toxic heavy metals can increase allergic reactions, cause genetic mutations, compete with essential trace metals for chemical binding sites, and acts as a broad spectrum antibiotics against both harmful and beneficial bacterial (Zhang *et al*., 2011; Chanda *et al*., 2011). Heavy metal toxicity and the danger of their bioaccumulation in the food chain pose a very dangerous health threats in the 21^{st} century generation (Ramesh *et al*., 2014).

Accumulation of heavy metals such as Cd and Pb in plants is particularly dangerous since plants and vegetables are at the bottom line of the food chain and are heavily consumed by animals and humans. When consuming the vegetables contaminated with heavy metals it has different detrimental effects on human health, this makes food safety in 21^{st} century a global problem. Therefore, monitoring contamination of heavy metals especially in plants that are used as food crops will alleviate unnecessary exposures of the heavy metals to humans (Keller *et al*., 2005).

Considering the health side effects of consuming contaminated vegetables by the public, it is necessary for responsible scientists and authorities to device proper measures, especially on the sites which are highly polluted with heavy metals especially Cd and Pb. In Kenya crops that are grown in soils highly polluted with heavy metal, especially those irrigated using sewage sludge have prompted public protests and uproots (Daily Nation, 2010). Crops grown in heavy metal contaminated soils bioaccumulates higher concentration levels of toxic heavy metals such as cadmium (Cd) and lead (Pb) thereby threatening food safety. The Cd and Pb are two heavy metals are of greatest concern due to their toxicity and cumulative nature in animals and humans consuming plants especially vegetables such as sukuma wiki (kales). Kales are some of the vegetables which accumulate heavy metals, thereby find its way to animal and human food chain to cause cancer and other diseases (Ma *et al*., 2010).

In many countries Kenya included, vegetable farming is an instrument of high importance in financial and economic terms (FAO, 2015). Kale is among the few crops harvested on leaf basis to earn the several people a living and improve the standard of living of small scale business persons (Zhang *et al*., 2011). However uptake of heavy metals by plants especially vegetables threatens food safety and such businesses. Scientists have used adsorbents to reduce heavy metal uptake in plants for many centuries. The uptake of heavy metal by the plants especially vegetables can be minimized through soil adsorbents. Various soil adsorbents, both inorganic and organic, have been used to immobilize heavy metals in polluted soils. Inorganic adsorbent materials that have been tested with success to reduce Cd availability to plants include rock phosphate, apatite, hydroxyapatite (HA), iron and manganese oxides and oxyhydroxides, and other liming agents (Keller *et al*., 2005). Similarly, organic ameliorates such as farmyard manure, cow manure and composites decrease bioavailability of heavy metals in soil and crops (Du *et al*., 2019; Ngorwe *et al*., 2013; Rattanawat *et al*., 2010). This adsorbent are low cost materials that add crop nutrients and improve soil water holding capacity and also helps in reducing heavy metal toxicity resulting in the increase in plant growth performance and survival (Du *et al*., 2019). Several studies have demonstrated the efficiency hydroxyapatite (HA) in not only reducing heavy metal uptake in plants but also boosting plant yields (Musico *et al*., 2013; Kumar *et al*., 2013; Rattanawat *et al*., 2011; Keller *et al.,* 2005). They are very effective in decreasing the metal bioavailability due to the introduction of addition binding sites for heavy metals and due to the pH effects (Helaly *et al*., 2014). Many of these amendments are by- products of industrial activities and therefore cheap and available in large amounts. Lime related adsorbents have been used for centuries to increase pH and decrease heavy metal uptake bioavailability and eventual uptake by crops (Kumar *et al*., 2013). Cadmium absorptive capacity of soil increases by a factor of three to one, when pH increases from 4 to 7.7. However, adding too much lime to the soil can lead to the immobilization of essential nutrients and the mobilization of harmful anions. (Li *et al*., 2012; Atlabachew *et al*., 2010).

However these adsorbents are even more effective in reducing uptake of Cd and Pb heavy metals with down scaling them into nanoparticles. The nanoparticles are encapsulated with NPK and DAP fertilizers to form nanofertilizers that have desired qualities such as increased affinity, adsorption capacity, and selectivity for heavy metals and other contaminants. (Kumar *et al*., 2013; Montazeri *et al*., 2010). Nanoparticles in nanofertilizers have become attractive as adsorbent materials because they have larger surface areas than traditional bulk particles. Moreover, some nanoparticles can be functionalized with various chemical groups to increase their affinity towards target compounds (Lahiani *et al*., 2013; Li *et al*., 2012; Song *et al*., 2011). These unique properties of nanoparticles have been recently exploited by several researchers to develop higher capacity and selective adsorbent for metal ion in making nanofertilizers (Nilwala *et al*., 2011; Montazeri *et al*., 2010; Ma *et al*., 2010). For example, carbon nano-tubes combine unique features of graphene-based materials and polymer materials to be one of the most promising and recent technological developments in one nano layered material (Ma *et al*., 2010). These nanoparticle materials show considerable improvement in properties that cannot normally be achieved using conventional fertilizer materials and polymers (Mahajan *et al*., 2011; Poinem *et al*., 2009). For instance, nanohydroxyapatites are very promising, since it has different ways of fabrication and dispersion. Hence if used to make nanofertilizers is likely to reduce heavy metal uptake as the metal ions are adsorbed and immobilized as noted by other several studies (Nadia *et al*, 2018 ;Musico *et al*., 2013; De la Rosa *et al*., 2013 and Lee *et al*., 2012;).

Excessive use of commercial fertilizers by farmers with the notion that some will be leached, lowers the soil pH thereby mobilizing toxic heavy metals such cadmium and lead increasing their uptake in crops to threaten food safety. It's only the adsorbents like zeolites, oxyapatites, composites, apatites, and hydroxyapatites that can used for to immobilize such heavy metals (Ngorwe *et al*., 2014; Khodakovskaya *et al*., 2013). Nanotechnology involves the design, characterization, production and application of structures, devices and systems by regulating the shape and size at nanometer scale (Helaly *et al*., 2014; Chinnamuthu and Boopathi, 2009). Nanoparticles have unique properties such as numerous adsorption sites and selective adsorption towards some substances. Nanoparticles unique properties have been found desirable for use in medicine for slow and controlled delivery of drugs to patients. Bone nanoparticles have been specifically hypothesized to be suitable than synthetic nano particles of hydroxyapatites by chemical precipitation for more controlled release of drugs to patients (Ramesh *et al*., 2014). Hence it can be used for slow and gradual release of nutrients to crops. Nowadays, it is practically used in the development of slow and gradual release of nutrient fertilizers, conditional release of pesticides and herbicides, making nanotechnology strategies to become critically important in promoting the development on environmentally friendly and sustainable agricultural devices (Raliya *et al*., 2015; Rai *et al*., 2012).

Nanoparticles have numerous adsorption sites that immobilize heavy metals like Cd and Pb thereby reducing uptake in crops while their controlled release of nutrients has longer interaction with crops to ensure higher crop yields to feed the sporadic ever growing population. But for commercial fertilizers they have higher solubility and higher release of nutrients to crops. Hence commercial fertilizer is not efficiently used by crops leading to poorer produce heightening the food crisis and lowering crop yields and quality (Juhel *et al*., 2011; Cui *et al*., 2010). This study made use of waste bone materials from streets that were otherwise regarded as an environmental menace to synthesize nanofertilizers which will not only reduce heavy metal uptake in crops but also minimize change of soil pH, reduce fertilizer wastage and reduce eventual heavy metal in crops. (Prasad *et al*., 2014; Srinivasan *et al*., 2010; Jaggard *et al*., 2010).

II. Material And Methods

Experimental design

Completely randomized block design was used in the study to grow kales. For this kales there were two set-ups each with eight treatments in triplicates. In the first set-up nanofertilizer treated pots were amended with synthetic nanoparticle, while set-up II experiments for kales, bone made nanofertilizers amendments was used. The experimental soil that was used was obtained 5m underground to ensure it had scarcity of nutrients to give a good control set-up. The experimental soil was artificially polluted with Cd/Pb by sprinkling with polluted water to concentration levels that are allowable by NEMA and then thoroughly mixed for homogenous distribution of Cd and Pb. In each set-up of kales eight treatments were set as follows; the non-treated control (CK), DAP added treatment, NPK added treatment, DAP-NPK added treatment, synthetic nanohydroxyapatite added treatment (nHA), DAP added nanofertilizer (nHA+DAP), NPK added nanofertilizer (nHA+NPK), DAP-NPK added nanofertilizer (nHA +DAP+NPK) respectively. The experiment was repeated using bone made nanoparticle fertilizers. All the commercial fertilizers and nanofertilizers were applied by basal dispersal one day before planting the seedlings and the soils were harrowed immediately into 5 cm deep soil layer.

The kale was transplanted into the treated pots with each seedling per pot. The kale vegetables were left to grow to maturity to a period of between one and three months (30-90 days) and then harvesting was done on monthly basis that's 30 days, 60 days and 90 days and then left to grow to full maturity. The Cd and Pb concentration levels were determined in the harvested leaves.

Preparation and characterization of hydroxyapatite nanofertilizers

The procedure described by Mateus *et al*. 2007 was used to chemically precipitated synthetic nanohydroxyapatites using aqueous solution of 2M calcium hydroxide $Ca(OH)_2$ and 0.6 M phosphoric acid (H_3PO_4). To $100cm^3$ of 2M calcium hydroxide solution in a conical flask 0.6M phosphoric acid (H_3PO_4) was added from the burette dropwise at different rates to vary the size of synthesized nanoparticles, while stirring vigorously under mechanical agitation of 1000 rpm for a period of 48 hours. The resulting synthetic nHA nanoparticles was thrice rinsed and washed with double distilled deionized water to remove excess phosphoric acid. The precipitated synthetic nanohydroxyapatites particles obtained were then oven dried at temperatures of $100\ ^0C$ for period of 6 hours.

The nanofertilizers were synthesized by dispersing synthetic nanoparticles in filtered saturated solutions of commercial NPK and DAP under ultrasonic mixing of 30 kHz for 2 hour. Then the resulting nanohydroxyapatites were dispersed and stirred in a saturated DAP-NPK solution at room temperature for 12 hours. Decantation was done after the resultant mixture was allowed to settle. The product was thrice washed with distilled deionized water to remove any loosely held NPK or DAP fertilizer and then resultant nanofertilizers was dried at 50 ^{0}C for a period of 8 hours.

For the bone nanoparticles, the bones were collected from streets and butcheries and any loose foreign tissues were physically removed, washed, dried and then cut into small pieces by grinder then the ground bones were put into the muffle furnace set at 600 ^{0}C for a period of 24 hours to obtain bone ash. The bone ash was ground into very fine sized particles, then it was mixed with water at a w/w ratio of 1:1 to form bone suspension which was then put into a vibratory miller for a period of 24 hours before filtering to get filter cakes. The filter cakes were oven dried at 100 ^{0}C for 6 hours then they were ground using mortar and pestle and they were then filtered to get nano sized particles. The bone made nanoparticles was used to make nanofertilizers using the same described procedure by Mateus *et al*. 2007 above.

Pot experiment

The greenhouse pot study was conducted at Nyakoe found at Kitutu Chache South sub-county, Kisii County, using the experimental soil that was collected, medially contaminated with Cd/Pb, air dried and then ground before being sieved to pass through 2 mm nylon mesh sieve. Pot experiments were conducted with eight levels of treatments as mentioned above. Three replicates were set in synthetic grown kales and bone grown kale plants. Single seedlings of kales were planted per pot and left to grow until maturity was reached with constant watering of thrice a week. The plant samples were harvested on monthly basis that's 30 days, 60 days and 90 days of growth, oven dried at temperature of 70 ^{0}C for 12 hours, then its ground before wet acid digested. The digested samples were analyzed by AAS.

Sample digestion

The soil, leave and seed samples were digested in accordance to the procedures described by Van Loon (Duruibe, 2007; L'vov, 2005). The digestions were done on accurately weighed 1.0 g soil samples which were digested with 70% $HClO_4$ acid in the ratio of 2:1 respectively. The excess nitric acid was used to avoid explosion of perchloric acid. The digestion was done at temperatures of 100 ^{0}C. After digestion, the samples were cooled and then diluted with 6% nitric acid. Then it was filtered into 50ml volumetric flask which was topped to the mark using distilled water. Then the digested samples were stored at 0 ^{0}C in a refrigerator before analyzing by AAS.

Data analysis

Student's t-test was used to compare concentration levels of Cd and Pb in soil samples. Analysis of variance (ANOVA) was used to compare concentration levels of Cd and Pb in kales leaves and other plant parts grown on various treatments as well as those grown without any soil treatments. Correlation analysis was used to compare concentration level of Cd and Pb in the polluted and unpolluted soil as well as concentration levels in soil and plant leaves.

III. Result

3.1 Levels of Cd and Pb in sukuma wiki leaves from synthetic and bone experiment

The concentration levels of cadmium and lead in sukuma wiki leaves harvested after first 90 days of growth in synthetic and bone experiment were statistically determined and compared using ANOVA and recorded in Table 1.

Table 1: Pb concentration levels for Sukuma wiki for the three harvests in Synthetic and bone experiments

TREATMENT	Synthetic Pb concentration levels			P-value	Bone Pb concentration levels			P-Value
	Harvest 1	Harvest 2	Harvest 3		Harvest 1	Harvest 2	Harvest 3	
Non-treated control	71.16 ± 1.61^{d} (62.72-77.88)	45.81 ± 2.78^{d} (33.4-55.32)	35.36 ± 2.16^{d} (25.69-42.55)	P<0.05	82.37 ± 1.15^{e} (75.12-87.07)	62.93 ± 0.76^{e} (57.78-65.21)	49.043 ± 0.76^{e} (44.45-52.1)	P<0.05
DAP Added	74.00 ± 1.78^{d} (67.31-84.31)	62.64 ± 6.34^{f} (36.18-81.37)	56.74 ± 5.75^{f} (32.89-73.97)	P<0.05	69.46 ± 1.13^{d} (65.48-75.58)	57.88 ± 0.94^{d} (54.57-62.98)	44.52 ± 0.73^{e} (41.98-48.45)	P<0.05
NPK Added	78.60 ± 1.63^{e} (72.83-86.15)	51.37 ± 0.92^{e} (46.91-56.09)	46.93 ± 0.81^{e} (42.65-50.99)	P<0.05	69.31 ± 2.15^{d} (63.18-80.64)	57.76 ± 1.79^{d} (52.65-67.20)	44.43 ± 1.38^{e} (40.50-51.69)	P<0.05
DAP+NPK Added	69.05 ± 6.98^{d} (37.45-94.88)	72.34 ± 2.20^{g} (62.22-81.37)	55.76 ± 2.00^{f} (56.56-73.97)	P<0.05	$56.33\pm.05^{c}$ (36.53-73.75)	46.95 ± 4.21^{c} (30.44-61.46)	36.12 ± 3.24^{d} (23.42-47.28)	P<0.05
nHA Added	43.42 ± 1.83^{a} (31.47-49.85)	24.66 ± 0.49^{a} (23.29-27.88)	14.60 ± 0.30^{a} (13.70-16.4)	P<0.05	34.54 ± 1.07^{a} (29.64-37.91)	19.19 ± 0.59^{a} (16.47-21.06)	15.91 ± 0.57^{a} (13.25-18.3)	P<0.05
nHA +DAP Added	46.07 ± 1.47^{b} (40.2-53.07)	40.95 ± 0.83^{c} (37.3-46.51)	21.42 ± 0.54^{b} (24.06-30.01)	P<0.05	38.21 ± 0.70^{b} (35.15-40.20)	35.21 ± 3.15^{b} (22.83-46.09)	27.09 ± 2.43^{c} (17.56-35.45)	P<0.05
nHA+ NPK Added	49.44 ± 1.13^{b} (43.88-55.83)	41.40 ± 1.88^{c} (30.82-48.53)	27.60 ± 1.25^{c} (20.55-32.35)	P<0.05	39.34 ± 0.94^{b} (33.77-42.96)	27.29 ± 0.50^{b} (25.11-28.71)	21.59 ± 0.74^{b} (19.32-26.7)	P<0.05
nHA+DAP+NPK Added	53.38 ± 1.73^{c} (46.18-62.26)	34.15 ± 0.77^{b} (29.98-36.78)	21.45 ± 0.50^{b} (18.74-22.99)	P<0.05	46.64 ± 2.11^{c} (38.83-58.12)	29.15 ± 1.32^{b} (24.27-36.33)	23.38 ± 1.12^{b} (18.67-27.95)	P<0.05
P-value	1.93×10^{-15}	1.44×10^{-18}	3.07×10^{-34}		3.15×10^{-26}	2.85×10^{-26}	2.39×10^{-17}	

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

3.1 Levels of lead in sukuma wiki leaves

3.1.1 Levels of lead in synthetic set-up sukuma wiki leaves

For harvest 1 in the synthetic experiment comparison on mean concentration of lead using ANOVA in sukuma wiki leaves between various treatments showed that they differed significantly ($p=1.93\times10^{-15}$). The treatment of synthetic ash (nHA added) recorded the lowest uptake of lead of 43.42±1.83µg g^{-1}, while NPK fertilizer added treatment recorded the highest levels of lead in sukuma wiki leaves of 78.6±1.63µg g^{-1}. However, there was no significance difference between DAP nanofertilizer that had Pb levels (46.07±1.47µg g^{-1}) and NPK nanofertilizer added treatments (49.44±1.13µg g^{-1}). In harvest 2, Pb uptake in the synthetic set-up the synthetic ash (nHA) had 24.66µgg^{-1}. Similarly, other synthetic nanofertilizers reduced uptake of Pb in kales drastically in comparison with commercial fertilizers and non-treated pots. After 90 days of growth in harvest 3, the Pb concentration levels differed significantly with each other among the eight treatments ($p<0.05$). The pots treated with synthetic nanofertilizers produced kale leaves with lower Pb concentration levels in comparison with the commercial fertilizer added pots. The synthetic ash (nHA) had the lowest Pb levels of 14.60±0.30 µgg^{-1} which is within the NEMA permissible levels and it was significantly different from Pb levels recorded by other synthetic nanofertilizer set-up. DAP synthetic nanofertilizer also recorded lower Pb levels of 21.42±0.54 µgg^{-1}which was not significantly different with NPK-DAP synthetic nanofertilizer with 21.45±0.50 µgg^{-1}. NPK nanofertilizers recorded significantly higher Pb levels of 27.60 µgg^{-1}. The DAP fertilizer treatment recording the highest Pb levels of 56.00±5.75 µgg^{-1}, non-treated control had slightly lower levels than the commercial fertilizer added pots.

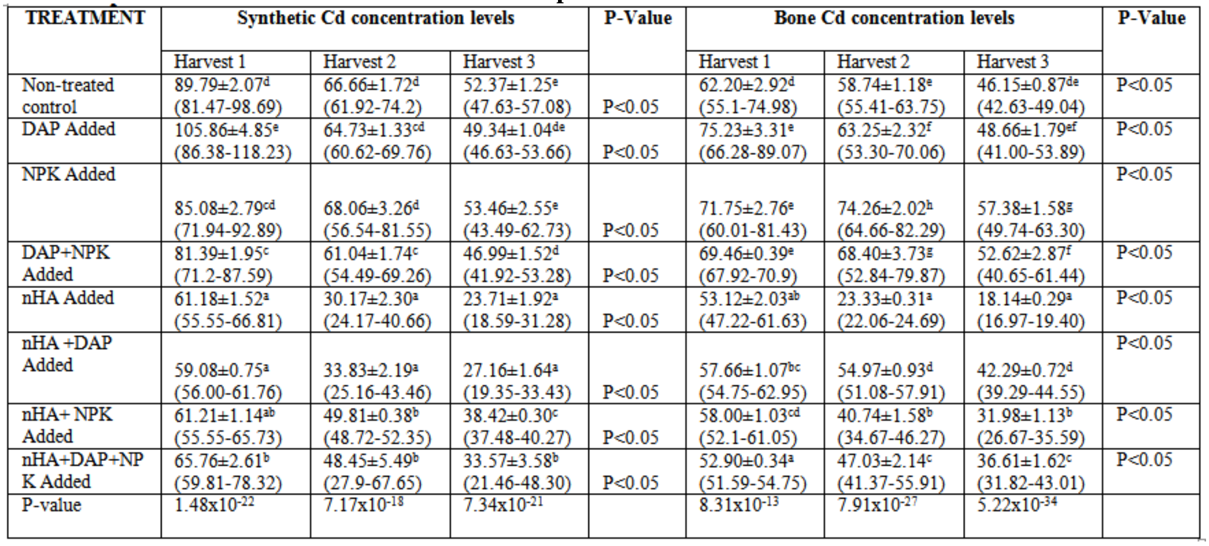
3.1.2 Levels of lead in bone set-up sukuma wiki leaves

The mean comparisons using statistical ANOVA for lead indicates that in the bone experiment there was significant difference of lead concentration between all the eight treatments in sukuma wiki leaves as evidenced by p-value in Table1 ($p=2.39\times10^{-17}$). In harvest 3, bone ash (nHA added) recorded the lowest uptake of lead of 15.91±0.57 µgg^{-1}, while non-treated control recorded the highest levels of lead in sukuma wiki leaves of 49.043±0.76 µg g^{-1}. The DAP nanofertilizers with lead concentration levels 27.09±2.43 µgg^{-1}. Fertilizer added bone experiments recorded relatively higher Pb concentration levels than nanofertilizer treated pots. The highest was DAP added treatments that recorded (69.46±1.13 µgg^{-1}) while NPK added treatments had 44.52±0.73^{e} µgg^{-1} which were not significantly different from each other. However NPK-DAP added treatment recorded slightly lower Pb levels (23.38±1.12 µgg^{-1}).

3.2 Levels of cadmium in sukuma wiki (kales)

3.2.1 Levels of cadmium in synthetic sukuma wiki (kales)

Table 2: Cd concentration levels for Sukuma wiki for the three harvests in Synthetic and bone experiments



TREATMENT	Synthetic Cd concentration levels			P-Value	Bone Cd concentration levels			P-Value
	Harvest 1	Harvest 2	Harvest 3		Harvest 1	Harvest 2	Harvest 3	
Non-treated control	89.79±2.07^{d} (81.47-98.69)	66.66±1.72^{d} (61.92-74.2)	52.37±1.25^{e} (47.63-57.08)	P<0.05	62.20±2.92^{d} (55.1-74.98)	58.74±1.18^{e} (55.41-63.75)	46.15±0.87^{de} (42.63-49.04)	P<0.05
DAP Added	105.86±4.85^{e} (86.38-118.23)	64.73±1.33^{cd} (60.62-69.76)	49.34±1.04^{de} (46.63-53.66)	P<0.05	75.23±3.31^{e} (66.28-89.07)	63.25±2.32^{f} (53.30-70.06)	48.66±1.79^{ef} (41.00-53.89)	P<0.05
NPK Added	85.08±2.79^{cd} (71.94-92.89)	68.06±3.26^{d} (56.54-81.55)	53.46±2.55^{e} (43.49-62.73)	P<0.05	71.75±2.76^{e} (60.01-81.43)	74.26±2.02^{h} (64.66-82.29)	57.38±1.58^{g} (49.74-63.30)	P<0.05
DAP+NPK Added	81.39±1.95^{c} (71.2-87.59)	61.04±1.74^{c} (54.49-69.26)	46.99±1.52^{d} (41.92-53.28)	P<0.05	69.46±0.39^{e} (67.92-70.9)	68.40±3.73^{g} (52.84-79.87)	52.62±2.87^{f} (40.65-61.44)	P<0.05
nHA Added	61.18±1.52^{a} (55.55-66.81)	30.17±2.30^{a} (24.17-40.66)	23.71±1.92^{a} (18.59-31.28)	P<0.05	53.12±2.03^{ab} (47.22-61.63)	23.33±0.31^{a} (22.06-24.69)	18.14±0.29^{a} (16.97-19.40)	P<0.05
nHA +DAP Added	59.08±0.75^{a} (56.00-61.76)	33.83±2.19^{a} (25.16-43.46)	27.16±1.64^{a} (19.35-33.43)	P<0.05	57.66±1.07^{bc} (54.75-62.95)	54.97±0.93^{d} (51.08-57.91)	42.29±0.72^{d} (39.29-44.55)	P<0.05
nHA+ NPK Added	61.21±1.14^{ab} (55.55-65.73)	49.81±0.38^{b} (48.72-52.35)	38.42±0.30^{c} (37.48-40.27)	P<0.05	58.00±1.03^{cd} (52.1-61.05)	40.74±1.58^{b} (34.67-46.27)	31.98±1.13^{b} (26.67-35.59)	P<0.05
nHA+DAP+NPK Added	65.76±2.61^{b} (59.81-78.32)	48.45±5.49^{b} (27.9-67.65)	33.57±3.58^{b} (21.46-48.30)	P<0.05	52.90±0.34^{a} (51.59-54.75)	47.03±2.14^{c} (41.37-55.91)	36.61±1.62^{c} (31.82-43.01)	P<0.05
P-value	1.48x10^{-22}	7.17x10^{-18}	7.34x10^{-21}		8.31x10^{-13}	7.91x10^{-27}	5.22x10^{-34}	

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

It is noted from Table 2 that levels of Cd for synthetic experiment for harvest 1 were significantly different with p=1.48x10^{-23} for all the eight different treatments, synthetic ash (nHA added) pots had sukuma wiki leaves that recorded the lowest mean concentration levels of 61.18±1.52µgg^{-1} however this was not significantly different from the cadmium concentration levels obtained by leaves harvested from pots treated with DAP nanofertilizer of 59.08±0.75 µgg^{-1}. Other synthetic nanofertilizer treatments recorded relatively lower cadmium concentration in comparison with the fertilizer added treatments. Similar trends were exhibited in harvest 2 and harvest 3.

3.2.2 Levels of cadmium in bone sukuma wiki (kales)

It is noted in Table 2 that levels of Cd for bone experiment for harvest 1 were significantly different with p=8.31x10^{-13} for all the treatments, bone ash (nHA added) pots had sukuma wiki leaves that recorded the lowest mean concentration levels of 53.12±2.03 µgg^{-1} however this was not significantly different from the cadmium concentration levels obtained by leaves harvested from pots treated with NPK-DAP nanofertilizer of 52.90±0.34µgg^{-1}. Other bone nanofertilizer treatments recorded relatively lower cadmium concentration in comparison with the fertilizer added treatments.

For harvest 2, NPK fertilizer added treatment recording the highest Cd levels of 74.26±2.02 µgg^{-1}. Other commercial fertilizer added treatments and non-treated control produced kales with also significantly higher Cd levels. The bone ash recorded the lowest Cd levels in harvest 2 of 23.33±0.31 µgg^{-1} which was significantly different from Cd levels recorded by other bone made nanofertilizers. In harvest 3, the Cd levels of the obtained leaves also differred very significantly with each other among the treatments as proved by p-value of p=5.22x10^{-34}. The NPK fertilizer added treatment gave kale leaves with the highest levels of Cd (57.38±1.58 µgg^{-1}), it was followed by DAP-NPK fertilizer pots (52.62±2.87 µgg^{-1}) which was not significantly different with DAP added fertilizer pot leaves with Cd levels of 48.66±1.79 µgg^{-1}. Bone ash recorde lowest Cd levels of18.14±0.29 µgg^{-1}.

3.3 Comparison of Cd and Pb levels between harvests in sukuma wiki (kales)

Table 3: Comparison of Cd concentration levels for kales between harvests in Synthetic and bone experiments

TREATMENT	Synthetic Cd Mean Concentration levels µgg^{-1}		P- value	Bone Cd Mean Concentration levels µgg^{-1}		P- value
	Harvest 1	Harvest 3		Harvest 1	Harvest 3	
Non-treated control	89.79±2.07^{d} (81.47-98.69)	52.37±1.25^{e} (47.63-57.08)	5.97x10^{-18}	62.20±2.92^{d} (55.1-74.98)	46.15±0.87^{de} (42.63-49.04)	1.58x10^{-17}
DAP Added	95.86±4.85^{e} (86.38-118.23)	49.34±1.04^{de} (46.63-53.66)	3.38x10^{-17}	75.23±3.31^{e} (66.28-89.07)	48.66±1.79^{ef} (41.00-53.89)	1.89x10^{-18}
NPK Added	85.08±2.79^{cd} (71.94-92.89)	53.46±2.55^{e} (43.49-62.73)	3.22x10^{-17}	71.75±2.76^{e} (60.01-81.43)	57.38±1.58^{g} (49.74-63.30)	2.18x10^{-17}

DAP+NPK Added	81.39±1.95^{c} (71.2-87.59)	46.99±1.52^{d} (41.92-53.28)	5.0x10^{-17}	69.46±0.39^{e} (67.92-70.9)	52.62±2.87^{f} (40.65-61.44)	9.0x10^{-18}
nHA Added	61.18±1.52^{a} (55.55-66.81)	23.71±1.92^{a} (18.59-31.28)	1.54x10^{-17}	53.12±2.03^{ab} (47.22-61.63)	18.14±0.29^{a} (16.97-19.40)	5.45x10^{-08}
nHA +DAP Added	59.08±0.75^{a} (56.00-61.76)	27.16±1.64^{a} (19.35-33.43)	1.48x10^{-17}	57.66±1.07^{bc} (54.75-62.95)	42.29±0.72^{d} (39.29-44.55)	2.54x10^{-06}
nHA+ NPK Added	61.21±1.14^{ab} (55.55-65.73)	38.42±0.30^{c} (37.48-40.27)	8.94x10^{-18}	58.00±1.03^{cd} (52.1-61.05)	31.98±1.13^{b} (26.67-35.59)	9.85x10^{-06}
nHA+DAP+NPK Added	65.76±2.61^{b} (59.81-78.32)	33.57±3.58^{b} (21.46-48.30)	6.71x10^{-18}	52.90±0.34^{a} (51.59-54.75)	36.61±1.62^{c} (31.82-43.01)	6.28x10^{-06}
P-value	1.48x10^{-22}	7.34x10^{-21}		8.31x10^{-13}	5.22x10^{-34}	

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

The comparison between Harvest 1 and Harvest 3 was significantly different in all the eight treatments as evidenced by $p<0.05$ shown in Table 3

3.4 Comparison of Pb concentration levels for Sukuma wiki between harvests

Table 4: Comparison of Pb concentration levels for Sukuma wiki between harvests in Synthetic and bone experiments

TREATMENT	Synthetic Pb Mean Concentration levels µgg^{-1}		P- value	Bone Pb Mean Concentration levels µgg^{-1}		P- value
	Harvest 1	Harvest 3		Harvest 1	Harvest 3	
Non-treated control	71.16±1.61^{d} (62.72-77.88)	35.36±2.16^{d} (25.69-42.55)	1.49x10^{-17}	82.37±1.15^{e} (75.12-87.07)	49.043±0.76^{e} (44.45-52.1)	1.5x10^{-08}
DAP Added	74.00±1.78^{d} (67.31-84.31)	56.74±5.75^{f} (32.89-73.97)	5.59x10^{-06}	69.46±1.13^{d} (65.48-75.58)	44.52±0.73^{e} (41.98-48.45)	2.68x10^{-08}
NPK Added	78.60±1.63^{e} (72.83-86.15)	46.93±0.81^{e} (42.65-50.99)	1.66x10^{-06}	69.31±2.15^{d} (63.18-80.64)	44.43±1.38^{e} (40.50-51.69)	2.69x10^{-08}
DAP+NPK Added	69.05±6.98^{d} (37.45-94.88)	55.76±2.00^{f} (56.56-73.97)	9.44x10^{-08}	56.33±.05^{c} (36.53-73.75)	36.12±3.24^{d} (23.42-47.28)	4.08x10^{-08}
nHA Added	43.42±1.83^{a} (31.47-49.85)	14.60±0.30^{a} (13.70-16.4)	8.03x10^{-08}	34.54±1.07^{a} (29.64-37.91)	15.91±0.57^{a} (13.25-18.3)	4.8x10^{-08}
nHA +DAP Added	46.07±1.47^{b} (40.2-53.07)	21.42±0.54^{b} (24.06-30.01)	2.74x10^{-08}	38.21±0.70^{b} (35.15-40.20)	27.09±2.43^{c} (17.56-35.45)	1.35x10^{-07}
nHA+ NPK Added	49.44±1.13^{b} (43.88-55.83)	27.60±1.25^{c} (20.55-32.35)	3.49x10^{-08}	39.34±0.94^{b} (33.77-42.96)	21.59±0.74^{b} (19.32-26.7)	5.29x10^{-08}
nHA+DAP+NPK Added	53.38±1.73^{c} (46.18-62.26)	21.45±0.50^{b} (18.74-22.99)	1.63x10^{-08}	46.64±2.11^{c} (38.83-58.12)	23.38±1.12^{b} (18.67-27.95)	3.08x10^{-08}
P-value	1.93x10^{-}	3.07x10^{-34}		3.15x10^{-26}	2.39x10^{-17}	

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

The concentration of lead in kale leaves harvested in various harvests in the bone and synthetic experimental treated soil in were determined by AAS to determine the effect of leaf maturity of uptake of lead in the kale leaves at different harvests and mean concentration levels recorded in Table 4. When comparing effects of leaf maturity (Table 4) between harvests in the bone experiment for lead concentration in kale leaves was significantly different ($p<0.01$). However, the first harvest had significantly higher concentration levels of lead as compared to the second and third harvest. Similarly, second and third harvest concentration levels of lead in kale leaves were significantly different.

3.5 Comparison of Cd and Pb levels between synthetic and bone grown kales

Table 5: Cd and Pb concentration levels of Sukuma wiki harvest 3

TREATMENT	Pb Mean Concentration levels µgg^{-1}		P- value	Cd Mean Concentration levels µgg^{-1}		P- value
	Synthetic nHA	Bone nHA		Synthetic nHA	Bone nHA	
Non-treated control	35.36±2.16^{d} (25.69-42.55)	49.043±0.76^{e} (44.45-52.1)	8.96x10^{-05}	52.37±1.25^{e} (47.63-57.08)	46.15±0.87^{de} (42.63-49.04)	0.000128
DAP Added	56.74±5.75^{f} (32.89-73.97)	44.52±0.73^{e} (41.98-48.45)	0.028196	49.34±1.04^{de} (46.63-53.66)	48.66±1.79^{ef} (41.00-53.89)	0.076759
NPK Added	46.93±0.81^{e} (42.65-50.99)	44.43±1.38^{e} (40.50-51.69)	0.017228	53.46±2.55^{e} (43.49-62.73)	57.38±1.58^{g} (49.74-63.30)	0.031807
DAP+NPK	55.76±2.00^{f}	36.12±3.24^{d}	2.88x10^{-07}	46.99±1.52^{d}	52.62±2.87^{f}	0.051629

Added	(56.56-73.97)	(23.42-47.28)		(41.92-53.28)	(40.65-61.44)	
nHA Added	14.60 ± 0.30^{a} (13.70-16.4)	15.91 ± 0.57^{a} (13.25-18.3)	0.034565	23.71 ± 1.92^{a} (18.59-31.28)	18.14 ± 0.29^{a} (16.97-19.40)	0.014949
nHA +DAP Added	21.42 ± 0.54^{b} (24.06-30.01)	27.09 ± 2.43^{c} (17.56-35.45)	0.000678	27.16 ± 1.64^{a} (19.35-33.43)	42.29 ± 0.72^{d} (39.29-44.55)	$4.1\text{x}10^{-05}$
nHA+ NPK Added	27.60 ± 1.25^{c} (20.55-32.35)	21.59 ± 0.74^{b} (19.32-26.7)	0.00053	38.42 ± 0.30^{c} (37.48-40.27)	31.98 ± 1.13^{b} (26.67-35.59)	$2.19\text{x}10^{-08}$
nHA+DAP+NPK Added	21.45 ± 0.50^{b} (18.74-22.99)	23.38 ± 1.12^{b} (18.67-27.95)	0.115454	33.57 ± 3.58^{b} (21.46-48.30)	36.61 ± 1.62^{c} (31.82-43.01)	0.012678
P-value	$7.44\text{x}10^{-27}$	$7.44\text{x}10^{-27}$		$7.34\text{x}10^{-21}$	$7.44\text{x}10^{-27}$	

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

The comparison of harvest 3 shown on Table 5 indicates that Pb concentration levels between synthetic and bone set-ups differed very significantly with each other in the seven treatments as they have p-value less than 0.05, while in NPK-DAP nanofertilizer treatment there was no significant difference between synthetic and bone NPK-DAP nanofertilizer leaves harvested after 90 days of kale growth with a p-value of 0.1155. In most treatments the bone set-up had lower Pb levels in harvest 3 such as in the bone ash treatment and NPK bone nanofertilizer treatment. The comparison of harvest 3 Cd levels in kales also differs very significantly in six treatments except in two treatments with $p>0.05$. There is no significant difference in leave Cd levels of DAP added treatment in two set-ups with $p=0.077$ and DAP-NPK added treatment with $p=0.052$.

Table 3.6 Percentage reduction of Cd and Pb uptake in kales

Table 6: Percentage reduction of Cd and Pb uptake in kales at maturity

TREATMENT	**% reduction in Pb uptake in kales**		**% reduction in Cd uptake in kales**	
	Synthetic nHA	Bone nHA	Synthetic nHA	Bone nHA
Non-treated control	0.0^{c}	0.0^{b}	0.0^{d}	0.0^{c}
DAP Added	-60.46^{a}	-14.04^{a}	-16.45^{c}	-5.44^{b}
NPK Added	-32.72^{b}	-13.81^{a}	-26.17^{a}	-24.33^{a}
DAP-NPK Added	-57.69^{a}	7.46^{c}	-10.90^{b}	14.01^{e}
nHA Added	58.71^{f}	59.25^{f}	44.04^{h}	60.69^{h}
nHA +DAP Added	39.42^{e}	30.61^{d}	35.90^{g}	8.39^{d}
nHA+ NPK Added	21.94^{d}	44.70^{e}	9.32^{e}	30.70^{g}
nHA+DAP-NPK Added	39.34^{e}	40.24^{e}	20.77^{f}	20.67^{f}
P-value	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$

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

Cadmium concentration levels in kales harvested from bone ash as given in Table 6, it recorded the highest reduction of 60.69% as compared with the non-treated control followed by bone NPK nanofertilizer with 30.70% cadmium uptake reduction. Lead concentration levels in kales harvested from synthetic ash recorded the highest reduction of 59.25% as compared with the non-treated control while DAP fertilizer added treatment increased Pb levels by with 60.46%.

IV. Discussion

There was variation in the lead concentration levels in Table 1, indicating that the type of nanofertilizer amended to the soil is a strong factor to influence experimental soil physico-chemical properties like the soil pH, % organic matter and electrical conductivity of the treated soil that influenced the uptake of lead in sukuma wiki leaves. But there was significant difference between synthetic ash and other nanofertilizer treatment on uptake of lead in sukuma wiki leaves because their effectiveness in reducing uptake of lead in sukuma wiki depends on the type of nanofertilizer added. But the amount added to the Cd/Pb contaminated soil determines the uptake factor of sukuma wiki leaves. The lower the amount Pb contamination in the planting soil the more effective is the efficiency of reducing heavy metal uptake in sukuma wiki (Ngorwe *et al*., 2014; Zhang *et al*., 2011). In harvest 2, synthetic nanofertilizer treatments had the highest impact in the reduction of Pb uptake in the synthetic set-up, with synthetic ash (nHA) having enough adsorption sites for chelation and complexation due to larger surface area to have the least Pb uptake of 24.66μgg^{-1} after 60 days of sukuma wiki growth. Similarly, other synthetic nanofertilizers reduced uptake of Pb in kales drastically in comparison with commercial fertilizers and non-treated pots. However harvest 2 Pb levels in sukuma wiki grown with commercial fertilizers and non-treated control had higher Pb levels.Commercial fertilizers lowers soil pH that mobilizes Pb^{2+} in experimental soil that's why they record higher levels, with DAP added treatment recording the highest Pb levels of 56.00±5.75 μgg^{-1}, non-treated control had slightly lower levels than the commercial fertilizer added pots due to lower biomass and vegetative growth.

The bone nanoparticle treatments recorded lower uptake of lead in the sukuma wiki leaves because they immobilized it by shifting pb^{2+} ions from available forms in the soil to fractions associated with organic matter, carbonates or metal oxides therefore rendering it unavailable for sukuma wiki uptake (Elmer *et al*., 2016). Also, the nHA nanoparticle amendment when added to the soil they have an ameliorative effect due to increase in surface area and an increase in the number of specific adsorption sites (Prabu, 2009). It could also have had a dilution effect to Cd/Pb contaminated soil as noted by other studies(Du *et al*., 2019 and Ma *et al*., 2010). Reduction of uptake of heavy metals such as lead and cadmium has been pointed out by various studies (Du *et al*., 2019; Musico *et al*., 2013; Rattanawat *et al*., 2010; Keller *et al*., 2005). The commercial DAP and NPK fertilizers lowers the soil pH and scorches the soil organic matter that increases mobility of Pb^{2+} to increases its uptake in sukuma wiki plants, that's why DAP added treatment (44.62±0.73 μgg^{-1}) recorded higher Pb levels that was not significantly different with NPK commercial fertilizers added treatment (44.43±1.38 μgg^{-1}).

In Table 2 the synthetic nanofertilizers recorded lower Cd levels in kale leaves due to increased number of adsorption, co-precipitation, chelation and complexation sites with synthetic ash (nHA) recording 23.71±1.92 μgg^{-1} which is within allowable limits by FAO in vegetables. The Cd levels in synthetic ash was significantly different from Cd levels obtained in DAP synthetic nanofertilizer treatment. The synthetic ash treatment produced sukuma wiki leaves that was within limit reported in Kenyan vegetables of below 20 ppm as noted Kumar *et al*. (2013). The uptake of Cd among pots differred significanly with each other indicating that each type of these nanofertilizers amendments added to the contaminated soil had a relatively different effect in reducing the uptake of cadmium in sukuma wiki leaves. The addition of bone ash in bone pot experiments resulted in lower uptake of cadmium as compared to the non-amended control. This was partly due to the dilution effect when bone nanohydroxyapatite was mixed with contaminated soil (Gomathi *et al*., 2019). In addition, the reduction of cadmium may be because of chelation, complexation, coprecipitation and more adsorption between organic matter and bone synthesized nanoparticles. The same results have been posted by other researchers such as Ma *et al*. (2013) and Zhang *et al*. (2011). However, if nano sized particles could have been reduced further better reduction effieciency could have been realised. Ma *et al*. (2013) recommended that most effective size of nHA in reducing uptake of cadmium in plants is below 20 nm. Zhang *et al*. (2011) also showed similar results with other heavy metals. They stated that the nanohydroxyapatite amendment was more efficient when cadmium concentration in the experimental soil was low or medially polluted. At higher concentrations of cadmium in experimental soil the amount of nanoparticle added could not be sufficient to immobilize all the cadmium available in the polluted soils.In bone experiment, fertilizer amended soil sukuma wiki leaves had higher levels of cadmium due to acidic effect caused by phosphatic fertilizers and nitrogenous fertilizers which mobilizes cadmium in the soil, thereby making it bioavailable for uptake by sukuma wiki plants (Ciu *et al.,* 2010). This was also demonstrated by Pidwirny (2006), who explained that commercial fertilizers add the heavy metals load to the soil which eventually is absorbed by the plants threatening the food safety.

In Table 3, the comparison of harvest 1 up to harvest 3 Cd concentration levels showed very significant difference in all the eight treatments ($p<0.05$) as shown in Table 3. However, the kales leaves obtained in the 1st and 2nd harvests in both bone and syntheticset-ups had significant difference in their cadmium concentration levels and they were relatively higher than in the third harvest which recorded the lowest mean concentration levels of cadmium, implying that Cd concentration in harvested leaves decreases with maturity period. Therefore the concentration of cadmium in the harvested kale leaves is greatly influenced by the leaf maturity bacause as the leaf matures the concentration levels of cadmium in the soil decreases due to many loss pathways such as uptake by weeds, leached to deep zones as cadmium has high mobility, cadmium being eroded together with soil. It can also be adsorbed and immobilized to make it unavailable for uptake by tobacco leaves. The minimum concentration of cadmium in kale leaves obtained in the second harvest was within allowed concentration levels of cadmium in kale (Du *et al*., 2019; Helaly *et al*., 2014; Li *et al*., 2012; Atlabachew *et al*., 2010).

In Table 4, The concentration of lead in kale leaves were to evaluate the effect of leaf maturity of uptake of lead in the kale leaves at different harvests and mean concentration levels recorded in Table 4. When comparing effects of leaf maturity (Table 4) between harvests in the bone experiment for lead concentration in kale leaves was not significantly different ($p<0.01$). However, the first harvest had significantly higher concentration levels of lead as compared to the second and third harvest. Similarly, second and third harvest concentration levels of lead in kale leaves were significantly different, hence pointing out that the number of adsorption sites were numerous in this nanofertilizer treatments implicating the adsorption, chelation, complexation sites varied widely between the harvests.

In Table 5, most treatments the bone set-up had lower Pb levels in harvest 3 such as in the bone ash treatment and NPK bone nanofertilizer treatment. This shows that the numbers of adsorption sites are higher and more stable in bone nanoparticles that guarantee better adsorption, chelation, co-precipitation and complexation of Pb to reduce its uptake in kale plants. The comparison of harvest 3 Cd levels in kales indicates that Cd levels of synthetic and bone set-up added with nanofertilizers differed significantly in the number of adsorption sites between synthetic and bone nanofertilizers. This is because lead metal is as mobile as cadmium when the soil pH is slightly lowered acidic conditions of between 6-7 although lead is less mobile. Research done by Pidwirny (2006) pointed out that lead was observed to be the less mobile heavy metal than cadmium which is leached to a depth of 30-40 mm in a soil pH of 7-8, in sandy loamy soil when compared with lead and zinc. There was no significance difference in comparison of DAP-NPK added experiments, indicating that concentrations levels of lead were affected by interaction added fertilizer with environmental variable factors predominant in the specially structured and stabilised bone nanoparticle experiment like lead leaching, soil erosion sprinkling irigated water and aeration, temperatures, moisture content in the experimental soil (Pidwirny, 2006).Several studies have demonstrated the efficiency of nanoparticles in reducing heavy metals like lead accumulation in plants (Zhang *et al.*, 2011; Ciu *et al.*, 2011; Ma *et al.*, 2010;). However if higher application rates of nanofertilizer is done some studies have indicated that 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 sukuma wiki (Helaly *et al*., 2014; Nwachukwu *et al*., 2008).

In Table 6, the results indicate that bone ash amendment was most effective in reducing cadmium uptake in kales by providing numerous binding sites for lead adsorption, chelation, coprecipitation and complexation. However kales harvested in fertilizer added soils had increased cadmium levels as compared to the control, with NPK fertilizer added treatment increasing Cd uptake in kales by 24.33%. Similarly synthetic ash (nHA) reduced cadmium uptake in the synthetic experiment by 44.04% while sukuma wiki harvested from NPK fertilizer added soils increased cadmium uptake by 26.17% as compared to control. Lead uptake in bone set-up was most efficiently reduced by bone ash (nHA) by 59.25% followed by bone NPK nanofertilizer with 44.70% lead uptake reduction. However fertilizer amendment increased lead uptake with DAP added treatment hiking lead uptake in kales by 14.04%. This is because soil pH was lowered mobilizing lead to readily bioavailable forms to be uptaken by sukuma wiki (Pidwirny, 2006). However fertilizer added soil increased lead uptake in this set-up with DAP added treatment increasing lead uptake by 60.46% as it lowers soil pH mobilizing lead to increase its uptake in kales. The percentage reduction of Pb in bone ash treatment in the bone experiment was 59.25% while reduction by same treatment in synthetic experiment was 58.71. Bone ash Cd percentage reduction was significantly higher (60.69%) than percentage Cd reduction in synthetic experiment (44.04%) as shown in Table 4. 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(Pidwirny, 2006). This proves that bone nanofertilizers are more multistructured and stable to immobilize the Cd. This shows that the numbers of adsorption sites are higher and more stable in bone nanoparticles that guarantee better adsorption, chelation, co-precipitation and complexation of Pb to reduce its uptake in kale plants.and Pb heavy metals.

V. Conclusion

The nanofertilizers treatment recorded lowest levels of Cd and Pb in sukuma wiki harvested leaves. The bone ash (nHA added) the lowest concentration levels of Cd (18.14±0.29) with NPK nanofertilizers also recording lower concentration levels of Pb (21.59±0.74) and lower levels of Cd and Pb to the permissible levels allowed by WHO/FAO in Kenyan vegetables. The bone ash nano treatment posted the lowest heavy metals concentration levels of Cd (18.14±0.29) and Pb (15.91±0.57) while in synthetic experiments leaves harvested from synthetic ash nanofertilizer treated pots had Cd (23.71±1.92) and Pb (14.60±0.30). Generally, sukuma wiki (kales leaves harvested from synthetic experiment had higher cadmium and lead concentration as compared to bone nanofertilizer harvested sukuma wiki (kales.. The results indicate that the nHA and nNPK soil amendments have a great efficiency in reducing uptake of Cd and Pb in sukuma wiki (kales) leaves. The bone ash was most effective in reducing uptake of cadmium with a range of 14.2-20.2 µg/g with a reduction of 44.04% in bone set-up and 60.69% in synthetic experiment set-up and lead within a range of 14.2-20.2 µg/g was reduced by 59.25% in bone set-up and 58.71% in synthetic experiment set-up. Therefore nanohydroxyapatite nanofertilizers can be used to reduce uptake of heavy metals in crops.

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