Genotoxicity of oil field wastewater in Nigeria

P. G. C. ODEIGAH, O. NURUDEEN and O. O. AMUND

Department of Biological Sciences, University of Lagos, Akoka, Lagos, Nigeria

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The general toxicity (root growth inhibition and malformation) and genotoxicity (induction of chromosome aberrations in root cells) of an oil field wastewater have been investigated by the Allium test. A series of 10 small bulbs of *Allium cepa* L. were cultivated in various concentrations of the wastewater, and after 48 h one root tip from each bulb was harvested and processed for cytological studies by the aceto-orcein squash technique. After 96 h, mean lengths of root bundles were obtained and the Effect Concentration (EC) values calculated. Treatment with wastewater resulted in significant dose-dependent root growth inhibition. EC_{50} (96 h) was 28.5 % while a total phytotoxic effect was induced by the undiluted sample. The wastewater is mitodepressive and increased significantly the frequency of chromosome aberrations in root cells (sticky chromosomes, c-mitosis, spindle multipolarity, bridges and fragments). At lower concentrations c-mitosis was the most common aberration. The suitability of the Allium test in genotoxicity screening is highlighted and the impact and significance of positive results on the environment and human health are discussed.

P. G. C. Odeigah, Department of Biological Sciences, University of Lagos, Akoka, Lagos, Nigeria

The discovery in Nigeria of petroleum and associated products certainly means very great benefits to the country; however, the exploration, production, refining and transportation of petroleum and petrochemical products have led to the hazardous deposition of hydrocarbons and heavy metals in the environment.

Of the major economic sectors, the ones most vulnerable to environmental pollution or degradation are agriculture and fisheries, mainly because they are directly dependent on natural systems and resources. Environmental degradation along with subsequent agronomic constraints is slowing down the growth in the world's food output. While dealing with environmental problems, studies on the biological effects of contaminants should form an integral complement to chemical analysis for potentially toxic components.

The operations involved in the production of crude oil generate waste streams that may discharge into the environment. The major waste stream, in terms of volume, during oil production, is "produced water", which is the technical term for water coproduced with the oil from the reservoir and separated by physical means before being discharged from production platforms and storage terminals. Studies world-wide have shown that produced water effluents have a relatively high biological oxygen demand (BOD) and Chemical oxygen demand (COD) largely derived from compounds such as fatty acids (MIDDLEDITCH 1984; WATKINSON and HOLT 1986). The concentration of total dissolved solids (salinity) in produced water ranges from less than 3 to about 300 g/l (RITTENHOUSE et al. 1969), and produced water thus contains more saline than normal sea water. Several potentially toxic metals, including arsenic, barium, copper, iron, lead, manganese, strontium, and zinc, have likewise been reported in produced water (COLLINS 1975; MIDDLEDITCH 1984). Small amounts of radionuclides are found primarily in the form of radium (^{226}Ra and ^{228}Ra), apparently derived from the normal concentrations of uranium and thorium associated with clay minerals and quartz sands that make up the matrix of a hydrocarbon/water reservoir.

Despite the potential toxicity of produced water effluents, there is a dearth of information on their actual effects on exposed biota. Toxicity bioassays have tested the effects of Brent produced water with oyster larvae, water flea (*Daphnia magna*), and shrimp. *Salmo gairdneri* was reported by SOMERVILLE et al. (1987) and the results indicated that Brent production water was only toxic at dilutions of less than 20-fold, thereby implying that the toxicity of produced water could be reduced by the dilution effect of sea water in offshore situations. However, the disposal of such effluents on land during onshore operations could pose a threat to exposed plant communities.

The effects of excess salt and high salinity on wetland plants are well documented. High salinity affects plant growth either osmotically, by direct toxicity or by creating nutrient imbalance (PARRONDO et al. 1978). According to PEZESHKI et al. (1987a), a common grass in freshwater marshes, *Panicum hemitomen*, for example, will die within four days after exposure to 10 parts per thousand (ppt) salinity, which is roughly one third the salinity of seawater (35ppt). PEZESHKI et al. (1986), also found that bald cypress (*Taxodium distichum*) seedlings can tolerate and recover from short-term exposure to salinity levels less than 3 ppt. Above that level, they cannot acclimate and ultimately die from reduced photosynthesis and metabolic stress. Even the slightest increase in salinity causes leaf injury and root damage to bald cypress seedlings (PEZESHKI et al. 1987b). In *Allium* unrestricted growth may occur only up to a salinity of 0.3 % (5×10^{-2}M; FISKESJÖ 1985a). In studies designed to simulate salt stress resulting from exposure to seawater and brine discharges associated with oil and gas operations, PEZESHKI et al. (1987a), found that photosynthetic rates in *Panicum hemitomen* declined between 20 % and 67 % within one day of saltwater exposure. Gas exchange rates were reduced between 55 % and 80 % within one day. Reduction in primary productivity of marsh plants by increased salinity will therefore affect carbon cycling and bring about reduction in the vertical accumulation of organic matter, directly resulting in wetland deterioration and habitat change.

Although the physiological effects of produced water discharges on plants are understood, there is practically no report on the genotoxicity of oil field discharges in Nigeria. Such studies are, however, necessary to determine the genetic basis of root damage and growth inhibition in exposed plant communities, as well as providing baseline data that are vital for the formulation of guidelines for pollution control with regard to wastewater discharges. We report herein the results of an investigation into the effects of a produced water sample on onion (*Allium cepa*) root meristem. The studies were designed to identify the chromosomal aberrations in treated meristematic cells and evaluate root growth inhibition of treated specimens.

MATERIALS AND METHODS

Test material

Equal-sized bulbs of the purple variety of the common onion (*Allium cepa*) purchased from a local market in Lagos, Nigeria, were our test materials.

Test effluent

A produced water effluent sample was obtained from the "North Apoi" production of Texaco Overseas Petroleum Company of Nigeria Limited, offshore the Niger Delta Coastline of Nigeria. The effluent was collected in a plastic container and stored in the refrigerator at 4°C pending use. Before each test was carried out, the effluent was equilibrated to room temperature (26 ± 2°C) and diluted with distilled water to produce the series of dilutions investigated.

Assay procedure

The method used was that described in INVITTOX protocol No. 8. The outer scales and the brownish bottom plates of healthy onion bulbs, 15–22 mm in size, were carefully removed leaving a ring of fresh root primordia. A series of 12 cleaned onion bulbs were then placed on top of containers each filled with 10 %, 20 %, 40 %, 60 %, 80 %, and undiluted aliquot of the wastewater and fresh tap water of good quality for controls. After the first day, the best 10 (in terms of root growth) were selected for continuation of the assay. The solutions were changed daily and after 48 h, one root tip from each bulb was harvested, fixed, and macerated in a solution of 9 parts 45 % acetic acid and 1 part 1N HCl at 50°C for 5 min. These were subsequently processed for cytological study by the conventional aceto-orcein squash technique. The mitotic index (M.I.) was determined by examination of 400 cells per slide and calculated as mitotic cells per 1000 cells. Characterization of mitosis and chromosome aberrations were scored in 100 cells per slide. All slides were coded and examined blind. Statistical analysis was performed using the χ^2-test for the chromosome aberrations. After 96 h, mean lengths of root bundles were obtained as described by FISKESJÖ (1985a) and the EC_{50} values and 95 % confidence interval (C.I.) were determined from a plot of root length, % of control against the sample concentrations using a Microsoft Excel computer programme.

RESULTS

Macroscopic effects

The results of the root lengths and morphology, parameters used in testing for general toxicity, are presented in Fig. 1, 2 (a, b and c) and Table 1.

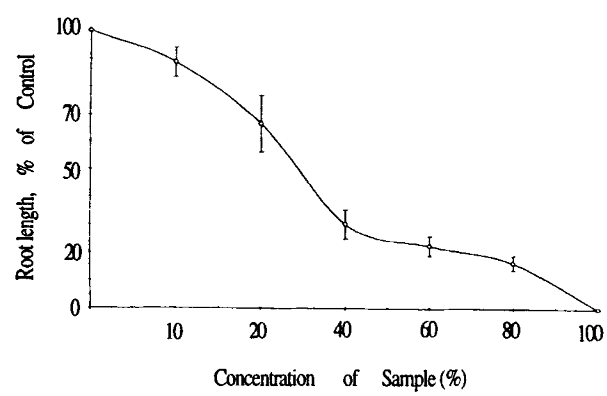

Fig. 1. Growth curve of Allium roots (in relation to control) after treatment with oil field wastewater (the curve, plotted by a computer, is based on mean values with the 95% confidence intervals shown as error bars).

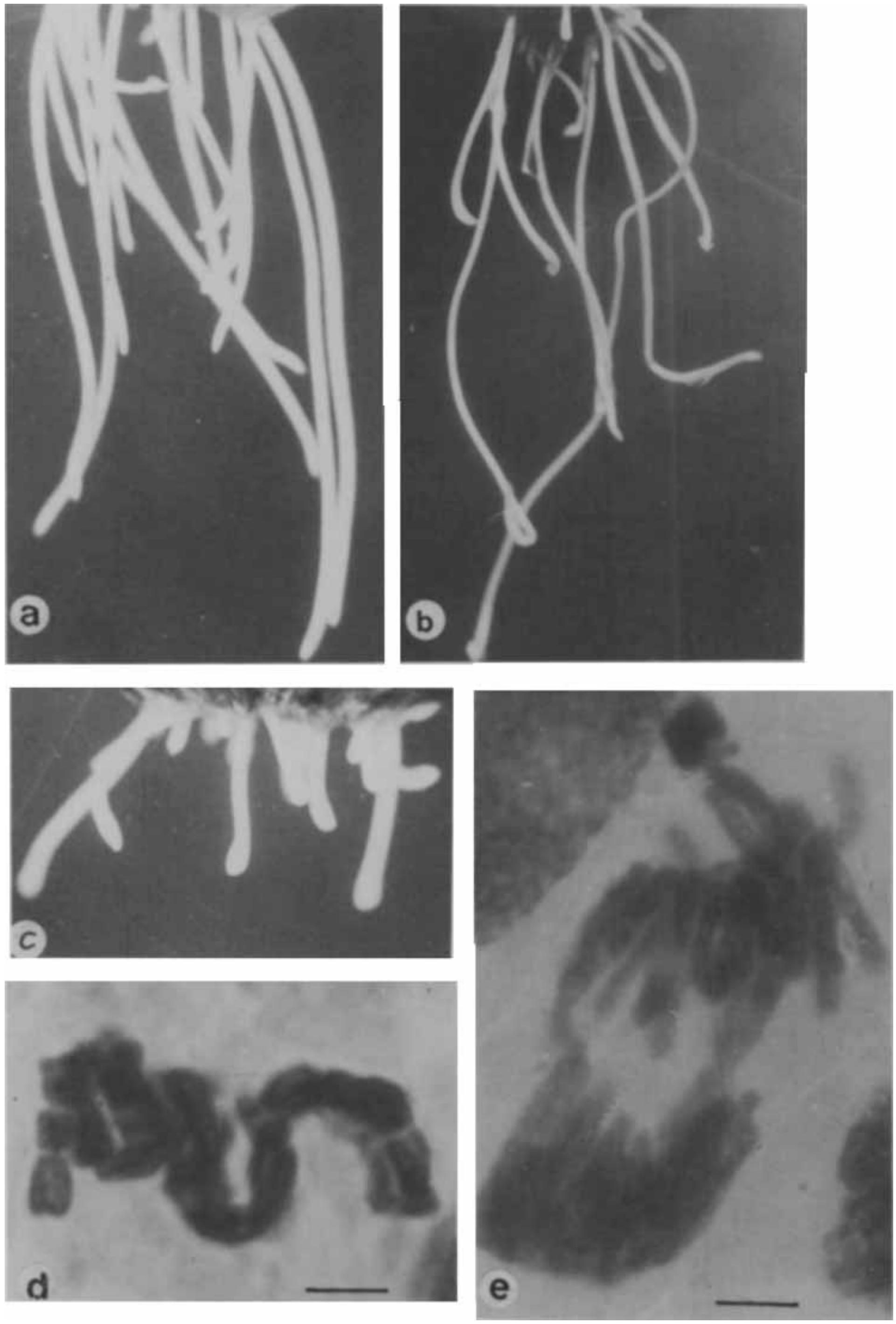

Fig. 2a–e. Examples of macroscopic and microscopic effects on Allium roots exposed to oil field wastewater. **a** Normal roots of one control bulb. **b** Crochet hooks (10 % concentration). **c** C-tumour (20 % concentration). **d** Sticky metaphase (60 % concentration). **e** Sticky anaphase with bridge and fragments (40 % concentration). Scale = 10 μm.

Strong growth retardation was observed in onion roots growing in high concentrations of the wastewater. The effects were less severe at low concentrations. At 10 % concentration, there was only about 11 % growth retardation in relation to root lengths in the control. The root growth retardation or inhibition is concentration-dependent with an EC_{50} value of 28.5 %, while a total phytotoxic effect (complete inhibition of root growth) was induced by the undiluted wastewater. Roots grown in the wastewater were also characterized by the presence of malformations. The presence of twists, root tips bent upwards resembling

Table 1. *Effects of treatments with different concentrations of the oil field wastewater*

Treatment Conc. (%)	Phenotypic indices			Chromosome aberrations[b]					Total ±SD
	Root length (% of control) 95% C.I.[c]	M.I.[a] ± SD	No. of cells	Stickiness	C-mitosis	Vagrant	Multipolar anaphases	Bridges fragments	
Control	100	48.4 ± 4.6	500	0	3	1	0	3	1.4 ± 0.2
10	88.7 (5.4)	34.1 ± 5.6	489	1	21	3	1	4	6.1 ± 0.4*
20	66.5 (10.4)	27.3 ± 7.3	471	6	28	4	0	5	9.1 ± 0.6*
40	30.1 (5.3)	20.6 ± 8.1	450	44	7	2	4	5	13.8 ± 1.2*
60	22.4 (3.5)	14.7 ± 4.5	410	38	2	1	1	1	10.5 ± 0.8*
80	16.3 (2.6)	0							
100 (undiluted)	0								

[a] The mitotic index was calculated as mitotic cells per 1000 cells (400 from each slide)
[b] Chromosome aberrations were scored on 500 cells/slide
[c] Confidence interval (see Assay procedure under Materials and methods!)
* $P < 0.001$ (χ^2-test)

hooks ('crochet hooks', Fig. 2b) and c-tumours (abnormalities appearing as swellings of the root tips, 2c) were observed especially at low concentrations of the wastewater.

Microscopic effects

The results of the microscopic effects are summarized in Table 1. There was a rapid decrease in the mitotic index with increasing concentration of the wastewater and the mitotic index was positively correlated to the root lengths, that is, decreasing with increasing concentration of the wastewater. Thus the mitotic index could be another endpoint for general toxicity. In no treatment was it possible to obtain as many as 500 mitoses for cytological screening, as was regularly the case in the controls. Onion bulbs cultivated in 80 % wastewater showed some root growth but had no mitoses in their root cells, only interphase stages. Analysis of the chromosomes showed wastewater-induced chromosome aberrations in the root cells at statistically significant levels ($p < 0.001$). The aberrations observed were sticky chromosomes, c-mitoses (c-metaphase and c-anaphase), vagrant chromosomes, multipolar anaphases and anaphase-telophase bridges with or without acentric fragments (Fig. 2d, e and Fig. 3). At lower concentrations (10 and 20 %), c-mitosis was the most common aberration observed, often with an incidence approaching 80 %. Some of the c-metaphases often observed at lower concentrations had fairly elongated centromeres (Fig. 3e) compared with normal metaphase. At higher concentrations (40 and 60 %), sticky chromosomes were the typical aberration observed (Table 1).

DISCUSSION

One of the aims of screening wastewater for toxicity is to identify polluting industries or other sources in the sewer systems and thus to reduce the toxic waste.

In the Allium test as presented in this study, two aspects of toxicity were evaluated: (1) general toxicity estimated by the inhibition of the growth of the root bundles, and (2) genotoxicity assessed by microscopic studies of the chromosomes of meristematic root cells. Thus the plant root is very useful in this testing because the root tips are often the first to be exposed to chemicals in the soil and water. Macroscopic and microscopic observations of the root tip system constitute a rapid and sensitive method of environmental monitoring (Fiskesjö 1988a). The relative values (% of control) rather than the exact values of the root lengths were considered most suitable for the demonstration of deleterious effects in environmental monitoring, since this procedure is considered to reveal other sources of damage, such as earlier treatments of the onions with herbicides during cultivation.

The growth curve of the onion roots grown in concentrations of the wastewater had generally a sigmoid shape, indicating a positive dose-response effect. There was no root growth at all in onion bulbs treated with the neat (undiluted) sample, while at 10 % concentration there was 88.7 % root growth relative to control, and the EC_{50} (96 h) was 28.5 % root growth relative to control. This clearly shows that the toxic compounds in the waste can be reduced by water leaching or dilution. In addition to root growth inhibition, wastewater induced 'crochet hooks' and c-tumours, malformations which have

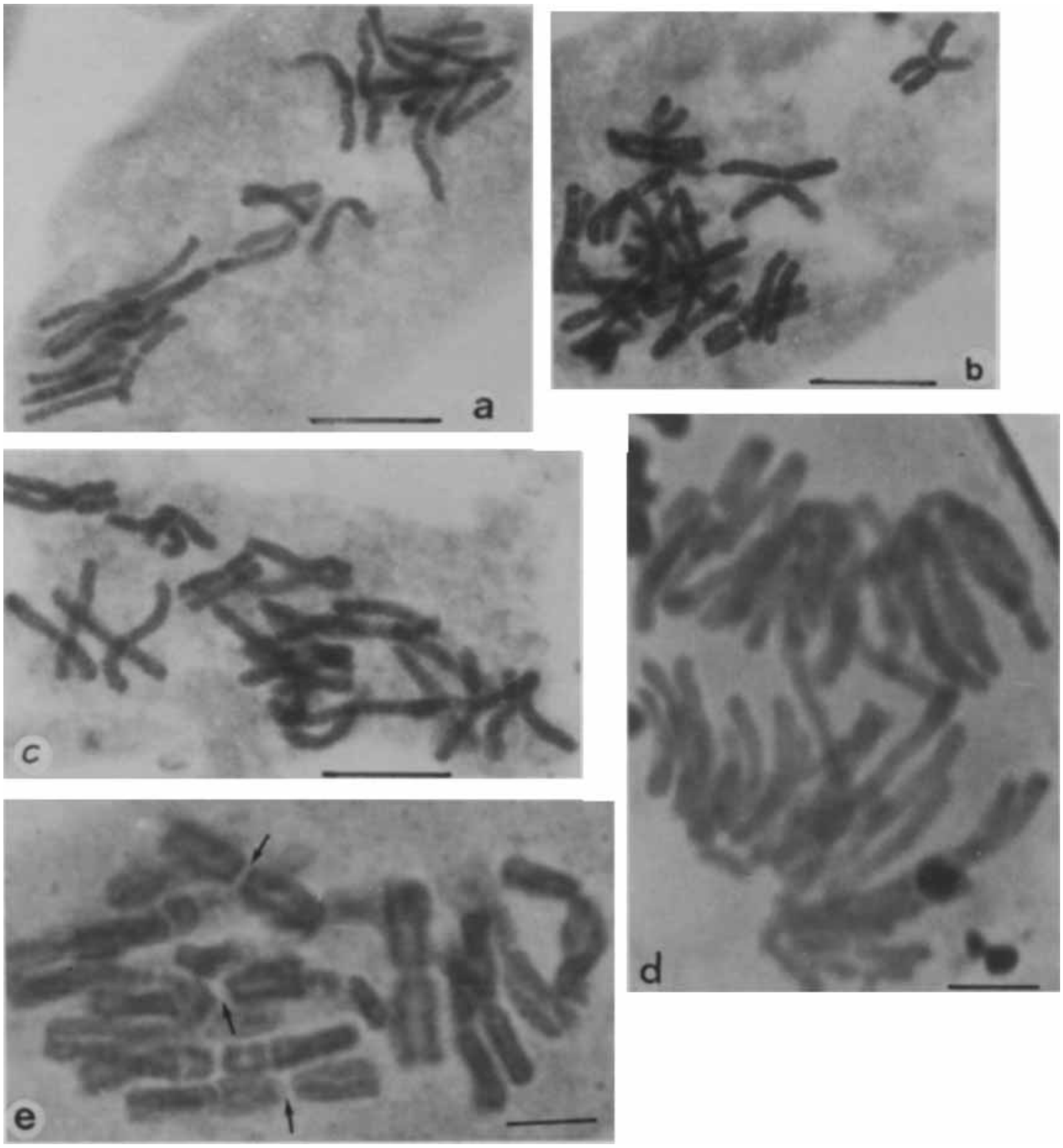

Fig. 3a–d. Mitotic aberrations caused by the oil field wastewater. **a** C-anaphase with laggards (20 % concentration). **b** Vagrant chromosome (10 % concentration). **c** C-metaphase. **d** Multipolar anaphase with bridges and fragments (40 % concentration). **e** C-metaphase chromosomes, three instances (arrowed) with elongated centromeres. Scale = 10 μm.

been shown by other studies (FISKESJÖ 1988a) to be useful signs of toxicity. The crochet hook reaction was first described by EHRENBERG et al. (1949) and it has been suggested that this effect may be a typical heavy metal effect. C-tumour effects are caused by several types of compounds, such as colchicine (LEVAN 1938), organic mercury compounds (FISKESJÖ 1969), and after treatment with growth-stimulating compounds such as naphthaleneacetic acid and indolylbutyric acid (LEVAN 1939), 2-4-D, and 2-4-5-T (CROCKER 1953).

The wastewater was mitodepressive and this effect was dose-dependent except at very high concentrations (80 % and the undiluted sample), where there were no mitoses due to the complete phytotoxic effects. Also the wastewater induced statistically significant chromosome aberrations. Thus, there was good correlation between the macroscopic and microscopic parameters. However, the types of chromosome aberrations varied somewhat with the concentration of the sample. At lower concentrations, c-mitosis (and, to some extent, vagrant chromosomes) was the most common aberration, whereas at higher concentrations sticky chromosomes were the most common. Similar observations have been made by other workers for other environmental chemicals, where c-mitosis is regarded as indicative of a weak toxic effect which may be reversible, vagrant chromosomes, a weak c-mitotic effect indicating risk of aneuploidy, while sticky chromosomes indicate a highly toxic, irreversible effect, probably leading to cell death (FISKESJÖ 1985a, 1988a). This probably accounts for why there were no mitoses at 80 % concentration and no root growth in the undiluted sample. Some of the c-mitoses observed at lower concentrations of the wastewater contained metaphase chromosomes with slightly elongated centromeres. Elongated centromeres have been observed earlier in various plant materials after oxiquinoline treatment (TJIO and LEVAN 1950) and also in *Allium cepa* root cells cultivated for two days at various distances from video display units for computer or TV set (FISKESJÖ 1988b).

There are several workers investigating the biological effects of low frequency electromagnetic field. Produced water or oil field wastewater samples are known to contain small amounts of radionuclides. To decide whether there is a link between the presence of radionuclides and the elongated centromeres, would require further study.

There was no simple positive relationship between frequency of chromosome aberrations and concentration of the waste water. For example, the frequency of chromosome aberrations at 40 % of the sample was higher than that at 60 % concentration of the sample. A possible explanation for this, is that with increasing concentration and consequent toxicity, there may be an inhibitory effect of the wastewater on cell division and consequent hindrance of the passage of affected cells into the mitotic cycle.

The Allium test has been used in the screening of other types of industrial waste and wastewater. PANDA et al. (1989) have reported the toxic effects of solid waste deposits of a chloroalkali plant, while FISKESJÖ (1985b) has reported the usefulness of the Allium test for monitoring the toxic and genetic effects of contamination of natural waters. Also RANK et al. (1993) have reported the genotoxicity of residues of a herbicide and its active ingredients. The genotoxicity of petroleum waste and other industrial and domestic waste or effluents have been demonstrated in other eucaryotic test systems (DONNELLY et al. 1985; ENGL et al. 1988; HOUK 1992; ODEIGAH and OSANYINPEJU 1995).

RANK and NIELSEN (1994) have evaluated the suitability of the Allium test in the screening of wastewater for genotoxicity. They compared the results of 15 chemicals screened with the Allium test, Ames test, and the microscreen assay (with the lambda prophage induction as endpoint). They found that the sensitivity of the Allium test is 82 % whereas it is 73 % for Ames test and 45 % for the microscreen assay. Thus, the high sensitivity, the fact that it is cheap, rapid, easy to handle and can be used on wastewater without pretreatment of the sample are major advantages of the Allium test. The results of this study have shown that the genotoxicity of oil field wastewater can be easily detected by the Allium test.

The impact of genotoxic wastewater on the environment and the significance to human health are difficult to predict, because wastewaters are complex mixtures of chemical substances. Complete interpretation of their effects often requires, in addition, chemical analysis of the constituents. Such an analysis may indicate the components of the wastewater that can persist and accumulate in exposed biota and thus potentially pose a hazard to human health.

Nevertheless, positive results in the Allium test should be considered a signal of warning, and also an indication that the sample tested may constitute a risk to environment and to human health.

Genotoxicity is by far the most important of toxic effects induced by environmental contaminants. Even though it is a sublethal effect, it poses a greater threat to the human population and to the natural ecosystems, because it leads to a higher genetic load. And an increased genetic load is potentially capable of producing a general decline in genetic health, and an abrupt, significant increase in the genetic load of any species is a serious threat to the survival of this species.

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