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Full Length Research Paper

Effect of *Archachatina marginata* feces and synthesize fertilizer on bioaccumulation and translocation of trace metals in some edible vegetables; a major threat to food safety

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ABSTRACT: The study investigated the uptake and translocation pattern of trace metals from different vegetables amended with *Achachatina marginata* feces and synthesize fertilizer. A total of 24 pots were used for the planting and were all filled with the same soil. The pots were divided into two groups, each group contains 12 pots. In the experimental pots, NPK fertilizer and snail faces were applied to group 1 and group 2 respectively. Before the fertilizer application and seeding, the initial soil sample and fertilizer sample (topsoils, 0-15 cm) were collected. Plant and soil samples were collected at week 7 after sowing. The plants were pulled out carefully, and soil samples were collected using a hand trowels to dig the soil around the plant position. Plant samples (leaves) were kept in separate polythene bags and properly labeled. The metal (Pb, Cd, As, and Hg) concentrations were determined by atomic absorption spectrophotometer (AAS). Results show that the concentration of metals in soil with NPK fertilizer was greater than the soil amended with snail faces which shows a significant ($P<0.05$) variation in the concentrations of metals. Vegetables amended with NPK fertilizer had higher heavy metals concentrations (As, Cd, Hg, and Pb) than vegetables amended with snail faces, which were all above the FAO/WHO permissible limit. The transfer factor indicates that the high presence of metals in the soil amended with NPK was also transferred to the vegetables. The more the presence of metals in the soil the more the bioaccumulation on the vegetables. The soil to plant TF in vegetables amended with NPK for As, Cd, Hg and Pb were all greater than one (>1), while snail faces were below one (<1). The present study revealed that vegetables grown in NPK amended soil bioaccumulate significantly high levels of trace metals that exceed the maximum prescribed limits for elements in vegetable, which toxicity disrupts natural ecosystems and affects the food chain, leading to deleterious health problems in humans and animals.

Keywords: Archachatina margenata, fertilizer, trace metals, translocation, vegetables

INTRODUCTION

The accumulation of trace metals by plants is one of the most serious environmental concerns lately due to translocation of these metals to edible part of the plants. Some consumers consider undamaged, dark green and big leaves as characteristics of good quality leafy vegetables. However, the external morphology of vegetables cannot guarantee safety from contamination especially when farming activities are carried out with application of fertilizer. Heavy metal contamination in agricultural environments can be attributed to atmospheric fall-out, pesticide formulations, chemical fertilizers and irrigation with waste water (Marcovecchio *et al*., 2007). Toxic trace element plays a vital role in chemical, biological, metabolic, and enzymatic reactions in the living cells of plants, animals and human (Hashmi *et al*., 2007; Oguh *et al*., 2019a). The uptake of metals by plants varies from the soil depends on different factors, such as their soluble content in it, soil pH, plant species, fertilizers, organic matter and soil type (Lubben and Sauerberck, 1991). Vegetables, especially leafy vegetables, accumulate higher amounts of heavy metals. Roots and leaves of herbaceous plants retain higher concentration of heavy metal than stems and fruits (Yargholi and Azimi, 2008).

Heavy metal is any metallic chemical element that has a relatively high density greater than 5 g/cm^3 and is toxic or poisonous at low concentrations (Oguh *et al*., 2019b). Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Ti) and lead (Pb). The densities of Pb, Hg and Cd are 11.4 gcm-^{3}, 13.6 gms/cubic cms and 8.65 gms/cubic cms respectively which make these metals to be classified under heavy metals. Recent researchers have found that even low levels of mercury, cadmium, lead, aluminum and arsenic can cause a wide variety of health problems (Hassaan *et al*., 2016). Heavy metals toxicity can result in damaged or reduced mental and central nervous function, lower energy levels and damage to blood composition, lungs, kidneys, liver and other vital organs. Long-term exposure may result in slowly progressing physical, muscular and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy and multiple sclerosis (Oguh *et al*., 2019a).

In Nigeria, farmers realize the need for soil amendments by using available resources such as crop wastes, farmyard manure and poultry waste (Adediran *et al*., 2003). This fertilizer type improves soil's physical, chemical and biological conditions, which in turns improve crop growing environment and culminate in the better production of economic plant parts (Pius, 1998). Snail grazing and feces production have been shown to be major components of nitrogen (N) in plants. *A. marginata* (AM) belongs to the group Phylum *Mollusca* and Family *Achatinidae* belonging to the class Gastropoda (Nkop *et al*., 2016). Apart from insects, mollusca are the largest invertebrate group in the animal kingdom (Yoloye, 1994). *A. marginata* are bilaterally symmetrical invertebrates with soft segmented exoskeleton, inhabiting mostly marine environments, tolerating varied environmental conditions and thrive best in temperate and tropical areas, where soil pH ranges from 4.5-8.0 (Adediran et al., 2003). Organic manure and dead decay plant, and sewage soil ultimately maximize snail productivity and economic returns (Oguh *et al*., 2019c). *A. margenata* also known as Dodon kodi in Hausa, Igbin in Yoruba and Ejule in Igbo.

Nutritionally, snails are of paramount important as source of high profile protein, low in fat and rich in iron food ideal for human nutrition especially for diabetic patients (Cobbinah, 1993, Awah, 2000). Snails serve as valuable sources of nutrition to human and animals with high levels of protein, iron, calcium, phosphorus and amino acid such as lysine, leucine, and arginine, relatively low amount of sodium, fat and cholesterol compared to poultry and other livestock (Wosu, 2003). Snail meat compares favourably with whole egg in all essential amino acids especially with regard to lysine, leucine, isoleucine and phenylalanine (Imebvore, 1990). The close contact of snails with dead decay matters, soil with high nutrient and their uncontrolled feeding pattern make the snail susceptible to produce nutrient rich manure or faces. Nitrogen is usually ascribed with the building up of leaf tissues. This is one of the essential elements most commonly used to increase crop yield. It is a constituent of all protein and chlorophyll. Plant tissue, usually contains more nitrogen than any other nutrients. Nitrogen application is used to produce rapid vegetative growth of vegetables (Ojetayo *et al*., 2011). It promotes luxuriant growth, and increases number of leaves.

Use of synthesis fertilizer to amend soil for cultivation of crops mainly accounts for decrease in the overall productivity and results in contaminated food grains and vegetables which adversely affects human health. Soil is also polluted through application of chemical fertilizers (like phosphate and Zn fertilizers). The main advantage associated with study of plants including crops, is their ability to accumulate metals, if grown on metal polluted land or irrigated with polluted water. Thus, plants serve as a good tool for phytoremediation. However, determination of the nature of toxicity, distribution of toxicants and level of accumulation in different plant parts would be essential before selection and cultivation of plants for phytoremediation (Barman and Ray, 1999; Barman *et al*., 2000, Barman *et al*., 2001).

Besides many essential macronutrients (N, P, K) and micronutrients (Fe, Mn, Ni, Cu, Co, B, and Mo), fertilizer also contains a number of toxic heavy metals such as Cd, Pb and Se (Rautaray et al., 2003). Sometimes, the concentration of trace metals in fertilizer exceeds the levels of these metals found in normal soil (Kalra *et al*., 1996). There is an inherent tendency of plants to take up toxic substances including the heavy metals that are subsequently transferred along the food chain. A principal source of nitrogen absorbed by upland crops is nitrate, large fraction of these reduced nitrogen is transformed into nitrate in the soil and move to the root rhizosphere and then absorbed by plant (Tadakatsu, 1991). The study aims to investigate the uptake and translocation pattern of trace metals from different vegetables amended with *Achachatina marginata* feces and synthesize fertilizer.

MATERIALS AND METHOD

Sampling

Four commonly consume vegetables; Eggplant (*Solanum melongena*), Green (*Amarathus cruentus*), water leaf (*Talinum triangulare*) and Jute mallow (*Corchorus olitorius*) were selected for the study.

Experimental field

Experimental plots were set up in Makolo farm, Chanchaga minna, Niger State during December 2019 to February 2020. The experimental field was top-dressed soil, cultivated about three years. A total of 24 pots were used for the planting and were all filled with the same soil. The pots were divided into two groups, each group contains 12 pots. The Experimental pots consisted of three replicate for each leafy vegetable and each replicate was separated and was left as barren. Seeds of Eggplant (*Solanum melongena*), Green (*Amarathus cruentus*), water leaf (*Talinum triangulare*) and Jute mallow (*Corchorus olitorius*) were sown on 17th December 2019 in all pots. NPK fertilizer and snail faces were applied to group 1 and group 2 respectively. Before fertilizer application and seeding, initial soil sample and fertilizer sample (top soils, 0-15 cm) was collected and analyzed in the laboratory for heavy metal.

Plant and soil sample collection

Plant and soil samples were collected at week 7 after sowing. The plants were pulled out carefully, and soil samples were collected using hand trowel to dig the soil around the plant position. Plant samples (leaves) were kept in separate polythene bags and properly labeled. Soil samples were collected at a depth of 0-15 cm from the same point of collecting plant samples. The samples were kept in polythene bags and labeled properly. The plant and soil samples were analyzed in the laboratory.

Preparation and preservation

The vegetable samples were washed in clean running water to eliminate dust, dirt, possible parasites or their eggs and then washed with deionized water. The clean vegetable samples were air-dried and placed in an electric oven at 65 °C for 72–96 hours depending on the sample size and thickness. The dried vegetables samples were homogenized by grinding using a ceramic coated grinder used for metal analysis. All soil samples were spread on plastic trays and allowed to dry at ambient temperature for 8 days. The dried samples of soils were ground with a ceramic coated grinder and sieved through a nylon sieve. The final samples were kept in labeled polypropylene containers at ambient temperature before analysis.

Digestion and metal analysis

Sample of 5 g was measured and transferred into a clean 25 ml conical flask. Exactly 5ml of concentrated H_2SO_4 was added followed by 25 ml of concentrated HNO_3 and 5 ml of concentrated HCL was then added to the sample for digestion. The samples were allowed to be evenly distributed in the acid by stirring with a glass rod; the flask was then placed on the digestion block in a fume cupboard for 2 hours at temperature of 150°C for digestion and then cooled to room temperature. The digested samples were then filtered into a 25 ml volumetric flask and made to mark with deionised water and allowed to settle for at least 15 hours. The digested samples were kept at 4°C prior to analysis. The metal (Pb, Cd, As, and Hg) concentrations were determined by atomic absorption spectrophotometer (AAS). Analysis of each sample was carried out three times to obtain representative results and the data reported in mg/kg (on a dry matter basis). Statistical differences were performed by ANOVA comparisons test by using SPSS version 23.

Translocation factor

The translocation factor was calculated by dividing the concentration of heavy metals in vegetables by the total heavy metals concentration in the soil for each metals. This index of soil – plant transfer or intake of metals from soil through vegetables was calculated using the following formular described by (Oguh *et al*., 2019b).

$$TF = \frac{C_{veg}}{C_{soil}}$$

Where; TF represent the translocation factor of vegetable
C_{veg} = metal concentration in vegetable tissue, mg/kg fresh weight
C_{soil} = metal concentration in soil, mg/kg dry weight.
TF > 1 indicates that the vegetable are en-riched in elements from the soil (Bio-accumulation)
TF < 1 means that the vegetables exclude the element from soil (excluder)

RESULTS

The levels of heavy metals investigated in the vegetable study were based on dry weight. The values are given as

Table 1: Soil Heavy metals Concentration.

Metals	Soil metal concentration			
	Before application	NPK soil	Snail faces soil	PL(WHO/FAO, 2016 Mg/kg
Cd	0.59 ± 0.07	1.36 ± 0.03	0.83 ± 0.02	3
Pb	0.95 ± 0.02	2.42 ± 0.04	1.28 ± 0.04	50
As	0.26 ± 0.08	2.75 ± 0.12	1.76 ± 0.10	20
Hg	0.09 ± 0.01	0.83 ± 0.19	0.35 ± 0.07	2.00

Table 2: Mercury concentration.

Vegetable samples	Mercury (Hg) concentration	
	Fertilizer (NPK)	Snail faces
Solanum melongena	1.07 ± 0.04	0.19 ± 0.02
Amaranthus cruentus	1.04 ± 0.13	0.16 ± 0.03
Talinum triangulare	1.02 ± 0.09	0.18 ± 0.01
Corchorus olitorius	1.08 ± 0.08	0.13 ± 0.05
PL of Hg in vegetable	0.1 mg/kg by (WHO/FAO, 2016)	

PL= Permissible limit

Table 3: Arsenic concentration.

Vegetable samples	Arsenic (As) concentration	
	Fertilizer (NPK)	Snail faces
Solanum melongena	2.76 ± 0.12	1.28 ± 0.01
Amaranthus cruentus	2.85 ± 0.09	1.34 ± 0.07
Talinum triangulare	3.01 ± 0.12	1.18 ± 0.02
Corchorus olitorius	2.82 ± 0.11	1.37 ± 0.12
PL of As in vegetable	0.5 mg/kg by (WHO/FAO, 2016)	

mean ± STD and the results are means of three replicates. The results obtained from the vegetable samples, and soil samples collected from the two groups for the mean concentration of As, Cd, Hg and Pb are summarized in the tables and chart below:

DISCUSSION

Heavy metals in soils have become an alarming threat to both the ecosystem and human health. The results of the soils showed that the concentration of heavy metals in the soil before amending with nutrients were below the permissible limit in soil. The concentrations of metals in soil with NPK fertilizer are greater than the soil amended with snail faces. The mean concentration of Cd and Pb in NPK soil (1.36, and 2.42 mg/kg) and snail soil (0.83 and 1.28 mg/kg) respectively were above the WHO/FAO, 2016 permissible limits of 3.0 mg/kg Cd and 50 mg/kg Hg for soil. The concentrations of As and Hg recorded were below the WHO/FAO, 2016 permissible limit of 20, and 2.0 mg/kg respectively for soil. The study revealed that the concentrations of all elements analysed were significantly ($P < 0.05$) higher at the soil amended with NPK compared to the soil amended with snail faces (Tables 1 to 5).

Heavy metals are considered the most important constituents of pollution from the terrestrial environment due to toxicity and accumulation by plant. The entire vegetable samples amended with NPK and snail faces contained detectable levels of the elements studied. The accumulation of these heavy metals by plants may represent a health risk, especially for populations with high consumption rates of vegetables. One-way Analysis of variance (ANOVA) revealed a significant ($P<0.05$) variation in the concentrations of metals in vegetable samples amended with NPK and snail faces, which is an indication of the extent of metal pollution from the application of nutrients. Generally vegetable amended with NPK fertilizer had higher heavy metals concentrations (As, Cd, Hg, and Pb) than vegetables amended with snail faces, which were all above the FAO/WHO, 2016 permissible limit. Heavy metals and nutrients absorbed by plants are usually translocated to different parts of the plants which could limit the concentrations in the soil. However, availability of metals in the soil and continuous absorption by the plant could lead to higher concentration in the leaves of the vegetables.

The high concentration of As, Pb, Hg and Cd in NPK amended vegetable may be due atmospheric deposition of the metal from non – ferrous metal activities, combustion, etc. which can be absorbed into foliage and translocated through the plant. The bio-accumulated metal on the vegetables may interact directly with biomolecules such as nucleic acid, protein, carbohydrate,

Table 4: Cadmium concentration.

Vegetable samples	Cadmium (Cd) concentration	
	Fertilizer (NPK)	Snail faces
Solanum melongena	1.42 ± 0.04	0.79 ± 0.09
Amaranthus cruentus	1.60 ± 0.03	0.66 ± 0.04
Talinum triangulare	1.53 ± 0.09	0.67 ± 0.08
Corchorus olitorius	1.38 ± 0.13	0.78 ± 0.02
PL of Cd in vegetable	0.20 mg/kg by (WHO/FAO, 2016)	

Table 5: Lead concentration.

Vegetable samples	Lead (Pb) concentration	
	Fertilizer (NPK)	Snail faces
Solanum melongena	2.62 ± 0.12	1.10 ± 0.03
Amaranthus cruentus	2.85 ± 0.12	1.13 ± 0.04
Talinum triangulare	2.68 ± 0.16	1.16 ± 0.01
Corchorus olitorius	2.92 ± 0.19	1.17 ± 0.10
PL of Pb in vegetable	0.3 mg/kg by (WHO/FAO, 2016)	

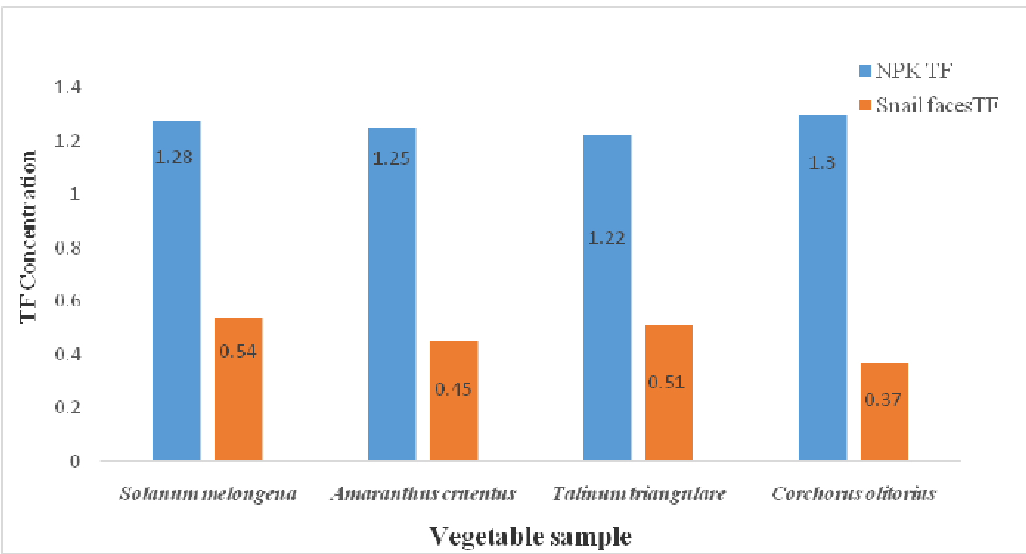

Figure 1: Mercury (Hg) Translocation Factor

disrupting critical biological processes, resulting in toxicity and the concomitant transfer of these metals through the food chain could ultimately pose risk to human life (Huang *et al*., 2017).

The concentration of Hg in *Solanum melongena, Amaranthus cruentus, Talinum triangulare,* and *Corchorus olitorius* show that plants amended with NPK fertilizer accumulate more metals which is higher than the WHO/FAO permissible limit of (0.1 mg/kg). Mercury poisoning symptoms include blindness, deafness, brain damage, digestive problems, kidney damage, lack of coordination and mental retardation. The ability of snails to accumulate essential metals equally enables them to acquire other nonessential metals from the soil. The concentration of As in vegetables show that plants amended with NPK fertilizer accumulate more metals which is higher than the WHO/FAO permissible limit of (0.5 mg/kg). Arsenic affects almost all organs during its acute or chronic exposure. Liver has been reported as target organ of arsenic toxicity. Toxicity is due to arsenic's effect on many cell enzymes, which affect metabolism, DNA repair and brain problem. The most prominent chronic manifestations of As involve the skin, lungs, liver and blood systems. The concentration of Cd in vegetables show that plants amended with NPK fertilizer accumulate more metals which is higher than the WHO/FAO permissible limit of (0.20 mg/kg). Cadmium is a dangerous element because it can be absorbed via the alimentary track; penetrate through placenta during pregnancy and damage membrane and DNA. Significant

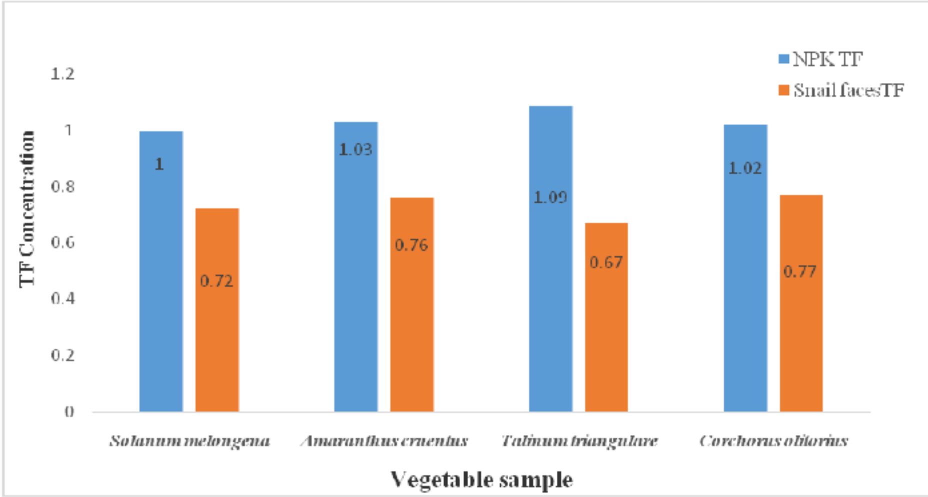

Figure 2: Arsenic (As) Translocation Factor

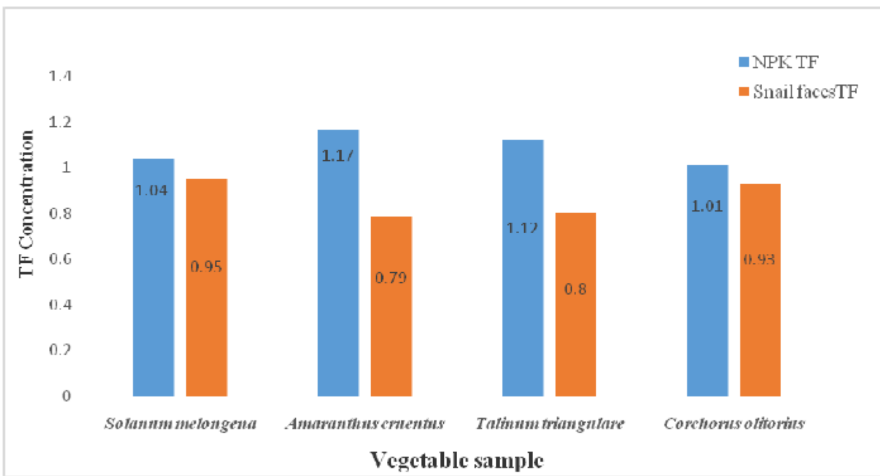

Figure 3: Cadmium (Cd) Translocation Factor

concentration of Cd may have gastrointestinal effect and reproductive effect on livestock (Maobe *et al.,* 2012). Ndukwe *et al.,* 2017 reported that cadmium causes both acute and chronic poisoning, adverse effect on kidney, liver, vascular and the immune system. The concentration of Pd in vegetables shows that plants amended with NPK fertilizer accumulate more metals which are higher than the WHO/FAO permissible limit of (0.3 mg/kg). Lead has no beneficial biological function and is known to accumulate in the body. Basapor and Ngabaza, 2015 reported that lead causes both acute and chronic poisoning and thus, poses adverse effects on kidney, liver, vascular and immune system. Lead can cause serious injury to the brain, nervous system, red blood cells, low IQ, impaired development, shortened attention span, hyperactivity, mental deterioration, decreased reaction time, loss of memory, reduced fertility, renal system damage, nausea, insomnia, anorexia, and weakness of the joints when exposed to high lead.

The soil to plant translocation factor TF is one of the key components of human exposure to toxic elements through the food chain. The TF in soil amended with NPK and soil amended with snail faces were significantly different. This indicates that the high presences of metals in the soil amended with NPK was also transferred to the vegetables. The more the presence of metals in the soil the more the bioaccumulation on the vegetables. The soil to plant TF in vegetable amended with NPK for As, Cd, Hg and Pb were all greater than one (>1), While the TF in vegetable amended with snail faces for As, Cd, Hg, and Pb were below one (<1). This indicates that the vegetable do not take up much toxic element from the soil amended with snail faces (Figures 1-4). Where TF > 1 indicates the vegetable are enriched in elements from the soil (Bio-accumulation). TF < 1 means that the vegetables exclude

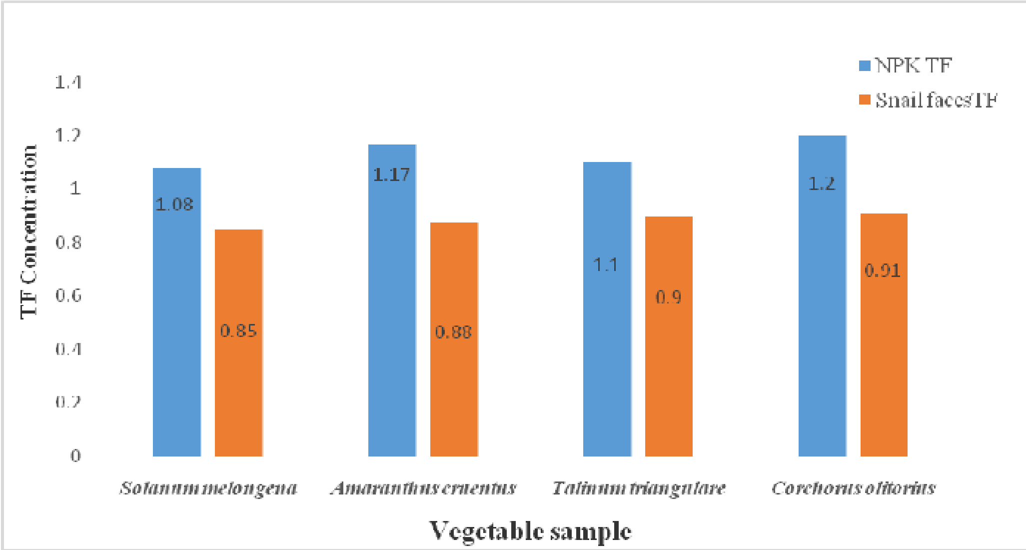

Figure 4: Lead (Pb) Translocation Factor.

the element from soil (Excluder).

Conclusion

The use of fertilizers to amend soil and increase yield of crops have shown to have a significant increase in the level of heavy metals concentration in the soil which is also bio accumulated and transfer to plants. The present study revealed that vegetables grown in NPK amended soil bioaccumulate significantly high levels of trace metals that exceed the maximum prescribed limits for elements in vegetable, which toxicity disrupts natural ecosystems and affects the food chain, leading to deleterious health problems in humans and animals.

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