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Use of tannery effluent for irrigation: an evaluative study on the response of antioxidant defenses in maize *(Zea mays)*

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Abstract: The present study aimed at studying the effect of tannery effluent, which contained chromium beyond non-permissible limits, on the antioxidant defenses of *Zea mays*. The seeds were treated with diluted (50%) and undiluted tannery effluent and the alterations in the germination physiology and enzymic antioxidant levels were studied. The seed germination percentage and seedling growth remain unaltered in the treated seeds when compared to the controls. However, the levels of the enzymic antioxidants namely superoxide dismutase, catalase and glutathione peroxidase were significantly elevated. Zymogram analysis confirmed the enhanced activity of catalase in the seeds treated with undiluted effluent. There was also a significant increase in starch and protein content of the treated seeds. In conclusion, irrigation using tannery effluent exerted oxidative stress in maize seeds. Nevertheless, the diluted effluent proved to be less toxic. The study also proved that catalase can be used as a biomarker of chromium-induced oxidative stress.

Keywords: Antioxidants, catalase, chromium, tannery effluent, *Zea mays*

Introduction

The disposal of sewage water is a major problem of big cities worldwide. Several toxic elements accumulate in soils as a consequence of industrial and urban activities and because of the use of untreated sewage sludge (Mehdi *et al*., 2003). But untreated waste water irrigation on urban and suburban lands has long been practiced in several parts of the world due to its high contents of plant nutrients and due to lack of infrastructure and facilities for safe disposal. The sewage effluents are not only a rich source of organic matter but also contain heavy metals like Fe, Mn, Cu, Zn, Pb, Cr and Ni. Continuous use of such effluents for crop production can result in accumulation of these metals in the soil as well as in plants, in concentrations that may become phytotoxic (Kirkhan, 1983). Accumulation of heavy metals over prolonged time becomes hazardous to animals and human health after entering in their body systems through food chain. Heavy metal pollution of agricultural soils is one of the most severe ecological problems faced worldwide (Shukry, 2001).

The environment is under increasing pressure from solid and liquid waste emanating from the leather industry. The byproducts of the leather manufacturing process cause significant pollution unless treated in someway prior to discharge (Karunya *et al*., 1994). Tanning industrial wastes are a serious threat when they pollute streams, fresh water bodies and land. The wastes from this industry rank among the most polluting of all industrial wastes (Javaid, 2000). Chemicals such as sodium chloride, sodium sulfite, lime and chromium used at different stages of tanning process are present in the discharged effluent. When used for irrigation, the effluent containing chromium in non-permissible limits can prove to be phytotoxic. Chromium exists in two oxidation states, trivalent (Cr^{III}) and hexavalent (Cr^{VI}) chromium. Both the forms are toxic to the plants and inhibit germination, reduce growth, generate oxidative stress, decrease protein content, inhibit photosynthesis and alter enzyme activities in the exposed plants (Panda and Choudhury, 2005; Shankar *et al*., 2005). Reactive oxygen species (ROS) are produced in plants in response to the damaging effects of environmental stresses and plants have evolved a variety of antioxidant defense mechanisms in response to stress (Jung and Kuk, 2003). The present work aimed at investigating the impact of diluted and undiluted tannery effluent on seed germination physiology of maize. Antioxidants were studied as biomarkers of the stress induced by treatment with effluent which contained chromium beyond permissible limits.

Materials and Methods

Effluent collection

The effluent (TE) was collected from a discharge of a tannery industry located near Chennai, Tamilnadu, India. The undiluted tannery effluent was analysed for various physico-chemical parameters (Table 1). The undiluted and 50% diluted effluent was used for seed treatment.

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Table 1. Physical and chemical examination of undiluted tanyard effluent

Parameters	Result
Physical Examination	
Appearance	Brownish
Total Dissolved Solids (mg/L)	17328
Electrical Conductivity (Sm^{-1})	2.3
Chemical Examination (mg/L)	
Sodium	3950
Potassium	350
COD	5174
BOD	1600
Zinc	0.198
Chromium	92.32

Seed germination and treatment

A local variety of maize (*Zea mays* L.) was used in the study. The seeds were surface sterilized by soaking in 1% sodium hypochlorite for 5 min and then washed thoroughly with distilled water. The seeds were divided in to three sets. One set of seeds were allowed to imbibe tap water for 3 hours, the second set of seeds were exposed to undiluted TE while the third set of seeds were allowed to imbibe diluted TE. All the sets of seeds were placed in Petri dishes layered with filter papers above and below the seeds. The filter papers were kept moist with either tap water or TE depending on the treatment.

Seed homogenate and biochemical assays

The emergence of radical was considered to be the first day of germination. All the control and exposed seeds were homogenized on the eighth day of germination. 10 seeds each of control and treatment groups were homogenized with 20 ml of 0.1 M phosphate buffer, pH 7.4 and the homogenates were vortexed well for 10 min. The homogenates were then centrifuged for 10 min and the supernatant was used for further studies. Starch was estimated by anthrone method according to Bau *et al*. (1970). Superoxide dismutase (SOD) was assayed by the method of Marklund and Marklund (1974). Catalase (CAT) activity was estimated by the method of Sinha (1972). Glutathione peroxidase (GPx) was assayed by the method of Rotruck *et al*. (1973) and total proteins were determined by Bradford (1976). The zymogram activity of catalase was performed as described by Woodbury *et al*. (1971).

Statistical analysis

All the experimental results were expressed as mean±S.D (n=10). The results were subjected to ANOVA using SPSS version 11.5 (SPSS Inc, New York, USA). A value of $p < 0.05$ and $p < 0.01$ were considered to be highly significant and significant respectively.

Results and Discussion

The impact of tannery effluent, which contained chromium beyond permissible limits, on the seed germination, seedling growth, starch and protein content and the response of antioxidants like SOD, CAT and GPx was assessed in maize (*Zea mays*) seeds. The germination percentage was maximum (95%) in both the control seeds and the seeds treated with 50% diluted TE. The germination was delayed by a day in the seeds treated with undiluted TE. Thangavel and Balagurunathan (2002) have reported an increase in germination percentage in seeds treated with 75 times diluted tanyard effluent. However, all the germinated seeds in our study exhibited no notable difference in the length of the radicle.

The starch content was found to increase in the treatment group when compared to the control seeds. Plants have been found to accumulate starch when exposed to environmental stress. The increase in starch content was highly significant in seeds treated with undiluted TE ($p < 0.05$), thus implicating the oxidative stress exerted by the chromium present in the TE. A significant decrease in protein content was observed in seeds exposed to undiluted TE ($p < 0.01$). This is due to increased mobilization of protein reserves of the plant under stress conditions. However, the increase in starch and protein content observed in seeds treated with diluted TE can be attributed to the organic sludge and microflora present in them. Shurky *et al*. (2001) have reported increase in soluble nitrogen content in wheat grains when irrigated with industrial effluents. Abiotic stresses are major factors which influence crop productivity. There are studies which report decrease in starch and protein content in seeds exposed to effluents (Singh *et al*., 2002; Trivedi and Shukla, 2002).

Detoxification of metals in plants is conducted by major antioxidant systems namely SOD and catalase. Therefore, the levels of antioxidant enzymes namely SOD, CAT and GPx were determined in the control and treated maize seeds. A highly significant increase ($p < 0.05$) in the levels of all the three antioxidant enzymes was observed in the seeds exposed to undiluted effluent. Nevertheless, the extent of elevation in the activity of anti-oxidants ($p < 0.01$) observed in the seeds treated with diluted TE was not detrimental as that obtained with undiluted effluent treatment (Table 2). Tolerance to environmental stress has been correlated with increased antioxidant levels. Even under optimal conditions, ROS such as superoxide radical (O_2^-), hydrogen peroxide ($H_2O_{2)}$ and the hydroxyl radical (OH), are generated as by-products of normal metabolism in different subcellular compartments including the chloroplasts, mitochondria, peroxisomes and the plasma membrane-linked electron transport system (Elstner,

Table 2. Alterations in biochemical parameters and antioxidant enzymes in seeds treated with tannery effluent (TE)

Parameter	Control	Undiluted TE**	Diluted TE*
Starch	9.25±0.72	11.58±1.70	9.71±0.64
Protein	89.96±3.99	72.74±7.7	97.95±1.3
SOD	23.88±6.18	35.99±6.04	25.92±7.5
CAT	0.148±0.046	0.289±0.093	0.156±0.046
GPx	0.022±0.003	0.0456±0.008	0.0266±0.004

All the values are expressed as mean ± S.D, n=10.
* $p < 0.05$; ** $p < 0.01$

1991; Asada, 1994; del Rio, 1998). These ROS can damage DNA, proteins, chlorophyll and membrane functions. Further more, the imposition of biotic or abiotic stress may give raise to an excessive concentration of ROS, resulting in oxidative damage at a cellular level. To migrate and repair damage initiated by ROS, plants have developed complex antioxidant systems (del Rio, 2002). The ROS formed as a consequence of exposure to TE are quenched by enzymic and non-enzymic antioxidant defenses. SOD is a prime enzyme which dismutates the superoxide anions to H_2O_2; the toxic H_2O_2 is utilised by CAT and GPx. Thus the cells are protected from damage that ensue oxidative stress exerted by ROS.

The zymogram study performed to study the activity of catalase showed increased catalase activity in the seeds treated with undiluted TE. Three isoforms of catalase are present in maize, CAT 1, CAT 2 and CAT 3. The expression of catalase isozymes is tissue and development specific; CAT 1 is the only isozyme expressed after imbibition and germination (Wadsworth and Scandalios, 1990). The activity staining performed with maize control and TE treated seeds showed a relatively increased level of catalase expression in the seeds treated with undiluted TE (Figure 1). This indicates the oxidative stress exerted by the undiluted effluent in the seeds. Evaluation of the effect of effluents on plant quality and yield has been well studied and reported (Bhati and Singh, 2003; Pandey, 2006; Singh and Singh, 2006). However, these documents do not report any analysis on plant antioxidants defenses as biomarkers.

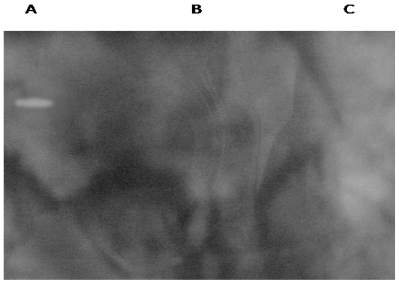

Figure 1. Zymogram exhibiting catalase activity
Lane A – undiluted TE: Lane B – control: Lane C – diluted TE. The achromatic band in lane A indicates catalase activity. Catalase activity was not detectable in lanes B and C

Conclusions

From our present study, we conclude that undiluted tannery effluents deteriorate the nutritional quality of plants when used for irrigation. Nevertheless, diluted effluents improve plant yield with a very low compromise with the nutrient quality and thus the metals and organic components of the effluent may be beneficial if present in permissible limits. The antioxidants present in the crops are good indicators of the oxidative stress exerted on them and catalase proved to be an excellent biomarker of chromium-induced oxidative stress.

References

Asada, K. 1994. Production and action of active oxygen species in photosynthetic tissue. In Foyer, C. H. and Mullmeaux, P. M. (Eds.) Causes of Photooxidative Stress and Amelioration of Defense System in Plants, p. 77-107. Florida: CRC Press.

Bau, L. C., Palmiano, E. P., Perez, C. M. and Jutliano, B. O. 1970. Enzymes of starch metabolism in the developing rice grain. Plant Physiology 46: 429-434.

Bhati, M. and Singh, G. 2003. Growth and mineral accumulation in *Eucalyptus camaldulensis* seedlings irrigated with mixed industrial effluents. Bioresource Technology 88: 221-223.

Bradford, M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry 72: 248–254.

del Rio, L. A., Corpas, F. J., Sandalio, L. M., Palma, J. M., Gomez, M. and Barroso, J. B. 2002. Reactive oxygen species, antioxidant systems and nitric oxide in peroxisomes. Journal of Experimental Botany 53: 1255-1272.

del Rio, L. A., Pastori, G., Palma, J. M., Sandalio, L. M., Sevilla, F., Corpas, F. J., Jimenez, J., Lopez-Huertas, E. and Heranandez, J. A. 1998. The activated oxygen role of peroxisomes in senescence. Plant Physiology 116: 1195-1200.

Elstner, E. F. 1991. Mechanisms of oxygen activation in different compartments of plant cells. In Pell, E. J. and Steffen, K. L. (Eds.) Active oxygen species, oxidative stress and plant, p. 13-25. Maryland: The American Society of Plant Physiologists.

Javaid, A., Ashraf, S. and Bajwa, R. 2000. Effect of tannery industrial effluents on crop growth and VAM colonization in *Vigna radiata* (L) *Wilczek* and *Zea mays* L. Pakistan Journal of Biological Sciences 3: 1292-1295.

Jung, S. and Kuk, Y. 2003. The expression level of a specific level of a catalase isozyme in maize mutants alters catalase and superoxide dismutase during norflurozon-induced oxidative stress in scutella. Journal of Pesticide Science 28: 287-292.

Karunya, L. S., Renuga, G. and Paliwal, K. 1994.

Effects of tannery effluent on seed germination, leaf area, biomass and mineral content of some plants. Bioresource Technology 47: 215-218.

Kirkhan, M. B. 1983. Study on accumulation of heavy metals in soils receiving sewage water. Agriculture Ecosystems and Environment 9: 251-255.

Marklund, S. and Marklund, G. 1974. Involvement of the superoxyde anion radical in the auto oxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry 47: 469-474.

Mehdi, S. M., Abbas, G., Sarfraz, M., Abbas, S. T. and Hassan, G. 2003. Effect of industrial effluents on mineral nutrition of rice and soil health. Pakistan Journal Applied Sciences 3: 462-473.

Panda, S. K. and Choudhury, S. 2005. Chromium stress in plants. Brazilian Journal of Plant Physiology 17: 131-36.

Pandey, S. N. 2006. Accumulation of heavy metals (Cd, Cr, Cu, Ni and Zn) in *Raphanuc sativus* L and *Spinacia pleracea* I. plants irrigated with industrial effluent. Journal of Environmental Biology 27: 381-384.

Rotruck, J. T., Pope, A. C., Ganther, H. E., Swansom, A. B., Hateman, D. G. and Hoekstra, N. G. 1973. Selenium biochemical role as component glutathione peroxidase purification and assay. Science 179: 558-590.

Shanker, A. K., Carlos Cervantes, T., Loza-Tavera, H. and Avudainayagam, S. 2005. Chromium toxicity in plants. Environment International 31: 739-753.

Shukry, W. M. 2001. Effect of industrial effluents polluting the river Nile on growth, metabolism and productivity of *Triticum aestivum* and *Vicia faba* plants. Pakistan Journal of Biological Sciences 4: 1153-1159.

Singh, A., Agrawal, S. B., Rai, J. P. N. and Singh, P. 2002. Assessment of the pulp and paper mill effluent on growth, yield and nutrient quality of wheat (*Triticum aestivum* L.). Journal of Environmental Biology 28: 283-288.

Singh, V. K. and Singh, J. 2006. Toxicity of industrial wastewater to the aquatic plant *Lemna minor* L. Journal of Environmental Biology 27: 385-390.

Sinha, A. K. 1972. Colorimetric assay of catalase. Analytical Biochemistry 47: 389-94.

Thangavel, P. and Balagurunathan, R. 2002. Effect of tannery effluent on germination and growth of certain crop plants. Bulletin of Pure and Applied Sciences 21: 21-25.

Trivedi, G. K. and Shukla, O.P. 2002. Role of microflora in environment monitoring and pollution control. (The) Botanica 52: 83-87.

Wadsworth, J. G. and Scandalios, J. G. 1990. Molecular Characterization of a Catalase Null Allele at the Cat3 Locus in Maize. Genetics 125: 867-872.

Woodbury, W., Spenser, A. K. and Stahmann, M. A. 1971. An improved procedure using ferricyanide for detecting catalase isoenzymes. Analytical Biochemistry 44: 301-305.