Salt and Oxalic Acid Content of Leaves of the Saltbush Atriplex halimus in the Northern Negev

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Highlight: Saltbush (Atriplex halimus L.) in the semiarid south of Israel was analyzed for leaf sodium, chlorine, and oxalic acid in order to identify and propagate low-salt bushes likely to be browsed more readily by range cattle and sheep. No correlation was found between leaf chlorine and growth habit factors like bush size and leafiness, or between chlorine and sodium. High-chlorine bushes had a lower Na/Cl ratio, and probably a substantial proportion of the Na⁺ and Cl⁻ ions are not linked as NaCl. Leaf oxalic acid was lower in high-chlorine bushes. The data suggest that moisture stress sharply reduced insoluble leaf oxalate. Values found are unlikely to cause toxicity problems in livestock.

The saltbush, *Atriplex halimus* L., grows wild mainly in the central and southern parts of the semiarid Negev, and also in the Jordan Valley. It is drought and salt resistant and remains green and succulent in late summer and autumn, when such forage is scarce in a Mediterranean environment. This has led the Israel Soil Conservation Service to plant it near settlements in southern Israel to provide ranchers with protein and mineral-rich browse to supplement stem-cured range forage, especially in drought years. However, the area planted to saltbush has failed to expand, largely because livestock and especially sheep often do not seem to browse it readily. The reason for this is not clear, but the situation makes the identification and propagation of saltbush more acceptable to stock an essential part of plant improvement.

Headden (1929), Bonsma and Maré (1942), Morrison (1956), Lachover and Tadmore (1965) and Dr. E. Eyal

The authors dedicate this study to the memory of "Kofish," as he was known to range scientists all over the world, (Prof. Naphtali Tadmor) of the Department of Botany, Hebrew University of Jerusalem, pioneer of Israeli range research and arid zone studies in Israel, who did much to inspire it.

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(personal communication) have expressed the view or provided data to show that high salt content of the leaves decreases livestock intake on the range.

Accordingly, in the present work, salt content of the leaves of a large number of saltbushes was determined (as chlorine), with the following aims:

1. To determine distribution of leaf chlorine content in the population.

2. To clarify whether leaf chlorine content is affected by location of bushes in the field.

3. To obtain high and low leaf chlorine propagating material for testing in a grazing experiment, and for plant improvement work.

4. To test for correlation of leaf chlorine with other parameters likely to be of importance in plant improvement, e.g. oxalate content, and growth habit factors such as bush size, prostrate habit (which makes for easier grazing by sheep), leafiness, and leaf size.

Materials and Methods

The saltbushes were planted 2 by 3 m apart at the Migda Experimental Range, 20 km NW of Beersheva, on non-saline sandy loam (loess) in March, 1964. They were nursery raised from seed collected from wild populations in the Negev and were 4.5 to 6 years old when sampled. Leaf chlorine on some 1,400 bushes in 1968-69 was determined by means of the Buchler Instrument Co.'s (1964) method of electrical titration with silver ions. On 85 bushes selected for further study, of which approximately half had a low leaf chlorine content, one quarter high, and one quarter medium, the following were determined in 1970 samplings: leaf chlorine, by titration as above, in July; leaf sodium and potassium, by ashing the leaves and using an "EEL" flame photometer on the aqueous extract, before the rains in November; total and water soluble oxalic acid (the latter on part of the samples only), using Moir's (1953) method, also in November.

Only fully expanded and non-senescent leaves within reach of cattle and sheep were sampled. Neither bases and tips of branches nor leaves on very high or very low branches were sampled. Preliminary tests had shown that leaves from the north and south sides of bushes sampled did not differ in chlorine content.

The soil of the Migda range is non-saline. Total soluble salts content was 0.08 - 0.22% of dry weight, and chlorine content 0.005 - 0.070% (Peleg and Lachover, 1963, sampling depth layers down to 180 cm). Our soil samples were taken from the 30 - 60 cm layer in which the roots of the adjacent bush

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seemed to be concentrated, 60 to 100 cm from the main stem. The growth habit parameters of bush size, prostrate habit, leafiness, and leaf size were visually estimated on a 0 to 10 scale (10 = maximum).

In analysing the data, the 85 bushes were divided into three groups according to leaf chlorine content (mean of 1968/69 and 1970 determinations): high content, > 11% C1 in dry matter; medium, 8 to 11%; and low, < 8%. For judging site effects (Fig. 2), only the low group was subdivided into low analysis bushes, 6 to 8%, and very low, < 6% C1 in dry matter. Weighted means (Table 4, footnote²) were based on the number of bushes in the population falling into each leaf chlorine group (Fig. 1; Table 2).

Results

Mean salt content of leaves of Atriplex halimus sampled in the rainless season in 1968/69, measured as % chlorine in dry matter, was $8.2 \pm (S.E.) 0.067\%$. Fig. 1 shows the frequency distribution of chlorine percentages estimated in single samplings of a total of some 1,400 individuals. The estimates were normally distributed at each of the sampling dates. In July 1969, 1,086 bushes were sampled with a mean of $8.3 \pm 0.066\%$ leaf chlorine; in November, 1968, 237 bushes with a mean of $7.9 \pm 0.070\%$; whereas the onset of rain caused the mean for the 83 bushes sampled in January, 1969, to drop to $6.3 \pm 0.190\%$.

The spatial distribution in the field of the very low, low, and high leaf chlorine bushes out of the 85 chosen for closer



Fig. 1. Distribution of chlorine content in leaves of Atriplex halimus L.

LEGEND



- - LOW 6-8% CI
- \triangle HIGH > 11% CI
 - H,L SOIL SAMPLES TAKEN



Fig. 2. Location of high and low leaf chlorine bushes of Atriplex halimus L. and of soil samples taken in the field.

study is shown in Figure 2. No connection could be found between mean leaf chlorine content of two samplings (1968/69; 1970) and location of bushes: low and high content bushes occupied adjacent sites. Table 1 shows data from soil samples taken adjacent to eight bushes high and eight low in chlorine content. Tests for soil chlorine and electrical conductivity showed the soil from these samples to be non-saline and did not reveal any site effect on leaf chlorine content.

Table 2 shows the range of values and means obtained from chemical analysis of leaves and visual ratings of growth habit of the bushes rated high, medium, and low in chlorine content. The ratio between leaf chlorine in the high and the low groups was about 2:1 for the group means, and 4:1 for the extreme values. Sodium values were much less variable, the comparable ratios being just over 1:1 and 2:1. This is reflected in the Na/Cl ratios of the three groups. The low content bushes contained twice as much sodium per unit of chlorine as the high group. Leaf potassium values, like those

Kind of bush and	Soil	sample	Saltbush leaves		
soil sample number	EC	Cl (ppm)	No.	Cl (%)	
Near high-chlorine bush	es				
й,	0.36	21.8	27/42	12.0	
H,	0.15	4.2	23/38	12.3	
H	0.34	8.1	11/39	11.3	
н	0.23	10.9	16/32	12.5	
H.	0.18	4.8	9/18	12.0	
HŽ	0.18	16.7	8/9	14.3	
н [°]	0.22	7.4	27/7	11.7	
H _s	0.18	5.4	27/8	13.0	
Near low-chlorine bushe	es				
L,	0.21	7.9	28/29	8.4	
L_2	0.26	6.2	28/21	6.3	
L	0.18	4.3	23/27	7.6	
$\frac{1}{L}$	0.20	7.7	25/19	7.0	
_4 L.	0.16	3.2	11/24	8.3	
L.	0.19	8.4	18/6	6.8	
L.	0.18	5.4	8/48	6.6	
\mathbf{L}_{8}^{-}	0.25	11.7	38/33	5.1	

Table 1. Saltbush leaf and soil chlorine (in dry weight) and soil electrical conductivity (EC, mmhos cm⁻¹) at 16 sampling sites.

for sodium, were much less variable than those for chlorine. In line with this relationship, Table 3, giving the correlation coefficients for the parameters of the 85 bushes, shows low correlations between leaf chlorine values on the one hand and those for sodium and potassium on the other.

High levels of both total and water soluble leaf oxalic acid were accompanied by low chlorine, and vice versa (Table 2). The correlation coefficient between chlorine and total oxalic acid was -0.54^* (Table 3), but for water soluble oxalic acid the coefficient was only -0.26. There was also a significant positive correlation between total and water soluble oxalic acid with a coefficient of 0.63^{**} . Less than half of the total oxalic acid was water soluble (Table 2).

No relationship could be found between the growth habit parameters of bush size, prostrate habit, leaf size, and quantity of leaves on the one hand, and leaf chlorine, sodium, and total oxalic acid on the other (Table 2). The appropriate correlation coefficients are low and non-significant (Table 3).

Discussion

The object of this study was to improve the prospects of increasing intake of Israel's native saltbush, *A. halimus*, by livestock, especially sheep, on semiarid range by locating and propagating bushes low in salt measurable as leaf chlorine.

The normal distribution of leaf chlorine content amongst the leaves means that to find bushes with extreme values, a large number must be screened and that the prospects of finding bushes either very low or high in leaf chlorine are roughly equal. Lachover and Tadmor (1965) found the seasonal changes in mean leaf chlorine content of three bushes of A. halimus in eastern Galilee to be closely paralleled by changes in leaf sodium. Our data do not exclude the possibility of most of the chlorine being present in the form of NaC1, but show that the Na/Cl ratio was smaller in high analysis bushes and that significant portions of the Na⁺ and Cl⁻ ions, in and on the leaves (Mozafar and Goodin, 1970) cannot, therefore, be linked as NaC1. Waisel and Eshel (1971) and Waisel (1972) reported that Na⁺ and C1⁻ in halophytes are distributed differently among plant tissues and even among parts of the cell.

The assumption that high leaf chlorine or salt content reduces saltbush intake by livestock has not been tested in a recent grazing experiment. Bonsma and Maré (1942) in South Africa with A. nummularia and Headden (1929) in Colorado with A. semibaccata, and the native saltbush A. argentia reported leaf chlorine or NaCl content of bushes avoided by sheep to be up to 20% higher than that of saltbush readily eaten. However, these data lack a test of significance.

The possibility of NaC1 (or C1) not being either the decisive or the only factor governing livestock preference on saltbush or mixed range cannot therefore be dismissed and must be considered when determining chemical criteria for saltbush improvement. On saltbush planted, as in Israel, to provide a protein and mineral supplement to stem-cured, non-halophyte herbaceous range, salt hunger might have the opposite effect to that assumed, and lead stock to seek out high-salt bushes (Dr. N. Seligman, personal communication). In determining saltbush improvement criteria, due weight

Table 2. Chemical and growth habit parameters of 85 bushes of Atriplex halimus, divided into 3 leaf chlorine content groups.

Parameter and group	Cl ¹ (%)	Na ¹ (%)	Na/Cl ¹ ratio	K ¹ (%)	Total oxalic acid ¹ (%)	Water soluble oxalic acid ¹ (%)	Bush size²	Degree of prostrate habit ²	Leaf size ²	Leafi- ness ²
Group No.	1:									
46 low chlo	orine bushes									
Mean	5.6	7.4	1.3	1.2	4.7	2.0	4.9	2.5	3.5	4.1
S. E.	1.0	0.3		0.1	0.1	0.1	0.3	0.3	0.1	0.2
Range	3.6-6.7	5.4-11.1		0.5 - 2.0	3.6-6.4	1.3-3.0	1.0-9.0	0.0-8.0	2.0-6.0	1.0 - 8.0
Group No.	2:									
19 medium	chlorine bush	nes								
Mean	8.8	8.9	1.0	1.4	4.4	1.8	4.2	2.2	3.3	4.2
S. E.	0.2	0.3		0.1	0.2	0.4	0.4	0.5	0.2	0.5
Range	6.6-9.8	6.6-10.4		0.7-2.3	2.8-5.7	1.2-2.7	1.0 - 8.0	0.0 - 8.0	2.0 - 5.0	1.0-9.0
Group No.	3:									
20 high chl	orine bushes									
Mean	12.5	8.3	0.7	1.4	3.5	1.6	4.2	3.4	3.2	4.6
S. E.	0.2	0.3		0.1	0.1	0.1	0.4	0.6	0.2	0.4
Range	10.9-14.4	6.6-11.1		0.6 - 2.1	2.5-4.2	1.0 - 2.1	2.0 - 8.0	0.0-9.0	2.0 - 7.0	2.0 - 8.0
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¹ Dry weight basis.

² Estimated on visual scale 0-10 (10 = maximum).

Table 3. Correlation coefficients (r) between chemical content (%) and growth habit parameters of saltbush (*Atriplex halimus* L.).¹

Chemical content	Chemical content ²			
growth habit parameters	Cl	Na	Total oxalic acid	
Na ²	0.28	_	-0.05	
K ²	0.26*	0.20	-0.13	
Total oxalic acid ²	-0.54*	-0.05	_	
Water soluble oxalic acid ²	-0.26	0.13	0.63**	
Bush size ³	-0.04	-0.22	0.10	
Degree of prostrate habit ³	0.18	-0.13	-0.07	
Leaf size ³	0.17	0.21	0.12	
Bush leafiness ³	0.10	-0.03	0.03	

*= significant at 5% level: ** = significant at 1% level.

¹ Based on 64-85 pairs of data (n); for data involving soluble oxalate : 35 pairs. ² Dry weight basis.

³Estimated on visual scale 0-10 (10 = maximum).

should therefore be be given to factors other than salt content. Oxalic acid content and the presence of sweet smelling volatile substances in saltbush and similar factors are likely to affect taste, flavour, and touch of the grazed portions, and hence, intake by sheep and cattle. Techniques such as Total Fluid Intake (Arnold and Hill, 1972) now make it possible to test directly for livestock acceptance or rejection of defined substances. Comparative studies of leaves from eaten and rejected bushes should therefore result in better data upon which to base saltbush improvement than those available until recently. While the low leaf chlorine content aimed at in this work should not be neglected, work now in progress did not confirm that in vegetative propagation of saltbush (Ellern, 1972) the leaf chlorine content of cuttings up to 1.5 years old is like that of the parent bushes. Improvement work in this direction is therefore dependent on further leaf chlorine tests of the cuttings, since the parent bushes were mature (about 5 years old) when tested.

Selecting saltbush for low salt content would be pointless if this were a soil rather than a plant effect. Our data do not indicate changes in soil chlorine or total soluble solids to account for the considerable variation in leaf chlorine (1:4). Workers with saltbush grown in nutrient solutions (Ashby and Beadle, 1957; Rosenblum and Waisel, 1969) reported a rise in leaf chlorine with rising substrate chlorine content. Beadle, Whalley and Gibson (1957), on the other hand, adduce clear evidence that in the field, leaf chlorine, though ranging from 9.6 to 13.6% C1 in dry matter, was completely unrelated to the enormous variation of soil chlorine ranging from 19 to 9,250 ppm. However, our analyses were carried out on only

Table 4. Mean anion and cation values in saltbush (*Atriplex halimus* L.) leaves sampled in semiarid (Negev) and subhumid (Galilee) environments.¹

Location	C1 (%)	Na (%)	Na/Cl ratio	K (%)	Total oxalic acid (%)	Water soluble oxalic acid (%)
Negev ² (Migda)	7.7	8.1	1.05	1.3	4.4	1.9
Galilee ³ (Moledet)	13.2	14.0	1.06	1.7	10.5	2.2

¹ Dry weight basis.

²Weighted means for 85 bushes (see "Methods").

³Means for three bushes.

one depth layer. They did reveal high soil chlorine or conductivity values near high leaf chlorine bushes in two analyses. Rosenblum and Waisel (1969) point out that roots of *Atriplex halimus* go down as much as 10 meters below the soil surface. Beadle et al. (1957) comment that "it is hard to know what soil layer to sample." Sharma and Tongway (1973) also stress the large variation in soil analyses taken a few meters apart in saltbush communities. The present study, therefore, does not clarify the effect of plant genetic versus soil factors in controlling leaf salt and chlorine content of saltbush.

The negative correlation between oxalic acid and chlorine in the leaves of saltbush may mean that selecting for both low leaf chlorine and low oxalic acid is not feasible. Association of low chlorine with high oxalic acid and vice versa was reported by Osmond (1963, 1967) in Australian saltbush (A. nummularia). Osmond found that "excess cations" such as Na⁺, K⁺ etc., are balanced by either organic and especially oxalic acid or the chlorine anion. Comparison of our Negev analyses with those of Lachover and Tadmor (1965) at Moledet in eastern Galilee shows higher values for leaf sodium, chlorine, and total oxalate in the Galilee analyses, whereas those for potassium and soluble oxalates were comparable to ours. The data suggest that in Galilee more oxalate was produced and that a major portion accumulated as insoluble calcium oxalate. Salguez (1962) found 40% more NaC1 in the ash of saltbush leaves in a coastal district of France than in an inland region. Soil and atmospheric moisture may well have played a part. Our analyses relate to bushes sampled in the 1968/69, 1969/70 and 1970/71 seasons, when rainfall was 224, 170 and 238 mm at the semiarid Negev location as against an ample 553 mm at the Galilee one in 1961/62. Osmond (1967) noted the association between nitrification and oxalate synthesis and quoted Bernstein and De Wit, who reported reduced organic acid synthesis in stressed plants. Mathams and Sutherland (1952) reported an immediate marked increase of oxalates in the salt bush A. semibaccata following rainfall. The low total oxalate content found in the Negev saltbush may, therefore, be due to soil moisture stress, possibly conducive also to lower nitrogen uptake. The level of oxalic acid found in the Negev saltbush is not likely to be toxic. Garner (1961) states that sheep's resistance to oxalic acid poisoning depends on state of nutrition and rumen bacteria. In a well-documented case of poisoning of cattle in Queensland (Jones, Seawright and Little. 1970) the grass Setaria sphacelata contained well over twice as much soluble oxalate as our Negev saltbush leaves. There does not, therefore, seem to be any need, because of toxicity, for saltbush selection to lower oxalic acid content. However, oxalic acid may affect taste and hence livestock intake.

Summary

A survey was made of some 1,400 saltbushes (Atriplex halimus L.) raised from seed collected from wild populations and planted 2 by 3 metres apart at the semiarid Migda Experimental Range. Leaf salt content was determined as chlorine with the aim of locating low-salt bushes likely to be more readily browsed by livestock and especially sheep. Eighty-five bushes, half with low, and one quarter each with medium and high leaf chlorine content, were studied in greater detail. Leaf chlorine, sodium, and potassium, as well as total and soluble oxalates, were determined. Growth habit was visually appraised on a 0 to 10 scale for size, prostrate habit, leaf quantity, and leaf size. Correlations between parameters were calculated.

Leaf chlorine was normally distributed in the population, and peak values were four times those of the lowest chlorine content. The Na/Cl ratio was lower in high chlorine bushes and no correlation between sodium and chlorine content was found. This suggests that a substantial portion of these ions are not linked as NaC1. There was a negative correlation between leaf chlorine and total oxalic acid. No correlation between growth habit and chemical composition was detected.

Comparison of these data with earlier work in Galilee, in a higher rainfall area, showed the Negev saltbush to be lower in leaf sodium and chlorine, somewhat lower in potassium, about the same in soluble oxalates, but markedly lower in total oxalates. Further work on saltbush should aim at comparative analyses of well and poorly grazed bushes, and include criteria other than leaf sodium and chlorine.

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