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Effects of Dump Site Soil on the Leaf Structures of *Luffa cylindrical* (Sponge gourd) and *Amaranthus viridis* (Green Amaranth)

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ABSTRACT: Previous researches have shown the presence of heavy metals (HMs) such as lead (Pb) at different levels in Olusosun dumpsite, Lagos. This study aimed at finding out if the anatomical differences found in *Luffa cyclindrical* and *Amaranthus viridis* grown on the dumpsite is an indication of the HMs and also determine the distribution of HMs on the dumpsite. The results showed the HMs detected in the dumpsite soil at three spots were significantly higher than the control ($p=0.5$). The concentrations of HMs were in sequence for soils A: Pb>Zn=Cr>Fe>Cu>Ni. Spot B: Fe>Cr>Pb>Zn>Cu>Ni, spot C: Fe>Pb>Cr>Zn>Cu>Ni and Control: Pb>Fe>Zn>Cu>Ni. All the HMs detected at the three spots and control were below the World Health Organization (WHO) and Dutch pollutant standard level, except for (Zn) which was at the exact limit (50.000Mg/kg). The plants obtained from both the dumpsite and control sites didn't show marked visible morphological changes. However, anatomical changes in the leaves epidermis such as irregular shape of the epidermis, absence of trichomes and decrease in quality and stomata size were all witnessed in the dumpsite plant as against the control. Hence, it is likely that all detected differences in the epidermal structures of the test plant grown on dumpsite soil were caused by the high level of HMs present in the dumpsites and therefore suggested that, changes in epidermal structures of *L. cyclindrical* and *A. viridis* grown on Olusosun dumpsite soils is an indication of HMs such as Zn and Pb present.

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Soil contamination by heavy-metals as a result of anthropogenic activities in dumpsites is a major environmental concern all over the world (Cortez and Ching, 2014). Urban areas are known for high level industrial activities, hence generate more pollutants and therefore subjected to the menace of resultant indiscriminate disposal of both domestic and industrial waste (Helmenstine, 2014). These wastes however contain heavy-metals such as lead (Pb) and Zinc (Zn) which are of great ecological significance due to their toxicity at certain concentrations. It is known to affect plants in various ways thereby affecting food quality, crop growth and also lead to death of some plants (Isak *et al.,* 2013). Because plants have a natural propensity to take up metals and also accumulate as the food chain progresses and show visible signs in their morphology and anatomy (Khan *et al.,* 2011), scientists have employed plants to be used as indicators of certain environmental stress (Patra and Sharma, 2000). The degree of impact in plants depends on pollutants concentration, location of entry into plant and species under consideration. Different species may present varying sensitivity/tolerance levels to different contaminating agents (Siva and Prasada, 2016). Toxic effects produced by pollutants on soil organisms and plants have usually been studied in laboratories under controlled conditions, but field studies have been scarce (Patra and Sharma, 2000). Plants tend to absorb and accumulate heavy metals such as Pb, Zn, Cd, Cu, Fe, Ca and Mg which are detrimental to their growth at high concentration (Buszewski *et al.,* 2000). The responses of plants to these pollutants were commonly investigated using morphological and anatomical parameters, such as stomata characteristics, trichome characteristics, epidermal features and leaf size (Wegh *et al.,* 2006). A dumpsite is a site for disposal of waste materials. Waste is any discarded or abandoned materials can be solid, liquid, or semi-solid and are always sourced from homes, schools, hospitals, and other business areas (Buszewski *et al.,* 2000). The use of dumpsites or their soil as farm land is a common practice in urban and sub-urban countries such as Nigeria because of the belief that decayed and composted wastes enhance soil fertility (Oguyemi *et al.,* 2003). Theses wastes often contain heavy metals in various forms and at different contamination levels. Heavy metal pollution is considered to be a worldwide threat now-a-days. Heavy metals are described as those metals with specific gravity higher or more than 5 g/cm. Some heavy metals, such as Fe and Ni are

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essential to the survival or all forms of life, if they are low in concentrations (Leah *et al.,* 2014). Amaranth originated in America and is one of the oldest food crops in the world (Raimondi *et al.,* 2015). *Amaranthus Viridis* is a member of Class Dicotyledonae and family Amaranthaceae. It is commonly known as green amaranth. It is an annual herb with an upright stem that grows to about 60-80cm in height. Numerous branches emerge from the base and the leaves are ovate 3-6cm long, 2-4cm wide with long petioles of about 5cm. In Nigeria, it is a common vegetable which goes with some carbohydrate dishes. It is also a very good source of vitamins including vitamin A, B6, and C; riboflavin, and foliate. It is also a major source of dietary minerals including calcium, iron, magnesium and phosphorus (Czerwinski *et al.,* 2004) it is also eaten as snacks or used in biscuits. It benefits people with hypertension and cardiovascular disease; hence regular consumption reduces blood pressure and cholesterol levels (Martirosyan *et al.,* 2007). L*uffa cylindrica* is a lignocellulose material composed mainly of cellulose, hemicelluloses and lignin (Rowell *et al.,* 2002). It's a member of order Cucurbititales and family Cucurbitaceae. It has alternate and palmate leaves comprising petiole. The leaf is 13 and 30 cm in length and width respectively and has the acute-end lobe. It is hairless and has serrated edges. The flower of *L. cylindrica* is yellow and blooms on August-September (Newton, 2006). Its fruit is green and has a large cylinder-like shape. It has anti-tussive, anti-asthmatic, cardiac stimulant, hepatoprotective properties (Khan *et al.,* 2011), analgesic (Velmurugan *et al.,* 2011) and anti-inflammatory (Khan *et al.,* 2011) are reported. The plant is cultivated in many countries, including Brazil and Nigeria (Muthumani *et al.,* 2010). This study was limited to these plants due to their abundance at Olusosun dumpsite in all the three spots selected.

MATERIALS AND METHODS

For this study, Olusosun dumpsite located in Ojota, Lagos state of Nigeria with geographical coordinates of latitude 6.441158ºN and longitude 3.417977º was used as the sample site for the contaminated soil. Three pots (13cm in height and 35cm in diameter) were filled with 9kg (7cm layer) of the dumpsite soil at three different spots (A, B and C) with 1km distance difference following (Musa *et al.,* 2017). The specific points where the three samples were collected was chosen at random. For the control soil, a pot of equal size was filled with equal soil obtained from botanical garden of University of Lagos (Unilag). The soil was unconatamինated and all the samples collected were made in triplicate. The soil samples were analyzed using air-acetylene flame (PerkinElmer model A-200, atomic absorption spectrophotometer) following (Ogunwande *et al.,* 2010).

Table 1: Showing geographical coordinates and distance of the three sample spots.

SPOT	Latitude	longitude	Distance
A	6˚35'12''N	3˚22'46''E	1km
B	6˚35'29''N	3˚22 '29''E	1km
C	6˚35'30''N	3˚22 ' 50''E.	1km

Experimental procedure: Seeds of *Luffa cylindrical* and *Amaranthus viridis* were obtained from Premier seed Nigeria Ltd, Zaria on 12th of October, 2018. (Ref: Ps233460). These seeds were surface sterilized in 0.1% $HgCl_2$ solution and washed with distilled water prior to germination at controlled temperature. The seeds were dispersed in experimental pot containing loamy soil from botanical garden of (Unilag). After five days, 24 best seedlings were transplanted into each experimental pot with 2 seedlings per replicate. 3 replicate were made for each samples A, B, C and Control at 2.5cm depth in a Randomized Complete Block Design (RCBD) following (Musa *et al.,* 2017). This experiment was carried out in a screen house that shielded the plants from rainfall and pests. Distilled water was used to irrigate the plant twice a week. The Experiments lasted for 60days during which the plants has matured and harvested. For each matured plant, epidermal strips were taken from the median portion of matured leaves. Each sample was macerated in concentrated trioxonitrate (v) acid for 2-4hours. The sample was then transferred to water pertridish, while adaxial and abaxial epidermises were carefully separated with forceps and dissecting needle. The inner parts (mesophyll tissue) of leaves were carefully cleared and the isolated epidermal layers were washed before being transferred to 50% alcohol for 1-2minutes for gardening. The tissue was then moved to a clear glass microscopic slide and stained with saffranin for less than 4minutes after excess water has been drained. Subsequently, the preparation was mounted in glycerine on a slide and examined under light microscope, while photomicrographs were taken (* 40) with Olympus E-330 digital SLR camera and E330- ADU 1.2 microscope adapter. The results obtained were statistically analyzed using a two-way ANOVA to check the Statistical differences among the soil samples obtained at Olusosun dumpsite (spot A, B, C) and Control. The multi-comparison analysis were done using Turkey post hoc test at $p<0.05$, $p<0.01$ and $p<0.001$. Also correlation analyses were used to compare the concentration of elements analyzed in soil samples. All analysis was performed with Graph pad 7 Softwar. The Stomata Index (SI) was estimated for the leaf surfaces using the following formulae as reported by (AbdulRahaman and Oladele, 2008).

SI = S÷ S + E ×100

Where: SI = Stomata Index, S = Number of Stomata per unit area and E = Number of Epidermal Cells in the same unit area.

RESULTS AND DISCUSSION

Elemental composition of soil samples (mg/kg): Result of this experiment indicated Iron (Fe), Zinc (Zn), Magnesium (Mg), Calcium (Ca), Chromium (Cr), Lead (Pb), Copper (Cu), Nickel (Ni), Nitrate (NO_3), Sulphur (S), Phosphorus (P) and Chloride (Cl^-) were present in Olusosun dumpsite soil at the three spots assayed. However for the control, only (Ni) and (Cr) were not detected. This study is in line with the work of (Hammed *et al.,* 2017) that discovered these heavy metals in Awotan and Ajakanga Dumpsite Ibadan, Oyo State of Nigeria. Furthermore, the result also indicated (table 2) that the heavy metals were found at different concentration at the three spots and the control. (Pb) was the highest metal detected in soil sample from spot A (75.00mg/kg), while (Ni) is the lowest (2.50mg/kg). (Cl^-) was the highest in spot B (88.50mg/kg) while Ni is the lowest (2.25mg/kg). in spot C, (NO_3) was the highest (136.30mg/kg) while (Ni) is the lowest (0.75mg/kg). For the control, (NO_3) was detected to be the highest (30.00mg/kg) while (Cu) is the lowest (2.50mg/kg). This may be likely due to leaching and run off going on in the dumpsite. A comparison was also made between the heavy metals level detected in the dumpsite soil and the World Health Organization (WHO) standard for permissible level of heavy metals in soil and also Dutch standard. The results showed that all the heavy metals detected were within the permissible rate.

Table 2: Elemental composition of soil samples (**mg/kg**).

Parameters	Fe	Zn	Mg	Ca	Cr	Pb	Cu	Ni	Cd	NO_3	S	P	Cl^-
Spot A	37.50	50.00	39.00	44.75	30.00	75.00	21.75	2.50	ND	67.80	113.40	41.00	72.10
Spot B	42.50	15.75	38.75	66.75	35.00	25.25	12.50	2.25	ND	96.90	71.76	28.00	88.50
Spot C	40.00	24.50	34.00	69.25	25.00	37.75	13.50	0.75	ND	136.30	37.03	45.53	62.00
Control	10.50	7.50	15.50	19.50	ND	15.00	2.50	ND	ND	30.00	15.50	17.14	15.00

The epidermal results of *Amaranthus viridis:* For the control, the epidermal of structures seen on both the abaxial and adaxial surface of *A. viridis* is polygonal in shape and its wall type is sinuous. Anisocytic type of stomata is present. The shape of the stomata is elliptical (oval). On its guard cells (kidney shape), chloroplast are evenly distributed. Meanwhile for the plant grown on spot A, B and C soil, the epidermal structures seen on both the abaxial and adaxial surface is also polygonal for spot A but irregular for spot B and C. The wall type for spot A is curvy, while for spot B and C is undulating. Anisocytic type of stomata is present in A, B and C while the majority of the stomata are closed. There is absent of cell inclusions in all the samples (table 3). The visible variation between the epidermal structures of the control and samples from the three spot is likely due to the presence of heavy metals. Furthermore, stomata size (bigger) in the abaxial surface. This may be due to foliar uptake through the stomata or leaf cuticle or both may be the principal route for accumulation of these airborne pollutants in plants growing around such polluted areas (Gostin, 2009). This implies that the leaf epidermal features of *A. viridis* are affected by the pollution level of the environment. The presence of numerous anisocytic stomata complex-types in *A. viridis* might suggest that it needs to transpire faster than normal to carry out biochemical activities, due to the dumpsite soil on the stomata pores. Earlier studies by (Oyeleke *et al.,* 2004) confirmed that more subsidiary cells surrounding the guard cells lead to faster opening of the stoma and vice versa. Also, the presence of the anomocytic stomata complex-type in large numbers may play a role in reducing the amount of toxic gases accumulating in the leaves, as (AbdulRahaman and Oladele, 2008) suggested that plants that possess stomata with many subsidiary cells (anomocytic types) play an important role in reducing greenhouse gases.

The epidermal results of Luffa cylindrca: For the control, the epidermal of structures seen on both the abaxial and adaxial surface of *A. viridis* is polygonal in shape and its wall type is curve. Anisocytic type of stomata is present. The shape of the stomata is elliptical (oval). On its guard cells (kidney shape), chloroplast are evenly distributed. Meanwhile for the plant grown on spot A, B and C soil, the epidermal structures seen on both the abaxial and adaxial surface is also polygonal for spot A, B and C. The wall type for spot A and B is straight, while for spot C is curve. Anisocytic type of stomata is present in A, B and C while the majority of the stomata are closed. There is absent of cell inclusions in all the samples (table 4). Stomata with many subsidiary cells tend to open more often than those with small number of subsidiary cells (AbdulRahaman and Oladele, 2008). These responses of *L. cylindrica* could be adaptive features to tolerate the high cement dust pollution of the area. Stomata with many subsidiary cells tend to open more often than those with small number of subsidiary cells (AbdulRahaman and Oladele, 2008).

Table 3: Foliar Epidermal Attributes Found on Both the Adaxial and Abaxial Surfaces of *Amaranthus viridis.*

Plant specie	Surface	Shape Of Epidermal Cell	Epidermal Wall Type	Stomata Type	Cell Inclusions
(Control)	Adaxial	Polygonal	Sinuous	Anisocytic	Absent
	Abaxial	Polygonal	Sinuous	Anisocytic	Absent
(Spot A)	Adaxial	Polygonal	Curve	Anisocytic	Absent
	Abaxial	Polygonal	Curve	Anisocytic	Absent
(Spot B)	Adaxial	Irregular	Undulating	Anisocytic	Absent
	Abaxial	Irregular and polygonal	Undulating	Anomocytic	Absent
(Spot C)	Adaxial	Irregular	Undulating	Anisocytic	Present
	Abaxial	Irregular	Undulating	Anisocytic	Present

Table 4: Foliar Epidermal Attributes Found on Both the Adaxial and Abaxial Surfaces of *Luffa cylindrca*

Plant specie	**Surface**	**Shape Of Epidermal Cell**	**Epidermal Wall Type**	**Stomata Type**	**Cell Inclusions**
(Control)	Adaxial	Polygonal	Curve	Anisocytic	Absent
	Abaxial	Polygonal	Curve	Anisocytic	Absent
(Spot A)	Adaxial	Polygonal	Straight	Anisocytic	Absent
	Abaxial	Polygonal	Straight	Anisocytic	Absent
(Spot B)	Adaxial	Irregular	Straight	Anisocytic	Absent
	Abaxial	Irregular and polygonal	Straight	Anomocytic	Absent
(Spot C)	Adaxial	Irregular	Curve	Anisocytic	Present
	Abaxial	Irregular	Curve	Anisocytic	Present

Conclusion: The findings of this study revealed the presence of different concentration of heavy metals in Olusosun dumpsite. The concentration determined were in sequence for soils
Spot A: Pb>Zn=Cr>Fe>Cu>Ni,
Spot B: Fe>Cr>Pb>Zn>Cu>Ni,
Spot C: Fe>Pb>Cr>Zn>Cu>Ni,
Control: Pb>Fe>Zn>Cu>Ni.
These metals were below the WHO and Dutch standard permissive levels. However, Zinc was found to be at the exact limit set by WHO. Also, the epidermal structures of the two test plants were affected as a result of the heavy metals and therefore may likely be used as bioindicator of heavy metals.

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