Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences 5 (2): 331-334, 2006
ISSN 1682-3974

The Effect of Waste Water on Root Growth and Mitosis in Onion (*Allium cepa*) Root Apical Meristem

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Abstract: In this study, the phytotoxic and genotoxic effects of waste waters and also mitotic index were investigated in onion (*Allium cepa*) root tip cells during germination. For this aim, samples of water from effluent channels of Olmuksa-paper, paddy and Textile-thread Factories were used in Edirne-Turkey on March 2002. The mineral content of the effluent was monitored by flame AAS and pH values of samples were measured. Tap water was used as control. The mitotic frequency of the onion root tip meristematic cells increased in samples of water from effluent channels. On the basis of present findings it may concluded that low concentrations of minerals in waters of effluent channels of textile-thread, paddy and Olmuksa-paper mill has some positive effects on root growth and mitotic divisions in onion root tip cells.

Key words: Effluent, *Allium cepa,* root elongation, mitotic index

INTRODUCTION

The essential sources of drinking and irrigation water often receive pollutants from industrial effluents, mines sewage, herbicides, pesticides, insecticides and various other chemicals and radioactive wastes. However, the extent of harm caused to biological systems in course of time, has been difficult to establish (Ray and Barman, 1987).

Waste water problem in the world gains importance due to industrial and social construction. Collecting of rain waters and let flow in channels is known for a long time but collecting of waste waters is started at the beginning of 17th century. Purification of waste waters in systematic way is started at the end of 17th century and at the beginning of 18th century.

Studies about phytotoxic effects of waste waters were started in 1970s. Samples of effluents from oil refinery, distillery, (Ray and Barman, 1987; Pandey and Neraliya, 2002, cycle, steel and tar factory (Ray and Barman, 1987), chlor alkali factory (Radha *et al*., 2002), pulp and paper mill (Singh *et al*., 2002), tannery (Chandra and Gupta, 2002; Thangaveland and Balagurunathan, 2002), thermal power station (Azad Shamim *et al*., 2002), flash light factory (Misra and Pandey, 2002), dyeing factory (Jothimani and Bhaskaran, 2002), sewage waste water (Dhankhar *et al*., 2002), etc. are used in research the effects of the contents to living organisms. In these studies, response of sugarcane to treated wastewater (Ahmad, 2003), genotoxic effect of smoke in *Cestrum diurnum* Linn. (Azad Shamim *et al*., 2002), effect of mixed industrial effluents on growth, in *Eucalyptus camaldulensis* (Bhati and Singh, 2003), inhibition of nitrate reductase activity in *Triticum aestivum* and *Brassica campestris* (Dhankhar *et al*., 2002), effects of dilution and dynamics of physical factors in cotton and *Sorghum* (Jothimani and Bhaskaran, 2002), effect of industrial sludge on the germination in *Cicer arietinum* (Misra and Pandey, 2002), toxic effect of solid waste on pigments and photosynthetic rate, respiration rate in rice (Radha *et al*., 2002), effect of industrial pollution on the rate of pollen germination in *Nerium odorum* (Salgare, 2002 a;b), germination and growth in crop plants and *Helianthus annus* (Thangaveland and Balagurunathan, 2002; Tomer *et al*., 2002) had been researched. Genotoxicity of waste water sludge using the *Allium cepa* anaphase-telophase chromosome aberration assay was also researched by Rank and Nielson (Rank and Nielson, 1998).

Onion (*Allium cepa*) is one of the chief horticultural crops of Turkey. Present production in Turkey is about 2.1 million tons of bulbs from 1.05.000 ha. Onion is cultivated under both irrigated and non-irrigated conditions. Portable sprinkler irrigation systems are commonly used to grow onion. Seasonal evapotranspiration ranges from 350 to 450 mm for optimum bulb yield depending on the environmental conditions of each year. Bulb yield ranges from 10 to 40 t ha^{-1} under non-irrigated and irrigated conditions, respectively (Anonymous, 1997).

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The aim of the present study was to investigate if the samples of water from effluent channels of Olmuksa-paper and Textile-thread and Paddy factories, had any phytotoxic effect on root growth and genotoxic effects on mitotic divisions and mitotic index in onion (*Allium cepa*) root tip cells during mitosis or not.

MATERIALS AND METHODS

Samples of water from effluent channels of Olmuksa-paper, paddy and textile-thread factories were collected from the middle of the flow and below the surface of water on March 2002 in Edirne-Turkey. *Allium cepa* was used as material to investigate phytotoxic, genotoxic effects and mitotic index.

The mineral content of the effluent was monitored by flame AAS (Atomic Absorption Spectrophotometer). A Unicam model 929 A Flame Atomic Absorption Spectrophotometer (FAAS) was used for metal determination. The instrumental conditions for each element were taken from the instrumental manual. All pH values were performed with an Orion 720A Model pH meter using a combined electrode. Standard solutions of the elements were prepared by appropriate dilution of 1000 μg mL^{-1} stock AAS solutions. Reference solutions were prepared daily by further dilution with distilled water.

All experiments were performed on adventitious roots of the onion *Allium cepa.* The onion used in the experiment had been prepared as described by Wierzbicka (Wierzbicka, 1987). They were placed in 60 mL tap water and grown in opaque containers at 22°C. When the roots reached a length of 1.5-1.7 cm, 28 onions were transferred to different samples of wastewater from effluent channels, the others 3 onions were left in previous solution and treated as controls. The plants were grown for 7 days. The root elongation toxicity test was performed according to Chang *et al.* (1997). At the end of 7 days the total root length was measured. The phytotoxicity results were based on the effective concentration that reduced root growth by 50% (EC_{50}) was extrapolated. The experiment was set up in a Completely Randomized Design with 4 replications. The significant difference between the treated and the control samples was analyzed by Student's-test. The values of root are means of 20 measurements.

After 1 week treatment, the root tips of onions were fixed in Carnoy fixative (3 alcohol:1 acetic acid) and hydrolyzed in 1 N HCl at 50°C for 5 min followed by squashing in a 2% aceto orcein stain. Slides were kept in a freezer and examined within a month. The Allium test was conducted according to Rank and Nielson with slight modification (Rank and Nielson, 1998). Slides were coded and scored for mitotic index (at least 500 cells/slides) and genotoxic effects (mitotic aberrations). The aberrations were characterized and classified in the following categories: fragments, bridges, lagging chromosome, c-mitosis, multipolarity and lack of cytokinesis. The slides were examined with an Olympus photomicroscope.

RESULTS AND DISCUSSION

Table 1 shows the mineral content and pH value of samples of water from effluent channels of Olmuksa-paper, Paddy, Textile-thread factories and tap water as control.

Table 2 shows the positive effects of samples of water from effluent channels of Olmuksa-paper, Paddy and Textile-thread factories on the development of the onion roots. General toxicity can't be determined from the table. The phytotoxic effects (EC_{50}) of waste water for root elongation were not found at any factories. We saw that after 7th day the development of the onion roots in waste water. There was positive change in the length of the adventive roots as we compared them with the control.

The effects of samples of water from effluent channels of Olmuksa-paper, Paddy and Textile-thread Factories on mitotic frequency are shown in Table 3. All the samples of waste water have positive effects on mitosis. The increase of mitotic frequency varies among waste waters (Table 3).

Table 1: The mineral content and pH values of the water samples (Olm: Olmuksa-paper mill, Txt: Textile-thread mill)

	Olm (mg L^{-1})	Txt (mg L^{-1})	Paddy (mg L^{-1})	Control (mg L^{-1})
Cd	-	-	-	-
Fe	0.499	0.465	0.221	-
Cu	-	-	-	-
Ni	0.133	0.196	-	-
Mn	0.286	0.123	-	-
Zn	-	-	-	-
Cr	-	-	1.471	-
Sb	-	-	-	-
Pb	-	-	-	-
Co	-	0.043	-	-
pH	8.12	7.9	7.6	7.5

Table 2: The effect on root elongation of *Allium cepa* of the effluent in 7 days

Water samples	Root elongation
Control	5.7 ± 0.7
Olmuksa-paper mill	7.8 ± 0.5
Textile-thread mill	7.6 ± 0.7
Paddy mill	7.2 ± 0.5

Table 3: The effect on mitotic index of root tip cells of *Allium cepa* of the effluent (Olm: Olmuksa-paper mill, Txt: Textile-thread mill)

	Control	Olm.	Txt.	Paddy
Cell counted	1000	1000	1000	1000
Cell divided	519	728	686	617
Cell undivided	481	272	314	383
% of cell divided	51.9	72.8	68.6	61.7

Olmuksa-paper > Textile-thread > Paddy > Control

In this study, the abnormalities of onion root tip cells during mitosis are negligible. Because we saw abnormalities in very small numbers for example 2 anaphase bridges in 1000 cells.

Elements such as C, H, O, N, K, Mg, Fe, S and P are major factors for plants' development. These are called as macro elements. There are some elements which are found in very small amounts and have important roles for cell metabolism are called microelements such as B, Zn, Mn, Cl, Cu, Al, Co etc. These elements generally taken up from soil and many of them are used in enzymatic reactions for plant cell metabolism. Fields deprived of these elements and water can't give good response to plants' development. In these situations, waters from effluent channels can be used for irrigation after refining.

In recently done studies are showed that small amounts of metals or heavy metals found in industrial effluents cause increases in mitotic index. Ahmad (2003) showed that sugarcanes had given better response to treated waste water of oil refinery than the ground water.

The root elongation is also relative to cell metabolism (Seregin and Ivanov, 2001). Water samples collected from effluent channels of three factories; Olmuksa-paper, Paddy and Textile-thread Factories are consist Fe, Mn and Co. Fe is the macro element and needed for electron carrier enzymes; cytochromes in oxidative phosphorylation. Mn and Co are enzyme activators for plant metabolism. In recently done studies it is found that Nickel was an essential trace element for plants (Brown *et al*., 1987). In higher plants it is a component of urease (Marcshner, 1998). Water samples which include these elements can cause cell metabolism better response as high values of root elongation and mitotic index in onion.

Pulp and paper mill effluents were used in another study made on wheat by Singh. Diluted effluent increased the chlorophyll content, plant height and root biomass, grain yield, protein, carbon hydrate and lipid contents in wheat grains, while undiluted effluent caused inhibition in plant growth resulting in a sharp decline of yield (Singh *et al*., 2002). A pot culture experiment was done with dying factory effluent on cotton and *Sorghum* (Jothimani and Bhaskaran, 2002).

pH is one of the important factors for water cultures. Optimum pH values are among 6-8. We can also see this from our results. All factories' effluents' pH values are near the optimum values. But we get minimum root elongation and mitotic index values from control and highest values from Olmuksa-paper mill. Fe and Mn contents of Olmuksa-paper mill's effluent are greater than other two factories. Ni content is greater in Textile-thread mill's effluent than Olmuksa paper mill.

In conclusion pH and mineral contents of effluent channels of paper, textile-thread and paddy mill are suitable for plant development especially physiology and cell metabolism. The water which is taken from effluent channels of these factories can be safely used for irrigation at proper dilutions after refinement.

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