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Biochemical tolerance of *Suaeda maritima* L. (Dumort) as a potential species for phytoextracting heavy metal and salt in paper mill effluent contaminated soil.

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ABSTRACT

Suaeda maritima is a halophyte that has been evolutionary adapted to cope with saline and heavy metal conditions. The aim of the present study was to examine the biochemical response of *Suaeda maritima* against phytoextracting heavy metals and salts from paper mill effluent. *Suaeda maritima* seedlings have been grown for 120 days with an irrigation solution of 250 ml of 75% raw paper mill effluent after four drenching. Analysis of biochemical parameters revealed that maximum synthesis of chlorophyll, protein, phenol, proline and glycinebetaine may be involved in their ability to cope with heavy metal and salt stress. The present study confirms that the accumulation and increase of biochemical constituents can be considered as an efficient ROS scavenger and also maintain cellular homeostasis and metabolic functions in photosynthetically active leaves in *Suaeda maritima*, during phytoextraction studies.

Keywords: phytoextraction, paper mill effluent, *Suaeda maritima*, biochemical, heavy metal and salts

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INTRODUCTION

With modern day urbanization and industrialization, heavy metal contamination has become a prime concern for today's society (Laghlimi *et al*., 2015). Industrial activities such as mining, smelting, burning of fossil fuels, dumping of municipal sewage sludge and agricultural practices related to long-term use of excessive fertilizers, fungicides, pesticides and sewage sludge have led to a dramatic increase in toxic heavy metals in numerous areas (European Environmental Agency 2007). Paper mills are chief industrial sectors utilizing a vast quantity of various cellulosic based materials and water through manufacturing process and discharge large amount of chlorinated lingo- sulphonic acids, chlorinated resin acids, phenols and hydrocarbons in the effluent (Sing 2007). The effluents which were discharged from paper mill contains huge amount of heavy metals which get accumulated and cause huge damage to plants and biological system (Mehta and Bhardwaj 2012).

Different physical, chemical and biological approaches have been employed to clean up of heavy metal and salt contaminated soil. Generally these methods suffer from limitations like high cost, intensive labour and disturbance of native soil microflora (Ali *et al*., 2013). Phytoremediation with native plants is an emerging technology and is considered as one of the best methods to remediate metal contaminated soils because of its visual advantage, extensive applicability, and cost effectiveness (Amna *et al*., 2015). The phytoremediation performance of halophytes is determined not only by its capacity to extract high metal concentration, but also by its ability to translocation the metals to aerial parts and simultaneously produce a high biomass (Ximenez-Embun *et al*., 2001). Halophytes are not only resistant to salt but also to heavy metals and resistance partly rely on common physicochemical mechanisms (Cong *et al*., 2013). Thus the present study aimed to explore the effect of paper mill effluent on biochemical parameters in order to study relationship between metal, salt toxicity and detoxification responses from *Suaeda maritima*.

MATERIALS AND METHODS

Selection of plant species

Suaeda maritima is an erect annual herb with many branches. Leaves are fleshy, long, linear and oblong and sickle shaped when young. Flowers are minute, axillary and usually hermaphrodite. Few flowers are clusters, elongated spike, stigma-3, bracteoles membranous entire, bisexual and buds are depressed. Seeds are brown and shining.

Experimental site

The experimental site was located at Botanical garden, Department of Botany, Annamalai University, Tamil Nadu, India. *Suaeda maritima* seedlings were collected from Pichavaram mangrove forest, located between Vellar and Coleroon estuaries (latitude 11^0 22' N–$11^0$30' and longitude 79^0 45' E–79^0 52') in Cuddalore District of Tamil Nadu, South India.

Collection of paper mill effluent

The raw paper mill effluent was collected from the Vadamangalam village, Villanur Taluk of Puducherry (state) in clean plastic canes and stored at 4^0C for physio-chemical analysis. The effluent was directly collected from the outlet of the paper mill industry. Analysis of physico-chemical characteristics of raw paper mill effluent and soil treated with effluent are given in Tables 1 and 2 respectively. All analytical methods followed procedures as described by American Public Health Association (APHA., 2005).

Design of the experiment

Red soil and sand (3:1 ratio) free from pebbles and stones were filled in polythene bags. The seedlings/cuttings from the selected species of similar size were transplanted from the nursery bed and planted at the polythene bags (35×20 cm). The experiment comprised of the following two set of treatments with five replicates and average values are reported.

1. Control - Without any treatment.
2. Effluent treatment- Halophytes were treated with 250 ml of 75% raw paper mill effluent for 4 times with a gap of 7 days.

The experiment was conducted in an open-air area with natural light, temperature, and humidity. Plants were watered for every 2-3 days, depending on the evaporative demand. To prevent leaching of heavy metals and salts from the polythene bags (35×20 cm) to the ground, plastic trays were placed under the each bag. Physical and chemical characteristic of paper mill effluent, soil and halophytes were determined before planting and harvesting. Plant samples were harvested for experimental purpose an intervals of 30, 60, 90 and 120 days.

Biochemical studies

Estimation of chlorophyll, soluble protein, total phenols, proline and glycinebetaine contents were estimated by following the methods of Moran and Porath (1980) using formulae suggested by Inskeep and Bloom (1985), Bradford (1976), Bray and Thorpe (1954), Bates et al., (1973) and Grieve and Grattan (1983) respectively.

Statistical analysis

All values described in this study are mean of five replicates. Analysis of variance (ANOVA) was done by using a statistical package, SPSS version- 21 and the calculated data were expressed in mean ± SD.

RESULTS AND DISCUSSION

Physico- chemical characteristics of effluent

Physico - chemical parameters of the effluent and BIS standard for tolerance limits of paper mill effluent discharge are given in Table 1. In the present study, pH was recorded as 9.40 which was above the tolerance limit of 6.5- 9.0 prescribed by bureau of Indian Standard Limit (2015). The average values of electrical conductivity (EC, 8.54 dS m^{-1}), biological oxygen demand (BOD, 138.o mg/L), chemical oxygen demand (COD, 720 mg/L) in the effluent were found significantly higher than the BIS limit which indicates the pollution load of effluent. Hazarika et al.(2007) reported that the acidity and alkalinity of paper mill effluent depends upon the various chemicals used in paper manufacturing processes.

The amount of sodium and chloride content in the effluent was 1173 and 1663 mg/L respectively. The level of potassium in the effluent was 1365 mg/L and magnesium was 437.4 mg/L. The calcium content present in the effluent was found at higher levels (900 mg/L). Chromium, Cadmium, copper and zinc contents in the effluent were found to 60.00, 56.00, 65.00 and 61.00 mg/L respectively. All these metals were present in higher concentrations compared to the prescribed limits of BIS (2015). The physico-chemical parameters of paper mill effluent revealed that paper mill effluent seems to be more polluted condition.

Table1: Physico- chemical characteristics of paper mill effluent

S.No	Parameters	Raw effluent	BIS Limit (2015)
1	Colour	Dark Brown	Dark Brown
2	Odour	Offensive	Offensive
3	pH	9.40	6.5-9.0
4	EC	8.40	NM
5	Chemical oxygen demand (mg/L)	720.00	250
6	Biological oxygen demand (mg/L)	138.00	30
7	Total dissolved solids (mg/L)	5460.00	500
8	Sodium (mg/L)	1173.00	8.00
9	Chloride (mg/L)	1663.00	NM
10	Potassium (mg/L)	1365.00	NM
11	Calcium (mg/L)	900.00	5.00
12	Magnesium (mg/l)	437.40	NM
13	Chromium (mg/L)	60.00	2.00
14	Cadmium (mg/L)	56.00	2.00
15	Copper (mg/L)	65.00	3.00
16	Zinc (mg/L)	61.00	5.00

NM: not mention

Physico-chemical characteristics of experimental soil treated by paper mill effluent

Table 2 shows, the physico- chemical characteristics of experimental soil treated by paper mill effluent. The pH and EC from treated soil was recorded as 8.10 and 5.43 dSm^{-1}. The amount of sodium and chloride content in the treated soil was found 44.31 and 40.45 mEq/L respectively. The level of potassium, calcium and magnesium in treated soil was found 26.00, 17.00 and 21.00 mEq/L respectively. The concentration of heavy metals such as chromium, cadmium, copper and zinc present in the paper mill treated soil shows 45.00, 40.00, 51.00 and 49.00 mg kg^{-1} respectively.

Table 2: Physico-chemical characteristics of paper mill effluent treated soil after four drenching

S.No	Parameters	Paper mill effluent treated soil
1	pH	8.10
2	EC (dSm^{-1})	5.43
3	Sodium (mEq/L)	44.31
4	Chloride (mEq/L)	40.45
5	Potassium (mEq/L)	26.00
6	Calcium (mEq/L)	17.00
7	Magnesium (mEq/L)	21.00
8	Chromium (mg kg^{-1})	45.00
9	Cadmium (mg kg^{-1})	40.00
10	Copper (mg kg^{-1})	51.00
11	Zinc (mg kg^{-1})	49.00

Growth and Morphology

During 120 days of cultivation, significant increase in growth characteristics was observed in *Suaeda maritima* cultivated in paper mill effluent treated soil when compared to control (figure 1).Our experimental results indicated that the presence of salts and heavy metals from the paper mill effluent negatively affects the growth of *Suaeda maritima* in terms of biomass production. Morphological analysis showed that the halophyte was more tolerant to heavy metals and salts. In the present study *Suaeda maritima* does not show any morphologica injury against paper mill effluent. In accordance to our result, Tauqeer *et al*. (2016) reported that the maximum concentration of cadmium and lead in shoots increased more than 100 mg kg^{-1} under 2.0 mM treatment suggested that increase in metal concentrations along with increase in biomass might be due to increase in the activities of antioxidant enzymes under cadmium stress. Bareen and Tahira (2011) reported that the highest biomass was observed in *Suaeda fruticosa* cultivated in field contaminated with tannery effluent. The work of Ghnaya *et al*. (2007) revealed high potential of *Sesuvium portulacastrum* to accumulate cadmium without growth retardation. In the present study, *Suaeda maritima* exhibit good adaptation to heavy metals and salts in terms of better growth, biomass and defense mechanism through accumulation of inorganic ions and development of succulence.

Figure 1: Growth characteristics of *Suaeda maritima* cultivated in control and paper mill effluent treated soil

Biochemical studies

In the present study, the biochemical parameters viz. chlorophyll, proteins, total phenol, proline and glycinebetaine showed an increasing trend in *Suaeda maritima* cultivated in paper mill effluent treated soil as compared to control. Chlorophyll and proteins was increased by (311% and 345%) respectively as compared to control (87% and 122%) as shown in figure 2. Similarly in figure 3, total phenol, proline and glycinebetaine were increased by (310%, 618% and 432%) respectively as compared to control (90%, 135% and 156%). However maximum increase was observed between 60- 90 days of cultivation period. Our results are in agreement with those reported previously in other halophytes that biochemical parameters increases with increasing salinity and heavy metals like Rastgoo *et al*. (2014) have proved that chlorophyll content in *Aeluropus littoralis* may reflect plants sensitivity to stress conditions. Bankaji *et al*. (2016) reported that leaves of *Suaeda fruticosa* treated with different concentrations of Zn^{2+} and Pb^{2+} did not cause any changes in the total chlorophyll concentration when compared to control. Tauqeer *et al*. (2016) studied an increase in protein contents in *Alternanthera bettzickiana* might be due to adaptations of this plant to metal stress. Diaz et al.(2001) reported that under heavy metals stress, proline accumulates because of their functions as intermediates in lignin biosynthesis, preserving the plant cells by building physical barrier. In *Aeluropus littoralis*, Rastgoo and Alemzadeh (2011) indicated the non enzymatic antioxidant compounds such as phenol significantly increased under higher concentrations of heavy metals and also suggested that halophytic plants have a high tolerance under high concentration of heavy metals. Shevyakova *et al*. (2003) considered that proline acts as an efficient reactive oxygen species scavenger in the facultative halophyte *Mesembryanthemum crystallinnum* exposed to Cd toxicity. Saiyood *et al* (2012) estimated that a high level of resistance to a complex mixture of inorganic pollutants in *Suaeda maritima* was directly related to a high capacity to accumulate protecting proline in the roots, stems and leaves.

Lefevre *et al*. (2009) found the presence of cadmium may trigger glycinebetaine over synthesis, which is the most efficient osmoprotectant synthesized in Chenopodiaceae. In *Kosteletzkya virginica*, NaCl and Cu have additional effects on synthesis of quaternary ammonium compounds (Han *et al*., 2013). Accumulation of glycinebetaine maintains membrane integrity and protection of other cellular structures. It stabilizes macromolecule structure and protects chloroplasts and photo system II (Hasegawa *et al*., 2000).

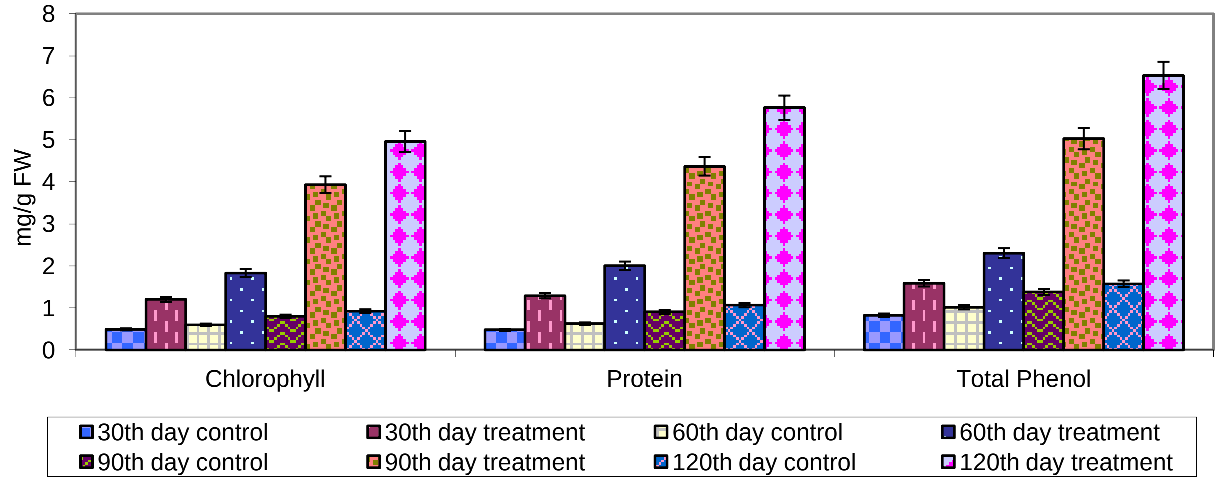

Figure 2: Chlorophyll, Protein and Total Phenol content of *Suaeda maritima* cultivated in paper mill effluent treated soil. The values are mean (±SD) of five replicates

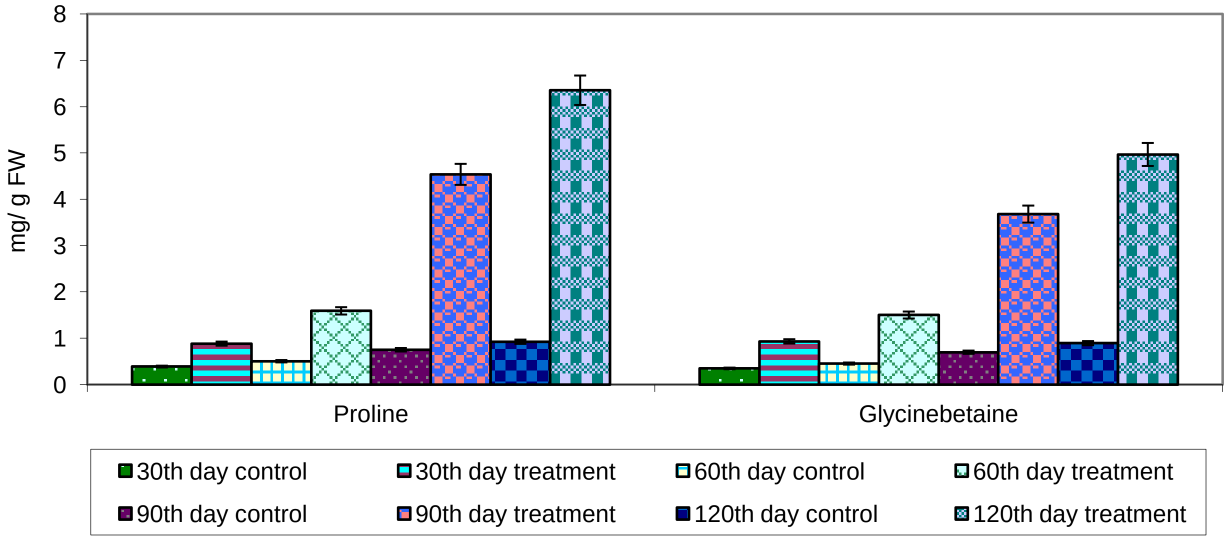

Figure 3: Proline and Glycinebetaine content of *Suaeda maritima* cultivated in paper mill effluent treated soil. The values are mean (±SD) of five replicates

CONCLUSION

Our result revealed that the accumulation of osmoprotectants (biochemical compounds) under paper mill effluent may indicate that these compounds plays an important role in osmoregulation to maintain its growth potentialities in saline and heavy metal stress conditions. Therefore the accumulation of osmoprotectants in *Suaeda maritima* can be considered as an indicator of tolerance to heavy metal and salt stress. Thus, in present study it may be conclude that non antioxidant enzymes may plays more important role in ameliorating the salt and heavy metal toxicity and provide a robust system to survive in toxic and extreme environments.

Conflict of interest

The authors have no conflict of interest with anyone

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