Experimental Study Determining Levels of Lead Contamination of Dioscorea Spp. (Yams) From Selected Regions of Kampala Capital City, Uganda.

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Abstract

Background:
Heavy metal toxicity is a growing concern and often an unknown root cause of a number of serious health issues. Due to bio-accumulation, heavy metals are passed up the food chain from plants to humans. The objectives of this study were to determine the quantity of lead in yams from selected swamps and upland gardens, to compare lead contamination in yams from swamps to those from upland gardens and compare the levels of lead in yams from swamps and upland gardens to the FAO/WHO acceptable limits.

Methods:
Ready to eat/mature yams were harvested from swamps and upland gardens respectively and their lead content determined using an atomic absorption spectrophotometer (AAS).

Results:
Yams collected from RS, a swamp site had the lowest amount of lead at a mean lead level of 1.110 mg/kg. Yams collected from KCU which was an upland garden had the highest amount of lead with mean lead concentration of 2.324mg/kg. Yams obtained from upland gardens contained a higher concentration of lead at a mean lead concentration of 1.7858 mg/kg compared to a mean lead concentration of 1.6172 mg/kg which was found in yams collected from the swamp sites. However, there was no statistically significant difference in lead contamination of the yams obtained from either site. The concentration of lead in both yams collected from swamps and those obtained from upland gardens was higher than the FAO/WHO acceptable limit of lead in food.

Conclusion: [a]
The level of lead contamination in yams is not dependent on the agricultural site where they are grown. Concomitantly, lead levels in yams regardless of their site of growth, were above the FAO/WHO maximum acceptable limits of lead in food and thus suggested that yams pose a significant route of human exposure to lead once they are consumed.

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1 Background

Heavy metals are elements with an atomic density greater than 6 g/cm3. They are one of the most common and persistent pollutants in waste water, with the most common toxic heavy metals being lead, cadmium, arsenic among others. The persistence of heavy metals in wastewater is due to their non-biodegradable nature[1].

The two main sources of heavy metals in wastewater are natural and human. The natural factors include soil erosion, volcanic activities, urban run offs and aerosols particulate while the human factors include metal finishing and electroplating processes, mining extraction operations, textile industries and nuclear power all ramifying from the rampant industrialisation that inevitably generates effluent.

Untreated or inadequately treated wastewater effluents contaminated with heavy metals can cause a variety of health and environmental impacts, when released into receiving water bodies. In aquatic ecosystems, heavy metals greatly depress the number of living organisms. Heavy metals have negative effect on the growth of aquatic organisms and can cause serious upsets in biological wastewater treatment plants.

Lead (Pb) is a heavy metal with atomic number 82, atomic weight 207.19 and a specific gravity of 11.34. It is a bluish or silvery-grey metal with a melting point of 327.5 °C and a boiling point of 1740°C at atmospheric pressure. It has four naturally occurring isotopes with atomic weights 208, 206, 207 and 204 (in decreasing order of abundance).

Lead occurs primarily in the inorganic form in the environment. Lead is a ubiquitous pollutant in the ecosystem. Following the industrialisation in Uganda, enormous quantities of industrial effluent is released into the environment, through the drainage channels, into water bodies that end up in wetlands. The industrial effluent is often heavily contaminated with heavy metals such as lead, arsenic, and cadmium among others accruing from the industrial waste.

Given the ubiquitous nature of heavy metals such as lead in the environment, it is inevitable that they end up in the food chain. Incidentally, the main route of exposure to heavy metals for most people is through the diet[18].

Wetlands especially in Uganda are a major catchment area for the industrial waste, unfortunately though coincidentally, these wetlands are at the same time used for agricultural purposes. A number of crops such as yams, rice, sugarcanes among others are grown in wetlands in Uganda.

There is an enormously great likelihood that such plants especially those that play an essential role in bioremediation, some of which are edible such as yams could accumulate the heavy metals in the different plant parts resulting into bioaccumulation and heavy metal toxicity along the food chain.

The WHO maximum limits of lead in cereals and legumes are 0.2 μg/g, and 0.3μg/g for vegetables[15].

The Commission of the European Communities (2001) and the Codex Alimentarius Commission (2001, 2004) set 0.1mg/kg as the Maximum allowable levels of Pb for vegetable crops[11].

According to Kooner, Mahajan, & Dhillon[13], the site of growth noticeably influences the heavy metal uptake by vegetables and contamination with lead occurs in vegetables grown on contaminated soils. Wastewater irrigation contributes significantly to the heavy metal contents of soils[5]. Irrigation by sewage water and industrial effluents has been identified as the main reason for accumulation of heavy metals in the vegetables[9].

Food is an important pathway of heavy metal exposure to humans and thus undertaking risk assessment is an important precautionary measure towards attainment of millennium development goals. This is more evident as the highest metal intake was observed in fruit Juice /wine drink as well as the carbohydrate food[17].

According to a study by Twinamatsiko, Mbabazi, & Twinomuhwezi[19] which was carried out to investigate the heavy metal contamination of Soil and plant samples from farms around the dump sites in Gulu Township Pece wet land while using samples from Katikamwe wet land in Bushenyi as a control site, it was found that lead levels in cocoyam and sugarcanes grown in the urban drainage system soils were significantly higher than those of the same crops grown in rural control sites.

Abnormally high levels of lead (474.14 mg/kg) were detected in Cocoyam (Colocasia esculentum) grown on Soil receiving Effluent from a Paint Industry. However, the study revealed that there was no significant correlation between lead levels in the soil and lead levels in the tubers[20].

According to a study by Kalagbor, Adooh, & James[12] undertaken to determine the concentration of five Heavy Metals in White Yam (Dioscorea Rotundata) and three- Leaved Yam (Dioscorea

Dumetorum) from Farms in Khana, Rivers State (Nigeria), lead and copper were found to be above the FAO/WHO acceptable limits of 0.1 mg/kg and 0.5 mg/kg respectively.

Continuous consumption of yams contaminated with lead results into Bioaccumulation in humans which poses health risks like; anaemia, body weakness, kidney and brain damage or even death[14]. High concentrations of lead can cause irreversible brain damage, seizure, coma, and death if not treated immediately. It has also been shown that lead may cause fatigue, irritability, memory problems, and reduction in sensory and motor reaction times, decision making impairment, low I.Q and lapses in concentration. In adults, lead is very detrimental to the cardiovascular system.

The heavy metal content of plants such as yams which are accumulator plants has often not been determined prior to consumption or distribution in the market which throws food safety into question. This study was therefore to determine the quantity of lead in yams from selected swamps and upland gardens, to compare lead contamination in yams from swamps to those from upland gardens, as well as compare the levels of lead in yams from swamps and upland gardens to the FAO/WHO acceptable limits.

2 Methodology

3 Ethical consideration

This study was exempted from ethical clearance by the research ethics committee of college of veterinary medicine, animal resources and bio security (COVAB) Makerere University, since it was neither a human nor an animal research study. The samples were collected with permission from the local authorities and the respective farmers. Only a modest payment was given to the farmers that provided us with the yams and this was given after collecting the samples to avoid bias in sample collection.

4 Study design

The study at hand was an experimental study where yam samples from different study sites were analysed to determine their lead content.

5 Study area

Yam gardens in wetlands and uplands within and around Kampala district were sampled. The samples were collected for six days from 14th October to 19th October 2018.

6 Study population

In this study, only yams were sampled and analysed for their lead content. The yams were collected from swamps and upland gardens of selected divisions in Kampala district. These included; KS: Kawempe swamp, RS: Rubaga swamp, KCS: Kampala Central swamp, NS: Nakawa swamp, MS: Makindye swamp, KU: Kawempe upland, RU: Rubaga upland, KCU: Kampala central upland, NU: Nakawa upland, and MU: Makindye upland

7 Inclusion and exclusion criteria

Only Ready/mature yams were harvested and analysed in this study. The yam samples analysed were freshly harvested from the garden; none of the yam samples pre-harvested by the farmers were included in this study. Gardens in kampala district were particularly selected because of the heavy industrialisation in the region happening simultaneously with agriculture and other economic activities.

8 Sampling and sample size

The stratified random sampling strategy was used to collect five samples of yams from gardens in the wetlands/swamps; similarly five samples of yams were collected from gardens in the dry lands/uplands.

In a sample collection site, five yams were randomly selected and these were pooled into a single sample until five samples were obtained for both categories of the sample collection sites. This made a total of 50 samples collected. These were harvested from points at least 100 metres apart in order to obtain representative samples. Thereafter, the freshly harvested samples were packed in labelled plastic bags and immediately transported to Natural Chemotherapeutics Research Institute (NCRI) Toxicology department for analysis.

9 Sample processing

The samples were thoroughly washed with distilled water to remove soil particles. Thereafter, they were cut into pieces of uniform size and then air dried. Air dried samples were then dried in a dehydrator for 3 days at 40◦C. These dried samples were then crashed into fine powder using an electric blender and stored in polythene bags prior to acid digestion.

From the finely ground sample, 2.0g were weighed into a clean dry 250 ml Pyrex conical flask and 5mls of distilled water added. To this, 25mls of concentrated nitric acid were then added. The contents were then digested in a water bath at 90◦C for4 hours after which they were left to cool for 30 minutes. Following that, 2 ml of concentrated hydrochloric acid were added and the volume made up to 100ml using distilled water. The lead calibration curve was made at 0, 1,2,3,4 and 8 ppm. The lead concentration in the samples was then determined using the GFA-EX71 SHIMADZU Graphite furnace atomizer based on the beer-lambert's law of absorbance.

10 Sample analysis

Standard samples of lead were prepared from the stock solutions and used to calibrate the Atomic Absorption Spectrometer**.** The clear supernatants from digestion of the yam samples were analysed for lead levels using the Atomic Absorption Spectrometer at a wavelength of 217nm.

11 Quality control

Only freshly harvested yams obtained from the garden were analysed in this study.

The yams were thoroughly washed and rinsed with distilled water prior to analysis in the machine. This was aimed that there was no heavy contamination arising from the soil on the tubers.

To test the efficiency of the techniques used, pure standard $1 \times 10{-}4$ M aqueous solutions of each of Pb^{2+}will be prepared by dissolving requisite amounts of Soluble salts of the metal in deionized/distilled water, making up to 1 litre, and diluting 100 ml of the solution ten-fold. 500 ml of this solution was subjected to a similar treatment as described before.

Additionally, analytical blanks were prepared by repeating the respective digestion procedures, minus the samples, and subsequently used to determine the instrument detection limits. In each case a read-out from the screen was taken as the concentration of the selected metals.

12 Data analysis

The raw data obtained from the AAS were entered into a Microsoft excel document, imported and analysed using SPSS version 25 in order to determine the mean lead concentration in the samples.

13 Results

Lead levels in yams collected from selected swamps and upland gardens

According to the findings of the study, yams collected from RS, a swamp site had the lowest amount of lead at a mean lead level of 1.110 mg/kg. It was also found that yams collected from KCU which was an upland garden had the highest amount of lead with mean lead concentration of 2.324mg/kg (Table 1).

14 Comparison of lead levels in yams collected from swamps to those collected from upland gardens

The study revealed that yams obtained from upland gardens contained a higher concentration of lead at a mean lead concentration of 1.7858 mg/kg compared to a mean lead concentration of 1.6172 mg/kg which was found in yams collected from the swamp sites. However, there was no statistically significant difference in lead contamination of the yams obtained from either site.

Considering that the 95% confidence interval of the mean difference in lead levels for both yams obtained from the swamps and yams obtained from upland gardens included a zero [-0.4515, 0.1143] and the p-value >0.05, the study implied that yams from either site could be equally contaminated with lead (Table 2).

Comparison of lead levels in yams collected from swamps and those obtained from upland gardens to the FAO/WHO acceptable limits of lead in food

The concentration of lead in both yams collected from swamps and those obtained from upland gardens was higher than the FAO/WHO acceptable limit of lead in food given the 95% confidence interval of the mean difference in lead levels; [1.2905, 1.7439] for yams obtained from the swamps and [1.5043, 1.8672] for yams obtained from upland gardens. The mean difference in lead levels found in yams from both the swamps and upland gardens,

Table 1. Mean lead (Pb) levels in yams collected from various swamps and upland gardens (mg/kg)

Site of sample collection	Mean ± Std. Error	95% Confidence Interval for Mean	
		Lower Bound	Upper Bound
KS	1.9100 ± 0.1646	1.4529	2.3671
RS	1.1100 ± 0.1676	0.6446	1.5754
KCS	2.1250 ± 0.2616	1.3986	2.8514
NS	1.3480 ± 0.2235	0.7274	1.9685
MS	1.5930 ± 0.1414	1.2004	1.9856
KU	1.5470 ± 0.2075	0.9710	2.1230
RU	1.6420 ± 0.1668	1.1790	2.1050
KCU	2.3248 ± 0.1473	1.9159	2.7337
NU	1.5080 ± 0.1065	1.2122	1.8038
MU	1.9070 ± 0.1136	1.5916	2.2224

Key: ±: plus or minus, KS: Kawempe swamp, RS: Rubaga swamp, KCS: Kampala Central swamp, NS: Nakawa swamp, MS: Makindye swamp, KU: Kawempe upland, RU: Rubaga upland, KCU: Kampala central upland, NU: Nakawa upland, MU: Makindye upland

Table 2. Comparison of the level of lead contamination in yams collected from swamps to those in yams obtained from upland gardens.

	Site where yams were collected	Mean± Std. Error	Mean difference	95% Confidence interval of the difference	
				Lower	Upper
Lead levels in yams (mg/kg)	Swamp	1.6172 ± 0.1098	-0.1685	-0.4515	0.1143
	Upland garden	1.7858 ± 0.0879			

Key: ± plus or minus, mg/kg: milligram/kilogram

and the acceptable limit of lead in food according to FAO/WHO was statistically significant (p-value < 0.001) (Table 3).

15 Discussion.

This study aimed at determining the level of lead contamination in yams obtained from selected swamps and upland gardens, comparing the lead levels between the two sample categories and also to the FAO/WHO acceptable limits of lead in food.

The study noted that one of the swamp sites, RS had the lowest lead concentration in its yams while an upland garden, KCU registered the highest amount of lead in its yams. This was much to the astonishment of the research hypothesis but was attributed to the complex chemical reactions that might occur in the dump site (swamp) resulting into conversion of most of the lead into a complex form which cannot be sufficiently absorbed by the plants hence the yams obtained from a swamp site having lower levels of lead than those from an upland garden.

It could also be thought that yams grown in swamps are more deep-rooted than those in upland gardens, possibly in a bid to evade water and get more firmly established in the soil and a reduction in lead uptake comes as a consequence of this since studies by Kachenko & Singh[11], and Mwegoha & Kihampa[16], demonstrated that lead levels decrease with soil depth.

This finding was congruent with that by Udosen, Akpan, & Sam[20], were abnormally high levels of lead (474.14 mg/kg) were detected in Cocoyam (Colocasia esculentum) grown on Soil receiving Effluent from a Paint Industry and that by Zhong, Ren, & Zhao[21], were the lead contents in tea leaves ranged from 0.48 mg/kg to 10.57 mg/kg although this study attributed the elevated lead levels detected in some samples of tea leaves to contamination during sample processing.

However, the study revealed that the soils or agricultural sites from where the yams were collected

Table 3. Comparison of lead levels in yams collected from swamps and from upland gardens to the FAO/WHO acceptable limits of lead in food

	FAO lead limit = 0.1mg/kg			
	p-value	Mean Difference	95% Confidence Interval of the Difference	
			Lower	Upper
Lead levels in yams from swamps (mg/kg)	<0.001	1.5172	1.2905	1.7439
Lead levels in yams from upland gardens (mg/kg)	<0.001	1.6858	1.5043	1.8672

Key: <: less than, mg/kg: milligram/kilogram, =: equals

could be significantly contaminated with lead as the lead levels found in yams from either site categories were found to be higher than those found In a study by Fion, Ustymowicz-farbiszewska, Górski, & Karczewski[6], where the level of lead (Pb) in cereal products ranged from 0.013mg/kg to 0.275mg/kg, and that in a study by Iweala, Olugbuyiro, Durodola, Fubara-Manuel, & Okoli, 2014 where lead was not detected in fried yam, yam flour, beans and herbal drinks.

The findings of this study suggested that there was no statistically significant difference in lead levels found in yams from swamps and yams obtained from upland gardens, more simply laid out; the yams from either site were equally contaminated with lead. This could possibly be because yams from either site were reaching their maximum lead uptake levels, where they cannot take up any more lead regardless of how much they are exposed to.

This finding concurred with that by Fuhrimann et al.,[7] which was carried out in Nakivubo wetland and the Lake Victoria ecosystem in Kampala, demonstrating that yams had a lead concentration similarly found in the sugarcanes despite finding abnormally high lead levels in the soil.

However, this finding contradicted with that by Twinamatsiko, Mbabazi, & Twinomuhwezi[19], which was carried out to investigate the heavy metal contamination of Soil and plant samples from farms around the dump sites in Gulu Township Pece wet land while using samples from Katikamwe wet land in Bushenyi as a control site and found out that lead levels in cocoyam and sugarcanes grown in the urban drainage system soils were significantly higher than those of the same crops grown in rural control sites.

This study made it evident that the yams collected from either sites were significantly contaminated beyond the maximum allowable limits set forth by the Commission of the European Communities (2001) and the Codex Alimentarius Commission (2001, 2004) and the FAO/WHO. This could be because the yams are naturally poised to take up lead from the environment regardless of the amounts to which they are exposed or the environment in which they are growing. Thus, they can be regarded as bio accumulator plants with the capacity to take up heavy metals from the environment to bring about environmental bioremediation.

This finding was congruent with those by Gupta et al.[9], Divya, George, & Midhun[3], and Goswami & Mazumdar[8] who also found lead levels in food to be above the National/international maximum allowable limits.

These findings contradicted with the rationale held by Kooner, Mahajan, & Dhillon[13], who believed that the site of growth noticeably influences the heavy metal uptake by vegetables and that generally, lead contaminations occur in vegetables grown on contaminated soils.

This research finding was also a clear contrast of a similar study by Doherty, Kanife, Ladipo, & Akinfemi[4] were there was lead detected and the other heavy metals analyzed were within the FAO/WHO maximum allowable limits, in the ten samples of flutted-pumpkins collected from different markets in Lagos-Nigeria.

16 Limitations of the study

The study at hand was not able to determine lead levels in the soil of the agricultural sites from which the yams were collected. The study was also not in position to determine the levels of other heavy metals in the yams collected. The study only focused on the vicinity of Kampala capital city and did not delve into distant areas away from the capital city.

17 Conclusion

The findings of this study suggest that the level of lead contamination in yams is not dependent on the agricultural site where they are grown. However, this requires further assertion by determining the lead levels in the soil samples from where the yam samples were collected. This will help establish whether the level of lead taken up by the yams is independent of the level of lead in the soil in which they are growing. There is need to further ascertain that there is no significant difference between lead levels of swamp soil and those in soils of the upland gardens.

The study findings however revealed that lead levels in yams regardless of their site of growth, were above the FAO/WHO maximum acceptable limits of lead in food and thus suggested that yams pose a significant route of human exposure to lead once they are consumed. It waits to be ascertained whether there is a significant reduction in these lead levels to within the FAO/WHO maximum acceptable limits, in yams once they are boiled and ready for consumption.

18 Recommendations

There is need to also determine lead levels in other parts of the yams in order to better understand the role of yams in phytoremediation, as this will broaden our understanding of the plant's physiology in line with where it actually stores the lead once it has been absorbed from the environment.

Since some studies have actually pointed out that different plants are suited for uptake of different heavy metals, it is worth labouring to find out the levels of other heavy metals in yams as they are equally toxic once consumed.

Similar studies in future should also aim at covering a wider scope beyond Kampala district in order to obtain a more detailed representation of the effect of soil contamination on human exposure to the different heavy metals.

19 List of abbreviations and acronyms

et al	And others
&	And
Dr.	Doctor
ml	millilitre
kg	kilogram
mg	milligram
g	Gram
cm^3	cubic centimetres
km	kilometres
km^2	square kilometres
Pb	lead
$^{\circ}C$	Degrees Celsius
%	percentage
μg	microgram
W	body weight
FEP	Free Erythrocyte Protopophyrin
m^3	cubic metres
CDC	Centre for Disease Control
EPA	Environmental Protection Agency
WHO	World Health Organization
FAO	Food and Agricultural Organization
ppm	parts per million
l	litre
ng	nanogram
±	plus or minus
≤	less or equal to
GAL	Government Analytical laboratories
AAS	Atomic Absorption Spectrophotometer
M	Molar
RU	Rubaga Upland garden
KU	Kawempe Upland garden
KCU	Kampala Central Upland garden
NU	Nakawa Upland garden
MU	Makindye Upland garden
MS	Makindye swamp garden
NS	Nakawa Swamp garden
KCS	Kampala Central Swamp garden
KS	Kawempe Swamp garden
RS	Rubaga Swamp garden

Declarations

Ethics approval and consent to participate

Not applicable

20 Competing interests:

The authors declare that they have no competing interests

Data availability statement

The original file/ source document as well as the raw data of this study are readily available with the corresponding author upon request.

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Authors' contribution

All authors contributed equally to this work.

Consent for publication

Not applicable

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