

## Original Research Article

# COMPARATIVE ANALYSIS OF HEAVY METALS CONCENTRATION IN SOIL AND VEGETABLE (*Capsicum annum*) COLLECTED FROM TWO SAMPLING SITES (FARMLAND AND DUMPSITE) AND THE EFFECT ON PLANT DNA

### ABSTRACT

The levels of heavy metals and its effect on DNA of *Capsicum annum* collected from dumpsite (in Ojota) and farmland (in Badagry) Lagos, Nigeria, was examined using standard procedures.

The results of concentration of heavy metals in the soil for the farmland and dumpsite respectively are: Zn ( $882.0 \pm 0.006\text{mg/kg}$ ,  $14316.8 \pm 1.009\text{mg/kg}$ ), Cr ( $2.006 \pm 0.002\text{mg/kg}$ ,  $3.888 \pm 0.002\text{mg/kg}$ ), Cd ( $0.098 \pm 0.001\text{mg/kg}$ ,  $0.644 \pm 0.002\text{mg/kg}$ ), Cu ( $0.206 \pm 0.001\text{mg/kg}$ ,  $0.997 \pm 0.001\text{mg/kg}$ ) and Pb ( $0.005 \pm 0.003\text{mg/kg}$ ,  $0.843 \pm 0.002\text{mg/kg}$ ) respectively. The values of N ( $3153.6 \pm 0.008\text{mg/kg}$ ,  $4191.2 \pm 0.006\text{mg/kg}$ ), P ( $7598.3 \pm 0.009\text{mg/kg}$ ,  $8794.5 \pm 0.003\text{mg/kg}$ ) and K ( $113.56 \pm 0.004\text{mg/kg}$ ,  $532.12 \pm 0.004\text{mg/kg}$ ) were recorded in the soil from farmland and dumpsite respectively. While the values of N, P and K in soil from dumpsite were higher ( $p < 0.05$ ) than that from farmland, only value of Zn in the soil of dumpsite was significantly higher than soil from farmland. Similarly, the values of all metals except Zn recorded in the leaf, stem and root of *C. annum* showed no significant difference between the sample collected from dumpsite and farmland. The values of Zn in leaf, stem and root of *C. annum* from farmland were significantly higher ( $p < 0.05$ ) than that of dumpsite. The DNA bands of the *Capsicum annum* from control site has clearer band spectrum than that from the dumpsite site, however, there was no DNA mutation. In conclusion, *C. annum* collected from both farmland (in Badagry) and dumpsite (in Ojota) contained minerals and heavy metals whose concentrations were still within standard permissible limits. Thus, *C. annum* from both sites are safe for human consumption.

**Keywords:** Farmland, dumpsite, *Capsicum annum*, DNA mutation, Heavy metals

### INTRODUCTION

*Capsicum annum* (sweet pepper) is native to Mexico and is one of the main vegetables worldwide (Mejía-Teniente *et al.*, 2013). It is an important agricultural crop, not only because of its economic importance, but also due to nutritional and medicinal value. They are excellent source of antioxidant compounds, natural colours, and vitamins (Idowu-Agida *et al.*, 2012, Ogunlade *et al.*, 2012).

Man-made activities are primarily responsible for the increasing concentrations of heavy metals in agricultural land. They may reach ground water through the soil and can be taken by plants causing profound effects on plant physiology and cytology (Siddiqui, 2013). In Nigeria, most peppers grown on farmland or dumpsite are not usually subjected to proper routine assessment to know the associated health and the ecological risks of the soil or plant that are grown in either area (Obasi *et al.*, 2012). The presence of heavy metals in the environment is of great ecological significance due to their toxicity at certain concentrations, translocation through food chains and non biodegradability which is responsible for their accumulation in the biosphere (Ikhouria *et al.*, 2010). Heavy metals have received the attention of researchers all over the world, mainly due to their harmful effects on plant and other living organisms (Tahar and Keltoum, 2011). The anthropogenic activities usually result in pollution,

especially in developing countries where low-end waste management is a common practice (Babajide and Dauda, 2009). According to Zengin and Munzuroğlu (2004), accumulation of heavy metals such as lead, cadmium and arsenic in plant could cause negative effects on the roots, stems and germination of seeds. Also, the DNA of plant is capable of being damaged via over accumulation of some metals such as lead (Silbergeld *et al.*, 2003).

Among various factors that could influence the growth and quality of plants, their physical environment is a major concern. Plants are expected to do well in an environment with a sufficient proportion of nutrients, adequate air conditioning and uncontaminated water supply. In Lagos state, especially within the study areas (Badagry and Ojota) selected for this study, there is currently dearth information on the nutrient and heavy metal concentrations in *C. annuum* grown on the soil. Also, the examination of effects of heavy metals bioaccumulation in leaf, root and stem of *C. annuum* from the two sites via DNA analysis has not been documented. However, present study opined to fill the information gap.

## **MATERIALS AND METHODS**

### **Sampling Stations**

Two sampling stations selected for this study were Ojota and Badagry. Ojota is a renown and highly populated town in Lagos state. Its geographical coordinates are 6° 35' 16" N and 3° 22' 56" E. It is located very close to Berger town which is a border point between Lagos state and Ogun state. The dumpsite in Ojota where the samples were collected for this study was spacious and has been in existence for over 20 years. In the foregoing, the second site- Badagry, is a coastal town that is bounded in the west by Republic of Benin and in south by the Atlantic Ocean (Mekuleyi *et al.*, 2019). The geographical coordinates of Badagry are 6.4183°N and 2.8901°E. Badagry is surrounded by creeks and island, which makes beaches and resorts one of the major attractions of the ancient town. Badagry town consist of less human population when compare to Ojota town. While most occupants in Ojota are Artisans and merchant, majority of occupant in Badagry are fishers, farmers and Artisans.

### **Collection of Samples and Preparation**

Samples of *C. annuum* used for this study were collected between December 2021 and February, 2022. The plant species (*C. annuum*) from each sampling sites were uprooted, while the soil particles were collected from the roots. The plant was then divided into leaf, stem, and root. They were kept in a paper bag, tied and labeled with a masking tape and a marker. The soil and plant samples were taken to the laboratory for heavy metal analysis, and plant for DNA examination. Analytical Reagent grade chemicals were used in all tests.

### **Pre-treatment Methods for Plant Samples**

The leaves, stems and roots of the plant samples were separately cleaned by gentle washing with distilled water. Thereafter, each sample was sub-sampled, and air-dried in the laboratory, at ambient temperature, for three days, and processed further by ashing and then used to quantify the heavy metals in the leaves, stem, root and fruits. A 5g of each of the dried sample was weighed into a porcelain crucible and ashed in a muffle furnace at 550 °C, for 4 hr, or until completely ashed. Thereafter, the residue was allowed to cool, and then dissolved with 5 ml of dilute (1:1) nitric acid. The mixture was diluted to 25 ml with distilled water. The solution was filtered through Whatman #1 filter paper. The filtrate was saved for the determination of the metals.

### **Pre-treatment Methods for Soil Samples**

5g each of the soil samples from the dumpsite and the control sites were separately weighed into labeled conical flasks. To each sample were added 10 ml of distilled water, and 5 ml of concentrated nitric acid. The mixture was heated on a hot plate, in fume cupboard, for 30 min. The mixture was allowed to cool to about 25°C and then filtered through Whatman #1 filter paper, and then made up to

25 ml, in a volumetric flask, with distilled water. The digest was then saved for the determination of the metals

### **Methods of Measurements of Heavy Metal Concentration**

The examined metals (Cd, Cr, Cu, Pb, Zn) were determined on filtrate of sample digestate by atomic absorption spectrometry while test results were validated with calibration curves obtained with certified metal standards (AccuStandard Inc, USA).

### **Reagent and Procedure for Preparation of 100mL SDS extraction buffer**

The following solutions were used for the preparation of 100mL SDS extraction buffer: 10mL of 1M Tris-HCl, 10mL of 0.5M EDTA, 10mL of 5M NaCl, 20% of SDS (20g), 1% PVP(1g), Mercaptoethanol-1% added immediately prior to use make up to 100ml with distilled water.

Samples were prepared by putting approximately 100mg of freeze dried tissues (leaf, root and stems) of *Capsicum annuum* into an extraction tube. Then, two steel balls were added each into the tube to enable grinding while the freeze dried tissue were grinded into fine powder by using Genogrinder-2000. After grinding, 450 $\mu$ l of pre-heated plant extraction buffer were added to the mixture, while the tubes were incubated at 65 $^{\circ}$ c for 20 min and mixed by occasionally inverting the tubes to homogenize the sample. Then, the tubes were removed to allow cooling for 2 min, and 200 $\mu$ l of ice-cold 5M Potassium acetate was later added. Also, the mixture was incubated on ice for 20 minutes to precipitate protein, follow by centrifuge at 10000rpm for 10 min and then transfer supernatant into freshly labeled tubes. Thereafter, 450 $\mu$ l of chloroform Isoamylalcohol (24:1) were added and mixed gently to further precipitate protein and lipids. The mixture was further centrifuged at 10000rpm for 10 min and then transfer with supernatant into freshly labeled tubes. This was followed by addition of 2/3 volume of ice-cold Isopropanol, which was mix gently and incubated in -80 $^{\circ}$ c for 15mins to precipitate the DNA.

### **Decantation and Centrifugation**

After the incubation periods, samples were cooled at room temperature, then centrifuged at 10000rpm for 10 min, while supernatant was decanted until the last drop. Then, 400 $\mu$ l of 70% ethanol was added to wash the DNA pellet. Centrifugation at 10000rpm for 10min was repeated, supernatant decanted until the last drop and while the pellet was air dry until ethanol smell disappears. Thereafter, addition of 60ul of ultra-pure water or low salt TE was done to re-suspend the DNA. Also, 2ul of RNase was added and incubate in 37 $^{\circ}$ c for 30-40 minutes.

### **Examination of Quality of DNA**

About 0.8% agarose gel was prepared for checking DNA quality and removal of RNA. This requires boiling 0.8gram of agarose in 100ml of 1X TBE, cool to about 60 $^{\circ}$ c and add 5ul ethidium bromide, and gently swim to mix and pour it on the gel tray before it polymerizes without allowing any air bubble in the middle of the gel. A 3 $\mu$ L of DNA and 3 $\mu$ L of loading dye were mixed, and briefly spin to collect to the bottom of the plate and load 6 $\mu$ l of this mix on to the 0.8% agarose gel. The gel was later run at 80volt for about 60 minutes. After this, the gel picture was saved. Also, check was made to ensure that the RNA is completely removed in order to proceed to the Nanodrop.

### **Quantification of DNA Concentration**

DNA concentration was quantified using DNA-50 option of the Nanodrop spectrophotometer. Approximately 1.8 ratios for sample absorbance at A260/280 is generally accepted as "pure" for DNA. A260/230 ratio was considered a secondary measure of nucleic acid purity for the presence/absence of co-purified contaminants while A260/230 ratio of 1.8-2.2 is generally acceptable.

### **PCR amplification using RBCL primers**

The DNA was subjected to the following cocktail mix and condition for the PCR. DNA samples from plant from each site were subjected to PCR amplification with a RBCL primer. The PCR cocktail mix consist of 2.5ul of 10x PCR buffer, 1ul of 25mM MgCl<sub>2</sub>, 1ul each of forward primer and reverse primer, 1ul of DMSO, 2ul of 2.5mM dNTPs, 0.1ul of 5u/ul Taq DNA polymerase, and 3ul of 10ng/ul DNA. The total reaction volume was made up to 25ul using 13.4ul Nuclease free water.

The PCR cycle was carried out with the initial denaturation for 5 min at 94°C, 9 cycles each of denaturation for 15s at 94°C, primer annealing for 20s at 65°C and 30s extension at 72°C. It was followed by 35 cycles of 94°C for 15s, 55°C annealing for 20s and 72°C for 30s. The final extension was at 72°C for 7 min, followed by cooling at 10°C until it finally cooled.

### **Statistical Analysis**

The data on the plant and soil samples from both dumpsites and farmland are computed using Statistical Package for Social Science (SPSS, Version 20) while the mean concentration of heavy metals and nutrients at both sites were analyzed using ANOVA for the plant's tissue (leaf, stem and root) and t-test (for difference between the two sites) while the level of significance at 95% confidence was set at  $p \leq 0.05$ .

### **RESULTS**

The summary of the heavy metals' concentration (Cd, Cr, Cu, Pb and Zn) and nutrients (N, P, K) in *Capsicum annuum* (pepper) and soil collected from Ojota's dumpsite and a farmland in Badagry, Lagos state, Nigeria is presented in Table 1. The results of concentration of heavy metals in the soil for the farmland and dumpsite respectively are: Zn (882.0  $\pm$  0.006mg/kg, 14316.8  $\pm$  1.009mg/kg), Cr (2.006  $\pm$  0.002mg/kg, 3.888  $\pm$  0.002mg/kg), Cd (0.098  $\pm$  0.001mg/kg, 0.644  $\pm$  0.002mg/kg), Cu (0.206  $\pm$  0.001mg/kg, 0.997  $\pm$  0.001mg/kg) and Pb (0.005  $\pm$  0.003mg/kg, 0.843  $\pm$  0.002mg/kg) respectively. The values of N (3153.6  $\pm$  0.008mg/kg, 4191.2  $\pm$  0.006mg/kg), P (7598.3  $\pm$  0.009mg/kg, 8794.5  $\pm$  0.003mg/kg) and K (113.56  $\pm$  0.004mg/kg, 532.12  $\pm$  0.004mg/kg) were recorded in the soil from farmland and dumpsite respectively. While the values of N, P and K in soil from dumpsite were higher ( $p < 0.05$ ) than that from farmland, only value of Zn in the soil of dumpsite was significantly higher than soil from farmland. Similarly, the values of all metals except Zn recorded in the leaf, stem and root of *C. annuum* showed no significant difference between the sample collected from dumpsite and farmland. The values of Zn in leaf, stem and root of *C. annuum* from farmland were significantly higher ( $p < 0.05$ ) than that of dumpsite.

**Table 1: Heavy Metals Concentrations in Rodo and soil from Farmland (Badagry) and Dumpsite (Ojota) in Lagos State, Nigeria**

Parameters	Farmland				Dumpsite			
	Leaf (mg/100g)	Stem (mg/100g)	Root (mg/100g)	Soil (mg/kg)	Leaf (mg/100g)	Stem (mg/100g)	Root (mg/100g)	Soil (mg/kg)
<b>Cd</b>	0.003 ± 0.001	0.004 ± 0.001	0.006 ± 0.001	0.098 ± 0.001	0.010 ± 0.002	0.006 ± 0.001	0.014 ± 0.004	0.644 ± 0.002
<b>Cr</b>	0.142 ± 0.012	0.096 ± 0.006	0.281 ± 0.004	2.006 ± 0.002	0.411 ± 0.002	0.130 ± 0.005	0.447 ± 0.006	3.888 ± 0.002
<b>Cu</b>	0.310 ± 0.001	0.110 ± 0.002	0.605 ± 0.006	0.206 ± 0.001	0.116 ± 0.001	0.125 ± 0.001	0.706 ± 0.009	0.997 ± 0.001
<b>Pb</b>	0.006 ± 0.001	0.004 ± 0.001	0.012 ± 0.004	0.005 ± 0.003	0.111 ± 0.003	0.121 ± 0.005	0.320 ± 0.017	0.843 ± 0.002
<b>Zn</b>	39.715 ± 0.474	23.505 ± 0.120	49.333 ± 0.936	882.0 ± 0.006	123.640 ± 3.338	98.601 ± 0.692	210.555 ± 2.029	14316.8 ± 1.009
<b>N</b>	418.795 ± 1.860	134.125 ± 1.252	984.490 ± 5.134	3153.6 ± 0.008	231.015 ± 2.850	410.520 ± 1.952	435.925 ± 5.282	4191.2 ± 0.006
<b>P</b>	105.630 ± 0.042	62.055 ± 2.737	177.180 ± 1.471	7598.3 ± 0.009	277.295 ± 1.294	98.910 ± 0.608	258.395 ± 2.581	8794.5 ± 0.003
<b>K</b>	299.800 ± 1.471	105.660 ± 4.723	343.700 ± 0.735	113.56 ± 0.004	555.200 ± 1.103	114.015 ± 1.464	610.380 ± 1.499	532.12 ± 0.004

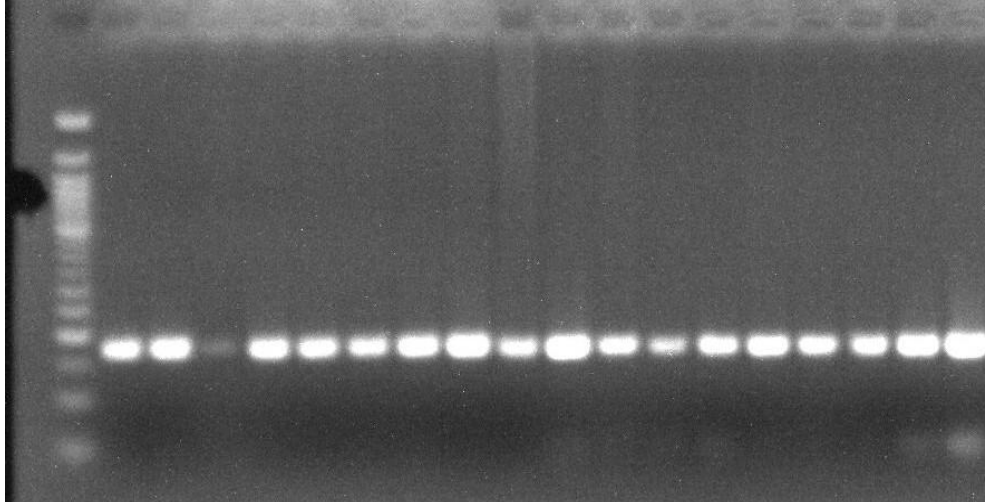
Values in the soil are in mg/kg. Mean value with same superscript in the row =not significant different ( $p>0.05$ )

The frequency distribution of heavy metals and nutrients in *Capsicum annuum* are shown in Figures 1 and 2 respectively. As shown in figure 1, Zinc (Zn) was the most abundant metal. Its greatest frequency was observed in root of *C. annuum* while the least was Cd. In comparison of the level of Zn in the root of the pepper from both sites, root of plant from dumpsite had more Zn than that from farmland. For the dumpsite, the most frequent nutrient in the leaf and root was K, while stem had more of N. On the other hand, the most frequent nutrients in the root, leaf and stem of the *Capsicum annuum* from farmland was N.

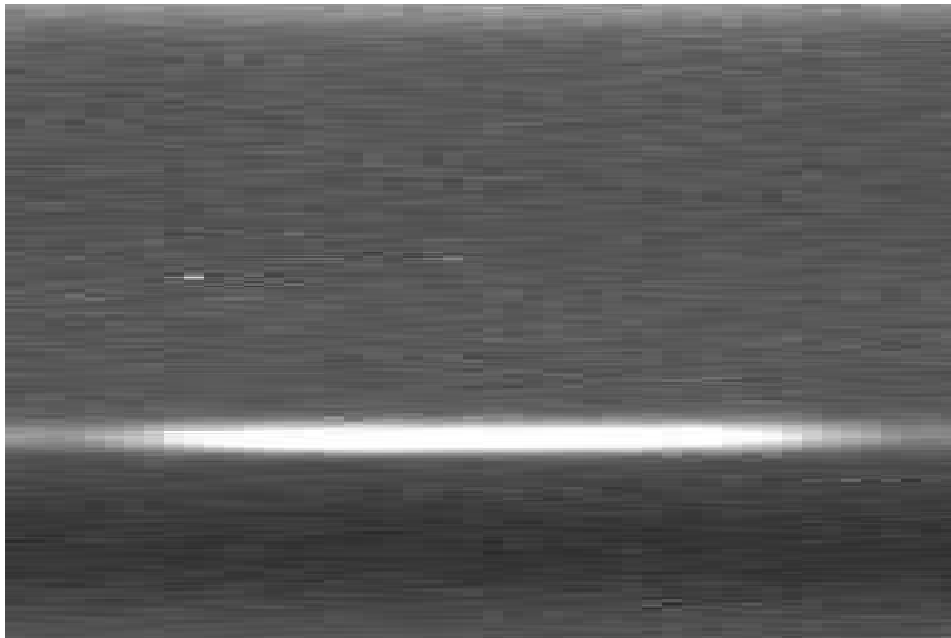
**Figure 1: Variation of heavy metals in the leaf, stem and root of *Capsicum annuum* from dumpsite (DS) and farmland (FM).**

**Figure 2: Variation of nutrients (N, P, K) in the leaf, stem and root of *C. annuum* from dumpsite (DS) and farmland (FM).**

The DNA bands of the *Capsicum annuum* from control site (Plate 1) has clearer band spectrum than that from the dumpsite site (Plate 2). Although, the DNA band of *Capsicum annuum* from the dumpsite seem to be positioned a little downward in comparison with that of the control, but there was no DNA mutation.



**Plate 1: DNA Bands of *Capsicum annuum* collected from Badagry (Control)**



**Plate 2: DNA Bands of *Capsicum annuum* collected from Ojo (Dumpsite)**

## **DISCUSSION**

The values of N, P and K in the soil from dumpsite were higher than that from farmland. From this observation, it is clear that soil of the dumpsite is more prone to N, P and K contamination. Such contamination might have resulted from indiscriminate burning of solid wastes as opined by Babayemi *et al.* (2017). However, the N, P and K contents in the soil from both sites were still below maximum permissible limits. Only Zn and Cr from the dumpsite's soil were higher than that from farmland. Similar results of metals contaminations from dumpsite and farmland's soil has been reported by Adu *et al.*

(2020). In this study, the metals recorded from soil of Badagry and Ojota in Nigeria were lower than that reported by Kimani (2010) in Kenya on Dandora waste dumpsite in Nairobi. However, level of metals in this study was similar to that reported by Hammed *et al.* (2017) from two dumpsites and a farmland at Idi-Ose in Akinyele Local Government in Nigeria.

The values of all metals except Zn recorded in the leaf, stem and root of *C. annuum* that showed no significant difference between the sample collected from dumpsite and farmland is an indication that pepper from both sites would supply similar level of metals needed for human consumption. However, Zn in leaf, stem and root of *C. annuum* from farmland were significantly higher than that of dumpsite. The levels Cd, Cr, Pb and Zn in vegetables (*Telfaria occidentalis*, *Talinum triangulare*, *Amaranthus hybridus*, *Vernonia amygdalina* and *Solmun nigrum*) in the vicinity of Enyigba Lead mine and Abakaliki investigated by Oti and Nwabue (2013) were above those recorded in this study. The K, N and P recorded in this study were above the values reported in *Annona squamosa*, *Psidium guajava*, *Anacardium occidentale*, *Ficussycomorus* and *Pomegranate* collected from Kwarin Gogaugarden, Airport road, Kano State (Baba and Mohammed, 2021). Also, the concentrations of all the minerals (N, P, K) and metals examined in this study were below maximum permissible limits recommended by World Health Organization-WHO (2005). Idera *at al.*(2017) and Hammed *et al.*(2017) stated that the recommended levels of metals such as Cd, Cu, Pb Cr and Zn in vegetables including pepper are 0.1 mg/kg, 73 mg/kg, 0.3mg/kg, 0.25mg/kg and 100 mg/kg respectively as opined by WHO/FAO (2016). The findings in this study on metals content in *C. annuum* was within this recommended level. Thus, the outcome of this study implies that *C. annuum* from both sites are safe for human consumption. In this study, Zn was the most abundant metal and its frequency was observed in root of *C. annuum*. The implication of this is that people with deficiency of Zn, could take more of pepper root to meet their need. The most frequent nutrient in the leaf and root of pepper from the dumpsite was K while stem had more of N. On the other hand, the most frequent nutrients in the root, leaf and stem of the *Capsicum annuum* from farmland was N. This observation could buttress the reason why some people choose to grow crop on dumpsite. Kihampa *et al.* (2011) and Musa and Ifatimehin (2013) had reported that for some supposed dietary and economic advantages, waste dumpsites in some parts of Nigeria and some other African countries have been converted to agricultural sites particularly for the cultivation of vegetables such as pepper.

The presence of metals in the root, stem and leaves of *Capsicum annuum* buttress the assertion of Nwoko and Egunobi(2002) that edible plants grown in polluted soils could be susceptible to heavy metal uptake. The medicinal value of *capsicum* annum such as being good sources of essential minerals such as Magnesium, Zinc, Iron, Phosphorous and Potassium, rich in proteins, lipids, carbohydrates, fibres, mineral salts vitamins etc has been documented in literatures (El-Ghoraba *et al.*, 2013). Therefore, *C. annuum* in this study with abundant of Zn confirmed the report of Christine *et al.* (2014) that *C. annuum* have health promoting properties and can also contribute significantly to the zinc and iron needed daily in the human diet.

It was observed in this study that the DNA bands of the *Capsicum annuum* from control site has clearer band spectrum than that from the dumpsite site. However, no DNA band mutation of *Capsicum annuum* from the dumpsite and control was recorded. This observation could suggest that plants from both sites have not over bio-accumulated heavy metals in their tissues. In same trend to the findings in this study, Carolyn *et al.*(2016) which investigated the effects of heavy metals on the DNA mutation on *Nepenthes* plant from abandoned mine, reported that the DNA band of the plant from the abandoned mine were located at the same location with the control. However, they divulged that their results of no DNA band

shift or alteration could be attributed to the level of Zn, Pb, Fe, Cd, Cr, Mn and Cu in the abandoned mine that were below the maximum permissible limits. On the other hand, DNA bands shift of stem and leaves of *V. amygdalina* due to Cu bio-accumulation was reported by Adu *et al.* (2021). DNA band shift often result in plant due to none healthy growth and development in the plants (Nicolia *et al.*, 2013) and Navari-Izzo, 2001). Akachuku (2001) had reported that land use for food production can be contaminated with heavy metals from natural and anthropogenic sources such as water smelt, irrigation with waste, disposal of solid waste, vehicle exhaust and adjacent industrial activities.

## **CONCLUSION**

The study have shown that *C. annuum* collected from both farmland (in Badagry) and dumpsite (in Ojota) contained minerals and heavy metals whose concentrations were still within standard permissible limits. There was no adverse effect of heavy metals on *C. annuum* from both sites as indicated by the DNA analysis. Thus, it could be concluded that *C. annuum* both sites are safe for human consumption.

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