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GENOTOXICITY OF PETROLEUM REFINERY WASTE WATER IN NIGERIA.

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ABSTRACT

The genotoxicity of petroleum refinery wastewater was investigated with the *Allium* test. 10 medium – sized *Allium cepa* L. bulbs of the pink variety were induced to sprout roots in distilled water and variously assaulted for 48 hours with different concentrations (v/v) of wastewater from the refinery. Serial dilutions of 20%, 40%. 60%, 80% and 100% were used while distilled water served as control. After the assault, roots were harvested from each treatment sample and assayed for genotoxic effects with the acetocarmine squash technique. Results showed that the wastewater induced statistically highly significant ($P \leq 0.001$) mitodepressive effects which were dose-dependent, culminating in total mitotic inhibition at 100% v/v concentration. Other aberrations including stickiness of chromosomes, erosion of chromatin, vagrant chromosomes, fragments and anaphase bridges were induced at higher concentrations (60%-80%) of wastewater. Lower concentrations (20%-40%) induced c-mitosis as the major aberration. The advantages of genotoxicity screening over general toxicity testing in environmental monitoring was highlighted.

KEYWORDS: *Allium* test, pollution, genotoxicity, chromosome, wastewater.

INTRODUCTION

The *Allium* test is one out of a battery of tests used in monitoring environmentally hazardous chemicals. A plethora of chemical species that impinge on biota have been assayed with this test with useful results. Such chemicals like: adriamycin, calcium salts, industrial effluents, refined petroleum products, used crankcase oil etc. have been reported to impact toxic and genotoxic effects on biota using the *Allium* test (Mercykutty and Stephen, 1980; Somashaker *et al.*, 1984; Fiskejo, 1988; Sinha *et al.*, 1989; Bellani *et al*, 1991; Rank *et al*, 1993; Odeigah *et al.*, 1997; Obute and Ekwoaba, 1999; Bakare *et al*, 2000).

Oil exploration and exploitation activities no doubt release toxic substances into the environment (Boesch *et al*, 1974) therefore, environmentally friendly companies pretreat their waste effluents before release. The efficacy of such pretreatment in Nigeria is doubtful because issues of compliance with internationally accepted standards are either at their rudimentary stages or entirely non-existent. It has been reported that refined fractions of petroleum products are toxic to biota at acute and chronic levels (Oladimeji and Onwumere, 1986; Cerniglia, 1992; Allen *et al.*, 1999; Obute and Ekwoaba, 1999). If the refined products can generate toxic and / or genotoxic consequences, it is expected that the wastewater of the refining process should be potentially toxic/genotoxic to biota as well.

This scenario has prompted environmental regulatory bodies to issue guidelines stipulating safe limits of chemicals permissible in effluents from such industries. For the refinery industry in Nigeria, the Federal Environmental Protection Agency (FEPA, 1991) has specified the permissible limits of constituents of refinery effluents.

It has, however, been observed that wastewater from the PHRC have levels that exceed the FEPA guidelines and as such the need to investigate the potential genotoxic hazards on biota on the route of refinery wastewater discharge. It is hoped that these findings would assist in formulation of guidelines for management and disposal of wastewater from the refinery.

MATERIALS AND METHODS

Bulbs of common onions (*Allium cepa L).* of the pink variety were purchased from the local markets in Port Harcourt city. Equal-sized ones (about 2cm in diameter) were carefully sorted out and used for the study. Wastewater sample was collected from the Refinery in a plastic container and stored in the refrigerator at 4^0C prior to use. At the onset of the experiment, a portion of the sample was decanted and equilibrated to room temperature (26 ± 2^0C) and serially diluted with distilled water to produce 20%, 40%, 60% and 80% (v/v) treatment samples. An undiluted aliquot and fresh tap water served as controls.

Assay procedure

The standard assay technique described in INVITOX protocol 8 was used to assay the test material. 12 healthy bulbs per treatment condition were cleaned by removing their scale leaves and dry crop of roots with a sharp razor blade, leaving a crop of root primordia. The bulbs were placed in fresh tap water and allowed to sprout roots 1 cm in length. 10 bulbs out of each batch with robust crops of roots were selected and assaulted with the various concentrations of wastewater. After 48 hours the roots were harvested for microscopic

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Table 1: Mitotic indices and frequencies of chromosomal aberrations induced by various concentrations of refinery wastewater.

Concentration (%)	M. I. (%)	Erosion (%)	Stickiness	C-mitosis	Fragments / Laggards (%)	Bridges (%)
Control	12.03	0.00	0.02	0.00	000	0.07
20	4.92	0.00	0.00	11.03	0.00	1.94
40	4.00	0.00	0.00	7.46	0.05	2.00
60	3.80	4.67	2.33	3.12	2.23	0.00
80	2.90	2.48	3.75	1.13	0.00	0.00
100	0.00	5.74	4.11	0.05	0.00	0.00

examination. Such roots were fixed directly in freshly prepared 1:3 glacial acetic acid: ethanol for at least 12 hours and stored in 70% ethanol in the refrigerator until needed for slide preparations. The root samples were then hydrolyzed in 18% HCl for 5-10 minutes and squashed in clean glass slides with the acetocarmine squash technique. Cells undergoing mitosis were scored and mitotic indices were determined by examining 400 cells per 1000 cells.

Gentoxic effects were characterized and scored in 100 cells per slide and 5 slides per treatment according to the O'Hare and Atterwill (1995) protocol. A Leitz Laborlux – 12 microscope fitted with photographic equipment was used for observations and photomicrography.

RESULTS

There was rapid decrease of mitotic index with increase in wastewater concentration as summarized in Table. 1 All the treatments but the control revealed this effect because it was in the control, only that up to 500 mitotic cells were encountered while others consistently showed less than 500 cells. Whereas most cells in the 80% treatment were in the interphase all the cells from the 100% showed none in the mitotic phase.

Chi-square tests showed that there was high significance ($P \leq 0.001$) in the chromosome aberrations the wastewater generated. At lower concentrations (20-40%) c-mitosis was the most common while stickiness, erosion of chromatin, anaphase bridges, vagrants and fragments were observed at higher wastewater concentrations (60-100%;

The frequency of these aberrations were rather not dose-dependent as some, like erosion, was observed at 60% concentration while it was less at 80% concentration (Table 1). More than one aberration were observed in the same wastewater concentration at the higher values. Erosion of chromatin for instance, c-mitosis and stickiness were mostly observed at the 60-100% concentration ranges.

DISCUSSION

Environmental monitoring requires rapid assays with higher predictive value to avoid arriving at false

negatives after tedious protocols. The *Allium* test not only meets these requirements but also is highly sensitive and specific (Ennerver, *et al.,* 1988) in detecting assaults to chromosome integrity of biota. Odeigah *et al.,* (1997) highlighted its suitability for screening genotoxic actions of wastewater and how its results co-related with other test systems as well as with the human system.

Since a dose-dependent mitodepressive effect was observed here (Table 1), it is an indication of the hazard posed to biota by the continued discharge of the wastewater in the present form. At 100% of wastewater mitosis stopped altogether. Perhaps the fact that some constituents of this wastewater outstripped the FEPA recommended limits premises this result Furthermore, statistically highly significant chromosomes aberrations were induced by the wastewater. Probably stickiness, erosion of chromatin and occurrence of fragments were cytotoxic effects that led to absence of mitosis at 100% concentration. Fiskejo (1985, 1999) reported that sticky chromosomes lead to cell death.

On the other hand, lower concentrations yielded mostly c-mitosis indicative of weak toxic effects (Fiskejo, 1988; Odeigah *et al.,* 1997; Obute and Ekwoaba, 1999). It is possible that inherent natural mechanisms that correct assaults on chromosome integrity operate to effect recovery of cells in c-mitosis to produce reversals at lower concentrations. Wang *et al* (1997) reported inhibitory effects of high concentrations of chemicals on mitosis. The absence of mitosis in the 100% concentration of wastewater may be an indication of genotoxicity equating to toxicity. However, as several workers agree genotoxic screenings are more sensitive than toxicity tests (Rank *et al,* 1993; Odeigah and Osayinpeju, 1995; Rand and Nielsen, 1994; Obute and Ekwoaba, 1999; Bakare *et al.,* 2000). This is because the conceptualization of toxicity tests to detect levels of pollutants that would be lethal to 50% of the population (EC_{50}) glosses over the sublethal concentrations of such chemicals. At such low concentration levels, chromosomes are damaged irreversibly a times before it manifests as toxic consequences.

The evidence available in this work is an early warning signal that though wastewater treatment before release is practised, there is an urgent need to ensure that such treatments are effective. Refinery wastewater apparently induces genotoxicity in biota in its present form of discharge into the environment.

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