PHOTOSYNTHETICA 43 (2): 217-221, 2005

Leaf characteristics and gas exchange of the mangrove *Laguncularia racemosa* as affected by salinity

M.A. SOBRADO*

Laboratorio de Biología Ambiental de Plantas, Departamento de Biología de Organismos, Universidad Simón Bolívar, Apartado 89.000, Caracas 1080A, Venezuela

Abstract

Under constant salinity we analysed the leaf characteristics of *Laguncularia racemosa* (L.) Gaertn. in combination with gas exchange and carbon isotopic composition to estimate leaf water-use efficiency (WUE) and potential nitrogen-use efficiency (NUE). NaCl was not added to the control plants and the others were maintained at salinities of 15 and 30 ‰ (S_0, S_{15}, and S_{30}, respectively). Leaf succulence, sodium (Na), nitrogen (N), and chlorophyll (Chl) contents increased under salinity. Salinity had a negative impact on net photosynthetic rate (P_N) and stomatal conductance (g_s) at high and moderated irradiances. Potential NUE declined significantly (p<0.05) with salinity by 37 and 58 % at S_{15} and S_{30}, respectively, compared to S_0 plants. Conversely, compared to S_0 plants, P_N/g_s increased under saline conditions by 12 % (S_{15}) and 50 % (S_{30}). Thus, WUE inferred from P_N/g_s was consistent with salinity improved short-term WUE. Long-term leaf WUE was also enhanced by salinity as suggested by significantly increased leaf δ^{13}C with salinity. Improved WUE under salinity explains the eco-physiological success of mangrove species under increasing salinity. Conversely, decline in NUE may pose a problem for *L. racemosa* under hyper-saline environments regardless of N availability.

Additional key words: leaf succulence; net photosynthetic rate; nitrogen use efficiency; sodium accumulation; stomatal conductance; water use efficiency; δ^{13}C.

Introduction

Competitive exclusion by fast-growing glycophyte species seems to limit mangrove species to saline habitats (Sternberg and Swart 1987). Mangrove species differ in morphological, phenological, and physiological responses to salinity, and the species differences are consistent with the particular zone they occupy within mangrove ecosystems (Ball 1996). Thus, competitive exclusion along spatial salinity gradients seems to operate within mangrove species as well (Duke *et al.* 1998). The conservative water use by leaves enhance salt tolerance of mangrove species. However, this is at expense of growth rates, so that species with a broad range of salinity tolerance tend to grow more slowly than less tolerant species (Ball 1996).

The mangrove species *Laguncularia racemosa* (L.) Gaertn. F. (Combretaceae) occupies the medium to high inter-tidal zone of mangrove forests. It can form pure stands in disturbed areas (Tomlinson 1986). Consequently, it can grow under contrasting salinities from near freshwater to above seawater salinity (Medina and Francisco 1997, Sherman *et al.* 1998, Lovelock and Feller 2003, Suárez 2003, Sobrado 2004). Leaf tissue osmolality of this salt-secreting species increases with increase in external salinity and remains constant as the leaves age (Biebl and Kinzel 1965, Medina 1999, Cram *et al.* 2000). Stomatal conductance is very responsive to changes in both air relative humidity and soil water salinity (Sobrado 2004). However, under field conditions, instantaneous leaf water use efficiency (WUE) estimated from gas exchange measurements is probably not greatly affected by soil salinity (Lovelock and Feller 2003), whereas integrated WUE, from carbon isotope determinations, increases in parallel to soil water salinity (Medina and Francisco 1997). Nevertheless, salinity in the field is highly variable, and usually does not affect all the roots simultaneously and/or with the same intensity. Thus, some roots are exposed to highly saline soil layers whereas other roots of the same plant grow in soil layers only slightly saline or not-saline at all (Waisel 1985). In addition, a trade-off between leaf WUE and nitrogen use efficiency (NUE) has been found in a number of species (Field and Mooney 1986). Thus, the objective of this

---

Received 20 September 2004, *accepted* 17January 2005.

*Fax: 58-212-906 3046, e-mail: msobrado@usb.ve

Acknowledgments: Financial support was provided by DID-USB (Fondo de Trabajo 2003-2004). I thank anonymous referees for their criticisms and helpful suggestions to improve this paper.

study was to assess the leaf characteristics, to combine leaf gas exchange and carbon isotopic composition analysis in order to estimate short- and long-term WUE, and to assess potential NUE of *L. racemosa* under constant salinity. This could lead to a better understanding of this species at the physiological level compared with results from field studies where salinity is highly variable.

Materials and methods

Plants: Seeds of *L. racemosa* were collected in the field in August 2001 and seedlings were grown for three years in pots with sand and nutrient solution in a glasshouse under natural sunlight and photoperiod. Afterwards, plants were treated with a solution prepared by dissolving NaCl in 50 % Hoagland solution. Pots were placed on trays 8 cm high in order to maintain a constant water supply without becoming waterlogged. Twelve plants per treatment were maintained for three months prior to measurements. NaCl was not added to the control (S_0) plants. The others were maintained at salinities of 15 and 30 ‰ (abbreviated as S_{15} and S_{30}, respectively), which corresponded to 257 and 514 mol(NaCl) m^{-3}. The best eight plants per treatment were used for all the measurements except for analysis of carbon isotopic composition where four replicates per treatment were used. Branches and leaves for measurements were young, fully expanded, and selected from the sunny part of the plant canopy of all the plants. Leaves were selected from the third and fourth pairs from the branch tip, which were comparable in developmental stage. This selection allows overcoming leaf-ageing effects, which are critical in this species (Cram *et al.* 2000).

Leaf characteristics: A total of 4–8 leaves were collected from each of eight plants per treatment. Immediately after collection, leaf fresh mass and area were determined. Samples were oven-dried (80 °C) and dry mass was determined. With this results the water content on leaf dry mass (W_c) and the leaf dry mass to leaf area ratio (S_w) were determined for each sample. Dried samples were ground and homogenised for subsequent analysis. Nitrogen content was determined by the micro-Kjeldahl method and lipids were quantified gravimetrically after being extracted with diethyl ether (Williams 1984). Concentrations of Na were obtained in hot water extracts by means of atomic absorption spectrometry. Four leaves of each of eight plants per treatment were collected for determination of pigment composition, and stored immediately after collection in a deep freezer. Chlorophyll (Chl_{a+b}) and carotenoids (x+c) were extracted with 80 % acetone for spectroscopic (model *UV 1201V*, *Shimadzu*, Tokyo, Japan) measurements (Lichtenthaler and Wellburn 1983).

Carbon isotope analyses ($\delta^{13}C$) were performed in leaf samples from four plants per treatment. These analyses were conducted in the Stable Isotope Research Facility for Ecological Research of the University of Utah (Salt Lake City, USA) by using an isotope ratio mass spectrometer (model *delta S*, *Finnigan MAT*, San Jose, CA, USA). For detailed description of the procedures see Ehleringer *et al.* (1992). The $\delta^{13}C$ was used as an indicator of long-term WUE.

Gas exchange was measured in six to ten leaves from each of eight plants per treatment using a portable gas analyser system model *LCA-2* (*Analytical Development Co.*, Hoddesdon, Herts, UK). Measurements were taken at three irradiances of 450, 1 300, and 1 800 μmol m^{-2} s^{-1} leading to leaf temperatures of 28.5, 33.5, and 35.0 °C, respectively. During the measurements, the air relative humidity and the ambient CO_2 concentration (C_a) were about 40–45 % and 350 μmol mol^{-1}, respectively. The parameters determined were net photosynthetic rate (P_N), stomatal conductance (g_s), and intercellular (C_i) CO_2 concentration following Caemmerer and Farquhar (1981). With the measurements taken at maximal irradiance (1 800 μmol m^{-2} s^{-1}), trends of P_N/g_s ratios as well as C_i/C_a were used as estimates of short-term WUE.

Statistical analyses: The significance of the differences between the means of each parameter at the three salinities was tested by using one-way ANOVA. For treatment comparisons, variance homogeneity was tested and least significant difference (LSD) determined. The details of the statistical procedures followed Sokal and Rohlf (1969).

Results and discussion

Leaf characteristics: Leaf mass to leaf area ratio (S_w) did not change significantly with salinity (Table 1). However, leaf succulence increased under salinity as shown through significant increase of water content on dry mass basis (W_c) by about 44–47 % compared to S_0 plants (Table 1). Consistent with this, leaf Na and N contents per dry mass unit increased significantly with salinity (Table 1). Conversely, increase in lipid content with salinity was not statistically significant (Table 1). Under high external salinity (high Na/K), Na inevitably enters the roots in large quantities *via* K channels (Amtmann and Senders 1999, Cram *et al.* 2002). Leaf osmolality of *L. racemosa* tended to increase in parallel with salinity, and Na represents an important contribution to this increase in intracellular solute concentration (Medina and Francisco 1987, Medina 1999, Suárez 2003, Sobrado 2004). On a leaf area basis, Na content increases due to cell accumulation in expanding leaves and due to leaf succulence in mature and old leaves (Cram *et al.* 2000). Thus, as in other vascular halophytic plants, leaf salt content and succulence increase in response to saline environments and this avoids excessive leaf Na concentration on a bulk water basis (Jenning 1976). Changes in leaf succulence induced by salinity may be the result of enlargement of the hypodermis and palisade parenchyma in cell size as well as in layer number.

Table 1. Leaf mass to leaf area ratio (S_w), water content on a dry mass basis (W_c), contents of sodium (Na), nitrogen (N), lipids, chlorophylls (Chl), and total carotenoids (Car). Means±SE of measurements of eight plants per salinity [‰] level (S_0, S_{15}, S_{30}). Significant differences ($p<0.05$) between treatments are indicated with different letters within the same row.

Parameter	S_0	S_{15}	S_{30}
S_w [g(d.m.) m^{-2}]	121 (2) a	129 (6) a	130 (5) a
W_c [g(H_2O) g^{-1}(d.m.)]	1.45 (0.04) a	2.14 (0.05) b	2.09 (0.08) b
Na [g kg^{-1}(d.m.)	1.12 (0.06) a	16.61 (1.22) b	24.67 (2.67) c
N [g kg^{-1}(d.m.)]	12.0 (0.5) a	12.3 (0.5) a	15.2 (0.7) b
Lipids [g kg^{-1}(d.m.)]	28 (3) a	23 (3) a	26 (2) a
Chl *a* [mg m^{-2}]	339 (15) a	372 (7) ab	400 (23) b
Chl *b* [mg m^{-2}]	89 (9) a	123 (4) b	140 (6) c
Chl *a*+*b* [mg m^{-2}]	428 (15) a	495 (9) b	540 (25) b
Chl *a/b*	3.9 (0.5) a	3.0 (0.1) b	2.9 (0.2) b
Car [mg m^{-2}]	92.5 (8.0) a	106.9 (8.9) a	117.4 (20.5) a

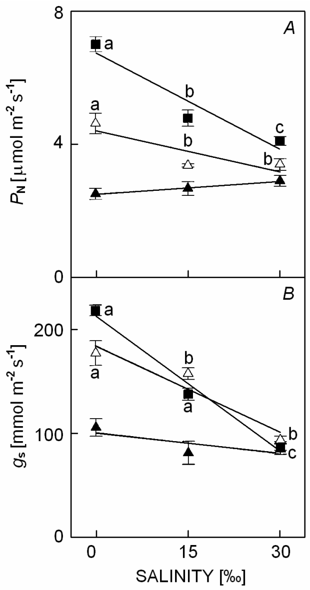

Fig.1. Net photosynthetic rate (P_N) and stomatal conductance (g_s) measured in plants grown without NaCl or at salinities of 15 and 30 ‰. Measurements at three irradiances of 450 (▲), 1 300 (Δ), and 1 800 (■) µmol m^{-2} s^{-1} leading to leaf temperatures of 28.5, 33.5, and 35.0 °C, respectively. Means for eight plants per treatment. *Bars* represent standard errors. Comparison of P_N and g_s among salinity treatments lead to statistical differences ($p\leq0.05$) at high and moderate irradiances.

Chlorophyll (Chl) *a* and *b* contents per unit leaf area were significantly higher under salinity compared to values of control plants (Table 1). A similar tendency was also observed in total carotenoids (Car), but the differences were not statistically significant. Despite this, control plants had significantly higher Chl *a/b* compared to plants under salinity. This may suggest greater photochemical capacity in control plants. Leaves in the three treatments appeared healthy. Chl/Car ratio remained about 4.6 in all treatments. Values of Chl *a/b* and Chl/Car were typical of photosynthetically active sun-exposed leaf tissue (Šesták 1985, Lichtenthaler 1987). However, Chl *a* content and Chl *a/b* ratio may decline in some species as a result of salinity (Singh and Dubey 1995, Ranjbarfordoei *et al.* 2002). The partition of N into Chl (Chl/N) remained between 0.27 and 0.31 in the three treatments. Under field conditions, Chl/N is between 0.17–0.23 (Medina and Francisco 1997, Suárez 2003). This may reflect N limitations under natural conditions.

Photosynthetic gas exchange: Salinity affected P_N at high and moderate irradiances (Fig. 1*A*). However, maximum P_N was found in S_0 plants at high irradiance and it declined significantly by 32 and 41 % at S_{15} and S_{30}, respectively. This was related to some extent to Na accumulation under salinity. In halophyte species, content of Na in the cytoplasm is about one-third of that in the vacuole, and this accounts for minimised cytotoxicity (Flowers and Yeo 1986). Cytoplasmic ion concentration may reach toxic levels at high salinity when a large content of intracellular salt occurs in *L. racemosa*. This may be ameliorated by enhanced gland secretion as salinity increases (Sobrado 2004). Salinity leads to reduction of P_N of mangroves due to limited inward diffusion of CO_2 (stomatal closure and low leaf internal conductance for water and CO_2), and also by salt effect on biochemical reactions of CO_2 fixation (Ball and Anderson 1986, Ball and Sobrado 1999, Sobrado 1999, Sobrado and Ball 1999, Kao *et al.* 2003). Despite P_N reduction and Na content increase with salinity, plants appeared healthy, and their leaf N and pigment contents increased (Table 1). Consequently, potential NUE (P_N per N content) declined significantly ($p<0.05$) with salinity by 37 and 58 % at S_{15} and S_{30}, respectively, compared to control plants (Fig. 2). Maximum P_N has been related to leaf N content in a number of species (Field and Mooney 1986, Evans 1989). Decline in potential NUE was followed by concomitant decline in P_N/Chl (Fig. 2*A*). Overall, NUE values were comparable to those found over a range in field grown *L. racemosa* over a range of N availability (Lovelock and Feller 2003).

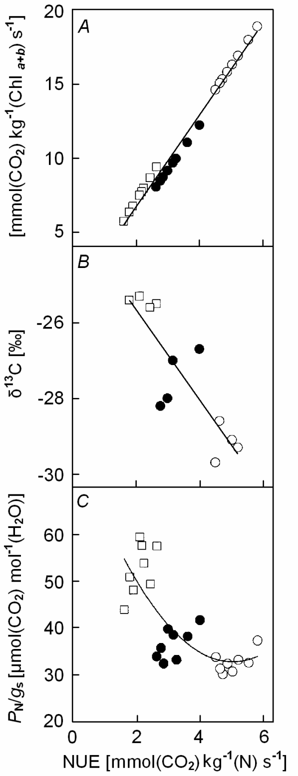

Fig. 2. P_N/Chl *a+b*, P_N/g_s, and $\delta^{13}C$ as a function of potential nitrogen use efficiency (NUE) in S_0 (○), S_{15} (●), and S_{30} (□) plants. P_N and g_s were measured at irradiance of 1 800 μmol m^{-2} s^{-1} in eight plants per salinity. The $\delta^{13}C$ was analysed in four plants.

g_s increased significantly with irradiance (Fig. 1*B*). However, we found that salinity negatively influenced g_s at high and moderated irradiances but not at low irradiance. Thus, at saturating irradiance, g_s declined by 37 and 60 % at S_{15} and S_{30}, respectively, compared to S_0 plants (Fig. 1*B*). Maximum P_N/g_s ratio increased under saline conditions by 12 (S_{15}) and 50 (S_{30}) % compared to control (Fig. 2*B*). The C_i/C_a was 0.70±0.02 in S_0 and S_{15}, but declined significantly ($p<0.05$) to 0.65±0.01 at S_{30}. Instantaneous WUE inferred from P_N/g_s suggested improved water use at high salinity in *L. racemosa*. Furthermore, long-term leaf WUE was also enhanced by salinity as suggested by increased leaf $\delta^{13}C$ (Fig. 2*C*). Increased leaf $\delta^{13}C$ agrees with higher WUE in plants from a broad range of provenance (Farquhar *et al.* 1982, 1989). Conservative water use by mangrove species aids the maintenance of favourable ionic balance (Ball 1988). In *L. racemosa*, enhanced long-term WUE ($>\delta^{13}C$) in this species was reported as salinity increased under field conditions from 0 to 40 ‰ (Medina and Francisco 1997). However, short-term WUE found in gas exchange measurements is comparable among plants thriving hyper-saline conditions between 32 and 54 ‰ (Lovelock and Feller 2003). Patchy soil salinity may bias salinity determination of actual water sources collected by roots (Waisel 1985). On the other side, WUE of mangroves may increase with salinity in sigmoidal fashion (Sobrado 1999). Thus, under hyper-saline conditions, WUE tends to reach a maximum.

In conclusion, salinity had a negative impact on leaf gas exchange parameters of *L. racemosa*. This species is attuned to salinity by controlling stomatal aperture, which in turn maintains carbon gain with improved WUE but also brings about a parallel decline in potential NUE. Improved WUE under salinity explains the eco-physiological success of mangrove species under increasing salinity. Conversely, decline in NUE may pose a problem for *L. racemosa* under hyper-saline environments regardless of nitrogen availability.

References

Amtman, A., Sanders, D.: Mechanisms of Na$^+$ uptake by plant cells. – Adv. bot. Res. **29**: 75-112, 1999.

Ball, M.C.: Salinity tolerance in the mangroves *Aegiceras corniculatum* and *Avicennia marina*. I. Water uses in relation to growth, carbon partitioning, and salt balance. – Aust. J. Plant Physiol. **15**: 447-464, 1988.

Ball, M.C.: Comparative ecophysiology of mangrove forest and tropical lowland moist rainforest. – In: Mulkey, S.S., Chazdon, R.L., Smith, A.P. (ed.): Tropical Forest Plant Ecophysiology. Pp. 461-496. Chapman and Hall, New York 1996.

Ball, M.C., Anderson, J.M.: Sensitivity of photosystem II to NaCl in relation to salinity tolerance. Comparative studies with thylakoids of the salt-tolerant mangrove, *Avicennia marina*, and the salt-sensitive pea, *Pisum sativum*. – Aust. J. Plant Physiol. **13**: 689-698, 1986.

Ball, M.C., Sobrado, M.A.: Ecophysiology of mangroves: challenges in linking physiological process with patterns in forest structure. – In: Press, M.C., Scholes, J.D., Baker, M.G. (ed.): Advances in Plant Physiological Ecology. Pp. 331-346. Blackwell Science, Oxford 1999.

Biebl, R., Kinzel, H.: Blattbau unter Salzhaushalt von *Laguncularia racemosa* (L.) Gaertn. f. unter anderer Mangrovebäume auf Puerto Rico. – Österr. bot. Z. **112**: 56-93, 1985.

Caemmerer, S. von, Farquhar, G.D.: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. – Planta **153**: 376-387, 1981.

Cram, W.J., Torr, P.G., Rose, D.A.: Salt allocation and leaf development and leaf fall in mangroves. – Trees **16**: 112-119, 2000.

Duke, N.C., Ball, M.C., Ellison, J.C.: Factors influencing biodiversity and distributional gradients in mangroves. – Global Ecol. Biogeogr. Lett. **7**: 27-47, 1998.

Ehleringer, J.R., Phillips, S.L., Comstock, J.P.: Seasonal variation in the carbon isotopic composition of desert plants. – Funct. Ecol. **6**: 396-404, 1992.

Evans, J.R.: Photosynthesis and nitrogen relationships in leaves of C_3 plants. – Oecologia **78**: 9-19, 1989.

Farquhar, G.D., Ball, M.C., Caemmerer, S. von, Roksandic, S.: Effect of salinity and humidity on $\delta^{13}C$ value of halophytes – evidence for diffusional isotope fractionation determined by the ratio of intercellular/atmospheric partial pressure of CO_2 under different environmental conditions. – Oecologia **52**: 121-124, 1982.

Farquhar, G.D., Ehleringer, J.R., Hubick. K.T.: Carbon isotope discrimination and photosynthesis. – Annu. Rev. Plant Physiol. Plant mol. Biol. **40**: 503-537, 1989.

Field, C., Mooney, H.A.: The photosynthesis-nitrogen relationship in wild plants. – In: Givnish, T.J. (ed.): On the Economy of Form and Function. Pp. 25-55. Cambridge University Press, Cambridge 1983.

Flowers, T.J., Yeo, A.R.: Ions relations of plants under drought and salinity. – Aust. J. Plant Physiol. **13**: 75-91, 1986.

Jenning, D.H.: Halophytes, succulence and sodium in plants. A unified theory. – New Phytol. **67**: 899-911, 1968.

Kao, W.-Y., Tsai, T.-T., Shih, C.-N.: Photosynthetic gas exchange and chlorophyll *a* fluorescence of three wild soybean species in response to NaCl treatments. – Photosynthetica **41**: 415-419, 2003.

Lichtenthaler, H.K.: Chlorophyll fluorescence signatures of leaves during the autumnal chlorophyll breakdown. – J. Plant Physiol. **131**: 101-110, 1987.

Lichtenthaler, H.K., Wellburn, A.R.: Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. – Biochem. Soc. Trans. **603**: 591-592, 1983.

Lovelock, C.E., Feller, I.C.: Photosynthetic performance and resource utilisation of two mangrove species coexisting in a hypersaline scrub forest. – Oecologia **134**: 455-462, 2003.

Medina, E.: Mangrove physiology: the challenge of salt, heat and light stress under recurrent flooding. – In: Yánez-Arancibia, A., Lara-Domínguez, A.L. (ed.): Ecosystemas de Manglar en América Tropical. Pp. 109-126. Instituto de Ecología A.C. Xalapa, México. UICN/ORMA – Costa Rica NOAA/NMFS, Silver Spring 1999.

Medina, E., Francisco, M.: Osmolality and $\delta^{13}C$ of leaf tissue of mangrove species from environments of contrasting rainfall and salinity. – Estuar. coast. Shelf Sci. **45**: 337-344, 1997.

Ranjbarfordoei, A., Samson, R., Lemeur, R., Van Damme, P.: Effects of osmotic drought stress induced by a combination of NaCl and polyethylene glycol on leaf water status, photosynthetic gas exchange and water use efficiency of *Pistacia khinjuk* and *P. mutica*. – Photosynthetica **40**: 165-169, 2002.

Šesták, Z.: Chlorophylls and carotenoids during leaf ontogeny. – In: Šesták, Z. (ed.): Photosynthesis during Leaf Development. Pp. 76-106. Academia, Praha; Dr W. Junk Publ., Dordrecht – Boston – Lancaster 1985.

Sherman, R.E., Fahey, T.J., Howarth, R.W.: Soil-plant interactions in a neotropical mangrove forests: iron, phosphorus and sulfur dynamics. – Oecologia **115**: 553-563, 1998.

Singh, A.K., Dubey, R.S.: Changes in chlorophyll *a* and *b* contents and activities of photosystems 1 and 2 in rice seedlings induced by NaCl. – Photosynthetica **31**: 489-499, 1995.

Sobrado, M.A.: Leaf photosynthesis of the mangrove *Avicennia germinans* as affected by NaCl. – Photosynthetica **36**: 547-555, 1999.

Sobrado, M.A.: Drought effect on photosynthesis of the mangrove, *Avicennia germinans*, under contrasting salinities. – Trees **13**: 125-130, 1999.

Sobrado, M.A.: Influence of external salinity on the osmolality of xylem sap, leaf tissue and leaf gland secretion of the mangrove *Laguncularia racemosa* (L.) Gaertn. – Trees **18**: 422-427, 2004.

Sobrado, M.A., Ball, M.C.: Light use in relation to carbon gain in the mangrove, *Avicennia marina*, under hypersaline conditions. – Aust. J. Plant Physiol. **26**: 245-251, 1999.

Sokal, R.R., Rohlf, F.J.: Biometry. – W.H. Freeman and Co., San Francisco 1969.

Sternberg, L., Swart, P.K.: Utilization of freshwater and ocean water by coastal plants of Southern Florida. – Ecology **68**: 1898-1905, 1987.

Suárez, N.: Leaf longevity, construction, and maintenance costs of three mangrove species under field conditions. – Photosynthetica **41**: 373-381, 2003.

Tomlinson, P.B.: The Botany of Mangroves. – Cambridge University Press, London 1986.

Waisel, Y.: The stimulating effects of NaCl on root growth of Rhodes grass (*Chloris gayana*). – Physiol. Plant. **64**: 519-522, 1985.

Williams, S.: Official Methods of Analysis. – AOAC, Arlington 1984.