PHYTOREMEDIATION OF SOIL USING SESUVIUM PORTULACASTRUM - PART I: REMOVAL OF NA⁺ AND CL⁻ FROM TANNERY WASTEWATER TREATED SOIL

by

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ABSTRACT

Salt tolerant halophyte Sesuvium portulacastrum (S. portulacastrum) is capable of absorbing sodium chloride. In the present study, the feasibility of using S. portulacastrum as a bioaccumulant for NaCl present in tannery wastewater has been studied. The plants were grown in soil fed with tannery wastewater with varying concentrations of NaCl. The growth parameters of the plants such as root and shoot length was studied. The amount of Na+ and Cl- accumulated by the plant in leaves and shoots were estimated. The accumulation of Na+ and Cl-increased with increase in plant density and salt concentration of tannery wastewater. However, it has been found that increase in salt concentration resulted in reduced growth of the plant. It has been observed that 34% of Na+ and 22% of Cl- were absorbed by the plant at a NaCl concentration of 15000 ppm at high plant density conditions. Maximum accumulation of Na+ and Cl- has been observed in leaves compared to stems and shoots. The results of the present work indicate that S. portulacastrum can be used for bioaccumulation of sodium chloride.

RESUMEN

La planta halófila tolerante a la sal Sesuvium portulacastrum (S. portulacastrum) es capaz de absorber cloruro sódico. En el presente estudio, la factibilidad de utilización S. potulacastrum como bioacumulador de NaCl presente en desechos líquidos de curtición fue estudiado. Las plantas crecieron en suelo irrigado con efluentes con concentraciones variables de NaCl. Los parámetros del crecimiento de las plantas tales como longitud de raíz y tallo fueron estudiados. La cantidad de Na⁺ y Cl⁻ acumulados por la planta en las hojas y tallos fueron estimados. La acumulación de Na+ y Cl⁻ aumentó con el aumento de la densidad de la distribución de plantación y la concentración de sal en los efluentes de la curtición. Sin embargo se encontró que con el aumento de concentración de la sal, se redujo el crecimiento de la planta. Se observó que 34% de Na+ y un 22% de Cl- fueron absorbidos por la planta a concentraciones 15.000ppm de NaCl bajo condiciones de altas densidades de plantación. Máxima acumulación de Na+ y Cl- se encontró en el follaje en comparación con los tallos y vástagos. Los resultados del presente trabajo indican que S. portulacastrum bien podría utilizarse para bioacumulación del cloruro sódico.

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Introduction

Sodium chloride (NaCl) used in the conventional curing method of raw hides and skins contribute to high total dissolved solids (TDS).^{1,2} The Soil salinity pose a threat to agriculture by reducing the fertility of soil and leading to low crop yield.³ The majority of the plants and crops do not grow at high salinity (600-1000 mM).³ Plants cannot tolerate large amounts of salt in the cytoplasm. Apart from hampering soil fertility, salinity leads to ground water contamination.³ It is essential that the disposal of saline waste water needs to be secured. Consequently, the disposal of the tannery wastewater with high salinity has become a major concern for the tanning industry.

S. portulacastrum belongs to Aizoaceae family and grows naturally in subtropical, mediterranean, coastal and warmer areas around the world. It is a salt tolerant, fast growing tropical marsh plant, which covers barren soil in a very short time.4 S. portulacastrum, can survive both in sea and fresh water. Whether the plants are transferred from seawater to fresh water or from fresh water to seawater, they wilt for a short time and recover to adapt themselves to the new conditions.⁵ The presence of some salt tolerant genes in S. portulacastrum is responsible for such changes.⁵ The thick fleshy leaves are borne on succulent reddish green stems that branch regularly forming dense stands close to the ground. S. portulacastrum produces decorative branches with pink purplish and white flowers. 6 S. portulacastrum is known to grow under the conditions of severe salinity and low nutrient availability.7 Plant growth in saline conditions depend on the level of the cytoplasmic Na+, which is kept low to protect the Na+ sensitive metabolic machinery. The ability of the plant to survive in high saline conditions is attributed to the presence of proline, an osmoprotectant in cytoplasm and active sequestration of Na⁺ in the vacuole.⁸ Large volume of tannery wastewater discharged from leather processing units has become a serious source of pollution. Untreated wastewater has reduced the irrigation potency of fertile lands. 10 The present study highlights the potential use of S. portulacastrum to absorb Na⁺ and Cl⁻ from the soil discharged with treated tannery wastewater.

EXPERIMENTAL

Collection of S. portulacastrum

S. portulacastrum, a halophyte, found in salt marsh creek lands of Ennore, Chennai, Tamil Nadu has been collected and used for the study.

Analysis of Composite Wastewater

Wastewater from commercial leather processing tannery has been collected, filtered and analyzed for NaCl as per the standard procedures. The wastewater has been suitably diluted to obtain 2500, 5000, 7500, 10000, 12500 and 15000 ppm of NaCl and used for the study.

Cultivation of S. portulacastrum

S. portulacastrum plants were collected and initially grown in pots containing sandy soil and organic manure from farm lands in the ratio of 1:1 and irrigated with commercial usage water. Soil conditions have been maintained neutral. These cultivated mother plants were used for experiments. Five centimeter long segments of the apical regions were taken from the mother plants and propagated by vegetative cuttings. These cuttings were grown in natural conditions in circular pots of 10 cm diameter, containing 1:1 ratio of mixture of soil and organic manure from farm yard and irrigated with tannery wastewater at various concentrations of NaCl. The plants have been grown in full sun with temperatures varying between 24°C (night) to 34°C (day). Experiment 1 consisted of 1-plant per pot, Experiment 2 consisted of 3-plants per pot and Experiment 3 consisted of 5-plants per pot. 100 ml of tannery wastewater containing NaCl concentration of 2500, 5000, 7500, 10000, 12500 and 15000 ppm has been fed on alternate days for a period of 30 days to all the experimental plants. Control for each experiment has been watered with commercial water containing 56 and 42 ppm of Na⁺ and Cl⁻. All the experiments were carried out in triplicate.

Monitoring the growth of S. portulacastrum

The plants were allowed to grow for a period of 30 days. After 30 days, the plants were harvested and washed by soaking in commercial water. After washing, the plants were quickly and carefully blotted dry with tissue paper for determining the fresh and dry weight. The root and shoot length of the plants were measured. Leaves, stem and root of the harvested plants were separated and the fresh weight (FW) and dry weight (DW) has been obtained. The leaves, stem and roots were oven dried at 60°C for 48 hrs.

Evaluation of Na⁺ and Cl⁻ ions absorbed by S. portulacastrum

The Na⁺ content of the finely ground dry plant was assayed by flame photometry⁹ after digesting the sample at 90°C with 0.5% HNO₃ for 2 h. Chloride has been determined using the same extract by standard argentometric method.⁹

Determination of % absorption of Na+, Cl- and TDS by S. portulacastrum

The Na⁺ and Cl⁻ were estimated in the five centimeter long experimental plantlets before plantation in the wastewater treated soil as given in the above section. The sodium and chloride content of the harvested plants were measured after the growth of the plants for 30 days. The percentage absorption of ions has been calculated as follows:

% absorption of Na+/Cl-/TDS =

[(Final – Initial) Na⁺/Cl⁻/TDS concentration in plants] X 100

Total ions offered from wastewater in 30 days

RESULTS AND DISCUSSION

Effect of salinity from tannery wastewater on the growth characteristics of *S. portulacastrum*

The extent of absorption of sodium chloride and growth characteristics of S. portulacastrum using varying concentrations of tannery wastewater at different plant density experiments has been studied. The growth of the plant in terms of root and shoot length has been monitored for a period of 30 days. The absorption of salt by the plant in terms of Na⁺ and Cl⁻ has been determined after 30 days. As seen in Figure 1, the root and shoot length of the plants decreased with an increase in the concentration of NaCl in the tannery wastewater offered. At lower levels of NaCl (2500 ppm) in experiment 1, the shoot length has been found to be 8.2 cm, whereas the root length has been 3.9 cm. However, shoot and root length of the plants in the experiment containing 15000 ppm of wastewater has been 3.6 and 1.8 cm, respectively. It is evident from the figure that the root and shoot length were found to decrease with increase in salt concentration. This observation indicates that the growth of the plant decreases as the osmotic (salinity) stress in the soil increases. It has been reported earlier that S. portulacastrum grows unaffected and survives in sea water containing 60,000 ppm of NaCl.11 The ability of the plant to adjust osmotically the ion balance is seen as an important determinant for growth characteristics. Succulence is considered to contribute to salt transportation by increasing the vacuolar volume available for ion accumulation. Also as seen in Figure 1, the growth of the plants has been further reduced with increase in plant density. As seen from Figure 2, the FW and DW of the plant decreased with an increase in the concentration of salt in the soil in all the experimental trials. At higher salt concentration of 15000 ppm in experiment 3, the FW has been 9.5 times higher than that of DW compared to experiment 2, which resulted in FW, 8.5 times greater than that of DW. However, the plant from control experiments showed FW 11 times higher values than DW for Experiment 3 and 9.4 times greater FW than DW for Experiment 2.

The decrease in the plant growth with an increase in salinity stress and plant density can be attributed to the osmotic stress that results mainly through the restriction of solute uptake in leaf expansion. Also, more energy could be spent by the plant in transpiring most of the water and salt accumulated in the vacuoles of the leaf. Therefore, the energy available for the plant growth may be low.

Accumulation of Na⁺, Cl⁻ and TDS by S. portulacastrum treated with tannery wastewater

As given in Table I, II and III, exposure to high salinity results in increased absorption of Na⁺ and Cl⁻ in the plant. Also *S. portulacastrum* plants grown in high plant density conditions accumulated high levels of sodium and chloride (Table I, II and III). As given in Table I, the uptake of Na⁺ and Cl⁻ by leaves of *S. portulacastrum* at 15000 ppm NaCl concentration of wastewater offered in experiment 3 (high plant density conditions) is higher by 30% as compared to

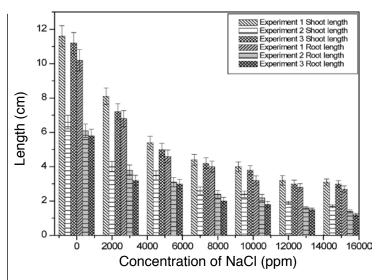


Figure 1: Effect of plant growth in terms of shoot and root length of S. portulacastrum grown at different plant density conditions at various NaCl concentration of tannery wastewater

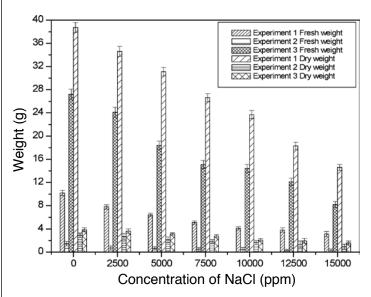


Figure 2: Effect of plant growth in terms of fresh and dry weight of S. portulacastrum grown at different plant density conditions at various NaCl concentration of tannery wastewater

its control experiment. As seen in Table I, II and III, Na⁺, is abundantly accumulated in leaves than in stems and roots. The accumulation of Na⁺ and Cl⁻ in the leaves and stems accounts for about 85% of the average weight of the plants. The accumulation of NaCl was found to be 60% in leaves and 25% in stems at higher concentrations of NaCl offered to the plants. The extremely succulent nature of the leaves in *S. portulacastrum* can be attributed to the requirement of abundance of Na⁺ in leaf tissues for metabolism. The increase in average Na⁺ concentration is also due to the elimination of leaf hydration in osmotically stressed plants. High uptake of Cl⁻ makes up for the cationic balance of Na⁺ and act as counter-ions in the vacuole. Also other negatively charged ions such as nitrate, phosphate, and sulfate depending on the pH could act as balance for accumulated Na⁺. Hence,

TABLE I
Composition of Na⁺ and Cl⁻ in leaves of S. portulacastrum grom at different plant density conditions at various NaCl concentration of tannery wastewater

S.	~NaCl	E	experim	ent 1			Experiment 3						
No	ppm	*FW	*DW	^Na+	^Cl-	*FW	*DW	^Na+	^Cl-	*FW	*DW	^Na+	^Cl-
1	Control	3.57	0.51	28	21	10.2	1.01	49	33	13.6	1.28	72	49
2	2500	2.73	0.25	74	64	9.1	0.96	156	120	12.4	1.21	188	180
3	5000	2.24	0.22	146	137	6.8	0.77	274	228	11.6	1.02	378	336
4	7500	1.76	0.15	285	217	5.4	0.62	486	338	10.3	0.95	634	565
5	10000	1.43	0.14	400	308	5.2	0.56	998	719	8.90	0.72	962	886
6	12500	1.33	0.12	698	560	4.6	0.48	1218	1017	5.87	0.67	1386	1363
7	15000	1.09	0.11	1168	826	3.1	0.36	1574	1372	4.75	0.61	1914	1889

^{* -} Values in g, % error is within 2% for all the experiments

TABLE II
Composition of Na⁺ and Cl⁻ in stem of S. portulacastrum grom at different plant density conditions at various NaCl concentration of tannery wastewater

S.	~NaCl		Exp	eriment			Experin	ment 2		Experiment 3			
No	ppm	*FW	*DW	^Na+	^Cl-	*FW	*DW	^Na+	^Cl-	*FW	*DW	^Na+	^Cl-
1	Control	5.10	0.71	22	15	13.1	1.2	32	21	18.7	1.68	39	33
2	2500	3.90	0.35	37	41	11.3	1.44	98	54	16.4	1.59	86	80
3	5000	3.20	0.32	60	58	8.4	0.98	154	104	15.3	1.48	148	152
4	7500	2.54	0.22	94	94	7.1	0.91	210	181	13.2	1.35	287	178
5	10000	2.04	0.21	186	122	7.0	0.74	347	284	11.8	0.98	413	306
6	12500	1.90	0.17	288	230	5.8	0.60	373	403	9.63	0.89	597	594
7	15000	1.55	0.16	302	412	4.1	0.41	656	572	7.05	0.74	756	745

^{* -} Values in g, % error is within 3.5% for all the experiments

FW - Fresh Weight, % error is within 2.6% for all the experiments

DW - Dry Weight, % errors is within 1% for all the experiments

^{^ -} Values in ppm, % error is within 5% for all the experiments

FW - Fresh Weight, % error is within 4% for all the experiments

DW - Dry Weight, % errors is within 1.4% for all the experiments

^{^ -} Values in ppm, % error is within 3% for all the experiments

TABLE III

Composition of Na⁺ and Cl⁻ in roots of S. portulacastrum grom at different plant density conditions at various NaCl concentration of tannery wastewater

S.	~NaCl	I	Experim	ent 1			Experi	ment 2		Experiment 3			
No	ppm	*FW	*DW	^Na+	^Cl-	*FW	*DW	^Na+	^Cl-	*FW	*DW	^Na+	^Cl-
1	Control	1.53	0.21	10	7	3.9	0.68	14	10	6.4	0.90	18	16
2	2500	1.17	0.11	26	18	3.7	0.65	34	39	5.8	0.84	47	44
3	5000	0.96	0.10	33	33	3.2	0.35	36	60	4.2	0.60	89	72
4	7500	0.80	0.07	67	54	2.6	0.30	102	75	3.1	0.55	110	144
5	10000	0.63	0.06	86	85	2.2	0.34	292	196	3.0	0.38	224	274
6	12500	0.57	0.04	186	154	1.7	0.23	298	275	2.8	0.37	256	252
7	15000	0.46	0.04	213	230	1.2	0.14	394	344	2.8	0.21	453	432

^{* -} Values in g, % error is within 1% for all the experiments

FW - Fresh Weight, % error is within 2% for all the experiments

DW - Dry Weight, % errors is within 1% for all the experiments

TABLE IV
Composition and absorption capacity of Na⁺ in S. portulacastrum grown at various
NaCl concentration of tannery wastewater

Amount of Na+ in plants

S. No	* Na+ offered to plant (mg)	Conc. of NaCl (ppm)		Inistial (mg)	Final (mg)				Na ⁺ Absorption (%)		
			Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp.3
1	137^	0	28±0.6	78±1.2	132±0.8	44±0.9	101±0.7	156±0.9	11.7±1.1	16.7±0.8	17.5±0.8
2	1477	2500	31±0.7	81±0.9	129±1.2	118±1.1	296±1.2	352±1.4	5.9 ± 0.9	14.5±0.7	15.±0.7
3	2955	5000	30±0.4	83±0.8	126±1.0	217±1.3	479±1.6	652±1.8	6.3 ± 0.7	13.4±1.2	17.8±1.2
4	4432	7500	27±0.3	84±0.3	133±0.8	421±1.6	812±1.9	1069±2.1	8.8±1.1	16.4±1.4	21.1±1.7
5	5910	10000	32 ± 0.5	86±1.2	124±1.3	656±2.2	1538±2.1	1626±3.9	10.5±0.9	24.6±1.6	25.3±2.2
6	7387	12500	31±0.6	86±0.7	129±1.5	1154±4.2	1998±2.9	2272±4.4	15.2±1.4	25.9±1.5	29.0 ± 2.4
7	8865	15000	36±0.1	83±1.0	136±1.2	1671±4.7	2632±3.6	3162±4.8	18.4±1.2	28.8±1.7	41.1±3.2

^{* -} Total Na+ offered from tannery wastewater to plant for 30 days

^{^ -} Values in ppm, % error is within 3% for all the experiments

^{^ -} Total Na+ offered from commercial usage water

Na⁺ and Cl⁻ are found to be systematically more abundant in the photosynthetic organs. The presence of small amounts of Na⁺ and Cl⁻ in commercial water resulted in smaller amounts of accumulation of NaCl in control experimental plants. This accumulation is required to maintain normal turgor dependent growth, because it leads to much higher tissue NaCl concentration than it would be necessary for an osmotic adaptation to the growth medium, low in salt.

The accumulation of Na⁺ in plants increased with an increase in NaCl concentration as given in Table IV. However, Na+ accumulation has been comparatively higher than the accumulation of Cl- in the plants (Table V). The plants treated with 15000 ppm of NaCl in experiment 3 (5 plants per pot) resulted in an increase of twofold the accumulation of Na+ and Cl- as compared to experiment 1 (1 plant per pot). Accumulation Na+ and Cl- in experiment 2 (3 plants per pot) has been 1.5 times higher than that of experiment 1. The significant increase in the average Na⁺ concentration is due to the elimination of leaf hydration in osmotically stressed plants.¹³ In general, the vacuole is the largest sink for Na⁺ ions in plant cells.^{14,15} The high succulent property of S. portulacastrum can be ascribed to the ability of plant to accumulate chloride ions in the vacuoles as counter ions to Na⁺. ^{12,16} The threshold capacity of Na⁺ sequestration and leaf metabolism will be inhibited when cytosolic Na+ concentration increases. Increase in the osmolality of the S. portulacastrum is due to an increase in the content of proline and glycine during NaCl treatment. Organic acids, amino acids and proteins accumulate with increasing Na⁺ concentrations in the vacuole and neutralize many charged ions.

The removal of salt in terms of TDS is presented in Table VI. In experiment 1, S. portulacastrum has been able to remove 14% of TDS from the tannery wastewater offered to the plant. However, under high plant density conditions as given in Experiment 2 and 3, there is an increase in the removal of TDS by 21 and 27% by the tannery wastewater (15000 ppm NaCl) treated plants. Sequestering salt from photosynthetic active tissues and cell compartments is a general phenomenon common in all plants. Tolerance levels of salt between plant species is quantitative than qualitative in nature.^{17,18} In an earlier study, it has been found that S. portulacastrum exhibited a maximal growth when it accumulated more than 4 mmol/g dry weight of Na+ in the leaf.7 The contribution of Na⁺ to total osmotic adjustment in S. portulacastrum is observed to be indirect through stimulation of soluble sugars and other osmolyte biosynthesis.¹⁹ The succulent halophytes are known to accumulate extremely high levels of chloride. Halophytes prevent Na+ accumulation in the cytoplasm either by reducing Na+ entry into the cell, or by active Na⁺ efflux from the cell or by active sequestration of Na⁺ in the vacuole.

TABLE V
Composition and absorption capacity of Cl in S. portulacastrum grown at various
NaCl concentration of tannery wastewater

Amount of Cl⁻ in plants

S. No	*Cl offered to plant (mg)	Conc. of NaCl (ppm)		Inistial Final Cl ⁻ Absor (mg) (mg)			Final (mg)			Absorption	(%)
			Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp.3
1	144^	0	19±1.8	49±1.9	93±0.9	34±0.6	67±1.9	118±1.2	10.4±0.9	12.5±0.9	17.4±1.3
2	2273	2500	21±1.9	51±1.4	90±1.9	111±0.9	217±1.5	325±1.7	3.9 ± 0.7	7.3 ± 1.9	10.3±0.9
3	4545	5000	18±1.5	52±1.8	93±1.7	214±1.9	394±0.9	584±1.5	4.3 ± 0.6	7.5 ± 1.5	10.8±0.8
4	6818	7500	22±1.3	50±1.9	95±1.2	357±1.7	594±1.4	907±1.9	4.9 ± 0.4	8.0 ± 1.2	11.9±1.1
5	9090	10000	17±1.2	54±1.6	98±1.8	499±2.3	1199±2.9	1494±3.4	5.3±0.9	12.6±0.9	15.3±0.8
6	11363	12500	20 ± 1.7	47±1.7	102±0.9	934±3.8	1695±3.2	2229±2.9	8.0 ± 0.8	14.5±1.2	18.7±1.6
7	13635	15000	20 ± 1.5	52±1.3	95±1.5	1458±4.9	2288±4.3	3068 ± 4.8	10.5±1.0	16.4±1.1	21.8±0.9

^{* -} Total Cl- offered from tannery wastewater to plant for 30 days

^{^ -} Total Cl⁻ offered from tap water

TABLE VI
Removal of NaCl in terms of TDS by S. Portulacastrum grown at various NaCl concentration of tannery wastewater

TDS absorbed by plants

S. No	TDS offered to plant (mg)	Initial (mg)				Final (mg)			TDS removal in tannery wastewater (%)		
		Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp.3	
1	281	47±0.9	127±1.9	225±1.3	78±0.9	168±1.5	274±1.9	11±0.9	14.6±0.9	17.4±1.9	
2	3750	52±1.3	132±1.2	219±1.6	229±0.5	513±1.3	677±1.9	4.7 ± 1.3	10.1±1.6	12.2±1.1	
3	7500	48±1.4	135±1.1	219±0.9	431±0.9	873±1.1	1236±1.7	5.1 ± 0.8	9.8 ± 1.7	13.5±1.6	
4	11250	49±1.4	134±1.6	228 ± 1.7	778±1.0	1406±0.8	1976±0.9	6.4 ± 0.6	11.3±1.3	15.6±1.7	
5	15000	49±1.0	140 ± 0.4	222±0.9	1155±0.9	2846±0.7	3117±1.0	7.4 ± 1.0	18.0±1.2	19.2±0.9	
6	18750	51±1.1	130±0.4	231±0.8	2088±1.1	3598±0.9	4501±1.6	10.8±0.9	18.5±1.7	22.8±0.9	
7	22500	56±1.0	135±0.9	231±1.3	3129±0.8	4920±1.2	6230±1.9	13.6±1.9	21.3±1.9	26.7±1.9	

The characteristic feature of *S. portulacastrum* is the presence of amino acids like proline, aspartic acid, glutamic acid, alanine, serine and glycine in the succulent stem and leaves.²⁰ Accumulation of free amino acids can be ascribed to a disturbed nitrogen metabolism due to high salinity, wherein the amino acids are not used further for the synthesis of protein. High concentration of proline in the leaves and stem of *S. portulacastrum* and in other halophytes seems to be a characteristic feature of this group of plants. Excessive proline in halophytes helps to maintain the intracellular osmotic potential, while it acts as a protective substance against the adverse effects of salinity in glycophytes. The accumulation of free proline in halophytes may also be due to inhibition of its incorporation into protein or to an inhibition of its transformation into glutamic acid and arginine²¹

The present study indicates that *S. portulacastrum* accumulates considerable amount of sodium chloride from the tannery wastewater treated lands. This accumulated salt is stored in vacuoles, which it absorbs when grown in saline conditions. *S. portulacastrum* is also known to have antibacterial, anti-fungal and anti-oxidant activity due to wide range of essential oils present in it.²² Part 2 of the present study explores the possibility of using this halophyte based product as an alternate to salt for the curing process.

CONCLUSIONS

In summary, *S. portulacastrum* a high salt tolerant halophyte, grown at tropical marsh land of India has the ability to vegetate and grow under high salinity. Harvesting of *S. portulacastrum* using plant-soil system has been effective in sequestering Na⁺ and Cl⁻ from the tannery wastewater. It accumulates substantial amount of Na⁺ and Cl⁻ to achieve osmotic balance across the soil-water-plant gradient. At high plant density conditions, removal of 40% TDS by *S. portulacastrum* has been observed. The present study offers a means for the removal of NaCl by *S. portulacastrum* from tannery wastewater. However, extensive studies at field level are essential for assessing the techno-economic feasibility for the reduction of TDS at tannery wastewater treated lands.

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