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Assessment of washing procedure for determination some of airborne metal concentrations

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This study was proposed to assess the suitability of washing technique to distinguish between airborne and soil borne several metal contaminants. For this reason, six plant species which grew under Mobarakeh Steel Company emissions were selected. Aluminum, iron, nickel, manganese, zinc, copper and lead concentrations were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) in washed and unwashed leaves of plants. The water washing reduced Al, Fe and Ni concentrations significantly ($p < 0.05$ and 0.01) in the most species. The highest reduction percentage of three metals was observed in *Nerium oleander* which was 76, 84 and 69% for Al, Fe and Ni, respectively. Washing had no significant effect for removing Mn, Zn and Cu from the leaf surfaces of most species. Influence of washing procedure on Pb concentrations was different. Washing effect varied with various physico-chemical characters of contaminants, plant species, primary level of contaminants and washing time. The results of this study show that the use of distilled water is a good technique for removing atmospheric deposition of Al, Fe and Ni from plant leaves. Also, there were a significant positive correlation between Al, Fe, Ni and Zn which had anthropogenic source.

Key words: Contaminants, airborne, soil borne, water washing, leaves, heavy metals, tree, shrub, washing effect.

NTRODUCTION

Leaf tissue has been used to study the role of plants as bioindicators and bioaccumulators of aerial pollution (Aksoy and Ozturk, 1997). On the other hand, chemical foliar analysis has also been employed to study the impact and the extent of air pollutants (Djingova et al., 1999; Ericsson et al., 1995; Huttl and Fink, 1991) and the pollutant accumulation capacity of different plants (Somsak et al., 2000). Leaves of higher plants have been used for heavy metals biomonitoring since 1950s (Al-Shayeb et al., 1995).Most plant contaminations arise from atmospheric particles accumulation through their foliage and leaves, and degree of pollution depends on be smooth of leaves, wind speed and on level of rainfall as Ward et al. (1977) has expressed that plant washing after sampling, decreased the element contents about 10

to 30% in comparison with unwashed plants (Ward et al., 1977). Sample cleaning is absolutely essential if the purpose is to distinguish between pollutants deposited on the surface of leaves and the composition of the internal tissues (McCrimmon, 1994; Alfani et al., 2000).

A number of different washing techniques have been used to investigate the location of elements in foliage such as shaking the leaves with washing agent, sample immersing in the washing solution, mechanical cleanings and etc. (Cercasov, 1985; Krivan et al., 1987; Rossini Oliva and Raitio, 2003). Also, various washing agents and combination of them have been used, such as distilled water or tap water only (Monni et al., 2000) or with chloroform (Krivan et al., 1987) or acetone (Carten and Hanson, 1990), hydrochloric acid (Salkl and Maeda, 1982), ammonium acetate solution (Martinez et al., 1971), weak acid solution (Rea et al., 2000) and etc. Washing agent selection depends on the aim of the study, plant species, leaf structure and properties of accumulated elements (Rea et al., 2000; Porter, 1986;

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Table 1. Structural properties of plant species leaves.

Plant species	Leaf type	Cuticle thickness	Cork	Roughness	Waxy layer
Pinus eldarica (T)	Acicular/needles	High	-	Low	Very high
Quercus brantii (T)	Spiny	Low	-	-	-
Elaeagnus angustifolia (T)	Lanceolate	Low	High	High	Low
Cercis siliquastrum (S)	Cordate	Very high	-	-	-
Nerium oleander (S)	Lanceolate	High	Low	Low	Low
Thuja orientalis (S)	Scale-like	Low	-	-	Moderate

T, Tree species; S, shrub species.

Kovacheva et al., 2000; Wyttenbach et al., 1985). Among different washing agents that have been used, distilled water is the most appropriate as it is similar to rain water, and it is safe, economic, and efficient (Bargagli, 1998). The water removable proportion of heavy metals provides information about both the prevailing metal source (natural or anthropogenic) and the leaf absorption amount of each metal (Yusuf and Oluwole, 2009). The results of speciation studies on air particulate suggest that metals from anthropogenic sources are mainly in water-soluble forms (Fernandez Espinosa et al., 2002).

Mobarakeh Steel Company is Iran's largest steel maker and one of the largest industrial complexes operating in Iran that is located at 65 km southwest of Isfahan, near the city of Mobarakeh, Isfahan Province, Iran.

Hoodaji and Jalalian (2003 a, b) showed that soil and plant near the Mobarakeh Steel Company were contaminated slightly with some heavy metals, such as Fe, Zn and Mn.

In the preliminary part of this research that was aimed to identify biomonitor plant species, obtained data about different levels of metal contaminations in this region (Ataabadi et al., 2010 a, b). The aim of this study was to assess the washing effect with deionized (DI) water on several metal concentrations in tree and shrub species in the vicinity of industrial zone for determination of contamination source.

MATERIALS AND METHODS

Site description

This study was carried out in botanic garden Mobarakeh Steel Company located in 32° 34' 15" N and 51° 25' 21" E, southwest of Isfahan in the center of Iran. The garden was approximately 3 ha in area with nine plots and three replications. This region has an arid climate with a mean annual rainfall of 140 mm. Wind direction was SW-NE.

Plant species selection

The six ornamental species which contain three tree species and three shrub species with various leaf structures were selected (Table 1). The species ages were 15 years.

Sampling, preparation and analysis of plant samples

The sampling of all species was performed in August 2007. Leaf sampling was done with wind direction (SW-NE) and from defined height of each species. After transferring the samples to a laboratory, the samples were divided into two groups. The first group was washed with distilled water to clean dust and deposited substances on leaves for 10 min while the second group was not washed. All samples air dried and then oven dried at 70°C for 48 h to achieve constant mass, milled and sieved through a 35 mesh screen. 1 g milled powder of each samples was weighed and after combusting in electrical furnace, were digested with 10 ml 2 N HCl (Chapman and Pratt, 1961). The metal concentrations were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (GBC Integra XL).

Washing time selection

The choice of washing time depends on plant species and properties of studied element (Steubing, 1982). Therefore, in the pre-experimental study, different washing times (5, 10, 15, 30 min) were tested. However in some cases, longer washing time was more effective than the shorter one, in this study, washing for 10 min was seem as suitable time.

The water-removable heavy metal was calculated as the difference between unwashed and washed leaf concentrations and then reduction percent was also calculated subsequently. All statistical analyses were processed using SPSS 14.0. Paired *t*-test was used to compare two sample groups (washed and unwashed). Relationship between elements was evaluated by Pearson correlation.

RESULTS AND DISCUSSION

Washing effect

Metal concentrations are affected by water washing differently. This treatment could remove different amounts of contaminants according to the plant species and element type. Metal concentrations in washed and unwashed leaves of six ornamental plants are shown in Table 2. According to these results, the washing treatment for Al, Fe and Ni show significant differences in the most species, but for Mn, Zn and Cu, there were no significant differences in the most cases. Influence of washing treatment on Pb concentrations varied with plant species. Reduction percents in metal contents due to

Table 2. Metal concentrations of plant species.

Plant species	Al		Fe		Ni		Mn		Zn		Cu		Pb	
	W	UnW	W	UnW	W	UnW	W	UnW	W	UnW	W	UnW	W	UnW
Pinus eldarica	122.32*	321.12	482.55*	1087.67	27.02*	58.02	58.49*	62.54	25.87	38.26	1.62	2.71	1.57	4.6
Quercus brantii	176.48*	367.62	471.02*	1001	60.08*	85.4	73.9	95.4	12.99	22.48	3.94	9.89	1.22	3.45
Elaeagnus angustifolia	130.64*	228.5	348.1*	539.75	35.58	47.73	206.6	219.75	10.79	14.14	1.56	5.66	1.39	3.52
Cercis siliquastrum	63.53*	155.3	193.63	380.33	24.95*	33.49	39.56	42.86	11.76	13.59	1.05	2.32	0.43*	0.96
Nerium oleander	38*	169.67	100.4*	632.83	11.80*	39.38	67.42	81.55	12.17**	14.13	4.47*	5.77	0.93*	4.15
Thuja orientalis	236.5*	373.33	862	1153.33	43.74*	61.1	44.97	51.6	19.41	23.9	1.6	2.11	1.5*	2.57

W, Washed; UnW, unwashed.

*, $p < 0.05$; **, $p < 0.01$; ***$p < 0.001$.

exerting washing treatment for each species are presented in Table 3.

Al concentration in all plant species is affected by washing significantly, and it can be presumed that the cause of difference in reduction percent is plant species. Lin and Schuepp (1996), McCrimmon (1994) and Rossini Oliva and Valdes (2004) also reported significant differences in aluminum content with washing treatment. The highest reduction percent was observed in *Nerium oleander* followed by *Pinus eldarica,* while in most previous studies (e.g. Zwolinski et al., 1998; Rautio, 2000) it was recommended to use the stronger washing agent for waxy surface leaves like Pine needles.

Fe content indicated significant differences before and after washing treatment except for *Cercis siliquastrum* and *Thenus orientalis.* According to the former studies, 60% to 80% of the Fe is removable and therefore assumed to be derived from surface contaminant (Wyttenbach et al., 1985; Porter, 1986).

Fe and Al are components of dust and they adhere on the leaf surface (Rossini Oliva and Valdes, 2003). For Ni, also the greatest washing effect was found in *N. oleander* followed by *P. eldarica*. The same result was reported by Salkl

and Maeda (1982) with 48% reduction for Ni deposition in Pine needles after washing treatment (Salkl and Maeda, 1982).

For Mn, Zn and Cu, there were no significant differences between washed and unwashed leaves except for Mn in *P. eldarica*, Zn and Cu in *N. oleander*. Cu and Zn are present in airborne particulates as water-soluble sulphate salts (Heal et al., 2005; Haggins et al., 2000; Karthikeyen et al., 2006) though, part of the Zn penetrates quickly in to the leaves, so it is not washable simply. Mn is not an important component of adhering dust (Wyttenbach et al., 1985) so washing procedure cannot cause significant differences. Rossini Oliva and Valdes (2004) also reported that there were no significant differences in concentrations of these metals after the washing treatment. Most studies have reported that there was no significant difference between washed and unwashed sample for Mn and Zn (Wyttenbach et al., 1985; Alfani et al., 2000); but Akosy and Sahin (1998) observed that washing significantly reduced Zn concentration in the leaves of *Elaeagnus angustifolia*.

Pb concentrations in cleaned and uncleaned samples indicated significant differences only in shrub species and the highest reduction percent

observed in *N. oleander* (80%). Romano and Abate (1995) also found significant difference for lead in washed leaves of *Lantana camera L.* (Romano and Abate, 1995).

Altogether with consideration of the differences in washing effect for various elements and even for an element among various species, it can be concluded that similar to other studies, removal efficiency is affected by physical and chemical characters of pollutants, plant species and subsequently properties of leaves and probably washing time (Lin and Schuepp, 1996; Rea et al., 2000; Porter, 1986). Singh and Kurmar (2006) also reported that the magnitude of heavy metal deposition on vegetable surface varied with morpho-physiological nature of the vegetables. In most studies, it is pointed that the washing of plants with thin cuticle and smooth surface is more effective but in this study, effective washing was observed commonly in *N. oleander* with thick cuticle and less or more rough surface in comparison with other species.

Results show that the primary level of metal on the leaves (unwashed leaves) and its relation with washing time of leaves is an important factor, as longer washing time is more effective than a shorter one (Rossini Oliva and Raition, 2003).

Table 3. Reduction (%) of metals in the studied plants.

Plant species	Al	Fe	Ni	Mn	Zn	Cu	Pb
Pinus eldarica	61	54	53	6	32	40	66
Quercus brantii	51	52	29	23	42	60	64
Elaeagnus angustifolia	42	36	25	6	24	73	61
Cercis siliquastrum	56	45	25	8	14	55	56
Nerium oleander	76	84	69	17	14	22	80
Thuja orientalis	36	24	28	13	19	24	55

Table 4. Pearson correlation of metal concentration in leaves of plant species.

Element	Al	Fe	Ni	Mn	Zn	Cu	Pb
Al	1						
Fe	0.87**	1					
Ni	0.88**	0.78**	1				
Mn	0.02	-0.16	0.08	1			
Zn	0.68**	0.73**	0.51*	-0.14	1		
Cu	0.19	0.03	0.47*	0.35	-0.17	1	
Pb	0.40	0.51*	0.43	0.36	0.48*	0.19	1

*, p-value < 0.05; **, p-value < 0.01.

Steubing (1982) reported that washing with distilled water for 1 min removed only a minor proportion of the pollutants, but washing for 15 min considerably increased the yield and increasing the washing time to 30 min did not remove any more pollutants (Steubing, 1982).

Correlation between metal contaminants

Inter-element relationships provide interesting information on heavy metal sources. Correlation coefficients are shown in Table 4. There are many high significant correlation coefficients between metals in plant species, such as Al vs. Fe, Ni, and Zn (r = 0.87, 0.88 and 0.68, respectively), Fe vs. Ni, Zn and Pb (0.78, 0.73 and 0.51, respectively), Ni vs. Zn and Cu (r = 0.51 and 0.47, respectively) and Zn vs. Pb (r = 0.48).
These results suggest that distribution of Al, Fe, Ni and Zn was mainly controlled by anthropogenic source. Principal component analysis of metal content in plant leaves also confirmed theses results (results of principal component analysis (PCA) not presented).

Conclusion

In this study, analysis of uncleaned leaves indicate the levels of environmental contaminations and the use of water washing method to distinguish contamination source in plant had significant effect on elimination of some studied metals. The effect of washing varied with plant species, physico-chemical contaminant characters,

primary level of contaminants and washing time. Water washing treatment led to significant reduction of Al, Fe and Ni in the most species. The highest reduction percentage of three metals was observed in *N. oleander* which was 76, 84 and 69% for Al, Fe and Ni, respectively. While washing mostly had no significant effect on removing of Mn, Zn and Cu from leaves surface. The results indicate that washing with distilled water is a good procedure for assessing Al, Fe and Ni in the atmospheric deposition of the industrial area. High correlation between Al, Fe, Ni and Zn also indicated that these elements arise via anthropogenic source.

REFERENCES

Akosy A, Sahin U (1998). *Elaeagnus angustifolia L.* as a biomonitor of heavy metal pollution. Trend J. Bot. 23: 83-87.
Aksoy A, Ozturk MA (1997). Nerium oleander L. as biomonitor of lead and other heavy metal pollution in Mediterranean environments. Sci. Total Environ. 205(2-3): 145-150.
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. Environ. Pollut. 109(1): 119-129.
Al-Shayeb SM, Al-Rajhi MA, Seaward MRD (1995). The date palm (*Phoenix dactylifera* L.) as a biomonitor of lead and other elements in arid environments. Sci. Total Environ. 168(1): 1-10.
Ataabadi M, Hoodaji M, Najafi P (2010a). Heavy metal biomonitoring by plants grown in an industrial area of Isfahanȯ Mobarakeh Steel Company. J. Environ. Stud. 35(4): 83-92.
Ataabadi M, Hoodaji M, Najafi P (2010b). Evaluation of airborne heavy metal contamination by plants growing under industrial emission. Environ. Eng. Manage. J. 9(7): 903-908.
Bargagli R (1998). Trace elements in terrestrial plants: An ecophysiological approach to biomonitoring and biorecovery', Springer-Verlag, Berlin, p. 313.

Carten Jr. CT, Hanson PJ (1990). Foliar retention of 15N-nitrate and 15N-ammonium by red maple (Acer rubrum) and white oak (*Quercus alba*) leaves from simulated rain. Environ. Exp. Bot. 30(3): 333-342.

Cercasov V (1985). Investigation of atmospheric particulates deposited on leaves using instrumental neutron activation analysis. Technical Note. Atmos. Environ. 19(4): 681-683.

Chapman HD, Pratt PF (1961). Methods of analysis for soils, plants and water. Univ. California, Berkeley, CA, USA.

Djingova R, Wagner G, Kuleff I (1999). Screening of heavy metal pollution in Bulgaria using Populus nigraitalica. Sci. Total Environ. 234 (1-3): 175-184.

Ericsson A, Walheim M, Norden LG, Nasholm T (1995). Concentrations of mineral nutrients and arginine in needles of Picea abies trees from different areas in southern Sweden in relation to nitrogen deposition and humus form. Ecol. Bull. 44: 147-157.

Fernandez Espinoza AJ, Ternero Rodriguez M, Barragan De La Rosa FJ, Jimenez Sanchez JC (2002). A chemical speciaion of trace metals for fine urban particles. Atmos. Environ. 36(5): 773-780.

Heal MR, Hibbsa LR, Agiush RM, Beverland JI (2005). Total and water-soluble trace metal content of urban background PM_{10}, $PM_{2.5}$ and black smoke in Edinburgh, UK. Atmos. Environ. 39(8): 1417-1430.

Hoodaji M, Jalalian A (2003a). Distribution of Iron, Zinc and Lead in soil and crops in the Mobarakeh Steel Plant Region. J. Environ. Stud. 30(36): 15-26.

Hoodaji M, Jalalian A (2003b). Distribution of Nickel, Manganese and Cadmium in soil and crops in the Mobarakeh Steel Plant Region. J. Sci. Technol. Agric. Natl. Resour. 8(3): 55-67.

Huggins FE, Huffman GP, Robertson JD (2000). Speciation of elements in NIST particulate matter SRMS 1648 and 1650. J. Hazard. Mat. 74(1-2): 1-23.

Huttl RF, Fink S (1991). Pollution, nutrition and plant function. In: Porter JR, Lawdor DW (Eds.). Plant growth: interaction with nutrition and environment. Cambridge University Press, Cambridge, pp. 207-226.

Karthikeyan S, Joshi UM, Balasubramanian R (2006). Microwave assisted sample preparation for determination water-soluble fraction of trace elements in urban airborne particulate matter Evaluation of Bioavailability. Anal. Chim. Acta. 576(1): 23-30.

Kovacheva P, Djingova R, Kuleff I (2000). On the representative sampling of plants for multielement analysis. Phytologia Balcanica. 6(1): 91-102.

Krivan V, Schaldach G, Hausbeck R (1987). Interpretation of element analyses of spruce-needle tissue falsified by atmospheric surface deposition. Naturewissenschaften. 74(5): 242-245.

Martinez JD, Nathany M, Dharmarajan V (1971). Spanish moss, a sensor for lead. Nature, 233: 564-565.

McCrimmon JN (1994). Comparison of washed and unwashed plant tissue sample utilized to monitor the nutrient status of creeping bentgrass putting greens. Commun. Soil Sci. Plant Anal. 25(7-8): 967-988.

Monni S, Uhlig C, Junttila O, Hansen E, Hynynen J (2000). Chemical composition and ecophysiological response of *Empetrum nigrum* to aboveground element application. Environ. Pollut. 112(3): 417-426.

Porter JR (1986). Evaluation of washing procedures for pollution analysis of *Ailanthus altissima* Leaves. Environ. Pollut. 12: 195-202.

Rautio P (2000). Foliar element analysis: a hand tool for both bioindication and plant nutrition studies, or is it? In: Lehtonen T, Salminen J, Pilhaja K (eds.). Proceeding Fifth Finnish Conference of Environmental Science, Finnish Society for Environmental Sciences, University of Turku, pp. 22-25.

Rea AW, Lindberg SE, Keeler GJ (2000). Assessment of dry deposition and foliar leaching of mercury and selected trace elements based on washed foliar and surrogate surfaces. Environ. Sci. Technol. 34(12): 2418-2425.

Romano D, Abate L (1995). Lead and Cadmium in leaves of ornamentals grown along urban roads. Agric. Medit. (Special volume): 189-193.

Rossini Oliva S, Raitio H (2003). Review of cleaning techniques and their effects on the chemical composition of foliar samples. Boreal Environ. Res. 8(3): 263-272.

Rossini Oliva S, Valdes B (2004). Influence of washing on metal concentrations in leaf tissue. Commun. Soil Sci. Plant Anal. 35(): 1543-1552.

Salkl H, Maeda O (1982). Cleaning procedures for removal of external deposits from plant surfaces. Environ. Sci. Technol. 16(8): 536-539.

Singh S, Kumar M (2006). Heavy metal load of soil, water and vegetables in peri-urban Delhi. Environ. Monit. Assess. 120(1-3): 79-91.

Somsak L, Simurdova B, Liptak J, Kromka M, Antonio J (2000). Accumulation of heavy metals by some forest tree species (*Tilia cordata* Mill., *Acer pseudoplatanus* L.). Ecologia. 19(3): 324-330.

Steubing L (1982). Problems of bioindications and the necessity of standardization. In: Steubing L, Jäger HJ (eds.). Monitoring of air pollutants by plants methods and problems. Dr. W. Junk Publishers, The Hague, Boston, London, pp. 19-24.

Ward NI, Brooks RR, Roberts E (1977). Heavy metal pollution from automotive emission and its effect on roadside soils and pasture species in New Zealand. Environ. Sci. Technol. 11(9): 917-920.

Wyttenbach A, Bajo S, Tobler L (1985). Major and trace element concentrations in needles of Picea abies: levels, distribution functions, correlations and environmental influences. Plant Soil, 85(3): 313-325.

Yusuf KA, Oluwole SO (2009). Heavy metal (Cu, Zn, Pb) contamination of vegetables in urban city: A case study in Lagos. Res. J. Environ. Sci. 3(3): 292-298.

Zwolinski J, Matuszczyk I, Zwolinski B (1998). Accumulation of sulfur and metals in and on pine (*Pinus sylvestries* L.) and spruce (*Picea abies* (L.) Karst.) needles in industrial regions in southern Poland. Foila Forestalia Polonica (A-Forestry). 40: 47-57.