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## THE EVALUATION OF GENOTOXICITY OF LEACHATE OF MUNICIPAL WASTE USING ALLIUM TEST

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**ABSTRACT:** *Municipal wastes are inevitable source of environmental pollution. Leachates from these wastes might contaminate the origin of potable water and affect human health. The study was carried out to investigate the possible genotoxic effect of leachate from the municipal waste site using Allium cepa chromosome aberration assay. The physicochemical characterization of the leachate samples viz. electrical conductivity, chemical oxygen demand, nitrate, total dissolved solids, total hardness and chloride, sulphate, cadmium, zinc, copper, nickel and lead were carried out. Morphological modification of the A. cepa roots, inhibition of root growth, cell division and chromosomal aberration were observed as compared to the control. The results confirm that leachate is a genotoxic agent in plant cells and imply that exposure to leachate in the aquatic environment may pose a potential genotoxic risk to organisms. The Allium anaphase –telophase test can be recommended as a monitoring system, which can serve as the first alert for the presence of genotoxic environmental pollution.*

**KEYWORDS:** *Leachate, root growth, chromosomal aberration, monitoring, Allium cepa.*

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### INTRODUCTION

The disposal of municipal solid waste in landfill is the most common disposal alternative in most countries. Landfill leachate is generated by excess rainwater and seepage water, percolating through the waste layers in landfill. There is a danger of ground water contamination by landfill leachate containing inorganic ions or organic components derived from the original refuse as organics produced by microbial decomposition. The pollution of water resources is a world wide problem (Vargus et al., 2001; Ohe et al., 2003). In addition to the direct health effects, pollutants also pose subtle dangers in that they may be mutagenic or toxic and lead to human afflictions such as cancer, cardiovascular diseases and premature aging. In 1938 the use of Allium cepa was introduced as a biological test system to evaluate the cytogenetic colchicines cells (Levan, 1938). Since then, A. cepa L. (onion) has been a biological material of wide use in the laboratory tests, due to the fast growth of its root and the response of genetic material to the presence of potential cytotoxic and genotoxic substances in the test liquids (Vesna et al., 1996). The Allium test has been applied to evaluate the quality of underground, surface waters and effluents in a simple way through the study of microscopic parameters, such as the values for root growth inhibition, cytological parameters such as aberrations of cellular metaphase and anaphase and cellular division inhibition (Vesna et al., 1996). Inhibition of root length is suitable for evaluation of substances in various concentrations. The simplicity of the Allium test procedure is the reason for its recommendation by international environmental protection agencies for estimating environmental pollution and toxicity caused industrial effluents, non-treated municipal or domestic waste water.

This work is an attempt to study the harmful compounds of leachates present in the municipal dumping site of Moraboroli, Tezpur, and its effect on the root tips of onion.

**MATERIALS AND METHODS****Study Area**

The study area chosen for this work is the municipality garbage dumping site of Morabharali, near Morabharali River, Tezpur, Assam, and India. The site is located in the ward no 12 with an area of 8 acre. Tezpur is an ancient city on the right bank of the river Brahmaputra between 97.47 degree East longitude and 26.37 degree North longitude.

**Test Material**

Commercial variety bulbs of the common onion (*Allium cepa* L.) were purchased locally. Only bulbs in good condition and of approximately 3.5 cm diameter were used.

**Leachate Sampling**

In this study, Leachate samples were collected in the first week of February 2009, from the dumping site at Morabharali in plastic bottles which were cleaned and washed with distilled water. Total three leachate samples were collected. Samples were collected and taken to the laboratory immediately to avoid any kind of change in the quality and thereafter analysis of the samples was performed as per the procedures in standard books.

**Parameter Studied**

pH, Temperature, EC, TDS, Total Alkalinity, Calcium, Magnesium, Chloride, Sulphate, Nitrate, Cadmium, Zinc, Copper, Nickle and Lead (Table1).

**TEST PROCEDURE****Planting of Onion**

The work was done during the cool monsoon season of the year. As cool monsoon season is favorable period for the growth of onions.

Apart from the raw leachate samples they were also being diluted by 20% and 40% respectively. Five onion bulbs each were immersed in each of the concentrated samples and also in the respective diluted samples upon in the small beakers. Also five onion bulbs were immersed in distilled water as control. On the 4<sup>th</sup> day when root tips became approximately half cm, they were harvested.

**ROOT LENGTH MEASUREMENT**

The root length of onion bulbs from each concentration was measured on 2, 3, and 4 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 leachate were fixed in Carnoy's Fluid (Glacial Acetic Acid and Ethanol, 1:3).

The root tips were put in 1N HCl for five minutes to soften the tissues. The tips were than macerated and stained with acetocarmine stain for 15 minutes. The stained root tips were covered with cover slips and squashed and later viewed in Trinocular Microscope.

**RESULTS AND DISCUSSION**

*Table 1: Physico-chemical parameters of the collected Leachate samples*

Parameters	WHO Std.	S1	S2	S3
pH	7.0-8.5	7.8	7.71	8.26
EC( $\mu$ s/cm)	600	366	418	580
TDS(mg/l)	500	1011	1034	1077
Chloride	200-600	62.48	352.16	48.28
T. Alkalinity	200-400	570	568	564

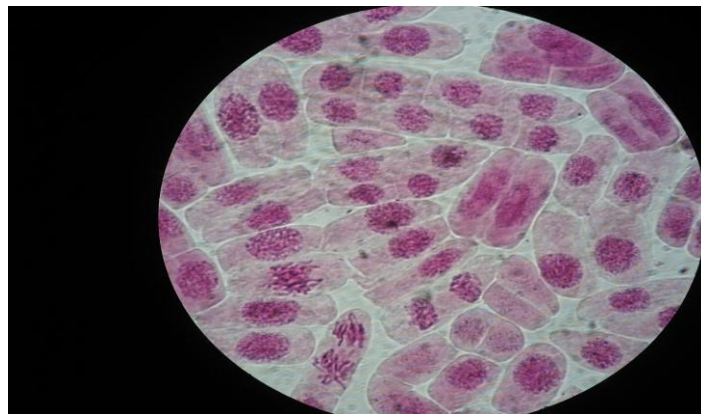
Nitrate	45	0.8	0.5	1.96
Sodium	-	35.5	33	39.5
Potassium	-	27.5	33	24.5
Hardness	150-500	511.8	518	520
DO	-	15.2	1.21	101.36
BOD	0.4	0.9	0.1	10.6
COD	-	24.53	20.26	28.80
Cd	0.01	0.016	0.067	0.01
Zn	3	0	0.009	0
Ni	0.02	0	0	0
Pb	0.1	0	0.3	0.23
Mn	0.05	0.01	0.07	0.21

**PHYSICOCHEMICAL CHARACTERIZATION**

The pH levels of the leachate samples were slightly alkaline in nature and varied between 7.8-8.26. TDS: TDS denote mainly the various kinds of solids present in the samples. The TDS values of the collected samples varies between 1011 to 1077 mg/l , which is very much higher than the permissible limits of ground water as stipulated by WHO(500mg/l).

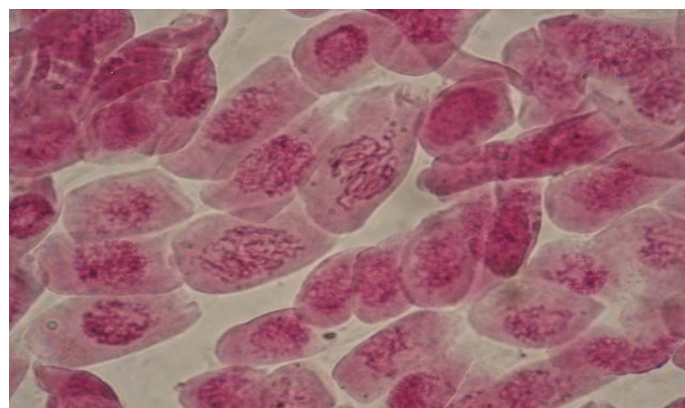
**ROOT LENGTH**

*Allium Test*

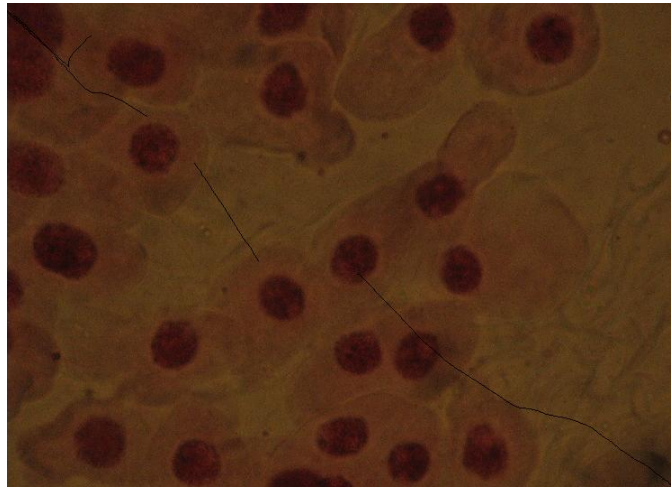


*Fig 1: Mitotic division in the control.*

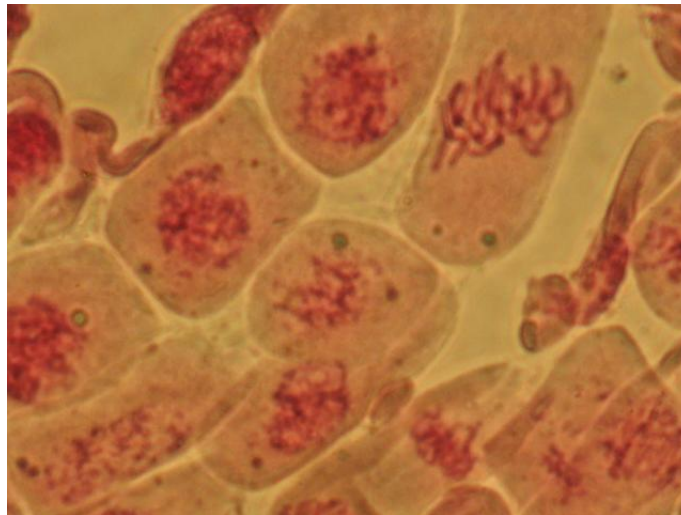
Treatment with leachate for 4 days lowered the mitotic activity significantly than the control.



A. 20% dilution of municipal leachate.



B. 40% dilution of municipal leachate.



C. 100% concentration of municipal Leachate

***Figure 2: Common chromosomal aberrations induced by municipal leachate.***

A variety of bioassays has been used to demonstrate the mutagenic activity of industrial and municipal waste waters (Zegura et al., 2009). In this study the toxic effects were evaluated by analyzing root growth and root morphology. Cyto and genotoxicity were estimated by observing cytological parameters such as the mitotic index and the number of chromosome abnormalities, including c-mitosis, laggards, chromosome breaks, anaphase bridges and stickiness.

Pronounced stickiness of the chromatin matrix often resulted in atypical metaphase and anaphase. The increased stickiness also leads to the formation of sticky bridges in anaphase and telophase, and thereby prevents normal cytokinesis. Sticky chromosomes indicate that the pollution is affecting the organization of the chromatin. This effect is related to a disturbed balance in the quantity of histone or other proteins responsible for controlling the proper structure of nuclear chromatin (Kuras, 2004). Sticky chromosomes have been reported in *Allium* roots after treatment with various heavy metals such as Hg, Ni and Cu (Fiskesjo, 1997).

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