The Genotoxic Effect of Sewage Effluent on *Allium Cepa*

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ABSTRACT: The genotoxicity of sewage effluent was investigated using both morphological and root chromosome assay. The mean root lengths of onions exposed to different concentrations of the effluent were measured every day for 5 days and EC_{50} values were determined from the growth curve as 47%.The result the mean root length was statistically evaluated by the analysis of variance and least significant difference. There was a significant decrease in root length of the experiment. Also the mitotic index decreased as concentration increased. Total aberrations increased significantly as concentration increased ($p<0.05$). These results demonstrate that the *Allium* test is a useful screening test for the evaluation of toxicity in sewage effluent. [Report and Opinion. 2009;1(6):36-41]. (ISSN: 1553-9873)

Key words: sewage, effluent, onions, genotoxicity, genetic damage.

INTRODUCTION

Sewage water has been used to support agricultural production in many countries over a considerable period of time. Grisolia *et al*, (2005), reported that in Basilia, capital of Brazil, sludge from sewage plant is recovered to be used to improve the organic content of agricultural soils. In Egypt, there are many examples of planned reuse of sewage effluents for irrigation of crops (El-Bagouri, 1999, Amin, 2002).

Effluent reuse can provide considerable benefit when used under controlled conditions to establish protection of health of both farm workers and consumers of the produce (Aleem and Malik, 2003).

The main components of sewage effluent that warrant consideration for irrigation include major and minor nutrients like nitrogen, potassium, phosphorous, calcium, iron, zinc, copper and manganese as well as heavy metals such as cadmium, lead, chromium and Mercury. There are also the presence of certain cations and anions which might cause adverse effect on soil properties and plant growth (Brown *et al.*, 1991, Amin and Migahid, 2000).

Friskesjo (1987) noted that with increasing interest in the use of cytological investigation in short term test (STIs) for environmental monitoring, higher plants have come into focus reviewing plant toxicity test. Wang (1992) has emphasised the sensitivity of simple root elongation test and recommends plants especially for toxicity testing for complex mixtures.

Several plant assay system have been used to monitor genotoxic substances in the environment. Bansal (1998) studied the impact of long term application of sewage on soil properties and residual affect of heavy metals uptake by barley and corn. The result revealed higher concentration of heavy metals when compared to fields irrigated with tube well water. Odeigah *et al*, (1997a) and (1997b) used the *Allium* test to evaluate the genotoxic effect of waste water and leachates from solid industrial wastes respectively. Their result showed that the contaminants have both macroscopic and microscopic effect on plants. However increased growth of olive trees and fodder grasses was observed for irrigation using sewage effluent (Saavedera *et al.*, 1984)

In Lagos State Nigeria, Sewage sludge is being used to cultivate vegetable crops like spinach, cabbage, carrot along Lagos- Badagry expressway. The choice of sewage sludge could be due to high cost of artificial fertilizer. An investigation has revealed that this sewage sludge were product of primary sedimentation (personal communication) thereby raising concern over the environmental implication of this practice.

The principal objective of this study is to assess the effect of untreated sewage water collected at Mile 2 dump site on the genetic material of *Allium cepa*. It is hoped that the result of this study may provide more information on the possibility of the use of treated waste water for irrigation farming.

MATERIALS AND METHODS

SEWAGE EFFLUENT:

The sewage effluent was obtained at mile 2 dump site in Lagos state, Nigeria. The samples were collected before their discharge into the coastal waters.

TEST MATERIAL

Onions bulbs were purchased at Tejuosho Market, Lagos. They were sun dried before use.

TEST PROCEDURE:

Planting of Onion

The outer scales of the bulbs and the brownish bottom plate were first removed. The rings of the root primordial were left intact. A series of cleaned small sized bulbs of onions, (*Allium cepa*) were first sprouted in water as described by Friskesjo (1987). After 24 hours, the best in terms of root growth were selected. Four each were placed on top of containers filled with 20%, 40%, 60%, 80% and undiluted aliquots of sewage effluent and fresh tap water for control and was termed as day one. The solutions were changed everyday during the experiment.

Root Length Measurement

The root length of onion bulbs from each concentration was measured on day 2, 3, 4 and 5 of the experiment using a calibrated ruler. The mean root length of each treatment in each concentration was calculated by dividing the total root length for each concentration by four. The root length of the control was also calculated and the result plotted on a graph.

Cytological Study:

The emerged root tips of the onion bulbs in the different concentration of sewage effluent were fixed in aceto-alcohol (1:3) after the second and fourth days of starting the experiment. The conventional feulgen-squash method (Sharma and Dphil, 1980) was used to prepare permanent slides of root meristems.

The root tips were put in 1 normal hydrochloric acid for five minutes to soften the tissue. The tips were them macerated and stained with aceto-orcein stain for 15 minutes. The macerated and stained root tips were covered with cover slip and squashed and later viewed in a microscope. The mitotic activity, rate and kind of aberrations were recorded using four replicates for each treatment.

The results of the mean root length were statistically evaluated by the analysis of variance and least significant difference (LSD) test at 5% significant level.

RESULT

MACROSCOPIC EFFECT

It was observed that sewage effluent suppressed root growth when compared with the control. At 100% concentration there was total growth inhibition and the roots decayed after 48 hours of the experiment while at 80% concentration the roots decayed after 72 hours. At 60% concentration their appeared crochet hooks and swellings at the root tips Signs of wilting started to appear on few of the treated roots after 48 hours especially at 80% concentration. The EC_{50} occurred at 47% (Figure 1).

MICROSCOPIC EFFECT:

Treatment with sewage effluent for 4 days lowered the mitotic activity significantly than the control. The mitotic index decreased with increasing the concentration of sewage effluent (Table 1). At 80% concentration and undiluted aliquot there was no mitosis. The aberration rate of non-dividing cells increased significantly as the concentration increased. ($p<0.05$) More than one kind of aberration was found per cell. The higher value was recorded in 20% concentration. Sticky metaphase chromosomes were the most frequent kind of aberration induced by sewage effluent. C-mitosis was only found in 20% concentration.

Abnormal chromosome orientation and movement such as multipolar anaphase and binucleus were found in 20% and 40% concentration (Plate 1). Dividing cells was highest in control experiment and decreased significantly as concentration increased (Table 1).

Table 1. Effects of treatment with different concentration of sewage effluent

	PHENOTYPIC INDICES				CHROMOSOME ABERRATIONS			
Concentration (%)	Root length % control	Mitotic index ±SD	Number of cells	Dividing cells	Stickiness	C-mitosis	Vagrant	Bridges fragments
Control	100	6.6 ± 0.46	500	33	8	0	0	0
20	97.5 + 5.8	3.9 ± 0.11	451	18	8	1	4	3
40	57.0+ 5.3	3.0 ± 0.10	396	12	7	0	3	5
60	29.1 + 3.2	2.9 ± 0.07	384	11	9	0	4	0
80	13.2 + 3.0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0

Mitotic index was calculated as mitotic cells per 1000 cells per slide;
Chromosomal aberrations were scored on 500 cells per slide

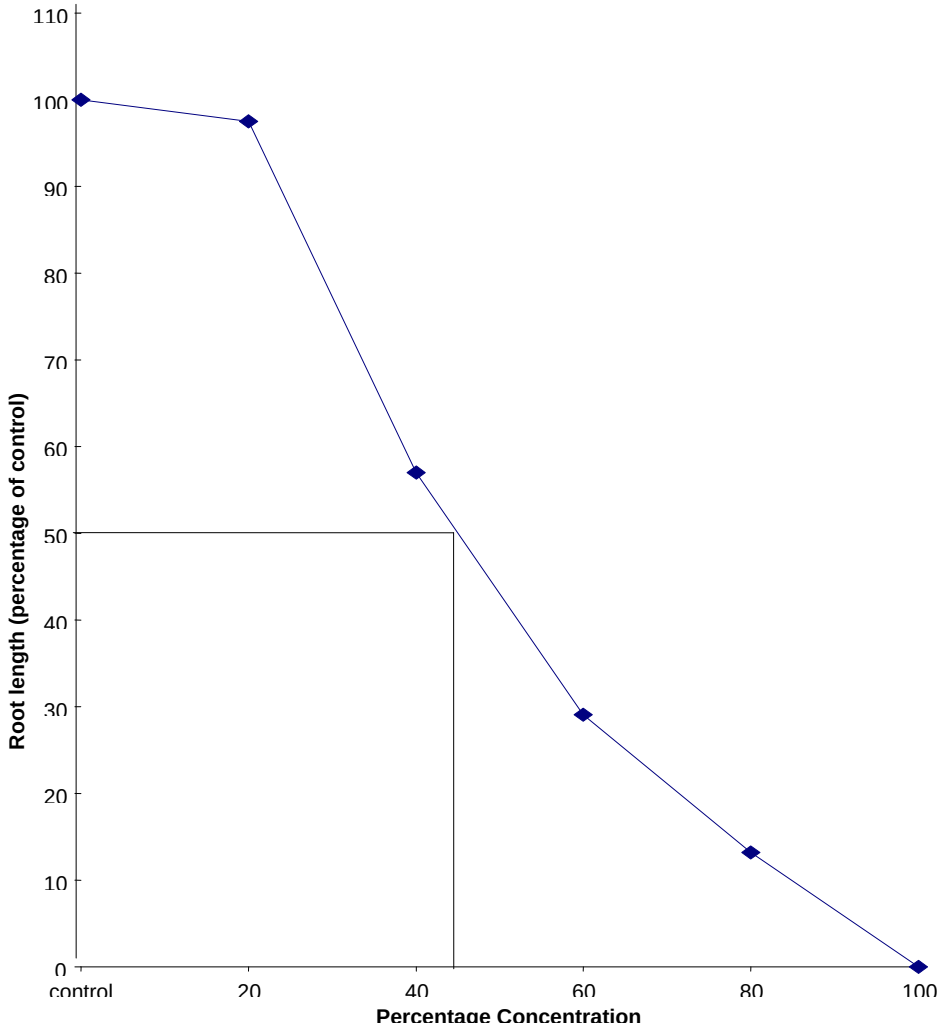


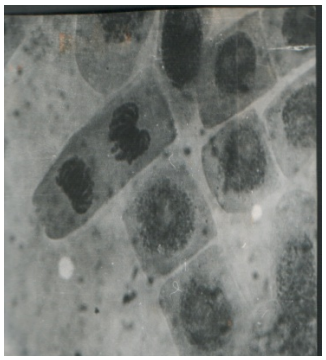
Fig. 1: Growth curve of Allium roots (in relations to control) after treatment with sewage effluents


Multipolar Telophase

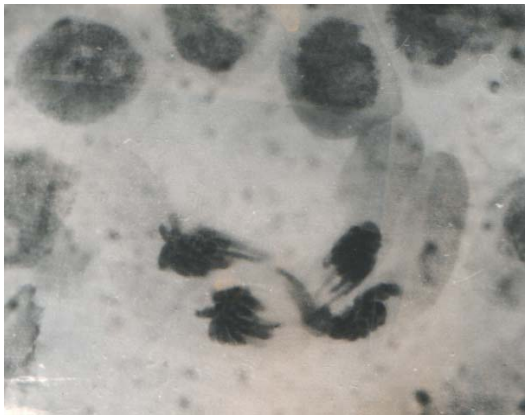

Binucleus


Vagrant and Multipolar Anaphase


Telophase with laggards


Sticky Telophase


Bridged and Fragmented Anaphase


Multipolar Anaphase


Vagrant Metaphase

Plate 1: Common chromosomal aberrations induced by sewage effluent.

DISCUSSION

Parameters such as root shape and growth, frequencies of mitosis and abnormal cell division can be used to estimate the cytotoxicity, genotoxicity and mutagenicity of environmental pollutant (Amin, 2002; Nielson and Rank, 1994). The *Allium* test has many advantages as genotoxicity screening assay, one being that *Allium* root cells posses the mixed function oxidase system which is capable of activating promutagens or genotoxic chemicals (Odeigah *et al*; 1997a). In the *Allium* test, inhibition of rooting and the appearance of stunted roots indicate retardation of growth and cytotoxicty, while root wilting explains toxicity (Odeigah *et al*; 1997b, Grant, 1982). Nevertheless both growth retardation and root wilting are accompanied by suppression of mitotic activity and occurrence of chromosomal aberration.

The present study provides evidence that sewage effluent inhibited root growth and caused growth retardation. The inhibition of growth may be due to high rate of chemical oxygen demand which affected certain physiological processes leading to the disturbance in the balance between promoter and inhibitors of endogenous growth regulator (Grover and Tejpar, 1981).

Growth inhibition was most marked at 100% concentration. There was also a marked decrease in root length when compared with the control. The suppression of mitotic activity was often used in tracing cytotoxicity (Smaka-Kinel *et al.*, 1996). This is usually accompanied by an increase in cells with c-mitosis, sticky and abnormal chromosome orientation (Amin and Migahed, 2000). In this study a decrease in the mitotic index was found as the concentration of effluent increased. This indicates the cytotoxic effect of sewage effluent. The rate of aberration increased as the concentration increased. However the type of chromosomal aberration varied. Sticky chromosomes were most frequent in all concentrations. According to Odeigah *et al*. (1997b), sticky chromosomes are indicative of a highly toxic usually irreversible effect, leading to cell death. This could be responsible for the completely decayed roots found in 80% and 100% concentration. Chromosome stickiness is caused probably through immediate reactions with DNA during its inhibition periods, causing DNA-DNA or DNA-protein cross linking (Amin, 2002). Sticky chromosome might also be the result of incomplete replication of chromosomes by defective enzymes (Bennet, 1977). Sewage water also induced crochet hooks and c-tumors, which have been shown by other studies to be useful sign of toxicity (Amin, 2002). Crochet hooks might be due to presence of heavy metals while c-tumors effects are caused by several types of compounds, such as colchicines (Odeigah *et al.*, 1997a).

Results obtained in this study show that sewage effluent induced chromosomal aberration through interactions with DNA and proteins leading to chromosome stickiness, mitotic disturbances and cell damage. This result is similar to the findings of Odeigah *et al. (*1997b) in which roots of *Allium cepa* exposed to effluent from industrial wastes resulted to cells with chromosomal aberration.

The impacts of using sewage effluent as fertilizer on the environment are difficult to predict. However positive result in *Alliums* test should be considered a signal of warning as this may constitute risk to environment and human health. Therefore it is recommended that sewage water be treated before they are used as fertilizer so that safety of humans would be achieved.

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