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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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The levels of heavy metals and their effect on the DNA of *Capsicum annuum* collected from dumpsite (in Ojota) and farmland (in Badagry) in Lagos, Nigeria, were examined using standard procedures.
The results of concentration of heavy metals in the soil for the farmland and dumpsite respectively are: Zn (882.0 ±0.006mg/kg, 14316.8±1.009mg/kg), Cr (2.006 ±0.002mg/kg,3.888± 0.002mg/kg), Cd (0.098 ± 0.001mg/kg, 0.644 ± 0.002mg/kg), Cu (0.206± 0.001mg/kg, 0.997 ± 0.001mg/kg) and Pb (0.005 ± 0.003mg/kg, 0.843± 0.002mg/kg) respectively. The values of N (3153.6± 0.008mg/kg, 4191.2± 0.006mg/kg), P (7598.3 ± 0.009mg/kg, 8794.5 ± 0.003mg/kg) and K (113.56 ± 0.004mg/kg, 532.12 ± 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, the only value of Zn in the soil of dumpsite was significantly higher than in 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 dumpsites and farmland. The values of Zn

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in leaf, stem, and root of *C. annuum* from farmland were significantly higher ($p<0.05$) than that of the dumpsite. The DNA bands of the *Capsicum annuum* from the control site have a clearer band spectrum than that from the dumpsite site, however, there was no DNA mutation. In conclusion, *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. Thus, *C. annuum* from both sites is safe for human consumption.

Keywords: Farmland; dumpsite; Capsicum annuum; DNA mutation; heavy metals.

1. INTRODUCTION

Capsicum annuum (sweet pepper) is native to Mexico and is one of the main vegetables worldwide [1]. It is an important agricultural crop, not only because of its economic importance but also due to its nutritional and medicinal value. They are an excellent source of antioxidant compounds, natural colors, and vitamins [2,3].

Man-made activities are primarily responsible for the increasing concentrations of heavy metals in agricultural land. They may reach groundwater through the soil and can be taken by plants causing profound effects on plant physiology and cytology [4]. 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 [5]. 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 nonbiodegradability which is responsible for their accumulation in the biosphere [6]. Heavy metals have received the attention of researchers all over the world, mainly due to their harmful effects on plants and other living organisms [7]. The anthropogenic activities usually result in pollution, especially in developing countries where the low-end waste management is a common practice (Babajide and Dauda, 2009). According to Zengin and Munzuroğlu (2004), the accumulation of heavy metals such as lead, cadmium, and arsenic in plants could cause negative effects on the roots, stems, and germination of seeds. Also, the DNA of a 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 an uncontaminated water supply. In Lagos state, especially within the study areas (Badagry and Ojota) selected for this study, there is currently a dearth of 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, the present study opined to fill the information gap.

2. MATERIALS AND METHODS

2.1 Sampling Stations

Two sampling stations selected for this study were Ojota and Badagry. Ojota is a renowned and highly populated town in Lagos state. Its geographical coordinates are 6^0 35' 16" N and 3^0 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 the Republic of Benin and in the south by the Atlantic Ocean [8]. The geographical coordinates of Badagry are 6.4183^0N and 2.8901^0E. Badagry is surrounded by creeks and islands, which makes beaches and resorts one of the major attractions of the ancient town. Badagry town consists of a less human population when compare to Ojota town. While most occupants in Ojota are Artisans and merchants, the majority of the occupant in Badagry are fishers, farmers, and Artisans.

2.2 Collection of Samples and Preparation

Samples of C. annum used for this study were collected between December 2021 and February 2022. The plant species (*C. annum*) from each sampling site 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 masking tape and a marker. The soil and plant samples were taken to the laboratory for heavy metal analysis and the plant for DNA examination. Analytical Reagent grade chemicals were used in all tests.

2.3 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, air-dried in the laboratory, at ambient temperature, for three days, 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 samples was weighed into a porcelain crucible and ashed in a muffle furnace at 550 ^{0}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.

2.4 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 a fume cupboard, for 30 min. The mixture was allowed to cool to about 25^{0}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.

2.5 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 (Accu Standard Inc, USA).

2.6 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 was ground into fine powder by using Genogrinder-2000. After grinding, 450µl of pre-heated plant extraction buffer was added to the mixture, while the tubes were incubated at 65^{0}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 200µl of ice-cold 5M Potassium acetate was later added. Also, the mixture was incubated on ice for 20 minutes to precipitate protein, followed by a centrifuge at 10000rpm for 10 min, and then transfer the supernatant into freshly labeled tubes. Thereafter, 450µl of chloroform Isoamylalcohol (24:1) was 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 the addition of 2/3 volume of ice-cold Isopropanol, which was mixed gently and incubated at -80^{o}c for 15mins to precipitate the DNA.

2.7 Decantation and Centrifugation

After the incubation periods, samples were cooled at room temperature, then centrifuged at 100000rpm for 10 min, while the supernatant was decanted until the last drop. Then, 400µl of 70% ethanol was added to wash the DNA pellet. Centrifugation at 10000rpm for 10min was repeated, the supernatant decanted until the last drop, and the pellet was air dry until the ethanol smell disappears. Thereafter, the 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^{o}c for 30-40 minutes.

2.8 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, cooling to about 60^{o}c and adding 5ul ethidium bromide, and gently swimming to mix and pour it on the gel tray before it polymerizes without allowing any air bubble in the middle of the gel. A 3µL of DNA and 3µL of loading dye were mixed, and briefly spin to collect to the bottom of the plate and load 6µl of this mix onto the 0.8% agarose gel. The gel was later run at 80volt for about 60 minutes. After this, the gel picture was saved. Also, the check was made to ensure that the RNA is completely removed in order to proceed to the Nanodrop.

2.9 Quantification of DNA Concentration

DNA concentration was quantified using the DNA-50 option of the Nanodrop spectrophotometer. Approximately 1.8 ratios for sample absorbance at A260/280 are 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 an A260/230 ratio of 1.8-2.2 is generally acceptable.

2.10 PCR Amplification using RBCL Primers

The DNA was subjected to the following cocktail mix and condition for the PCR. DNA samples from the plant from each site were subjected to PCR amplification with an RBCL primer. The PCR cocktail mix consists of 2.5ul of 10x PCR buffer,1ul of 25mM MgCl2, 1ul each of forwarding primer and reverses primer, 1ul of DMSO, 2ul of 2.5mMDNTPs, 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 the 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.

2.11 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 $p \leq 0.05$.

3. 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 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 ±0.006mg/kg, 14316.8±1.009mg/kg), Cr(2.006 ±0.002mg/kg, 3.888± 0.002mg/kg), Cd (0.098 ± 0.001mg/kg, 0.644 ± 0.002mg/kg), Cu (0.206± 0.001mg/kg, 0.997 ± 0.001mg/kg) and Pb (0.005 ± 0.003mg/kg, 0.843± 0.002mg/kg) respectively. The values of N (3153.6± 0.008mg/kg, 4191.2± 0.006mg/kg), P (7598.3 ± 0.009mg/kg, 8794.5 ± 0.003mg/kg) and K (113.56 ± 0.004mg/kg, 532.12 ± 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, the 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 dumpsites 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 the dumpsite.

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

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
Cd	0.003 ± 0.001^{a}	0.004 ± 0.001^{a}	0.006 ± 0.001^{a}	0.098 ± 0.001^{a}	0.010 ± 0.002^{a}	0.006 ± 0.001^{a}	0.014 ± 0.004^{a}	0.644 ± 0.002^{a}
Cr	0.142 ± 0.012^{a}	0.096 ± 0.006^{a}	0.281 ± 0.004^{a}	2.006 ± 0.002^{b}	0.411 ± 0.002^{a}	0.130 ± 0.005^{a}	0.447 ± 0.006^{a}	3.888 ± 0.002^{ab}
Cu	0.310 ± 0.001^{a}	0.110 ± 0.002^{a}	0.605 ± 0.006^{a}	0.206 ± 0.001^{a}	0.116 ± 0.001^{a}	0.125 ± 0.001^{a}	0.706 ± 0.009^{a}	0.997 ± 0.001^{a}
Pb	0.006 ± 0.001^{a}	0.004 ± 0.001^{a}	0.012 ± 0.004^{a}	0.005 ± 0.003^{a}	0.111 ± 0.003^{a}	0.121 ± 0.005^{a}	0.320 ± 0.017^{a}	0.843 ± 0.002^{a}
Zn	39.715 ± 0.474^{a}	23.505 ± 0.120^{aa}	49.333 ± 0.936^{ab}	882.0 ± 0.006^{b}	123.640 ± 3.338^{bb}	98.601 ± 0.692^{bc}	210.555 ± 2.029^{c}	14316.8 ±1.009^{ac}
N	418.795 ± 1.860^{a}	134.125 ± 1.252^{ab}	984.490 ± 5.134^{b}	3153.6 ± 0.008^{bb}	231.015 ± 2.850^{aa}	410.520 ± 1.952^{ad}	435.925 ± 5.282^{ac}	4191.2 ±0.006^{bc}
P	105.630 ± 0.042^{a}	62.055 ± 2.737^{ab}	177.180 ± 1.471^{b}	7598.3 ± 0.009^{bb}	277.295± 1.294^{aa}	98.910 ± 0.608^{ad}	258.395 ± 2.581^{ac}	8794.5 ± 0.003^{bc}
K	299.800 ± 1.471^{a}	105.660 ± 4.723^{ab}	343.700 ± 0.735^{b}	113.56 ± 0.004^{bb}	555.200 ± 1.103^{aa}	114.015 ± 1.464^{ad}	610.380 ± 1.499^{ac}	532.12 ± 0.004^{bc}

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

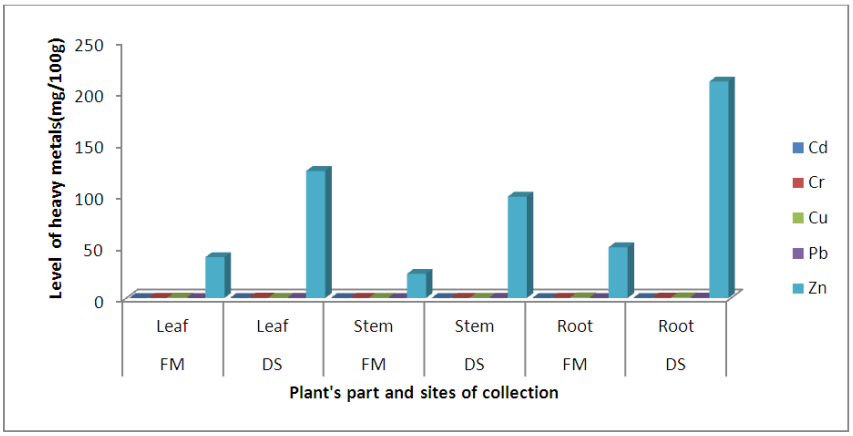

Fig. 1. Variation of heavy metals in the leaf, stem, and root of *Capsicum annuum* from dumpsite (DS) and farmland (FM)

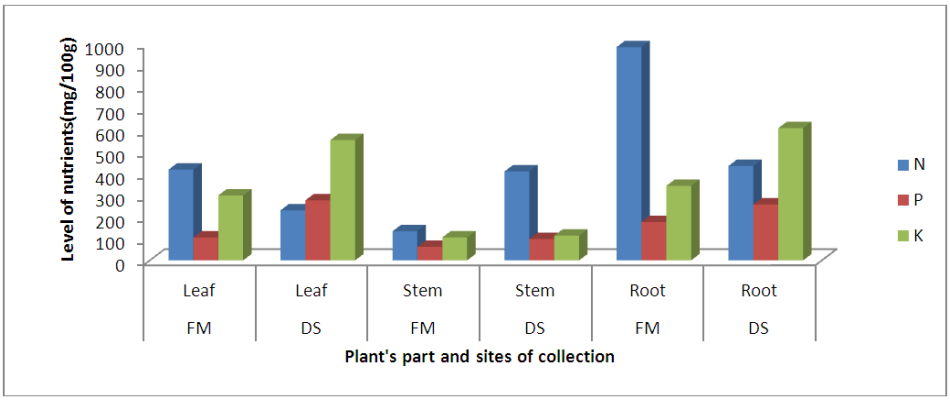

Fig. 2. Variation of nutrients (N, P, K) in the leaf, stem, and root of *C. anuum* from dumpsite (DS) and farmland (FM)

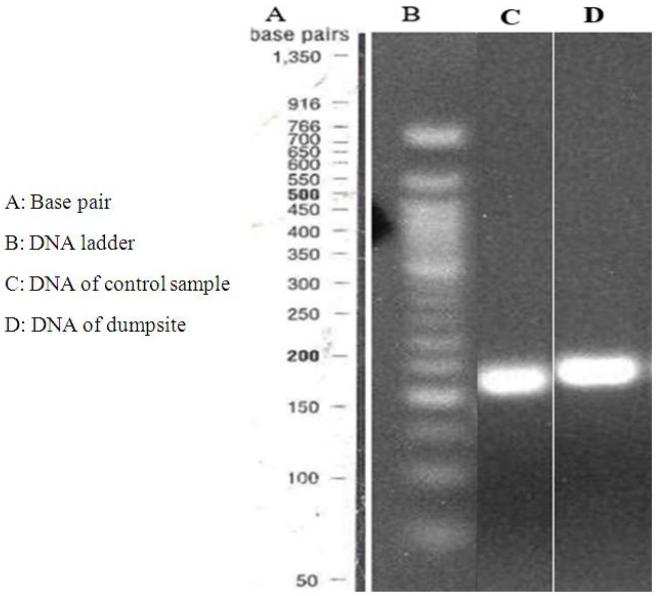

Fig. 3. DNA-fragmentation analysis of *C. argentea* collected from the farmland and dumpsite

The DNA fragmentation analysis of the *C. annuum* collected from the farmland and dumpsite (Fig. 3) showed that there was no significant fragmentation in the DNA of the two samples.

4. 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 the 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. [9]. 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 soil have been reported by Adu et al. [10]. In this study, the metals recorded from the soil of Badagry and Ojota in Nigeria were lower than that reported by Kimani (2010) in Kenya on the Dandora waste dumpsite in Nairobi. However, the level of metals in this study was similar to that reported by Hammed et al. [11] from two dumpsites and 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 a similar level of metals needed for human consumption. However, Zn in leaf, stem, and root of *C. annuum* from farmland was significantly higher than that of the 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 the maximum permissible limits recommended by World Health Organization-WHO [12]. Idera et al. (2017) and Hammed et al. [11] 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* were within this recommended level. Thus, the outcome of this study implies that *C. annuum* from both sites is safe for human consumption. In this study, Zn was the most abundant metal and its frequency was observed in the root of *C. annuum.* The implication of this is that people with a deficiency of Zn, could take more pepper root to meet their needs. The most frequent nutrient in the leaf and root of pepper from the dumpsite was K while the 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 the crop on the dumpsite. Kihampa *et al*. (2011) and Musa and Ifatimehin [13] 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* buttresses 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 source of essential minerals such as Magnesium, Zinc, Iron, Phosphorous, and Potassium, rich in proteins, lipids, carbohydrates, fibers, mineral salts vitamins, etc have been documented in literature (El-Ghoraba et al. 2013). Therefore, C. annuum in this study with abundant of Zn confirmed the report of Christine et al. [14] that C. annuum has health-promoting properties and can also contribute significantly to the zinc and iron needed daily in the human diet.

In this study that there was no observable DNA fragmentation in *C. annuum* samples obtained from the farmland and dumpsite. This observation suggests that plants from both sites have not over bio-accumulated heavy metals in their tissues. In the same trend to the findings in this study, Carolyn et al. [15] who investigated the effects of heavy metals on the DNA mutation on Nepenthes plant from an abandoned mine, reported that the DNA band of the plant from the abandoned mine was 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 was below the maximum permissible limits. On the other hand, DNA bands shift in the stem and leaves of V. amygdalina due to Cu bio-accumulation was reported by Adu et al. [10]. DNA band shifts often result in plants due to none healthy growth and development in the plants (Nicolia et al. 2013) and Navari-Izzo, 2001). Akachuku [16] 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 [17,18].

5. CONCLUSION

The study has 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* at both sites is safe for human consumption.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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