Original Research Article

Comparative Analysis of Heavy Metals Concentration in Soil and Vegetable (*Vernonia Amygdalina)* Collected From Two Sampling Sites (Farmland And Dumpsite) And The Effect On Plant DNA

ABSTRACT

Different studies have shown that *Vernonia amygdalina* is enriched with proteins, fats, fibres, amino acids, minerals vitamins, and carbohydrates but no record yet on the level of heavy metals *Vernonia amygdalina*from Ojota (dumpsite) and Badagry(control) in Lagos state. Samples of *V. amygdalina*were collected and analyzed for metals using standard method, while differences in concentrations were analyzed using t-test analysis at level of significance set atp=0.05. The results recorded in mg/100g for the metals content (Cd, Cr, Cu, Pb and Zn) in the plant across the two stations were not significantly different ($p>0.05$). The Zn in leaf and stem (100.157 ±1.351,45.743± 3.664) respectively from dumpsite were significantly higher than that of farmland (91.468 ± 2.386, 35.505 ±1.662). Also,Zn content in the rootfrom farmland (73.671 ±2.205) was higher than that of dumpsite (69.515 ± 2.312). All the values of N, P and K recorded in the leaf, stem and roots of bitter from both sites differs significantly ($p<0.05$). The most frequent nutrient in the leaf, stem and root of the bitter leaf was K, followed by N while the least was P. In contrast, the root contained the highest K, followed by the leaf and then the stem. The DNA band of *V. amygdalina*from the both sites showed no alteration. In conclusion,bitter leaf from both sites are rich in minerals(N,P,K) though their values were higher at the dumpsites. However, heavy metals contents in *V. amygdalina*at both sites being within standard permissible limits imply it is safe for human consumption.

Keywords: *Vernonia amygdalina, Heavy Metals, Farmland, Dumpsite, DNA, Bioacumulation, Lagos*

1.0 INTRODUCTION

***Vernonia amygdalina*(Bitter leaf) is one of the most famous plants found in Africa and Asia, and is a highly appreciated vegetable in West and Central Africa that are often consumed in various dishes (Farombi and Owoeye, 2011; Ijeh and Ejike, 2011).The nutritional compositions of *V. amygdalina* leaf, root and stems varied from one study to another, probably due to different geographical location, genetic, environmental, harvest conditions, and ecology of the plant. Most plants and animals depend on soil as a growth substrate for their sustained growth and development. In many instances the sustenance of life in the soil is adversely affected by the presence of deleterious substances or contaminants (Adu *et al.*, 2020). The contamination of soils with heavy metals or micronutrients in phytotoxic concentrations generates adverse effects not only on plants but also poses risks to human health (Murugesan *et al.*, 2008).**

Plants including *Vernonia* species are sessile organisms and are often confronted with a variety of stress factors simultaneously, which can dramatically decrease their yield and quality. The accumulation of heavy metals such as lead (Pb), nickel (Ni), chromium (Cr), cadmium (Cd), mercury (Hg), aluminium (Al), zinc (Zn), copper (Cu), and tin (Sn) etc in the soil over a long period of time inadvertently becomes part of the biogeochemical cycle (Afolayan, 2018; Ayeni *et al.*, 2017). Over time, the environmental hazardous metals accumulated in the soils resulting in high metals concentration level uptake in plants (Afolayan, 2018).Similarly,the contaminated farmlands used for the cultivation of crops and plants in some way negatively influence physiological activities of the

plants (plant growth, dry matter accumulation and yield) (Oyelekee*t al*., 2016). Studies by Rattan *et al*. (2005) have shown that plant species and plants absorbing levels determine heavy metal accumulation, which has adverse effects on plants. The consumption of crops contaminated by heavy metals contaminated agricultural lands have been identified to be the leading causes of health dysfunctional, which are but not limited to impaired psychosocial faculties, intrauterine growth retardation, low immunological defences, upper gastrointestinal cancer, bloody urine, icterus, kidney failure, stomatitis etc (Duruibe *et al*., 2007). Indiscriminate dumping of wastes (domestic, industrial, hospital, agriculture etc) on free and unoccupied land spaces create both long and short term environmental and health problems. Unfortunately, indiscriminate waste dumping and open dumpsite burning are the most prevalent forms of waste disposal practice in Nigeria. To this end, there is a growing sense of global urgency regarding the pollution of our environment by an array of chemicals used in various activities (Palaniappan *et al*., 2009). With the increasing health risks associated with the presence of metals in the soil, exposure to contaminant type, exposure pathway and its vulnerability, it is therefore, necessary to monitor the contamination level in soil constantly (Ogunkule and Fatoba, 2014; Adekola *et al*., 2012).

The use of plants' DNA as bioindicators of genetic toxicity of environmental pollutants such as heavy metals has been reported in several studies (Valavanidis and Vlachogianni, 2010). Mustapha *et al.* (2019) assessed thelevels of heavy metals in plant and soils from dumpsite, mechanic workshop and metal scrap site in Lagos and their effects on the plants using their DNA as biomarker. Their findings indicated that plant in polluted sites had more DNA yield and fragmentation than those from non-polluted sites; more smearing of DNA was also identified in polluted plants than those from control sites. Weixuan *et al.* (2019) elucidated heritable changes in gene expression and DNA methylation in rice upon exposure to heavy metal stress and their results showed that gene component of the plant were alter with high heavy metal uptake from the soil. Study carried out by Carolyn *et al*.(2016) on the effect of heavy metals on the DNA mutation on Nepenthes plant from abandoned mine indicated that the DNA band of the plant from the abandoned mine were located at the same location with the control Nepenthes which implies that no mutation occur for the plant collected from the abandoned mine. 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.

Based on this background, this study aimed to investigate the concentrations of heavy metals (Cd, Cr,Cu, Pb, Zn) and nutrients (N, P, K) in leaf, stem, and root of *Vernonia amygdalina* and soil collectedfrom dumpsite at Post Service in Ojo and Farmland in Badagry town, and as well investigate effects of the selected metals on *Vernonia amygdalina* collected from both sites by using DNA analysis.

2.0 MATERIALS AND METHODS

Sample collection and Preparation

Samples of *Vernonia amygdalina*used for this study were collected from Post service (dumpsites) and Badagry (Farmland) in Lagos, Nigeria between December 2021 and February, 2022. The plant species 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, while the effects of heavy metal on the plant were investigated via DNA examination.

Calibration of Metals (Cd, Cr, Cu, Pb, Zn)

Calibration standards were prepare by diluting the appropriate certified reference standard solutions (100 mg/L) with deionized water to contain0.050 - 1.00mg/Lofeachofthe multi-element mix of the metals standards from AccuStandardInc.The certified reference standard calibration mix is a 100 mg/L multi-element standardpurchasedcommercially(AccuStandard, Inc, USA).

Pre-treatment Methods for Plant and Soil Samples

The leaves, stems and roots of the*Vernonia amygdalina* 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 ℃, 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.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

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.

Procedure for Preparation of 100mL SDS extraction buffer

Samples were prepared by putting approximately 100mg of freeze dried tissues (leaf, root and stems) of *Vernonia amygdalina*into an extraction tube. Then, two steel balls were added each into the tube to enable grinding while the 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.

Centrifugation and Decantation

After the incubation periods, samples were cooled at room temperature, 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 incubate in 37^{o}c for 30-40 minutes.

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^{o}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 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 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 MgCl2, 1ul each of forward primer and reverse 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 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 t-test and the level of significance at 95% confidence was set at $p \leq 0.05$.

3.0 RESULTS

The results recorded in mg/100g for the metals content (Cd, Cr, Cu, Pb and Zn) in the leaf, stem and root of bitter leaf, as well as nutrients (N,P and K) in bitter leaf across the dumpsite(Post service) and farmland (Badagry) is presented in Table 1.The values of Cd, Cr, Cu, Pb recorded in the leaf, stem and root of bitter leaf from the two sampling sites were not significantly different($p>0.05$). But, the concentrations of the zinc (Zn) in the leaf, stem and root of between the dumpsite and farmland were significantly different ($p<0.05$). While the values of Zn in leaf and stem(100.157±1.351,45.743± 3.664) respective from dumpsite were significantly higher than that of farmland(91.468 ± 2.386, 35.505 ±1.662), the Zn content in the root was higher in sample from farmland (73.671±2.205) than that of dumpsite (69.515 ± 2.312). However, all the values of N, P and K recorded in the leaf, stem and roots of bitter from both sites differs significantly ($p<0.05$). Samples from dumpsite had higher P in the leaf, stem and root, higher N in the stem and higher K in the leaf. On the other hand, farmland's samples had higher N in leaf and root, and higher K in stem and root.

Table 1: Heavy Metals Concentration in bitter leaf from Dumpsite (Post Service) and Farmland (Badagry) in Lagos State, Nigeria

	Dumpsite			Farmland			WHO (2005) Permissible Limits(mg/100g) for human consumption
Parameters (mg/100g)	**Leaf**	**Stem**	**Root**	**Leaf**	**Stem**	**Root**	
Cd	0.003± 0.002[a]	0.002 ±0.001[a]	0.009 ±0.001[a]	0.002 ±0.001[a]	0.002 ±0.001[a]	0.008 ±0.001[a]	0.1
Cr	0.171± 0.009[a]	0.061 ± 0.007[a]	0.223± 0.004[a]	0.335± 0.003[a]	0.095 ±0.003[a]	0.207 ±0.004[a]	1
Cu	0.127± 0.008[a]	0.099 ±0.001[a]	0.104± 0.010[a]	0.074 ±0.004[a]	0.048 ±0.006[a]	0.043 ±0.007[a]	40
Pb	0.006± 0.001[a]	0.016 ±0.005[a]	0.016 ±0.005[a]	0.004 ±0.001[a]	0.006 ±0.001[a]	0.005 ±0.001[a]	0.3
Zn	100.157 ±1.351[a]	45.743 ± 3.664[aa]	69.515 ±2.312[bb]	91.468 ±2.386[ab]	35.505 ±1.662[b]	73.671 ±2.205[bc]	600
N	213.285±2.242[a]	252.717 ±3.697[b]	320.566 ±0.772[c]	584.502 ±5.122[bb]	143.155 ±1.649[ab]	663.375 ±4.629[bc]	1200
P	117.334 ±3.752[a]	57.540 ±2.063[aa]	199.988 ±0.145[bb]	109.872 ±2.133[b]	36.489 ± 2.109[c]	167.010 ±2.659[ac]	1500
K	416.327 ±5.496 [a]	307.361 ± 2.559[b]	526.260 ±6.491[bb]	610.214 ±2.042 [c]	399.205 ±1.490[ab]	784.730 ±7.623[ac]	1800

Mean value with same superscript in the row =not significant different (p>0.05)

The result of the concentration of heavy metals and nutrient in soil is presented in figure 1. The respective values of heavy metals (mg/kg) in soil at farmland and dumpsite are: Cd (0.098 ± 0.001, 0.337 ± 0.001), Cr (2.006 ± 0.002, 1.778 ± 0.002), Cu (0.206± 0.001, 3.983 ± 0.002), Pb (0.005± 0.003, 3.008 ± 0.002), Zn (882.0± 0.006, 1023.8 ±1.008) respectively. On the other hand, values of N (3153.6 ± 0.008,3243.5 ± 0.006), P (7598.3 ± 0.009,7598.2 ± 0.003) and K (113.56 ± 0.004, 670.12 ± 0.004) were recorded in the soil from farmland and dumpsite respectively. For the metals in the soil, only values of Cu, Pb and Zn was significantly higher ($p<0.05$) in sample of dumpsite when compared with that from farmland. However, values of N and K in soil from dumpsite were higher ($p<0.05$) than that from farmland.

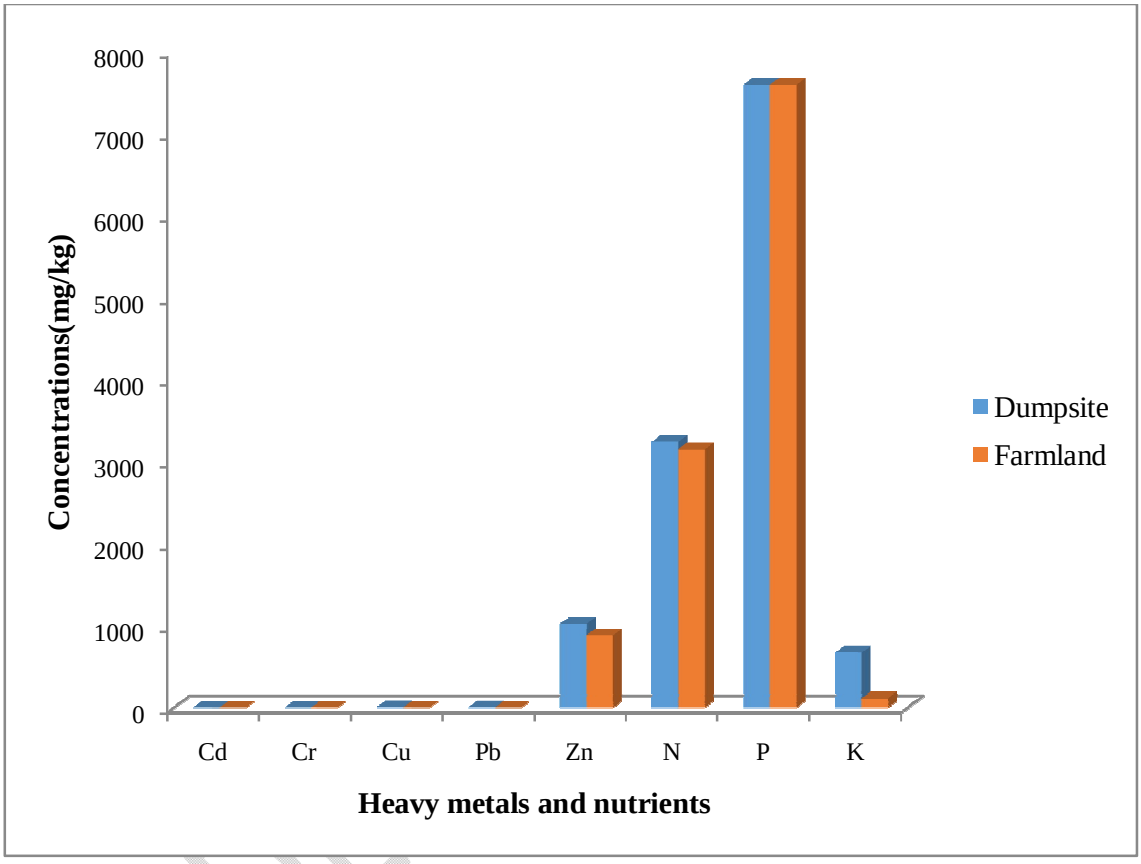

Figure 1: Concentrations of heavy metals and nutrients in soil from the dumpsite and farmland

The frequency distribution of heavy metals and nutrients in bitter leaf are shown in Figures 2 and 3 respectively. As observed in figure 2, Zinc (Zn) was the most abundant metal in the leaf, stem and root of bitter leaf while the least was Cd. In comparison of the level of Zn on the part of the plant, leaf had the highest Zn, followed by root and then stem. The most frequent nutrient in the leaf, stem and root of the bitter leaf was K, followed by N while the least was P. In contrast to what was observed in figure 2, the root contained the highest K, followed by the leaf and then the stem as shown in figure 3.

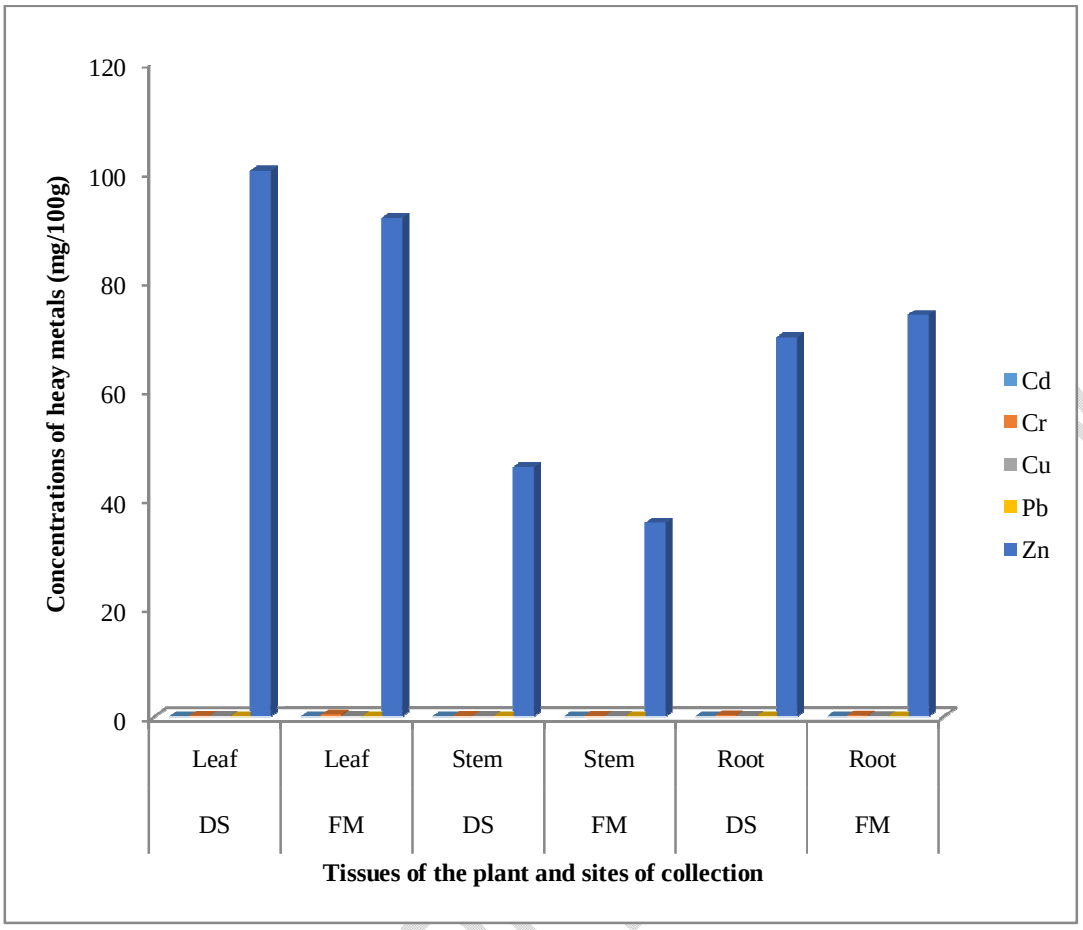

Figure 2: Variation of heavy metals in leaf, stem and root of bitter leaf from dumpsite (DS)and farmland (FM)

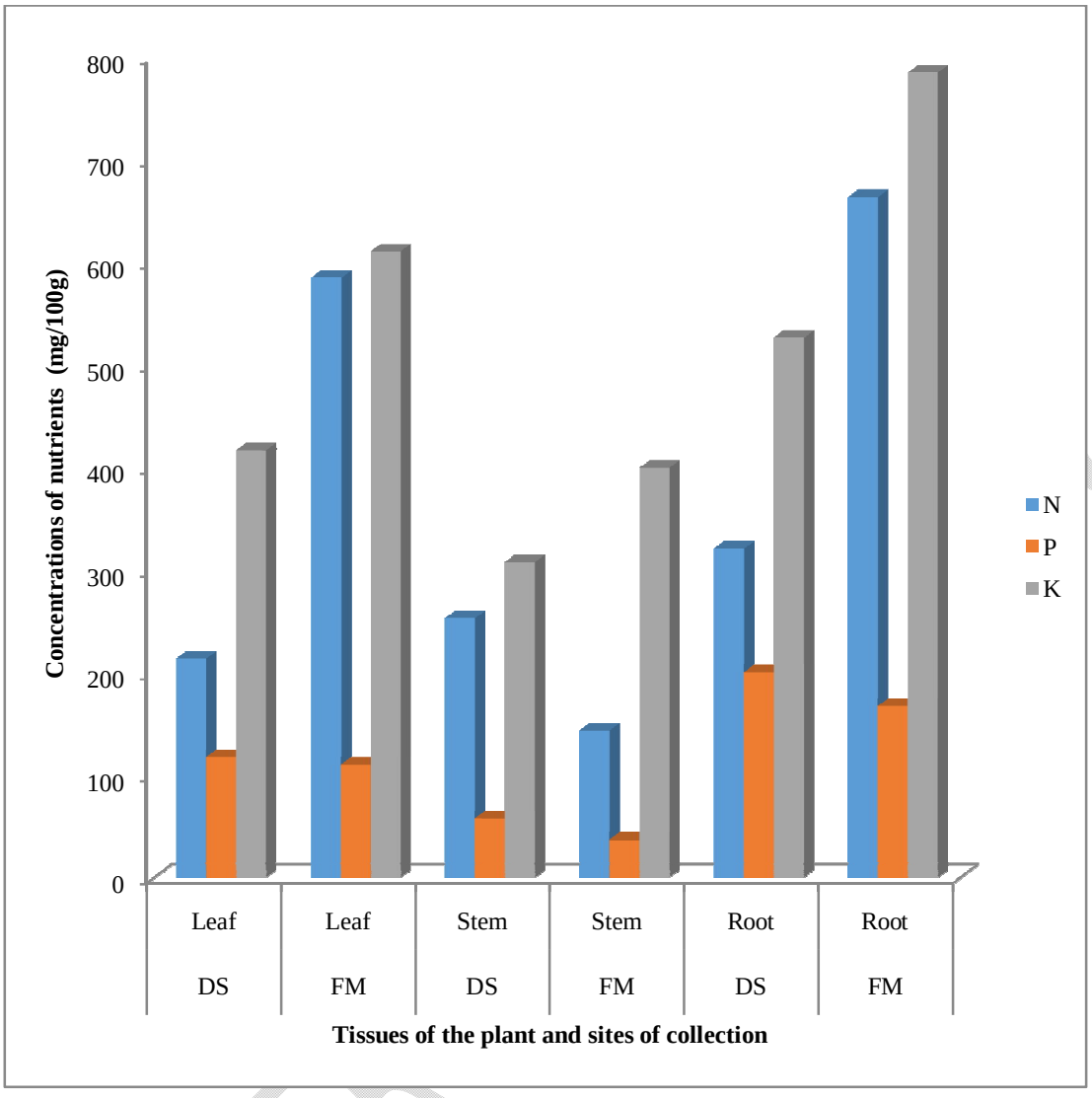

Figure 3: Variation of nutrients (N, P, K) in leaf, stem and root of bitter leaf from dumpsite (DS) and farmland (FM)

The result of the probable DNA fragmentation of *V. amygdalina* collected from both the farmland and dumpsite is presented in Plate 1.There was no vivid damage or changes to the base pairs of the two samples.

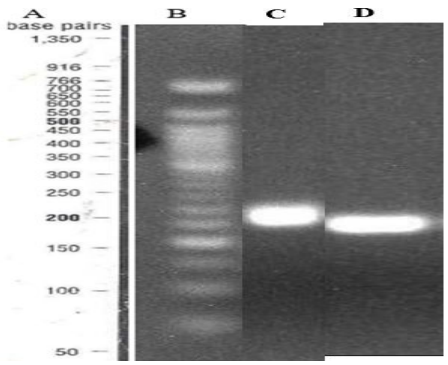

Plate 1: DNA-fragmentation analysis of *V. amygdalina* collected from the farmland and dumpsite

DISCUSSION

In this study, only level of zinc (Zn) in the leaf, stem and root of *Vernonia amygdalina*collected from the dumpsite and farmland were significantly different. However, all the values of N, P and K recorded in the leaf, stem and roots of *V. amygdalina* from both sites differs significantly. This observation of indifference in the metal loads of *V. amygdalina* across the two sampling stations could suggest that rate of metals discharge at the dumpsite is still moderate at present. Also, the similarity in the metals values for the dumpsite and farmland could be that the dumpsite is not an industrial dumpsite. As opined by Hang *et al.* (2016) which worked on plants grown at Eagle Island /Agip and Eneka / Igwuruta both of industrial dumpsites and domestic dumpsite, higher concentrations of heavy metals were found at the industrial dumpsite than the domestic dumpsite.The findings of Okorosaye-Orubite and Igwe (2017) was not similar to the observation in this study, as they reported heavy metals (Pb, Cd, Cu, Ni, Cr, As, Hg, and Zn) in *Vernonia amgdylina*, *Telfairia occidentalis*, *Amaranthus hybridus*, *Abelmoschus esculentus* and *Amaranthus viridis* harvested at some dumpsites that were significantly higher and varies from the results for the farmlands. However,zinc in the leaf, stem and root of *V.* being the most abundant in this study, connoted with their findings.The values of all metals recorded in this study were lower than that reported in *V.amygdalina* from Ibeno in Akwa Ibom State (Emmanuel *et al.*, 2020).All the values of heavy metals and minerals examined in *V. amygdalina* for this study did not exceed recommended permissible limits. (World Health Organization). In contrast to the present findings, Amah *et al*. (2018) which investigated the heavy metals (Hg, Cd, Mn and Pb) contents in *Vernonia amygdalina* collected from Eke Okigwe Market in Imo State reported that only values of Mn and Cd were still within recommended permissible limits for consumption. High level of metals in plant has been attributed to the occurrence of metallic waste deposited from industrial and municipal wastes in the areas (Adefemi and Awokunmi, 2013).

For the metals in the soil, only value of Cu, Pb and Zn were significantly higher in sample of dumpsite when compared with that from farmland. The study by Oluyemi and Awokunmi (2013) and Akintan *et al.* (2019) also showed elevated level of Cu and Cr in the dumpsites compared to the farmlands. Also, values of N and K in soil from dumpsite were higher than that from farmland. All the values of detected metals in the soil from both sites were below standard maximum permissible limits. Similar results of soil whose metals load were within permissible limits have been reported in Ado Ekiti dumpsite (Ayeni *et al.*, 2017). However, Zhang and Wang (2007) reported that high amount of heavy metals in polluted soil could slow down the mineralization rate of soil organic carbon and increase the amount of hardly biodegradable carbon. The range of minerals (N,K,P) from both sides in this study were similar to the findings of Linkon *et al.* (2015) on vegetables samples from three sites in Bangladesh. The similarity of results at both sites could be attributed to dumping of agricultural waste which later turned to manure at the dumpsites and applications of fertilizer and other organic products on farmlands could also increase fertility of the farmlands.

There was no mutation in the DNA structure of *Vernonia amygdalina* from both sites. This implied that the level of metal contamination at both sites had no adverse effects on the plant yet. In contrast to the present observation, Weixuan*et al.*(2019) reported that there were heavy metal-induced DNA methylation changes in rice. According to Mustapha *et al* (2019), the DNA content of plants collected from polluted and control sites in their study differs per site. This was not so in this study.

Conclusion

It is clear in this study that bitter leaf from both farmland and dumpsite are rich in minerals (N, K, P) while their values were higher at the dumpsites, it was low in values at farmlands However, the non significant difference in heavy metals contents at both sites could mean that the dumpsite is less polluted with metals. However, the values of metals in the soil and *Vernonia amygdalina* at both sampling stations that were within the acceptable limit indicated that the vegetable is safe for human consumption.

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