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Variations in spatial distribution of heavy metals in soil along a continuum and uptake effects on field infestation of okra, *Abelmoschus esculentus* (L.) Moench by *Dysdercus superstitiosus* (Hemiptera: Pyrrhocoreidae)

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Running Head: Heavy metal contamination along a continuum in an urban agro-ecosystem

ABSTRACT

This two year study investigated spatial distribution of heavy metal contaminants in soil along a continuum in an urban agricultural site. The study also assessed uptake bio-concentrations of the toxicants in okra, Abelmoschus esculentus and its effects on field infestation by its major pest, Dysdercus superstitiosus. Two hypotheses tested in the study were: a. the concentration and distribution of heavy metals at each 25m away from the hill crest are the same and random along the continuum; and b. percentage uptake bio-concentration of the toxicants at different strata along the continuum is not different. Results showed that the level of soil contamination with the heavy metals: lead, cadmium and mercury differed significantly (p<0.05) with each 25m distance away from the gently sloping hillcrest along the continuum. The middle stratum (25-50m away from the hillcrest) was the most contaminated (p<0.05) with elemental lead (8.5 – 8.6 mg/kg soil) followed by the Hill-base (≥50m away from the hillcrest) (6.7– 7.3 mg/kg soil) and the hill-crest (6.5 – 7.1 mg/kg soil) compared to the control. There was no significant difference (p>0.05) between concentrations of cadmium at each sampled stratum except with the control site. The concentration of mercury was significantly higher at the hill crest (1.34 - 1.68 mg/kg soil) followed by the middle stratum (1.02 – 1.04 mg/kg soil) and the hill base (1.02 mg/kg soil) compared to the control site (0 mg/kg soil). Mean infestation of okra fruits by D. superstitiosus was significantly (p<0.05) higher at the hill base (58.6 – 63/plant) where mercury concentration was lowest followed by the middle stratum (43.1 – 47/plant) and least at the hill crest area (19.4 – 21.2/plant). The most concentrated heavy metal in the insect body tissue was lead followed by cadmium. Mercury was not found in tissue of the insects. Field infestation was comparatively higher (p<0.05) in okra grown on soils with Lead and Cadmium (48.7%) only than in soils with different concentrations of Lead and Cadmium and Mercury.

Keywords: spatial contamination, bioaccumulation, infestation, heavy metal, mercury

INTRODUCTION

Encouragement of farming activities by the Nigerian government coupled with the global economic down turn has led to a great expansion in the utilization of urban and peri-urban lands for agriculture in recent times. However, these soils and the crops grown on them are under intense risks of toxic metal contamination (Ogunyemi *et al*, 2003). The sources of these contaminations are diverse, from industrial, automobile, domestic to municipal wastes (Botkin and Keller, 1987; Ogunyemi *et al*, 2003).

Okra, *Abelmoschus esculentus* L. Moench. is one of the most important vegetables in Nigeria with general domestic production often exceeding the daily demands of the consuming population (Ogunyemi *et al*, 2003). Vegetables are generally important for their flavour, and desired taste appeal with proven very high nutritive values (Hills and Waller, 1988; Botkin and Keller, 1987; Cummingham and Saigo, 1999; Babalola, *et al*., 2002). Okra is important for its fruits and fresh leaves which are very rich in vitamins A and B as well as protein, mineral salt, phosphorous and iron. The proteins contained in okra fruits generally have higher amino acid ratio compared to many cereals and some leguminous crops and so okra is considered nutritionally better (FAO/WHO, 1978; Food and Agricultural Organization, 1992).

Production of okra is constrained mainly by pests and climatic factors like many other vegetable crops (Rehm, and Espig, 1991). This crop is commonly planted alongside other crops on vacant lands in and around metropolitan cities where in most cases, they accumulate heavy metal elements (Ogunyemi *et al*, 2003;). Heavy metals are known to be very toxic when consumed along with food or feed of man and livestock beyond certain limits. However, the safety of use of urban and peri-urban sites for agricultural activities has remained a subject of increasing interest (Shapiro *et al.,* 1989; Yagdi *et al.,* 2000; Marshall *et al.,* 2003). Availability of such urban soils attracts peasant farmers. However, the sites could actually be fertile to support crops but they are often contaminated with toxic heavy metals. It is known that plants absorb and accumulate heavy metals in the edible portions of crops and this has serious consequences to the consumer (Marshall *et al.,* 2003). For example, heavy metals such as mercury, lead, cadmium and arsenic among others have been indicted by the World Health Organization (WHO) as primary causes of cancer, neo-natal mortality, nerve damage (Minata disease in Japan) and other serious human and livestock health problems (Ananda, 1976). Thus, the vegetable crops planted on such contaminated soils along with the insects that live on them retain these toxicants in their tissues with serious implication on bioaccumulation in the food chain (WHO/UNEP, 1976; United State Environmental Protection Agency, 2003). Despite these, it is obvious that the level of contamination within an agricultural site would vary from one point on a field to another. Therefore, two hypotheses were tested; a. the concentration and distribution of heavy metal toxicants at each 25m distance away from the hill crest (=stratum) are the same and random along the continuum; and b. percentage uptake bio-concentration of the toxicants in living tissues of okra and *Dysdercus superstitiosus* from different strata along the continuum are not significantly different. This is with a view to ensuring production and consumption of safe vegetable that is devoid of toxic chemical contamination beyond codex safe limits.

MATERIALS AND METHODS

Study sites and history

The study was carried out in two years between July and December 2004 and 2005 at two urban agricultural sites located at Oke – Afa Area, Isolo Local Government Area (LGA), Lagos – Nigeria and the University of Ibadan main campus, Ibadan Nigeria. The Lagos site was selected based on history of contamination by municipal and industrial wastes as typical of an urban agricultural site. The 75 x 35m (2625m^2) area selected for the study at the site sloped down to a canal at about 35^o. A major road bounds the site westward while the Oke-Afa canal, where liquid effluents from the Lagos-Ikeja Industrial Estate are emptied; bounds it northward. Lagos is located on the latitude 6^{o}28^1 N and longitude 3^{o}24^{1}E at a relatively low altitude of 100m above sea level. Mean annual rainfall is 1829mm with bimodal peaks in June and October. Natural infestation of okra by *D. superstitiosus* was therefore not constrained by weather variables. This site was originally a marshland dumpsite, which became filled with waste matter particularly industrial and municipal domestic wastes in the early 1950s and 60s. The site thus became a gently sloping hill which transformed to an agricultural site in a continuum. Over the years, peasant farmers cultivated different crops such as maize and cowpea during rainy season and vegetables in the dry season. At the time of this study, okra was the vegetable crop planted. The second study site, the Practical Year Training Programme (PYTP) site of the Faculty of Agriculture and Forestry located within University of Ibadan, Ibadan served as the check. This site has a similar topography and physical characteristics with a perennial stream at the base of the gently sloping (35^o) hill. This site also has a history of cultivation of arable crops especially maize, cowpea and vegetables with careful management. Day neutral dwarf okra variety, NHAe 47-4 obtained from the National Horticultural Institute, Ibadan, Nigeria was planted and used for the study.

Assessment of Spatial distribution of heavy metals along the continuum

Areas for soil sampling were systematically mapped out along the continuum from 0-25m, 25-50m and 50-75m distances away from the hillcrest down the slope at each site. Each sampling point was divided into 20 grids from which 500g soil was taken from the top 10 cm depth using a soil auger and bulked. The soil samples were pre-digested following the method of Allen *et al.* (1974) before analysis for heavy metals using the Atomic Absorption Spectrophometer (AAS). Chemical analysis of the soil samples was done using the wet-digestion method in which 1gram of soil sample was weighed into a conical flask to which was added 10ml of perchloric acid and nitric acid in ratio 1:5. The mixture was heated gently to avoid frothing after which the temperature was increased till clear white fume was emitted. The digest was thereafter washed into 50ml flask and made up to mark with de-ionized water. The heavy metals (lead, cadmium and mercury) were detected by aspiration on the AAS. Data collected was concentration of lead, cadmium and mercury from each sampling stratum.

Assessment of uptake concentration of heavy metals at different trophic levels on infestation of Okra by *Dysdercus superstitiosus*

Ten (10) Okra plants with intact fruits were randomly selected from each of the identified 3 sampling strata. All adults and nymphs of the cotton stainer, *D. superstitiosus* found on each plant (= a replicate) were collected using the drop cloth method (Omoloye *et al*., 2002). The total number of adults and nymphs of the cotton stainer was counted and mean infestation per plant was determined. Each okra plant was cut into pieces and separated into stems and fruits and then sun dried. The insects collected were also dried. Thereafter, the plant samples and the insects collected per replicate were pre-digested following the method of *Allen et al.* (1974) and analyzed for heavy metals using the AAS at different wave bands. The following data were collected: a. uptake bio-concentration of each of lead, cadmium and mercury in the okra plant stem, fruit and the insect. b. population of *D. superstitiosus* infesting plant per stratum.

Data Analysis

Bio-concentration data were square-root transformed $(x+1)^{1/2}$ before analysis of variance (ANOVA) and mean separation using the Tukey Honestly Significant Difference test, (Tukey HSD) at 5% level of probability (SAS, 1987).

RESULTS

Spatial contamination of urban soils and organisms by heavy metals

This study has shown that the spatial occurrence and concentrations of the heavy metals: lead, cadmium and mercury along a sloping hill with an angle of elevation of about 35^o in typical urban agricultural soils highly varied ($p<0.05$) with each 25m distance away from the hillcrest (Tables 1 and 2). The middle stratum (25-50m away from the hillcrest) of the continuum was the most contaminated ($p<0.05$) with elemental lead (8.5 – 8.6 mg/kg soil) followed by the Hill-base (>50m away from the hillcrest) (6.7 – 7.3 mg/kg soil) followed by the hill-crest (6.5 – 7.1 mg/kg soil) compared to the control. There was no significant difference ($p>0.05$) in the level of soil contamination by cadmium at each sampled stratum except with the control (Tables 1 and 2). The concentration of mercury was significantly higher at the hill crest (1.34 - 1.68 mg/kg soil) followed by the middle stratum (1.02 – 1.04 mg/kg soil) and the hill base (1.02 mg/kg soil) compared to the control (0 mg/kg soil).

Uptake bio-concentration of each heavy metal investigated in the stem and fruit of okra at each stratum of the continuum also differed ($p<0.05$) and reflected the same spatial pattern of distribution found in the soil. For example, concentration of lead was highest in the okra stem planted at the middle stratum (3.3 – 3.4 mg/kg) and this translated to ca, 39.1% of the amount of lead in the soil at this stratum (Table 3). The next highly significant concentration of lead in the stem was recorded in okra planted at the Hill-base (>50m away from the hillcrest) with 2.9 - 3.0 mg/kg soil (about 42.5% of soil lead) and the least being in okra planted at the hill-crest (2.7 – 2.8 mg/kg soil) compared to the control (0.7 – 0.9 mg/kg soil) (Tables 1 and 2). Similarly, uptake concentration of lead in the fruit was highest in the okra planted at middle stratum of the continuum (3.1 – 3.2 mg/kg) followed by the Hill-base (>50m away from the hillcrest) (2.6 - 2.8 mg/kg soil) and least in that planted at the hill-crest (2.5 – 2.6 mg/kg soil) compared to the control (0.7 – 0.9 mg/kg soil) (Tables 1 and 2). However, the differences in the bio-concentration values obtained for the stem, fruit and insect body at each stratum along the continuum of each site were not significant ($p>0.05$). Similarly, there was no significant difference in the uptake concentration of cadmium with regard to spatial distribution along the continuum in soil, stem and fruits as well as in the insect (Tables 1 and 2). However, uptake concentration of mercury was significantly higher at the hillcrest (1.3 – 1.68 mg/kg) than the Middle stratum (1.02-1.04 mg/kg) and the hill base (1.02 mg/kg) compared to the control (0 mg/kg).

The stratum along the continuum and the level of contamination not withstanding, uptake concentration of each heavy metal investigated in this study decreased progressively from the soil up the stem to the fruit of okra (Table 3). The decrease also varied with each heavy metal investigated. For example, uptake bio-concentration of lead relative to the concentration in the soil ranged between 36.7 – 42.5% in the stem. In the fruit, it ranged between 36.9 and 38.7% and did not differ significantly ($p>0.05$) at each stratum along the continuum at the experimental site but differed significantly ($p<0.05$) with the control (Fig.1). Also, percentage accumulation of cadmium in the okra stem compared to the level in soil did not differ ($p>0.05$) at each stratum but differed significantly ($p<0.05$) with the control. In the insect body tissue, although percentage concentration of cadmium ranged between 3.3 – 4.99%, this was not significantly ($p>0.05$) different from the control site where cadmium was not detected at any stratum in the insect body. Uptake concentration of mercury that was detected in the stem and fruits of okra was comparatively very low (<1%) compared to the concentrations in the soil. Mercury was not detected at all in both the fruit of okra and in the body of insects irrespective of the site.

Field infestation of okra by *D. superstitiosus* varied significantly ($p<0.05$) with spatial strata at both experimental and the control site investigated in this study. Comparatively, field infestation was significantly lower at the experimental site control (21.2 – 58.6 insects per plant) than in the control (54.2 – 76.4 insect per plant). Spatially, there was significantly higher number of insects per plant at the hill base of each site than other strata, the site notwithstanding (Tables 1 and 2).

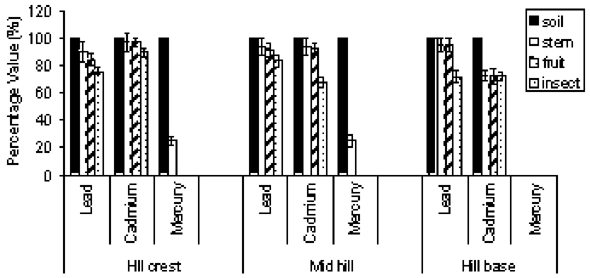

Fig.1: Uptake retention of three heavy metals in tissues of okra, *Abelmoschus esculentus* and its major insect pest, *Dysdercus superstitiosus* as percentage of soil concentration along the continuum in an urban vegetable site, Lagos, Nigeria

Table 1: Mean infestation intensity of Okra, *Abelmuschus esculentus* by *Dysdercus superstitiosus* in relation to heavy metal contamination at an urban agricultural site, Oke Afa – Lagos and Practical Year Training programme (PYTP) site, University of Ibadan Nigeria. Dry season crop 2002

Sampling points (Distance from Hill Crest)	Mean Infestation per plant (no./plant) n=10	Mean Concentration (mg/kg) *											
		Lead (Pb)				Cadmium (Cd)				Mercury (Hg)			
		Soil	Stem	Fruit	Insect	Soil	Stem	Fruit	Insect	Soil	Stem	Fruit	Insect
Oke-Afa, Lagos													
A - Hill Crest area (0-30	19.4± 6.4d	7.10b	2.80a	2.6a	1.3a	9.34a	1.30a	1.30a	0.20a	0.08a	0.02	0a	0a
B - Middle Stratum (30-60)	47.0±6.7c	8.60a	3.40a	3.2a	1.0a	9.40a	1.31a	1.29a	0.20a	0.04b	0.01	0a	0a
C- Base of hill (60-90m)	63.1±5.5b	7.30b	3.04a	2.6a	0.9a	9.60a	1.20a	1.20a	0.20a	0c	0a	0a	0a
PYTP site, U.I. Ibadan													
A - Hill Crest area (0-30	48.6± 5.5c	2.09c	0.72b	0.47 b	0.21a	4.26b	0.18b	0.12b	0a	0c	0a	0a	0a
B - - Middle Stratum (30-60)	67.5±4.8b	2.12c	0.81b	0.54 b	0.34a	4.32b	0.19b	0.14b	0a	0c	0a	0a	0a
C- Base of hill (60-90m)	88.1±6.2a	2.20c	0.91b	0.72 b	0.36a	4.48b	0.27b	0.21b	0a	0c	0a	0a	0a

Means transformed $(x+1)^{1/2}$ before

DISCUSSION AND CONCLUSION

This study showed that the spatial distribution of the heavy metal contaminants investigated in this study did not reflect any distinctive spatial pattern at different strata along the gently sloping continuum. This suggests a random spatial distribution of the heavy metals along the continuum especially in such slope that is set at an elevation of about 35^o. It is therefore inferred that the spatial distribution of heavy metals especially lead, cadmium and mercury at each stratum along a continuum depends to a large extent on contaminations from anthropogenic sources especially by direct localized discharge of industrial emissions and effluents instead of localized concentration at particular stratum along a continuum.

The study also showed that the urban agricultural site, Oke-Afa, Lagos that was investigated in this study was a contaminated site with lead, cadmium and mercury. Except for lead in which the level of contamination was comparatively lower than the maximum permissible concentration limits of potential toxic elements (PTE) (EC, 2001), the level of soil contamination with cadmium and mercury exceeded the International Standard Tolerable Limits of 3.0 mg/kg and 1.0 mg/kg respectively. Such contamination has been attributed to a wide range of sources including wastes from small-scale industries, re-suspended road dust, vehicular emissions and gaseous emissions from generator sets (Marshall *et al.,* 2003**).** Onianwa and Ajayi (1987) reported that the high contamination level of cadmium in highly populated urban centre could be attributed to urban activities such as large scale refuse burning, welding and small metal works.

The heavy metal with the highest concentration in soil and the living tissues of okra plants and the fruit insect pest, *D. superstitiosus* was lead followed by cadmium, although the concentrations in the plant and insect tissues were below the recommended maximum concentration limit of 2mg/kg for lead and a tolerable daily intake of 0.0057-0.0071mg/kg body weights for cadmium (EC, 2001). High concentration of heavy metals in living tissues particularly of lead in the stem and fruit of okra is known to be characteristic of malvaceous plants (Ogunyemi *et al.,* 2003). However, mercury was not detected in the fruit and the insect tissues investigated in this study. It is known that high concentrations of heavy metals have serious implications for bio-magnification in human and other large animal systems and are thus of serious environmental concerns (Tucker, 2003). The liver and kidney are known to harbour half the total cadmium in the body and its bioaccumulation has been related to hypertension and cardiovascular diseases of humans (Onianwa and Ajayi, 1987; Ogunyemi *et al.*, 2003). Lead has been implicated in constipation, vomiting, anaemia, swelling of the brain, paralysis and even death when ingestion is acute (Shapiro *et al.*, 1989). Chang (1992) reported that lead interferes with the function of mitochondria, thereby impairing respiration. However from this study, it appears that the concentrations of the heavy metals in the okra fruit investigated were below the potential toxic level and this suggests that okra planted at the site is comparatively safe for human and livestock consumption.

Although infestation of okra by *D. superstitiosus* was comparatively lower at the contaminated site compared to the control site in Ibadan, there was no tangible reason to suggest that the occurrence of the heavy metals was responsible for the comparatively low infestation level. This is because there was a comparatively low infestation from hill crest and progressively down the slope along the continuum at both sites. This implies that the distance away from water source rather than heavy metal contamination could be the reason for higher infestation at the hill bases. It is known

Table 2: Mean infestation intensity of Okra, *Abelmuschus esculentus* by *Dysdercus superstitiosus* in relation to heavy metal contamination at an urban agricultural site, Oke Afa – Lagos and Practical Year Training programme (PYTP) site, University of Ibadan Nigeria. Dry season crop 2003

Sampling points (Distance from Hill Crest)	Mean Infestation per plant (no./plant)	Mean Concentration (mg/kg) *											
		Lead (Pb)				Cadmium (Cd)				Mercury (Hg)			
	n=10	Soil	Stem	Fruit	Insect	Soil	Stem	Fruit	Insect	Soil	Stem	Fruit	Insect
Oke-Afa, Lagos													
A- Hill Crest area (0-30)	21.2± 3.6e	6.53b	2.7a	2.5b	1.21a	6.31a	1.18a	1.12a	0.3a	0.04a	0.01a	0b	0a
B - Middle Stratum (30-60)	43.1±5.2d	8.53 a	3.3a	3.1a	1.74a	6.38a	1.29a	1.13a	0.5a	0.02b	0.01a	0a	0a
C- Base of hill (60-90m)	58.6±5.5c	6.7b	2.9a	2.8b	1.81a	6.61a	1.27a	1.21a	0..3a	0c	0b	0b	0a
PYTP site, U.I. Ibadan													
A- Hill Crest area (0-30	54.2± 4.8c	2.16c	0.91b	0.66c	0.41a	4.46b	0.26a	0.15a	0b	0c	0a	0a	0a
B - Middle Stratum (30-60)	68.2±5.6b	2.32c	0.82b	0.69c	0.42a	4.44b	0.29a	0.14a	0b	0c	0a	0a	0a
C- Base of hill (60-90m)	76.4±8.2a	2.39c	0.88b	0.78c	0.52a	4.68b	0.49a	0.24a	0b	0c	0a	0a	0a

Means transformed $(x+1)^{1/2}$ before analysis of variance
Means within the same column in each season followed by the same alphabet are not significantly different ($p<0.05$). Tukey HSD Test
* n=3

that high water table due to proximity to stream at the areas around the hill base could facilitate better water uptake by the okra plant, thus making the plants therein to be lush and healthy and therefore attractive to insect pest. It is also inferred that the favourable environment caused by the ameliorated atmospheric temperature around the hill-base, coupled with the less dust-coating of plant and insect surface due to the distance away from the roads predispose the okra plants at the hill-base to heavy infestation.

It is therefore concluded from this study that heavy metal contamination at the study sites at Oke-Afa, Lagos and the PYTP site, University of Ibadan was random; and that infestation of okra by *D. superstitosus* was not determined by level of heavy metal contamination. The study also affirms that the concentration of heavy metals in the okra fruits from the sites used in this study was below the potentially toxic levels and is therefore safe for human and livestock consumption.

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Table 3: Percentage uptake of heavy metals in living tissues of plant and insect herbivore at different strata along the continuum from soil

Spatial stratum along continuum	Mean uptake accumulation of heavy metals compared to concentration in soil (%)							
	Lead			Cadmium			Mercury	
	Stem	Fruit	insect	Stem	Fruit	insect	Stem	Fruit
Oke-Afa, Lagos								
Hill crest area	40.39a	37.45a	18.42a	16.31a	15.84a	3.45a	0.97a	0a
(0-30m)	(39.43-41.35)	(36.62-38.28)	(18.31-18.53)	(13.92-18.70)	(13.92-17.75)	(2.14-4.75)	(0.75-1.19)	
Middle stratum	39.11a	36.93ab	15.78a	17.08a	15.72a	4.99a	0.97a	0a
(30 – 60m)	(38.69-39.53)	(36.34-37.21)	(11.63-19.93)	(13.94-20.22)	(13.72-17.71)	(2.13-7.84)	(0.96-0.98)	
Hill base	42.46a	38.71a	19.67a	15.86a	15.41a	3.31a	0a	0a
(60 – 90m)	(41.64-43.28)	(35.62-41.79)	(12.33-27.01)	(12.5-19.21)	(12.5-18.31)	(2.08-4.54)		
PYTP site, UI, Ibadan								
Hill crest area	38.29a	26.53c	14.52a	5.03b	3.09b	0b	0a	0a
	(34.45-42.13)	(22.49-30.56)	(10.05-18.98)	(4.23-5.83)	(2.82-3.36)			
Middle stratum	36.78a	27.61c	17.07a	5.47b	3.20b	0b	0a	0a
	(35.34-38.21)	(25.47-29.74)	(16.04-18.10)	(4.40-6.53)	(3.15-3.24)			
Hill base	39.09a	32.69bc	19.06a	8.25b	4.91b	0b	0a	0a
	(36.82-41.36)	(32.73-32.64)	(16.36-21.76)	(6.03-10.47)	(4.69-5.13)			

Treatment means followed by the same letter in the same column are not significantly different (Tukey HSD $p<0.05$)

Range in parenthesis

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