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RESEARCH ARTICLE

BIOCHEMICAL PARAMETERS OF TWO HALOPHYTES (*Suaeda maritima* (L.) Dumort. AND *Sesuvium portulacastrum* (L.) TO THE APPLICATION OF TANNERY EFFLUENTS

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ABSTRACT

Heavy metal pollution due to industrial effluents is gaining worldwide attention. Tannery industry is common in many parts of the world and major sources of heavy metals, it pollutes groundwater and ecosystems and produce major heavy metals and sodium chloride. Our study showed the effect of tannery effluents on two halophytes *Suaeda maritima* (L.) *Dumort.* and *Sesuvium portulacastrum* L. grown in pot experiment conditions. The aim of this work was to investigate some biochemical systems response of these plants to tannery effluent treatments. Analysis was carried on biochemical constituents, photosynthetic pigments, carotenoids, protein, phenol, amino acids and proline contents of *S. maritima* and *S. portulacastrum* leaves. Four months after sowing, plants were subjected to different concentrations of tannery effluents (0, 30%, 60% and 90%) and samples were analyzed at intervals of 30, 60, 90, 120 days. Results demonstrate that all the biochemical parameters increased progressively with increasing concentrations of tannery effluent. These results indicate that stress of tannery effluents induced biochemical changes in both halophytes with stimulation of heavy metal concentration. This study suggests that, when compared to *Sesuvium portulacastrum*, *Suaeda maritima* plants can be a good source for the phytoremediation of heavy metal polluted tannery effluent contaminated areas.

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INTRODUCTION

Urbanization and industrial development are major causes of heavy metal pollution (Fakoyade and Onianwa, 2002 and Fakoyade and Olu-Owolabi, 2003). Leather tanning industries have cropped up in India over the past three decades. A total number of 2161 tanneries are located in India and spread across the states of Tamil Nadu, West Bengal, Maharashtra, Punjab, Karnataka, Andhra Pradesh, Bihar and Uttar Pradesh. At present more than 568 tanneries are well established in Dindigul, Erode and Vellore districts of Tamil Nadu (Murali and Rajan, 2012). The major metals at these sites are lead (Pb), zinc (Zn), copper (Cu) and cadmium (Cd) and chromium (Cr) (Baskar and Abdul Raheem, 2011). In addition, salinity is also common in different parts of the world. Therefore, investigating the survival of salt- tolerant halophytes under heavy metal stress seems pertinent (Vahedi, 2013). To preserve the natural environment, new methods of remediation using physical, chemical and biological principles are being studied (Cunningham and Berti, 1993 and Basha and Jha, 2008). Some plants have a proven potential for removing the heavy metals from contaminated soil.

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A great deal of recent studies strongly indicates that halophytic plants could be more suitable for heavy metal extraction mainly from saline soil than glycophytes (Manousaki and Kalogerakis, 2011 and Milić *et al*., 2012). Many halophytes often have high metal tolerance that is strongly linked to traits for salt tolerance (Duarte *et al*., 2013). In this paper we have explored the potential for the different concentrations of tannery effluents on *Suaeda maritima* and *Sesuvium portulacastrum* to characterize the biochemical parameters for phytoremediation of tannery effluent contaminated soil.

MATERIALS AND METHODS

Plant material: Two species of fast growing salt marsh halophytic herbs like *Suaeda maritima (L.)* Dumort. And *Sesuvium portulacastrum L.* were selected for the characterization and screening for phytoremediation of heavy metals from tannery effluents with special reference for biochemical studies. The experimental site was located at Panampattu Village, Villupuram District of Tamil Nadu, India.

Tannery effluents collection

The raw effluent was collected from the tannery industry situated at Vaniyambadi near Vellore District in clean plastic cans and stored at 4^0C for further studies.

Pot culture experiments

The experiment was conducted in an open-air area with natural light, temperature, and humidity. 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. The experiment comprised of the following three set of treatments with five replicates and average values are reported. Plants were watered for every 2-3 days, depending on the evaporative demand. Plants were harvested for experimental purpose at intervals of 30, 60, 90, 120 days. During each and every sampling day, samples were randomly collected, washed thoroughly with tap water followed by distilled water.

S.No	Treatment	Method
1.	Control	Without any treatment (Plants are irrigated with tap water only)
2	Effluent treatment	30%, 60% and 90% of tannery effluents was treated 250 ml for 4 times with a gap of 7 days intervals.

Photosynthetic pigments

Chlorophyll was estimated according to Arnon (1949). Tissue for chlorophyll estimation was washed three times with deionized water and blotted on tissue paper. Plant tissue (0.1 g) was homogenized in 80% acetone and incubated in the dark for 6 h. The homogenate was centrifuged at 10,000 rpm for 10 min. The supernatant obtained was read at 645 nm, 665 nm in Spectra Max plus (Molecular Device, USA). The total chlorophyll amount was then calculated according to Arnon (1949).

Estimation of carotenoids

The same chlorophyll extract was measured at 480nm, in spectrophotometer to estimate the carotene using the method of Kirk and Allen, 1965.

Estimation of protein

Fresh tissue weighing 0.5 g was macerated in 20 per cent trichloroacetic acid using mortar and pestle. The homogenate was then centrifuged at 600 rpm 30 minutes and the supernatant was discarded. To the pellet, 5 ml of 0.1N NaOH was added and centrifuged for 30 minutes. The supernatant was saved for the estimation of protein by using the method of Lowry *et al*. (1951). The absorbance was read at 660 nm in a spectrophotometer against an appropriate blank. Bovin serum albumin was used as the standard.

Estimation of total phenols

One gram of leaf tissues was boiled in 25 ml of 80 per cent ethanol for 25 minutes, centrifuged and again reextracted with 20 ml of 80 per cent ethanol. Supernatants were pooled and made up to 25 ml. From this, 1 ml was taken in a test tube and 1 ml of Folin-Ciocalteau reagent and 2 ml of 20 per cent Na_2CO_3 were added. The solution was placed at 60°C in a water bath for 1 minute. After this, the volume was made up to 25 ml with distilled water and the absorbance was read at 640 nm by using the method of Bray and Thorpe (1954).

Estimation of amino acids

Free amino acid was measured by the method prescribed by of Moore and Stein (1948) also called as ninhydrin method and is widely used. Absorbance was recorded at 570 nm using Spectrophotometer (Spectrascan UV 2700). Leucine was used as the standard. Be5

Estimation of proline

Proline was extracted from the leaves and estimated by the methods of Bates *et al*. (1973). Homogenates of the leaf samples were prepared in 3% sulphosalicylic acid. Pink colour was developed by a reaction with glacial acid and ninhydrin. The colour was separated in toluene layer and intensity of the colour was measured at 529 nm spectrophotometrically.

Statistical analysis

Each experiment was repeated five times and the mean values and standard deviations were then calculated (Snedecor and Cochran, 1967).

RESULTS

Present investigation, observations of biochemical characters (Photosynthetic pigments, carotenoids, protein, phenol, amino acid and proline) were observed after 30^{th}, 60^{th}, 90^{th} and 120^{th} days of *Suaeda maritima* and *Sesuvium portulacastrum* plants treated with tannery effluents of 0%, 30%, 60% and 90% concentrations. Apparently, both the plants showed no symptoms of chlorosis in the surviving range of tannery effluent treatment. When compared to control plants the highest photosynthetic pigment content was observed in tannery effluent treated plants. The maxium chl *a*, chl *b* and total chlorophyll content was observed in (1.152, 0.586 and 1.738 mg/g.fr.wt respectively) in *Suaeda maritime* followed by *Sesuvium portulacastrum* (1.113, 0.526 and 1.639 mg/g.fr.wt respectively) plant treated with 90% of tannery effluents after 120 days cultivation period. The lowest chl *a*, chl *b* and total chlorophyll content was observed in (0.111 0.082 and 0.193 mg/g.fr.wt respectively) in *Sesuvium portulacastrum* plants in control at 30 days (Table 1). Table 2 shows carotenoid content of *Suaeda maritima* and *Sesuvium portulacastrum* plants treated with tannery effluents. The maximum carotenoid (0.411 mg/g.fr.wt) was observed in plants treated with 90% of tannery effluents after 120 days cultivation. The minimum carotenoid content (0.003 mg/g.fr.wt) was recorded in *Sesuvium portulacastrum* in control plants after 30 days of cultivation. The protein content of *Suaeda maritima* and *Sesuvium portulacastrum* plants treated with tannery effluents are represented in the table 3. Similar to chlorophyll and carotenoid, when compared to control plants the highest protein content was observed in tannery effluent treated plants. The maximum protein content (1.563 mg/g.fr.wt) was observed in *Suaeda maritima* plant treated with 90% of tannery effluents after 120 days cultivation period followed by 1.363 mg/g.fr.wt in *Sesuvium portulacastrum*. The lowest protein content was observed in *Sesuvium portulacastrum* (0.236 mg/g.fr.wt) control plants in 30 days. The results showed that phenolic compounds significantly increased between up to 90% comparing to control.

Table 1. Effect of different concentrations of tannery effluents on photosynthetic pigments content (mg/g.fr.wt) of *Suaeda maritima* and *Sesuvium portulacastrum*

S.NO	Plants	Concentrations (%)	30 DAS			60 DAS			90 DAS			120 DAS		
			Chl 'a'	Chl 'b'	Total Chl.	Chl 'a'	Chl 'b'	Total Chl.	Chl 'a'	Chl 'b'	Total Chl.	Chl 'a'	Chl 'b'	Total Chl.
1.	*Suaeda maritima*	Control	0.126± 0.0063	0.089± 0.0044	0.215± 0.0107	0.172± 0.0086	0.109± 0.0054	0.281± 0.0140	0.211± 0.0105	0.136± 0.0068	0.347± 0.0173	0.311± 0.0155	0.201± 0.0100	0.512± 0.0256
		30%	0.252± 0.0126	0.159± 0.00759	0.411± 0.0205	0.316± 0.0158	0.186± 0.0093	0.502± 0.0251	0.573± 0.0286	0.231± 0.0115	0.804± 0.0402	0.758± 0.0379	0.282± 0.0141	1.040± 0.0520
		60%	0.288± 0.0144	0.177± 0.0088	0.465± 0.0232	0.377± 0.0188	0.233± 0.0116	0.610± 0.0305	0.611± 0.0305	0.289± 0.0144	0.900± 0.0450	0.914± 0.0457	0.388± 0.0194	1.302± 0.0651
		90%	0.318± 0.1159	0.496± 0.0098	0.514± 0.0257	0.418± 0.0209	0.251± 0.0125	0.669± 0.0334	0.686± 0.0343	0.354± 0.0177	1.040± 0.0520	1.152± 0.0576	0.586± 0.0293	1.738± 0.0869
2.	*Sesuvium portulacastrum*	Control	0.111± 0.0055	0.082± 0.0041	0.193± 0.0096	0.163± 0.0081	0.098± 0.0049	0.261± 0.0130	0.208± 0.0104	0.112± 0.0056	0.320± 0.0160	0.289± 0.0144	0.196± 0.0098	0.485± 0.0242
		30%	0.199± 0.0099	0.142± 0.0071	0.341± 0.0170	0.298± 0.0149	0.173± 0.0086	0.471± 0.0235	0.488± 0.0244	0.213± 0.0165	0.701± 0.0350	0.719± 0.0350	0.273± 0.0136	0.992± 0.0496
		60%	0.263± 0.0131	0.168± 0.0084	0.431± 0.0215	0.316± 0.0158	0.216± 0.0108	0.532± 0.0266	0.599± 0.0299	0.265± 0.0132	0.864± 0.0432	0.888± 0.0444	0.368± 0.0184	1.256± 0.0628
		90%	0.298± 0.0149	0.183± 0.0091	0.481± 0.0240	0.400± 0.0200	0.249± 0.0124	0.649± 0.0324	0.643± 0.0321	0.319± 0.0159	0.962± 0.0481	1.113± 0.0556	0.526± 0.0283	1.639± 0.0819

Table 2. Effect of different concentrations of tannery effluents on carotenoid content (mg/g.fr.wt) of *Suaeda maritima* and *Sesuvium portulacastrum*

S.NO	Plants	Concentrations (%)	30 DAS	60 DAS	90 DAS	120 DAS
1.	*Suaeda maritima*	Control	0.004 ± 0.00022	0.005 ± 0.00025	0.007 ± 0.00035	0.009± 0.00045
		30%	0.009 ± 0.00045	0.123 ± 0.00610	0.182 ± 0.0091	0.285± 0.0142
		60%	0.156 ± 0.0078	0.189 ± 0.0094	0.258 ± 0.0129	0.356 ± 0.0178
		90%	0.168 ± 0.0084	0.222 ± 0.0111	0.352 ± 0.0176	0.411 ± 0.0205
2.	*Sesuvium portulacastrum*	Control	0.003 ± 0.00015	0.004± 0.0002	0.006 ± 0.0003	0.008 ± 0.0004
		30%	0.107 ± 0.0035	0.118 ± 0.0059	0.171 ± 0.0085	0.271 ± 0.0135
		60%	0.143 ± 0.0071	0.173 ± 0.0086	0.223 ± 0.0111	0.341 ± 0.0170
		90%	0.161 ± 0.0080	0.200 ± 0.0100	0.311 ± 0.0155	0.400 ± 0.0200

Table 3. Effect of different concentrations of tannery effluents on protein content (mg/g.fr.wt) of *Suaeda maritima* and *Sesuvium portulacastrum*

S.NO	Plants	Concentrations (%)	30 DAS	60 DAS	90 DAS	120 DAS
1.	*Suaeda maritima*	Control	0.252 ± 0.0126	0.286± 0.0143	0.322 ± 0.0161	0.463± 0.0231
		30%	0.396 ± 0.0198	0.483 ± 0.0241	0.577 ± 0.0288	0.722± 0.0361
		60%	0.454 ± 0.0227	0.563± 0.0281	0.688 ± 0.0344	0.963 ± 0.0481
		90%	0.499 ± 0.0249	0.611 ± 0.0305	0.733 ± 0.0366	1.563 ± 0.0781
2.	*Sesuvium portulacastrum*	Control	0.236 ± 0.0118	0.271± 0.0135	0.300 ± 0.0150	0.418 ± 0.0208
		30%	0.382 ± 0.0191	0.463 ± 0.0231	0.540 ± 0.0170	0.716 ± 0.0358
		60%	0.422 ± 0.0211	0.555 ± 0.0277	0.635 ± 0.0317	0.916 ± 0.0458
		90%	0.491 ± 0.0245	0.600 ± 0.0300	0.704 ± 0.0352	1.363 ± 0.0681

Table 4. Effect of different concentrations of tannery effluents on phenol content (mg/g.fr.wt) of *Suaeda maritima* and *Sesuvium portulacastrum*

S.NO	Plants	Concentrations (%)	30 DAS	60 DAS	90 DAS	120 DAS
1.	*Suaeda maritima*	Control	0.263 ± 0.0131	0.498± 0.0249	0.996 ± 0.0498	1.354± 0.0677
		30%	0.654 ± 0.0327	1.856 ± 0.0928	2.339 ± 0.1169	3.964± 0.1982
		60%	0.842± 0.0421	1.992± 0.0996	2.522 ± 0.1261	4.188 ± 0.2094
		90%	1.000 ± 0.0500	2.456 ± 0.1228	2.888 ± 0.1444	4.593 ± 0.2296
2.	*Sesuvium portulacastrum*	Control	0.260± 0.0130	0.482± 0.0241	0.990 ± 0.0495	1.253 ± 0.0626
		30%	0.612 ± 0.0306	1.739 ± 0.0869	2.185 ± 0.1092	3.662 ± 0.1831
		60%	0.811 ± 0.0405	1.786 ± 0.0893	2.353 ± 0.1176	3.964 ± 0.1982
		90%	0.922 ± 0.0461	2.384 ± 0.1192	2.711 ± 0.1355	4.393 ± 0.2066

Table 5. Effect of different concentrations of tannery effluents on amino acids content (mg/g.fr.wt) of *Suaeda maritima* and *Sesuvium portulacastrum*

S.NO	Plants	Concentrations (%)	30 DAS	60 DAS	90 DAS	120 DAS
1.	*Suaeda maritima*	Control	0.456 ± 0.0228	0.682± 0.0347	0.711± 0.0355	0.986± 0.0403
		30%	0.582 ± 0.0291	0.782 ± 0.0371	0.800± 0.0400	1.240± 0.0620
		60%	0.633 ± 0.0316	0.816± 0.0408	0.988 ± 0.0494	2.116 ± 0.1058
		90%	0.782 ± 0.0391	0.999 ± 0.0499	1.632 ± 0.0816	2.858 ± 0.1429
2.	*Sesuvium portulacastrum*	Control	0.400 ± 0.0200	0.616± 0.0308	0.700 ± 0.0350	0.918 ± 0.0459
		30%	0.516 ± 0.0258	0.718 ± 0.0359	0.713 ± 0.0356	0.988 ± 0.0494
		60%	0.611 ± 0.0305	0.788 ± 0.0394	0.916 ± 0.0458	1.882 ± 0.0941
		90%	0.719 ± 0.0359	0.928 ± 0.0464	1.468 ± 0.0734	2.368 ± 0.1184

Table 6. Effect of different concentrations of tannery effluents on proline content (mg/g.fr.wt) of *Suaeda maritima* and *Sesuvium portulacastrum*

S.NO	Plants	Concentrations (%)	30 DAS	60 DAS	90 DAS	120 DAS
1.	*Suaeda maritima*	Control	0.113 ± 0.0050	0.196± 0.0098	0.283 ± 0.0141	0.352± 0.0176
		30%	0.281 ± 0.0140	0.394± 0.0197	0.568 ± 0.0284	0.694± 0.0347
		60%	0.365 ± 0.0180	0.500± 0.0250	0.718 ± 0.0359	0.958 ± 0.0479
		90%	0.388 ± 0.0190	0.688 ± 0.0344	0.814 ± 0.0407	1.319 ± 0.0659
2.	*Sesuvium portulacastrum*	Control	0.110 ± 0.0050	0.182± 0.0091	0.265 ± 0.0132	0.321± 0.0160
		30%	0.271± 0.0130	0.365 ± 0.0182	0.417 ± 0.0235	0.622± 0.0311
		60%	0.341 ± 0.0170	0.471 ± 0.0235	0.688 ± 0.0344	0.858 ± 0.0426
		90%	0.380 ± 0.0190	0.616 ± 0.0108	0.765 ± 0.0382	1.186 ± 0.0593

Also results indicated that the increase of phenolic compounds was dependent on the concentration of heavy metals present in tannery effluents. The highest phenolic compounds accumulation was observed in 90% of tannery effluents after 120 days cultivation (4.593 mg/g.fr.wt) was observed in *Suaeda maritima* followed by 4.393 mg/g.fr.wt in *Sesuvium portulacastrum*. The lowest phenol content was observed in *Sesuvium portulacastrum* (0.260 mg/g.fr.wt) in 30 days of control plants (Table 4). In comparison to control, the highest amount of amino acids content was observed in plants treated with tannery effluents. The maximum amino acid content (2.858 mg/g.fr.wt) was observed in *Suaeda maritima* plant treated with 90% of tannery effluents after 120 days cultivation period followed by 2.368 mg/g.fr.wt in *Sesuvium portulacastrum*. The lowest protein content was observed in *Sesuvium portulacastrum* (0.400 mg/g.fr.wt) control plants after 30 days (Table 5).

The results showed a positive relationship between tannery effluent concentration and proline accumulation in all treatments (table 6). The results showed that proline accumulation significantly increased up to 90% of tannery effluent treatments. The maximum proline accumulation (1.319 mg/g.fr.wt) was observed in plants treated with 90% of tannery effluents after 120 days cultivation. The minimum proline accumulation (0.110 mg/g.fr.wt) was recorded in *Sesuvium portulacastrum* in control plants after 30 days of cultivation.

DISCUSSION

The present study indicated, after 120 days of cultivation of halophytes treated with tannery effluent, showed the maximum growth. All the biochemical parameters such as photosynthetic pigments, carotenoids, protein, phenol, amino acid and proline of both the plants were increased with an increasing concentration of tannery effluents. The highest increase was recorded in 90% of tannery effluent treatment. The lowest values are observed in 30% of control plant in both the experimental plants. Maximum chlorophyll synthesis was observed in halophytes treated with tannery effluent when compared to control. These results suggested a positive effect of tannery effluent on chlorophyll synthesis in halophytes. Manousaki and Kalogerakis (2009) reported in *Atriplex halimus*, that the chlorophyll content was increased in plants treated with both metals and salinity. Jayakumar *et al*. (2008) studied that effect of phytochemical changes such as chlorophyll *a*, and chlorophyll *b* content on green gram (*Vigna radiata*) under phytoremediation of cobalt stress. Jayakumar (2009) reported that effect the various treatment of cobalt pigment content of soybean plant. Thamayanthi and Sharavanan (2011) reported the phytoremediation of cadmium on growth and pigment contents of *zinnia* plants. Saadet (2013) reported that effects of lead on chlorophyll content in duckweed (*Lemna minor*). Effect of mercury and cadmium on pigment contents of pigeon pea (*Cajanus cajan)* was reported by Aruna and Mohanty (2014). The increase in total soluble protein content in the present study under heavy metal stress

may be related to induce the synthesis of stress proteins such as enzymes involved in Krebs cycle, glutathione and phytochelatin biosynthesis and some heat shock proteins (Mishra *et al*., 2006). Nedjimi *et al*. (2006) observed in *Atriplex halimus* that proline concentration substantially increased with an increase in salinity. In addition to its role as an osmolyte for water economy, proline helps to stabilise sub-cellular structures (e.g. membranes and proteins), scavenge free radicals and buffer cellular redox potential under stress conditions (Ashraf and Orooj, 2006). Navarro *et al*. (2006) showed an increased total phenolic content with moderately saline level in red peppers. Huang *et al*. (2010) studied the physiological and biochemical responses in the leaves of two mangroves *Kandelia candel* and *Bruguiera gymnorrhiza* exposed to multiple HMs (Cd^{2+}, Pb^{2+} and Hg^{2+}) and concluded that Proline, GSH and PCs-SH may play a more important role in ameliorating the effect of HM toxicity.

Sharma *et al*. (2010) observed that accumulation of heavy metals and its biochemical responses in *Salicornia brachiata*, an extreme halophyte. One – month –old plants were treated with different concentrations (50µM and 100µM) of heavy metals for one week and the content of photosynthetic pigments, phenolic compounds, proline, soluble proteins and the activities of enzymatic antioxidants (superoxide dismutase, guiacol peroxidase and catalase) as marker for oxidative stress were investigated by Rastgoo and Alemzadehs (2011). Manivasagaperumal *et al*. (2011) indicated that low concentration of zinc (10 and 25 mgl^{-1}) showed a significant increase in biochemicals (proline and protein) content in *Cyamopsis tetragonoloba*. In *Aeluropus littoralis*, Rastgoo and Alemzadeh (2011) reported that the non enzymatic antioxidant compounds 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 concentration of heavy metals. In the present study, both halophytes have the capacity for osmotic adjustment and accumulate all the biochemical and that maintains the osmotic balance disrupted by the presence of ions in the vacuole. When compared to control seedlings, proline, glycinebetaine and phenol showed an increasing trend in halophytes cultivated in tannery effluent soil. Proline is considered to be the major cytoplasmic osmoticum and it also protects the various enzymes in the cytoplasm. It is hypothesized that glycinebetaine serves as a balancing osmoticum in the cytoplasm and protects the photosystem II complex by stabilizing the association of extrinsic PS II complex proteins in the presence of heavy metals and salts. The main role of many plant phenolics may be the protection of leaves from photo damage and they can achieve this by acting as antioxidants.

Conclusion

It was concluded that, when compared *to Sesuvium portulacastrum* plants *Suaeda maritima* plants showed a high biochemical adaptations and biomass production. It plays a maximum role in phytoremediation of heavy metals and NaCl. So, repeated cultivation of these halophytes is required for high removal of heavy metals and ions from tannery effluent contaminated areas.

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