Int. J. Environ. Res., 4(1):1-10,Winter 2010
ISSN: 1735-6865

Testing *Nerium Oleander* as a Biomonitor for Surfactant Polluted Marine Aerosol

Gonthier, P. [1], Nicolotti, G .[1*], Rettori, A.[1] , Paoletti, E.[2] and Gullino, M. L.[3]

[1] Plant Pathology, University of Torino, Via Leonardo da Vinci 44, I-10095 Grugliasco (Torino), Italy
[2] Plant Protection Institute – CNR, Via Madonna del Piano 10, I-50019 Sesto Fiorentino (Firenze), Italy
[3] Center of Competence for the Innovation in the Agro-environmental field, University of Torino, Via Leonardo da Vinci 44, I-10095 Grugliasco (Torino), Italy

Received 1 April 2009; Revised 12 Aug. 2009; Accepted 19 Aug. 2009

ABSTRACT: Oleander was tested as biomonitoring plant for surfactant polluted marine aerosol. Potted plants in the greenhouse were sprayed once a week for 5 weeks with seawater containing sodium dioctyl sulfosuccinate (SDS) at the following concentrations: 5, 10, 15, 30, 60, 120, 250, 500 mg/L. A significant correlation was found between SDS concentration in the spray and surfactant deposit on the leaves at the end of the 5 weeks. At that time and two months later, we assessed: leaf visible injury, foliar chloride content, damage to stomatal crypts, water potential, net photosynthesis and stomatal conductance. Relative to controls (not sprayed and sprayed either with deionized water or with seawater without surfactants), all the parameters were affected ($P<0.05$) by the presence of surfactant. Furthermore they were correlated ($P<0.05$) with the concentration of surfactant. Visible injury occurred after treatments containing concentrations of surfactant exceeding 30 mg/L.

Key words: Sea pollutants, Detergents, Bioindicator, Physiopathology, Stomata

INTRODUCTION

Studies concerning the deterioration of coastal vegetation in densely-populated areas and in proximity to river mouths started, in Italy, around the end of the 1960s (Gellini and Paiero, 1969; Lapucci *et al.*, 1972). According to these and subsequent studies, the role of pollution caused by surfactants, i.e. the active ingredients in commercially-available detergents, is crucial in determining environmental damages to the coastal flora. Surfactants reach the vegetation carried by marine aerosols, particularly frequent when seas are heavy. Surfactants cause the leaves to absorb more sea salt, leading to degeneration of the epicuticular waxes and alteration of photosynthetic processes (Itoh *et al.*, 1963; Helenius and Simons, 1975; Badot and Badot, 1995; Badot *et al.*, 1995). Along with desert dust, marine aerosol represents the highest percentage of aerosol emitted into the atmosphere each year (Giorgi, 1996).

*Corresponding author E-mail: giovanni.nicolotti@unito.it

Recently the phenomenon has been reported to assume worrying proportions in some coastal areas both in Italy (Bussotti *et al.*, 1997; Nicolotti *et al.*, 2001; Paoletti 2001; Rettori *et al.*, 2005) and in other Countries such as Australia, France, Spain, Turkey and Tunisia (Astorga *et al.*, 1993; Badot and Garrec, 1993; Garrec and El Ayeb, 2001; Marull *et al.*, 1997). Despite the ban on non-biodegradable surfactants, the situation has not improved in the last years and these substances have been persisting in marine and coastal environments. Some investigations carried out in Israel and Turkey confirm the presence of surfactants in the Mediterranean sea and rivers (Vural *et al.*, 1997; Zoller and Hushan, 2001).

Damages by surfactant-polluted marine aerosols, while found on a worldwide scale, strike well-defined areas, located either by the mouth of rivers near highly populated centers or in other

remote locations, when the marine currents carry a surfactant load along the coastline (Nicolotti *et al.*, 2005). Damages are generally found within 500-800 m inland.The classical analytical systems for the environmental monitoring have been successfully employed for the most common pollutants, i.e. those impacting on human health. On the contrary, compounds at very low concentrations are rarely analysed, including those having a potential impact on vegetation (Bargagli, 1998). For these reasons, the use of plants for environmental monitoring should be regarded as a suitable tool to integrate classical analytical methods.Among the most impacting pollutants, surfactants within marine aerosols are not detected by the classical systems. In addition, there are a very few reports about the biomonitoring of surfactants through plants (Nali *et al.*, 2009). Hence there is a need to identify a specific biomonitor, sensitive, rustic and ornamental, when placed in urban environments, and useful to set up a wide and not expensive monitoring net. Preliminary field observations indicated that Oleander (*Nerium oleander* L.) is affected by foliar symptoms of abiotic origin in densely populated areas with spillage of high quantities of surfactant into the sea (Nicolotti *et al.*, 2005). The above symptoms occur as a progressively-spreading yellowing of the foliar lamina which may necrotize, leading to partial leaf fall, especially on the side exposed to sea winds. The phenomenon is extremely evident all the year round as Oleander is an evergreen species. Even if surfactant polluted marine aerosols can induce aspecific symptoms on many broadleaves and conifers species, Oleander was studied as a possible surfactant biomonitor, since it is sensitive to detergents, rustic and widespread along the Mediterranean coasts. This species has been used as a bioindicator to assess the presence of airborne heavy metals in urban environments (Oliva and Mingorance, 2006; Mingorance *et al*., 2007).

The main aim of this study was to test Oleander as a biomonitoring species for surfactant polluted marine aerosol. In particular we studied: 1) how realistic concentrations of surfactants affect the onset of aerosol toxicity in Oleander under controlled conditions, 2) which is the concentration threshold over which visible injury occurs, and 3) which physiological parameters are better correlated with the surfactant concentration.

MATERIALS & METHODS

Plant material and growing conditions

Fifty-five healthy 3-year-old Oleander plants (*Single Salmon* variety, Cultivar group 4, single flower Pagen, 1988), 1 to 1.20 m height, growing in 5 dm^3 pots were used. Plants were watered daily. Treatments were carried out in a greenhouse where air temperature and relative humidity were 20 ± 2°C and 40–60 %, respectively. The seawater used for the treatments, collected at a depth of 2 m off the Ligurian coast, was analysed in the laboratory using the Methylene Blue Active Substances (MBAS) method (Longwell and Maniece, 1955), in order to verify the absence of surfactant. The seawater was stored at 5 °C until use.

Plant crowns were sprayed once a week, for a period of 5 weeks, with seawater containing sodium dioctyl sulfosuccinate (SDS) at the following concentrations (Polluted Seawater; PSW): 5, 10, 15, 30, 60, 120, 250, 500 mg/L. Such concentrations were chosen to ensure foliar deposits comparable with those reported in the field (Nicolotti *et al.*, 2005). Non-sprayed (NW) plants, plants sprayed with deionized water (DW) and plants sprayed with seawater and no surfactant (SW) were used as controls. For each treatment, 5 plants were sprayed until dripping point. A completely randomized block design was used.Before the treatments, in order to avoid contaminations of the soil, the pots were covered with a polythene film that was removed after each spray and replaced before the next one. Sprays were performed with a 100-l air compressor that was connected to a spray gun with a reservoir of 1 l, and regulated at an air exit pressure of 4 atm. The flow was regulated as to obtain drops of 70–150 µm diameter, as measured by using hydrosensitive sheets of paper (Guidi *et al.*, 1988). For each plant, the following parameters were measured: 1) visible injury, 2) surfactant deposits on leaves, 3) foliar content of chloride (Cl^-), 4) damages to the stomatal crypts, 5) water potential at midday, and 6) gas exchange. Visible injury was assessed three times, as suggested by Horsfall and Barratt (1945): at the end of the 5 weekly treatments (time I), one month (time II) and two months later (time III). The other parameters were assessed twice, immediately after the 5 treatments and two months later, except surfactant deposit on leaves that was determined only after the period of treatments.

Visible injury was assessed on all leaves of three randomly selected plants per treatment. The mean percentage of injured surface area per plant (IA) was visually assessed by using the scale in Granett (1982). The percentage of injured leaves per plant (IP) was calculated. A Plant Injury Index (PII) was then calculated by combining the two parameters: PII = (IA * IP) / 100 (Paoletti *et al*., 2009).For the assessment of surfactant deposit on leaves, 50 g of fresh leaves were sampled from each plant, washed in 1 l of deionized water, and analyzed using the Methylene Blue Active Substances (MBAS) method (Longwell and Maniece, 1955).For the determination of chloride content, 10 g of fresh leaves per plant per treatment were sampled. To remove chloride deposited above the leaves, the samples were washed 5 times in deionized water, each washing lasting 5 min. Content of Cl^- was calculated by the volumetric method (American Public Health Association, 1992).

As Oleander stomata are inside crypts, i.e. invaginations of the epidermis in which several stomata are located, and crypts are covered by trichomes (Fig. 1). it was not possible to assess alterations of individual stomata. Thus, alterations of stomatal crypts as a whole were assessed on 15 randomly selected fully-developed leaves per treatment, 3 leaves per plant. The leaves were gathered with tweezers, in order to avoid damaging the cuticles during handling, and were dried as recommended by Kurhu and Huttunen (1986). Three square-shaped areoles of 2-3 mm^2 were collected from the apical portion and from the centre of each leaf, fixed on an aluminium stub, metalized (sputter coater E5000C PS3), and observed with a 15 kV Cambridge Stereoscan 200 Scanning Electron Microscope. For each leaf areole, 50 crypts were assessed by using a modified version of the damage classes described by Crossley and Fowler (1986) (Fig. 1). An index of damage (ID) was finally calculated by applying the formula of Raddi *et al*. (1992).Measurements of midday water potential (MWP) were performed in the hottest hours of the day (from 11 a.m. to 2 p.m.) by using a portable pressure chamber (SKPM 1400, Skye Instruments). The crowns of three randomly selected plants per treatment were ideally subdivided into three portions (upper, middle and lower). Water potential was measured on three randomly chosen mature leaves per crown portion.

Gas exchange was measured with a portable gas exchange system (ADC/LCA-3) on three plants per treatment. The measurements were taken randomly in the top third of the crown since most of the photosynthesis and transpiration occur in that portion (Turner, 1981). To reduce variability due to the stage of foliar development, only fully-developed leaves (three per branch on three branches per plant) and with good exposure to the sun were chosen (Wong and Dunin, 1987). Measurements were carried out in clear blue skies (from 10.30 a.m. to 3.30 p.m.), at saturating light, i.e. 1300 $\mu mol\ m^{-2}\ s^{-1}$ of photosynthetically active photon flux density (PPFD).Data were analyzed either with parametric or non-parametric methods depending on their normal distribution, as assessed through the Kolmogorov-Smirnov and Lilieford tests. Analysis of variance (ANOVA) and Tukey HSD test were used to test differences among treatments for gas exchange parameters (net photosynthesis and stomatal conductance) and surfactant deposit on leaves. For the other parameters, differences were tested through the non-parametric Kruskal-Wallis test. Cut-off for significance was set at 5%. After a significant Kruskal-Wallis, the Dunn test was performed as a post hoc test. The Spearman rank order correlation test was used to test correlations between parameters and concentration of surfactant in the treatment sprays. Statistical analyses were performed using the software Statistica (StatSoft Inc., Tulsa, Oklahoma).

RESULTS & DISCUSSION

At the end of treatments (time I), visible injury occurred at concentrations equal or greater than PSW30 (Fig. 2). corresponding to a surfactant deposit on the leaf surface of about 1 mg/L/kg fw (Fig. 3). At this time, PSW d" 120 did not differ significantly from controls while PSW250 and PSW500 did. At time II and III, injury occurred on the foliage of all plants treated with PSW, irrespective of SDS concentration. Controls did not show necrosis. Severity of symptoms was higher in plants treated with PSW containing higher concentrations of SDS, and became significantly higher with respect to controls after treatments with PSW 30. Necrosis always started at the apical portion of leaves and then progressively developed to the edges, spreading rapidly towards the inner leaf edge without affecting the veins. Injury

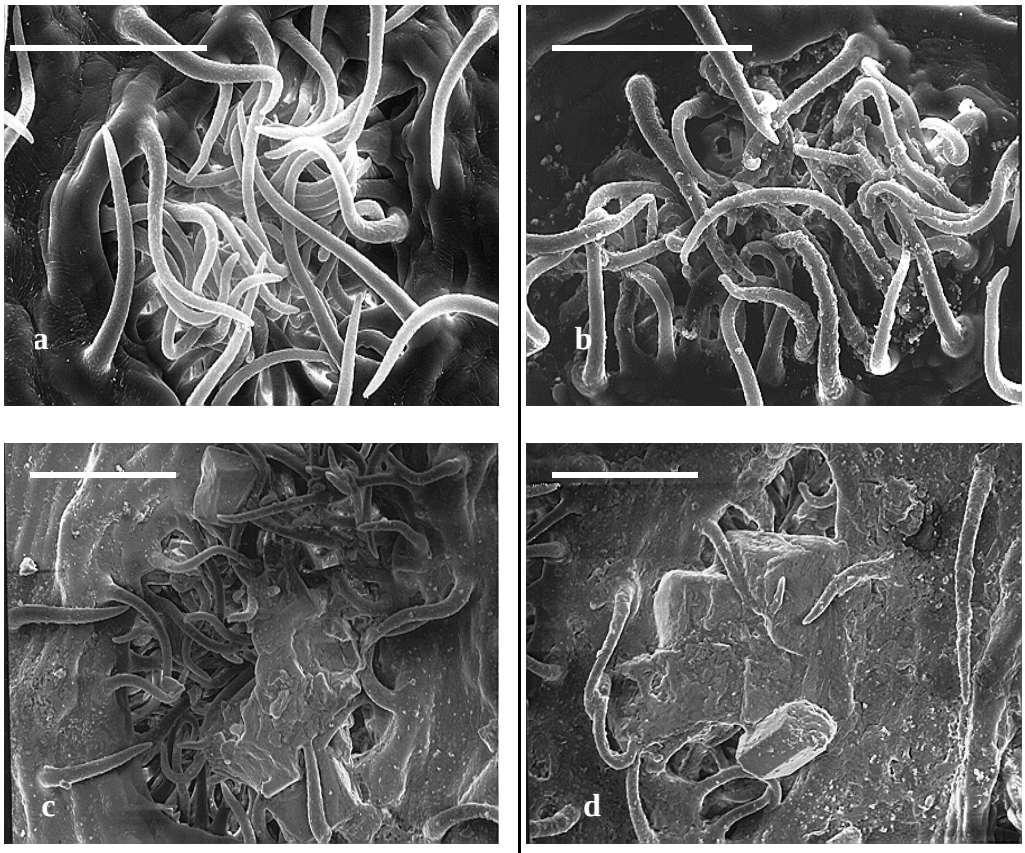

Fig. 1. Stomatal crypt damage classes: a = 0 (Perfect structures; no sign of alteration; no wax granules or crystals present on the stomatal chamber); b = 1 (Slight signs of alteration, such as wax granules or crystals (2-5 µm), piliferous layer still intact and separate, or with a few coalesced elements); c = 2 (Moderate signs of alteration; formation of wax granules and crystals (10-15 µm) that may obstruct even 50 % of the stomatal crypt; about 50 % of piliferous layer is coalesced); d = 3 (Severe alterations; formation of wax granules and crystals (20-30 µm) that totally obstruct the crypt; piliferous layer is more than 80 % coalesced). Bar = 10 µm

was very evident on both the upper and lower side of leaves.A significant increase in MBAS deposits on leaves (mg/L/kg fw) was observed when raising the concentration of surfactant in the aerosol ($F = 2535.4539$; $P < 0.0001$) (Fig.3). After the treatments (time I), chloride content increased linearly from DW to PSW10 and then was nearly stable, ranging from 0.95 to 1.23 % d.w., up to PSW500 (Fig . 4). Chloride contents in NW and DW leaves did not significantly differ at any time. At time III, chloride content increased linearly from DW to PSW30 and then was nearly stable, ranging from 1.28 to 1.55 % d.w. Chloride contents at time III were significantly higher than at time I in the treatments from PSW30 to PSW500.

Alterations to stomatal crypts occurred after treatments with PSW e”10 mg/L (Fig. 5). With the exception of controls and PSW5, ID values were lower at time I than at time III, and such differences were significant in most treatments. The maximum ID value (1.45) was observed with PSW30 two months after treatments. PSW containing 30 mg/L or higher concentrations of surfactant resulted in damages twice as severe than PSW with lower concentrations of SDS.Water potential showed similar trends at time I and III (Fig. 6). However, water stress was greater two months after the end of treatments. Such a difference was significant from PSW5 to PSW60. The minimum water potential was observed, in both the surveys, in plants treated with PSW500.

Treatments did not affect significantly the net photosynthesis of plants after the period of treatments ($F = 1.6810$; $P = 0.0941$), but they did two months later ($F = 7.1985$; $P < 0.0001$). The lowest values of net photosynthesis, ranging from 1.37 to 1.61 µmol $m^{-2} s^{-1}$, were measured on plants treated with PSW30, PSW120, PSW250 and PSW500. These were the only treatments showing values of net photosynthesis significantly

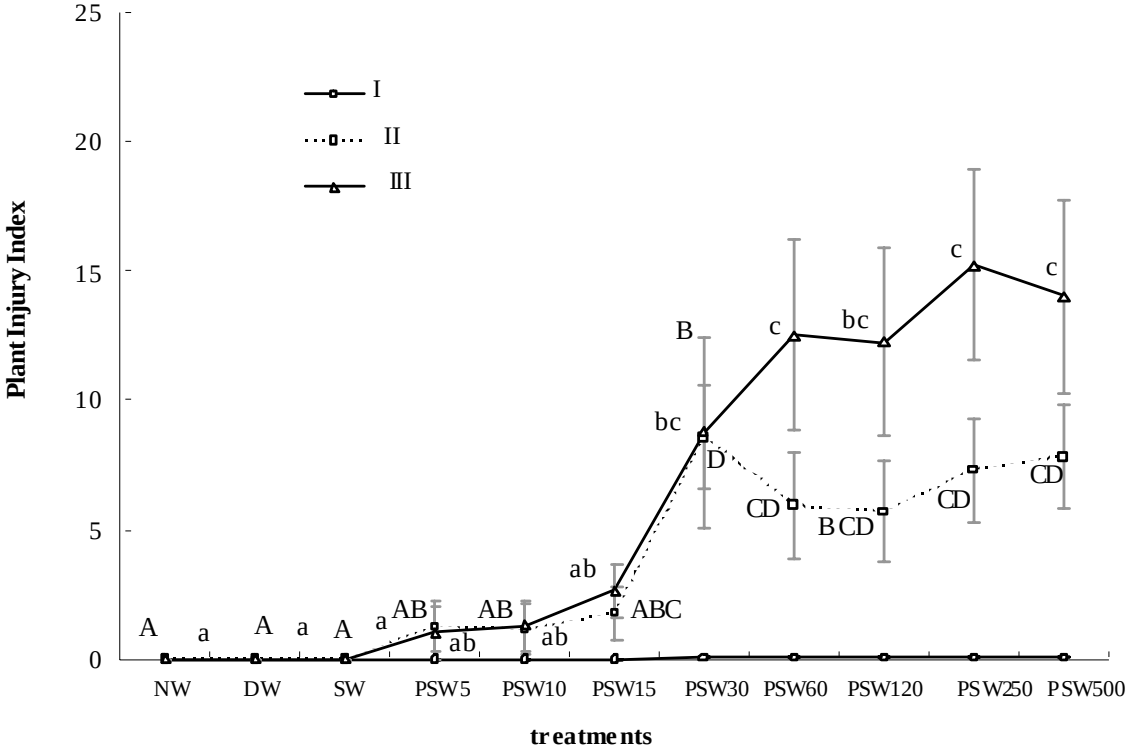

Fig. 2 . Plant Injury Index (PII) (±SD) at the end of treatments (I), one month later (II) and two months later (III). NW = no water; DW = deionized water; SW = seawater; PSW5 = 5 mg/L ; PSW10 = 10 mg/L; PSW15 = 15 mg/L; PSW30 = 30 mg/L; PSW60 = 60 mg/L; PSW120 = 120 mg/L; PSW250 = 250 mg/L; PSW500 = 500 mg/L. Treatments with the same letters did not differ significantly at P≤0.05 according to the Dunn test after Kruskal-Wallis (capital letters and small letters for the comparisons at time II and III, respectively). Statistics is shown only for time II and III. At time I, PSW d" 120 did not differ significantly from controls while PSW250 and PSW500 did

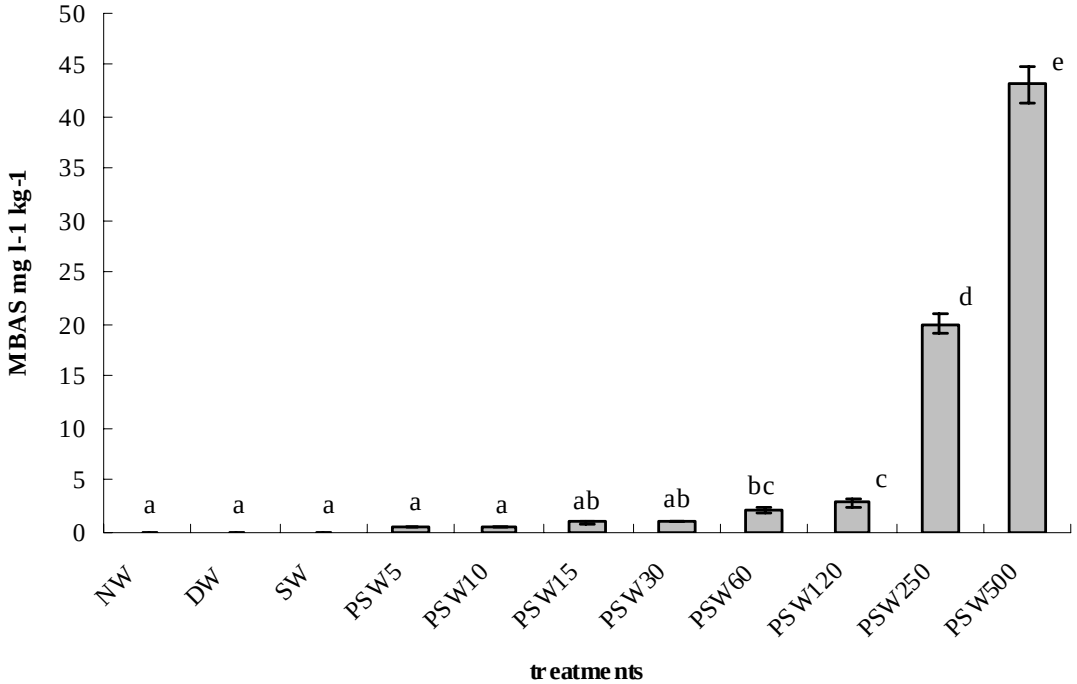

Fig. 3. Mean deposit of MBAS (±SD) on the leaves. NW = no water; DW = deionized water; SW = seawater; PSW5 = 5 mg/L ; PSW10 = 10 mg/L; PSW15 = 15 mg/L; PSW30= 30 mg/L; PSW60 = 60 mg/L; PSW120 = 120 mg/L; PSW250 = 250 mg/L; PSW500 = 500 mg/L. Bars with the same letters

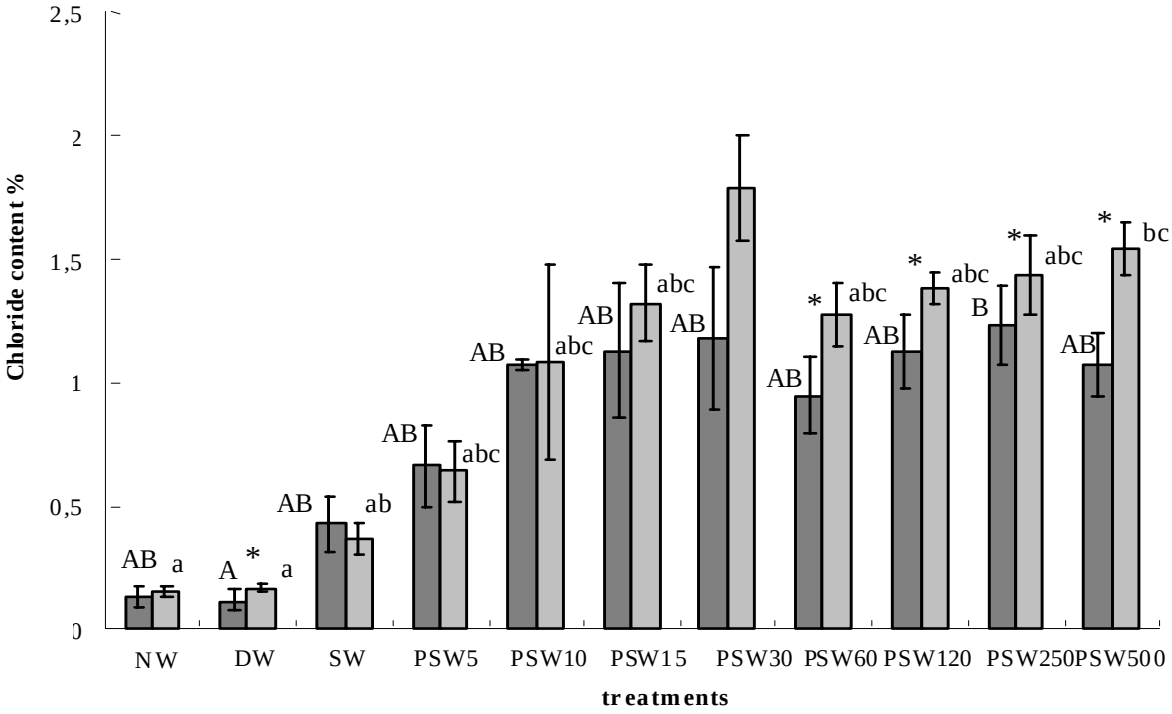

Fig. 4. Chloride content as mean % d.w. (±SD) at 0 (■, time I) and 60 (■, time III) days after the treatments (DW, deionized water; SW, seawater; PSW5-500, 5 to 500 mg/L surfactant in seawater). Treatments with the same letters did not differ significantly at P≤0.05 according to the Dunn test after Kruskal-Wallis (capital letters and small letters for the comparisons at 0 and 60 days after treatments, respectively). Treatments whose values differed significantly at 0 and 60 days after treatments are marked with an asterisk

lower than controls. Photosynthetic rates of the three controls were similar and did not differ significantly.Significant differences among treatments in terms of stomatal conductance (g_s) were observed both after the period of treatments ($F = 2.0554$; $P = 0.0342$) and two months later ($F = 6.2055$; $P < 0.0001$). At time I, the only two treatments showing significant differences amongst them were PSW30 and PSW500 (Fig. 7). At time III, a raise in SDS concentration resulted in a progressive decrease in stomatal conductance.

All parameters were significantly correlated with the concentration of surfactant in seawater at time II and III (Table 1). At time I, correlations between the concentration of surfactant in seawater and chloride content in leaves, net photosynthesis and stomatal conductance were not significant. Damage to stomatal crypts and water potential showed higher correlations with the concentration of surfactant in seawater at time I than at time III.

CONCLUSION

The results obtained in this study highlight the sensitivity of Oleander to surfactant polluted marine aerosols. Injury under controlled conditions was similar to injury observed in areas with spillage of high amounts of surfactant into the sea during preliminary field investigations (Nicolotti *et al.*, 2005). Significant toxicity of surfactant and seawater depositions on leaves was previously reported on *Chamaerops humilis* L., *Genista* spp., *Juniperus communis* L., *J. phoenicea* L., *Phoenix canariensis* Chabaud, *Quercus ilex* L., *Rosmarinus officinalis* L. and *Tamarix* spp. (Guidi *et al.*, 1988; Badot *et al.*, 1995; Nicolotti *et al.*, 2005; Rettori *et al.*, 2005).Increasing amounts of surfactant in the seawater resulted in an increase in phytotoxicity, associated with the presence of chloride within the foliar tissues. The increased chloride uptake into the tissues is likely due to the lowering of surface tension of the seawater caused by the surfactant (Latif and Brimblecombe, 2004).The low amount of chloride in leaves treated with seawater (SW), as well as the absence of visible injury and the very low level of damage to stomata, suggest that Oleander is a species relatively tolerant against sea aerosol, as it may be equipped with efficient natural defenses (e.g., thick cuticles, stomata protected into crypts). Our data suggest that the presence of surfactant

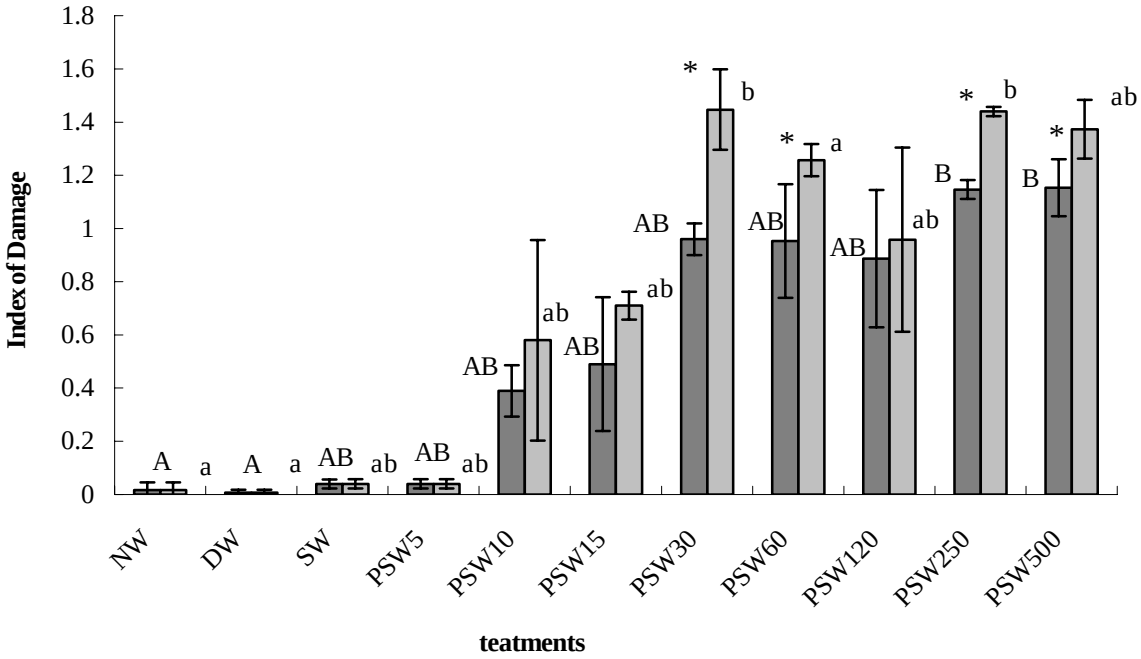

Fig. 5. Stomatal index of damage (±SD) at 0 (■, time I) and 60 (■ , time III) days after the treatments (DW, deionized water; SW, seawater; PSW5-500, 5 to 500 mg/L surfactant in seawater). Treatments with the same letters did not differ significantly at P≤0.05 according the Dunn test after Kruskal-Wallis (capital letters and small letters for the comparisons at 0 and 60 days after treatments, respectively). Treatments whose values differed significantly at 0 and 60 days after treatments are marked with an asterisk

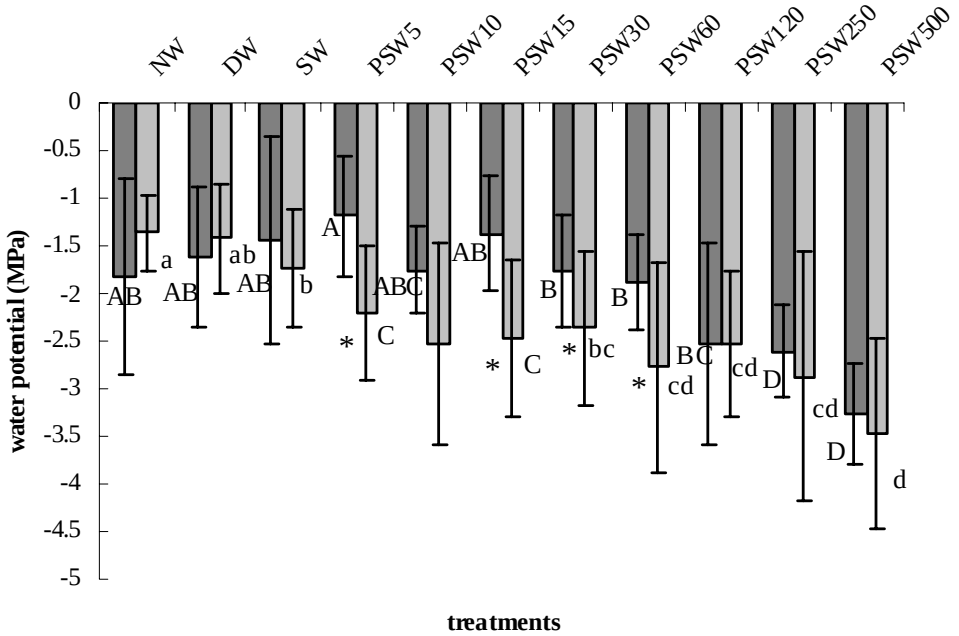

Fig. 6. Midday water potential (±SD) at 0 (■, time I) and 60 (■, time III) days after the treatments (DW, deionized water; SW, seawater; PSW5-500, 5 to 500 mg/L surfactant in seawater). Treatments with the same letters did not differ significantly at P≤0.05 according to the Kruskal-Wallis test (capital letters and small letters for the comparisons at 0 and 60 days after treatments, respectively). Treatments whose values differed significantly at 0 and 60 days after treatments are marked with an asterisk

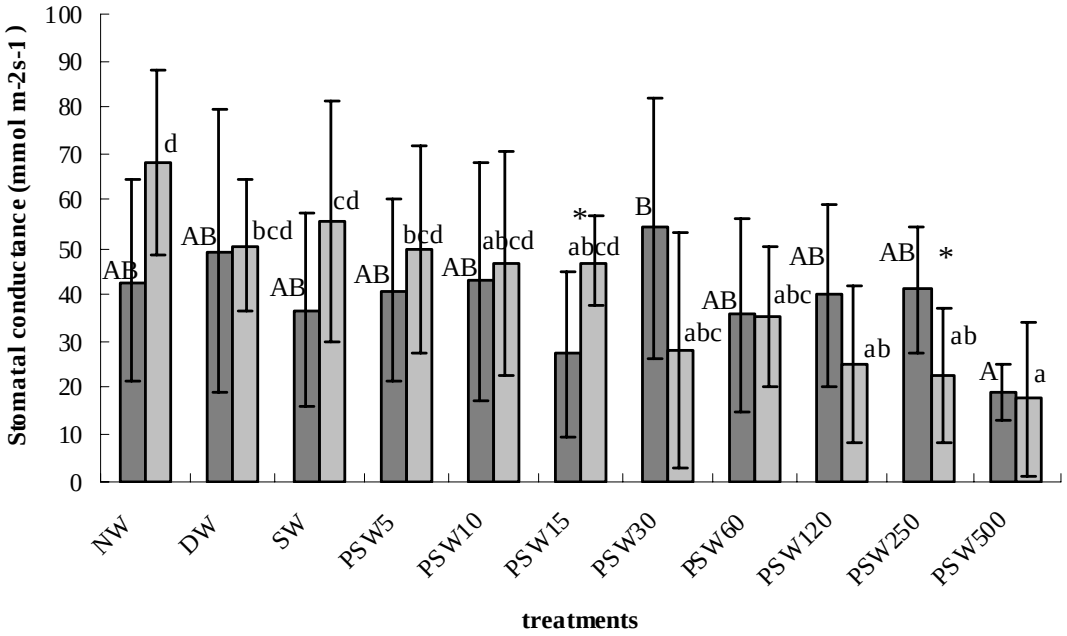

Fig. 7. Stomatal conductance (±SD) at 0 (, time I) and 60 (, time III) days after the treatments (DW, deionized water; SW, seawater; PSW5-500, 5 to 500 mg/L surfactant in seawater). Bars with the same letters did not differ significantly at P≤0.05 according to the Tukey HSD test (capital letters and small letters for the comparisons at 0 and 60 days after treatments, respectively). Treatments whose values differed significantly at 0 and 60 days after treatments are marked with an asterisk

Table 1. Summary of the Spearman rank order correlation analysis between the concentration of surfactant in seawater and the parameters measured on Oleander

Parameter	Period of assessment	Spearman's rho	t (N-2)	P-level
Visible foliar injury				
	end of treatments	0.8660	4.5826	0.0025
	after 1 month	0.8000	3.5277	0.0096
	after 2 months	0.7500	3.0000	<0.0001
Deposit of MBAS on the leaves				
	end of treatments	1.0000	-	-
Foliar chloride content				
	end of treatments	0.5667	1.8196	0.1116
	after 2 months	0.8000	3.5277	0.0096
Damage to stomatal crypts				
	end of treatments	0.9167	6.0685	0.0005
	after 2 months	0.7950	3.4673	0.0104
Water potential				
	end of treatments	-0.9167	-6.0685	0.0005
	after 2 months	-0.8500	-4.2691	0.0037
Net photosynthesis				
	end of treatments	-0.1667	-0.4472	0.6682
	after 2 months	-0.8667	-4.5962	0.0025
Stomatal conductance				
	end of treatments	-0.2333	-0.6349	0.5457
	after 2 months	-0.9667	-9.9890	<0.0001

is crucial in the lowering of plant defense leading to phytotoxicity. Such interaction among salt, surfactant and plants was first documented on some pine species (Townsend, 1989).

The fact that the maximum damage to stomatal crypts (ID), the maximum visible injury (PII) at time II, and the highest content of chloride were found in plants treated with intermediate (e.g., PSW30) rather than highest concentrations of

surfactant could be explained by the rapid phylloptosis which occurred much more frequently in plants treated with high concentrations of SDS. Immediately after the period of treatments, significant differences between controls and polluted treatments were noticed for most of the parameters. Two months later, such differences became even more evident, especially for treatments with high concentration of surfactant. The PSW30 concentration, equivalent to a surfactant deposit on leaves of about 1 mg/L/kg fw, is suggested as a threshold above which visible injury occurs and is easily recognizable. Furthermore, at that concentration we observed the highest content of chloride in leaves and a marked worsening of the alteration of stomatal crypts. The aforementioned value may thus be considered as a threshold of sensitivity for Oleander to surfactant-polluted marine aerosols. Similar values have been recorded in field conditions. In the Tuscany shore, deposits of MBAS ranging from 0.1 to 50 mg/L/kg fw were found on crowns of *Pinus pinea* directly exposed to sea winds (Bussotti *et al*., 1995). Thus, the Oleander proved to be a species sensitive to surfactants up to a threshold (1 mg/L/kg fw) which may be considered a reasonable alarm signal.Although visible injury was always correlated with the concentration of surfactant in seawater, including right at the end of the treatments, we believe that a reliable prediction of SDS concentration based on visible injury could be achieved only through the assessment of symptoms one month or two months after the exposure of plants. Based on the correlation analysis, delayed rather than immediate measurements would be better also for chloride content in leaves, net photosynthesis and stomatal conductance. In contrast, the damage to stomatal crypts is a very sensitive parameter even when assessed immediately after exposure. These findings suggest that polluted marine aerosol primarily affect the functionality of stomatal crypts, then induce accumulation of chloride (cuticular penetration) and physiological alterations in leaves, and visible injury development. Water potential is a very good predictor for marine polluted aerosol both after exposure than two months later.

According to Tingey's description (1989), Oleander may be classified as a reactor organism, i.e. very sensitive to environmental stresses. We propose Oleandear as a biomonitor for assessing surfactants in marine aerosol. The dense and evergreen crown, both in mature and young plants, makes this species suitable to intercept aerosols all the year round. Plants small in size could be easily transported and exposed in risky areas and routinely analyzed, as previously recommended for the assessment of heavy metals depositions (Sawidis *et al.*, 1995). Our results may help a correct planning, management and protection of coasts and shoreline vegetation.

ACKNOWLEDGEMENT

Work is partially supported by the Italian Ministry of Environment, Land and Sea.

REFERENCES

American Public Health Association (1992). Standard methods for the Examination of Water and Wastewater. 18th Ed., WPCF, 1-18.

Astorga, T., Lopez, D., Carazo, N., and Savé, R. (1993). Efecto del viento marino en la vegetación urbana del nuevo litoral barcelonés. Actas del 2° Congreso Ibérico SECH, Zaragoza (Spain), 539-545.

Badot, P. M. and Garrec, J. P. (1993). Dépérissement local du Pin d'Alep (*Pinus halepensis* Mill.) le long du littoral méditerranéen. Revue Foréstière Francaise, **45**, 1345-1401.

Badot, P. M. and Badot, M. J. (1995). Dépérissement du pin d'Alep (*Pinus halepensis* Mill.) sous l'effet des embruns marins pollués. Symptômes macroscopiques et mise en évidence de perturbations hydriques dans les aiguilles. Ann. Sci. Univ. Fr.-Comté, Besançon, Biologie-Ecologie, **3**, 37-43.

Badot, P. M., Richard, B., Lucot, E., Badot, M. J. and Garrec, J. P. (1995). Water disturbances and needle surface alterations in *Pinus halepensis* Mill. trees exposed to polluted seaspray. Proceedings of the International Symposium Ecotoxicology of Air Compartment, Rouen, France, 179-189.

Bargagli, R. (1998). Trace elements in terrestrial plants, an ecophysiological approach to biomonitoring and biorecovery. Springer, Berlin, New York.

Bussotti, F., Grossoni, P., and Pantani, F. (1995). The role of marine salt and surfactants in the decline of Tyrrhenian coastal vegetation in Italy. Annals of Forest Science, **52**, 251-261.

Bussotti, F., Bottacci, A., Grossoni, P., Mori, B. and Tani, C. (1997). Cytological and structural changes on *Pinus pinea* L. needles following the application of an anionic surfactant. Plant Cell and Environment, **20**, 513-520.

Crossley, A. and Fowler, D. (1986). The weathering of Scots pine epicuticular wax in polluted and clean air. New Phytologist, **103**, 207-218.

Garrec, J. P. and El Ayeb, N. (2001). Il problema degli aerosol marini inquinati in Francia e in Tunisia. Linea Ecologica, **1**, 51-54.

Gellini, R., and Papero, P. (1969). Osservazioni preliminari sulle cause del deperimento di alcune pinete litoranee toscane. Bollettino degli Ingegneri, **12**, 24-27.

Giorgi, F. (1996). Climate modeling over the Mediterranean region. An overview. Stefano Guerzoni & Roy Chester (Ed.), The impact of desert dust across the Mediterranean (pp. 1-14). Norwell (USA): Kluver Academic Publishers.

Granett, A. L. (1982). Pictorial keys to evacuate foliar injury caused by hydrogen fluoride. Hortscience, **17**, 587-588.

Guidi, L., Lorenzini, G., and Soldatini, G. F. (1988). Phytotoxicity of sea water aerosols on forest plants with special reference to the role of surfactants. Environmental and Experimental Botany, **28**, 85-94.

Helenius, A. and Simons, K. (1975). Solubilization of membranes by detergents. Biochimica Biophysica Acta, **415**, 29-79.

Horsfall, J. G. and Barratt, R. W. (1945). An improved grading system for measuring plant disease. Phytopathology, **35**, 655.

Itoh, M., Izawa, S. and Shibata, K. (1963). Disintegration of chloroplast with dodecylbenzene sulfonate as measured by flattering effect and size distribution. Biochimica and Biophysica Acta, **69**, 130-142.

Karhu, M. and Huttunen, S. (1986). Erosion effects of air pollution on needle surface. Water, Air and Soil Pollution, **31**, 417-423.

Lapucci, P. L., Gellini, R. and Papero, P. (1972). Contaminazione chimica dell'acqua di mare di moria dei pini lungo le coste tirreniche. Annali Accademia Italiana Scienze Forestali, **21**, 323-358.

Latif, M. T., and Brimblecombe, P. (2004). Surfactants in atmospheric aerosols. Environmental Science & Technology, **38**, 6501-6506.

Longwell, J. and Maniece, W. D. (1955). Determination of anionic detergent in sewage effluents and river water. Analyst, **80**, 167-171.

Marull, J., Savé, R. and Bayona, J. M. (1997). Efectos de la contaminaciòn quìmica sobre la vegetación del fronte litoral de Barcelona. Retema, nov-dec, 25-35.

Mingorance, M. D., Valdes, B. and Oliva, S. R. (2007). Strategies of heavy metal uptake by plants growing under industrial emission. Environment International, **65 (2)**, 177-182.

Nali, C., Balducci, E., Frati, L., Paoli, L., Loppi, S. and Lorenzini, G. (2009). Lettuce plants as bioaccumulators of trace elements in a community of central Italy. Environmental Monitoring and Assessment, **149**, 143-149.

Nicolotti, G., Rettori, A., Paoletti, E., Patetta, A. and Gullino, M. L. (2001). Inquinamento da tensioattivi ed effetti sulle pinete liguri. Economia Montana - Linea Ecologica, **1**, 35-42.

Nicolotti, G., Rettori, A., Paoletti, E., and Gullino, M. L. (2005). Morphological and physiological damage by surfactant-polluted seaspray on *Pinus pinea* and *Pinus halepensis*. Environmental Monitoring and Assessment, **105**, 175-191.

Oliva, S. R. and Mingorance, M. D. (2006). Assessment of airborne heavy metal pollution by aboveground plant parts. Chemosphere, **65 (2)**, 177-182.

Paoletti, E. (2001). Indagine preliminare sul deperimento delle pinete costiere di pino d'Aleppo in Puglia. Linea Ecologica, **1**, 43-50.

Paoletti, E., Ferrara, A. M., Calatayud, V., Cerveró, J., Giannetti, F., Sanz, M. J. and Manning, W. J. (2009). Deciduous shrubs for ozone bioindication: *Hibiscus syriacus* as an example. Environmental Pollution, **157**, 865-870.

Raddi, P., Moricca, S., Gellini, R. and Di Lonardo, V. (1992). Effects of natural and induced pollution on the leaf wax structure of three cypress species. European Journal of Forest Pathology, **22**, 107-114.

Rettori, A., Paoletti, E., Nicolotti, G. and Gullino, M. L. (2005). Ecophysiological responses of Mediterranean pines to simulated sea aerosol polluted with an anionic surfactant: prospects for biomonitoring. Annals of Forest Sciences, **62**, 351-360.

Sawidis, T., Marnasidis, A., Zachariadis, G. and Stratis, J. (1995). A study of air pollution with heavy metals in Thessaloniki city (Greece) using trees as biological indicators. Archives of Environmental Contamination and Toxicology, **28 (1)**, 11-124.

Tingey, D. T. (1989). Bioindicators in air pollution research – applications and constraints. In: Committee on Biologic Markers of Air- Pollution Damage in Trees (Ed.), Biologic Markers of air-pollution stress and damage in forests (pp. 73-80). Washington, USA: National Academy Press.

Townsend, A. M. (1989). The search for salt tolerant trees. The Arboricultural Journal, **13**, 67-73.

Turner, N. C. (1981). Techniques and experimental approaches for the measurement of plant water status. Plant Soil, **58**, 339-365.

Vural, N., Duydu, Y. and Kumbur, H. (1997). Monitoring of anionic surfactants in Ankara stream. Review International Contaminated Ambient, **13**, 47-50.

Wong, S. C. and Dunin, F. X. (1987). Photosynthesis and transpiration of trees in a Eucalyptus forest stand: CO_2, light and humidity responses. Australian Journal of Plant Physiology, **14**, 619-632.

Zoller, U. and Hushan, M. (2001). The nonionic surfactant pollution profile of Israel Mediterranean sea coastal water. Water Science Technology, **43**, 245-250.