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Comparative Analysis of Heavy Metals Concentration in Soil and Vegetable (*Celosia argentea*) Collected from Two Sampling Sites and Their Effect on the Plant's DNA Stability

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ABSTRACT

This study investigated the quantities of heavy metals including cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn)in soil samples and in the organs (leaf, stem and root) of *Celosia argentea* collected from a dumpsite (at Ojota) and a farmland (at Badagry) in Lagos, Nigeria. Additionally, the macroelement content of the soils and the effect of heavy metal soil contamination on *C. argentea* DNA stability was analysed. Heavy metals and NPK concentrations in the soil samples and organs of *C. argentea* were evaluated using standard techniques. The results of this study revealed that the microelement was more abundant in the soil collected from the dumpsite. Likewise, all the heavy metals (Cd,Cr,Cu,Pb and Zn) investigated in this study were detected in higher quantities in the dumpsite soil. Notably, the concentration of the metals was within the WHO/FAO limits in the soils collected from both sampling sites save Zn which was 660.260 mg/kg and 449.180mg/kg in the dumpsite and farmland respectively. The concentration of heavy metals in the *C. argentea* organs in this research were higher in samples collected from the dumpsite and Zn was the most accumulated on both the dumpsite and farmland. The highest amount (95.987 mg/kg) of Zn was by *C. argentea* root collected from the dumpsite while the least amount (15.380 mg/kg) was detected in *C. argentea* stem collected from the farmland. The DNA base pairs were not visibly fragmented in the samples *C. argentea* collected from the control and dumpsite sites. Although most of the heavy metals detected in *C. argentea* were in negligible quantities, the soils and the vegetable appear to be Zn-contaminated and unsafe for consumption.

Keywords: Heavy Metal, DNA, vegetables, biomagnifications, Bioaccumulation, Dumpsites Farmlands

INTRODUCTION

Urban agriculture, otherwise known as the production, processing and distribution of food varieties, including vegetables, within urban centres, has gained momentum in the recent past. It is perceived as a coping approach adopted by the poor to survive the harsh economic and social conditions in developing cities.[1] Over the years, urban land cultivation has metamorphosed into social realities and has become a response to various crises. Thus, efforts are geared toward integrating urban agriculture into cities, especially in developing nations (Adedeji et al., 2009). Nevertheless, the unorganised nature of urban and industrial developments coupled with improper waste management has led to the elevation of heavy metals in the soils of these metropolitan cities. [2]

The accumulation of heavy metals in the ecosystem evolves around the geochemical and biological cycles. It is majorly

influenced by human activities, including improper waste management, agricultural practices and industrial activities [3] Heavy metal pollution has become an environmental problem and is a crucial talking point as industrialisation continues globally. [4] Approximately 0.235 billion hectares of cultivatable land, which represent about 13.58% of the world's total cultivated land, are affected by heavy metal contamination. [5] The chief sources of heavy metals in the ecosystem include mining, fuel refining processes, energy, fertiliser and pesticide production and metal treatments [6] Heavy metal contamination has raised health concerns due to its immeasurable spread and effects on the ecosystem. Heavy metals such as lead and chromium can bioaccumulate in living organisms and are believed to be carcinogenic, toxic, mutagenic and teratogenic in certain quantities.[7]

Moreover, heavy metal contamination also negatively influences plants' physiological and metabolic status. Plants grown in heavy metal-contaminated sites often show reduced growth, lower biomass production, altered metabolism, and high metal accumulation. Heavy metals can cause various physiological, biochemical, and molecular changes in plants. [8] Molecular changes may include DNA damage in plants that directly interact with DNA or indirectly inhibit the DNA-repairing enzymes by producing ROS compounds. [9]

Vegetables such as *Celosia argentea* L. are essential components of diets in many households. They contain vitamins, minerals and several phytocompounds, which are usually of health benefits to humans. [10] Vegetables act as buffers to acidic substances produced during food digestion [11] Although there has been a public push for an increase in the cultivation and consumption of fresh vegetables, growing vegetables have become one of the major concerns for urban agriculture due to heavy metals and other toxic chemicals contamination and bioaccumulation invegetable [12] Urban farmers who are involved in cultivating vegetables within the municipality are left with no option but to cultivate their crops on road verges, river valleys, waste dumpsites, abandoned areas and wetlands whose heavy metal records are not documented due to the paucity of resources, including land .[12] Therefore, this study aimed to assess the safety of *C. argentea* cultivated in a typical dumpsite in Lagos and the likely harmful effect of heavy metal pollution on its DNA fragmentation.

MATERIALS AND METHODS

Site Description

The two sampling sites purposively selected for this study were farmland at Badagry town latitude (6.4183^0N and longitude 2.8901^0E) in Badagry Local Government Area and Olusosun dumpsite situated at Ojota town (6^0 35' 16" N and longitude 3^0 22' 56" E) in Kosofe Local Government Area of Lagos State. *C. argentea* plants obtained from the farmland were cultivated on a farmland, while *C. argentea* collected at the dumpsite were grown on soils around the Olusosun dumpsite.

Sample Collection and Preparation

Samples of *C. argentea* used for this study were collected between December 2021 and February 2022. *C. argentea* collected from each sampling site was carefully harvested, while the soil particles around the root zones were collected and carefully labelled. The collected plants were then divided into leaf, stem, and root and stored in a -20^0C freezer until they were used.

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, oven-dried in the laboratory at 40^0C for five days, processed further by ashing and then used to quantify the heavy metals in the leaves, stem, and root. A 5g of each dried sample was weighed into a porcelain crucible and ashed in a muffle furnace at 550^0C for 4 hr.

Thereafter; the residue was allowed to cool and 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 used to determine 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 labelled 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 minutes. The mixture was allowed to cool to about 25^0C, filtered through Whatman #1 filter paper, and then made up to 25 ml in a volumetric flask with distilled water. The digest was then used to determine the metals

Methods of Measurements of Heavy Metal Concentration

The examined metals (Cd, Cr, Cu, Pb, Zn) were determined on the filtrate of samples by atomic absorption spectrometry. At the same time, test results were validated with calibration curves obtained with certified metal standards (AccuStandard 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:

$$\text{Concentration of metal (mg/100g)} = \frac{\text{mg/L} \times 0.1 \times V_{final}}{W}$$

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

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.

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 *Solanum lycopersicum* into an extraction tube. Then, two steel balls were added to the tube to enable grinding while the freeze-dried tissues were ground into a fine powder using Genogrinder-2000. After grinding, 450µl of pre-heated plant extraction buffer was added to the mixture. The mixtures were then incubated at 65^0C for 20 min and mixed by occasionally inverting the tubes to homogenise 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, centrifuge at 10000rpm for 10 min and then transferred supernatant into freshly labelled tubes. After that, 450µl of chloroform Isoamyl alcohol (24:1) were added and mixed gently to precipitate protein and lipids further. The mixture was centrifuged at 10000rpm for 10 minutes and then transferred with supernatant into freshly labelled tubes. This process was followed by adding 2/3 volume of ice-cold Isopropanol, which was mixed gently and incubated at -80^{o}C for 15mins to precipitate the DNA.

Further Centrifugation and Decantation

After following the incubation periods, samples were cooled at room temperature, then centrifuged at 10000rpm 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-dried until the ethanol smell disappeared. Thereafter, 60ul of ultra-pure water or low salt TE was added to re-suspend the DNA. Also, 2ul of RNase was added and incubated in 37^{o}c for 30-40 minutes.

Checking for Quality of DNA

About 0.8% agarose gel was prepared to check DNA quality and RNA removal. Briefly, 0.8gram of agarose in 100ml of 1X TBE was boiled, cooled to about 60^{o}C, and 5ul Ethidium bromide was added and gently swam to mix and spread on the gel tray before it polymerised without allowing any air bubble in the middle of the gel. A 3µL of DNA and 3µL of loading dye were mixed and briefly spun 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 80V for about 60 minutes. After this, the gel picture was saved.

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. The primer sequences for RBCL were: RBCL-F535: CTTTCCAAGGCCCGCCTCA and RBCL-R705: CATCATCTTTGGTAAAATCAAGTCCA

RESULTS

The heavy metals and macronutrients (N, P, K) concentrations of the soils collected from the two sampling sites are presented in Table 1. The highest quantities of N (2097.19±2.48), P (4397.83±2.81) and K (278.85±7.65) were detected in the soils collected from the dumpsite. The concentration of Cr (2.017±0.03) and Zn(660.26±10.14) were also higher in the soil samples obtained from the dumpsite. Similarly, the amount of Pb (0.425±0.07), Cu (0.506±0.04) and Cd (0.328±0.05) were also detected in higher quantities in the soil samples collected from the dumpsite (Table 1).From these results, the concentration of Cd, Cr, Cu and Pb in the soil collected from the two sampling sites fell below the permissible quantities of heavy metals advised by FAO/WHO (2011), while Zn, on the other hand, was found to be above the standard value.

Macronutrients/ Heavy metals mg/kg	Sampling sites		FAO/WHO Permissible limits for soils (2011)
	Farmland	Dumpsite	
Nitrogen (N)	1584.28±2.85	2097.19±2.48	
Phosphorus (P)	3800.50±3.38	4397.83±2.81	
Potassium (K)	60.640±9.36	278.85±7.65	
Cadmium (Cd)	0.500±0.20	0.328±0.05	1.40
Chromium (Cr)	1.075±0.01	2.017±0.03	100.00
Copper (Cu)	0.160±0.00	0.506±0.04	100.00
Lead (Pb)	0.040±0.00	0.425±0.07	70.00
Zinc (Zn)	449.180±21.68	660.260±10.14	300.00

Table 1: The macronutrients and heavy metal composition of the soil collected from the two sampling sites

The heavy metal concentration in the different parts of *C. argentea* collected from the two sampling sites varied significantly. From our results presented in Fig 1a-c. Zn

was the most accumulated heavy metal in the leaves, stem and root of *C. argentea*. Zn was superiorly accumulated in the root of *C. argentea* collected from the dumpsite. For Pb, the highest accumulation was recorded in the stem of *C. argentea* obtained from the dumpsite, followed by the root, whereas the least quantity of Pb was detected in the leaves of *C. argentea* collected from the farmland. The concentration of Cu was accumulated most in the root of *C. argentea* obtained from the farmland, and the lowest concentration was detected in the stem. The concentrations of Cd and Cr were also generally higher in the organs of *C. argentea* collected from the dumpsite. However, the leaves of *C. argentea* obtained from the farmland contained more Cr.

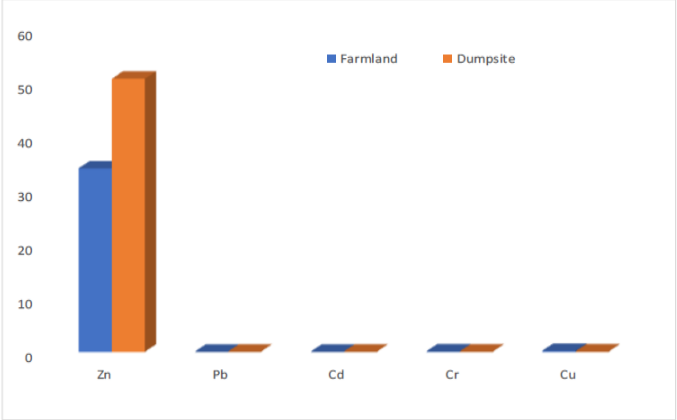

Figure 1a: Concentrations of heavy metals detected in the leaf samples of *C. argentea* obtained from farmland and dumpsite

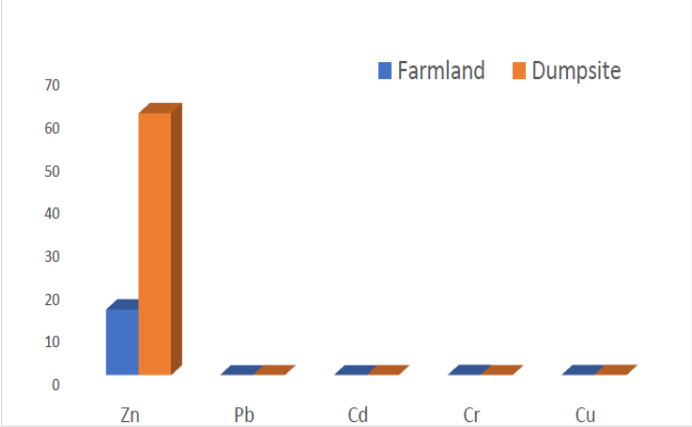

Figure 1b: Concentrations of heavy metals detected in the stem samples of *C. argentea* obtained from farmland and dumpsite

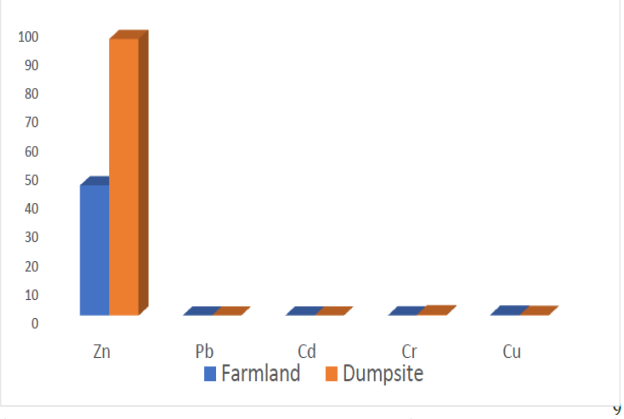

Figure 1c: Concentrations of heavy metals detected in the root samples of *C. argentea* obtained from farmland and dumpsite

The result of DNA damage analysis (DNA fragmentation) as evaluated by Agarose gel electrophoresis is presented in Fig. 2. No fragmentation was observed along the base pairs of *C. argentea* collected from both farmland and dumpsite.

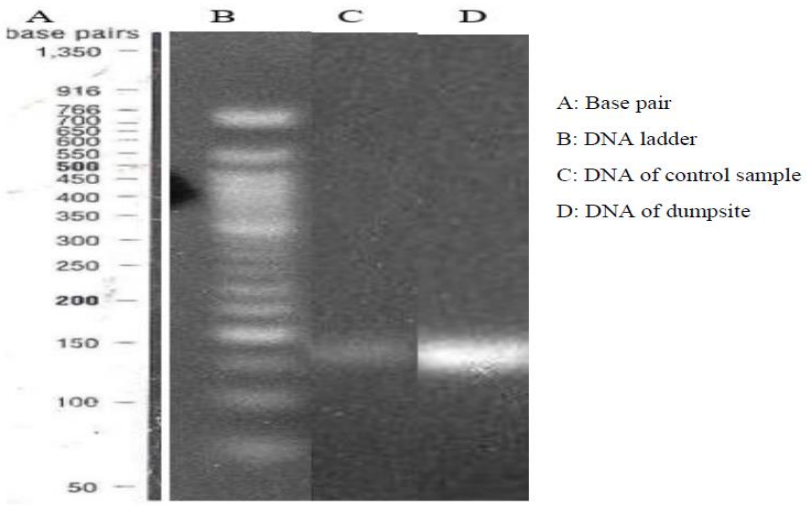

Fig 2: DNA-fragmentation analysis of C. *argentea* collected from the farmland and dumpsit

DISCUSSION

The result of the macronutrients and heavy metals content of soils collected from the dumpsite and farmland in this study revealed that the dumpsite soil is richer in macroelement and more heavy metal contaminated than the farmland soil. The macronutrient content of the dumpsite soil may lend credence to usual luxuriant plant growth and common farming activities conducted by locals around the dumpsite. [13] The higher K, P and N contents found in the dumpsite soil compared to the farmland soil disagree with the earlier findings of [14] who reported lower macronutrient content in the soil of a typical dumpsite when compared to adjacent farmland soils.

Previous findings have suggested that dumpsites and refuse landfills in urban cities may damage the integrity of surrounding soils. In this study, aside from Cd, the other heavy metals investigated were slightly higher in the dumpsite soil sample than in the farmland soil. This result is similar to the findings of [15] and [16]. Although most of the heavy metals investigated in the two sampling soils were within acceptable WHO/FAO concentration, Zn was detected in quantities above the recommended limit. This result is not in line with the findings of [17] and [18] who all reported higher Zn concentration on dumpsite soils. The quantity of heavy metals in soils varies from one soil to the other, and the age of a typical dumpsite appears to be one of the determining factors [18]

Over the years, researchers have beamed light on the effect of heavy metal toxicity in vegetables due to the neurotoxic and carcinogenic effects of these metals. [19] Bioaccumulation and biomagnification of heavy metals through food chains may pose serious health challenges including kidney and liver failures in human. [20] In this research, all the investigated heavy metals were found to be slightly higher in root than the aerial part of the plant. This result is consistent with the earlier report of [21] who reported that *Alhagi maurorum* and *Malva sylvestris* accumulated higher heavy metals in their below ground parts. Additionally, Zn was found in high quantities exceeding the [22] limit in the plant organs which is in agreement with the earlier published work of [23] and [24] The high concentration of Zn in the plant organs of both sampling sites can be easily attributed indiscriminate dumping of domestic refuse, wastes from construction materials, motor emissions and motor vehicle wear.

There is an increasing concern about possible ecological and genotoxic consequences of heavy metal pollution in agricultural soils. [25] Result from this study revealed that there was noticeable damage in the DNA of *C. argentea* collected from the two sampling sites. This perhaps may be due to the low concentration of heavy metals detected on both sites. A similar result was reported by [26] who did not notice any damage in the DNA of *Sinapis alba* under heavy metal stress. Several authors have reported the mutagenic effects of many abiotic stress factors including temperature and heavy metal heavy metals on plants [27]; [28] [29] further demonstrated that the genotoxicity of heavy metals in plants can be connected with industrial loads and their concentrations.

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