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Original paper

Heavy Metal Analysis and Chromosome Aberrations Induced by Soil from Spare Parts Dumpsite at Meme Bridge, Lokoja, Kogi State

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ABSTRACT: Using an *Allium cepa meristem* assay, this study examined the heavy metal concentrations and chromosome aberrations caused by soils taken from a spare part waste site. The soils taken from the spare part disposal site at Meme Bridge, Lokoja, Kogi State, Nigeria, were analyzed for Zinc (Zn), Chromium (Cr), Copper (Cu), Manganese (Mn), Nickel (Ni), Lead (Pb), Cobalt (Co), and Arsenic (As) using the Atomic Absorption Spectrophotometer (AAS). For 48 hours, roots from onion (*Allium cepa*) sets were allowed to grow in beakers containing varying concentrations of soil filtrate (i.e. 25%, 50%, 75%, and 100%) and the control. The tips were harvested for chromosomal observations between 8:00 and 9:00 a.m. West African Time (WAT). Data for mitotic phases and chromosomal aberrations were pooled under X400 magnification of the light microscope and analyzed using Analysis of Variance (ANOVA), while means with significant differences between concentrations were separated using Duncan Multiple Range Test (DMRT). The results show that the levels of zinc (Zn), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), cobalt (Co), and arsenic (As) in the soil exceeded the World Health Organization's minimal acceptable standards. This study also discovered vacuolated cells, binucleate cells, bridging chromosomes, defective polarity, fragmented chromosomes, variant chromosomes, C-mitotic cells, spindle disruption, and unipolar movement of chromosomes, indicating that the soil is genotoxic. A 50% concentration was discovered to stimulate dividing cells. The practice of dumping spare parts along the Meme River should be avoided due to the health and environmental consequences.

Keywords: Heavy metals, chromosomes, aberrations, *allium cepa* , stimulatory

INTRODUCTION

Airoboman and Onobhayedo (2022) singled out environmental pollution as the major challenge confronting man today. The environment is exposed to multiple sources of pollutions (Iqubal *et al.,* 2020). Sherene (2010) reported that soil is the most polluted part of the environment. According to Sabeen *et al.* (2020) the rapid urbanization and industrialization going on across the globe have caused serious contamination of soils with toxic metals. Sankhla *et al.* (2016) reported that the toxic, easy bioaccumulation and non-degradable nature of these metals pose serious threat on environmental and human health (Kolawole *et al.*, 2022). Pollution from anthropogenic activities, according to Mishra *et al.* (2019), is one of the greatest public health dangers of worldwide concern, accounting for around 9 million fatalities each year. According to Alotaibi *et al.* (2022), heavy metals are the most important environmental contaminant threatening human health.

Khan *et al*. (2004) observed that heavy metal mobilization and transportation have increased since the 1940s. This according to Wieczorek Dbrowska *et al*. (2013) placed living beings at serious risk since then.

According to Azeez *et al*. (2011), the significant prevalence of heavy metals in most Nigerian cities is due to indiscriminate dumping of non-biodegradable material. Automobile parts are examples of non-biodegradable goods. According to Muze *et al*. (2020), non-biodegradable wastes generated by vehicle workshops and spare part dumpsites include metal scraps and lead batteries. According to Osakwe *et al*. (2010), heavy metals from scraps at various states of corrosion, discarded motor batteries, and worn out tyres contaminate soil around automobile dumpsites.

Eventually, these heavy metals in the soil find their way into the food chain. Hashem *et al* (2017) noted that recent human health problems have been attributed to the consumption of food contaminated with heavy metals. According to Makwara and Snodia (2013), this situation has been exacerbated by poor waste management, where materials are recklessly dumped on undeveloped land, rivers and byways, polluting the environment.

According to Orusun *et al*. (2020), the usual practice of dumping spare parts in a specific spot in town is a substantial source of pollutants entering the earth. Poverty, a lack of investment in contemporary technology, and inadequate environmental legislation, according to Miah *et al*. (2021), are the reasons why this practice of dumping scraps at various areas in developing countries persists.

Lokoja, Kogi State, is not immune to the practice of dumping spare parts at a specific spot. A large spare part dump is located near Meme River, where contaminants from discarded spare parts enter the soil as well as the neighbouring river, damaging water bodies and agricultural plants.

In view of the limited studies on the toxic nature of the soil at the spare parts dumpsite at Meme Bridge, Lokoja, Kogi State, this study was carried out to determine the heavy metal composition and genotoxicity caused by the soil.

MATERIALS AND METHODS

Study location

The study area is in Lokoja, the capital city of Kogi State. Lokoja is located on the Greenwich Meridian at 7°47' 3"N and 6°33'47"E. The Meme River is a tributary of the Niger River, with a basin area of 667560.5 square kilometres. The vacant terrain surrounding the River is being exploited as a dump site for vehicle and other metal spare parts. Vegetable irrigation cultivation is also practiced along the river's bank.

Collection of samples

Soil samples were taken at random at a depth of 15cm from three distinct locations at the spare part dump site near Meme Bridge, Lokoja, using clean sterile beakers. Soil composite samples were transferred to a clean, labeled polythene bag for heavy metal analysis in the laboratory. For this investigation, little onion (*Allium cepa*) bulbs weighing between 3.00g and 3.50g were obtained from the International market Lokoja in Kogi State.

Preparation of filtrates from soil

The collected soil sample was spread on brown papers to dry under room temperature in the Laboratory of Department of Biology. The soil sample was sieved and 2.0g from the fine samples was poured into 100 ml of distilled water. This was left to stand for 48 hours after which the contents were carefully decanted to get the filtrate. This filtrate was considered for heavy metals and genotoxic studies.

Heavy metal analysis

The filtrate was taken to the laboratory of Department of Chemistry, Federal University of Technology, Akure for heavy metal analysis. Eight heavy metals (Cobalt, Chromium, Copper, Manganese, Nickel, Lead, Arsenic and Zinc) were analyzed using Atomic Absorption Spectrophotometer (AAS) method described by Tiwari *et al.* (2016).

Genotoxicity study

The loose outer scales of the onion bulbs were removed and the root primordials (in the reduced stems) were allowed to touch the distilled water in beakers until roots developed. Ten bulbs of onions were grown per sample in this manner while five with satisfactory root growth were selected for this study. The developed roots were thereafter transferred into labeled beakers containing 100%, 75%, 50% and 25% concentration of the filtrates from the soil. Onion roots grown in distilled water served as the control for this study. The method outlined by Udebuani *et al.* (2016) was adopted for this study. Each treatment was replicated five times and arranged in Completely Randomized Design (CRD). The roots were removed from the treatments after 48hours of exposure to the different treatments. Thereafter, 1cm of the root tips were cut into labeled specimen bottles between 7:30am and 8:30am West African Time (WAP) for chromosome examinations. The methods outlined by Akinyele (2007) modified by Alege *et al.* (2022) were adopted for fixation, hydrolysis, squashing and staining of root tips.

Chromosome observation

The 4X, 10X, 40X objectives of the Digital model of the light microscope was used to observe the slides. Photomicrographs of the normal mitotic stages and aberrant cells were taken at 400X magnifications.

Data analysis

The means obtained from triplicate values of each heavy metal were expressed in bar chart. Five slides were prepared for each sample while ten microscopic views were considered per sample to give a total of fifty microscopic views per sample. The following counts were recorded from each microscopic view at 1000X magnification: total number of dividing cells, number of cells at interphase, number of cells at prophase, number of cells at metaphase, number of cells at anaphase, number of cells at telophase, and number of the different aberrant cells observed. This was done according to the methods described by Ping *et al.* (2012) with slight modifications. Altogether, a total of 1,000 cells were considered per sample. Data generated were analyzed using Analysis of Variance (ANOVA) and separation of significant means was done using the Least Significant Difference (LSD). The percentage of cells with chromosomes irregularities, Mitotic Index (MI) and Relative Division Rate (RDR) for cells exposed to herbal infusions in relation to the control were determined by formulae outlined by Malode *et al.* (2012) as given below:

Percentage of Aberrant Cells (PAC) was calculated using the formula:

$$(PAC) = \frac{Total\ number\ of\ abnormal\ cells}{Total\ numberof\ cells\ examined}\ X\ 100$$

$$Mitotic\ Index\ (MI) = \frac{Total\ number\ of\ dividing\ cell}{Total\ numberof\ cells\ examined}\ X\ 100$$

The numbers of prophase, metaphase, anaphase and telophase were summed to represent the total number of dividing cells. The calculation of Relative Division Rate (RDR) was carried out as follow:

$$(RDR) = \frac{\%\ of\ dividing\ cells\ in\ treated\ root\ tips - \%\ of\ dividing\ cells\ in\ control\ root\ tips}{100 - \%\ of\ dividing\ cells\ in\ control\ root\ tips}\ X\ 100$$

RESULTS

It was observed from the (Figure 1) that the order of abundance of the eight heavy metals is as follow: Zn >Cu >Mn> Cr > Ni >Pb> Co > As. Seven out of the eight analyzed heavy metals occurred beyond the WHO permissible level. Only Chromium (Cr) occurred below the WHO permissible level.

Five out of the six mitotic division parameters showed significant differences among the treatments studied (Table 1 and Plate I). The attributes with significant differences include the total number of cells, number of interphase, number of prophase, number of metaphase, and number of cells at anaphase. Only numbers of cells at telophase stage did not show statistical significant differences (P≥0.05) across treatments. It was also observed from the (Table 1) that there is significant increase in the total number of cells for treated root tip cells compared to control. 50% concentration of the filtrate showed significant high numbers of prophase (12.07), metaphase (6.64) and Anaphase cells (5.79). It was observed that nine chromosomal aberrations were induced in *Allium cepa* root tip cells exposed to different concentrations of filtrate obtained from soil collected from spare part dumpsite (Table 2 and Figure II). The nine chromosomal aberration recorded in this study are vacuolated cells, binucleate cells, bridged chromosome, faulty polarity, fragmented chromosomes, variant chromosomes, C- mitotic cell, spindle disturbance and unipolar movement of chromosomes. No chromosomal abnormality was recorded in roots treated with distilled water (control). The number of cells with unipolar movement of chromosome did not show statistical significant differences among the treatments.

It can also be observed from (Table 2) that only two chromosome aberrant conditions (i.e, vacuolated cells and binucleate cells) were recorded in 25% concentration of the filtrate. Significantly high values of variant chromosome (0.13), C-mitosis (0.40) and Spindle disturbance (0.27) were recorded for 100% concentration of the filtrate. Similarly, variant chromosomes and unipolar movement of chromosomes were observed only in 100% and 50% filtrates obtained from soil collected at spare part dumpsite. The highest total number of aberrant cells was recorded in 25% filtrate from soil collected at spare part dumpsite (16.73) while the least number of aberrant cells was recorded in onion roots tips exposed to 50% filtrate from soil collected at spare part dumpsite (14.05).No aberrant cell was recorded in untreated (control) root tips (Figure 2).

Table 3 showed that cells from 25% concentration also had the highest percentage aberrant cells of 25.17%. Control had mitotic index of 33.91% which is higher than the mitotic indices of observed for 25% concentration (22.39%), 75% concentration (28%) and 100% concentration (25.80%). Only 50% concentration of filtrate from soil collected from spare part dumpsite produced mitotic index value (35.99%) that is higher than that of the control (33.91%). Similarly, when the rates of

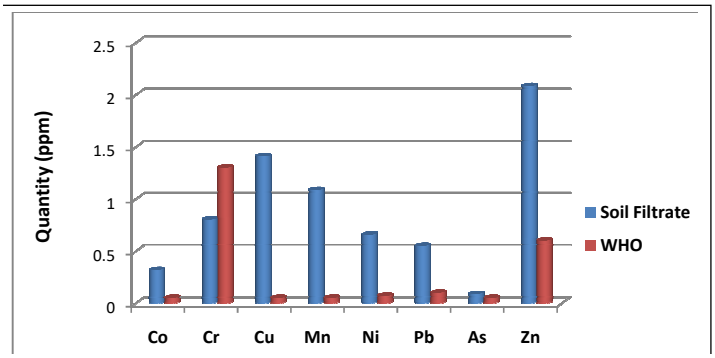

Figure 1: Heavy Metal Contents of Filtrates Obtained from Soil from the Spare Part Dumpsite Compared to WHO Permissible Level
Source: WHO (2011).

Table 1: Effects of Filtrate from Soil Collected at Spare Part Dumpsite on Mitotic Cell Division of Allium cepa

Conc.	ANC	I	P	M	A	T
Control	45.45^{b}	30.05^{b}	6.73^{bc}	4.00^{b}	3.50^{b}	1.18
25%	66.67^{a}	35.00^{a}	6.33^{c}	3.93^{b}	3.60^{b}	1.07
50%	71.43^{a}	31.64^{ab}	12.07^{a}	6.64^{a}	5.79^{a}	1.21
75%	71.43^{a}	36.07^{a}	10.07^{ab}	5.36^{ab}	3.86^{b}	0.71
100%	66.67^{a}	34.53^{ab}	7.13^{bc}	5.07^{bc}	3.80^{b}	1.20
LSD Value	1.707	0.732	0.563	0.239	0.217	NS

- Means with the same alphabets will are not significantly different from P≤0.05

Key:
ANC – Average Number of Cells per View
I - Interphase
P - Prophase
M- Metaphase
A- Anaphase
T - Telophase

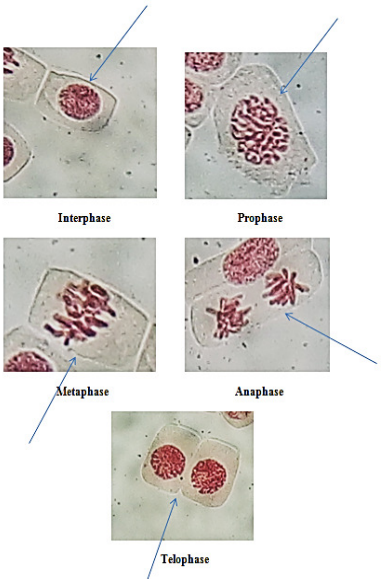

Plate I: Normal stages of mitotic division in *Allium cepa* root tip cells treated with filtrate from soil collected at spare part dumpsite

Table 2: Chromosome Abnormalities induced by filtrate from soil collected from spare part dumpsite.

Conc.	VC	BIC	BRC	FP	FRC	VRC	CMT	SPD	UPM
Control	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00
25%	16.60^{a}	0.13^{ab}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00
50%	13.21^{a}	0.07^{ab}	0.14^{ab}	0.21^{a}	0.14^{ab}	0.00^{b}	0.07^{b}	0.14^{ab}	0.07
75%	14.29^{a}	0.31^{a}	0.29^{a}	0.00^{b}	0.29^{a}	0.00^{b}	0.21^{ab}	0.00^{b}	0.00
100%	13.13^{a}	0.20^{ab}	0.33^{a}	0.27^{a}	0.20^{ab}	0.13^{a}	0.40^{a}	0.27^{a}	0.00
LSD Value	0.885	0.038	0.039	0.032	0.036	0.018	0.037	0.030	NS

- Means with the same alphabets will are not significantly different from P≤0.05

Key:
VC –Vacuolated Cells
BIC – Binucleate Cells
BRC – Bridged Chromosome
FP – Faulty Polarity
FRC – Fragmented Chromosomes
VRC – Variant Chromosomes
CMT – C- mitotic Cell
SPD – Spindle Disturbance
UPM– Unipolar Movement of Chromosomes

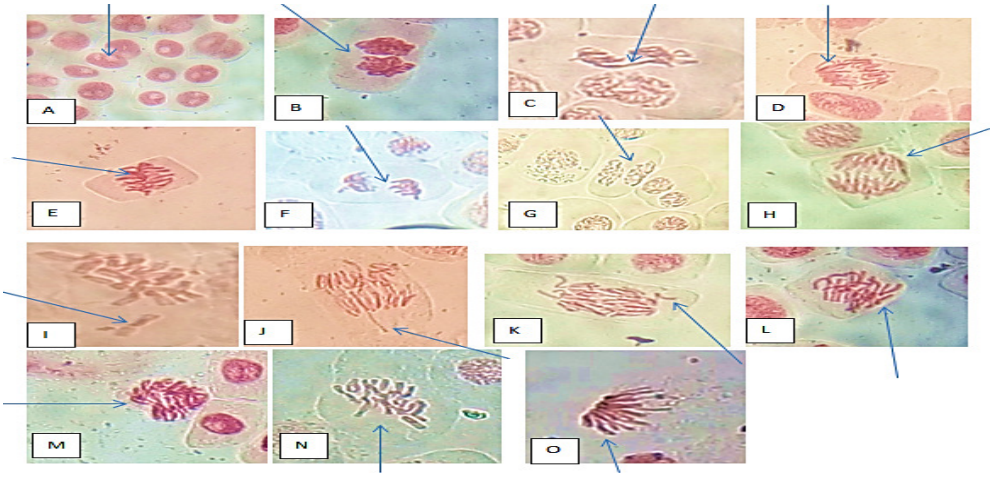

Plate II: *Allium cepa* root tip showing aberrant cells induced by filtrate from soil collected at spare part dumpsite
KEY:
A: Vacuolated Cells, B: Binucleate Cells, C-D: Bridged Chromosomes, E-H: Faulty Polarity, I: Fragmented Chromosomes, J-K: Variant Chromosomes, L-M: C-mitosis, N: Spindle Disturbance, O: Unipolar Movement of Chromosomes.

cell division in the different concentrations of the filtrates were compared with control, negative values were observed in 25%, 75% and 100% (i.e., -17.43%, -8.94% and -12.27% were recorded for 25%, 75% and 100% respectively) while 50% concentration had a positive value (3.15).

DISCUSSION

The fact that soil filtrates exceeded the minimal values advised by the World Health Organisation for Zn, Cu, Mn, Ni, Pb, Co, and As suggests that soil is dangerous. According to Orosun *et al.* (2020) high concentration of heavy metals can lead to poisoning of human tissues. This poisoning according to them may result from inhaling of air containing high concentration of these metals, drinking water containing these contaminants, or ingestion via food chain. Mehar *et al.* (2023) reported that heavy metals bio-accumulate in the human body which constitutes serious health issue.

The high Zn content compared to other heavy metals observed in this study is in conformity with the report of Orosun *et al.* (2020) that automobile spare part and recycling market in Ilorin contained significant amount of

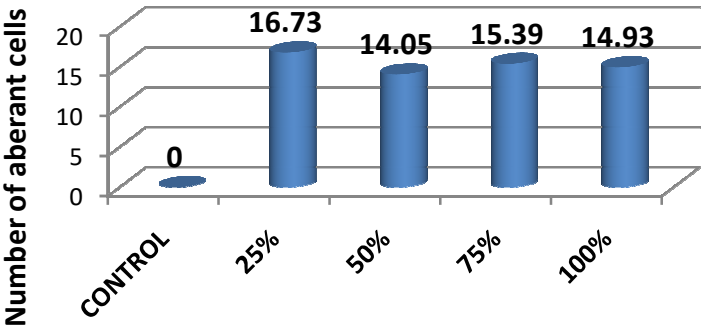

Figure 2: Number of Aberrant Cells Induced by Soil from Spare Part Dumpsite

Table 3: Cell division frequencies of *Allium cepa* root tip cells treated with filtrate from soil collected from spare part dumpsite.

Conc.	PAC (%)	MI (%)	RDR (%)
Control	0	33.91	----
25%	25.17	22.39	-17.43
50%	19.67	35.99	3.15
75%	21.55	28.00	-8.94
100%	22.39	25.80	-12.27

Key:
PAC = Percentage of Aberrant Cells
MI = Mitotic Index
RDR =Relative Division Rate

Zn. The Chromium (Cr) content is relatively high but not higher than the WHO permissible level which indicates that the soil is not heavily polluted with Chromium.

The fact that the total number of cells, number of interphase, number of prophase, number of metaphase, and number of anaphase varies significantly with different concentrations of the filtrates suggests that the contents of the filtrate disrupts the process of cell division and cell cycle. Similar report was given by Sharma *et al.* (2021) on the effects of paper mill effluent on *Allium cepa* root tip cells. The filtrate did not significantly affect the progress of telophase in the *Allium* root tip cells which according to Alege *et al.* (2022) indicates stability of the cells at telophase stage.

This study revealed that the contents of filtrates from the spare part dump site soil are capable of inducing chromosomal aberrations in *Allium cepa* root tip cells. The observed vacuolated cells, binucleate cells, bridged chromosome, faulty polarity, fragmented chromosomes, variant chromosomes, C- mitotic cell, spindle disturbance and unipolar movement of chromosomes indicates mutagenic potential of the soil. Kovalchuk *et al.* (1998) reported Chromosome Bridge, fragmented chromosomes, variant chromosomes and C-mitosis from soil contaminated with Chernoby.

The fact that all the different concentrations considered in this study produced aberrant cells indicates genotoxic nature of the soil. The negative relative division rates recorded for 25%, 75% and 100% concentrations suggest the inhibitory effects of the filtrates. Although 50% concentration produced stimulatory effect on treated *Allium cepa* root tip cells. This observation is further buttressed by the high Mitotic Index values recorded at 50% concentration is this study. This finding suggests that intermediate concentration of the filtrate had stimulatory effects on cell division while the extreme concentrations had suppressive effects on cell division. Kumari and Tripathi (2019) reported that reduction in Mitotic Index in chemical-treated *Allium cepa* root cells could be attributed to the disruptive effect of toxic chemicals in the substance on cell division through the process of DNA or protein synthesis.

Conclusion

This study revealed that soil from the spare part dump site is highly contaminated with heavy metals like Zn, Cr, Cu, Mn, Ni, Pb, Co and As. Only the Cr content is within the permissible level recommended by World Health Organization. It can be concluded that contents of soil from the spare part dump site is capable of inducing chromosome aberration and is therefore genotoxic to the cell. Particularly 50% concentration has been found to have stimulatory effect on dividing cells. This study revealed that the practice of dumping spare part around the Meme River obviously have serious health and environmental implications and should be discouraged. Proper monitoring by the concerned agencies to ensure compliance is highly recommended.

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