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# Studies on phyto-genotoxic assessment of tannery effluent and chromium on *Allium cepa*

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**Abstract**

Tannery effluent contributes significantly to pollution of the environment. In this study, phytotoxic and genotoxic effects of tannery effluent and chromium (Cr) were investigated in *Allium cepa*. For this purpose, tannery effluent was collected from "Up flow Anaerobic Sludge Blanket" (U.A.S.B) Jajmau, Kanpur. *A. cepa* were exposed to various concentrations of tannery effluent (0.0, 3.125, 6.25, 12.50, 25.0%) and Cr (0.0, 1.0, 2.0, 4.0, 8.0 mg $l^{-1}$) for 48 and 168 hr. The perusal of data revealed that the physico-chemical characteristics of tannery effluent viz. pH (8.5), EC (11.94 $dSm^{-1}$), BOD (499 mg $l^{-1}$), COD (1382 $mgl^{-1}$) and Cr content (2.32 mg $l^{-1}$) were much higher than the prescribed permissible limit for industrial effluent discharged into inland waters. These substances provoked phytotoxic and genotoxic effects in *A. cepa*. Total chlorophyll and protein content in leaves of tannery effluent and Cr treated plants decreased significantly in dose-duration dependent manner. A maximum decrease of 86.29 and 84.26% in total chlorophyll and 81.27 and 76.16% in protein content was observed after 168 hr of exposure while carotenoid content increased up to 6.25% effluent and 2.0 $mgl^{-1}$Cr treatment and decreased further. In all treated plants, a significant ($p \leq 0.05$) reduction in root length, mitotic index (MI) and induction in chromosomal (CA)/mitotic (MA) aberration and micronuclei (MNC) were observed as compared to unstressed plants. A maximum reduction of 81.15 and 79.71% in MI, and induction of 6.8 and 4.8% in CA, 29.24 and 26.66% in MA and 0.52 and 0.43% in MNC were found at 12.50% effluent and 4 $mgl^{-1}$ Cr treated plants as compared to unstressed plants, however at highest effluent and Cr concentration both the plants showed pyknosis condition after 168 hr.

**Key words**

Genotoxicity, Tannery effluent, *Allium cepa*, Chromosomal aberrations

## Introduction

Indiscriminate discharge of untreated or partially treated waste water directly or indirectly into aquatic bodies may render water resources unwholesome and hazardous to man and other living systems (Bakare *et al.*, 2009; Olorunfemi *et al.*, 2010). Tannery effluents are ranked as the highest pollutants among all industrial wastes. India is the third largest producer of leather in the world having about 3000 tanneries with annual processing capacity of 0.7 million tonnes of hides and skin. In Uttar Pradesh (India) Jajmau, Kanpur is a major industrial town (about 400 tanneries) located on the bank of river Ganga specialized in processing hide into heavy leather (Sinha *et al.*, 2008; Gupta *et al.*, 2010). Nowadays, majority of tanning industries favour chrome tanning for processing leather. Unfortunately only fraction of chromium (Cr) is utilized in tanning process and rest is discharged as byproduct of wastewater treatment (Hafez *et al.,* 2002). Therefore, the treated wastewater discharged from tanning industries contains high level of BOD, COD, electrical conductivity and heavy metals especially Cr above permissible limit as recommended by various regulatory agencies making it potentially toxic (Lal, 2009). Usually tanning industries discharge their wastewater into nearby rivers and indirectly is being used for irrigation of crops and vegetables. This practice has ultimately led to movement of potentially toxic metals

from water to plant system and ultimately to human beings (Sinha *et al*., 2008). It is well known that Cr (VI) is a potent carcinogen to humans and animals as it enters cells via surface transport system and gets reduced to Cr (III) inducing genotoxicity (Matsumoto *et al.,* 2006). Thus, Cr loaded effluent used for irrigation disrupts several physiological and cytological processes in cells.(Shanker *et al*., 2005; Chidambaram *et al*., 2009) leading to reduced root growth, biomass, seed germination, early seedling development (Irfan and Akinici, 2010), and induces chlorosis, photosynthetic impairment and finally leading to plant death (Seoccianti *et al* .,2005; Akini and Akini, 2010). Previous studies have shown tannery effluent and Cr induced various chromosomal abnormalities in plant cells thereby severely reducing mitotic index and root growth (Olorunfemi *et al*., 2010).

The use of plant bioassay is a sensitive and reliable test for assessing genotoxicity and *in situ* monitoring (Grant, 1994). Higher plants such as *Allium cepa, Vicia faba* and *Zea mays* are good genetic models for the assessment of environmental pollutants. Due to thier sensitivity in evaluation of the genotoxicity of dangerous / harmful chemicals, but also due to potential of assessing numerous genetic end points, varying from point mutation to chromosome aberrations and micronucleus formation in cells (Hoshina, 2009). Because of the potential environmental and human health impact connected with the heavy use of Cr, the aim of the present study was to investigate the physico-chemical properties of tannery effluent and evaluate the phytotoxic, cytotoxic and clastogenic effects of tannery effluent and Cr using *Allium cepa* bioassay.

## Materials and Methods

Tannery effluent collected in acid washed plastic containers from the main discharge outlet of Up Flow Anaerobic Sludge Blanket (USAB) Jajmau, Kanpur (U.P.) were analyzed for physico-chemical characteristics following standard methods of APHA (2005).

Healthy onion bulbs of *Allium cepa* (2n = 16) were procured from the local market and their outer scales were peeled and kept under running tap water for half an hour prior to tests. To demonstrate possible dose-duration dependent effect of effluent and Cr, the onion bulbs were treated with multiple concentrations of $EC_{50}$ of tannery effluent (3.125, 6.25, 12.50, 25%) and chromium (1.0, 2.0.4.0, and 8.0 $mgl^{-1}$) as potassium dichromate salt ($K_2Cr_2O_7$). A set of plants grown in tap water served as control. The plants were harvested after 48 and 168 hr to study phytotoxic and genotoxic parameters. To determine bioaccumulation of Cr, the roots were dried at 80°C and digested in mixture of $HNO_3$ : $HClO_4$ (3:1 ratio) using Microwave Digestion System MDS 2000 and Cr content was estimated by GBC Avanta S Atomic Absorption Spectrophotometer using air acetylene gases at 357.9 nm wavelength.

For phytotoxic assessment, chlorophyll and carotenoids were estimated following the method of Arnon (1949) and Duxbury and Yentsch (1956). Protein content was assayed by the method of Lowry *et al.* (1951). To evaluate genotoxic effect and mitotic index, the root tips of *Allium cepa* were fixed in Carnoys fixative (3 alcohol: 1 acetic acid), hydrolyzed in 5N HCl for 10-15 min and stained with haemotoxylin (Fiskejo, 1985). The slides were sored for mitotic index (MI), chromosomal/mitotic aberrations (CA/MA)and micronuclei (MNC). For MI, dividing cells were counted from 5000 to 6000 interphase cells along with scoring MNCs' and the data were expressed in percent. For CA 200-300 well spread metaphase cells, and for MA 500-600 dividing cells were scored.

The experiment was set up in a completely randomized design with three replications. The significant difference between the treated and the control samples was analyzed by chi - square test and analysis of variance (ANOVA) (Gomez and Gomez, 1984).

## Results and Discussion

The UASB treated tannery effluent was dark brown in colour, alkaline in nature (pH 8.5), having foul odour, high BOD (499 mg $l^{-1}$) and COD (1382 mg $l^{-1}$). It was contaminated with high concentrations of Cr (2.32 mg $l^{-1}$) and Fe (3.02 mg $l^{-1}$). Physico-chemical properties and Cr content in the effluent were fairly above the discharge limit (0.05 mg $l^{-1}$) as recommended Environment Protection Agency (USEPA, 2002) revealing toxic nature of tannery effluent.

**Table 1:** Effect of various concentrations of tannery effluent on total chlorophyll, carotenoid and protein content in *Allium cepa* after 168 hr of treatment.

| Concentrations (%) | Total Chl (µg $g^{-1}$ f.wt.) | Carotenoid (µg $g^{-1}$ f.wt.) | Protein (µg $g^{-1}$ f.wt.) |
|---|---|---|---|
| Control | 1.97± 0.64 | 0.57 ± 0.07 | 13.51±1.8 |
| 3.125 | 1.44±0.31$^{NS}$ | 0.62±0.06$^{NS}$ | 11.27±1.6$^{NS}$ |
| 6.25 | 1.30±0.24* | 0.68±0.05* | 8.41±1.7* |
| 12.50 | 0.93±0.07* | 0.41±0.07* | 5.18±1.5* |
| 25.0 | 0.27± 0.03* | 0.23±0.02* | 2.53±1.2* |

All values are mean of triplicates ± S.D. ANOVA ($p<0.01$); LSD: *Significant ($p<0.01$) compared to control, NS: Non significant as compared to control.

**Table 2:** Effect of various concentrations of Cr metal on total chlorophyll, carotenoid and protein content in *Allium cepa* after 168 h of treatment.

| Concentrations (mg $l^{-1}$) | Total Chl (µg $g^{-1}$ f.wt.) | Carotenoid (µg $g^{-1}$ f.wt.) | Protein (µg $g^{-1}$ f.wt.) |
|---|---|---|---|
| Control | 1.97±0.64 | 0.57±0.08 | 13.51±1.8 |
| 1.0 | 1.74± 0.53$^{NS}$ | 0.68±0.04$^{NS}$ | 12.13± 1.5$^{NS}$ |
| 2.0 | 1.43±0.49* | 0.74±0.09* | 10.05±1.2* |
| 4.0 | 1.08±0.37* | 0.49±0.05* | 6.38±1.3* |
| 8.0 | 0.31±0.01* | 0.30±0.02* | 3.22±1.4* |

All values are mean of triplicates ± S.D. ANOVA ($p<0.01$); LSD: *Significant ($p<0.01$) compared to control, NS: Non significant as compared to control.

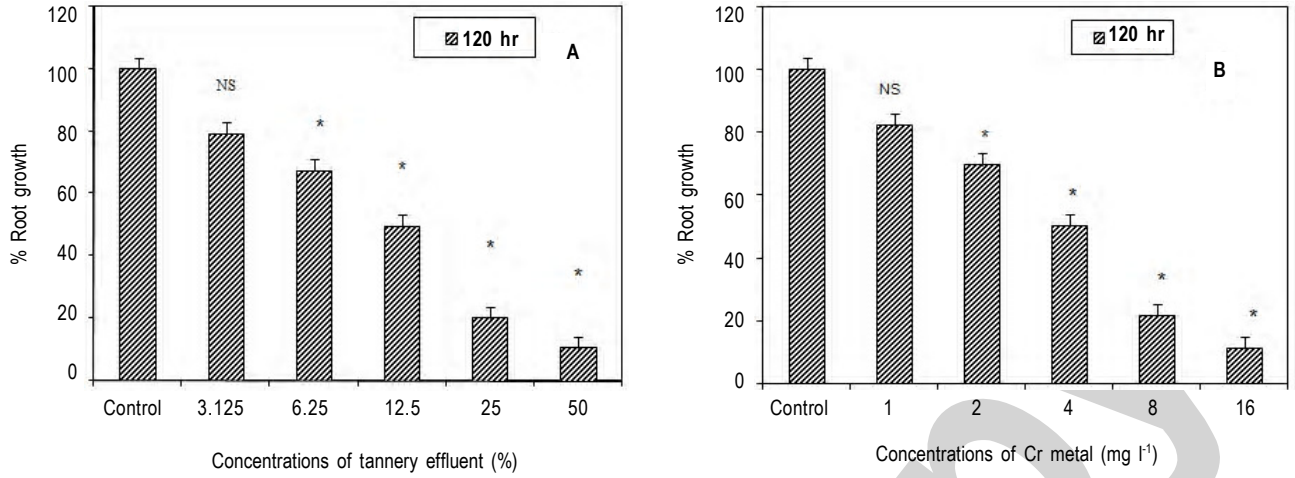


**Fig.1:** Effect of various concentrations of (A) tannery effluent and (B) Cr on root growth of *Allium cepa* after 120 hr exposure to determine $EC_{50}$. All the values are mean of triplicates ± S.D. ANOVA ($p<0.01$). LSD: *Significant ($p<0.01$) compared to control, NS: Non significant as compared to control

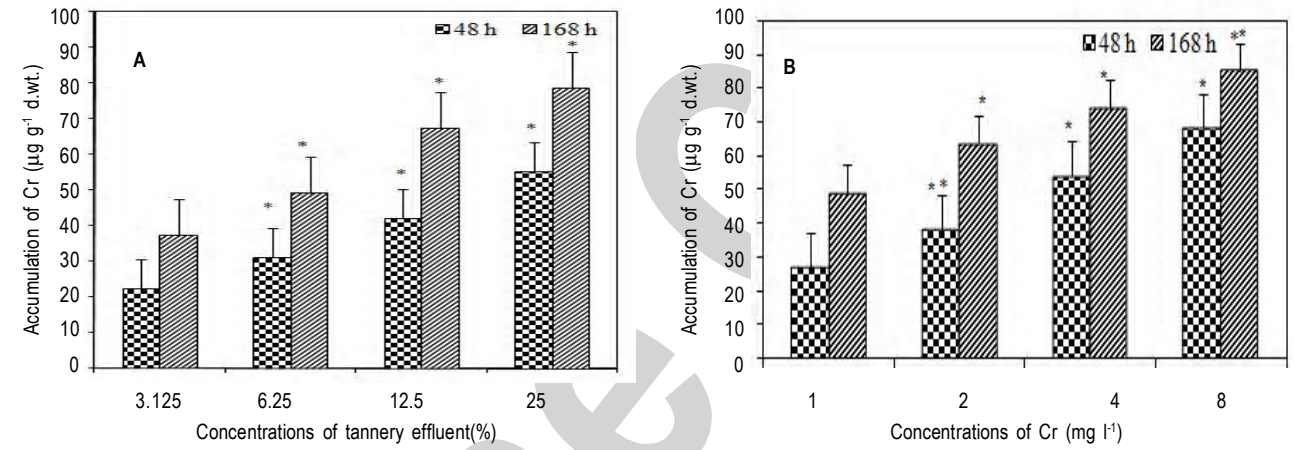


**Fig.2:** Accumulation of Cr in roots of *Allium cepa* at different concentrations of (A) tannery effluent and (B) Cr after 48 and 168hr exposure. All the values are mean of triplicates ± SD. ANOVA significant at $P \leq 0.01$. LSD: *Significant ($P<0.01$) compared to control, NS: Non significant as compared to control.

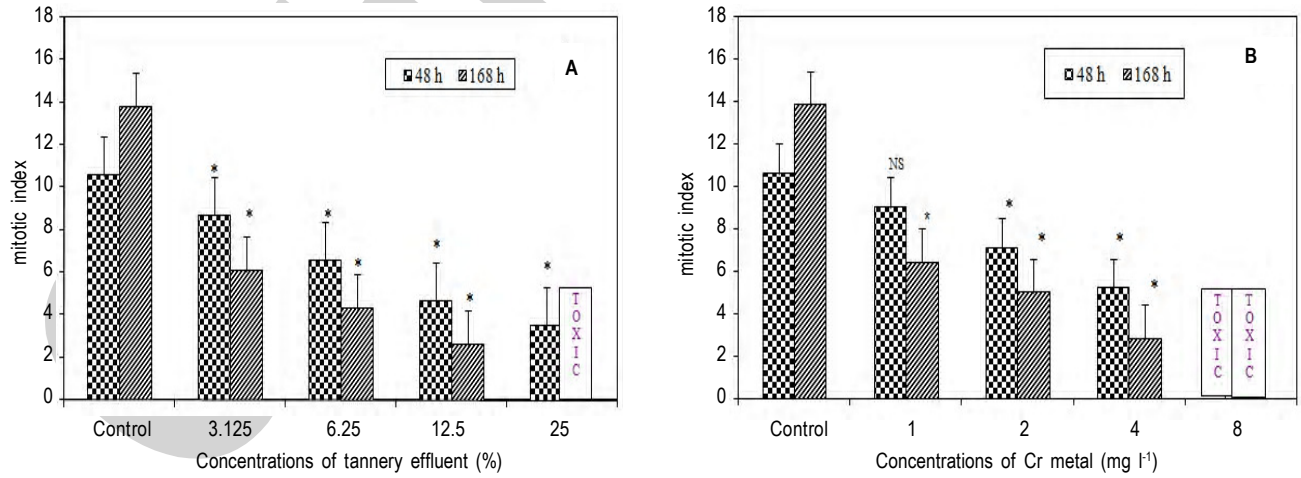


**Fig. 3:** Effect of various concentrations of (A) tannery effluent and (B) Cr solution on mitotic index of *Allium cepa* after 48 and 168 hr exposure. All the values are mean of triplicates ± S.D. ANOVA ($p<0.01$). LSD: *Significant ($p<0.01$) compared to control, NS: Non significant as compared to control.

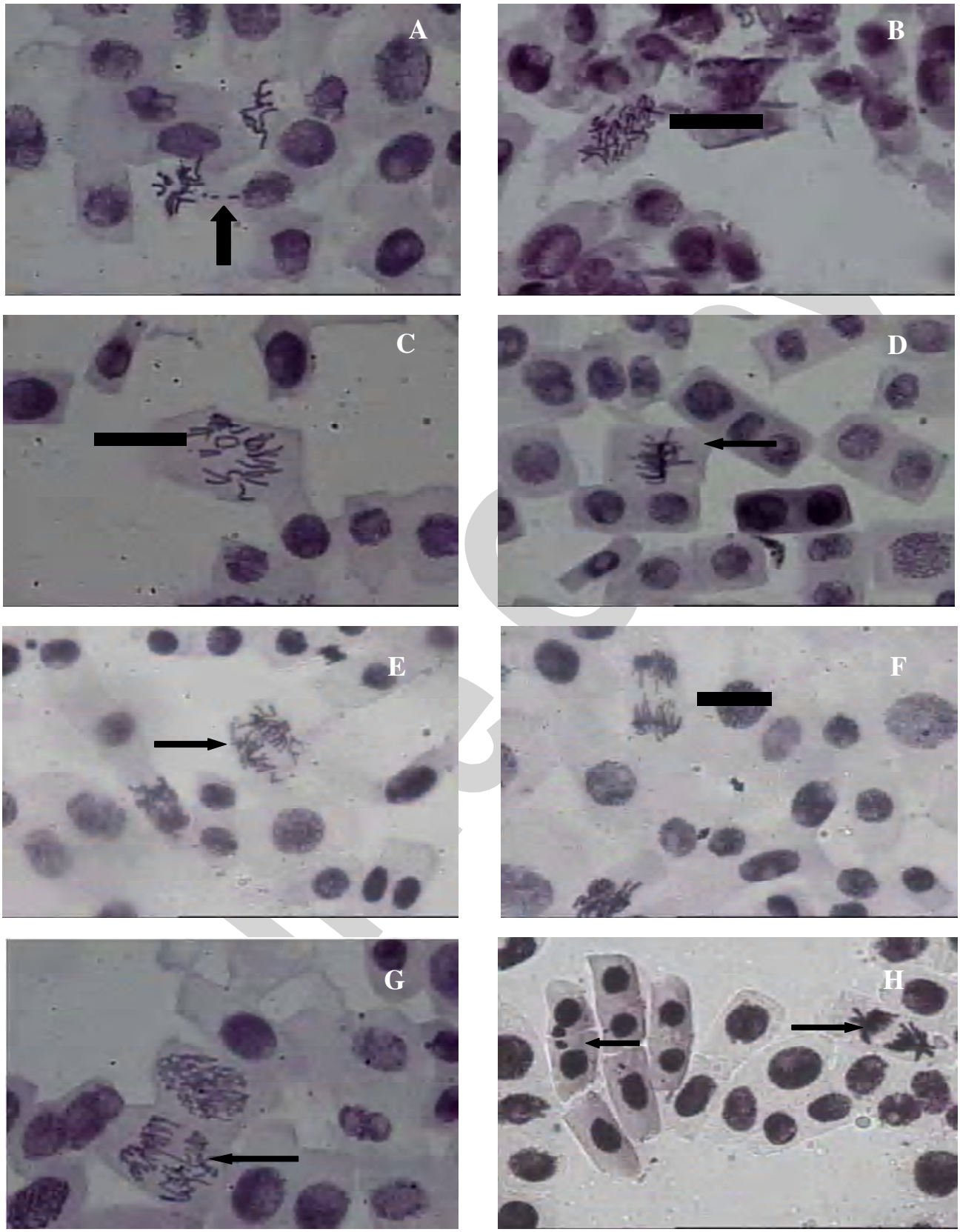


**Fig. 4** Chromosomal abnormalities observed in mitosis following tannery effluent treatment: (A) Break (B) Fragments (C) C-metaphase (D) Stickiness (E) Multipolar, (F) Laggard, (G) Bridge and (H) Condensation and Micronuclei.

**Table 3 :** Chromosomal aberration and micronucleus (MNC) assays in root meristem of *Allium cepa* exposed to different concentrations of tannery effluent (T.E.) and chromuim (Cr) after 48 and 168 hr

| | T.E. | | Chromosome aberrations | | | | | | Mitotic aberration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time (hr) | Conc. (%) | No of metaphase | Break | Fragments | Total aberrant cells | % Chromosome aberrations | No of scored cells | No of ÷ cells | C - Metaphase | Stickiness | Multipolar | Laggards | Bridge | Conden-sation | % Abberant cells | % Micro nucleated cells |
| | C | 250 | 2 | 0 | 2 | 0.8 | 2800 | 240 | 1 | - | - | - | - | - | 0.80 | ND |
| | 3.125 | 250 | 2 | 5 | 7 | 2.8$^{NS}$ | 2650 | 122 | 1 | 3 | - | 1 | - | 3 | 6.50* | 0.01$^{NS}$ |
| **48** | 6.25 | 250 | 6 | 7 | 13 | 3.6** | 2910 | 132 | 3 | 2 | 3 | 2 | 2 | 4 | 12.12** | 0.05$^{NS}$ |
| | 12.50 | 250 | 8 | 3 | 11 | 4.4** | 2593 | 80 | 2 | 2 | 1 | 1 | 3 | 6 | 18.75** | 0.14** |
| | 25.0 | 250 | 4 | 5 | 9 | 5.2** | 2505 | 85 | 1 | 6 | 1 | 1 | 3 | 8 | 23.52** | 0.28** |
| | C | 250 | 2 | 1 | 3 | 1.2 | 2700 | 336 | - | 2 | - | - | - | 2 | 1.2 | 0.01 |
| | 3.125 | 250 | 6 | 4 | 10 | 2.0$^{NS}$ | 2625 | 103 | 3 | 2 | 2 | 1 | 1 | 2 | 10.67* | 0.16** |
| **168** | 6.25 | 250 | 9 | 8 | 17 | 4.0* | 2600 | 130 | 4 | 3 | 2 | 8 | 3 | 5 | 19.23** | 0.31** |
| | 12.50 | 250 | 2 | 3 | 5 | 6.8** | 2500 | 106 | 6 | 9 | 5 | 3 | 2 | 6 | 29.24** | 0.52** |
| | 25.0 | 250 | - | - | - | Toxic | - | - | - | - | - | - | - | - | Toxic | Toxic |
| **Cr concentration (mg l$^{-1}$)** | | | | | | | | | | | | | | | | |
| | 1.0 | 250 | 0 | 3 | 3 | 1.2$^{NS}$ | 2650 | 122 | 1 | 3 | - | 1 | - | 3 | 5.10* | 0.07 |
| **48** | 2.0 | 250 | 2 | 3 | 5 | 1.6* | 2905 | 121 | 2 | 4 | 1 | 1 | 1 | 2 | 9.09** | 0.19** |
| | 4.0 | 250 | 4 | 8 | 12 | 2.0* | 2862 | 136 | 3 | 5 | 2 | 3 | 3 | 4 | 14.70** | 0.28** |
| | 8.0 | 250 | 2 | 2 | 4 | 3.2** | 2500 | 102 | 3 | 4 | 2 | 2 | 2 | 8 | 20.58** | 0.32** |
| | C | 250 | 2 | 1 | 3 | 1.2 | 2892 | 336 | 0 | 2 | 0 | 0 | 0 | 2 | 1.2 | 0.01 |
| | 1.0 | 250 | 2 | 4 | 6 | 2.0$^{NS}$ | 2918 | 153 | 3 | 2 | 2 | 2 | 1 | 3 | 8.49* | 0.14** |
| **168** | 2.0 | 250 | 3 | 3 | 6 | 2.4* | 2700 | 112 | 4 | 3 | 2 | 1 | 2 | 3 | 13.39** | 0.27** |
| | 4.0 | 250 | 4 | 1 | 5 | 4.8** | 2800 | 105 | 6 | 9 | 3 | 3 | 2 | 5 | 26.66** | 0.43** |
| | 8.0 | 250 | 0 | 0 | 0 | Toxic | - | - | - | - | - | - | - | - | Toxic | Toxic |

Significant ($p<0.05$); **Significant ($p<0.01$) compared to control

Fig. 1 shows the dose-duration dependent effects of tannery effluent and Cr on root growth of *Allium cepa* as compared to control. The growth response curve obtained between root length and different concentrations of tannery effluent and Cr metal determined the $EC_{50}$ which was 12.50% for tannery effluent and 4 mg $l^{-1}$ for Cr treated plants after 120 hrs of exposure. The roots of *A. cepa* accumulated significant ($p \leq 0.05$) amount of Cr in a concentration and duration dependent manner (Fig. 2). Such a high accumulation of metal has been reported earlier reported in several crop plants treated with industrial effluent and metal solution (Srivastava *et al.*, 2005).

Total Chl content in leaves of *A. cepa* treated with tannery effluent and Cr concentrations progressively decreased in dose dependent manner with respect to their control (Table 1, 2). Maximum inhibition of 86.29 and 84.26 % in total Chl content was observed at 25% tannery effluent and 8 $mgl^{-1}$ Cr concentration after 168 hr of treatment. Such a reduction in total Chl content may be due to disruption of thylakoid membrane or inhibition of $\alpha$-amino levulenic acid dehydrates, a key enzyme in Chl biosynthesis or Chl degradation due to increase chlorophyllase activity (Gupta *et al.*, 2010; Srivastav *et al.*, 2005). In contrast, carotenoid content in effluent and Cr treated plants increased up to 6.25 % effluent (19.30%) and 2.0 mg $l^{-1}$ Cr (29.82%) treatment after 168 hr of exposure. Increase in level of carotenoids in treated plants may be attributed for protection from free radicals formation against environmental stress (Hou *et al.*, 2007) whereas decrease at higher concentration and duration may be due to toxicity of effluent (Gupta *et al.*, 2010).

A dose and time dependent significant ($p \leq 0.05$) reduction in protein content was observed in the leaves of *A. cepa* treated with effluent (81.27 %) and Cr (76.16 %) (Table 1,2). The reduction in protein content may be due to lipid peroxidation leading to damage of membranes and inhibition of membranes proteins. Further, oxidative stress caused by the metal may also denature or damage the proteins (Srivastava *et al.,* 2005).

The effect of various concentrations of effluent and Cr concentrations on mitotic index (MI) of *A.cepa* after 48 and 168 hr treatment is shown in Fig. 3. All the test concentrations significantly ($p \leq 0.05$) inhibited MI in a concentration and duration dependent manner. A maximum reduction of 81.15 and 79.71% ($p<0.01$) in MI was observed in root cells of *A. cepa* treated with 12.25 % effluent and 4.0 mg $l^{-1}$ Cr concentrations after 168 hrs of exposure. However, at highest concentrations after168 hrs of treatment cells showed toxicity due to pyknosis. Thus, decline in MI reflects cytotoxicity that directly affects root growth and elongation. Besides organic and inorganic salts, tannery effluent contains several heavy metals, especially Cr that is cytotoxic and inhibits cell division in root tips of plants as observed in the present study (Chandra *et al.,* 2005).

These results are in well corroborated with earlier studies conducted on *A. cepa* and *V. cepa* exposed to tannery waste leachates that inferred Cr and Ni compounds jointly affects amino acids and / or DNA biomolecule which led to significant decrease of MI and root growth (Chandra and Gupta, 2002; Chandra *et al.*, 2004).

Table 4 and 5 summarize the percentage of chromosomal/ mitotic aberrations (CA/MA) and micronucleated cells (MNC) in root meristem cells of *A. cepa* exposed to various concentrations of effluent and Cr solution for 48 and 168 hr respectively. The frequency of aberrant cells significantly ($p \leq 0.05$) increased in a dose- duration dependent manner in all treated plants. Further, the types of chromosomal as well as mitotic aberrations were similar in both effluent and Cr treated *A. cepa*. Chromatid breaks and fragments were frequent CA whereas C- metaphase, stickiness, multipolar arrangements, laggards, bridges and condensed chromosome at anaphase and metaphase stage of cell division were found to be frequent MA (Fig. 4, 5). The highest frequency of MA/CA aberrations was 29.24/6.8 and 26.66/4.8 in 12.50% effluent and 4 mg $l^{-1}$ Cr treatment after 168 hrs of exposure, however after 168 hr of treatment pyknosis was observed at highest concentration of effluent and Cr solution. Both effluent and Cr treatment significantly ($p \leq 0.05$and $p \leq 0.01$) induced MNC's in root meristem cells of *A. cepa*. The frequency of MNC's were relatively highest in 12.50% effluent and 4 $mgl^{-1}$ Cr treated cells after 168 hrs exposure. O' Brien *et al* (2001) reported the potential of tannery effluent to damage the DNA of test organisms. Induction of chromosomal/ mitotic aberrations including micronucleus formation indicates the genotoxic potential of tannery effluent and Cr metal (Matsumato *et al.*, 2006). The induction of aberrant mitotic stages may be the outcome of spindle poisoning that cause chromosome disturbances during mitotic cell division. Various heavy metals are known to induce chromosome breaks, fragments, and micronucleus formation in plants and their effects were emphasized to be the result of formation of DNA–DNA and DNA-protein cross link (Costa *et al.*, 1994; Chandra *et al.*, 2004). Micronucleus induction involves the mitotic spindle and consequent production of laggard chromosomes during anaphase and loss of a complete chromosome (Sudhakar *et al.*, 1998; Matsumato *et al.*, 2006).

It may be concluded that tannery effluent which contains Cr may cause phyto-genotoxic effects on *Allium cepa* and the use of sensitive plant bioassay may be an amenable tool to screen the phytotoxicity and genotoxicity of wastewater.

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## References

Akinici, I.E. and S. Akinci: Effect of chromium toxicity on germination and early seedling growth in melon (*Cucumis melo* L.). *Afri. J. Biotech.*, **9**, 4589-4594 (2010).

American Public Health Association (APHA): Standard methods for examination of water and wastewater 21st Ed. APHA, AWWA, WFF,

Washington DC. (2005).
Arnon, D.I.: Copper enzymes in violated chloroplast polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.*, **24**, 1-15 (1949).
Bakare, A.A., A.A. Okunola, O.A. Adetunji and H.B. Jenmi: Genotoxicity assessment of a pharmaceutical effluent using four bioassays. *Genet. Mol. Biol.*, **32**, 373-381(2009).
Chandra, S., L.K.S. Chauhan, R.C. Murthy, P.N. Saxena, P.N. Pandey and S.K. Gupta: Comparative biomonitoring of leachates from hazardous solid waste of two industries using *Allium* test. *Sci. Total Environ.,* **347**, 46-52 (2005).
Chandra, S. and S.K. Gupta: Genotoxicity of leachates of tannery solid waste in root meristem cells of *Allium cepa*. *J. Ecophysiol. Occup. Hlth*., **2**, 129-133 (2002).
Chandra,S., L.K.S. Chauhan, P.N. Pandey and S.K. Gupta: Cytogenetic effects of leachates from tannery leachates from tannery solid waste on the somatic cells of *Vicia faba*. *Environ. Toxicol.*, **19**, 129-133 (2004).
Chidambaram, A., P. Sundaramoorthy, A. Murugan, K. Sanker Ganes, L. Baskaran: Chromium induced cytotoxicity in black gram (V*igna mungo* L). *Iran. J. Environ. Hlth. Sci. Eng.,* **6**, 17-22 (2009).
Costa, M., K. Salnikov , S. Coostentino, C.B. Klien, X. Huang, and Z. Zhuang: Molecular mechanism of nickel carcinogenesis. *Environ. Hlth. Perspec*., **102**, 127-130 (1994).
Duxbury, A.C. and C.S. Yentsch: Plankton pigment monographs. *J. Marine Res.*, **15**, 92-101 (1956).
Fiskejo, G.: The *Allium* test as a standard in environmental monitoring. *Hereditas,* **102**, 99-112 (1985)
Gomez, K.A. and A.A. Gomez: Statistical procedure for agricultural research. John Willey and Sons, New York (1984)
Grant, W.F.: The present status of higher plant bioassays for the detection of environmental mutagens. *Mutat. Res.*, **31**, 175-185 (1994).
Gupta, K., S. Gaumat and K. Mishra: Chromium accumulation in submerged aquatic plants treated with tannery effluent at Kanpur, India. *J. Environ. Biol.*, **32**, 591-597 (2011).
Hafez, A.I., M.S. El-Manharawy and M.A. Khedr: RO membrane removal of unreacted chromium from spent tanning effluent. A pilot scale study, part 2. Desalination, **14**, 237-242 (2002).
Hoshina, M.M. and M.A. Marin-Morales: Micronucleus and chromosome aberrations induced in onion (*Allium cepa*) by a petroleum refinery effluent and by river water that receives this effluent. *Ecotoxicol. Environ. Saf.*, **72**, 2090-2095 (2009).
Hou, W., X. Chen, G. Song, Q. Wang and C.C. Chang: Effect of copper and cadmium on heavy metal polluted water body restoration by duckweed (*Lemna minor*). *Plant Physiol. Biochem.*, **45**, 62–69 (2007).
Irfan, E.A. and S. Akinici: Effect of chromium toxicity on germination and early seedling growth in melon (*Cucumis melo* L.). *African J. Biotech*, **9**, 4589-4594 (2010).
Lal, N.: Biochemical alterations due to acute tannery effluent toxicity in *Lemna minor* L. *J. Phytology*, **1**, 361-368 (2009).
Lowry, O.H., N.J. Rasebrough, A.L. Farr and R.J. Randall: Protein measurement with folin-phenol reagent. *J .Biol. Chem.*, **193**, 265-275 (1951).
Matsumoto, S.T., S.M. Mnlovani, M.I.A. Malaguttii, A.L. Dias, I.C. Fonseca and M.A.M. Morales: Genotoxicity and mutagenicity of water contaminated with tannery effluent, as evaluated by the micronucleus test and comet assay using the fish *Oreochromis niloticus* and chromosome aberrations in onion root tips. *Genetics Mol. Biol.*, **29**, 148-158 (2006).
O' Brien, T., J. Xu and S.R. Patierno: Effects of glutathione on chromium induced DNA cross linking and DNA polymerase arrest. *Mol. Cell Biochem.*, **222**, 173-182 (2001).
Oleurnfemi, D.I, G.E Okoloko, A.A. Bakare and A. Akinboro: Cytotoxic and genotoxic of effects of cassava effluents using the *Allium cepa* assay. *Res. J. Mut.*, 1994-7917 (2010).
Seoccianti, V., R. Crinelli, B. Tirillini, V. Mancin and A. Speranza: Uptake and toxicity of Cr (III) in celery seedlings. *Chemosphere,* **64**, 1695-1703 (2006).
Shanker, A.K., C. Cervantes, H. Loza-Tavera and S. Avudainayagam: Chromium toxicity in plants. *Environ Intl.*, **31**, 739-753 (2005).
Sinha, S., S. Singh and S. Mallick: Comparative growth response of two varieties of *Vigna radiata* L. (var. PDM 54 and var. NM 1) grown on different tannery sludge applications: Effects of treated wastewater and ground water used for irrigation. *Environ Geochem Hlth.,* **30**, 407–422 (2008).
Srivastava, S., S. Mishra, S. Diwedi, V.S. Baghel, S. Verma, P.K. Tondon, U.N. Rai and R.D. Tripathi: Nickel phytoremediation potential of broad bean, *Vicia faba* L. and its biochemical responses. *Bull. Env. Contam. Toxicol*., **74**, 715-724 (2005).
Sudhakar, D., K.K. Panda and B.B. Panda: Biomonitoring of low levels of mercurial derivatives in water and soil by *Allium* micronucleus assay. *Mutat. Res.,* **203**, 11-23 (1998)
USEPA: United States Environmental Protection Agency, Effluent Guidelines and Standards. Sub-Chapter N, Parts 400-424, USA (2002).