ORIGINAL RESEARCH PAPER

Ecotoxicology

KEY WORDS: *Allium cepa*, Cytotoxicity, Mitotic index, Industrial effluents, Phase indices

THE IMPACT OF INDUSTRIAL EFFLUENTS ON THE CELL DIVISION OF *ALLIUM CEPA*

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ABSTRACT

Objective: is to study the cytotoxic effect of milk, insulin and paper industrial wastewater effluents on growth of Allium cepa root cells.

Materials and Methods: The onion bulbs were exposed to the effluents of milk processing industry, paper industry and insulin industry. After treatment of 48, 72, 96 and 120 hours the length of the root was measured and compared with the onion root maintained in the control. The root tips of treated onions were squashed to estimate the mitotic index and phase index.

Results and discussion: The onion root length measured highest in control (5.3cm) followed by in the effluents of milk industry (3.625cm), paper industry (1.75cm) and least in insulin industry (0.83cm). The highest mitotic index observed in control or tap water treated onion root (12.47%) followed by milk industry (11.45%), paper industry (9.3%) and least in insulin industry (7.2%) effluent treated onion roots. The highest phase index recorded for prophase in three different effluents and least for teleophase on the three different effluents.

Conclusion: The industrial effluents of milk, paper and Insulin industry suggested negative impact on the root growth of allium cepa. The highest cytotoxic effect observed in the effluent of insulin industry followed by paper industry and least in milk processing Industry.

INTRODUCTION

As population increases which leads to influencing industrialization process which cause adverse effect on environment. The industrial effluent contains pollutants which are cytotoxic and genotoxic, those pollutants once enter into our biological process. It's really difficult to eliminate them from the environment and disturb various biochemical processes. Industrializations process which mainly cause adverse effect on water, soil and air. When the effluents disposed indiscriminately into rivers, streams, lakes, ponds responsible for the ecological imbalance, those were too polluted and they are not suitable for basic uses like drinking supply, fishing etc.., and also decrease the level of dissolved oxygen, there by disrupting the water's ecological balance.

The *Allium* test is a very good tool for evaluation of toxicity [1]. The industrial effluents effects are mainly detected through reduction of cell division in the meristem cells of *Allium* cepa because the root tips are often first to be exposed to the chemicals in the soil and water. *Allium test* has successfully been used in evaluating cytotoxic and genotoxic effects of effluents. Oil industrial wastewater revealed the general toxicity on root growth and genotoxicity of chromosomes aberration by the *Allium* test. The research work revealed the industrial effluent is mitodepressive and increased significantly the frequency of chromosomal aberration in root cells of *Allium* cepa (sticky chromosome, c-mitosis, spindle multipolarity, bridges fragments) [2].

Study on the impact of different concentration of biological and chemical wastewater treatment on the root meristemetic tissue of Allium cepa revealed the mitotic division and the plant growth was interrupted. The chromosomal aberrations such as high frequency of lagging chromosomes, polar slips and sticky chromosomes were observed 3. Different concentrations of olive oil and milk industrial wastewater solutions (100%, 75%, 50%, 25%) treated with onion root tip germination. The impact of waste water evaluated during mitotic division. According to this study, olive oil industrial wastewater was more toxic than milk industrial waste water. Several mitotic abnormalities were observed in mitotic cell division and decrease the mitotic frequency. Chromosomal bridges, laggard chromosome, polar slip and lack of cytokinesis and some other chromosomal abnormalities were observed in different phases of mitosis[4].

The study on genotoxic potential of blitox (fungicide) was investigated by using chromosome aberration in *Allium* cepa root tip cells. *Allium* cepa roots were treated with different concentration of blitox (2g/lt, 3g/lt and 4g/lt) for different treatmental duration. The result indicated that blitox significantly increases the genetic abnormalities at all concentrations and decreased the mitotic index compared to controlled condition [5].

Allium cepa root chromosomal aberrations have been the most used to detect genotoxicity/ antigenotoxicity along the years. The mitotic index and chromosomal abnormalities are used to evaluate genotoxicity and micronucleus analysis used to verify mutagenicity of different chemicals [6].

Cytotoxicity of the paper mill effluent was investigated by treating onion root with different concentrations of effluent (25%, 50%, 75% and 100%) for 24, 42 and 72 hours. The effluents inhibited cell division at strong dosage effect was observed from a decline in mitotic index with increase in effluent concentrations and duration of treatment. The effluent was also induced various mode of mitotic anomalies, such as laggard, fragmentation, stickiness and bridge etc. The investigation revealed that the Paper-mill effluent acts as a cytotoxic/mutagenic agent at high concentration [7]. The objective this paper is to evaluate the impact of industrial wastewater of milk industry, paper industry and insulin industry.

MATERIAL AND METHOD

The onion bulbs were placed on the top of plastic containers which contain sand and diluted (25%) industrial effluents of milk industry, paper industry and insulin industry. The control with tap water was maintained in another plastic container. After treatmental duration of 48, 72, 96 and 120 hours, the length of the onion root was measured and squash preparation was carried out to study the chromosomal arrangements during mitotic division. 1000 cells observed for dividing cells and non-dividing cells on a slide to estimate mitotic index. . Among the dividing cells number of cells counted for prophase, metaphase, anaphase and telophase to estimate phase indices[4,8]

$$Mitotic\ index\ (\%) = \frac{number\ of\ dividing\ cells}{total\ number\ of\ cells} \times 100$$

$$Phase\ indices\ (\%) = \frac{number\ of\ pro/meta/ana/\ telophase}{total\ number\ of\ mitotic\ cells} \times 100$$

RESULTS AND DISCUSSION

According to the results in the table 1, the length of the onion root length was measured. In the control after 48, 72, 96 and 120 hours exposure, the germinated root length was 2.1, 4.3, 6.9 and 8.1cm respectively. In the diluted effluent (25%) of milk industry after 48, 72, 96 and 120 hours of treatmental duration, the root length was 1.6, 2.8, 4.3 and 5.8cms respectively. In the paper

industrial effluent at 25% concentration, after 48, 72, 96 and 120 hours of exposure, the root length was 0, 0.8, 2.3 and 3.2cms respectively. In the insulin industry effluent at 25% concentration, treatment of onion bulb root meristem after 48, 72, 96 and 120 of exposure, the root length was 0cm, 0.1cm, 0.9cm, 1.3cm respectively. These results suggest that, in the 120 hour exposed root length was highest (8.1cm) in control followed by milk industry effluent (5.8cm), paper industry effluent (3.2cm) and least in insulin industry effluent (1.3cm). According to these results, the insulin industry effluent is responsible for the higher growth retardation of Allium cepa than either milk industry effluent or paper industry effluent.

According to results in table 2, in the control, the mitotic index of Allium cepa was 9.9%, 11.5%, 13.2% and 15.3% during 48h, 72h, 96h and 120h exposed root tips of meristematic cells. In the milk industrial effluent during the 48h, 72h, 96h and 120h the mitotic index was 8.1%, 10.1%, 12.9% and 14.7% respectively. In the paper industry effluent during 48h, 72h, 96h and 120h the mitotic index was 0, 7.0%, 9.4% and 11.5% respectively. And in insulin industry effluent during 48h, 72h, 96h and 120h the mitotic index was 0, 0, 5.9%and 8.5% respectively. Impact of toxicity level of a test compound can be determined based on the increase or decrease in the mitotic index 9, 10. In compression of the root tip which is treated with tap water (control) showed highest mitotic index (12.47%) followed by milk industry effluent (11.45%), paper industry effluent (9.3%) and lowest in insulin industry effluent (7.2%).

Comparative result of phase indices indicated in the table 3. In the control the prophase phase indices was highest (35.77%) followed by metaphase (26.91%), anaphase (21.49%) and lowest was teleophase (15.65%). In milk industry effluent prophase the phase indices was highest (34.73%) followed by metaphase (30.55%), anaphase (21.75%) and lowest in telophase (13%). In paper industry effluent the prophase phase indices was highest (39.83%) followed by metaphase (29.16%), anaphase (17.84%) and lowest in telophase (12.95%). In insulin industry effluent the prophase phase indices was highest (39.45%) followed by metaphase (25.3%), anaphase (21.32%) and lowest is telophase (13.80%). In conclusion, the insulin industry effluent showed highest cytotoxic effect than paper industry and milk processing industry effluents.

Table. 1: Root length of an *Allium cepa* exposed to 25% industrial effluents and control for different duration

Duration (Hours)	Length of onion root (cm) in diluted (25%) effluents			
	control	Milk processing industry	Paper industry	Insulin industry
48	2.1	1.6	0	0
72	4.3	2.8	0.8	0.1
96	6.9	4.3	2.3	0.6
120	8.1	5.8	3.2	1.3

Table. 2: Mitotic index of *Allium cepa* root meristemetic cells during germination at different treatmental duration and in different diluted industrial effluents (25%).

Duration (Hours)	Mitotic Index (%)			
	Tap water	Milk industries	Paper industries	Insulin industries
48	9.9	8.1	-	-
72	11.5	10.1	7.0	-
96	13.2	12.9	9.4	5.9
120	15.3	14.7	11.5	8.5
Average	12.47	11.45	9.3	7.2

Table. 3: Phase indices of *Allium cepa* treated in different industrial effluents (25%)

	Tap water	Milk industry	Paper industry	Insulin industry
Prophase index (%)	35.86	34.70	39.97	39.55
Metaphase index (%)	26.96	30.55	29.24	25.33
Anaphase index (%)	21.53	21.75	17.84	21.32
Teleophase index (%)	15.65	13.00	12.95	13.80

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