

## RESEARCH ARTICLE II

# Bioprospecting Halophytes for Reclamation of Paper and Pulp Mill Effluent Irrigated Soil

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### ABSTRACT

Paper and pulp mills use a huge quantity of water in the manufacturing process and release a substantial quantity of salts in the effluent. The use of industrial effluents for irrigating crops due to the nutrient content in arid zones is under practice. The long-term use of paper and pulp mill effluent as a source of irrigation accumulates salts in the soil surface and rhizospheric zone. In this study, halophytes, namely *Sesuvium portulacastrum*, *Salicornia brachiata*, and *Suaeda maritima* were evaluated for their phytodesalination potential in paper and pulp mill effluent irrigated soil. Among these *Sesuvium portulacastrum* recorded the highest dry matter production (8.21 g pot<sup>-1</sup>), and the sodium accumulation was 6.7% greater than *S. brachiata* on 120<sup>th</sup> day. Furthermore, the soil EC was also 7.7 % lower in pots cultivated with *S. portulacastrum* than pots cultivated with *S. brachiata*. Hence, *Sesuvium portulacastrum* could effectively be used to phytodesalinate saline soils and ensure the sustainable cultivation of crops using paper and pulp mill effluent.

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### INTRODUCTION

India has a sound base in several core industries, like metals, chemicals, petroleum products, fertilizers, textiles, pulp and paper among which the pulp and paper industries are economically vital (Singh *et al.*, 2013) and is third most water-intensive industry worldwide. Paper mill treated effluent often has a high sodium concentration which deteriorate physical and chemical characteristics of soil (Kumar and Chopra, 2012). Irrigation with this treated effluent for long duration results in a significant salt buildup in the rhizosphere and higher salinity and exchangeable sodium levels (Balusamy *et al.*, 2019). In India, the land affected with saline, sodic, and saline-sodicity was 3.26, 4.12 and 4.62 million ha, respectively (Kumar and Thiyageshwari, 2018). The salinity and sodicity reclamation process is unceasing due to the high cost and time-consuming techniques. Salinity has an initial osmotic and an

ionic effect on plants, limiting their ability to absorb water and micronutrients (Leogrande and Vitti, 2019). The use of halophytes in saline agriculture has been explored since the early 21<sup>st</sup> century. Hence more research for an ecologically appropriate halophyte with multiple uses and efficient cultivation in a wide range of climatic conditions is the key to a sustainable future. *Sesuvium portulacastrum*, a halophyte, has succulent leaves that sequester excess Na<sup>+</sup> in the vacuole and allow it to thrive in a saline environment (Lokhande *et al.*, 2013). This halophyte is a salt accumulator, capable of growing profusely at 100–400 mM NaCl and tolerating up to 1,000 mM NaCl without toxicity to the leaves (Messedi *et al.*, 2004). *Salicornia brachiata* of Chenopodiaceae family *Suaeda maritima* (L.) of Amaranthaceae family are annual halophytes predominantly found in the Pichavaram mangroves of Tamil Nadu (Benjamin *et al.*, 2020) and grows

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well at 200 mM NaCl salt concentration. *Salicornia brachiata* is a leafless annual succulent with a dry weight of 30–40% sodium chloride (Sharma *et al.*, 2010). To exploit these unique characteristics, a pot culture experiment with coastal halophytes was conducted to assess their potential for phyto-desalination of paper and pulp mill effluent irrigated soil.

## MATERIAL AND METHODS

### Experimental setup

*Sesuvium portulacastrum*, *Suaeda maritima*, and *Salicornia brachiata* were collected from halophyte germplasm, MSSRF (M.S. Swaminathan Research Foundation), Vedaranyam, India (Figure 1.). The halophytes were propagated through vegetative methods except for *Suaeda maritima*, which was propagated through seeds. A 15 day old propagated seedlings with an initial fresh weight of 1.5 – 2.5 g were used for the pot experiment. The *Sesuvium portulacastrum* and *Salicornia brachiata* were propagated as cuttings of 5 – 7 cm length with 2 internodes. The soil for the experiment was collected from Pandipalayam village, Karur district, Tamil Nadu, India (11°01'24.9"N and 77°59'59.3"E), a long term paper and pulp mill effluent irrigated soil. The initial soil was slightly alkaline with pH at 8.25 and Electrical Conductivity (EC) at 2.31 dS m<sup>-1</sup>. The soil organic carbon content was about 0.32 percent. Paper and pulp mill effluent of alkaline pH ranging from 7.20 - 8.32 and electrical conductivity of 2.1 - 3.1 dS m<sup>-1</sup> was used for irrigation. The cations like calcium, magnesium, and sodium were also present in the effluent at the range of 203 - 258, 48 - 60.2, and 320 - 385 mg L<sup>-1</sup>, respectively. Since the effluent also contains nutrients (Sharma, Tripathi and Chandra, 2020) no additional nutrients were supplied during the experiment. The pots were irrigated once in three days to field capacity and maintained weed-free throughout the experimental period.

### Sample collection and analysis

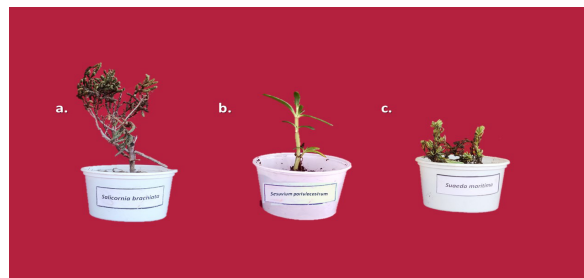
Soil samples were collected and analyzed every 30 days after planting up to the 120<sup>th</sup> day. The plant samples were collected after 120 days, oven-dried at 70 °C, and ground into a fine powder. Plant samples (0.5 g) were digested with 13 mL nitric acid and 2 mL H<sub>2</sub>O<sub>2</sub> using a microwave digestion unit (Ethos Easy, Milestone Srl, Italy) (Ximénez-Embún *et al.*, 2002). The concentration of ions was measured using MP-AES (MP-AES, Agilent Technologies, United States). The EC in soil was estimated by saturation paste

extract (Rhoades *et al.*, 1989). Phyto desalination potential of halophyte is determined by the following equation Eqn. 1

$$\text{Phytodesalination Capacity} = \text{Na}^+ \text{ concentration (\%)} \times \text{DMP (kg ha}^{-1}\text{)} \quad (1)$$

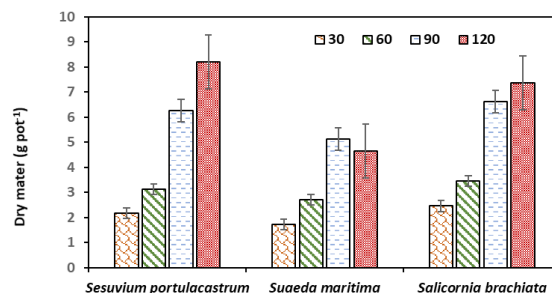
### Statistical analysis

All the treatments were replicated five times and using SPSS 19.0 (Chicago, USA), a statistical software, and the collected data were evaluated using one-way analysis of variance (ANOVA). Duncan's tests were used to assess the significant difference at P 0.05. (Gerber *et al.*, 1997).

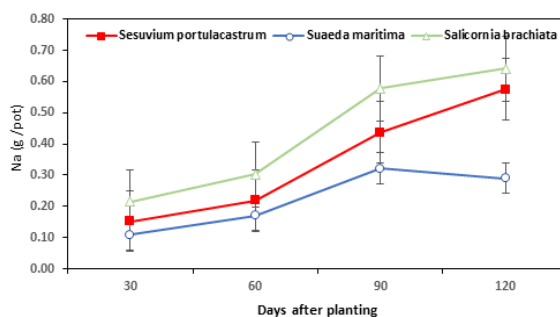


**Figure 1. Halophytes collected from the halophyte germplasm, MSSRF, Vedaranyam, India**

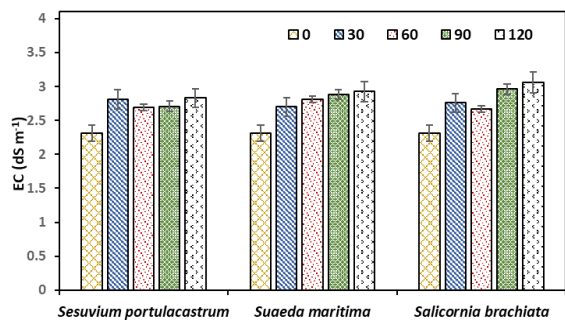
a. *Salicornia brachiata*, b. *Sesuvium portulacastrum*, c. *Suaeda maritima*



**Figure 2. Dry matter production (g pot<sup>-1</sup>) by halophytes in paper and pulp mill effluent irrigation**



**Figure 3. Sodium content in the biomass (mg g<sup>-1</sup>) of halophytes at 30 days interval**



**Figure 4. Reduction in soil EC due to phytodesalination of paper and pulp mill effluent irrigated soil using halophyte**

## RESULTS AND DISCUSSION

### Biomass production

Biomass production is an important character to evaluate the phytoremediation potential of the plants. It depends on the climate, soil condition, and stress (Lokhande *et al.*, 2013). Under paper and pulp mill effluent irrigated soil, the highest dry matter production was recorded in *S. portulacastrum* (8.21 g pot<sup>-1</sup>) followed by *S. brachiata* (7.16 g pot<sup>-1</sup>) (Figure 2). However, the growth rate of *S. maritima* reduced after 60 days and started to shed its leaves; hence, the dry matter content decreased on 120<sup>th</sup> day. *S. maritima*, unlike the other two halophytes, requires sandy soil for its growth (observation during the survey) but paper and pulp mill effluent irrigated soil tend to accumulate organic content, reducing pores in the soil. This explains the reduced growth of *S. maritima* (Tsfai *et al.*, 2002; Iniyalakshimi *et al.*, 2019). Due to its perennial, succulent nature and fast growth, *S. portulacastrum* and *S. brachiata* can be harvested after 90 days (end of the vegetative stage), making this a viable option to remove salt at a faster rate compared to *S. maritima*. It also prevents the flowering and unsupervised dispersal of seeds.

### Phytodesalination potential

The highest phytodesalination capacity was observed in *S. portulacastrum*, due to the high biomass and highest Na<sup>+</sup> content (Figure 3). *S. portulacastrum* and *S. brachiata* accumulated 0.57 and 0.64 g pot<sup>-1</sup>, respectively. When this observation is theoretically redressed for phytodesalination per hectare, it is, 140-150 kg Na, which is lower than the findings of (Sundararaj *et al.*, 2014), where *S. portulacastrum* could remove 400 kg of NaCl from one hectare of saline soil. Similarly, *S. brachiata*

had the potential to remove 130 to 160 kg, which was also lower than the earlier reports (334 kg ha<sup>-1</sup> of Na ions in its shoot) by (Sundararaj *et al.*, 2014). However, the highest salt accumulation was recorded on 90<sup>th</sup> and 120<sup>th</sup> day in *S. portulacastrum*. *Suaeda maritima* was known to accumulate high salt content in the vacuoles, even up to 500 – 800 mM (Hasanuzzaman *et al.*, 2014). On the other hand, its Na uptake potential was recorded as 0.29 g pot<sup>-1</sup> in this study. The drop in Na<sup>+</sup> concentration in *S. maritima* on 120<sup>th</sup> day shows the hindrance in growth due to paper and pulp mill effluent irrigation. Hence, cultivation and harvest of the halophytes, *S. portulacastrum* and *S. brachiata* could effectively reduce the soil Na<sup>+</sup> content below toxic levels (EPA, 2020). In addition, the application of amendments like gypsum and farmyard manure to release the Na<sup>+</sup> bound to the soil matrix may increase the potential of phytodesalination (Mahmoodabadi *et al.*, 2013; Zoghdan and Ali, 2019).

### Changes in Soil EC

The reclamation of saline soil by halophyte depends not only on the halophyte species but also on soil texture, contaminants, weather, water source, and availability of nutrients (Imadi *et al.*, 2016). The initial EC of the soil was 2.31 dS m<sup>-1</sup> which is due to irrigation of paper and pulp mill effluent for long duration (Rezende *et al.*, 2010). The soil EC significantly increased from 0<sup>th</sup> day to 120<sup>th</sup> day after planting (Figure 4). In correspondence to the Na<sup>+</sup> uptake by the halophyte, the EC reduction is evident after 60 days of planting in the soil with *S. portulacastrum* and *S. brachiata*. This reduction in EC is positively correlated with the Na<sup>+</sup> uptake by the *S. portulacastrum* (R<sup>2</sup> = 9.65) and *S. brachiata* (R<sup>2</sup> = 8.95). On the other hand, in spite of growth upto 90<sup>th</sup> day, the reduction of soil EC was not significant in *S. maritima*. The reduction in soil EC is more pronounced when applied with soil organic and inorganic amendments (Sadiq *et al.*, 2007), which should be evaluated field level and biomass utilization after phytodesalination needs special attention.

## CONCLUSION

Remediation using halophyte requires a choice of elite species with stable results. In this study, three halophytes were evaluated, of which *S. portulacastrum* had the highest biomass production with phytodesalination potential. Compared to the other two, *S. portulacastrum* serves

as a suitable phytoremediant of soils salinized by long-term irrigation of paper and pulp mill effluent and can be promoted for cultivation at industrial and farmer's scale.

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### Ethics statement

No specific permits were required for the described studies because no human or animal subjects were involved in this research.

### Competing interests

There was no conflict of interest in the publication of this manuscript.

### Conflict of interest

The authors here by declare no conflict of interest.

### Consent for publication

All the authors agreed to publish the content.

### Author contribution

Research grant – Dr. P. Thangavel, Dr. M. Maheswari; Conceptualization- Dr. P. Thangavel, Experiments- Mr. Joseph Ezra John, Guidance – Dr. G. Balasubramanian, Dr. T. Kalaiselvi, Dr. E. Kokiladevi; Writing original draft- Mr. Joseph Ezra John, Dr. P. Thangavel; Reviewing & editing- Dr. P. Thangavel, Dr. M. Maheswari

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