Asian Journal of Chemistry Vol. 16, No. 1 (2004), 476–482

Comparative Study of *Ficus microcarpa* L.f. and *Nerium oleander* L. as Bioindicators of Metal Pollutants in an Urban Area

S. ROSSINI OLIVA*, B. VALDÉS AND J. MÄKKINEN†
Department of Plant Biology and Ecology, Faculty of Biology, University of Sevilla, Avda. Reina Mercedes s/n Apto 9045, 41080 Sevilla, Spain
Tel.: +34-954557057; Fax +34-954557059, E-mail: bisabina@libero.it

The ability of *Ficus microcarpa* L.f. with respect to bioindication of atmospheric metal pollutants has been studied and the data have been compared with the ability of *Nerium oleander* L. as bioindicator of atmospheric metal pollutants. Leaves were collected at six sampling sites (urban roads, urban parks and a rural area) around the city of Palermo (Sicily, Italy) every two months, from April 1998 to June 2000. The concentrations of Ba, Cd, Cu, Fe, Pb, Mg, and Zn were measured in the leaves by using inductively coupled plasma/mass spectrometry (ICP/MS). Differences were found in the ability to retain some pollutants like Ba, Cu, Pb and Zn in the leaves of the two plant species suggesting that *N. oleander* is a more suitable bioindicator than *F. microcarpa* in Mediterranean urban areas.

Key Words: Atmospheric contaminants, bioindication, deposition, urban areas, ornamental plants, Palermo.

INTRODUCTION

Bioindicators represent organisms or communities of organisms whose content of certain elements or compounds and/or whose morphological, histological or cellular structure, metabolic-biochemical processes, behaviour or population structure(s), including changes in these parameters, supply information on the quality of the environment or the nature of environment changes. The definition of biomonitors is the same as the above, but in a quantitative sense[1]. It is a well established fact that air pollutants are adsorbed and accumulated by higher plants, and they are therefore suitable for monitoring trace elements in the atmosphere. Plants can react to pollutants in different ways.

Higher plants have been used in many studies for monitoring atmospheric pollution[2–6]. Interest in biomonitoring by plants increased when urban atmosphere started to became hazardous to human health in many cities owing to an increase in anthropogenic activities. Plants can take up pollutants through both roots and

†Finnish Forest Research Institute, Parkano Research Station, Kaironiementie 54, FIN-39700 Parkano, Finland

leaves, from where they can be transported to the xylem. Pollutants can also be deposited on the leaf surface, and a part of them will penetrate into the leaf tissues. In leaves the cuticle provides an effective barrier against penetration of pollutants. Pollutants that have passed through the stomata may move along intercellular spaces, where they are partly scavenged by a range of chemical and biochemical reactions; once they reach the cytoplasm, they can interfere with the cellular metabolism[7]. The foliage is a part of plants that is generally easy to sample; since 1970 more than 92 studies have been carried out using leaf analysis as an indicator of environmental pollution[8].

Previous studies[2, 9] demonstrated a positive relationship between atmospheric heavy metal deposition on leaves and their concentrations in certain plant species.

Palermo is the largest city in Sicily, with about one million inhabitants[10]. The city has a Mediterranean climate and the main anthropogenic activities affecting air quality are associated with traffic and heating systems, industrial activities being very limited. This paper presents the results of a study undertaken during a period of two years (1998–2000) on the possibility of using two ornamental species, Indian laurel (*Ficus microcarpa* L.f.) and oleander (*Nerium oleander* L.) as bioindicators of atmospheric pollutants and to compare their bioindicator capacity. The main reason for selecting these species was their wide use as ornamental plants in the city of Palermo. Other reasons suggesting that they could be valuable as environmental bioindicators are that they are long-lived, evergreen broadleaved species which are easy to identify and well adapted to urban environments. The evergreen leaves should allow for monitoring of long-term deposition much like with needles in conifers.

Hitherto no studies on bioindication capacities of *Ficus microcarpa* have been published. The focus of this paper is to compare the role of these two species as bioindicators, and to investigate whether they could be used to improve the atmospheric conditions in urban areas. In addition, results about the ability of *F. microcarpa* to act as biomonitors are discussed in this paper.

EXPERIMENTAL

The leaves of the two species (*Ficus microcarpa* and *Nerium oleander*) were collected at six sampling stations in the city of Palermo every two months during two years, giving a total of 72 samples for each species.

Three of the sites were chosen as best representing the most contaminated parts of the city:

(a) Amendola Square (Amendola), which is a location with a high traffic density;

(b) G. Cesare Square (Cesare) in the centre of the city is located near to the main railway station and a petrol station;

(c) Palmerino Street (Palmerino), a long avenue with high urbanization and traffic densities.

The fourth and fifth sites were urban parks: Normanni Palace (Normanni) is a historical palace surrounded by an attractive garden in an area with a low traffic density; Villa Trabia (Trabia) is a historical house with a garden to which no cars are admitted. The sixth sampling site, Villa Igiea (Igiea) was chosen as a control site as it is located far from the city centre in a sparsely populated area with a low traffic density.

At each sampling station several branches from two or three plants of the same species were cut off from all sides of the canopy at 3–5 m above the ground level in trees (*F. microcarpa*) and at 1.5–3 m in shrubs (*N. oleander*). About 500 g of fresh leaves, including the petioles, were separated from the branches in the laboratory. Samples were dried overnight at 70–90°C and ground with an electric mixer. 300 mg subsamples were digested in open vessels in a microwave oven system (PROLABO A 301) using a mixture of 8 mL of 60% HNO_3 and 6 mL of 70% $HClO_4$, at a power level of 45 W. The concentrations of Ba, Cd, Cu, Fe, Pb, Mg and Zn were determined by simultaneous inductively coupled plasma mass spectrometry (ICP/MS) (Mod. FISONS 3410). The overall procedure was tested with reference material (BCR 62 olive leaves). The data were analysed by SPSS® Base 8.0 software package. The results of all the elements were tested by two-way analysis of variance, or by the Brown-Forsythe test if the variances were not homogeneous. The statistical significance of differences between sampling site and between species was tested using the multiple comparison Tukey HSD-test, non-parametric multiple tests or by Dunnett's T3 test when the variances were not equal. A correlation analysis was done between Al and all the other elements to discern which elements can come from soil resuspended material.

RESULTS AND DISCUSSION

The results of the analysis of the reference material (BCR 62 olive leaves) are shown in Table-1. There were no significant differences between the certified values of the reference material and the measured values of this sample, which proves the validity of the methodology used. Each species had different leaf metal concentrations at the individual sampling sites. Results of the metal concentrations in the leaves of *N. oleander* were recently published[11] and results of spatio and temporal variation of *F. microcarpa* are under publication.

TABLE 1.
MEASURED AND CERTIFIED ELEMENT CONCENTRATIONS (mg/kg) IN THE REFERENCE MATERIAL

Elements	Measured values	Certified value
Cu	45.80	46.6 ± 1.8
Cd	0.09	0.10 ± 0.02
Mn	56.50	57 ± 2.4
Pb	24.00	25 ± 1.5
Zn	15.10	16 ± 0.7

Ficus microcarpa: The highest mean concentrations of Cd, Cr, Cu, Fe, Pb and Zn were detected in leaves sampled in Amendola (Tables 2 and 3). The highest mean concentration of V was found in Igiea, Amendola and Trabia. The highest mean concentration of Mg, Mn and Ni (Table-3) was observed in samples from Normanni. The mean concentration of Ba was the highest in leaves sampled in Igiea (Table-2), but the difference was significant only when compared to samples from Cesare (p = 0.000***). The results of the multiple comparison tests indicate that there were no significant differences between the highest concentrations of Cd, Ni and V, respectively, at the different sites.

TABLE-2
MEAN CONCENTRATION (±SE) OF Ba, Cd, Cr, Cu AND Fe IN LEAVES OF *FICUS MICROCARPA* (FIC.) IN THE DIFFERENT SAMPLING SITES: 1, AMENDOLA; 2, CESARE; 3, NORMANNI; 4, PALMERINO; 5, TRABIA; 6, IGIEA

Sampling site	Ba	Cd	Cr	Cu	Fe
1	19.4 ± 1.25	0.18 ± 0.05	1.18 ± 0.27	14.1 ± 1.66	279 ± 32.6
2	13.3 ± 0.95	0.15 ± 0.05	0.23 ± 0.06	7.51 ± 0.57	183 ± 22.1
3	22.2 ± 2.18	0.12 ± 0.04	0.77 ± 0.56	6.36 ± 0.79	176 ± 20.9
4	20.8 ± 0.87	0.09 ± 0.03	0.21 ± 0.06	5.13 ± 0.4	117 ± 8.05
5	22 ± 1.43	0.11 ± 0.04	0.43 ± 0.09	6.52 ± 0.54	247 ± 20.9
6	23.1 ± 1.61	0.14 ± 0.04	0.24 ± 0.07	3.92 ± 0.60	120 ± 13.4

TABLE-3
MEAN CONCENTRATION (±SE) OF Pb, Mg, Mn, Ni, Zn AND V IN LEAVES OF *FICUS MICROCARPA* (FIC.) IN THE DIFFERENT SAMPLING SITES: 1, AMENDOLA; 2, CESARE; 3, NORMANNI; 4, PALMERINO; 5, TRABIA; 6, IGIEA

Sampling site	Pb	Mg	Mn	Ni	Zn	V
1	7.60 ± 0.93	4142 ± 288	18.26 ± 2.56	0.99 ± 0.44	34.1 ± 3.70	0.31 ± 0.06
2	4.49 ± 0.98	6294 ± 405	15.3 ± 2.40	0.29 ± 0.13	22.6 ± 3.00	0.21 ± 0.07
3	3.41 ± 0.81	6726 ± 815	24.7 ± 4.64	2.45 ± 2.07	21.4 ± 3.72	0.28 ± 0.06
4	3.49 ± 0.65	2942 ± 295	23.4 ± 1.14	0.73 ± 0.34	22.9 ± 3.18	0.16 ± 0.06
5	5.17 ± 0.95	5094 ± 438	18.6 ± 1.69	0.23 ± 0.11	26.6 ± 3.94	0.30 ± 0.07
6	2.26 ± 0.65	3824 ± 228	15.6 ± 1.73	0.44 ± 0.15	20.6 ± 2.27	0.32 ± 0.09

TABLE-4
BACKGROUND VALUES FOR THE ELEMENTS STUDIED

Element	Mean (mg/kg)
Ba	40
Cd	0.05
Cr	1.5
Cu	10
Fe	150
Mg	2000
Mn	80–200
Pb	1
V	0.5
Zn	15–50

The differences for Cr were significant only between samples from Amendola and Palmerino. The Cu concentration was significantly lower between samples from Amendola and the other sites ($p < 0.05$), except for Cesare where no differences were found. The mean concentration of Fe was always significantly higher in samples from Amendola than from the other sampling sites.

The only difference for Mn was between the leaf concentrations from Cesare and Palmerino, which were higher at the latter site.

The Mg concentrations in samples from the control site were lower than those from Normanni ($p < 0.0005$***).

The results of the Tukey HDS test showed that the leaf Pb concentrations were lower at Amendola and the control site than at the other sites ($p = 0.000$***), and also lower at Normanni ($p = 0.013$) and Palmerino ($p = 0.016$*) compared to those obtained for Amendola, while there were no trends for the other sites.

The Zn leaf concentration was significantly lower in the samples from the control site ($p = 0.037$*), but no statistical differences were found for samples from the other sites.

Differences in metal removal between the three species

All the elements investigated are constituents of plants; the background values published by Markert[12] and Baker[13] are shown in Table-4. These values show that *F. microcarpa* is not able to remove Ba, Cr, Mn, Ni and V through deposition on the leaves.

Baker *et al.*[14] demonstrated that Cr, Cu, Fe, Pb and Al are fixed in the roots, and there is almost no transportation to the shoots. This means that the high concentrations of these elements in the leaves are derived from atmospheric deposition. Table-2 shows that *F. microcarpa* is not able to retain Ba, whilst according to the Tukey HSD test Ba leaf concentration in *N. oleander* was significantly higher than in *F. microcarpa* ($p < 0.000$***).

Cadmium is one of the most dangerous pollutants for humans. It is a nonessential element for plants[15], and it is taken up by the roots and readily transported within the plant and distributed to all plant organs[16]. Because Cd is readily available to plants from both atmospheric and soil sources, its concentration rapidly increases in plants grown in polluted areas[15]. According to Markert[12] and Baker[13], the normal Cd concentration in plants is 0.05 mg/kg; according to Table-2, *F. microcarpa* had higher concentrations. According to multiple comparation, however, there were no significant differences between the concentrations of *F. microcarpa and N. oleander.*

Chromium is not an essential element for plants, either. As the two species had lower leaf concentrations than the normal values, this means that they are not able to remove this element. The Cu concentrations in both species were similar to those found in *Tilia cordata* leaves in the Slovak Republic[17]. Copper is a microelement that is essential for plants[12], and it is an important constituent of the enzymes involved in oxidation-reduction reactions[16]. Its concentration in plants (10 mg/kg) tends to be relatively constant, and is relatively independent of the Cu composition of the substrate[18]. Higher concentrations were found in *F. microcarpa* at some of the sites. According to the Tukey HSD test, Ficus microcarpa had a significantly lower removal capacity of Cu than *N. oleander* ($p = 0.031$*).

Table-2 shows that the Fe leaf concentration of *F. microcarpa* at almost all the sites was higher than the background value and it was the same for Mg. The Tukey HSD test also showed that *N. oleander* did not have a different removal capacity for these elements than *F. microcarpa*.

There is no evidence that Ni is an essential element in plants, although the reported beneficial effects of Ni on plant growth stimulated speculations that this metal may have some beneficial role in plants. But likewise hormesis (low-dose effects of toxic substances) may be involved in the above observation. Nickel is likely to be accumulated in leaves[15]. There is not much information on Ni in plants, and the background values are only generic. In ecosystems where Ni is an airborne pollutant, the upper parts of plants are likely to have the highest Ni concentrations[15]. No differences were found between the leaf concentrations in the two species, and all the values were below the background values for both species[11]. This means that they are not able to remove this element through deposition on the leaves.

Lead has not been shown to play any essential role in plants[15], but it receives considerable attention because it is a major environmental pollutant (in fact, Pb is adverse to photosynthesis for inhibiting production of porphyrines and thus of chlorophyll from aminolevulinic acid). Airborne Pb, which is a major source of Pb pollution, is readily taken up by plants through the foliage. According to Table-3 and to Rossini Oliva and Valds[11], the Pb concentrations in both species were higher than the normal values, which indicates that they are all able to remove this dangerous pollutant. A significantly higher Pb leaf concentration was found only in samples of *F. microcarpa* from Amendola (Tables-3) compared to the control site ($p = 0.001^{**}$), and Amendola was namely the site where the mean concentration was higher than the normal value (7.6 mg/kg). This means that this site is more contaminated than the others, but that only this species is able to indicate this. As the Tukey HSD test showed that there were differences between the two species ($p = 0.001^{**}$), we can conclude that *F. microcarpa* is a better suitable species as indicator of Pb environmental pollution.

The mean V and Mn concentration was always lower in *F. microcarpa* than the normal level, and no differences were found between the species. The low V and Mn concentrations indicates that this species does not have a good removal capacity of this element, therefore making it unsuitable as indicator of environmental pollution by both elements. Zinc is an essential element in all living beings, and it plays an important role in the biosynthesis and functioning of enzymes, auxins and proteins[16]. It can be concluded from the results in Table-4 that *F. microcarpa* is able to remove this element, and Dunnett's test showed that no differences exist between the two species.

Conclusions

The two species had different abilities to retain pollutants, presumably owing to differences in their physical and chemical foliar characteristics. Both species have the same ability to indicate the presence of Cd, Fe, Mg and Zn. *N. oleander* is the only bioindicator species of Ba and it is a more sensible bioindicator of Cu than *F. microcarpa*. Furthemore, the results suggest that *F. microcarpa* is the only species investigated suitable as bioindicator of Pb. The variations in leaf concentrations of metal pollutants in *F. microcarpa* suggests that biomonitoring here may reflect lower gradient concentrations only for Cu, Fe, Mg, Mn, Pb and Zn at the urban road sites relative to the control site, while no differences were found for Ba, Cd, Ni and Zn. This indicates that the control site did not have lower pollution levels, and that sources other than traffic may contribute to the increase in the latter elements or that other factors, like climatic ones, can have a major effect.

REFERENCES

1. B. Markert, J. Oehlmann and M. Roth, General Aspects of Heavy Metal Monitoring by Plants and Animals, in: K.S. Subramanian and G.V. Iyengar (Eds.), Environmental Biomonitoring-Exposure Assessment and Specimen Banking, ACS Symposium Series 654, American Chemical Society, Washington DC, pp. 18–29 (1997)
2. B.O. Berthelsen, E. Steinnes, W. Solberg and L. Jingsen, *J. Environ. Qual.*, **24**, 1018–1026 (1995)
3. M. Kovács, K. Penksza, G. Turcsányi, L. Kaszab and E. Ötvös, *Acta Bot. Hung.*, **39** (1–2), 3 (1995).
4. G. La Malfa, D. Romano and C. Leonardi, *Agronomia*, **3** (Suppl. Luglio-Settembre), 461 (1996)
5. S. Anderson, A.H. Chappelka, K.M. Flynn and J.W Odom, *Water, Air and Soil Pollut.*, **118**, 1–11 (2000)
6. F. Monaci, F. Monni, E. Lanciotti, D. Grechi and R. Bargagli,. *Environ. Pollut.*, **107**, 321 (2000)
7. R. Bargagli, Trace Elements in Terrestrial Plants: An Ecophysiological Approach to Biomonitoring and Biorecovery, Springer-Verlag, Berlin-Heidelberg-New York (1998)
8. S. Luyssaert, Spatial variability of the Cadmium Concentration in the Crown of *Salix fragilis L.* and Its Implications for Leaf Sampling, PhD Thesis, Faculteit Landbonwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent (2000)
9. M. Salemaa, I. Vanha-Majamaa and J. Derome, *Environ. Pollut.*, **112**, 339 (2001).
10. D. Caltabellotta, A. Drago, B. Lo Bianco and M. Lombardo, Climatologia della Sicilia, Regione Siciliana, Ass. Agric. Foreste, Gruppo IV Servizi allo Sviluppo, Unitá di Agrometereologia, Palermo (1998)
11. S. Rossini Oliva and B.Valdés, *Inf. Bot. Ital.*, **32** (2002) (in press).
12. B. Markert, Instrumental Element and Multi-Element Analysis of Plant Samples, John Wiley & Sons Ltd., Chichester-New York-Brisbane-Toronto-Singapore (1996)
13. A.J.M. Baker, *Biorecovery*, **1**, 81 (1989)
14. A.J.M. Baker, R.D. Reeves and A.S.M. Hajar, *New Phytol.*, **127**, 61 (1994)
15. S.A. Kabata-Pendias and H. Pendias, Trace Elements in Soil and Plants, CRC Press Inc., Boca Raton, Florida (1984).
16. W Dmuchowski and A. Bytnerowicz, *Environ. Pollut.*, **87**, 87 (1995)
17. L. Šomšak, B. Šimurdová, J. Lipták, M. Kromka and J. Antoni, *Ekológia (Bratislava)*, **19**, 324 (2000).
18. A.J.M. Baker, R.R. Brooks and W.J. Kersten, *Taxon.*, **34**, 89 (1985).

(*Received*: 16 June 2003; *Accepted*: 7 November 2003) AJC–3211