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Cytotoxic Effects of Distillary Waste on *Allium cepa* L.

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Water pollution is a very serious hazard to which the common man is exposed. Industrial and domestic discharges have been recognized as one of the major sources of toxic chemicals present in the environment. Therefore, in recent years much emphasis has been given to develop simple and reliable biological methods to evaluate the degree of pollution.

The Allium test employing rooted bulbs of Allium cepa has been used as a standard short term test in environmental monitoring and as a tool for evaluation and ranking of chemical with reference to their toxicity by various agencies (Royal Swedish Academy of Science, 1973; Stichet et al., 1975). This test has frequently been used by many pollution biologist (Levan, 1938; Clarkson, 1965; Kihlman, 1966; Fiskesjo, 1981; Linn et al., 1978). It has many advantages like low cost, easy to handle, good chromosome condition to help study chromosome abnormalties during cell division, evaluation of aneuploidy and has good correlation with prokaryotic and other eukaryotic test systems. This test system comprises a rapid and sensitive method at macroscopic level by measuring growth inhibition of roots as well as at microscopic level comprising harmful qualitative and quantitative effects on cell and tissues. The microscopic level also includes the Allium micronucleus assay which has been used in studying genotoxicity and bioavailability of solid waste deposits (containing mercury) from chloralkali plant (Dash et al., 1988; Panda et al., 1989; Panda et al., 1990).

The present study was undertaken to monitor and assess cytotoxic effects of distillary waste discharged from a sugarcane factory, Kanpur, India, employing modified Allium test (Fiskesjo, 1985). Modified Allium test employs direct treatment of unrooted bulbs with test liquids instead of rooted ones. The effluent was analysed for its physico-chemical properties and its cytotoxic responses have been studied in relation to pollution hazards.

MATERIALS AND METHODS

Equal-sized bulbs were chosen from a commercial variety of Allium cepa (2n=16). The outer scales of the bulb and brownish

plate were removed. The ring of the root primordia being left intact. The peeled bulbs were put into fresh water during the cleaning procedure to protect the primordia from drying. Thereafter, the bulbs were placed directly in 0.5%, 1.0%, 1.5%, and 2.0% concentration of test liquids, a method which mimics natural conditions. Ten onion bulbs were set up in each series. Normal tap water free from toxic ions was used for the control. Experiment was performed at about 20 ± 2°C and was protected against direct sun light. Test liquids were changed everyday.

The experimental and control root tips were fixed on 2 day in ethanol: acetic acid (3 : 1, v/v) and squashed according to the conventional haematoxylin method (Darlington and La Cour, 1976) for chromosomal analysis. On 4 day the root length were measured. The length of the whole root bundle was measured outside the test tubes by a ruler.

The physico-chemical properties of the effluent were determined in accordance with the standard method (APHA AWWA 1971).

RESULTS AND DISCUSSION

Results of the analysis of the physico-chemical parameters in effluent sample is presented in Table 1. The value of some

Table 1. Physico-chemical analysis of distillary waste and tap water

Parameters*	Distillary waste	Tap water
Colour	Dark Brown	Colourless
Total Solid	2800.0	500
Dissolved solid	800.0	nil
Suspended solid	2000.0	nil
Oil and Grease	583.8	nil
pH	6.00	7.5
B.O.D.	78000.00	70
C.O.D.	1,20.000.0	100
Total Nitrogen	227.4	100.0
Chloride	155.0	500
Sulphate	31.3	250
Nitrate	0.15	0.05
Phenols (ppb)	48.0	-
Fluoride	1.14	0.3
Phosphate	1.05	.01

*All values are in ppm

parameters which impart toxic nature of effluent like COD, BOD and phenols were found at levels higher than the reccomended permissible limits (WHO, 1985). The fluoride level in the

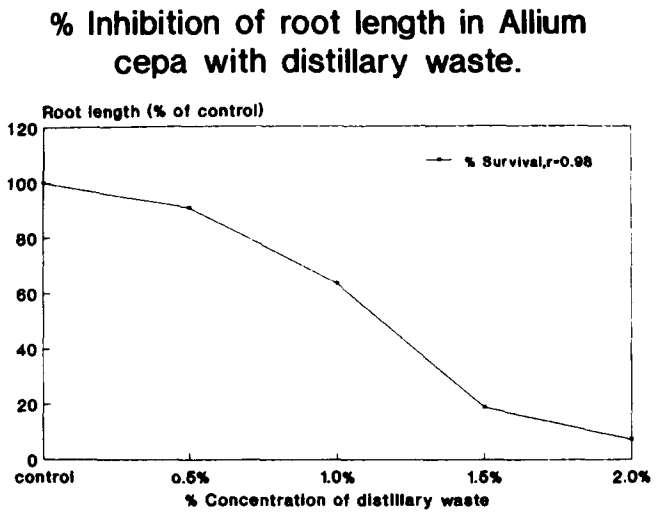

Figure 1. Percent inhibition of root length in Allium cepa with distillary waste.

effluent was also alarming. The nitrate, phosphate and fluoride content was much higher in distillary waste as compared to tap water. As evidenced by the growth curve, there was a gradual inhibition of root length with increasing effluent concentration, however, a sudden fall at concentration 1.5% was observed. A negative correlation was found between effluent concentration and root length inhibition (r=-0.98) (Fig. 1). The EC-50 of distillary waste being 1.175% indicated that it had moderate toxicity. The 1.5% and 2.0% concentration gave clear macroscopic growth retardation effect. Restriced growth response showed the toxicity of treatments.

During the experiment root tips turned brownish which may be due to the dark brown colour of effluent. Table - 2 shows the result from microscopic studies. The effluent concentrations of 0.5% and 1% were not much effective with respect to mitotic index and number of counted cells. Figure 2 (a-f) represents the cytotoxic abnormalities after effluent treatment. At 1.5% and 2.0% concentration, there occur microscopical damage effects namely the displacement in number of normal stages of mitosis. At the cytological screening during the persent experiment a very low degree of C-metaphase (Fig. 2b) was recorded with 1.5% and 2.0% concentration which were consequences of inactivation of spindle apparatus connected with delay in the division of the centromere. Stickness (Fig. 2c) was very much frequent with 1.5% concentration is a generalized type of toxic effect. The frequency of chromosomal fragment (Fig. 2e) were higher with higher concentration at anaphase giving a indication of mutation

Table 2. Cytological effects of 2 day of treatment with different concentrations of distillary waste.

Conc. (%)	Mitotic Index	Number of classified cells						
		Number of counted cells	Normal meta-phase	Normal ana-phase	C-Meta phase	Sticky chromo-somes at meta-phase	Fragments at ana-phase	Laggards at ana-phase
Control	55	440	250	190	-	-	-	-
0.5	52	429	238	184	1	5	1	-
1.0	39	378	199	170	1	6	2	-
1.5	15	120	20	46	4	42	6	2
2.0	8	62	15	22	3	15	4	3

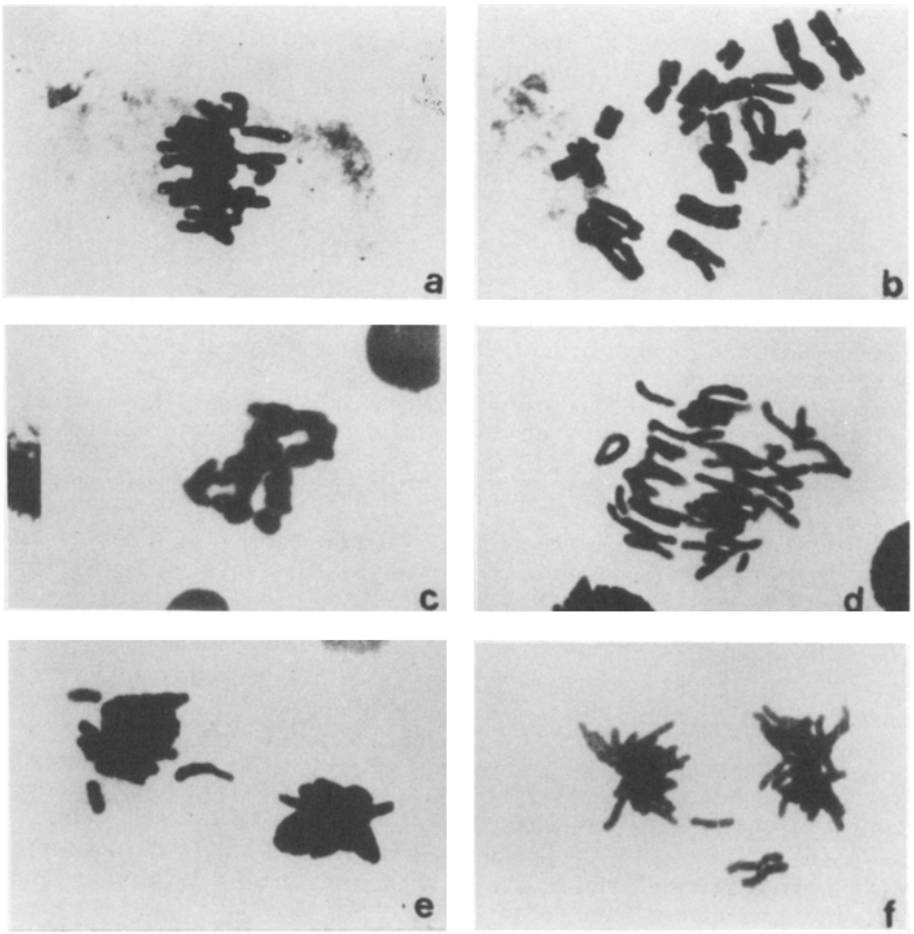

Figure 2 a-f. Microscopical parameters in the Allium test as effected by effluent treatment. a, metaphase control; b, C-metaphase; c, metaphasic chromosome stickness; d, anaphase control; e, anaphasic fragmentation; f, anaphasic laggards.

risks. Such chromosome breaks are highly correlated to mutagenic events (De Serres, 1978). Laggards were observed at anaphase (Fig. 2f) with a low frequency.

Results are indicative of a linear relationship between macroscopical and microscopical parameters. However, the effect on the macroscopic parameter seem to be more apparent. Growth restriction seems to be cumulative response of all damage effect.

Therefore, the present Allium test indicate that the distillary waste which was tested in the present experiment can be ranked to moderate toxic environmental chemical with reference to its toxicity. The cytological abnormalities presently observed indicate that the contamination of water bodies by the sugar

cane waste water may be hazardous to both flora and fauna. However, to standardized the Allium test system some more work would be required in relation to different environmental factors.

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