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Research Article

STUDIES ON BIO-CHEMICAL EFFECTS OF CHROMIUM ON THE PLANTS GROWING AT TANNERY EFFLUENT DISPOSAL SITE BY MOLECULAR TECHNIQUES

[*1]P. Natarajan, [1]B. Kaleeswaran and [2]R. Jenni

[1]Department of Zoology and Bio-Technology, AVVM Sri Pushpam College, Poondi-613 503, Tamil Nadu, India

[2]Department of Zoology Thiru. Vi. Ka. Govt Arts College, Tiruvarur-610 003, Tamil Nadu, India

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ABSTRACT

Understanding the status of heavy metal pollution is the basic idea for remediating the environment. Effluents discharged with heavy metals from tanneries are seriously concerned with the physiological and bio-chemical processes. Plants growing on industrial effluent–contaminated soul accumulate potentially toxic elements from the soil. The present study deals with the chemical composition of tanneries and various tests like Bio-chemical as pay plants growing on tannery effluent site were tested by various test like Bio-Chemical Assay, DNA isolation, Protein isolation, Genonic DNA were also conducted.

Keywords: Bio-chemical, Chromium, Plants growing, Tannery effluent disposal site, Molecular techniques.

INTRODUCTION

Environmental pollution has become an urgent problem throughout the world over the recent decades. Many industrial processes are major contributors to extensive environmental pollution (Cunningham *et al.*, 1995); Excessive agricultural and industrial activities adversely affect biodiversity, threatening the survival of species in a particular habitat as well as posing disease risks to humans. One of the most serious environmental stresses is the harmful effect of heavy metals as it affects various physiological and biochemical processes, modifying many metabolic pathways.

Heavy metal pollution is not only affects the production and quality of crops, but also influences the quality of the atmosphere and water bodies, and threatens the health and life of animals and human beings by way of the food chain. Most severe is that this kind of pollution is covert, long term and non-reversible (Zhang, 1998). Removal of the heavy metals from the environment in order to avoid their entrance into the food chain is important for protecting the health of animals and human beings. But, understanding the status of heavy metal pollution is the basic idea for remediating the environment.

Plants growing on industrial effluent-contaminated soil accumulate potentially toxic elements (PTE) from the soil and to some extent from air. Lead (Pb), Chromium (Cr), Nickel (Ni), and Cadmium (Cd) are toxic even at very low concentrations.

Variation in nutrient absorption among crop varieties was most likely not caused by a single biological mechanism (Chang *et al.*, 1982). Metal variation in plants is due to availability of metals to plants that depend on total concentration in the soil and firms in which they occur (Roberts and Johnson, 1978).

Therefore, plants are of special importance in a chemically polluted environment, excessive amounts of toxicants are involved into biological cycles. The role of the formation of biogenic cycles of chemical elements is determined by a number of factors, including the concentrations of elements in aboveground and underground organs, their biomass, and their mineralization rates.

Leather is a major industrial product made from the natural fabric (the skin) and its processing has evolved from a traditional artisanal practice to an industrial activity. Intensive leather production in small clusters has caused environmental concern. An environmental challenge from

*Corresponding author address: Associate Professor, Department of Zoology and Bio-Technology, AVVM Sri Pushpam College, Poondi-613 503, Tamil Nadu, India, Email: natrajpushpam@gmail.com, Mobile: +91 9443421546.

leather processing arises from the nature and quantity of the chemicals used along with the amount of wastes generated and discharged (Cooman *et al*., 2003). The use of tannery wastewater is a major problem due to the presence of pollutants particularly metals, however, it is being used for the irrigation of agricultural crops and vegetable. Long term irrigation can induce changes in the quality of agricultural soil and trace element inputs which sustained over long period (Barman *et al*., 2000; Gothberg *et al*., 2002; Sinha *et al*. 2005, 2006).

Environmental pollution during leather processing is an industrial problem in some countries, such as China and India, where leather is a major export commodity. Hazardous chemicals such as lime, sodium sulphide, solvents, etc., arise mainly from the pre-tanning stages of leather processing. Therefore, to overcome these hazards, cleaner and more eco-friendly technology for leather processing and effluent treatment need to be established.

A variety of environmental stresses like soil salinity, drought, extremes of temperature and heavy metals are known to cause oxidative damage to plants either directly or indirectly by triggering an increased level of production of reactive oxygen species (ROS). These ROS include superoxide radical (O_2 +J, hydroxyl radical (OH+) and hydrogen peroxide (H_2O_2) that are produced as by products during membrane linked electron transport activities as well as by a number of metabolic pathways and in turn cause damage to the bimolecular such as membrane lipids, proteins, chloroplast pigments, enzymes, nucleic acids, etc. To combat the oxidative damage plants have the antioxidant defense system comprising of enzymes.

The harmful nature of industrial effluents in relation to plant growth and development is well recognized owing of the presence of toxic chemicals in it. Agricultural production in several countries is being heavily affected by the reckless discharge of the effluents to the water bodiesnear the industrial establishments, which are the main sources of irrigation. Research studies to lessen the deleterious effects of industrial effluent on the environment, and to evolve suitable treatments prior to its disposal are generally recommended.

Chromium is usually associated with oxygen as chromate (or) dichromate oxyanions. Chromium (III) is less mobile, less toxic and is mainly found bound to organic matter in soil and aquatic environment. It was suggested by Becquerel *et al* (2003).

Jing dong (2007) recorded that increasing chromium levels to 200-800 jtm induced a significant decrease in plant height and biomass, but no significant injury was detected. Also it induced significant increase in super oxide dismutase (SOD) and peroxides activities Kumkum Mishra *et al* (2009) reported that the lower doses of chromium had stimulatory effect on various metabolic activities in plants including chlorophyll, protein, nitrate reductase and mitotic index.

Chromium is most toxic form and mutagenic because of its high solubility, ability to penetrate the cell membranes and strong oxidizing ability. This was studied by Shanker *et al.* (2005).

The present study was aimed to investigate the effect of tannery effluent site growing plants tested by various parameters like biochemical assay, DNA isolation, protein isolation and genonic DNA.

MATERIALS AND METHODS

Collection of Samples

The study involves a site contaminated with tannery effluent of Gundur near Bharathidasan University, Tiruchirappalli. The soil in the study site has been prone to effluent contamination over a period of 10 years. The plants *Ficus religiosa* (Figure 1), neem tree (Figure 2) and five-leaved tree (Figure 3), around the study area within a radius about 100 meters are collected. Control samples were also collected for analysis. It is then transported to the laboratory to carry out further analysis.

Figure 1. Leaves of *Ficus religiosa.*

Figure 2. Leaves of neem tree.

Figure 3. Leaves of five-leaved chaste tree.

Biochemical assay

The plants were tested for their antioxidant enzymes particularly SOD, LPO and Catalase (Kakkar *et al.*1984; Bergmeyer, 1983).

Protein isolation

Protein estimation was carried out by Lowry *et al.* (1951) method.

DNA isolation

DNA of the samples was isolated by means of the described procedure. Analysis of the isolated DNA is done by using a Gel documentation system. The DNA is run on an Agarose gel electrophoresis and the purified DNA is used for further analysis (Taylor and Powell, 1982).

Genonic DNA isolation

Spinned 1.5 ml culture for 30-60 sec, and decanted thesupernatant leaving 50-100micro liter in tube, then vortex to resuspended. Added 300 micro liter TENS and vortex for 5 sec. Added 150 micro liter of sodium acetate and vortex for 5sec, and then spinned for 2 min in micro centrifuge. Transfered supernatant to a tube and add 0.9 m1 of pre chilled 100% ethanol and spinned for 5 min. Then discarded the supernatant and added 90% ethanol, then air dried the pellet. Resuspended pellet in 30 micro liter distilled water and keep it at 4°C/20°C. (Taylor and Powell, 1982).

RESULTS AND DISCUSSION

Biochemical Assays

The Central Pollution Condition Board has devised strict regulations for the discharge of Tannery effluent. Depending on the types of Tannery industry, the discharge standards are as follows. The levels of major physio-chemical parameters observed in the tannery effluent are shown in the Table 1. Chromium is the most abundant heavy metal present in the tannery effluent. It was found to be present in the concentration of 2 mg/1 while the maximum permissible limit is 0.1mg/l.

The amounts of antioxidant enzymes particularly SOD, LPO and Catalase in the plant I. LPO levels were found to increase with the increase in time. Compared to the control plants, LPO Level was found to be much higher in test sample (TI).

SOD level in Test sample T2 was found to be 10 times higher than the control. This might be due to the increased production of LPO and to counter them. The level of Catalase was also found to be higher than the control. Being free radical scavengers the amount of antioxidants are found to be present in elevated levels.

Table 1. Chemical characterization of tannery effluent.

Type of tanneries	Chrome tanneries / combined Chrome & Vegetable tanneries	Vegetable Tanneries	Chrome tanneries
pH	6.5-9.0	6.5-9.0	6.5-8.5
SS	Below 600	Below 600	-
Chromium Concentration	45	-	-
BOD	100	-	100
TDS	100	-	45
Total Chromium	2.0	-	0.10

Concentration limit not exceed, mg/1 (except pH).

Protein Isolation

Extraction of proteins and its subsequent running on PAGE confirmed the presence of different banding pattern (Figure 4).

A 48 KDa protein submits has been indentified from the Plant. 1. Earlier reports suggesting its involvement in the remediation process has been known. Further detailed work on the protein subunit could be carried out. The different proteins that are present could be extracted, purified and used for further analysis. The importance of any subunit of protein differing from the normal could be further investigated for its involvement in the remediation process.

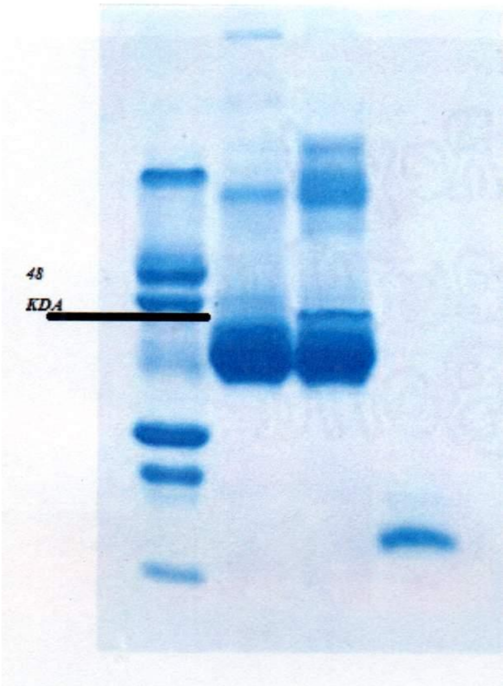

Figure 4. Protein Isolation.

DNA Isolation

The Figure 5 shows the bands of DNA isolated from the plants.

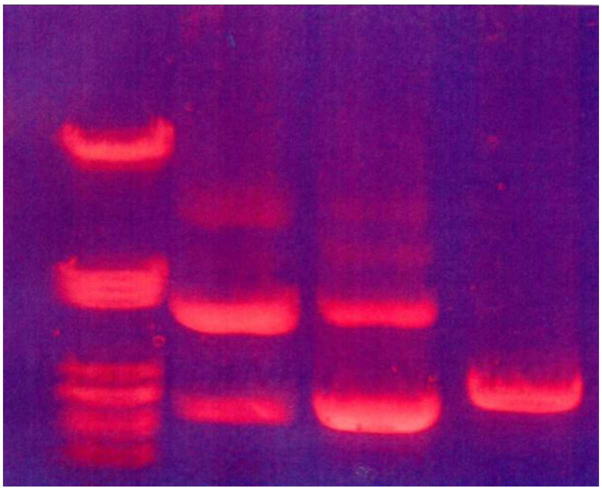

Figure 5. SDS PAGE.

CONCLUSION

Three plants growing in the vicinity of Tannery effluent contaminated site has been used for the present study. The response of antioxidants like SOD, LPO and Catalase has been observed with respect to heavy metal stress. The increase in LPO could be linked to the release of free radicals. RFLP analysis by HinIII shows different fragments of the species indicating its diversity. Protein studies show the resultant changes in banding pattern of the species.

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