African Journal of Biotechnology Vol. 10(20), pp. 4127-4132, 16 May, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2452
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Biomonitoring of some heavy metal contaminations from a steel plant by above ground plants tissue

M. Ataabadi, M. Hoodaji* and P. Najafi

Islamic Azad University, Khorasgan Branch, Isfahan, Iran.

Accepted 21 March, 2011

Soil and plants growing in the vicinity of industrial areas display increased concentrations of heavy metals and give an indication of the environmental quality. The contamination source for aluminum, iron, nickel and lead in the Botanical garden of Mobarakeh Steel Company was recognized by analyzing the leaves and topsoil of two evergreen species: *Quercus brantii* and *Ligustrum vulgare*. Availability of the studied elements for plants was investigated by measuring their total and extractable concentration inthe soils. For ensuring air borne source of these metals, plant tissues were washed with distilled water. Plant available heavy metals of the soils were few in comparison with the total values which were related to the high pH value and the $CaCO_3$ percentage in the soils around each plant. On the other hand, significant differences were obtained between the washed and unwashed leaves in both species indicating metal contaminations from the atmosphere. Significant differences were detected between the contaminated and background site samples in both plants for Al, Fe and Ni. In addition, the leaves of *L. vulgare* accumulated more elements partially than *Quercus brantii*. Difference in the accumulation potentials of the plants were related to the diversity of the physical and chemical properties of the leaves and the type of elements. The enrichment ratio of plants ($C_{contaminated}/C_{background}$) was calculated and it indicated that, the Fe in both plants was moderately enhanced by anthropogenic activities.

Key words: Industrial contamination, *Quercus brantii*, *Ligustrum vulgare*, enrichment factor.

INTRODUCTION

Human activities cause the slow extermination of plant and animal species in nature through toxic pollution due to industrial and technological advancement in recent decades (Ives and Cardinale, 2004). Many heavy metals emitted mostly from anthropogenic sources, have now exceeded or equaled their natural emissions (Biney et al., 1994) and have been posing a serious threat to the ecosystems (Wen Kuang et al., 2006).

Soil and plants growing in the nearby zone of industrial areas display increased concentration of heavy metals, serving in many cases as biomonitors of pollution loads. Plants take up large quantities of pollutants and translocate them into vegetative and generative organs at various rates (Kovács et al., 1993), which make clear the quality of the environment. Calzoni et al. (2007) in their studies on the ability of *Rosa rugosa* for biomonitoring of heavy metal indicated that, leaf accumulation was due to atmospheric deposition rather than to soil uptake (Calzoni et al., 2007). Chemical foliar analysis has also been employed to study the impact and extent of air pollutants (Djingova et al., 1999; Ericsson et al., 1995; Hüttl and Fink, 1991) and the pollutant accumulation capacity of different plants (Bicchiega et al., 1994; Somsak et al., 2000). Leaves of higher plants have been used for biomonitoring heavy metals since the 1950s (Al-Shayeb et al., 1995). Pine needles for the determination of airborne pollutants are also a suitable technique for monitoring purposes (Holoubek et al., 2000).

Parts of the deposited particles are not removed by rainfall and become irreversibly adsorbed or incorporated into the hydrophobic wax layer of the foliage (Rossini and Raitio, 2003). Therefore, cleaning has an effect on the foliar concentrations of Al, As, At, Br, Cd, Cr, Fe, Ni, Pb, V, T and other heavy metals (Rossini and Raitio, 2003). Aksoy and Öztürk (1996), for example, used date palm leaves to monitor the distribution of airborne Pb, Cd, Zn and Cu in the city of Antalya in Turkey. A comparison of

*Corresponding author. E-mail: M_Hoodaji@Khuisf.ac.ir Tel: 0311-5354061, 09131172651. Fax: 0311-5354061.

Table 1. Mean values of soil properties.

Site	pH	O.C (%)	CEC (meq/100 g)	$CaCO_3$ (%)	Texture
Contaminated					
Q. brantii	8.1	0.3	11.2	68	Sandy loam
L. vulgare	8.3	0.2	10.5	70	Sandy loam
Background					
Q. brantii	8.1	1.3	29.1	26	Clay loam
L. vulgare	8	1.2	28.5	23	Clay loam

O.C, organic carbon; CEC, cation exchange capacity.

washed and unwashed samples showed that, leaf analyses gave a reasonably reliable measure of the total aerial fallout of heavy metals in the studied area.

Plants contamination in most cases arises from atmospheric particles accumulation through their foliage and leaves and the degree of contamination depend on be smoothness of leaves, wind speed and on the value of rainfall. Ward et al. (1977) expressed that plant washing after sampling, decreased the element contents to about 10 to 30% in comparison with unwashed plants (Ward et al., 1977).

Hoodaji and Jalalian (2003 a,b) showed that, soil and plant near the Mobarakeh Steel Company were slightly contaminated with some heavy metals, such as Fe, Zn and Mn; for example they also reported that, topsoils (0 to 5 cm) and shoot of rice, wheat and bean were enriched in Fe (Hoodaji and Jalalian, 2003a, 2003b). The plant contamination was assessed using the enrichment factor. The enrichment factor (EF) was based on the standardization of a tested element against a reference one (Reinmann et al., 2001). The aim of this study was to assess the leaves of *Quercus brantii* and *Ligustrum vulgare* as possible biomonitors of heavy metal contamination in the vicinity of industrial area.

MATERIALS AND METHODS

Site description

Mobarakeh Steel Company (MSC) is located at 65 km south west of Isfahan, near the city of Mobarakeh, Isfahan Province, Iran. It is Iran's largest steel maker and one of the largest industrial complexes operating in Iran and is one of the main contamination sources in this region. This study was done in the Botanical garden of Mobarakeh Steel Company located in 32°34'15'N and 51°25' 21, E southwest of Isfahan, Iran. This region has an arid climate with a mean annual rainfall of 140 mm and wind direction of SW-NE. The region's type of soil, according to information obtained from profile description was Typic Haplocalcids. The garden was approximately 3 ha in area, with 9 plots and 3 replications. The background site was located at 50 km away in a village with low traffic in the Bagh Bahadoran region. Minimum and maximum annual temperatures of this location were 7.6 and 24.33°C, respectively. Relative humidity was 40% and mean wind speed was 1.77 km/h.

Two species, *Q. brantii* and *L. vulgare* were selected. These species are widely distributed in landscape and can survive under a wide temperature range and grow in almost any type of soil. They have different behaviors to metal contaminants due to various morphological and physico-chemical characteristics of their leaves. *L. vulgare* is a shrub with oval to lance-shaped sub-shiny dark green leaves, thick cuticle and moderate epicuticular waxy layer, whilst *Q. brantii* is a tree with spiny shape of the lightly lobed leaves and thin cuticle.

Sampling, preparation and analysis of plant samples

The sampling of both species was performed in August 2007 from the studied and background sites. Leaf sampling was done with wind direction (SW to NE) and from approximate height of 190 and 140 cm for *Quercus* and *Ligustrum,* respectively, along 9 plots in 3 replications. After transferring the samples to a laboratory, leaves were divided in to two parts. One part was washed with de-ionized water to clean dust and any deposited substances on the leaves and another part was not cleaned. At first, all samples were air dried and then oven dried at 70°C for 48 h to constant mass, milled and sieved through a 35 mesh screen. 1 g milled powder of each samples were digested with 10 ml 2N HCl (Chapman and Pratt, 1961).

Sampling, preparation and analysis of soil samples

Soil samples were also collected randomly from a depth of 0 to 30 cm around each plant. Soil samples were air dried, ground and passed through sieve (2 mm).The main soil chemical properties were determined by laboratory analysis. Organic carbon was determined by a modified wet oxidation (Nelson and Sommers, 1982). Soil pH was measured using potentiometric titration of the soil extract. Cation exchange capacity (CEC) was measured by ammonium acetate extraction (Rhoads, 1982). Calcium carbonate was determined by back titration (FAO, 1974). Soil texture was measured by the Hydrometer method (Gee and Bauder, 1986). The total metal concentrations (Al, Fe, Ni and Pb) were determined after digestion with 10 ml HNO3 70% (Pyatt, 1999; Soon and Abbund, 1993). The plant available heavy metals in soils were extracted with diethylen triamine penta acetic acid (DTPA) solution (0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA at pH = 7.3) (Lindsay and Norvell, 1978). Finally, heavy metal concentrations in plant and soil samples were determined by ICP- AES (GBC Integra XL model). Some chemical and physical properties of soils are given in Table 1.

To identify the possible origin of metals in the plants, an enrichment factor of plant (EF_{plant}), was calculated with Equation 1 for each metal as follows:

Table 2. Total and plant available mean metal concentration (mg kg^{-1}) ± Standard error in topsoil around each plant.

Location/species	*Q. brantii*				*L. vulgare*			
	Al	Fe	Ni	Pb	Al	Fe	Ni	Pb
Contaminated site								
Total	17651.3 ± 1399.9	22693.3 ± 956.7	40.1 ± 3.3	16.6 ± 0.7	15492 ± 1189.7	20005.3 ± 1364.7	36.7 ± 1.9	18.6 ± 4.1
Plant available	0.4 ± 0.2	9.3 ± 1.1	2 ± 0.2	1.1 ± 0.1	0.3 ± 0.1	12.7 ± 3.3	1.5 ± 0.1	0.8 ± 0.4
Available percent*	0.005	0.04	5.07	6.62	0.002	0.06	3.98	4.31
Background site								
Total	19250 ± 1000	30000 ± 680	57.2 ± 1	15.9 ± 1	18750 ± 788	30400 ±1500	44.8 ± 5.5	21.5 ± 2
Plant available	2.3 ± 0.1	18.9 ± 3	1.3 ± 0.09	3.3 ± 0.2	2 ± 0.3	30.3 ± 0.75	1.2 ± 0.1	2.9 ± 0.08
Available percent*	0.01	0.06	2.3	19.8	0.01	0.1	2.71	13.5

*Available percent: (plant available / total) × 100.

$$EF_{plant} = \frac{M_{plant}}{M_{control}} \quad (1)$$

Where, M_{plant} and $M_{control}$ are the concentrations of the examined elements of the plant in the contaminated and background sites, respectively (mg kg^{-1}) (Reinmann et al., 2001).

Data analysis was done by a statistical package SPSS 14.0. To evaluate the effect of washing treatment on element concentration, the statistical significance ($p < 0.05$) of the differences between unwashed and washed samples was determined using t-test.

RESULTS AND DISCUSSION

Relationship between soil properties and heavy metal uptake by plant

The total and plant available heavy metal values in the soils are given in Table 2. The total metal concentrations were high, but only a little fraction of them were available for the plants. These results can be explained with the consideration of the chemical properties of the soils in Table 1.

Total soil heavy metal concentration is commonly used to indicate the degree of contamination (Karaca, 2004), although, DTPA-extractable concentration provides a more suitable chemical evaluation of the amount of metals available for plant uptake (Lindsay and Norvell, 1978; Pretuzzelli, 1989; Zufiaurre et al., 1998). Loading and accumulation of heavy metals in the soil depend on different factors such as chemical form of elements, pH, organic matter content, texture and cation exchange capacity (CEC) of the soil (Logan and Chaney, 1983). With increasing pH, organic matter content, CEC and clay percentage and availability of the metals reduced. In addition to the existence of carbonate, sulfate, phosphate and sulfide forms of elements in soil causes an increase in the metal precipitation and consequently decrease their availability for plant (Shuman, 1985 and Forstner, 1985).Therefore, in calcareous soils ($CaCO_3$ > 60%) mobility and uptake of heavy metals were very low. Kabata-Pendias and Pendias (1999) also expressed that, uptake and disuptake of elements depend on plant species, growth stage and composition of soil solution, especially Ca (Kabata-Pendias and Pendias, 1999).

Consequently, in the contaminated site, the existence of more than 60% CaCO3 with pH >8 led to decrease in mobility and availability of these elements in the soils.

Consideration of the available percents of the metals also confirmed the results, therefore, it can be concluded that the source of these elements in plants cannot be the soil.

Concentration of elements in plants

Leaf samples from contaminated site (MSC) had significantly higher concentrations of Al, Fe and Ni than the background site for both species. This result showed the direct effect of atmospheric contamination in industrial regions. The effects of industrial activities on heavy metal pollution have been reported by many authors (El-Hassan et al., 2002; Rossini and Mingorance, 2006). Comparison

Table 3. The mean values of $EF_{plant.}$

Plant species	Al	Fe	Ni	Pb
Q. brantii	1.5	2.6	1.9	0.9
L. vulgare	1.5	2.9	1.9	1.5

Table 4. Elemental concentrations ± standard error in leaves of *Q. brantii* compared by t-test.

Element	Al		T-test	Fe		T-test	Ni		T-test	Pb		T-test
Site	Unwashed	Washed		Unwashed	Washed		Unwashed	Washed		Unwashed	Washed	
Contaminated	367.6 ± 28.7	176.5 ± 27.4	*	1001 ± 157.9	471 ± 41.8	*	85.4 ± 5.2	60.08 ± 9.1	*	3.5 ± 0.7	1.2 ± 0.2	*
Background	250.3 ± 25.3	146.8 ± 16.1	*	380.8 ± 45.25	157.08 ± 28.8	*	45.2 ± 6.85	29.6 ± 5.49	ns	3 ± 0.0.1	1.3 ± 0.5	*
T-test	*	ns		**	*		*	*		ns	ns	

Significance: *p < 0.05, **p < 0.01. ns: not significant.

Table 5. Elemental concentrations ± Standard error in leaves of *L. vulgare* compared by t-test.

Site	Al		T-test	Fe		T-test	Ni		T-test	Pb		T-test
	Unwashed	Washed		Unwashed	Washed		Unwashed	Washed		Unwashed	Washed	
Contaminated	415 ± 85.2	266.7 ± 34.6	ns	1024 ± 156.6	486.32 ± 51.7	*	85 ± 10.2	45.1 ± 3.9	*	4.7 ± 0.6	1.1 ± 0.2	*
Background	267.2 ± 35.1	60.9 ± 9.2	*	350.3 ± 55.6	100 ± 9.25	*	43.6 ± 2.80	15.3 ± 1.34	*	3 ± 0.2	05 ± 0.08	*
T-test	*	*		**	*		ns	*		ns	ns	

*p < 0.05; **p < 0.01; ns, not significant.

of the normal content of Fe in plants (100 to 500 mg kg^{-1} of dry weight) (Pais and Benton, 1997) with the detected concentrations, showed that the contamination of Fe in the study area was considerable.

Enrichment factor of plant

EF_{plant} showed the different ratio of metal bio-accumulation in each part of the plant in relation to the control site. EF > 2 indicates sample enrichment (Mingorance et al., 2007). 1<EF<2 for nearly all the studied elements (Table 3) indicated that the element concentration in the contaminated site were relatively higher than the background site, but not for the enrichment threshold, except for Fe.

EF_{Fe} of about 3 in both species arise from airborne Fe contamination from industrial activities, so the probability for accounting the soil as the contamination source for plants is incorrect with regard to Table 3.

The comparison between plant species

The mean values for Al, Fe, Ni and Pb concentration in washed and unwashed leaves samples of *Quercus* and *Ligustrum* species in both contaminated and background sites are presented in Table 4 and 5 respectively. Each table shows the concentration values for each element expressed in mg kg^{-1}. Regard to the data in these tables, elements was found at higher concentration in the leaves of *Ligustrum*. Differences those existed in the metal concentration between these plants influenced by characteristics of leaf surface. However *Ligustrum vulgare* has a thicker cuticle but due to the moderate waxy surfaces of its leaves is better candidate for accumulation of airborne contamination.

Investigation of atmospheric source of elements with water washing treatment

The ability to distinguish airborne and soil borne contamination was assessed by washing the leaves. Washing with water was an effective procedure for the removal of Pb, Fe, Al, Cr and V from the leaves of ornamental species (Rossini Oliva and Raitio, 2003) General comparison between the element concentrations before and after washing in the contaminated site indicated significant differences in both species except for Al in *Ligustrum vulgare* (Table 4 and 5). In the contaminated site, after washing, element contents of leaves decreased about 29-64 and 28-74 percents for Quercus and Ligustrum, respectively. Therefore, it can be concluded that metal concentrations in the plants in the studied area were due to atmospheric dust from industrial activities. Removal efficiency of the studied elements after washing with water were much higher in comparison with Ward et al ´s results (1977), which reported reduction in element content about 10-30 percents with washing (Ward et al., 1977). Reduction in element content after washing with water is different by physical and chemical characters of pollutants, plant species and subsequently properties of leaves (Rea et al., 2000, (Rossini Oliva and Raitio, 2003)

Conclusions

The findings of this study showed that, both species were suitable biomonitors for Al, Fe and Ni contamination in the studied area. *L. vulgare* acted better than the *Q. brantii* in some cases which confirmed that the accu-mulative ability of plant tissues depend on their surface properties (Tables 4 and 5). Both species were mode-rately enriched in Fe due to the $EF_{plant} > 2$. Considerable differences between the total and DTPA-extractable heavy metals in soils due to high pH value and $CaCO_3$ percentage disproved the probability of plant contamination through the soil and fortified the theory of atmospheric source of these elements in the industrial area. On the otherhand, the use of water-washing procedure which led to significant differences in the heavy metals content of the leaves in both plants in the contaminated site, demon-strated the atmospheric source of the metals.

REFERENCES

Aksoy A, Öztürk M (1996). *Phoenix dactylifera* L. as a biomonitor of heavy metal pollution in Turkey. J. Trace Microprobe Tech. 14(3): 605-614.

Al-Shayeb SM, Al-Rajhi A, 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.

Bicchiega M, Biondi E, Manes F, Campanella L, Cardarelli E, Achilli M (1994). Studi di bioindicazione su metallic pesanti (cromo, ferro, manganese, nichel, rame) in due siti a differente livello di impatto ambientale., Atti XI Convegno Societa Italiana Chimica Agraria, Patron Editore, Roma, pp. 569-576.

Biney C, Amuzu AT, Calamari D, Kaba N, Mbome IL, Naeve H, Ochumba PBO, Osibanjo O, Radegonde V, Saad MAH (1994). Review of heavy metals, in: Calamari D, Naeve H (Eds.). Review of pollution in the African aquatic environment. Rome, FAO, pp. 33-60.

Calzoni GL, Antognoni F, Pari E, Fonti P, Gnes A, Speranza A (2007). Active biomonitoring of heavy metal pollution using Rosa rugosa plants. Environ. Pollut. 149(2): 239-245.

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.

El-Hassan T, Al-Omari H, Anwar J, Al-Nasir F (2002). Cypress tree (*Cupressus semervirens L.*) bark as an indicator for heavy metal pollution in the atmosphere of Amman City, Jordan. Environ. Int. 28(6): 513-519.

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.

FAO (Food and Agriculture Organization) (1974). The Euphrates Pilot Irrigation Project. Methods of soil analysis, Gadeb Soil Laboratory (A laboratory manual). Rome, Italy.

Forstner U (1985). Chemical forms and reactivities of metals in sediments. In: Leschber R, Davis RD, Hermite PL (Eds.). Chemical methods for assessing bioavailable metals in sludge and soils. Elsevier. London, pp. 1-10.

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.

Holoubek I, Kofinek P, Seda Z, Schneiderova E, Holoubkova I, Pacl A, Tfiska J, Cudlin P, Caslavsky J (2000). The use of mosses and pine needles to detect persistent organic pollutants at local and regional scales. J. Environ. Pollut. 109(2): 283-292.

Hüttl 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.

Ives AR, Cardinale B (2004). Food web interactions govern the resistance of communities after non-random extinctions. Nature, 429: 174-177.

Gee GW, Bauder JW (1986). Particle size analysis, in: Klute A (Eds.), Methods of soil analysis. Part 1-(2nd-ed) Agron. Mongr. 9. ASA and SSSA, Madison, WI. pp. 383-411.

Kabata-Pendias A, Pendias H (1999). Biogeochemistry of Trace Elements, 2nd. Ed. Wyd. Nauk. Pwn. Waraw. 400p.

Karaca A (2004). Effect of organic wastes on the extractability of cadmium, copper, nickel, and zinc in soil. Geoderma. 122(2-4): 297-303.

Kovács M, Turcsányi G, Szõke P, Penksza K, Kaszab L, Koltay A (1993). Heavy metal content in cereals in industrial regions. Acta Agron Hung. (42): 171-183.

Lindsay WL, Norvell WA (1978). Development of a DTPA test for zinc, iron, manganese and copper. Soil Sci. Soc. Am. J. 42: 421-428.

Logan TJ, Chaney RL (1983). Utilization of municipal waste water and sludge on land-metals, In: Page AL, Gleason TL, Smith JE, Iskandar Jr IK, Sommers LE (Eds.), Proceeding of the 1983 workshop on utilization of municipal wastewater and sludge on land. University of California, Riverside. pp. 235-326.

Mingorance MD, Valdés B, Rossini Oliva S (2007). Strategies of heavy metal uptake by plants growing under industrial emission. Environ. Int. 33 (4): 514-520.

Pais IJ, Benton Jr. J (1997). The handbook of trace elements. Publishing by CRC Press, Boca Raton Florida. p. 221.

Pretuzzelli G (1989). Recycling wastes in agriculture: heavy metals bioavailability. Agric. Ecosyst. Environ. 27 (1-4): 493-503.

Pyatt FB (1999). Comparison of foliar and stem bioaccumulation of heavy metals by Corsican pines in the Mount Olympus area of

Cyprus. Ecotoxicol. Environ. 42(1): 57-61.
Reinmann C, Koller F, Frengstad B, Kashulina G, Niskavaara H, Englmaier P (2001). Comparison of the element composition in several plant species and their substrate from a 150000-km2 area in Northern Europe. Sci. Total. Environ. 278(1-3): 87-112.
Rhoads JD (1982). Cation exchange capacity, in: Page AL (Eds.), Methods of soil analysis, pp. 149-157. Agron. No. 9, Part 2: Chemical and mineralogical properties. Am. Soc. Agron. Madison, WI, USA.
Rossini OS, 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 OS, Mingorance MD (2006). Assessment of airborne heavy metal pollution by aboveground plant parts. Chemosphere, 65(2): 177-182.
Shuman LM (1985). Fractionation method for soil microelement. Soil Sci. 140(1): 11-22.
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.
Soon YK, Abboud S (1993). Cadmium, Chromium, Lead and Nickel, in: Carter MR (Eds.). Soil sampling and Method of Analysis. pp: 101-108. Canadian Society of Soil Science, Lewis Publishers, Boca
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.
Wen Kuang Y, Yi Zhou G, Zhi Wen D, Zhong Liu S (2006). Heavy metal in bark of Pinus massoniana (Lamb.) as an indicator of atmospheric deposition near a smeltery at Qujiang, China. Environ. Sci. Pollut. Res. Int. 14(4): 270-275.
Zufiaurre R, Olivar A, Chamorro P, Nerin C, Callizo A (1998). Speciation of metals in sewage sludge for agricultural use. Analyst. 123(2): 255-259.