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Comparative analysis of heavy metals concentration in soil and vegetable (*Solanum lycopersicum)* collected from two sampling sites (Farmland and Dumpsite) and the effect on plant DNA

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Abstract

One of the most popular crops with a unique role in human diets especially in Nigeria is *Solanum Lycopersicum*. The study investigated the levels of heavy metals (cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb)and zinc(Zn) and nutrients(nitrogen (N), phosphorus(P) and potassium(K) in the leaf, stem and root of *Solanum lycopersicum* and soil collected from dumpsite (in Ojota) and Farmland (in Badagry) in Lagos, Nigeria and their effects on *S. lycopersicum* using DNA analysis. The results recorded in mg/100g showed that only the value of Zn in stem of *S. lycopersicum* from farmland (77.671 ± 2.193) was significantly higher than that of dumpsite (72.169 ± 1.656) while Zn in the root *S. lycopersicum* from farmland (66.556 ± 0.613) was significantly lower than that of dumpsite (71.760±2.699). The values of K in the stem and root, P in the leaf, and N in the stem and root were higher in samples from farmland, while samples from dumpsite had higher value of N in leaf, P in stem and root and K in leaf. Only, Zn in the soil was significantly higher (p<0.05) at dumpsite than farmland. The values of N, P and K in soil from dumpsite were higher (p<0.05) than that from farmland. The DNA bands of the *Solanum lycopersicum* from control site and dumpsite site have no alteration. In conclusion, all the metal levels did not exceed World Health Organization limits and thus *S. lycopersicum* is safe for human consumption.

Keywords: Heavy Metals; *Solanum lycopersicum*; DNA; Dumpsite; Farmland; Ojota; Badagry

1. Introduction

One of the most popular crops with a unique role in human diets especially in Nigeria is *Solanum lycopersicum.* They contain various health-promoting factors including vitamins, minerals, proteins, fiber, antioxidant molecules, and some essential minerals like iron, calcium, manganese, potassium, phosphorus, nitrogen, sodium and other trace elements [1] They are largely consumed and so cultivated almost all year round in different parts of Northern Nigeria by irrigation [2]

Like every other crops, *S. lycopersicum* are frequently exposed to a variety of abiotic stresses including salinity, drought and heavy metal pollutions. Several industrial activities, urban waste, spraying and fertilization held in agriculture and uses of heavy metal-containing pesticides are some of the sources of heavy metal pollution. These pollutants causes decrease in the quality of agricultural products [3]; [4] [5] disclosed that dumpsite's soils in south-western Nigeria and other part of the country support plant growth and biodiversity and as such they have been extensively used for cultivating varieties of edible vegetables and plant based food stuffs. However, the content of edible portions of crops has been known to directly reflect soil contents. The aetiology of many diseases including cancers has been associated

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with environmental factors such as exposures to known toxicant metals and pathogens in the environment. Unregulated refuse disposal, refuse dump sites close to residential areas, rivers and farm lands have also contributed largely to increased exposure to toxic heavy metals in Nigeria [6]). Studies have shown that some metals such as lead apart from contaminating soil are capable of inactivating enzymes, permit or enhance carcinogenic events involved in DNA damage, DNA repair and regulation of tumour suppressor and promoter genes [7] Universally, *S. lycopersicum* are commonly used vegetables by humans in their daily consumption thus their level of heavy metals should be very minimal if not absent. Therefore it is highly pertinent at this point in time to investigate the health status of *S. lycopersicum* cultivated in dumpsite (Ojota) and farmland (Badagry) in Lagos State, Nigeria and as well as investigates the effects on the metals on the DNA of *S. lycopersicum.*

2. Material and methods

2.1. Site Description

The two sampling sites purposively selected for this study were farmland at Badagry town in Badagry Local Government Area, and dumpsite situated at Ojota town in Oshodi Local Government Area of Lagos state. Ojota town is situated on latitude $6^0 35' 16"$ N and longitude $3^0 22' 56"$ E, while Badagry town is on latitude 6.4183^0N and longitude 2.8901^0E.The *Solanum Lycopersicum* at the farmland are grown by Horticulturist under a well suitable conditions, while plant at the dumpsite grow naturally on their own as a results of some waste of the plant species that might have been dumped there along with domestic refuse.

2.2. Sample Collection and Preparation

Samples of *Solanum Lycopersicum* (tomato) used for this study were collected between December 2021 and February, 2022. The plant species 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 a masking tape and a marker. The soil was taken to the laboratory for heavy metal and nutrient analysis, while the plant samples were analyzed at the laboratory for heavy metal, nutrient and DNA examination.

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, 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 550^0C, 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 while 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 fume cupboard, for 30 min. The mixture was allowed to cool to about 25^0C 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 digested by atomic absorption spectrometry while test results were validated with calibration curves obtained with certified metal standards (Accu Standard Inc, USA). Quantitation of metal levels in the vegetable samples was obtained with Perkin Elmer WinLab AA software. Conversion of instrument data from mg/L to mg/100g of vegetable is given by:

$$\text{Concentration of metal (mg/100g)} = \frac{\text{mg/L x 0.1 x Vfinal}}{W}$$

$$= \frac{\text{mg/L x 0.1 x 25}}{5}$$

Where;

mg/L = concentration of the metal in the digest,

V_{final} = final volume of the digested solution (ml) = 25

W = weight of sample digested (ashed)

0.1= Factor for conversion to mg/ kg.

2.6. Reagent 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.

2.7. Procedure for Preparation of 100mL SDS extraction buffer

Samples were prepared by putting approximately 100mg of freeze dried tissues (leaf, root and stems) of *Solanum Lycopersicum* 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, 450µl of pre-heated plant extraction buffer were added to the mixture, while the tubes were incubated at 65^0c 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, follow by centrifuge at 10000rpm for 10 min and then transfer supernatant into freshly labeled tubes. Thereafter, 450µl 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^oc for 15mins to precipitate the DNA.

2.8. Further Centrifugation and Decantation

After following the incubation periods, samples were cooled at room temperature, and then centrifuged at 100000rpm for 10 min, while 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, 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 incubated in 37^oc for 30-40 minutes.

2.9. Checking for 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^oc 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 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 on to the 0.8% agarose gel. The gel was later run at 80 volt 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.

2.10. 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.

2.11. 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 MgCl2, 1ul each of forward primer and reverse primer, 1ul of DMSO, 2ul of 2.5mMDNTPs, 0.1ul of 5u/ulTaq 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. The primer sequences for RBCL were: RBCL-F535: CTTTCCAAGGCCCGCCTCA and RBCL-R705: CATCATCTTTGGTAAAATCAAGTCCA

3. Results

Table 1 showed the heavy metals' concentration and nutrients (N, P, K) in tomato grown on Ojota's dumpsite and a farmland in Badagry, Lagos state, Nigeria. The results recorded in mg/100g showed that there are no significant difference ($p>0.05$) in the level of cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) recorded in the leaf, stem and root of tomato across the two sampling sites. However, the concentrations of the zinc (Zn) in the stem and root of tomato between dumpsite and farmland were significantly different ($p<0.05$). While the value of Zn in stem of tomato from farmland (77.671 ± 2.193) was significantly higher than that of dumpsite (72.169 ± 1.656), on the other hand, zinc in the root of tomato plant from farmland (66.556 ± 0.613) was significantly lower than that of dumpsite (71.760 ± 2.699). However, there was significant different ($p<0.05$) in the values of nitrogen, phosphorus and potassium recorded in the leaf, stem and roots of tomato from the dumpsites and farmland. Specifically, the values of N,P and K in the leaf, stem and root of tomato from farmland were: (312.845 ± 3.373, 209.095 ± 0.177, 237.545 ± 3.458); (94.771 ± 2.179, 56.931 ± 3.847, 106.137 ± 5.496) and (994.489 ± 4.600, 436.150 ± 5.699, 1072.995 ± 8.337) respectively, while the trend of N, P and K in the leaf, stem and root of tomato from dumpsite were: (316.331 ± 8.302, 113.897 ± 2.357, 207.122 ± 0.648); (92.460 ± 1.090, 129.071 ± 5.587, 111.738 ± 1.010) and (998.850 ± 1.569, 286.176 ± 0.232, 859.719 ± 7.451) respectively. The values of K in the stem and root, P in the leaf, and N in the stem and root were higher in samples from farmland, while samples from dumpsite had higher value of N in leaf, P in stem and root and K in leaf.

The results of concentration of heavy metals and nutrient in soil are presented in figure 1 and 2 respectively. The respective values of heavy metals (mg/kg) in soil at farmland and dumpsite are: Cd (0.098 ± 0.001, 0.644 ± 0.002), Cr (2.006 ± 0.002, 3.888 ± 0.002), Cu (0.206 ± 0.001, 0.997 ± 0.001), Pb (0.005 ± 0.003, 0.843 ± 0.002), Zn (882.0 ± 0.006, 14316.8 ± 1.009) respectively. On the other hand, values of N (3153.6 ± 0.008, 4191.2 ± 0.006), P (7598.3 ± 0.009, 8794.5 ± 0.003) and K (113.56 ± 0.004, 532.12 ± 0.004) were recorded in the soil from farmland and dumpsite respectively. For the metals in the soil, only values of Zn was significantly higher ($p<0.05$) in sample of dumpsite when compared with that from farmland. However, all the values of N, P and K in soil from dumpsite were higher ($p<0.05$) than that from farmland.

Table 1 Heavy Metals Concentration in Tomato from Dumpsite (Ojota) and Farmland (Badagry) in Lagos State, Nigeria

Parameters (mg/100g)	Dumpsite			Farmland		
	Leaf	Stem	Root	Leaf	Stem	Root
Cd	0.002 ± 0.001[a]	0.002 ± 0.001[a]	0.009 ± 0.001[a]	0.005 ± 0.001[a]	0.003 ± 0.001[a]	0.008 ± 0.002[a]
Cr	0.207 ± 0.004[a]	0.126 ± 0.006[a]	0.492 ± 0.008[a]	0.207 ± 0.003[a]	0.117 ± 0.004[a]	0.117 ± 0.006[a]
Cu	0.130 ± 0.005[a]	0.118 ± 0.007[a]	0.764 ± 0.005[a]	0.124 ± 0.003[a]	0.073 ± 0.008[a]	0.093 ± 0.008[a]
Pb	0.003 ± 0.002[a]	0.006 ± 0.001[a]	0.030 ± 0.004[a]	0.003 ± 0.002[a]	0.005 ± 0.001[a]	0.009 ± 0.001[a]
Zn	82.493 ± 3.056[a]	72.169 ± 1.656[b]	71.760 ± 2.699[b]	82.970 ± 3.734[a]	77.671 ± 2.193[ab]	66.556 ± 0.613[bb]
N	316.331 ± 8.302[a]	113.897 ± 2.357[b]	207.122 ± 0.648[bb]	312.845 ± 3.373[aa]	209.095 ± 0.177[ab]	237.545 ± 3.458[ac]
P	92.460 ± 1.090[a]	129.071 ± 5.587[b]	111.738 ± 1.010[ac]	94.771 ± 2.179[ab]	56.931 ± 3.847[aa]	106.137 ± 5.496[bb]
K	998.850 ± 1.569[a]	286.176 ± 0.232[b]	859.719 ± 7.451[ac]	994.489 ± 4.600[ab]	436.150 ± 5.699[aa]	1072.995 ± 8.337[bb]

Mean value with same superscript in the row =not significant different ($p>0.05$)
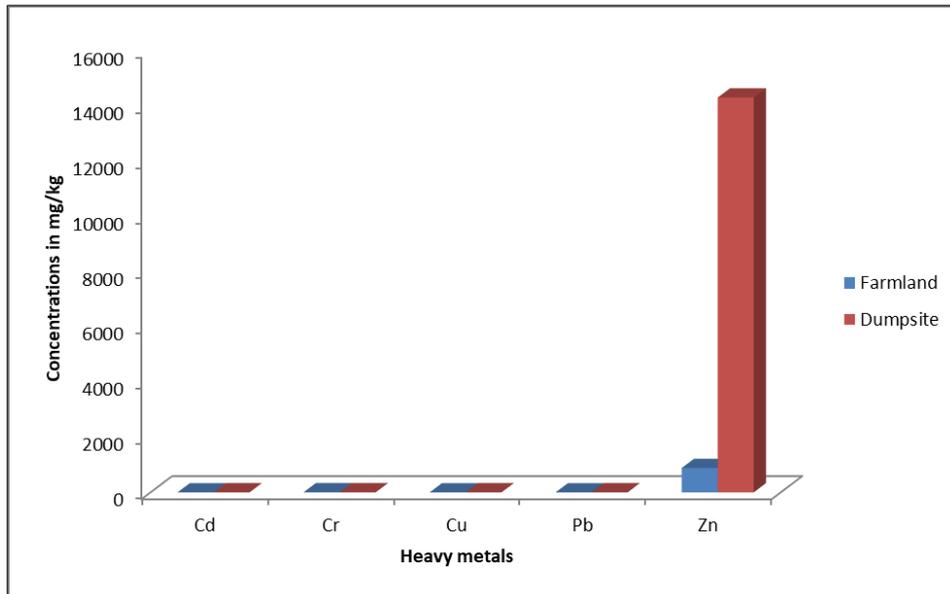
Figure 1 Heavy metals concentrations in soil from the dumpsite and farmland
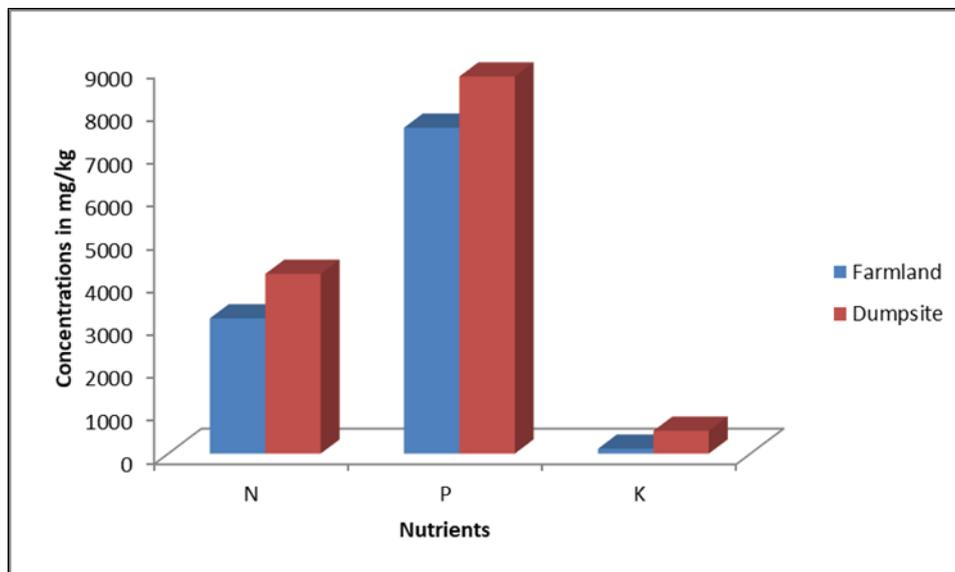

Figure 2 Concentrations of nutrients in soil from the dumpsite and farmland

The frequency of the examined heavy metals (Cd, Cr, Cu, Pb and Zn) and nutrients (N, P and K) in tomato from the dumpsite and farmland are presented in figures 3 and 4 respectively. At both dumpsite and farmland, the most frequent metal in the leaf, stem and root of tomato was zinc (Zn), however, their trend was in the order: leaf > stem >root. Metal with least value at both sites was Cd which was recorded in leaf and stem at the dumpsite and stem at the farmland respectively. As shown in figure 4, K was the most abundant nutrients recorded in tomato across the two sites. At dumpsite, highest value of K was obtained in the leaf while root had the peak value of K at the farmland. On the other hand, the nutrient with the least values was P that was recorded in stem and root of tomato from farmland and dumpsite respectively.
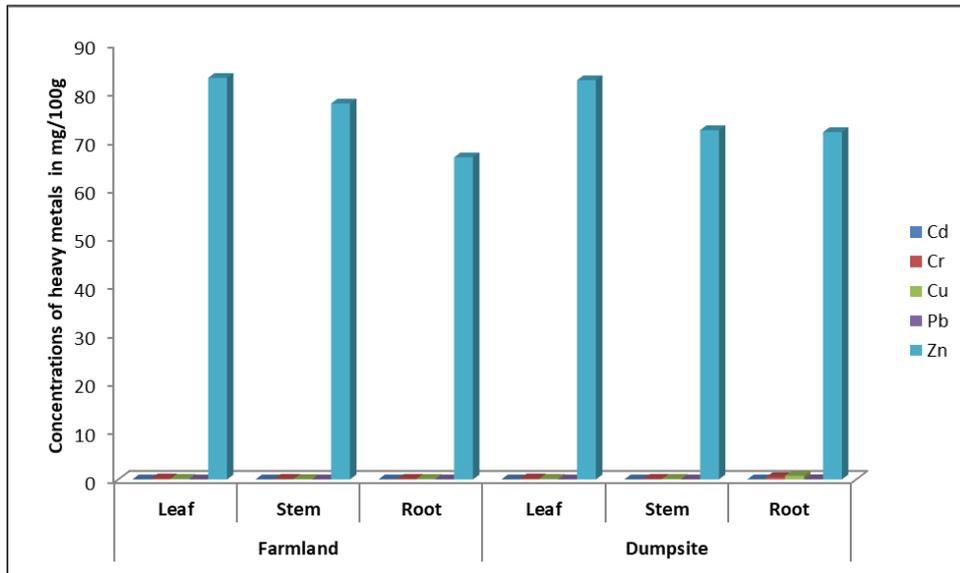
Figure 3 Variation of heavy metals in the leaf, stem and root of tomato from the two sites
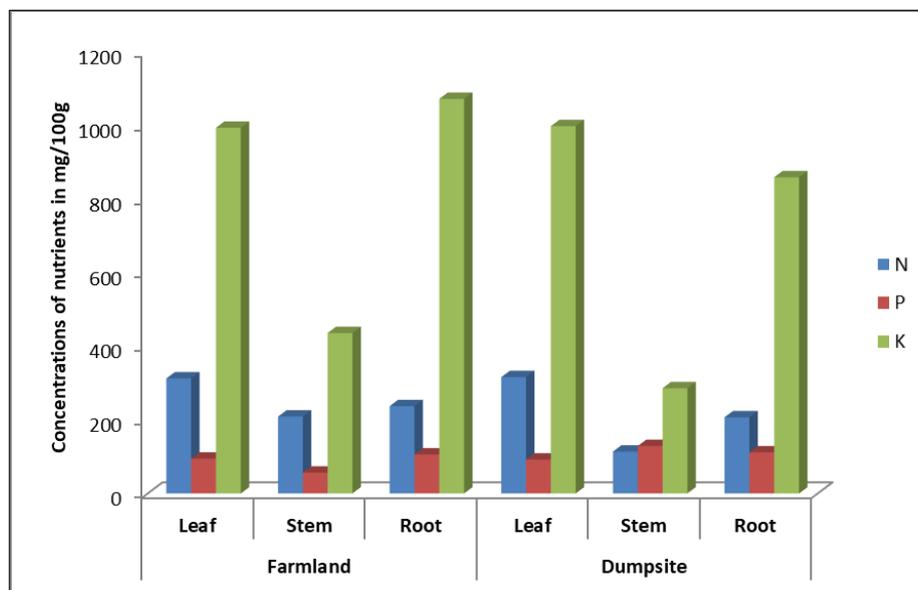

Figure 4 Variation of nutrients in the leaf, stem and root of tomato from the two sites

The DNA bands of the *S. lycopersicum* from farmland and dumpsite is presented in Plate 1. There was no apparent fragmentation in the DNA nucleotide bases of the two samples,
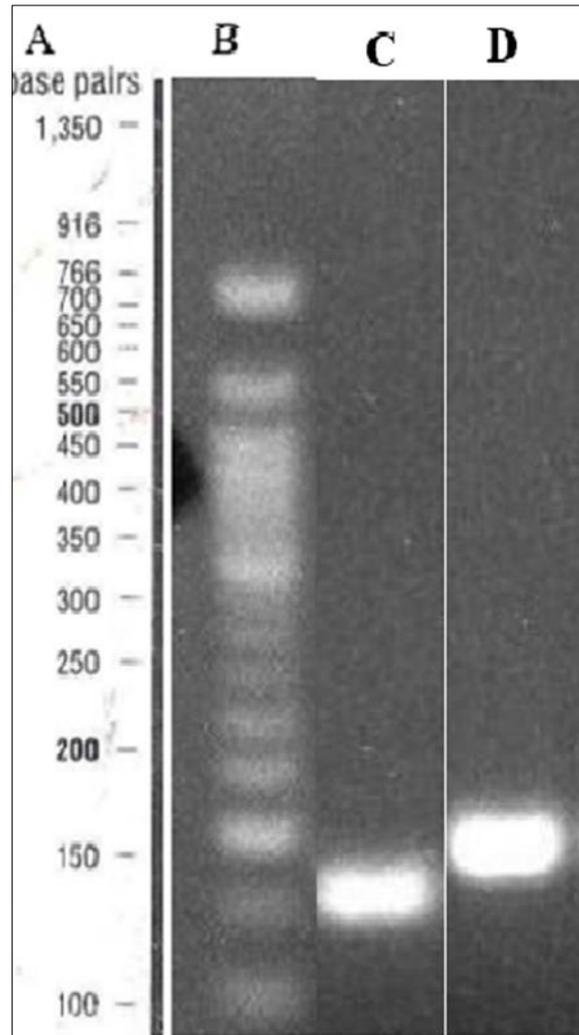
Figure 5 DNA-fragmentation analysis of *S.lycopersicum* collected from the farmland and dumpsite

4. Discussion

In this study, there was no significant difference in the level of cadmium, chromium, copper, and lead recorded in the leaf, stem and root of *S.lycopersicum* between the sampling sites. This could suggest that level of metals contamination at both site are similar and this observation negates the general notion that dumpsites are often more polluted than conventional farmland. Also, this observation could imply that less human activities exist on the sampled dumpsites. [8] had opined that the composition of waste dumps varies widely with many human activities located close to dumpsites. However, the concentration of the zinc in stem of *S. lycopersicum* from farmland for this study was significantly higher than that of dumpsite, while zinc in the root of tomato plant from dumpsite was significantly higher than that of farmland. Zinc is known as major metal component and activator of several enzymes involved in metabolic activities and biochemical pathways. It is a functional, structural or regulatory co-factor of a large number of enzymes. It is required in a large number of enzymes and plays an essential role in DNA transcription [9]

However, the concentrations of all metals recorded in the tissues (leaf, stem, root, fruit and shoot) in *S. lycopersicum* for this study were all within [10] permissible limits. Similar reports of level of metals that were not above permissible limits have been reported in *Hibiscus sabdariffa, Letuca sativa* and *Amaranthus caudate* cultivated in Sabon Tasha Yola, Adamawa State [11] [12] also reported results that connotes with the current findings but exceeded recommended standards maximum value for fruit vegetables set by [13]

As recorded in this study, the values of K in the stem and root, P in the leaf, and N in the stem and root were higher in samples from farmland, while samples from dumpsite had higher value of N in leaf, P in stem and root and K in leaf. This observation indicated that *Solanum lycopersicum* from both sites are rich in N, P and K. The levels of N, K and P in this

study were lower than that reported in *Solanum lycopersicum* by [1] and [13] but was similar to that reported by [14] All the levels of metals in *Solanum lycopersicum* for this study was within permissible limits.

The concentration of metals in the soil from both sites were above that reported by [15] and exceeded the limits recommended by [10] in soils. However, values of metals in soil for this study were similar to that reported in Ekiti state [16] The trend of metals content in the leaf, stem and root of *S. lycopersicum* for this in which root has the higher content of metals, was synonymous to the observation of [17] which worked on levels of heavy metals (Cd, Co, Cr, Cu, Ni, Pb and Zn) in roots and shoots of *Amaranthus spinossus* nd *Talinum triangulare* collected from three dumpsites.

As shown in this study, K was the most abundant nutrients recorded in tomato across the two sites. While at dumpsite, highest value of K was obtained in the leaf, root had the peak value of K at the farmland. On the other hand, the nutrient with the least values was P that was recorded in stem and root of tomato from farmland and dumpsite respectively. The present of K and P at reasonable amount in the *Solanum lycopersicum* from both sites and their values being below maximum permissible, confirmed that the plant is rich in mineral. [1] had reported appreciable concentrations of nitrogen, phosphorus, and potassium in *Solanum lycopersicum.* Also, in the study carried out by [18] on peel byproduct of tomato potassium presented the highest concentration. The present findings was in line with the work of [18] and [13]

As revealed in this study, there was no mutation in the DNA constituents of *S. lycopersicum* collected from both the dumpsite and farmland. This observation was different from the results gotten by [19] which showed that plant in polluted sites had more DNA yield and fragmentation than those from non-polluted sites. Also, the findings of [19] indicated that dumpsite, mechanic workshop and metal scrap sites are potential sources of toxic metals, which can pose serious human health and ecological risks. However, the work of [20] indicated that solid waste pollution gave the highest reduction in DNA and RNA content of Olive plants. In line with the present findings, [21] reported that there was no DNA mutation on Nepenthes plant from abandoned mine and farmland.

5. Conclusion

It has been established in this study that there was no deleterious effect of heavy metals on the examined plant from both dumpsite and farmland. Also, the level of metal contamination of the examined plant which did not exceed World Health Organization limits indicated that the plant is safe for human consumption. However, efforts must be made by farmers and all users of soil around the study area to discharge metals responsibly.

Compliance with ethical standards

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Disclosure of conflict of interest

All the authors that contributed in this research have no conflict of interest whatsoever.

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