

# Influence Of Tannery Wastewater Irrigation On Growth And Enzymatic Activities Of Ipomoea Pes-Caprae Sweet And Clerodendron Inerme (L.) Gaertn.

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**Abstract:** The present study deals with the exogenous addition of various concentrations of tannery wastewater on growth and enzymatic activity of Ipomoea pes-caprae and Clerodendron inerme was assessed. The morphological parameter such as shoot and root length, fresh and dry weight of both plants organ increased upto extreme level of 90% when compared to that of control on both the sampling day. The enzymatic studies such as peroxidase, catalase, polyphenoxidase, phytochelaton and glutathion increased with increasing tannery effluent at 90% on both the sampling day. The aim of this study to analyze these two halophytic plants accumulate more heavy metal stored in plant organ.

**Index Terms:** Catalase, Wastewater, Phytochelaton, Seedlings.

## 1 INTRODUCTION

Halophytic species accumulate high concentration of salt in cells and tissues and overcome salt toxicity by developing succulence, environmentally, the plant has a remarkable ability to service under different abiotic stress including salinity, heavy metals and drought (Slama et al., 2008). Halophytes usually have high tolerance to heavy metals compared with common plants. Tolerance of halophytes to salt and heavy metal may atleast in part, possess common physiological mechanisms. Since heavy metals induce both secondary water stress and oxidative stress in plants and the capability of halophytic plants to synthesize organic compatible solutes may allow them to tolerate heavy metals (Manousaki and Kalogerakis, 2011b). The accumulation of heavy metals and its biochemical responses in *Salicornia brachiata* induced changes in amino acids and protein levels (Sharma et al., 2010). In *Aeluropus littoralis* reported that the non-enzymatic antioxidant compound such as phenol and proline significantly increased under higher concentrations of heavy metals (Cd and Pb) and also suggested that halophytic plants have high tolerance under high concentrations of heavy metals (Rastgoo and Alemzadeh, 2011). Accumulation of heavy metals leads to stress condition in the plant system by interfering with the metabolic activities and physiological functioning of plants. Heavy metal stimulate the formation of reactive oxygen species (ROS) such as superoxide radicals ( $O_2^{\cdot-}$ ), hydroxyl radicals ( $OH^{\cdot}$ ) and hydrogen peroxide either by transferring electron involving metal cations or by inhabiting the metabolic reactions controlled by metals. In order to survive under the conditions, plant have enzymatic and non-enzymatic antioxidants to scavenge free radicals and reactive oxygen species (Gratao et al., 2005). The activities of CAT, POD and SOD are usually enhanced by heavy

metal stress such as Cd,  $Cr^{6+}$ , Hg, Ni, Pb and Fe in low concentration but as the concentrations of heavy metals increases the activities of enzymes. Polyphenoloxidase is an oxidoreductase that catalyses the oxidation of phenols to quinones and its activity has been shown to increase under heavy metal stress and has been associated to some form of defence mechanism. Increase polyphenoloxidase activity along with peroxidase in generally reported is species under various environmental stress such as *Aegiceras corniculatum* (Parida et al., 2004b), *Suaeda fruticosa* (Hameed et al., 2012) and *Salicornia brachiata* (Venkatesan and Sridevi, 2009). Phytochelatin are synthesized non-translationally from reduced glutathione (GSH) in a transpeptidation reaction catalyzed by the enzyme phytochelation synthase (PCS). This reactivity along with the relative stability and high water solubility of GSH makes it an ideal biochemical to protect plant against stresses including oxidative stress, heavy metal stress and certain exogenous and endogenous organic chemicals (Rausch et al., 2007). Several studies have indicated that exposure of plants to high level of heavy metals induces ROS, either directly or indirectly by influencing metabolic processes. GSH participate in the control of  $H_2O_2$  level of plant cells (Shao et al., 2005). Change in the ratio of its reduced (GSH) to oxidized (GSSG) from during degradation of  $H_2O_2$  is important in certain redox signaling pathways. The aim of the present study to critically evaluate the fast growing halophytes to assess the heavy metal and NaCl bioaccumulation from tannery wastewater and also evaluate the growth, enzymatic activities of these two halophytic species.

## 2 MATERIALS AND METHODS

The experiment was conducted in an open air area with natural light, temperature and humidity. Red soil and sand in the ratio of 3:1, free from pebbles and stones were filled polythene layer. The uniform sized cuttings/seedlings of Ipomoea pes-caprae Sweet and Clerodendron inerme Gaertn. were collected from Pichavaram area, Cuddalore district, Tamilnadu. The seedlings were transplanted from nursery bed with maintain polythene bags. The experiment comprised of the following three set of treatments with five

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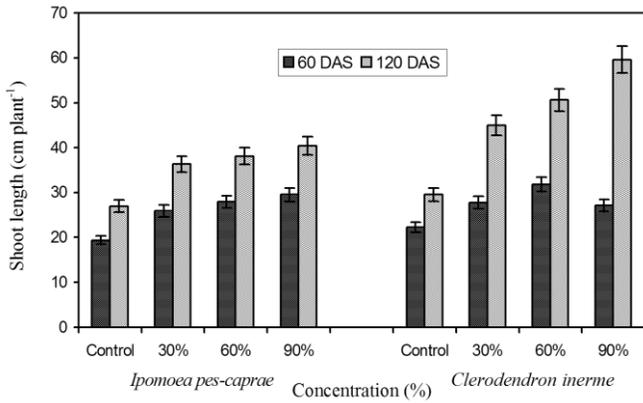
replicates and average values are reported. The plants were treated for every 2-3 days depending on the evaporative conditions. The plant samples were harvested for experimental purpose at bimonthly intervals (60 and 120 days). Physical and biochemical characteristics of tannery wastewater, soil and halophytes were determined before planting and harvesting. The control plants treated without addition of effluent (only tap water). The effluent treatment for various concentration of 30, 60 and 90% were prepared in 1 litre and allow 250 ml for four times. The shoot and root length were measured by scale ( $\text{cm plant}^{-1}$ ) and the fresh and dry weight of the plant organs weighed by electronic balance.

The catalase (EC 1.11.1.16) activity was analyzed by the method of Maechly and Chance (1967). The peroxidase and polyphenol oxidase analyzed by the method of Kumar and Khan (1982). The phytochelatin enzyme (EC 2.3.2.15) assay was followed by Glazer et al. (1975). The glutathione enzyme assay (EC 6.3.2.2) was followed by Anderson (1985).

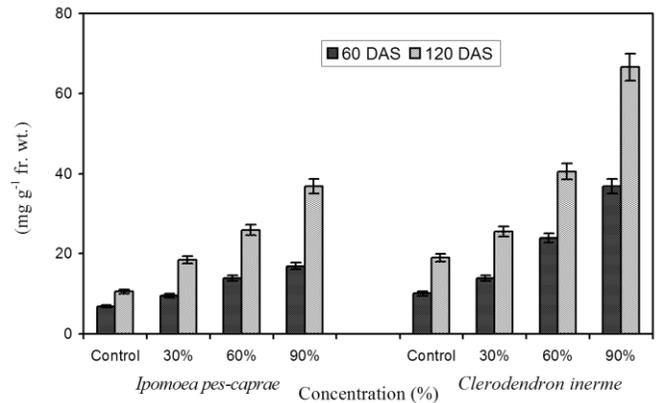
### 3 RESULTS AND DISCUSSION

The results on the effect of various concentration of tannery wastewater for two fast growing halophytes at 60 and 120 day after treatments (Figs. 1 and 2). The maximum shoot and root length was observed in *Ipomoea pes-caprae* ( $40.4$  and  $10.6 \text{ cm plant}^{-1}$ ) and *Clerodendron inerme* ( $59.6$  and  $8.6 \text{ cm plant}^{-1}$ ) increased with increasing concentrations upto 90% on 120 day sampling. The similar trends also observed in the fresh weight and dry weight of *Ipomoea pes-caprae* ( $99.86$  and  $36.88 \text{ g plant}^{-1}$ ) and *Clerodendron inerme* ( $168.55$  and  $66.58 \text{ g plant}^{-1}$ ) in 120 day after treatment (Figs. 3 and 4). Significant increase in growth of both the plants in possibly due to the utilization of heavy metals and NaCl by experimental plants in trace elements. The primary toxicity mechanism may be due to heavy metals were shown as altering the anabolic function of enzymes, increasing number of cell division, increasing the activity of photosynthesis, protein synthesis and respiration (Kupper et al., 1996). Increase in fresh weight in the leaves was mainly due to an increase in tissue water content and can be a good reason for tissue succulence. In the present study increase in dry weight may be attributed to the accumulation of inorganic and organic constituents in plant tissue. The similar observation was recorded some other reviewers such as *Sesuvium portulacastrum* (Lokhande et al., 2012); *Suaeda altissima* (Meychik et al., 2013), glycophytic species *Raphanus sativus* (Chitra, 2017) and *Ipomoea pes-caprae* (Venkatesan, 2019). The data on the effect of tannery wastewater increase the enzyme activity of catalase, peroxidase, phytochelatin and glutathione level upto extreme concentrations of 90% on both the sampling days (Figs. 5 to 8). The maximum activity was found in *Ipomoea pes-caprae* (catalase 2.958; peroxidase 1.688, polyphenoloxidase 1.289, phytochelation 0.711 and glutathione  $0.501 \text{ units min}^{-1} \text{ mg}^{-1} \text{ protein}$ ) and in *Clerodendron inerme* (catalase 2.499, peroxidase 1.569, polyphenol oxidase 1.112, phytochelation 0.699, glutathione  $0.465 \text{ units min}^{-1} \text{ mg}^{-1} \text{ protein}$ ) on 120<sup>th</sup> day after wastewater treatment when compared to control. Catalase is one of the most important components of plant protective mechanism that exist in mitochondria and peroxidase and has important role in scavenge free radicals specially  $\text{H}_2\text{O}_2$

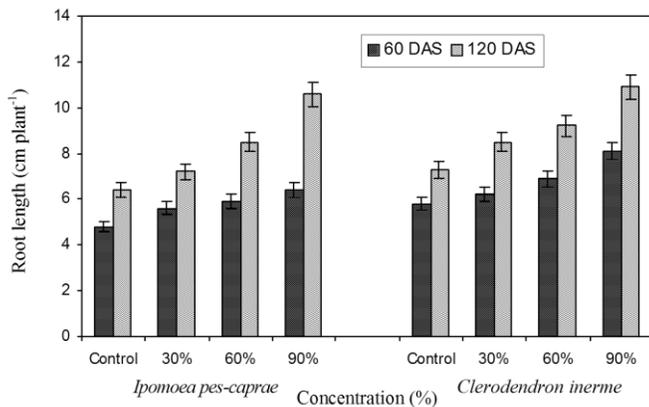
generated during photorespiration and stress conditions. In the present work, increase in catalase activity can be considered as a circumstantial evidence for the role of catalase in detoxification of  $\text{H}_2\text{O}_2$  that induced under heavy metal stress. Similar report on increased catalase activity was made in at higher salinities *Sesuvium portulacastrum* (Lokhande et al., 2012), *Salicornia brachiata* (Venkatesan and Sridevi, 2009), *Zygophyllum simplex* (Sharma and Ramahat, 2014). POD is considered to be a marker of heavy metal toxicity, having broad specificity for phenolic substrate and higher affinity for  $\text{H}_2\text{O}_2$  than CAT (Radwan et al., 2010). The increased POD and PPO activity under high saline condition has been reported in the halophyte under various environmental stress in *Salicornia brachiata* (Venkatesan and Sridevi, 2009) and *Halimione portulacoides* (Durcrate et al., 2012). A strong antioxidant defense system along with efficient ion regulation, production of compatible solutes and the maintenance of photosynthesis is attributed to salt tolerance in halophytes. The high antioxidant capacity to halophytes in the present study has been suggested as one of the important reasons for the superior ability to tolerate high levels of salinity stress. Phytochelation of heavy metals is a ubiquitous detoxification on strategy described in a wide variety of plants. A principle class of heavy metals chelator known in plants in phytochelatin (PCs), a family of Cys-rich peptides. Therefore, availability of glutathione is very essential for PCS synthesis in plants atleast during the exposure of heavy metals. Chelation and sequestration of metals by particular ligands are also mechanisms used by plants to cope with metal stress (Morlos et al., 2014). Estrella-Gomez et al. (2009) suggested that the accumulation of PCs in *Salvinia minima* is a direct response to  $\text{Pb}^{2+}$  accumulation and phytochelatin participate as one of the mechanism to cope with  $\text{Pb}^{2+}$  of this Pb-hyperaccumulator aquatic fern. PCs can transport Pb to vacuoles in vascular tissues of root and shoot. Glutathione reductase is ubiquitous NADPH dependent enzyme which catalyses the reduction of oxidized glutathione present in cells. It has been well documented that the activity of glutathione reductase altered due to metal stress. Glutathione plays fundamental role in many cellular detoxification processes of xenobiotics and heavy metals. The mechanism of Pb tolerance in coontail (*Ceratophyllum demersum*) plants has also been found to be mediated by PCs (Mishra et al., 2006). An increase in cysteine and GSH content has been observed at moderate exposure of Pb. PCs were synthesized to significant level upon exposure to Pb with concomitant decrease in GSH levels. The production of PCs seem to be important for the detoxification of heavy metals. Over production of PCs may lead to the depletion of GSH and consequently causes oxidative stress. Finally, it was concluded that the two fast growing halophytes tolerate and accumulate more heavy metals and NaCl. So repeated cultivation of these halophytes is required for high removal of heavy metals and ions from tannery wastewater contaminated soil, these sedimented soil is utilized for further crop improvement.



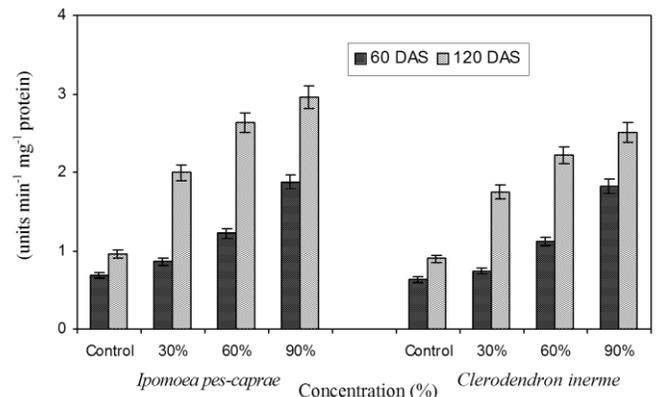
**Fig. 1.** Effect of different concentrations of tannery effluents on shoot length of *Ipomoea pes-caprae* and *Clerodendron inerme*



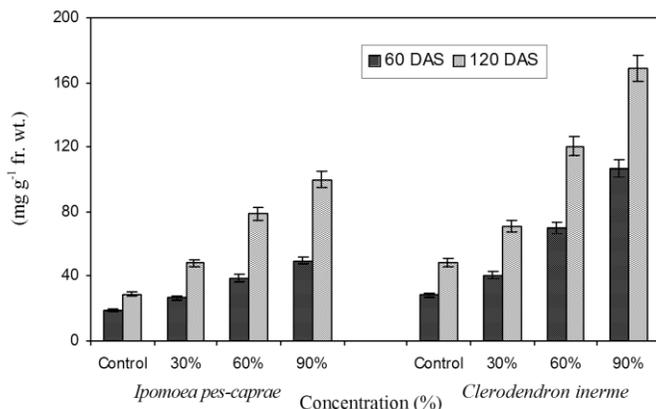
**Fig. 4.** Effect of different concentrations of tannery effluents on dry weight of *Ipomoea pes-caprae* and *Clerodendron inerme*



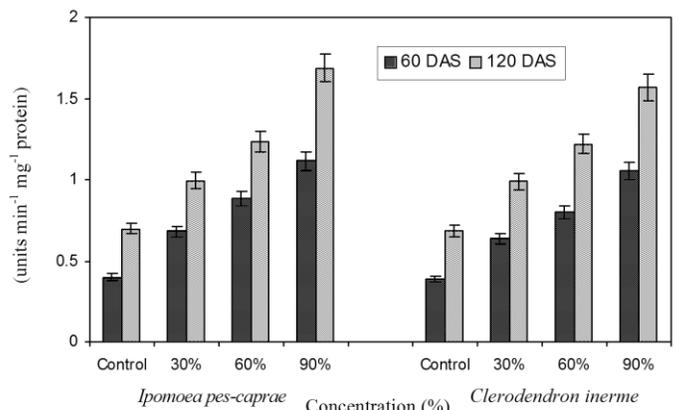
**Fig. 2.** Effect of different concentrations of tannery effluents on root length of *Ipomoea pes-caprae* and *Clerodendron inerme*



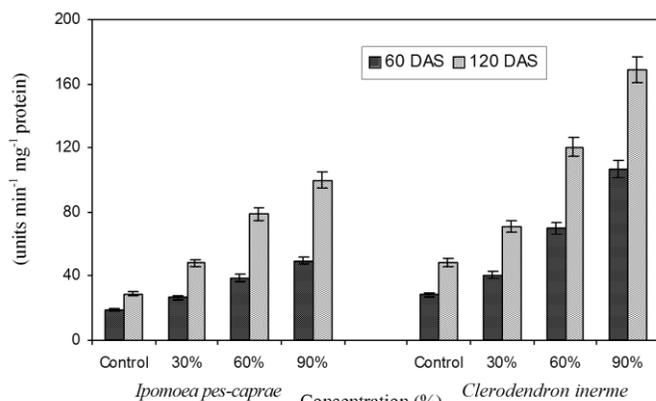
**Fig. 5.** Effect of different concentrations of tannery effluents on catalase of *Ipomoea pes-caprae* and *Clerodendron inerme*



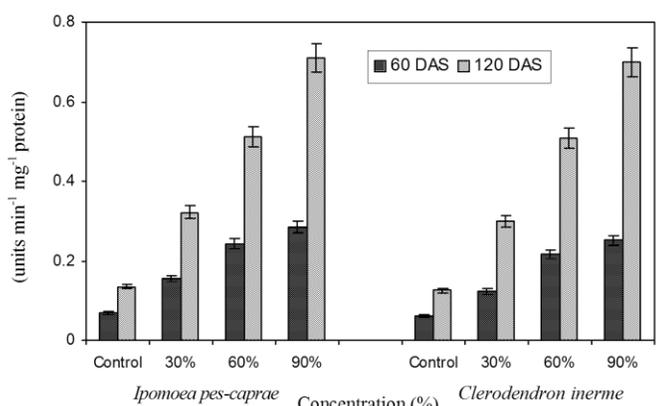
**Fig. 3.** Effect of different concentrations of tannery effluents on fresh weight of *Ipomoea pes-caprae* and *Clerodendron inerme*



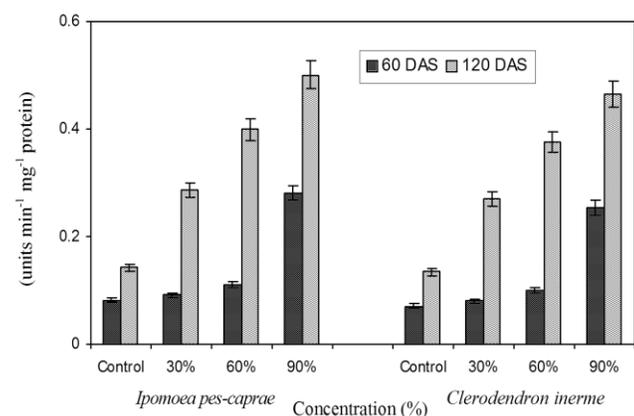
**Fig. 6.** Effect of different concentrations of tannery effluents on peroxidase of *Ipomoea pes-caprae* and *Clerodendron inerme*



**Fig. 7.** Effect of different concentrations of tannery effluents on polyphenol oxidase activity of *Ipomoea pes-caprae* and *Clerodendron inerme*



**Fig. 8.** Effect of different concentrations of tannery effluents on phytochelation of *Ipomoea pes-caprae* and *Clerodendron inerme*



**Fig. 9.** Effect of different concentrations of tannery effluents on glutathione of *Ipomoea pes-caprae* and *Clerodendron inerme*

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