

*Full Length Research Paper*

# The genotoxic effect of lead and zinc on bambara groundnut (*Vigna subterranean*)

E. O. Oladele\*, P. G. C. Odeigah and I. A. Taiwo

Department of Cell Biology and Genetics, University of Lagos, Nigeria.

Accepted 3 December, 2012

The effects of lead and zinc treatments on the chromosomes of bambara groundnut was investigated. The seeds of bambara groundnut were placed in Petri dishes in three replicates and allowed to germinate for five days in different concentrations: 25, 50 and 100 mg/L of both lead and zinc nitrates while the control group had distilled water. The total aberrations were examined. The mitotic index was calculated and the results were statistically evaluated by the analysis of variance at 5% significant level. The mitotic index decreased as the concentration increased ( $p < 0.05$ ). The highest mitotic index value was  $3.40 \pm 0.88$  for the control while the least was  $1.30 \pm 0.48$  for the 100 mg/L Pb treatment. The results show the most frequent chromosomal anomalies induced by these heavy metals as stickiness and bridges. Pb is much more genotoxic than Zn, as it induced more aberrations having percentage abnormalities of 92.3% while Zn had 30.0% for the highest concentration tested. Increased metal pollution can lead to some irreversible cytogenetic effects in plants and higher organisms. The study is an attempt to corroborate the toxic effect of lead and zinc on the chromosomes of plants. These results will be useful in environmental monitoring of the cytotoxicity of metals.

**Key words:** Heavy metal, aberrations, genotoxic, cytogenetic, mitotic index.

## INTRODUCTION

Pollution by metals varies from air, water to soil and heavy metals are found to stay much longer in soil than other parts of the biosphere (Lasat, 2002). Most industrial operations release various toxic heavy metals in their effluents which eventually find their way into water sources such as lakes, rivers and streams. The alarm of heavy metal pollution started with the effects of Minamata disease caused by consumption of mercury. Liu et al. (1994) reported that high concentrations of heavy metals have been found to be chromotoxic and mutagenic to a large number of plant species. These metals including Pb characteristically inhibit root growth and cell division in plants like *Allium cepa* (Liu et al., 1994) as well as *Zea mays* L. (Jiang and Liu, 2000). Ademoroti (1996) has shown that Pb occurs more in the stem than in the root tips of *Abelmoschus esculentus* (Okro) and bitter leaf

plants. Plants grown in contaminated soil have higher levels of Pb (as well as other heavy metals) than those grown in an uncontaminated soil (Bearington, 1975, Gingell et al., 1976). Zinc pollution occurs as a result of natural and anthropogenic processes and these can lead to the deposition of Zn on land. Ademoroti (1996) and Odiete (1999) noted that Zn arises as industrial effluents and such industries that produce Zn as effluents include electroplating industries, metallurgical industries, dye, paint and pigments, pharmaceuticals and mining industries. Rajesh and Rahda (2011) found that copper and zinc altered the nucleic acid contents and the nutritional status of *Vigna mungo* (L.) Hepper. Fragašová (2001) found that lead and zinc were very toxic to *Sinapsis alba* L. seedlings. According to Mittler (2002), the toxicity of heavy metals including Pb and Zn may arise as a result of the generation of reactive oxygen species that may cause wide-ranging damage to proteins, nucleic acids, lipids and eventually apoptosis (cell death).

The bambara groundnut is important because it fixes

\*Corresponding author. E-mail: [esteromitiran@yahoo.com](mailto:esteromitiran@yahoo.com). Tel: +2348054317299.

atmospheric nitrogen as nitrates into the soil which improves soil fertility. It is high in methionine, an essential amino acid. The beans are eaten fresh after harvest and can be dried and stored for later use and consumption (NRC, 2006). The young fresh seeds may be boiled and eaten as a snack in a manner similar to boiled peanuts and could be made into pudding locally called moi moi or okpa (bean porridge) in some parts of Nigeria. It has been reported that in Zambia, bambara groundnut is used for bread making (Brough et al., 1993) while Poulter and Caygill (2006) also reported that it could be used for milk making. The great genetic diversity potential of bambara groundnut has been described by Wazael et al. (2004). Aremu et al. (2006) carried out an assessment of heavy metals including Pb and Zn in bambara groundnut planted along some roads in Nasarawa state, Nigeria. They found the levels of these two metals to be high especially Pb at 19.2 mg/100 g which is about 10 times the recommended daily intake. W.H.O (1972), recommends daily intake to be 0.430 mg per person. If this plant was to be consumed, it would have been harmful. Studies on the treatment effects of lead and zinc on this important legume are scarce.

The objective of the study was therefore to determine the toxicity of these two metals on the chromosomes found at the root tips of the bambara groundnut. This investigation is an attempt to corroborate the toxic effect of lead and zinc on the chromosomes of plants. The results obtained may help in understanding the possible constraints in the role of Pb and Zn as pollutants in plants.

## MATERIALS AND METHODS

Dry seeds of Accessions of bambara groundnut (*Vigna subterranea*): Tvsu 102 was collected from the International Institute of Tropical Agriculture (I.I.T.A.), Ibadan. Seeds were spread uniformly in Petri dishes lined with filter paper. The Petri dishes were divided into three replicates and the seeds were divided into two sets of treatment. Equal volumes of the different concentrations of lead and zinc nitrates solutions (25, 50 and 100 mg/L), respectively were administered while the control group had distilled water. The seeds were allowed to germinate within the Petri dishes and were treated with the different concentrations of each of the metals and distilled water, respectively at a temperature of 25°C for five days. Growing root tips which were brittle, translucent and gently tapering were selected from the three plants grown in the effluents of different concentration and from the control. About 2 to 3 mm terminal root tips were cut off using a sharp blade, then placed on a clean glass slide and macerated with the aid of two dissecting needles, and the remaining portion discarded. A drop of 1 N hydrochloric acid (HCL) was added to the root tip and left for 5 min; this softens the root tissue breaking up the middle lamellae. The excess acid was sucked up with a filter paper and the softened tissue is further macerated with dissecting needles so that the cells easily absorb the stain and spread adequately for microscopic observation. Then, a drop of lactic acetic orcein stain (2%) was placed on the macerated root tip and allowed to stand for 20 min for clearer viewing of the mitotic stages using the microscope (Michelle-Frainer et al., 2006). Each slide was covered with a cover slip and pressed down to allow the tissue spread out and also to

allow the excess stain seep out at the edges of the cover slip. This was removed by placing the slide between the folds of the filter paper and squashing was done with the base of the dissecting set. All slides were examined under the light microscope with high power magnification (x40 objective): then the good slides were preserved by sealing the edges of the cover slip with nail varnish to prevent the stain from evaporating. The photomicrographs of good slides were then taken under the oil immersion lens (x1000 objective) using a Wild M20 microscope with MPS 55 photoautomat attachment. The mitotic index was calculated according to Balog (1982) using the formula:

$$\text{Mitotic index (M/I)} = \frac{\text{Number of cells in mitosis per field} \times 100}{\text{Total Number of cells per field } 1}$$

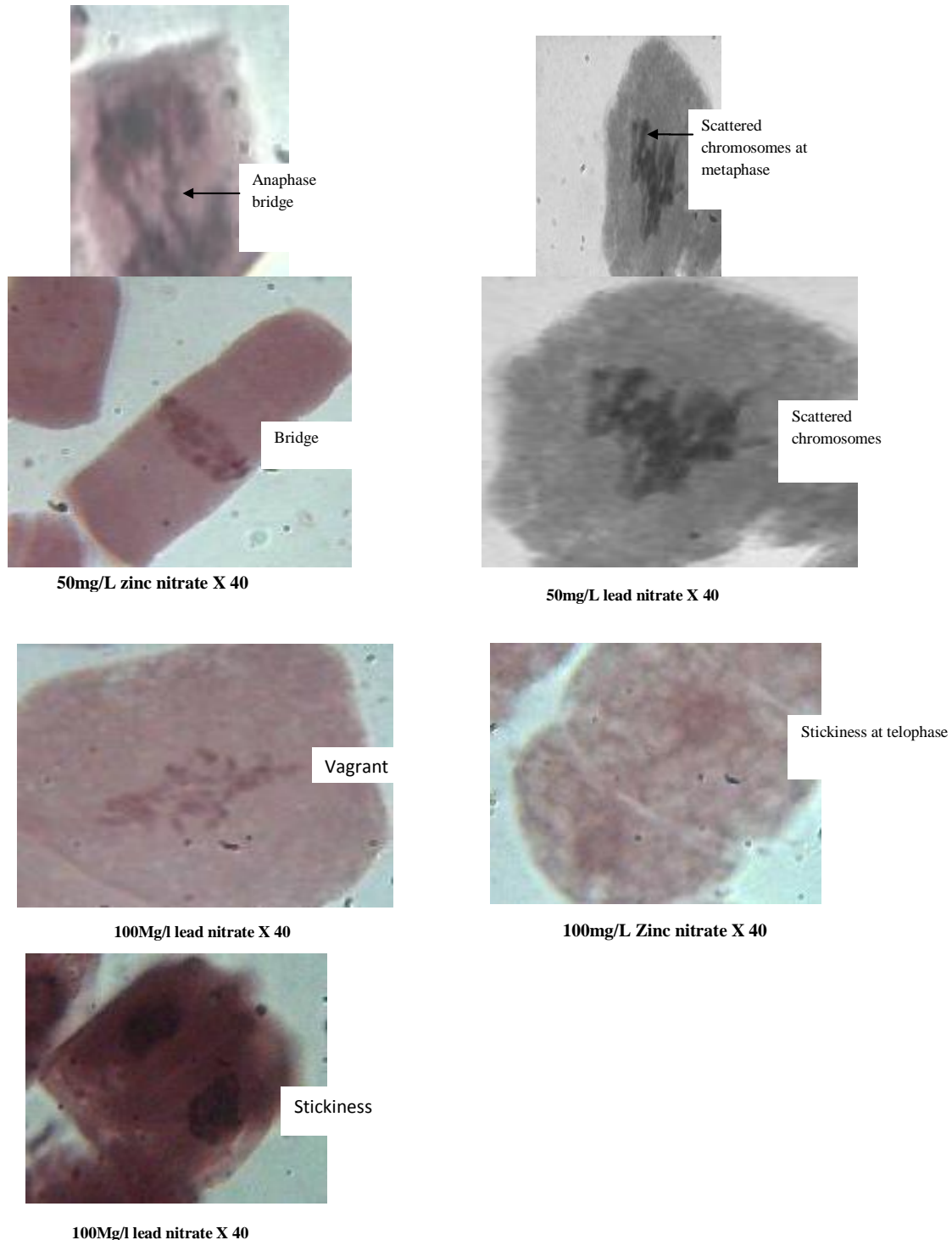
The results of the mitotic index were statistically evaluated by the analysis of variance at 5% significant level using Microcal Origin 5.0 software.

## RESULTS AND DISCUSSION

The results for genotoxicity assay of the treated bambara groundnuts are shown in Figure 1. 25 and 50 mg/L of lead and zinc nitrates resulted in anaphase bridges, fragmented and scattered chromosomes while the 100 mg/L concentrations mostly resulted in stickiness and vagrant chromosomes.

Table 1 shows the total number of cells analyzed, mitotic index values and the number of chromosome aberrations observed in bambara groundnut treated with different concentrations of lead and zinc nitrates. The results show that there was decrease in mitotic index with increase in treatment concentrations. The control had 3.40 while 25, 50 and 100 mg/L of Pb had mitotic index of 2.10, 1.50 and 1.30, respectively. The aberrations observed include: anaphase bridges, stickiness, vagrants, laggards and fragments. The highest treatment concentration of 100 mg/L was observed to cause mostly stickiness and vagrants especially with lead. The most frequent chromosomal aberrations were anaphase bridges, stickiness and vagrants.

A concentration-dependent increase in chromosomal abnormalities was observed for Zn and a decrease for Pb except for the highest concentrations in both cases. At 100 mg/L of Pb, the highest chromosomal aberration was observed while Zn treatment at this concentration resulted to a decrease in percentage chromosomal aberration. There was a linear correlation between the concentration of metals and the percentage of abnormalities in the treated sets especially for Pb. The total aberration (%) increased after 50 mg/L Pb exposure and a decrease was observed after 50 mg/L Zn exposure. This was probably due to increased metal concentration and subsequent detrimental effect especially for Pb. A concentration-dependent decrease in number of dividing cells was observed. This indicates an interference with cell division. There was a highly significant difference between both metal-treated set and the control set ( $P < 0.05$ ). Scattering, bridges, stickiness



**Figure 1.** Chromosomal aberrations induced by Pb and Zn.

were the most frequent aberrations observed for the two heavy metals (Pb and Zn). Similar observations were shown by other studies (Fiskesjo, 1993) and (Ukaegbu and Odeigah, 2009). All the concentrations were capable of inducing different types of chromosomal abnormalities. This provides a case for comparison of the deleterious

effects of these metals on the concerned plant. These aberrations were probably caused mainly by the interference of Pb and Zn with cell division at the root tips (meristems). Several researchers performed similar studies on the genotoxic effects of different chemicals on different plant materials (Ahmad and Yasmin, 1991;

**Table 1.** Chromosome aberrations in bambara groundnut root tips cells treated with different concentrations of lead and zinc nitrate.

Concentrations	TCN	ND	ST	CM	BF	VG	LG	MA	TA(%)	MI	MI ± SD	P-value
Control	1000	34 (P <sub>3</sub> M <sub>16</sub> A <sub>12</sub> T <sub>4</sub> )	0	0	0	0	0	0	-	3.40	3.40±0.88	0.7376**
25 mg/L Pb	1000	21 (P <sub>2</sub> M <sub>8</sub> A <sub>5</sub> T <sub>6</sub> )	0	0	3	5	0	0	38.10	2.10	2.10±0.76	0.0014*
50 mg/L Pb	1000	15 (P <sub>0</sub> M <sub>5</sub> A <sub>6</sub> T <sub>4</sub> )	0	0	2	0	0	0	13.3	1.50	1.50±0.63	0.0028*
100 mg/L Pb	1000	13 (P <sub>6</sub> M <sub>2</sub> A <sub>2</sub> T <sub>3</sub> )	3	0	4	2	3	0	92.3	1.30	1.30±0.48	<0.0001*
25 mg/L Zn	1000	26 (P <sub>6</sub> M <sub>8</sub> A <sub>6</sub> T <sub>6</sub> )	0	0	5	1	1	0	26.92	2.60	2.60±0.81	0.0081*
50 mg/L Zn	1000	24 (P <sub>0</sub> M <sub>0</sub> A <sub>0</sub> T <sub>24</sub> )	6	0	6	0	0	0	50.0	2.40	2.40±0.80	0.0036*
100 mg/L Zn	1000	20 (P <sub>1</sub> M <sub>9</sub> A <sub>7</sub> T <sub>3</sub> )	2	1	2	0	1	0	30.0	2.00	2.00±0.73	0.0010*

TCN, Total cell number; P, prophase; M, metaphase; A, anaphase; T, telophase; ND, number of dividing cells; ST, stickiness; CM, C-mitosis; BF, bridges fragment; VG, vagrant; LG, laggard; MA, multipolar anaphase; TA, total aberrations; SD, standard deviation; MI, mitotic index. Data were expressed as mean ± SD. \*P<0.05, Significantly different from control; \*\*P>0.05, not significantly different from control.

Kumar and Tripathi, 2007). Metal-induced chromosomal stickiness has been reported to interfere with cell division (Kumar and Srivastava, 2006). Kumar and Tripathi (2007) reported the interference of Pb with cell division in grass pea. Pb was also found to inhibit root growth and cell division in *Zea mays* L. (Jiang and Liu, 2000). Metal-induced chromosomal stickiness accompanied by pyknosis and chromatin degeneration has also been reported in maize by Caetano-Pereira et al. (1995), *Allium cepa* (Kumar and Tripathi, 2003) and *Helianthus annuus* (Kumar and Srivastava, 2006), these metals include Pb and Zn. Chromosome stickiness which was observed can lead to sticky metaphase and precocious separation of chromosomes. This may be due to breaking of the protein moiety of the nucleoprotein backbone by these heavy metals (Pb and Zn). Patnaik et al. (1984) had similar observations with chemicals tested on *Allium cepa*. Scattering which involves chromosomes spreading irregularly over the cell may be due to disturbance of the spindle apparatus by the metals. Anaphasic bridges formed were probably due to unequal exchange or dicentric chromosomes. Fragments observed at metaphase may be due to the failure of broken chromosomes to recombine. Fragments might be due to the stickiness of the chromosomes and the consequent failure of the arrival of chromatids at the poles. The characteristic behaviour of laggard chromosomes is that they generally lead to micronucleus formation (Kumar and Rai, 2007). Agarwal and Ansari (2001) noted that fragments caused by Pb may also be due to acentric chromosomes formed as a result of inversion. Accumulation of these abnormalities affects gamete formation and can lead to non-viable gametes, which considerably reduces plant fertility. Studies on different plant species have shown that declines in seed production are correlated with meiotic irregularities (Kumar and Rai, 2007).

From this study, it can be said that both heavy metals are capable of inducing chromosomal anomalies. Pb is much more genotoxic than Zn, as it induced more abnormalities having percentage abnormalities of 92.3% while zinc had 30.0% for the highest concentration tested. The statistical values (P < 0.05) obtained for Pb

treated sets are much lower than those for Zn treated sets. Also, Zn treated sets had higher number of dividing cells compared to Pb treated sets. Increased soil pollution can lead to some irreversible cytogenetic effects in plants and higher organisms. Thus, mutagenic data from plant assays are very important for genetic research and for maintaining a stable ecosystem.

## REFERENCES

- Ademoroti CMA (1996). Environmental chemistry and toxicology. Ibadan Foludex Press Ltd., London. 215 p.
- Agarwal R, Ansari MYK (2001). The effect of aniline on root tip cells of *Vicia faba* L. J. Cytol. Genet. 2(NS) 129-134.
- Ahmad S, Yasmin R (1991). Effects of methyl parathion and trimltox on the mitosis of *Allium cepa*. Cytologia 57:155-160.
- Aremu MO, Olonisakin A, Ahmed SA (2006). Assessment of heavy metal content in some selected agricultural products planted along some roads in Nasarawa State, Nigeria. J. Eng. Appl. Sci. 1(3):199-204.
- Balog C (1982). The mitotic index in diploid and triploid *Allium* roots. Cytologia 47:689-697.
- Brough SH, Azam-Ali SN, Taylor AJ (1993). The potential of Bambara groundnut (*Vigna subterranea*) in vegetable milk production and basic protein functionality systems. Food Chem. 47:277-283.
- Caetano-Pereira CM, Pagliarini MS, Brasil EM (1995). Influence of aluminium in causing chromosome stickiness in maize microsporocytes. Maydica 40:325-330.
- Fargašová A (2001). Phytotoxic effects of Cd, Zn, Pb, Cu and Fe on *Sinapsis alba* L. seedlings and their accumulation in roots and shoots. Biologia Plantarum 44:471-473.
- Fiskesjo G (1993). "The Allium test in waste water monitoring". Environ. Toxicol. Water Qual. 8:291-298.
- Gingell SM, Campbell R, Martin RH (1976). The effect of Zinc, Lead and Cadmium pollution on the leaf surface microflora. J. Environ. Pollut. 11(1):25-37.
- Jiang W, Liu D (2000). Effects of Pb<sup>2+</sup> on root growth, cell division and nucleolus of *Zea mays* L. Bull. Environ. Contam. Toxicol. 65:786-793.
- Kumar G, Rai P (2007). Genotoxic potential of mercury and cadmium in soybean. Turk. J. Biol. Sci. 31:13-15.
- Kumar G, Srivastava S (2006). Mechanism of Aluminum cytotoxicity in meristematic cells of *Helianthus annuus* L. J. Phytol. Res. 19:175-178.
- Kumar G, Tripathi A (2003). Comparative mitotoxicity and mutagenicity of two heavy metals in *Allium cepa* L. J. Cytol. Genet. 4(NS):169-173.
- Kumar G, Tripathi R (2007). Lead induced cytotoxicity and mutagenicity in Grass Pea. Turk. J. Biol. Sci. 32:73-78.
- Lasat HA (2002). Phytoextraction of toxic metals: a review of biological mechanisms. J. Environ. Qual. 31(1):109-120. (PubMed)
- Liu D, Jiang W, Zhao FM, Lu C (1994). Effects of lead on root growth, cell division and nucleolus of *Allium cepa*. Environ. Pollut. 86:1-4.

- Michelle-Frainer K, Antonio CF, Thais S, Solange BT (2006). Effects of *Pterocaulon polystachyum* DC. (Asteraceae) on Onion (*Allium cepa*) Root-tip cells. Genet. Mol. Biol. 29:539-542.
- Mitler R (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7:405-410.
- National Research Council (NRC) (2006). "Bambara Bean". Lost Crops of Africa: Volume II: Vegetables. Lost Crops of Africa 2. National Academies Press. ISBN 978-0-309-10333-6. [http://books.nap.edu/openbook.php?record\\_id=11763](http://books.nap.edu/openbook.php?record_id=11763) and page=53. Retrieved 2008-07-15.
- Odiere WO (1999). Environmental physiology of animals and pollution. Diversified Resources, Ltd., Lagos, Nigeria. 261 p.
- Patnaik S, Saran BL, Patnik SM (1984). Effect of zarda (processed tobacco leaf) extract on the chromosomes of *Allium cepa*. Cytologia 49:807-814.
- Rajesh D, Radha S (2011). Effect of copper and zinc on physiological and biochemical parameters in *Vigna mungo* (L.) Hepper. Int. J. Pharm. Biosci. (2)554-565.
- Ukaegbu MC, Odeigah PGC (2009). Genotoxic effects of sewage effluent on *Allium cepa*. In: Report and Opinion. 1(6):36-41 (ISSN:1553-9873).
- Wazael HN, Inga CB, Jorgen LC, Sven BA (2004). Analysis of genetic diversity in bambara groundnut [*Vigna subterranea* (L.) Verdc] landraces using amplified fragment length polymorphism (AFLP) markers. Afr. J. Biotechnol. 3:220-225.
- World Health Organization (WHO) (1972). Evaluation of certain food additives and the contaminants: Mercury, Lead and Cadmium. WHO Tech. Rep. Ser. No. 505; FAO Nut, Meet Rep. Ser. No. 51.