NJBAS

Available online at http://www.ajol.info/index.php/njbas/index
Nigerian Journal of Basic and Applied Science (June, 2015), 23(1): 31-37
DOI: http://dx.doi.org/10.4314/njbas.v23i1.5

ISSN 0794-5698

Full Length Research Article

Toxicity Evaluation and Cytogenetic Screening of Process Water Using a Plant Bioassay

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ABSTRACT: The effect of toxic substances on aquatic lives from a wastewater indiscriminately discharged into the environment during oil and gas exploration activities in Nigeria is the focus of this study. A plant bioassay, the *Allium cepa* test, was used for the cytogenotoxicity screening of process water on root growth inhibition and chromosome abnormalities in the meristematic roots of the plant. Results of the physicochemical analysis of the wastewater collected from Nigerian Agip Oil Company (NAOC) facility at Ogboinbiri in Bayelsa State at the point of discharge into the environment showed that it had an unpleasant odour, was slightly alkaline (pH 7.72) and had high electrical conductivity (2793.33 µS/cm). Lead, manganese, iron, chromium and nickel were present at amounts (0.13, 0.33, 4.47, 0.17 and 0.10 mg/l respectively) above national (NESREA) and international (USEPA) limits for effluent discharge. Macroscopic evaluation of *A. cepa* cultivated in the wastewater resulted in significant ($p<0.05$) concentration-dependent root growth inhibition with an EC_{50} value of 14.8 % at 96 h. Root tip cells of the onion bulbs processed for cytological studies by the aceto-orcein squash technique after exposure to the wastewater for 48 h at concentrations of 0.5, 1.0, 2.5, 5.0, and 10% (v/v; wastewater/tap water) showed chromosomal aberrations at all concentrations. Statistical analysis of microscopic results show significant ($p<0.05$) concentration-dependent frequency of aberrant chromosomes and reduction of mitotic index. The findings in this study calls for proper treatment of process water before its discharge into water bodies to avoid cyto-genetic damages to aquatic lives.
Keywords: Toxicity, chromosome aberration, *Allium cepa*, process water

INTRODUCTION

Large quantity of water is associated with hydrocarbon production during oil exploration activities. Water is used for drilling, hydraulic fracturing, completion and well treatment; it is one of the most commonly used liquids injected into the reservoirs through specific wells (injection wells) for oil production support. This is done during so-called "secondary" recovery in order to compensate for the drop in pressure inside the reservoir after it has started production. It is also used to improve the efficiency of oil displacement and extraction [water flooding, Enhanced Oil Recovery (EOR)] (Zenon Environmental, 1987).

With respect to the Oil and Gas industry, process water is defined as water that has been in intimate contact with hydrocarbons in the refinery. Water that is generated in the process units is represented by desalter effluent, sour water, tank bottom draws and spent caustic (IPIECA, 2010). Quality tolerances for process water vary widely with the purpose for which it is used; in general, process water should be clear, colourless and free from iron, manganese, hydrogen sulfide, and organic growths. It should typically have a conductivity ranging from 0,1 to 50 uS/cm (Nordell, 1951).

Although progress has been made over the last few years by the oil refining sector to make improvements to the way in which water is managed, the extent of compliance with standards and global best practices in the treatment and discharge of wastewaters still remains a challenge (Isehunwa and Onovae, 2011).

We are not aware of any documented study conducted on the evaluation of the toxicity of process water by physicochemical analysis or genotoxicity bioassay. Since the complexity of contaminated water makes it almost impossible to carry out a hazard assessment based on chemical and microbial analysis alone (WHO, 1976), a comprehensive approach involving the use of plants as standard bioassays alongside physicochemical analysis and other animal tests has been advocated (Arkhipchuk *et. al*., 2000).

Among the seven plant bioassays reviewed by the US Environmental Protection Agency EPA Gene-Tox program in 1980, the *Allium* root tip chromosome aberration assay was one of the protocols adopted and standardised by the International Program on Plant Bioassays (IPPB) for monitoring or testing environmental pollutants, which is currently in operation under the auspices of the United Nations Environment

Program UNEP (Ma, 1999). The advantage of this test in comparison with others is that it does not require preliminary processing of water samples for establishing toxicity and genotoxicity (Grant, 1994).

Toxicity endpoints obtained with this plant assay compare well with animal and fish lethality bioassays in their relative sensitivity toward environmental pollutants (Grover and Kaur, 1999; El-Shahaby *et al*., 2003; Junior *et al*., 2007; Abdel-Migid *et al*., 2007; Sık *et al.,* 2009, Olorunfemi *et al*. 2011; 2012). In the light of the above, this study was undertaken to ascertain and blind, the mutagenicity of process water on the chromosomes of *A. cepa*.

MATERIALS AND METHODS

Collection of Samples

The process water used for this study was obtained from the Nigerian Agip Oil Company (NAOC) facility at Ogboinbiri (4°50'0"N, 5°58'0"E) in Bayelsa State in March, 2012. The wastewater was collected at the point of discharge with a funnel into 10-litre plastic containers which were previously washed and rinsed with distilled water. They were kept in an ice chest for onward transport to the laboratory and stored in the refrigerator at 4°C and analysed for physicochemical parameters within 24 h of collection

Physicochemical analysis

The wastewater was analyzed for a number of standard physicochemical parameters including pH, hardness, total dissolved solids, conductivity, alkalinity, chloride, nitrates, ammonia, sulphates, phosphates and 13 metals and heavy metals namely: Ca, Na, K, Mg, Fe, Cu, Zn, Al, Cr, Pb, Ni, Mn and Cd using standard analytical methods (USEPA, 1999; APHA, 2005).

Allium cepa Assay

The plant material used in this study was the purple variety of the common onion (*Allium cepa* L, 2n=16). Equal-sized (15-22 mm diameter) onion bulbs were obtained commercially at the Lagos Street market in Benin City, Nigeria (6°15'N, 5°25'E) and those infected by fungi were discarded at the beginning of the experiment. Several onion bulbs (about four times the total number of the onion bulbs required) were purchased and sun-dried for 2 weeks before the commencement of the study to enable replacement of any bulb that may dry up, rot or damaged by mould (Fiskesjö, 1985). The same batch of onions were used throughout to evaluate the cytogenotoxic potentials of the produced water samples using root growth inhibition and induction of chromosomal aberration as the assay end points.

The outer scales of the onion bulbs were carefully removed without tampering with the primordial root ring. The concentrations of the test samples used were 0.5, 1.0, 2.5, 5 and 10 % (v/v; wastewater/tap water). As suggested by Fiskesjö (1985), tap water used as negative control should be of good quality having a pH 7.1 and relatively high hardness (Ca+Mg = 50-70 mg/l) and free from any chlorine compounds and toxic ions. This was obtained from *UNIBEN* Enterprises, University of Benin, Ekenhuan Campus, Benin City. The base of twelve onion bulbs were suspended in the wastewater and tap water inside 100 ml beakers containing about 75 ml of the test sample at 27±1°C in the dark. The process water samples were changed daily.

For the evaluation of *in vivo* induction of chromosomal aberration, the test samples were cultivated for 48 h. Root tips from two onion bulbs were cut and fixed in ethanol:glacial acetic acid (3:1, v/v) inside universal bottles after the exposure periods and kept at 4°C for 24 h before use. The already fixed root tips were hydrolysed in 1N HCl at 60 °C for 5 minutes. The hydrolysed root tips were washed several times with distilled water. Two root tips were squashed on each slide and stained with aceto-orcein for 10 min. Excess stains were removed and the edges of the cover slips were sealed as suggested by Grant (1982). Five scorable slides were prepared per sample and examined for different mitotic stages and occurrence of aberrant cells at 1000x magnification using a Nikon Eclipse (E400) light microscope. The mitotic index was calculated as the number of dividing cells per 1000 observed cells (Fiskesjo, 1985; 1997). The frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored at each concentration of each the wastewater.

For the evaluation of root growth inhibition, root lengths of 20 roots of ten onion bulbs were removed with a forceps after 96 h and measured using a meter rule. From the weighted averages for each sample and the control, the percentile root growth inhibition in relation to the negative control and EC_{50} value was interpolated from a plot of root lengths as percent of control against the log concentrations for the wastewater. Photographs of morphological changes induced by the wastewater on the *A. cepa* root tips were taken.

STATISTICAL ANALYSIS

Data were analysed using SPSS 15® software. Statistical significance of the differences in mean±SE values between treated and control group, were determined with the Student's t-test at $p<0.05$ level.

RESULTS

The result of the physicochemical analysis of process water is presented in Table 1.

Table 1: Physicochemical Properties of Process Water

Parameter	Process water (Mean± S.E.)	NESREA (2009) Limit	USEPA (2009) Limit
pH	7.72±0.12	6-9	6.5.-8.5
Total hardness	8.12±1.46	-	0-75
Total Dissolved Solids	0.05±0.01	500	500
Conductivity	2793.33±20.46	-	-
Alkalinity	0.05±0.01	150	20
Ammonia	0.33±0.18	1	0.03
Sulphates	88.50±6.93	250	250
Nitrates	0.06±0.03	10	10
Phosphates	90.67±14.5	2	-
Potassium	123.97±12.1	-	-
Sodium	105.53±8.69	-	-
Calcium	82.37±5.55	-	-
Magnesium	00.00±0.00	-	-
Chloride	8.57±4.28	250	250
Iron	4.47±0.41	-	0.3
Lead	0.13±0.03	0.05	0.02
Copper	0.20±0.12	0.5	1.3
Zinc	0.00±0.00	-	0.12
Cadmium	0.00±0.00	0.02	0.002
Manganese	0.33±0.18	0.2	0.05
Aluminum	0.00±0.00	-	-
Chromium	0.17±0.09	0.05	0.1
Nickel	0.10±0.06	0.05	0.005

All values are expressed in mg/l except conductivity (µS/cm) and pH (no units).

The wastewater had an unpleasant odour and slightly alkaline with pH 7.72. The wastewater was characterized by relatively high values of conductivity (2793.33±20.46 µS/cm), phosphate (90.67±14.5 mg/l), Na (105.53±8.69 mg/l) and potassium (123.97±12.1 mg/l). The amounts of Fe, Pb, Mn and Cr in the wastewater were above permissible limits of national (NESREA) and international (USEPA) regulatory bodies for effluent discharge. Cd and Al were not detected in the process water sample. The exposure of *A. cepa* roots to the wastewater did not cause any change in colour of roots at any of the concentrations, however, there was concentration-dependent root growth inhibition. The onion bulbs exposed to process water showed greater root inhibition compared to the negative control. For example, the mean root length of *A. cepa* grown in process water at 0.5% and 10% were 4.30±0.20 cm and 2.50±0.11 cm respectively compared to the control 5.01±0.45 cm (Figure 1).

Results of the microscopic evaluation are presented in Table 2. Process water induced a concentration-dependant, significant ($p<0.05$) decrease in the mitotic index (MI) as concentration increased. It also induced chromosomal aberrations in root tips of onions at all tested concentrations compared to the negative control. The most frequent aberrations were bridges, sticky chromosomes, vagrants and C- mitosis (Plate 1).

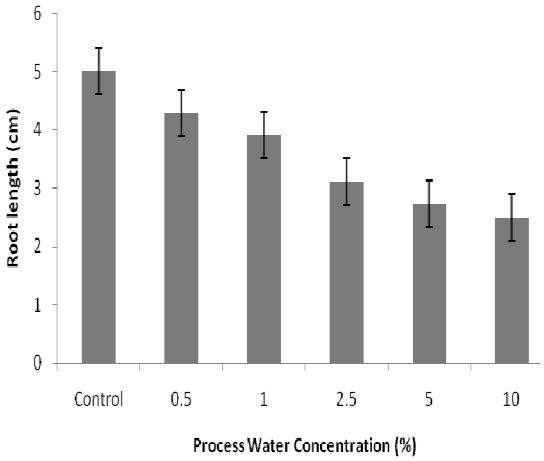
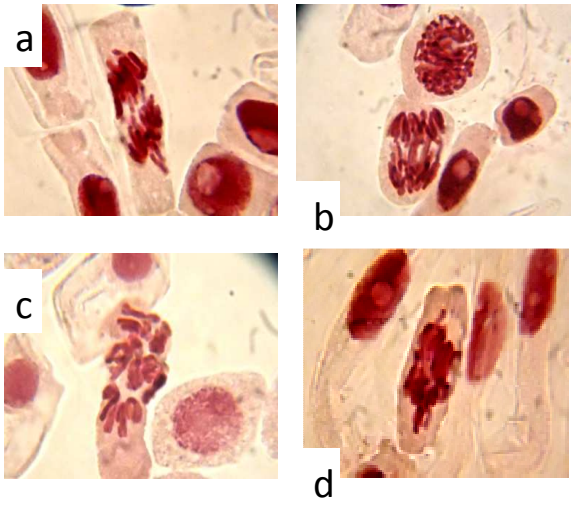


Figure 1: Root length (cm) of *A. cepa* roots exposed to different concentrations of process water

Table 2: Cytological Effects of Process Water on Cells of *Allium cepa*

Conc. (%)	No of dividing cells	Mitotic index (Mean ±SE)	Mitotic inhibition (Mean ±SE)	Aberrant cells (%)
0	124	12.4±0.43	-	-
0.5	95	9.45±0.21*	23.8	5.1
1	72	7.21±0.26*	41.7	7.4
2.5	56	5.64±0.21*	54.5	11.1
5	36	3.53±0.02*	65.1	12.3
10	15	1.51±0.01*	87.8	13.7

*Significant difference between control and treatment ($p<0.05$)



P**late 1:** Aberrations observed in *Allium cepa* root tips cells exposed to process water. (**a**) vagrant chromosome (**b**) multiple bridges (**c)** C- mitosis (**d)** sticky chromosome

DISCUSSION

One of the aims of monitoring wastewater for toxicity is to identify sources of pollution and contamination in the ambient spheres of biotic organisms and suggest possible measures of abatement to reduce levels of toxicity of the wastewater.

In the *A. cepa* test, there is usually a relationship between root growth retardation, mitotic indices (cytotoxicity) and chromosomal damage (genotoxicity). Whenever chromosome aberrations occur, there were almost always certain growth restriction and reduction in the number of dividing cells (i.e. mitotic indices). The mitotic index is considered to be reliable in identifying the presence of cytotoxic pollutants in the environment (Smaka-kinel, *et al.*, 1996; Grover and Kaur, 1999; Chandral and Kulshrestha, 2004). The concentration-dependent decrease in mitotic index and increase in the number of aberrant cells of processed water in this study is an indication of toxicity. This is in agreement with observations in earlier related studies (El-shahaby *et al*., 2003; Olorunfemi *et al.* 2011; 2012). It can be inferred that the inhibitory effects of the tested water sample on root growth and cell proliferation in *A. cepa* could have been by inhibition of DNA synthesis at S-phase (Sudhakar, *et al.,* 2001; Glinska *et al.,* 2007), complete destruction of metabolic activities that prevented the cell from entering mitosis (Metin and Burun, 2010) or disturbances of cell cycle or chromatin materials (Glinska *et al*., 2007).

Chromosomal aberrations provide a sensitive endpoint for assessing the genotoxicity of chemicals (Topashka-Ancheva *et al.*, 2003). The most frequent abnormalities in this study were stickiness, vagrants, C-mitosis and multiple bridges which resulted from chromosome and/or chromatid breaks in the wastewater. Stickiness of chromosomes may be due to increase chromosome contraction and condensation or DNA depolymerization (Ahmed and Grant, 1972; Klasterska *et al*., 1976) and nucleoproteins dissolution (Kaufman, 1958). Stickiness is considered a common sign of toxic effect of pollutants on chromosomes probably leading to cell death (Fiskesjo, 1997). Metaphase bridges are probably formed during breakage and fusion of chromosomes and chromatids (Halıem, 1990), suggesting that the constituents of processed water has clastogenic effect on the genetic materials of the exposed *A. cepa* (Leme and Marin-Morales, 2009). Vagrants arise as a result of irregular separation and dislocation of chromosomes: thereby constituting a risk of aneuploidy (Maluszynska and Juchimiuk, 2005). C-mitosis is one of the consequences of inactivation of spindle apparatus connected with delay in the division of centromere (Mann, 1977)

Trace elements and other chemical pollutants acting singly or in their combined states have been implicated in induction of genetic abnormalities in biological systems (Godet *et al*., 1993; Chauhan *et al*., 1999; Kong and Ma, 1999; Babatunde and Bakare, 2006). Lead has been known to cause reduction in root growth and frequency of mitotic cells in the meristematic zone of onions. It also induced chromosome damage and disturbance of mitotic processes in onions (Lerda, 1992). Results obtained from the physicochemical analysis of process water in this study showed that it contained Pb, Mn, Fe, Cr and Ni in amounts above national (NESREA) and international (USEPA) limits for effluent discharge. Sticky chromosomes have been reported in *Allium* roots after treatment with various heavy metals such as Hg, Ni and Cu (Fiskesjo, 1993, 1997). In a study conducted by Shahın *et al.* (1991) they attributed the induction of C-mitosis to chemicals in the tested sample. In a recent study, Olorunfemi *et al.* (2013), attributed the presence of Cr, Fe, Mn, Zn and other inorganic compounds in borehole water supplied to hostels in a tertiary institution to the growth inhibition, low mitotic indices and chromosome abnormalities induced in *A. cepa* root meristems. The presence of these trace elements could probably account for the poor root growths, low number of dividing cells and the

chromosomal abnormalities induced in the Allium roots exposed to the wastewater.

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