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The effect of sea brine and bittern on survival and growth of mangrove *Avicennia marina* (Dicotyledones : Avicenniaceae)

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The effect of 25.5°B′e brine and 28.5°B′e bittern prepared from 3.5°B′e seawater was studied on the juvenile plants of the mangrove *Avicennia marina* (Forsk.) Vierh under greenhouse conditions. The maximum in elongation growth and percentage increase in number of leaves in 5% concentration of brine was 35.0 mm and 200% respectively. However for 5% bittern they were 33.0 mm and 160% respectively. The 50% concentrations of brine and bittern were growth inhibitory while 100% concentration was lethal during 8 hours continuous exposure for 10 days. The highest growth of the *Avicennia marina* was observed when brine and bittern were diluted between 20 and 200 times. The undiluted and 50% diluted brine and bittern were lethal and growth inhibitory, respectively. The leaves were the best indicator of the inhibitory or lethal effects on the plants. The 1 to 5% brine promoted the growth of the leaves while other concentrations were inhibitory or lethal. However, the bittern in the range of 9 ml l^{-1} and 1 to 50% were growth inhibitory for leaves, while 100% was lethal. The results indicate that discharge of brine and bittern in the marine environment, having semi diurnal tide cycle, will not be toxic or lethal to the plants.

[**Key words:** Brine, bittern, *Avicennia*, mangrove, pollution, salt tolerance]

The tidal zones of tropical seas are frequently lined with great mangrove forests. The dominating species belong to such genera as *Avicennia, Rhizophora, Bruguiera* and *Sonneratia*[1]. India has a mangrove cover of 4827 km^2 consisting of 50 species and in Gujarat, 9 species including *Avicennia marina*(Forsk.) Vierh have been reported growing over 1324.4 km^2 area[2]. The major regions of mangrove formations in Gujarat are Gulf of Kachchh, Gulf of Cambay and Saurashtra coast. Most degraded mangrove formations have been reported from Gulf of Kachchh and Gulf of Cambay[2-4].

In India, Gujarat state produces the highest quantity of edible and industrial grade common salt (sodium chloride). In this region the common salt is produced by solar evaporation of seawater in salt farms. The concentrated seawater or any solution of sodium chloride in water containing other salts is called as brine. The solution of bromide, magnesium and calcium salts that remain after sodium chloride has been crystallized by concentration of seawater or brine is known as bittern. This is usually achieved when the concentration of seawater reaches 28.5° B′e and more. The Baum′e (B′e) is an arbitrary scale of specific gravity and normally used for the graduation of hydrometers used for finding out the specific gravity of high concentration of salt solutions (one B′e is equal to 10000 mg l^1 salt concentration)[5,6]. The bittern is useful for recovery of marine chemicals but it is normally discharged to the coastal waters. Fate of mangrove forest vegetation due to discharge of brine and bittern to the marine environment is not known so far. There is a paucity of information on the effect of sea brine and bittern on *Avicennia marina*. However, the effect of sodium chloride solution and different lower concentration of normal seawater on growth and some of the physiological functions of *Avicennia marina* have been studied[7-9]. The aim of this study is to quantify the good or bad effects of brine and bittern on *Avicennia* in case they are discharged to the marine environment.

Materials and Methods

The juvenile plants of *Avicennia marina* (Forsk.) Vierh (Spermatophyta: Dicotyledones: Avicenniaceae) (14.44 cm ± 2.22 cm long) were collected from their natural habitat at Ghogha near Bhavnagar Port (lat. 21°45′N, long.72°14′E). One plant was planted in one litre plastic beakers containing sediment obtained from the same habitat of these plants. The plants were acclimatized for a week under greenhouse condition utilizing natural seawater. The brine (25.5° B′e) and

bittern (28.5° B′e) were obtained from the salt farm of a chlor-alkali industry.

In the first set of experiments different concentrations of brine and bittern (1,5,50 and 100% corresponding to 100.0, 20.0, 2.0 and 0.0 times dilution) were prepared separately using 3.5° B′e seawater collected from Ghogha. In the second set of experiments, *Avicennia marina* was treated with 1,3,5,7 and 9 ml bittern l^{1} of seawater corresponding to 1000.0, 333.3, 200.0, 142.8 and 111.1 times dilution of bittern by seawater. Triplicate sets of *Avicennia marina* were treated with each concentration of brine and bittern. Natural seawater of 3.5°B′e was used for control sets. Brine and bittern in 200 ml quantity were applied above the mud in each beaker while the same quantity of seawater was applied to control sets. These treatments were given for 8 hours everyday while during the remaining 16 hours of the day the different concentrations of brine and bittern were replaced with natural seawater. This sequence was continued till the end of the experiments. The plants in all the treatments were dipped for many times up to one hour in normal seawater to remove excreted salt. Only aerial portion of the plants were dipped avoiding contact of seawater with soil. This procedure and treatment of plants with brine and bittern for 8 hours was done to partially simulate tidal effect on the plants.

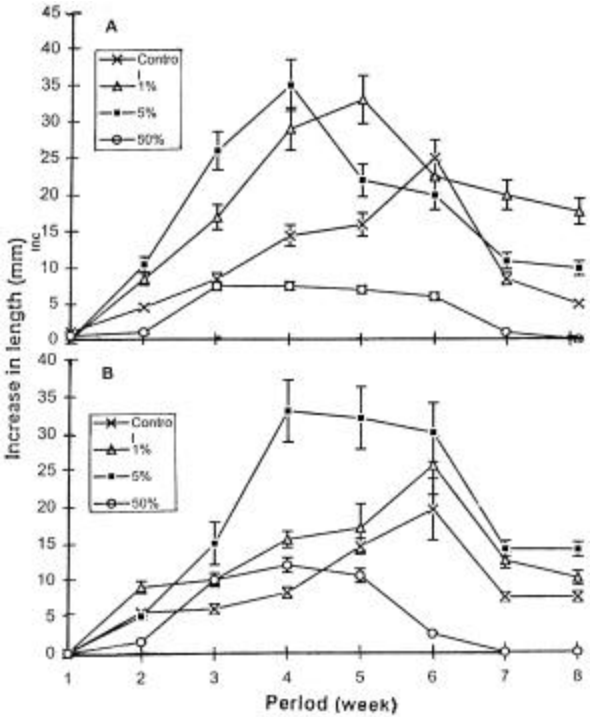

Fig. 1—The effect of different concentrations of brine (A) and bittern (B) on the elongation growth of *Avicennia marina*

The experiments were conducted in a greenhouse where the photon irradiance ranged from 31.0 ± 6.39 to 70.62 ± 13.86 µ mol m^{-2} s^{-1} and temperature from 18.52 ± 2.43 to 32.87 ± 4.42°C during the course of experiments. The growth of the plants in each replicate was measured in terms of increasing length and number of leaves per plant at weekly intervals. The growth of the leaves was calculated as percentage increase or decrease in number of leaves over initial number at the beginning of the experiment. However, percentage increase of length in treatment was calculated considering the control as 100% at different periods of growth. The brine and bittern were analyzed[10,11] for sodium, potassium, calcium, magnesium, chloride, sulphate, PO_4-P, NO_2-N, NO_3-N, NH_4-N, total nitrogen, and total phosphorus.

Results and Discussion

The effect of different concentrations of sea brine on the elongation growth of *Avicennia marina* is presented in Fig. 1A. The maximum growth was observed in 5% concentration ($P < 0.05$) during the 4th week of growth followed by 1% brine ($P < 0.05$) during the 5th week. The growth of the plants in these two concentrations were 141 and 100% more as compared to the control. The 50 and 100% concentrations were growth inhibitory. The effect of different concentrations of brine on growth (increase in number) of leaves of *Avicennia marina* is shown in Table 1A. The maximum increase in the number of leaves (200%) was observed in 5% ($P < 0.05$) brine followed by 160 % increase in 1% ($P < 0.05$) brine during the 4^{h} week of growth. The 50% concentration was inhibitory for formation and growth of leaves while 100% concentration was lethal.

The effect of different higher concentrations (1 to 50%) of bittern on increase in length of *Avicennia marina* is depicted in Fig. 1B. The increase in elongation growth of the plants in bittern also was maximum in 5% bittern ($P < 0.05$) followed by 1% bittern ($P < 0.05$) during the 4th and the 6th week of growth respectively. It was 288 and 31% more in these two concentrations as compared to the control. The effect of different higher concentrations of bittern on growth (increase in number) of leaves of *Avicennia marina* is depicted in Table 1B. The growth of the leaves in bittern at 50% concentration was inhibited by 80 to 60% during 3^{d} and 8^{h} week of growth, while 100% concentration was lethal after one week.

The effect of different lower concentrations (1 to 9 ml l^{-1}) of bittern on *Avicennia marina* is depicted

in Table 2A. The maximum length (97.5 mm) of the plant was observed in 5 ml l^{-1} (200 times dilution; $P<0.05$) concentration of bittern followed by 3 ml l^{-1} concentration (27.5 mm) (333.3 times dilution; $P<0.05$) during the 6th week of growth. The growth of the plants was inhibited by 9 ml l^{-1} concentration (111.1 times dilution). The effects of lower concentrations of bittern on the growth of the leaves (expressed as percentage decreased on increased in number of leaves) are presented in Table 2B. The maximum number of leaves was observed in 5 ml l^{-1} ($P<0.05$) followed by 3 ml l^{-1} ($P<0.05$) during the 6th and 8th week respectively. Concentration of bittern between 1 ml and 7 ml l^{-1} promoted the growth of the leaves ($P<0.05$ for each of these concentrations). However, 9 ml l^{-1} concentration was inhibitory for the growth of leaves.

The growth of the plants in 5% brine and bittern increased continuously from the first week to the 4th week and later on declined. However, the rate of decline in brine was faster than in bittern. The elongation growth of the plant in brine during the 4th week of growth, was slightly higher (6%) than in bittern. The growth of *Avicennia marina* was highest in 5% concentration followed by 1% concentration of bittern and brine. The lethal effect on the growth of plants in 100% bittern is probably due to high salt concentration and ionic imbalance as compared to seawater.

The colour of leaves changed from dark green to pale yellow on third day of treatment in 100% concentration of brine and bittern. The entire upper surface of the leaves and whole stem were covered with tiny crystals of excreted salts during this period. The

Table 1 — The effect of different concentrations of brine (A) and bittern (B) on growth of the leaves (percent increase or decrease over initial)

Concentration	Period (weeks)								
	0	1	2	3	4	5	6	7	8
(A) Brine									
Control	100	100	100	116.6	115.3	133.3	133.3	116.6	116.6
1%	100	100	90	90	160	120	116	110	110
5%	100	100	150	150	200	200	200	200	200
50%	100	100	120	90	80	60	60	60	60
100%	100	100	0	0	0	0	0	0	0
(B) Bittern									
Control	100	100	125	137.5	150	162.5	175	175	175
1%	100	100	100	100	120	120	140	140	160
5%	100	100	110	120	140	140	160	160	170
50%	100	100	100	80	80	80	80	80	60
100%	100	100	0	0	0	0	0	0	0

Table 2 — The effect of different lower concentrations of bittern on the elongation growth (mm) (A) and increase in number of leaves (B) of *Avicennia mari*

Concentration	Period (week)							
	1	2	3	4	5	6	7	8
(A) Elongation growth								
Control	2.0	4.0	8.0	9.0	3.0	2.5	2.5	2.0
1 ml l^{-1}	6.0	11.5	12.5	16.0	21.5	8.0	7.5	5.0
3 ml l^{-1}	1	13.0	17.5	22.0	27.5	28.5	8.0	6.0
5 ml l^{-1}	2.5	19.5	22.5	41.0	60.0	97.5	14.5	8.0
7 ml l^{-1}	1.0	12.0	13.0	16.5	22.5	9.5	3.0	2.0
9 ml l^{-1}	4.0	17.0	7.5	5.0	5.0	5.0	4.0	2.0
(B) Increase in number of leaves								
Control	100	100	100	100	100	100	100	120
1 ml l^{-1}	100	100	100	100	100	100	120	120
3 ml l^{-1}	100	100	150	150	150	150	150	150
5 ml l^{-1}	100	100	150	150	150	150	175	175
7 ml l^{-1}	100	100	125	125	125	125	125	125
9 ml l^{-1}	100	100	100	100	100	110	110	110

Table 3 — Physio-chemical analysis of seawater, brine and bittern used for experiments with *Avicennia marina*

	Seawater	Brine	Bittern
Specific gravity	1.03	1.22	1.24
*p*H	8.15	7.13	6.95
Baum′e (°B′e)	3.50	25.50	28.50
Na (ppm)	10880.00	102890.00	77890.00
K (ppm)	394.00	3560.00	7630.00
Ca (ppm)	423.00	340.00	150.00
Mg (ppm)	1311.00	11910.00	31740.00
Cl (ppm)	19655.00	185500.00	191160.00
SO_4 (ppm)	2742.00	16730.00	43230.00
PO_4-P (μ mol l^{-1})	1.73	0.05	1.00
NO_2-N (μ mol l^{-1})	0.47	0.08	0.21
NO_3-N (μ mol l^{-1})	5.38	0.032	0.22
NH_4-N (μ mol l^{-1})	0.63	1.50	1.50
Total Nitrogen (μ mol l^{-1})	87.38	17.23	21.32
Total Phosphorous (μ mol l^{-1})	7.68	7.32	9.27

lower surface of leaves were not covered with salt. At this stage such leaves droped while other normal leaves were normal. The excretion of the salt on the leaves was minimum during day time and maximum during night time. All leaves dried and shed within a week, however, stems still remained green up to 10 days and later on died. The length of the stem between the two whorls of leaves were longer in 5% and 5 ml l^{-1} concentration as compared to other concentrations of brine and bittern and control.

The physio-chemical analysis of seawater, brine and bittern is presented in Table 3. The values for specific gravity, Baum′e, potassium, magnesium, chloride, sulphate, PO_4-P, NO_2-N, NO_3-N, total nitrogen and total phosphorous were higher in bittern as compared to brine. However, the values for *p*H, sodium and calcium were higher in brine as compared to bittern. The concentrations of *p*H, calcium, PO_4-P, NO_2-N, NO_3-N and total nitrogen were higher in seawater as compared to brine and bittern. The concentration of NH_4-N did not show any variation in brine and bittern.

Ungar[9] has reported the reduction in growth of *Atriplex patula* L. — a halophyte with increase in NaCl salinity from 0-2%. However, in the present study the growth of *Avicennia marina* is up to 5% concentration of brine and bittern was more than in the controls. Therefore, the results indicate that the salinity caused by NaCl alone is more toxic than the higher salinity caused by the mixture of other salts containing higher concentration of NaCl. The 5% concentration of brine and bittern contains 3.92 and 3.59% NaCl respectively. The 5% concentration of brine and bittern gave maximum growth for *Avicennia marina*. However, the decline in growth after the peak period was more in brine than bittern. This is due to the higher concentration of sodium chloride in brine than bittern (Table 3). Drennan & Pammenter[7] have reported that the excretion of salt from *Avicennia marina* was greater in 100% than 50% seawater and the excretion was low during day time as compared to night time. Similar results have also been observed in our studies. It is reported that the *Avicennia marina* eliminates large quantity of salts through special glands in the leaves, a property which other species of mangrove like *Rhizophora* and *Sonneratia* do not possess[1]. Although *Avicennia* species have a mechanism to excrete the salt at normal seawater concentrations, in the present study the growth of the plant was considerably reduced or the plants died at 50% and 100% concentrations of brine and bittern. Therefore, the results indicate that this range of concentration is toxic or lethal to *Avicennia marina* even though it has a salt excretory mechanism. The concentrations of brine and bittern in the range of 10-100% are normally observed in the intertidal belt or tide pools where some part of *Avicennia* population grows naturally. The 5% concentration or less are observed in the subtidal region or in the intertidal region during high tides. However, considering the semi-diurnal nature of the tide on the Gujarat coast the discharge of large quantity of bittern and brine into the natural *Avicennia* forest area might not be toxic. These are the results obtained with juvenile plants of *Avicennia*. However, a study should be made of the effect of brine and bittern on mature plants of *Avicennia* forest in *in situ* conditions.

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