

Research Article

Bromide Tolerance in Plants: A Case Study on Halophytes of Indian Coast

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Many industrial effluents contain occasionally various toxic elements. Many a time, bromide forms a constituent of the effluents especially originating from coastal regions. Plant materials have been effectively employed as tools to remediate this situation. Some halophytes are the best choice for reducing the toxic levels from affected salinised soils. This paper deals with the bromide uptake and its accumulation effect on the growth of *Salicornia brachiata*, *Suaeda nudiflora*, and *Salvadora persica*, the common halophytes of Indian coast. The species were grown with NaBr solution along with other essential nutrients. The growth in *S. brachiata*, *S. nudiflora*, and *S. persica* was more or less same except for some apparent morphological differences in NaBr grown plants as compared to that in NaCl-fed plants. The bromide present in various parts of these plants was determined by simple and eco-friendly techniques for the first time. A reliable spectrophotometric method was developed and employed to estimate the bromide composition in all plant extracts. The bromide levels were about 0.086–0.2 g in the root, 0.175–0.443 g in stem and 0.287–0.432 g in leaves per g of dry plant material and at higher levels it affected the photosynthetic activity. Cultivation of these plants for reclamation of bromide affected soils has been advocated as an alternative.

1. Introduction

Many of the halophytic species (~2,500) reported worldwide have been used for their high economic value like fodder, oil, fuel, and so forth. and also for ecological interests like soil desalination, dune fixation, phyto-remediation, landscaping, and ornamental purpose [1, 2]. Further, domestication of halophytes as potential crop plants for biomass production is an emerging field towards rehabilitation of salinised lands in semi-arid and arid areas, [3]. Soils adjacent to the coastal areas under irrigation or otherwise get enriched with various materials including hazardous elements from the discharges of industrial effluents, which in turn pose a great problem for the reclamation of soils and for their use in cultivation. Some industrial effluents comprise occasionally various toxic elements like Pb, Cd, Zn, Hg, Cu, Al, and so forth. Even bromide also forms a constituent of these effluents especially coming from bromine recovery plants,

organo-bromine manufacturing units, and salt producing centers, which generally are located near or on the coastal regions, the habitat of many halophytes. To overcome this situation, phytoremediation, an eco-friendly process, is the only alternate solution. For this purpose, materials of plant origin, either living or dead, have been effectively employed as tools. Among the living organisms, some halophytes of economic importance are the best choice for reducing the levels of toxic materials from salinised soils. In this attractive technique, the contaminants are ingested through roots and stored in the biomass and/or transported to the stem or leaves till the harvesting period, thereby reducing their concentration levels in the soil to a significant extent and thus this technique has been suggested to clean up the polluted surroundings [4, 5]. Hence, this method seems to be promising in transforming thousands of acres of hostile land or soil to make them fit for further vegetation or reuse [6–10].

Massive storage of sodium chloride in tissues of many halophytes grown on saline soils/waters has been reported [11–16]. The halophytes occurring abundantly on Indian coast viz. *Salicornia brachiata* (*S. brachiata*), *Suaeda nudiflora* (*S. nudiflora*), and *Salvadora persica* (*S. persica*) have been selected for this study since these plants have been used as a source of biological salt (*S. brachiata* and *S. nudiflora*) or oil (*S. persica*), and these plants have also shown that they can accumulate and sustain the salinity levels nearly double that of seawater [11–14]. Generally these halophytes are sensitive to bromide thereby causing physiological changes like inhibition of photosynthesis, and disturbance in ion accumulation and metabolic functions primarily obstructing the water relations. To overcome the stress induced by bromide accumulation, some of them develop succulence thereby increasing the water content in tissues that dilute the accumulated toxins in the cells. Finally, the metabolic functions of these plants will get altered to cope up with the prevailing ambient atmospheric conditions, which is a common feature observed in halophytes of saline environment. The selected species for this study are inhabiting on the seacoast of Bhavnagar where bromide concentration is reported to be relatively high. The recent analyses on nutrient rich vegetable salt recovered from the biomass of *S. brachiata* and *S. nudiflora* have indicated NaCl content about 88%–92%, KCl about 5%–8%, Ca²⁺ about 0.02%–0.4%, Mg²⁺ about 0.02%–0.08%, and iodine about 7–10 ppm [17].

The present study is aimed at evaluating the growth of *S. brachiata*, *S. nudiflora*, and *S. persica* plants by feeding them with half-strength Hoagland's minerals containing 0.2 M NaBr or 0.2 M NaCl. A comparison of the results of this study revealed that these plants could be cultivated in the bromide affected soils for reclamation.

2. Experimental

2.1. Culture Conditions and Samples Preparation. The seeds of *S. brachiata*, *S. nudiflora*, and *S. persica* were collected from the seacoast of Bhavnagar (21°75' N and 72°14' E), sown in plastic pots having 20 cm diameter at the top and a depth of 18 cm, filled with 2.5 Kg of horticulture grade soil and watered initially with distilled water. After 15 days of germination, the pots were irrigated with half-strength nutrient solution [18] for another 15 days. The plants were then fed with 500 mL of 0.2 M NaBr solution on alternate days for one month. Simultaneously, another set of plants were also irrigated according to the procedure mentioned above using 500 ml of 0.2 M NaCl solution for comparison.

The well-grown three-month old NaBr- and NaCl-fed plants were uprooted slowly and dissected carefully into root, stem, and leaves. The individual plant materials were then washed thoroughly with tap water followed by distilled water to remove the adhering particles and then air-dried. The materials were dried by heating at 70°C in an oven to obtain a constant weight and powdered to 200-(BSS) mesh size. Simultaneously, the irrigated soil fed with NaBr and NaCl individually was collected, dried, powdered, and sieved through 200-(BSS) mesh.

2.2. Materials and Chemicals. Analytical grades KBr, KBrO₃, KI, NaCl, NaBr, (SD's chemicals, India), and H₂SO₄ (Ranbaxy Fine Chemicals, India) were used in these investigations. Double distilled water was used to prepare all the solutions. About 1 g of dry KBr was dissolved in 100 or 250 mL of double distilled water and used in preparing all experimental solutions. 1% (w/v) of KBrO₃ solution was prepared and used as a stock. A solution of 50% (v/v) H₂SO₄ was used to adjust its composition to 4% (v/v) in all experimental solutions. The glassware was soaked in chromic acid, washed with detergent, and then rinsed with distilled water before use.

2.3. Extraction Procedures. Samples for bromide estimation in different plant parts (root, stem and leaves) were made by different extraction procedures as detailed in the following sections.

2.4. Hot Water/0.1 N HNO₃ Extraction. Powdered plant material (0.2 g) was suspended in 70 mL distilled water/0.1 N HNO₃, heated on a hot water bath for 2 hours, and then filtered. The residue was washed with 10–15 mL of distilled water and the filtrate was diluted to 100 ml with distilled water/ 0.1 N HNO₃ for bromide analysis.

2.5. Extraction by Ashing/Fusion/Acid Digestion. Powdered sample (0.2 g) was directly ashed/fused along with 1 g of 1 : 1 NaOH-Na₂CO₃ mixture at 550°C or digested in 10 mL of concentrated HNO₃. The ashed/fused/digested material was treated with 20 mL of water, filtered, and washed with hot water. The filtrates were collected, diluted to 100 mL with distilled water, and analysed for bromide content.

2.6. Soil Extract. About 200 g of soil sample fed with sodium bromide-nutrient solution were treated with 50 mL of 30% H₂O₂ for 24 hours and soaked in 200 mL distilled water for another 24 hours to extract all inorganic bromide. The soil-water mixture was filtered and the filtrate was diluted to one liter with distilled water, and the bromide content in it was measured. Similar procedure was adopted for the chloride estimation in the soil.

2.7. Analytical Procedures. The extracts of the plant materials (shoot of *S. brachiata*/leaf of *S. nudiflora*/*S. persica*) obtained by digestion with concentrated HNO₃ were used to quantify the cationic elements present in both NaCl and NaBr-fed plants by employing inductively coupled plasma atomic emission spectrometer (ICP-AES).

2.8. Calibration Plot for Bromide Ion. Working standards for Br⁻ estimation in the range from 0.01 to 28 mM (0.8 to 224 µg/mL) were prepared from KBr stock solution. To an aliquot (1–10 mL) of KBr stock solution in a standard flask containing 2.5 mL of 1% KBrO₃, 2 mL of 50% H₂SO₄ was added under cooling to give 0.1% and 4%, respectively, after dilution to 25 mL with distilled water. Absorption spectra between 200 and 600 nm of these solutions were recorded after 10–15 minutes of equilibration. In the case

TABLE 1: Ion accumulation data of ICP in shoot (*S. brachiata*)/ leaf (*S. nudiflora*, *S. persica*) grown independently by feeding with 200 mM each of NaCl and NaBr solutions.

Halophyte	Feed	Ion accumulation (mg/g DW)			
		Na	K	Ca	Mg
<i>S. brachiata</i>	NaCl	101.85 ± 1.24	18.48 ± 0.47	17.47 ± 0.54	17.80 ± 0.96
	NaBr	93.74 ± 0.87	13.51 ± 0.50	12.09 ± 0.23	9.06 ± 0.32
<i>S. nudiflora</i>	NaCl	83.71 ± 2.93	19.93 ± 1.02	17.93 ± 1.25	16.47 ± 1.54
	NaBr	77.24 ± 2.84	15.11 ± 0.83	12.66 ± 1.68	10.60 ± 0.55
<i>S. persica</i>	NaCl	64.76 ± 2.33	15.12 ± 0.66	9.38 ± 0.54	16.47 ± 0.57
	NaBr	54.8 ± 2.98	10.03 ± 0.60	5.68 ± 0.37	4.90 ± 0.38

of bromide estimation in plant (10 mL) or soil (2–5 mL) extracted sample, a mixture of 2.5 mL of 1% KBrO₃ and 2 mL of 50% H₂SO₄ was added constantly and the absorbance at 390 nm was noted after diluting to 25 mL in each case.

2.9. Bromide Estimation in Plant or Soil Extracts. To 10 mL of the plant extract or 2–5 mL of the soil extract, 2.5 mL of 1% KBrO₃ and 2 mL of 50% H₂SO₄ were added and made up to 25 mL. The absorbance data at 390 nm was noted and used in the determination of Br⁻ content. The deviation was calculated from the average of at least five experimental readings as ±0.025% (w/w).

2.10. Chloride Ion Estimation. To 10 mL of the plant extract, 4–5 drops of K₂CrO₄ indicator were added and then titrated against 0.01 N AgNO₃ solution in the burette until a red color forms by adding of every drop and begins to disappear more slowly, which is an indication that most of the chloride has been precipitated. The addition of AgNO₃ solution was continued drop wise until a faint but distinct change in color occurred [19].

2.11. Instrumentation. In all cases, the absorption spectral data of the samples were recorded between 200 and 600 nm on Shimadzu UV-160 spectrophotometer (Germany) with a built-in thermostat (25 ± 0.1°C) cell compartment coupled to a temperature controlling unit using a 10 mm quartz cuvette [20]. Inductively coupled plasma atomic emission (ICP-AES) spectrometer of Perkin Elmer model Optima 2000 DV (Germany) was used to quantify the cationic elements (Na, K, Ca, and Mg) present in the concentrated HNO₃ digested plant materials.

3. Results and Discussion

3.1. Plant Growth and Morphology. The growth in *S. brachiata*, *S. nudiflora*, and *S. persica* was more or less quite similar in both the NaCl- and NaBr-fed plants. However, marked morphological differences were noted as illustrated in Figure 1. The appearance of the plants grown with NaBr had a pale green in case of *S. brachiata* and a light pale green in *S. persica*, while it was normal in color with NaCl fed plants. The morphological differences in case of *S. nudiflora* was less significant. This implied that NaBr feeding had more visible impact on morphology incase of *S. brachiata* and *S. persica*

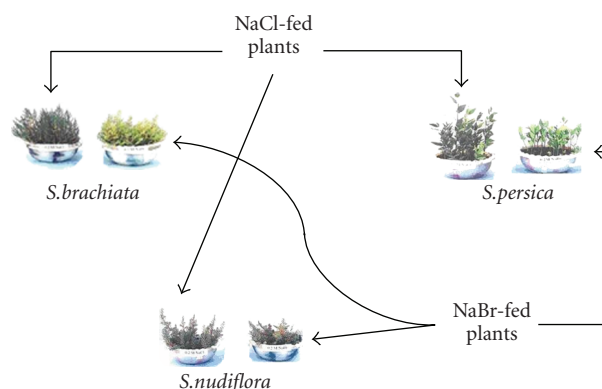


FIGURE 1: Visible morphological differences of three-month old *S. brachiata*, *S. nudiflora*, and *S. persica*.

and less on *S. nudiflora*. This probably could be attributed to the reduced chlorophyll content effecting photosynthesis. To have more understanding of these morphological differences, the hot water extracts of leaf/shoot/root were analyzed by ICP-AES for quantification of elements (Na, K, Ca, and Mg) and also by spectrophotometry for bromide/Mohr's method for chloride estimations.

3.2. ICP-AES Measurements. The data given in Table 1 indicated that these metal ions accumulate relatively more in NaCl fed plants than in NaBr-fed ones. The data revealed that the accumulation (% on dry weight basis) of Na, K, Ca, and Mg was more by 8–18, 32–50, 41–65, and 55–236, respectively in NaCl fed plants. Interestingly, these differences go on increasing in the order of *S. nudiflora* < *S. brachiata* < *S. persica*. However, a significant difference was noted in case of Mg content between NaCl- and NaBr-fed plants. About 55% less Mg accumulation was noticed in *S. nudiflora*, 91% in *S. brachiata*, 236% in *S. persica* in NaBr-fed plants, which were reflected in the morphological changes. Similarly, the NaCl-fed plants favored the accumulation of Ca more than that of NaBr-fed ones. The data reiterated that about 44% of reduction in Ca accumulation in *S. brachiata*, 42% in *S. nudiflora*, and 65% in *S. persica* were noted when NaBr-fed plants were used for irrigating the plants. It is interesting to note that the accumulations of Ca and Mg which were nearly comparable in these halophytes except in *S. persica*, when grown in NaCl, deviated to a considerable

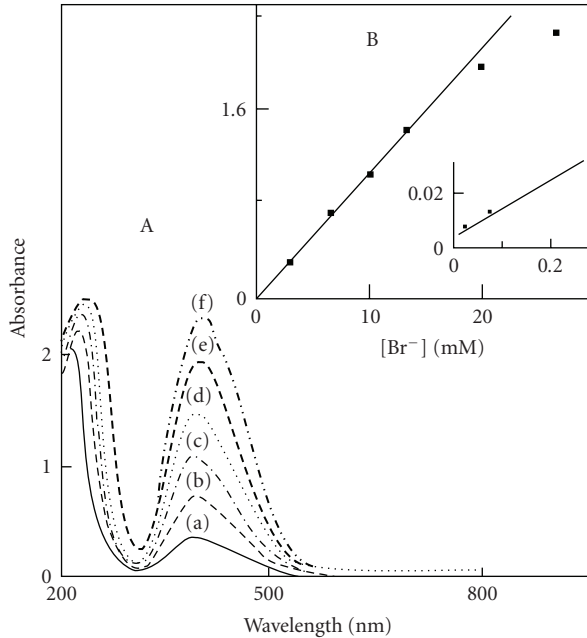


FIGURE 2: Absorption spectra of (A) 0.1% KBrO_3 -4% H_2SO_4 mixture containing (a) 3.42; (b) 6.83; (c) 10.25; (d) 13.61; (e) 20.49; (f) 27.32 mM KBr at 25°C . (B) Plot of $[\text{Br}^-]$ versus Absorbance at 390 nm. The data between 0.02–17 mM fits to the linear equation $\text{Abs}_{390} = 105 [\text{Br}^-] + 0.003$ ($r = 0.9998$).

extent in NaBr solution. The Ca accretion with NaBr solution exceeds that of the Mg by about 33% in *S. brachiata*, 27% in *S. nudiflora*, and 16% in *S. persica*.

3.3. Spectrophotometric Estimation of Bromide Ion Calibration Plot. Estimation of Br^- ion seems to be more difficult, but an interesting problem [19, 21–29]. Earlier methods based on Spectrophotometry consisted of transformation of Br^- to Br_3^- via BrO_3^- by employing hypochlorite solution [24] or the oxidation of Br^- with chloramines-T and the subsequent bromination of phenol red [25] which had serious limitations like incongruity observed on the molar absorptivities of Br_3^- at 267 nm [25–28], involvement of carcinogenic materials [29], or dependence of accuracy and precision of results on reaction time [30]. Very recently, an easy and reliable spectrophotometric method had been developed to enable the determination of bromide ion in 0.1% KBrO_3 -4% H_2SO_4 medium in the presence of high chloride ion concentrations [19]. A similar method was developed (as discussed below) for the first time in the present study for estimation of bromide in all studied samples containing negligible quantities of chloride.

Absorption spectra of 0.02–30 mM (1.6–2400 ppm) Br^- were studied in 0.25% KBrO_3 -4% H_2SO_4 solutions. The spectral results of Br^- in the concentration range of 3–30 mM are explained in Figure 2(a). In all cases, the charge transfer band at 390 nm matching with that of aq-Br_2 in 2% NaBrO_3 -4% H_2SO_4 mixture was noted. The intensity of the band at 390 nm increased with the increase in the concentration of Br^- . However, the plot of $[\text{Br}^-]$ versus

TABLE 2: Data on bromide and chloride in NaBr and NaCl fed halophytes, respectively.

Halophyte	^a Part	g per g of plant material/soil		Br^- to Cl^- holding ratio
		^b Br^-	Cl^-	
Soil	—	0.063	0.300%	—
<i>S. brachiata</i>	Root	0.200	0.135	1.48
	Stem	0.443	0.225	1.97
<i>S. nudiflora</i>	Root	0.155	0.119	1.30
	Stem	0.332	0.162	2.05
Leaf	0.432	0.200	2.16	
	Root	0.086	0.089	0.97
<i>S. persica</i>	Stem	0.175	0.103	1.70
	Leaf	0.287	0.127	2.26

^aHot water used for extraction of plant parts and normal water for soil extracts.

^bThe deviation calculated from the average of at least five experimental readings is $\pm 0.025\%$ (w/w).

absorbance at 390 nm (Figure 2(b)) showed that the increase was linear between 0.02 and 16 (1.6 or 1280 ppm) mM satisfying,

$$\text{Abs}_{390} = 105 [\text{Br}^-] + 0.003. \quad (1)$$

The nonlinearity of the plot (Figure 2(b)) at higher Br^- concentration is attributed for its loss during analysis caused by its high volatility. The lower and higher detection limits as estimated from the data under the present set of conditions for Br^- are $5\mu\text{M}$ (0.4 ppm) and 16 mM (1280 ppm), respectively.

3.4. Bromide Composition in Plant Extracts. Substituting the absorption (Abs_{390}) data in (1), the concentration of Br^- ion in all hot water extracts of different parts of NaBr -fed plants was determined and tabulated in Table 2. Interestingly, the data revealed that the soil fed with 0.072 g (8%) of Br^- has retained 0.063 g (6.3%) allowing the rest (1.7%) for consumption to the plant on irrigation. Comparison of Br^- content in the plant material showed that the accumulation of Br^- ion in the plant *S. brachiata* was 1.33 and 2.53 times more as compared to those in *S. nudiflora* and in *S. persica*, respectively. This signifies that their succulent property decreases in the order of *S. brachiata* > *S. nudiflora* > *S. persica*. This implies that the Br^- accumulation is related to the succulence property in these plants. Further, the data on leaf indicated that the accumulation of Br^- seemed to be 1.3 to 1.6 and 2.8 to 3.3 times more as compared to those in stem and root, respectively. More deposition in leaf is accounted for the maintenance of osmotic potential flow of water along with the dissolved salts within the plant against gravity.

The Cl^- ion concentration in individual plant extracts of NaCl fed plants had also been estimated and compared (Table 2) with the bromide content of the respective extracts of NaBr -fed plants. The data exhibited a decrease in chloride ion uptake (*S. brachiata* > *S. nudiflora* > *S. persica*) and also

TABLE 3: Bromide composition in NaBr fed *S. brachiata* obtained by different extraction procedures.

Extraction method	³⁵ Br ⁻ g per g of plant material
Hot water	0.443
0.1 M HNO ₃	0.442
Conc. HNO ₃	ND
Direct ashing	0.387
Fusion (NaOH-Na ₂ CO ₃)	0.404

in its accumulation in different plant parts (0.98 to 1.23 and 1.43 to 1.68 times higher in leaf than those in stem and root, resp.), which were similar to those found with NaBr-fed plants.

Comparison of bromide content with that of chloride in individual plants (Table 2) showed that these halophytes accumulated 2.16–2.26 times more bromide than that of chloride in leaf. Similarly, in stem and root, they store about 1.70–2.05 and 0.97–1.30 times more bromide than that of Cl⁻. Overall, the Br⁻ accumulation in these plants seems nearly to be double to that of Cl⁻. Significantly, the Br⁻ to Cl⁻ uptake ratio, as a whole, is closely comparable to that of their atomic mass (2.25). Although, the Br⁻ to Cl⁻ atomic ratio in these plants is almost same, the data in Table 2 together with the visual observations in Figure 1 indicates that these plants could tolerate bromide ion to a considerable level. However, it affects moderately the chlorophyll activity at relatively high levels, unlike in the case of chloride ion.

The concentration of Br⁻ ion in different parts of plants obtained by different extraction methods was also calculated and the data on NaBr-fed *S. brachiata* plant (stem) is shown in Table 3. The data indicated that of all the extraction procedures adopted in the present study, the hot water and dilute nitric acid extracts, contained maximum bromide. The negligible bromide content in case of concentrated HNO₃ extract could be due to possible oxidation of Br⁻ to elemental Br₂ and its loss to atmosphere due to high volatility. Further, the low yields by direct ashing and fusing methods at 550°C might have caused the loss of bromide as HBr. The loss of halide by direct burning was reported earlier [31, 32].

4. Conclusions

In conclusion, the excessive uptake of bromide by the coastal halophytes, *S. brachiata*, *S. nudiflora*, and *S. persica*, was established. An easy and eco-friendly technique was developed for the first time for the extraction of bromide in different parts of the plants. A reliable spectrophotometric method of estimation was employed to derive the concentration of bromide in them. The plants absorbed high quantity of bromide, nearly double to that of chloride from soil and stored bromide about 0.086–0.200 g in the root and transported the excess to 0.175–0.443 g into the stem and 0.287–0.432 g to leaves per gram of dry plant material, confirming that these species could tolerate bromide as good as that of chloride and could be used in phytoremediation. Unlike Cl⁻ ion, these plants could tolerate bromide ion to a

considerable level; however, they were moderately affected at higher levels in their photosynthetic activity.

As demonstrated in the present study these halophytes, namely, *S. brachiata*, *S. nudiflora* and *S. persica*, could be cultivated/grown for their economic importance even on highly polluted soils for reclamation, wherein the halide, namely, chloride and bromide in the soil, can be effectively removed making the soil suitable for normal plantation/cultivation. Moreover, the plants thus cultivated could be utilized for their economic purpose/end products. Resorting to this type of cultivation in the coastal salinised lands leads to alleviation of poverty stricken population and thereby increasing their living standards.

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