Metal concentrations in Seville orange (*Citrus aurantium*) fruits from Seville (Spain) and Palermo (Italy)

Sabina Rossini Oliva* & Benito Valdés

*Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Avenida de Reina Mercedes, s/n, Apartado de Correo 1095, ESP-41080 Sevilla, Spain (*e-mail: bisabina@libero.it)*

Five samples of Seville orange (*Citrus aurantium*) fruits from trees growing in the cities of Seville (Spain) and Palermo (Italy) were analysed for metal concentrations. Ten elements (Al, Ba, Cd, Cr, Cu, Mn, Ni, Pb, V and Zn) were determined by simultaneous inductively coupled plasma mass spectrometry. A sample from a rural area was also analysed for comparison. The effect of washing on accumulation capacity was also investigated, as well as the correlation between contaminants. The data show that Seville orange fruits do not retain high concentrations of heavy metals, except for barium, cadmium and zinc. Mesocarp accumulates less heavy metals than epicarp. Washing has a different effect on accumulation depending on the element. There is a positive correlation between lead and manganese concentration values.

Key words: atmospheric contaminants, heavy metals, Rutaceae, urban pollution, washing effect

Introduction

Seville or bitter orange (*Citrus aurantium*) is a member of Rutaceae originating from Asia and cultivated as an ornamental plant for centuries. The Arabs introduced it in Sicily and Spain (Schirarend 1998), where it has been incorporated in gardens, parks and streets. Seville is the Spanish city with the highest number of orange trees (over 25 000 according to Barbera *et al.* 1998), which are primarily used as ornamentals. However, the fruits are yearly harvested and used to make marmalade and to distil the volatile compounds to prepare liquors and perfumes.

There is not much information on heavy metal accumulation in fruits (Stewart & Ross 1969), but it is a well established fact that part of the heavy metals accumulated in other parts of the plants is sometimes found also in fruits (Scintalan & Tuba 1992). For instance, Stewart and Ross (1969) found that in apple trees there is some transportation of mercury, nickel and cadmium from leaves to fruits.

This study was undertaken to check whether contaminants in the urban atmosphere can be retained in Seville orange fruits, and whether the levels of this potential absorption could be harmful to man, as the destination of fruits harvested in Seville is the elaboration of marmalade.

Five fruit samples were collected in areas with a rather high atmospheric contamination, three in the city of Seville (Spain) and three in

Palermo (Sicily), and were used to study concentrations of Al, Ba, Cd, Cr, Cu, Mn, Ni Pb V and Zn. These concentrations were compared with those in the fruits from an area far away from any potential source of contamination (Alcalá del Río).

Material and methods

Fruits of *Citrus aurantium* were collected in March and April 1999, from three sampling sites of Seville (Spain) and Palermo (Sicily), presenting a high range of traffic and urbanization densities, and consequently subjected to high potential contamination. Two of the sites in Seville were chosen as best representing the most contaminated parts of the city: Palmera (Palmera) and Avenida Carrero Blanco (Blanco). Alcalá del Rio (Seville) was chosen as a control site to be used for comparison. This site is a field far away from any potential source of contamination (road or urban traffic, industries, heating systems, etc), At this site, samples of fruits from five trees were collected in April 2001. Two sites in Palermo were chosen as representing localities with high traffic densities: Piazza Agata (Agata) and Piazza Politeama (Politeama). A control site was Parco della Favorita (Favorita). Fruits were collected when fully ripe. At each sampling station, fruits from three trees were collected from all sides of the canopy at 3–5 m above ground level.

Fruits were mixed and parts of them were taken at random to prepare the material to be studied. Pericarp samples were generated from each fruit cutting the piece with a stainless steel knife. Two parts of the fruit were chosen: the epicarp (peel) and the mesocarp (pulp of the divisions). Each epicarp sample was separated into two parts taken from different fruits. One was washed several times with distilled water to eliminate all solid particles deposited on the surface. The other was left unwashed. Six replications were done for each site.

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 Al, Ba, Cd, Cr, Cu, Fe, Pb, Mg, Mn, Ni, Zn and V were determined by simultaneous inductively coupled plasma mass spectrometry (ICP/MS) (Mod. FISONS 3410).

The overall procedure for plant analysis was tested with reference material (BCR 62-olive leaves), which produced results that were within the certified concentration ranges for all metals analysed. The data were analysed with SPSS® Base 8.0 software package. One-way analysis of variance (ANOVA) was carried out to determine the mean concentration values of each element at each sampling portion which represent the arithmetic mean values for the six samples collected in Seville and Palermo. Furthermore, it provides understanding of whether washing has a significant effect on heavy metal accumulation in the epicarp and if there are statistical differences between the sampling sites. A Tukey test was performed to find out in which location significant differences exist for metal concentration. A correlation analysis was carried out to study relations between metals. Pearson's correlation coefficients were calculated.

Results and discussion

Metal concentrations

It is very difficult to establish in which cases Al and Mn concentrations found in Seville orange fruits come from atmospheric pollution alone, as they are components in the soil and consequently there is a translocation of these metals from roots to fruits. Background concentrations for *Citrus sinensis* (orange) indicated by Kabata-Pendias and Pendias (1984) were 15 mg kg^{-1} for Al and 1.3–1.5 mg kg^{-1} for Mn. The values for Al found in all samples can be considered as normal except for those in the Agata samples (Table 1). Values of Mn are higher in all sampling sites (Table 1).

In most samples, Ba concentration was higher than the background value given by Kabata-Pendias and Pendias (1984) for *Citrus sinensis*: 3.1 mg kg^{-1}. However, values found in Favorita, a park in the centre of Palermo, are considerably lower. A lower Ba concentration was also observed in Seville in samples from Blanco (Table 1).

Cadmium concentration increases quickly in plants grown in polluted areas and the highest content in polluted areas were reported for roots and leaves (Kabata-Pendias & Pendias 1984). Mean concentration in orange fruits reported by Kabata-Pendias and Pendias (1984) is 0.14 mg kg⁻¹, i.e. lower than values found in the present study (Table 1). The concentration found in the standard sample (Table 1: Alcalá del Río) agrees with the background values given by Baker (1989) and Markert (1996) for plants: 0.005 mg kg⁻¹.

Lead and Cr deposition in the epicarp is negligible in the samples taken from urban places, but the Cr content is significantly higher in the control sample (Alcalá del Rio). According to Kabata-Pendias and Pendias (1984), background value of Pb in fruits is 3 mg kg⁻¹. The Pb concentrations in all samples were lower than the background value (Table 1).

According to Baker (1989) and Markert (1996), background concentration of Cu in plants is 10 mg kg⁻¹, which means that fruits of Seville orange do not accumulate this metal. The concentration found in samples from urban areas does not differ from the control sample (Table 1).

Data for Ni and V are difficult to interpret because background values for both elements are not known for fruits of Seville orange, although they could be similar to those indicated for *Citrus sinensis* by Kabata-Pendias and Pendias (1989): 0.39 mg kg⁻¹ for Ni and 0.029 mg kg⁻¹ for V. The background values given by Baker (1989) and Markert (1996) for Ni and V in plants are 1.5 mg kg⁻¹ and 0.5 mg kg⁻¹. Accordingly, the concentrations of these two metals have to be considered low or not significantly high (Table 1).

According to Baker (1989) and Markert (1996), background concentration of Zn in plants is 100 mg kg⁻¹. Kabata-Pendias and Pendias (1984) reported a background concentration of 5 mg kg⁻¹ in fruits of *Citrus sinensis*, and accordingly it can be assumed that this will also be the normal value for *C. amara*, as this concentration is similar to that found in the control sample (Table 1). This means that fruits of *C. amara* growing in urban areas retain Zn. The samples with higher Pb concentration come from Piazza

Table 1. Mean concentrations of elements (mg kg⁻¹) with standard deviation in epicarp of samples from the different sampling sites. A = unwashed, B = washed, PA = Palermo, SE = Seville. Significant differences between locations are indicated. n.s. = not significant; * $p < 0.01$; ** $p < 0.05$.

Sites		Al*	Ba**	Cd[n.s.]	Cr[n.s.]	Cu[n.s.]	Mn**	Ni[n.s.]	Pb[n.s.]	Zn[n.s.]	V[n.s.]
Blanco (SE)	A	19.82 ± 14.62	2.59 ± 1.32	0.59 ± 0.32	0.17 ± 0.09	4.25 ± 2.98	4.67 ± 0.45	1.08 ± 0.98	0.92 ± 0.76	27.58 ± 19.34	0.33 ± 0.18
	B	14.06 ± 5.26	2.16 ± 1.16	0.25 ± 0.18	≤ 0.005	3.66 ± 2.23	4.50 ± 0.35	0.92 ± 0.34	≤ 0.05	19.13 ± 11.23	≤ 0.01
Favorita (PA)	A	9.90 ± 4.68	2.25 ± 1.05	0.58 ± 0.23	0.083 ± 0.076	3.91 ± 2.04	3.75 ± 1.98	0.91 ± 0.27	1.08 ± 0.98	10.49 ± 4.56	0.42 ± 0.28
	B	13.50 ± 4.10	1.99 ± 0.89	0.24 ± 0.16	≤ 0.005	2.59 ± 1.98	2.25 ± 1.23	0.92 ± 0.22	≤ 0.05	18.07 ± 11.45	0.24 ± 0.17
Palmera (SE)	A	9.16 ± 3.97	9.91 ± 5.67	0.42 ± 0.12	≤ 0.005	2.58 ± 2.03	3.24 ± 1.53	1.33 ± 1.05	≤ 0.05	34.68 ± 18.98	≤ 0.01
	B	7.49 ± 5.48	8.23 ± 5.23	0.33 ± 0.12	≤ 0.080	2.50 ± 2.23	3.50 ± 1.15	0.33 ± 0.15	≤ 0.05	14.83 ± 7.56	0.25 ± 0.19
Agata (PA)	A	21.31 ± 10.15	5.24 ± 1.42	0.33 ± 0.16	≤ 0.005	2.58 ± 2.07	6.16 ± 2.34	0.33 ± 0.13	0.50 ± 0.39	30.3 ± 16	0.17 ± 0.11
	B	20.14 ± 7.82	3.91 ± 0.82	0.33 ± 0.14	≤ 0.005	1.83 ± 1.34	5.07 ± 1.67	0.59 ± 0.32	1.25 ± 1.05	11.4 ± 6.56	0.25 ± 0.13
Politeama (PA)	A	17.64 ± 1.77	4.99 ± 1.42	0.33 ± 0.24	≤ 0.005	2.50 ± 2.13	4.58 ± 1.24	0.91 ± 0.54	≤ 0.05	9.07 ± 3.29	0.42 ± 0.18
	B	17.31 ± 2.34	4.67 ± 1.30	0.42 ± 0.32	≤ 0.005	2.25 ± 1.89	4.24 ± 1.65	0.08 ± 0.07	≤ 0.05	18.06 ± 3.45	≤ 0.01
Alcalá del Rio (SE)	A	15.61 ± 1.88	5.08 ± 2.34	≤ 0.05	3.25 ± 2.78	3 ± 2.3	7 ± 2.75	1.16 ± 0.98	≤ 0.05	5.91 ± 1.78	≤ 0.01
	B	13.65 ± 2.23	4.16 ± 2.88	≤ 0.05	2.75 ± 2.1	2.83 ± 1.89	6.75 ± 2.05	1.33 ± 0.75	≤ 0.05	6.75 ± 2.34	≤ 0.01

Table 2. Mean concentrations of elements (mg kg⁻¹) with standard deviation in mesocarp of samples from the different sampling sites. SE = Seville, PA = Palermo.

Sampling sites	Al	Ba	Cd	Cr	Cu	Mn	Ni	Pb	Zn	V
Blanco (SE)	7.65 ± 5.67	1.75 ± 1.53	0.50 ± 0.32	≤ 0.005	2.16 ± 1.54	2.50 ± 1.54	≤ 0.01	≤ 0.05	23.04 ± 9.42	≤ 0.01
Palmera (SE)	7.50 ± 5.73	5.83 ± 2.60	0.25 ± 0.20	≤ 0.005	4.58 ± 2.55	1.41 ± 1.02	1.16 ± 1.04	≤ 0.05	19.73 ± 12.2	≤ 0.01
Agata (PA)	9.58 ± 5.03	1.33 ± 1.73	0.42 ± 0.22	≤ 0.005	2.58 ± 1.54	2.58 ± 1.43	0.59 ± 0.53	0.33 ± 0.24	10.74 ± 5.80	≤ 0.01
Politeama (PA)	14.04 ± 9.49	2.16 ± 1.24	0.42 ± 0.27	≤ 0.005	3.99 ± 0.86	2.25 ± 1.26	0.50 ± 0.49	≤ 0.05	40.85 ± 23.7	0.25 ± 0.18
Alcalá del Rio (SE)	14.38 ± 6.27	2.83 ± 1.14	≤ 0.05	3.83 ± 1.49	3.16 ± 0.59	3 ± 0.58	1.33 ± 1.04	≤ 0.005	8.5 ± 1.96	≤ 0.01

Agata (PA) and Palmera (SE), which are sites where traffic intensity is very high. This is in agreement with the origin of Zn in an urban atmosphere, which derives mainly from abrasion of tyres and brake disks (Angoletta *et al.* 1993, Bargagli 1998, Nimis *et al.* 1999).

Significant differences exist between locations only for Al, Ba and Mn (Table 1).

No statistically significant differences in the concentration values found in mesocarp (pulp) of the samples were found between the locations (Table 2). When these data are compared with those for washed samples of epicarp (Table 1), it can be verified that with the exception of Cu, metal concentration in the mesocarp is lower or similar to that in the epicarp for most metals. In the case of Al, Ba and Zn, these differences are rather significant and seem to demonstrate that the highest quantity of these elements taken from the atmosphere is retained in the epicarp.

Washing effect

Metal concentrations are affected by washing, which can remove different amounts of pollutants, but this varies according to the species and physical and chemical characters of pollutants (Lin & Schuepp 1996, Rea *et al.* 2000). Analysis of washed samples provides data on contaminants incorporated into tissues, while analysis of unwashed samples gives the values of surface contamination including particles (Alfani *et al.* 2000). Concentrations of elements in washed and unwashed epicarp sections are shown in Table 1.

Results for ANOVA analysis for washed and unwashed samples are presented in Table 3. It

Table 3. *F* and *p* values of analysis of variance (ANOVA) between washed and unwashed samples.

Elements	*p*	*F*
Al	0.86	0.033
Ba	0.97	0.000
Cd	0.07	4.23
Cu	0.49	0.517
Mn	0.86	0.030
Ni	0.76	0.093
Zn	0.85	0.034

was not possible to compare washing effects for Cr, Pb and V because most values for these elements were below detection limits.

All *p* values are not statistically significant ($p > 0.05$), therefore there are no significant differences in any metal concentrations between unwashed and washed samples. Thus concentrations are not affected by washing and elements are not deposited on the surface or they are not in soluble form.

Some studies have demonstrated that washing does not affect Zn concentration (Wyttenbach *et al*. 1985, Worley 1993, Alfani *et al*. 2000). However, other investigations showed that Zn can be eliminated by washing (Little 1973, Moraghan 1991, Worley 1993, McCrimmon 1994), and this suggests that the effect of washing varies from one species to another. In a study of Scots pine (*Pinus sylvestris*) needles significant differences were found for Cu, as washing eliminates Cu deposition (Zwoliński *et al*. 1988). This is not the case, however, for the samples of the studied Seville orange.

As Mn is not an important component of adhering dust (Wyttenbach *et al*. 1985), washing does not make any significant difference. In some cases, including the control sample (Alcalá del Río), there is an even higher concentration in washed than in unwashed samples, which agrees with previous studies (Wyttenbach *et al*. 1985, Porter 1986, Worley 1993, Alfani *et al*. 2000).

Correlation between metal contaminants

The only positive correlation found is that between Pb and Mn: $r = 0.58$, $p > 0.05$. This could be explained by the close anthropogenic origin of both contaminants. Manganese and Pb are additives in fuel, and both are produced and liberated into the atmosphere by combustion of fuel and petrol (Monaci & Bargagli 1995, Nimis *et al*. 1999).

Conclusions

Seville orange fruits do not retain large amounts of pollutants, except for Ba and Zn, although for Ba it is difficult to establish whether the concen-

trations found could be harmful to man. All the other metals show concentrations much below levels that could be dangerous to health.

There are differences in metal concentration levels in epicarp and mesocarp, the concentration in the epicarp being higher for most elements studied. Pollution level only affects metal variation in epicarp portion and only for Al, Ba and Mn. Washing has no significant effect on removing surface contamination for all elements studied. Lead and Mn are the only two metals showing a significant correlation due to their anthropogenic source.

References

Alfani, A., Baldantoni, D., Maisto, G., Bartoli, G. & Virzo De Santo, A. 2000: Temporal and spatial variation in C, N, S and trace element contents in the leaves of *Quercus ilex* within the urban area of Naples. — *Env. Poll*. 109: 119–129.

Angoletta, M., Bentivoglio, A. & Giusto, D. 1993: Accumulo di metalli pesanti in piante erbacee. — *Inquinamento* 5: 74–79.

Baker, A. J. M. 1989: Terrestrial higher plants which hyperaccumulate metallic element — a review of their distribution, ecology and phytochemistry. — *Biorecovery* 1: 81–126.

Barbera, G., Rossini Oliva, S. & Bonell, E. J. 1998: Arancio amaro, nuova specie arborea per ambiente urbano. — *Acer* 2: 38–41.

Bargagli, R. 1998: *Trace elements in terrestrial plants: an ecophysiological approach to biomonitoring and biorecovery*. — Springer-Verlag, Berlin.

Csintalan, Zs. & Tuba, Z. 1992: The effect of pollution on the physiological process in plants (the fundamentals of plant physiology indication of pollutants at individual level). — In: Kovacs, M. (ed.), *Biological indicators in environmental protection*: 169–185. Ellis Harwood, New York etc.

Kabata-Pendias, A. & Pendias, H. 1984: *Trace elements in soils and plants*. — CRC Press Inc., Boca Raton.

Lin, Z. Q. & Schuapp, P. H. 1996: Concentration and distribution of metal elements at the surface of balsam fir foliage by micro-proton induced X-ray emission. — *Env. Sci. Technol*. 30: 246–251.

Little, P. 1973: A study of heavy metal contamination of leaf surfaces. — *Env. Poll*. 5: 159–172.

Markert, B. 1996: *Instrumental element and multi-element analysis of plant samples*. — John Wiley & Sons Ltd., Chichester etc.

McCrimmon, J. N. 1994: Comparison of washed and unwashed plant tissue samples utilised to monitor the nutrient status of creeping bentgrass putting greens. — *Comm. Soil Sci. Plant* 27: 967–988.

Monaci, F. & Bargagli, R. 1995: Biomonitors as tool to evaluate trace element deposition in complex urban environments. — In: Anttila, P., Kämäri, J. & Tolvanen, M. (eds.), *Proceedings of the 10th World Clean Air Congress, Espoo, Finland*: 238–241. The Finnish Air Pollution Society, Helsinki, Finland.

Moraghan, J. T. 1991: Removal of endogenous iron, manganese and zinc during plant washing. — *Comm. Soil Sci. Plant* 22: 323–330.

Nimis, P. L., Skert, N. & Castello, M. 1999: Biomonitoraggio di metalli in traccia tramite licheni in aree a rischio del Friuli-Venezia Giulia. — *Studi Geob.* 18: 3–49.

Porter, J. R. 1986: Evaluation of washing procedures for pollution analysis of *Ailanthus altissima* leaves. — *Env. Poll.* 12: 195–202.

Rea, A. W., Lindberg, S. E. & Keeler, G. J. 2000: Assessment of dry deposition and foliar leaching of mercury and selected trace elements based on washed foliar and surrogate surfaces. — *Env. Sci. Technol.* 34: 2418–2425.

Schirarend, C. 1998: La botanica degli Agrumi. — In: Raimondo, F. M. & Lark, H. W. (eds.), *Le mele d'oro:* 26–62. Ed. Grifo, Palermo.

Stewart, D. K. R. & Ross, R. G. 1969: Nickel residues in apple fruit and foliage following a foliar spray of nickel chloride. — *Can. J. Plant Sci.* 49: 375–377.

Worley, R. E. 1993: Pecan leaf analysis is not improved by washing. — *Hort. Sci.* 28: 956.

Wyttenbach, A., Bajo, S. & Tobler, L. 1985: Major and trace element concentrations in needle of *Picea abies*: levels, distribution functions, correlations and environmental influences. — *Plant & Soil* 85: 313–325.

Zwoliński, J., Matuszczyk, I. & Zwolińska, B. 1998: Accumulation of sulphur and metals in and on pine (*Pinus sylvestris* L.) and spruce (*Picea abies* (L.) Karst.) needles in industrial regions in southern Poland. — *Folia For. Polon.* Ser. A 40: 47–57.