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EVALUATION OF THE DIFFERENTIAL GROWTH CONDITIONS, HEAVY METAL COMPOSITION AND CYTOGENETICS OF *Allium cepa* BY THE USE OF CHROMOSOME ABERRATION BIOASSAY.

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ABSTRACT

Comparative study of the suitability of onion bulbs grown in different environmental conditions for the *Allium cepa* bioassay was undertaken using the chromosome aberration test. Onion bulbs were obtained from Gombe, Kano, Maiduguri, and Sokoto. Cadmium, nickel, selenium, and lead were not detected in Gombe onions. Kano and Maiduguri contained high levels of lead (0.30±0.01 and 0.20±0.01 mg/l) and nickel (0.30±0.01 and 0.30±0.01 mg/l) respectively. The highest zinc and selenium contents were detected in Maiduguri onions (7.40±0.01 and 0.40±0.01 mg/l respectively). Results of cytological studies using the aceto-orcein squash technique showed that Gombe onions had the highest mitotic index while Kano had the least. In the same vein, Gombe onions had the lowest percentage aberrant cells, while Kano had the highest. The mean root lengths of the onion bulbs grown in tap water at 96 h were of the increasing order: Gombe>Sokoto>Maiduguri>Kano. The results of the *Allium* test indicate Gombe onions were the best and most suitable for *A. cepa* bioassay as they were relatively free from heavy metal contamination. The findings also show that health risks could be caused to the unsuspecting public by the daily intake of heavy metals through the contaminated vegetables with heavy metals.

Key words: *Allium cepa*, metallic contents, mitotic index, environmental conditions

INTRODUCTION

Onion (*Allium cepa* Linn.) cultivation in Nigeria is confined to the semi-arid northern guinea and Savanna zones. The bulk of onion production is from the dry season cropping system particularly under irrigation in the Northern States (Amans, 1996). In this light, the greater part of onion production in Nigeria is undertaken in the north of the country specifically in Kaduna, Kano, Jigawa, Katsina, Sokoto, Plateau and Bauchi States. The natural features of these regions, especially the presence of flood prone plains and river basins and above all the development of vast irrigated lands, create conditions that greatly favour the development of this crop (Ojo *et al*., 2009). In comparison with other fresh vegetables, onions are relatively high in food value and antioxidant phytochemicals (Hussaini *et al*., 2000; Smith, 2003). The crop is second only to tomatoes in importance among the vegetables in Nigeria and fifth in the world market and is mainly grown for its bulbs (Cramer, 2000; Hussaini *et al*., 2000).

Trace metals may enter the food chain from soil through mineralization by crops or environmental contamination as in application of agricultural inputs such as pesticides, fertilizers, herbicides or use of polluted river (from industrial effluents) for irrigation to water crops (Miller, 1996; Onianwa *et al*., 2001; Audu & Lawal, 2005; Awofolu *et al.,* 2005; Francis, 2005).

Apart from its nutritional value, the common onion is one of the most outstanding higher plants recommended by United States Environmental Protection Agency (USEPA) and the American Society for Testing and Materials (ASTM) in 1982 and 1994 respectively for use as an excellent and alternative first–tier indicator for safety evaluation of cytogenetic and mutagenic effects of drinking water and environmental pollutants. The use of *A. cepa* for root length inhibition and chromosome aberration bioassay as a sensitive, cost effective and valid indicator of toxicity test for the routine monitoring of water pollution is due to the important activation enzymes the root tip cells possess (Fiskesjö, 1993) and they have shown good correlation with other test systems involving genotoxicity (Fiskesjö, 1985a, 1985b, 1997; Grant, 1982, Rank, 2003, Rank & Nelson, 1998, Babatunde & Bakare, 2006, Olorunfemi *et al*., 2011).

In Nigeria, most of the *Allium* bulbs available in local markets and used by most workers for genotoxicity studies are obtained from irrigated farms in the northern states which have been shown to contain large amounts of toxic heavy metals (Binns *et al*., 2003). The objective of this study was to determine the trace and heavy metals in onion bulbs cultivated in some northern states, as well as the validity and consistency of their use as biomonitors of environmental mutagens of wastewaters in cytogenetical studies.

MATERIALS AND METHODS

Test Materials: Onion bulbs *(Allium cepa* L., 2n=16) of the purple variety of average size (15-22 mm diameter) were purchased locally in Benin City, Edo State in Nigeria ($6^{0}15^{1}$ N and $5^{0}25^{1}$ E). The bulbs were purchased directly from vendors who bring them from Gombe, Kano, Sokoto and Maiduguri. They were stored in dry and well aerated conditions for several few weeks before use in. Bulbs that were dried and mouldy were all discarded. The study was carried out in 2010.

Macroscopic Evaluation: The dried roots present at the base of the onion bulbs were carefully shaved off with a sharp razor blade to expose the fresh meristematic tissues. The bulbs were then placed in freshly prepared distilled water to protect the primordial cells from drying up. The bulbs were removed from the distilled water and placed on a blotting paper to remove excess water. For root growth inhibition evaluation, the bulbs were exposed directly in the test sample (normal tap water of good quality). The tap water is ascertained to be of good quality with pH around 7 and has relatively high hardness (Ca + Mg= 50-70 mg/l) and free from any chlorine compounds and toxic ions (Fiskesjö, 1985a). To account for a number of bulbs in the population that would be naturally slow or poor growing (Fiskesjö, 1988), seven replicate bulbs were used for the test sample. The base of each of the bulbs was suspended on the tap water inside 100 ml beakers in the dark for 96 h. Test effluents were changed daily. At the end of the exposure period, the roots of five onion bulbs with the best growth were removed with a forceps and their lengths measured (in cm) with a metre rule. The effect of the tap water on the morphology of growing roots was also examined.

Microscopic Evaluation: For the evaluation of induction of chromosomal aberration, five onion bulbs were suspended in the tap water for 48 h at the end of which root tips from these bulbs were cut and fixed in ethanol:glacial acetic acid (3:1, v/v). These were hydrolyzed in 1N HCl at 60 ^{o}C for five minutes after which they were washed in distilled water. Two root tips were then squashed on each slide, stained with aceto-carmine for 10 min and cover slips carefully lowered on to exclude air bubble. The cover slips were sealed on the slides with clear fingernail polish as

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suggested by Grant (1982). This is to prevent drying out of the preparation by the heat of the microscope (Sharma, 1983). Six slides were prepared for each test sample out of which five were analyzed at ×1000 magnification for induction of chromosomal aberrations. The mitotic index and the frequency of aberrant cells (%) were calculated as in previous studies (Olorunfemi *et al*., 2011).

Analysis of Onion Bulbs for Metallic Composition: Three sets of ten whole onion bulbs were analyzed for fifteen metals (including eleven heavy metals) namely lead, cadmium, molybdenum, selenium, vanadium, silver, copper, iron, zinc, nickel, and manganese according to standard analytical methods (APHA, 1998). Briefly, 100 ml of the effluents were digested by heating with concentrated HNO_3, and the volume reduced to 3-5 ml. This volume was made up to 10 ml with 0.1 N HNO_3. Concentrations of the metals were estimated by using an Atomic Absorption Spectrophotometer (AAS, Unicom 969).

Statistical Analysis: The means, with 95 % confidence limits and the standard errors for results of the root inhibition and chromosome aberrations of each test sample were calculated. Data were expressed as Mean ± Standard Error. All statistical analyses were carried out using SPSS®14.0 statistical package.

RESULTS

The metallic contents of the onions obtained from the different geographical locations are shown in Table 1. Lead was not detected in Gombe onions, but substantially high values were found in Kano and Maiduguri onions (0.30±0.01 and 0.20±0.01 mg/l) respectively. Cadmium, molybdenum and silver were only detected in Maiduguri and Kano onions. Selenium was not detected in Gombe onions but high values were found in onions from Kano and Maiduguri (0.20±0.01, 0.30±0.01 and 0.40±0.01 mg/l) respectively. Vanadium was not detected in Gombe onions. Compared with Gombe, Sokoto and Kano, the levels of zinc, manganese and copper were highest in onions from Maiduguri.

TABLE 1. METALLIC CONTENT OF THE *Allium cepa* SAMPLES FROM DIFFERENT LOCATIONS

Parameter (mg/l)	Gombe (± S.E)	Sokoto (± S.E)	Maiduguri (± S.E)	Kano (± S.E)
Potassium, K	51.60(0.02)	85.20(0.04)	93.60(0.05)	74.20(0.03)
Sodium, Na	70.60(0.03)	98.10(0.01)	110.50(0.05)	80.20(0.04)
Calcium, Ca	70.50(0.03)	55.50(0.02)	61.90(0.05)	48.90(0.01)
Magnesium, Mg	80.50(0.04)	90.50(0.04)	81.30(0.01)	101.50(0.05)
Iron, Fe	7.50(0.01)	3.50(0.01)	5.90 (0.01)	3.90(0.01)
Copper, Cu	0.40(0.01)	0.30(0.01)	0.50(0.01)	0.40(0.01)
Manganese, Mn	0.40(0.01)	0.40(0.01)	0.90(0.01)	0.60(0.01)
Zinc, Zn	2.10(0.01)	1.90(0.01)	7.40(0.01)	5.10(0.01)
Nickel, Ni	ND	0.20(0.01)	0.30(0.01)	0.30(0.01)
Cadmium, Cd	ND	ND	0.10(0.01)	0.10(0.01)
Selenium, Se	ND	0.20(0.01)	0.40(0.01)	0.30(0.01)
Vanadium, Va	ND	0.10(0.01)	0.10(0.01)	0.20(0.01)
Molybdenum, Mo	ND	ND	0.10(0.01)	0.10(0.01)
Silver, Ag	ND	ND	0.10(0.01)	0.20(0.01)
Lead, Pb	ND	0.10(0.01)	0.20(0.01)	0.30(0.01)

Values are means of 3 replicates (± S.E)
ND: Not Detected.

Table 2 shows the mean root lengths of the onion bulbs grown in tap water. It was observed that the root mean lengths of the onion bulbs was in increasing order Gombe>Sokoto>Maiduguri>Kano (2.80±0.28 cm, 2.32±0.16 cm, 2.18±0.16 cm and 1.87±0.17 cm) respectively.

The growth response of the onion bulbs grown in tap water after 96 hr is presented in Plate 1. There was no colour change in any of the onion roots; however, root malformations such as short roots, twists and 'crotchet hooks' were predominant in onions obtained from Maiduguri and Kano. The level of growth of response was in the increasing order: Gombe>Sokoto>Maiduguri>Kano.

TABLE 2. ROOT LENGTH OF ONION SAMPLES GROWN IN TAP WATER

Sample Source	Total length of overall No. of root (cm)	Overall No. of roots	Mean root length (±S.E.)
Gombe	794.30	283	2.80 (0.28)
Sokoto	543.40	234	2.32 (0.16)
Maiduguri	393.20	180	2.18(0.43)
Kano	177.70	95	1.87 (0.17)

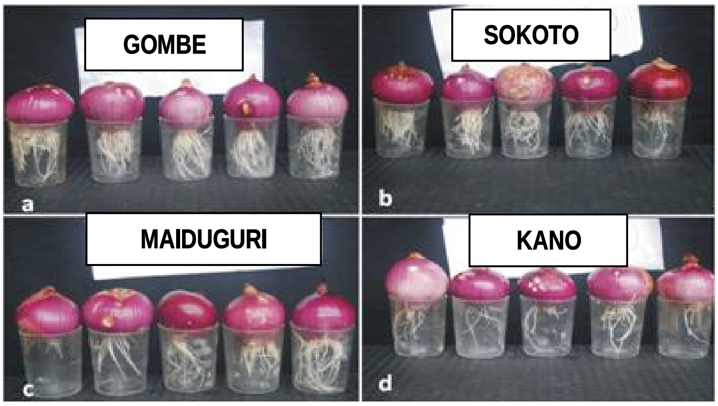

PLATE 1. GORWTH RESPONSE OF ONION BULBS SAMPLES GROWN IN TAP WATER AFTER 4 DAYS.

The cytological effects of the different onion bulbs after two days of growth in tap water are presented in Plate 2 and Table 3. It was observed that mitotic index was in the increasing order Gombe>Sokoto>Maiduguri>Kano (97.80, 92.40, 90.20 and 88.00) respectively.

The types and frequency of chromosomal aberrations induced in the onion root tips were most predominant in the Kano onions. In the same vein, the percentage aberrant cells was in the decreasing order Gombe<Sokoto<Maiduguri<Kano (7.73, 21.24, 34.73 and 40.75 %) respectively.

TABLE 3. MITOTIC INDEX OF SAMPLES ROOT TIP GROWN IN TAP WATER

Sample Source	Mitotic index (M.I)	Chromosome Aberration per 1000 cells							% Aberrant cells
		Polar Deviation	Laggards	Fragments	Vagrants	Stickiness	Bridges	Micronucleus	
Gombe	97.80	-	-	-	2	-	2	-	1.10
Sokoto	92.40	5	3	3	25	12	30	-	14.16
Maiduguri	90.20	39	5	5	28	34	44	2	34.81
Kano	88.00	35	5	6	38	50	45	6	42.04

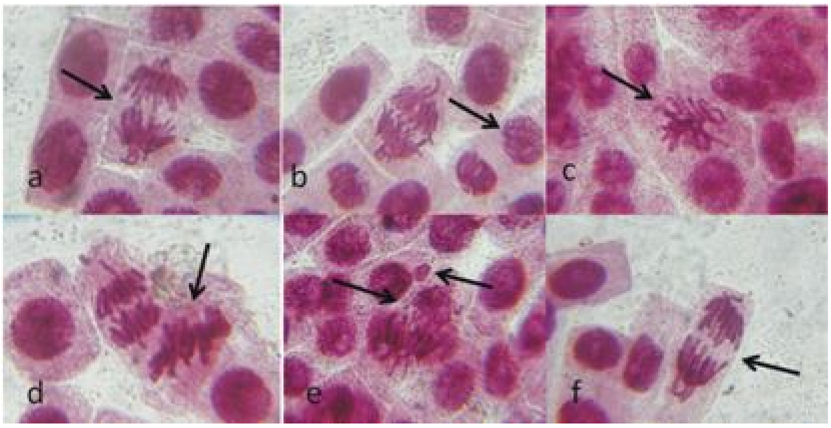

PLATE 2. CHROMOSOME ABERRATIONS OBSERVED IN THE ROOT TIPS OF DIFFERENT ONION SAMPLES GROWN IN TAP WATER.

(a) bridges (b) stickness at prophase (c) disoriented chromosomes (d) stickness at metaphase (e) chromosome breaks and micronucleus (f) bagrants (x1000 magnification).

DISCUSSION

The bulk of onion production in Nigeria is from the dry season cropping system particularly under irrigation in the Northern States. Repeated application of poor quality irrigation water can strongly influence the speciation of heavy metals, especially if the metal input is anthropogenic and make it more available for plant utilization (Shridhara Chary *et al*., 2008). Results obtained from this study shows that there were varying amounts of heavy metals in the onion bulbs obtained from different parts of northern Nigeria. This is probably due to uptake of these metals in poorly irrigated water and inefficient farming techniques. For instance, large amounts of industrial wastes, domestic and abattoir wastewaters been reported to be discharged into Kano city streams and used for irrigation, for which Binns *et al*., (2003) found to contain large amounts of toxic heavy metals. Similar reports of high levels of heavy metal accumulation in onion leaves in irrigated fields have been reported (Abdullahi *et al*., 2008, 2009).

Even though production of onion using irrigation practices has contributed significantly to poverty alleviation, food security, and improvement of the quality of life for rural populations, a study conducted in Sokoto by Ojo *et al*., (2009) observed that the farmers were technically inefficient with the use of fertilizers and agrochemicals due to their level of education.

The *A. cepa* material used for this work is a genetic population (not a clone) of onions, meaning that there is a natural variability in the material; so in a sense it is advantageous to purchase the onions in the market since the variation within the population will be compensated for by the use of a series of onions for each test. However, this is on the assumption that the onions are not at the risk of treatment with herbicides (Fiskesjö, 1985a). In the light of the above literature on the differential conditions of cultivation of onions found in our markets, the differential root growths of the different onions in tap water obtained in this study could be attributed to the differential quantity of toxic heavy metals accumulated in the plant materials themselves.

In *Allium* test, there usually seems to be a certain correspondence between root growth retardation (toxicity) and certain chromosomal deviations (genotoxicity) reduction. When chromosomal aberrations occur, there are almost always some growth restrictions (Fiskesjö, 1985b). The linear relationship between the macroscopic growth and microscopic parameters in this study infers the quantity of toxic heavy metals accumulated in the plant material is responsible for the differential percentage aberrant cells obtained in the different onions. In the same vein, in *Allium cepa,* whenever there is root growth inhibition, there is always reduction in the number of dividing cells (Bakare 2001;

Fiskesjo, 1997; Bakare & Wale-Adeyemo, 2004). Various types of induced chromosome aberrations have been associated with the heavy metals present during treatment of the onions with herbicide during cultivation (Natarajan *et al.,* 1996). In this study, the onion bulbs induced chromosome aberrations; however the types varied with the source of the onion bulbs. Chromosomal aberrations such as vagrants, stickiness, bridges, laggards, fragmentations, polar deviation and even micronuclei were found in the onion bulbs. The implication of the presence of these chromosome aberrations found in supposed controls in cytogenetic studies is that such results cannot guarantee the authenticity and reproducibility of findings where contaminated onion bulbs are utilized. Onion bulbs obtained from Gombe had the least heavy metal contamination; they also had the highest root growth and the highest mitotic index value.

On the other hand Kano onions with the most heavy metal contamination had the least root growth and the highest percentage aberrant cells. The high levels of heavy metal accumulation in onion leaves in irrigated fields in Kano (Abdullahi *et al*., 2008, 2009) may have been responsible for this development. The findings in this study suggest that onion bulbs obtained from Gombe are probably the most suitable for *Allium cepa* bioassay.

Vegetables accumulate heavy metals in their edible and non edible parts. Although some of the heavy metals such as Zn, Mn, Ni and Cu act as micro-nutrients at lower concentrations, they become toxic at higher concentrations. Health risk due to heavy metal contamination of soil has been widely reported (Satarug *et al*., 2000; Eriyamremu *et al*., 2005; Muchuweti *et al*., 2006). Crops and vegetables grown in soils contaminated with heavy metals have greater accumulation of heavy metals than those grown in uncontaminated soil (Marshall *et al*., 2007; Sharma *et al*., 2006, 2007). Intake of vegetables is an important path of heavy metal toxicity to human being.

Results of this study has shown that the contents of lead and other heavy metals in the onion bulbs obtained from Kano, Maiduguri and Sokoto were appreciably high. The likely health risks that could be caused to the unsuspecting public by the daily intake of heavy metals through these contaminated vegetables should not be overlooked by relevant health care and agricultural regulatory authorities.

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