POTENTIAL BIOMONITORS FOR ATMOSPHERIC POLLUTION

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Undesirable influence of air pollution on the human health and the ecosystem has led to an increased interest in toxic emissions monitoring programs. To fingerprint the countrywide and regional transboundary pollution profiles, extensive direct monitoring network is needed, which is very expensive and laborious. Plant leaves, mosses and epiphytic lichens are regarded as potential alternate tools for monitoring levels of atmospheric pollution. Mosses and epiphytic lichens, unlike higher plants have no roots and waxy cuticle nor stomata; hence for mineral nutrition they are largely dependent on wet or dry deposition from the atmosphere. This unique feature has been exploited to predict the pollution levels of the areas in which they are grown by analyzing the embodied toxic elements. Sampling of such naturally growing bioindicators is relatively easier and no sophisticated equipment are needed. The aim of this study was to obtain insight into the response of such plants, when exposed in the vicinity of highly polluted areas and subsequently to confirm the agreement between elemental levels in bioindicators and those measured in atmospheric deposition collected directly with air samplers. INAA and AAS techniques were employed for multielement analysis of exposed samples. Definite influence of pollution on the studied bioindicators was observed for some of the heavy metals like Cd, Cu, Pb and Zn. Results obtained in this work indicate the feasibility of using Broussonetia Papyrifera leaves and moss as bioindicators for environmental pollution monitoring. This pattern was also confirmed by analyzing the air particulate matters (APM) collected on filters using Gent air sampler.

Keywords: Biomonitors, Neutron activation analysis (NAA), Air particulate matter (APM), Air sampler, Lichen, Mosses

1. Introduction

Adverse effects of uncontrolled air pollution emissions on human health and bionetwork have activated global concern to establish monitoring and emission management programs [1-3]. To identify the critical emissions and actual atmospheric occurrences for transboundary pollution migration studies and risk assessment; extensive sampling is a prerequisite on long-term basis and at a large number of sites. So far long-term sampling and measurement of air particulate matter at large number of sites using technical equipment has been very limited, mainly due to the high cost and highly cumbersome procedure [4-5]. Lately uses of bio-organism/ materials to obtain information on certain characteristics of the biosphere have been explored and are being used in certain countries [6-13]. Among the most commonly used atmospheric pollution bio-monitors are the lichens and mosses due to the reason that they are organisms without root system and pick up nutrition and other chemical substances mainly via wet and dry atmospheric deposition. They have very high capacity to accumulate trace elements; which can reflect a time-integrated picture of their life span. Present study was undertaken to observe the accumulation behavior of locally grown species of bioindicators towards air pollution and subsequently compare the elemental concentration levels obtained in the samples collected by dichotomous air sampler at the same time and same site. Direct air sampling at limited sites is ongoing under IAEA-sponsored project RAS-014/7 [14]. Elemental analyses of the collected samples of bioindicators as well as air filters were carried out using instrumental neutron activation analysis (INAA) and atomic absorption spectrometric (AAS) techniques.

1.1. Meteorological data

Metrological data of thirty years was obtained from Pakistan Meteorological Department to foresee the seasons and growth pattern of the studied biomonitors. Average of the temperature, humidity and rainfall of the sampling site is presented in Fig. 1.

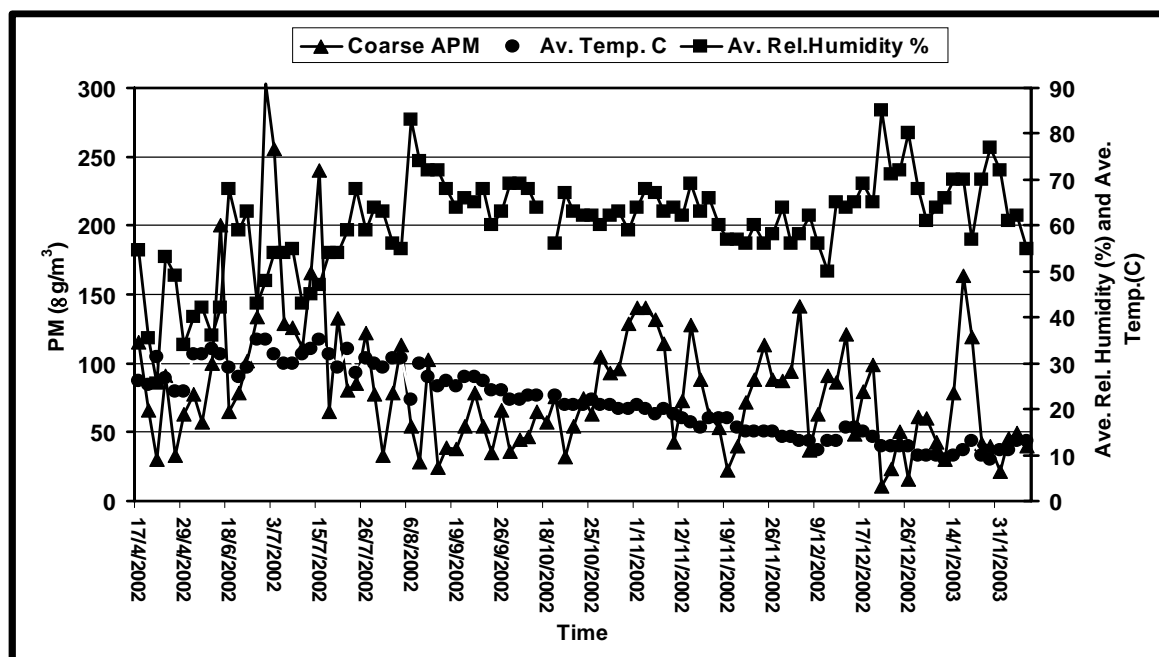* Corresponding author : shujaat@pinstech.org.pk

Figure 1:   Pollution and trends with meteorological parameters in Islamabad.

2.   Experimental

2.1.   Sample collection

Samples were collected from selected locations in Lahore and Islamabad. Two sites were selected within a 10 km radius around the city centre and one in the rural area at a distance of 21 km. At each site mosses were either grown or exposed for 1-2 months. For exposure, specimen were tied with nylon thread on a wooden holder at a height of 1.5-2 m above the ground, equivalent to average height of the inhabitant of the area. For exposure, tree branches covered with the species were collected at the remote areas and transported on the same day to the site. Exposed samples were transported to the laboratory for elemental analyses alongwith grass and soil of the area as a reference. Samples were moistened with distilled water and carefully separated from the bark of the branches using nylon tweezers. The soil and grass samples were collected without roots from the sampling area. In case of soil collection two-centimeter layer was gathered after clearing the top lose surface with the blower. Collected soil was pulverized and then homogenized with twin tub shaker.

Plants leaves of Broussonetia Papyrifera commonly known as Mulberry have been sampled on the same day from different areas in Islamabad. The locations selected for the collection of leaves samples were the remote area Nilore (Site-1), urban area of Islamabad (Site-5) and Industrial area I-9, Islamabad (Site-9).

2.2.   Samples treatment

Samples and grass were washed thoroughly in a glass or porcelain dish to remove sand and dust particles with deionised water. Cleaned sample alongwith grass were dried at room temperature to obtain fresh weight and then dried at 80 °C in an oven until constant dry weight was obtained. Dry weight constant was calculated and values were 60-70% depending on the nature of the sample and the season of sample collection. The dried samples were pulverized and homogenized and then stored in screw topped pre-cleaned polyethylene bottles. Four aliquots of 100 mg from each bottle were randomly analyzed using INAA for their Mn contents to establish their homogeneity. In case of diverse results grounding and homogeneity procedure was repeated till the variation became lower than 5%.

2.3.   Standard preparation

The standards were prepared from the stock solutions of the respective elements under investigation containing 1 mg/ml ultrapure spectrographically standardized solutions

(Johnson, Matthey & Co. UK). Solutions were further diluted to expected range. In case of INAA technique, appropriate solutions were dried on ashless filter paper (Watman-40). Blank filter paper was also analyzed to determine its contribution [15].

2.4. Sample preparation for INAA

Two portions of around 200 mg of the each sample were packed in separate precleaned polyethylene vials alongwith appropriate amount of standards and SRM (NIST-1572) citrus leaves and IAEA-SRM soil-7 were used as control materials. Subsequently all the packed samples were inserted into irradiation rabbits specially designed to reach into the core of the reactor through pneumatic channel. Three-group irradiation protocol, established on the previous findings for the analysis of biological materials [16] was used to get maximum elemental information with minimum irradiation efforts as shown in Table 1.

2.5. Irradiation

All the irradiations were conducted in 27 kw miniature neutron reactor (MNSR) having thermal neutron flux $1x10^{12}$ cm^{-2} s^{-1} at PINSTECH. After specified irradiation and cooling times the samples were transferred into preweighed fresh counting vials and reweighed to get the actual weight of the sample [17].

3. Instrumentation

3.1. Gamma ray spectrometry setup for INAA

All the measurements were made with gamma spectrometric system comprising high purity germanium (HPGe) detector with the resolution of 1.9 kev for 1332.5 kev peak of ^{60}Co and peak to Compton ratio of 40:1, coupled with PC based multichannel analyser (MCA) programmed with "INTERGAMMA" software for analysis.

3.2. Atomic absorption spectrometry (AAS) setup

Hitachi atomic absorption spectrometer (AAS) Model Z-8000 was used for measurement of elements known to give comparatively better results with this technique e.g. Cu, Pb and Cd. These marker elements are very important from pollution source identification point of view. The system is also equipped with a graphite atomizer and optical temperature controller. Instrumental parameters used are listed in Table 2.

3.2.1. Sample dissolution for AAS

For AAS studies 250 mg of sample was digested in a beaker with 10 ml of purified nitric acid at 60 ^{o}C for 30 minutes. After cooling, 2 ml of concentrated HClO$_4$ (70%) was added and reheated at 250 ^{o}C with occasional shaking till white fumes evolved. The solution was filtered through sintered glass crucible and volume was made to 25 ml with de-ionized water in a volumetric flask.

3.2.2. Soil dissolution

About one gram soil sample was slurried with 2 ml of water in 150 ml long stem beaker, then 10 ml of distilled HNO$_3$ was added and heated at 70 ^{o}C for 2 hours. The mixture was cooled then 5 ml of 30% H$_2$O$_2$ was added dropwise. Heating was continued for another hour with intermittent stirring by gentle swirling of the beaker. The cooled solution was filtered through fine pored sintered glass crucible by applying a slight pressure above the solution with the help of atomizer bulb. The solution volume was made upto 25 ml with distilled water.

3.3. Airborne particulate matter (APM) collection

To identify and confirm the elemental distribution pattern APM samples were also collected with the IAEA 'GENT' stacked filter unit (SFU) designed by the Institute for Nuclear Sciences, University of Gent, Belgium with two type of polycarbonate filter collecting 2.2 and 10 μm fractions [19].

4. Results and Discussion

In the present investigation, moss samples as well as soil and grass as reference samples were collected from urban, industrial and rural areas of Lahore. At the same time Broussonetic papyrifera leaves, a potential bioindicator in temperate zone, were also collected from three different sites of Islamabad. The concentrations of 34 elements were determined in the leave samples using INAA technique. AAS technique was used as complementary technique for quantification of Cd, Cu and Pb and results are presented in Table 3. Concentrations of 12 elements in moss samples collected from Lahore area are shown in Table 4.

INAA has been shown; particularly appropriate for the analysis of air pollution biomonitors as it is non-destructive, multielemental, reliable, extremely sensitive for many trace elements,

Table 1.   Irradiation regime for INNA.

Isotope used	Half life	γ-ray used (keV)	Irradiation time	Cooling time	Counting time
Sequential irradiation conditions					
^{28}Al	2.24 min	1778.9	2 min	3 min	100 s
^{52}V	3.75 min	1434.0	2 min	3 min	100 s
^{51}Ti	5.75 min	320.1	2 min	3 min	100 s
^{49}Ca	8.72 min	3084.0	2 min	3 min	100 s
Short irradiation conditions					
^{28}Mg	9.46 m	843.8	2 min	3 min	100 s
^{56}Mn	2.578 h	846.8	2 min	2 h	300 s
Intermediate irradiation conditions					
^{42}K	12.36 h	1524.7	5 h	2 d	900 s
^{24}Na	14.96 h	1368.6	5 h	2 d	900 s
^{76}As	26.3 h	559.1	5 h	2 d	900 s
^{82}Br	35.4 h	554.3	5 h	2 d	900 s
^{140}La	40.2 h	1596.5	5 h	2 d	900 s
^{153}Sm	46.7 h	103.2	5 h	2 d	900 s
^{122}Sb	2.7 d	564.1	5 h	2 d	900 s
Long irradiation conditions					
^{177}Lu	6.71 d	112.9	5 h	2 w	10 h
^{147}Nd	10.98 d	91.1	5 h	2 w	10 h
^{131}Ba	11.8 d	469.3	5 h	2 w	10 h
^{117m}Sn	13.61 d	158.5	5 h	2 w	10 h
^{86}Rb	18.6 d	1076.6	5 h	2 w	10 h
^{233}Th	27.0 d	311.9	5 h	2 w	10 h
^{51}Cr	27.7 d	320.1	5 h	2 w	10 h
^{141}Ce	32.501 d	145.4	5 h	2 w	10 h
^{181}Hf	42.39 d	482.2	5 h	2 w	10 h
^{59}Fe	44.6 d	1099.3	5 h	2 w	10 h
^{160}Tb	72.1 d	1178.0	5 h	2 w	10 h
^{46}Sc	83.8 d	889.3	5 h	2 w	10 h
^{182}Ta	114.4 d	1221.4	5 h	2 w	10 h
^{75}Se	119.8 d	264.7	5 h	2 w	10 h
^{65}Zn	244.0 d	1115.5	5 h	2 w	10 h
^{134}Cs	2.06 y	795.8	5 h	2 w	10 h
^{60}Co	5.27 y	1173.2	5 h	2 w	10 h
^{152}Eu	12.7y	344.3	5 h	2 w	10 h

matrix independent and suitable for all concentration ranges (i.e. nanogram to percent levels) [16-18]. Reliability and accuracy of the NAA regime used for this study is reflected in Table 3, where the quality assurance (QA) data for NIST-SRM-1572 and IAEA-SRM soil-7 are listed. Certified values validate our values and are in very good agreement.

Elemental concentrations in the leaves shown in Table 4 indicate higher concentration of most of the anthropogenic pollution elements in the

Table 2.   AAS parameters for the determination of Cu, Cd and Pb.

ANALYTICAL CONDITIONS	Cu	Pb	Cd
Lamp current (mA)	7.5	7.5	7.5
Wavelength (nm)	324.8	283.3	228.8
Slit width (nm)	1.3	1.3	1.3
Burner height (mm)	7.5	-	-
Oxidant Gas (Air) Pressure (kg.cm^{-2})	1.6	-	-
Fuel Gas (Acetylene) Pressure (kg.cm^{-2})	0.3	-	-
Carrier Gas (Argon) Flow (ml/min)	-	100	100
Sample Volume (μL)	-	10	10
HEATING PARAMETERS			
Drying Temperature (^{o}C)	-	80-120	80-120
Time (s)	-	30	30
Ashing Temperature (^{o}C)	-	400	300
Time (s)	-	30	30
Atomizing Temperature (^{o}C)	-	2100	1700
Time (s)	-	7	7
Cleaning Temperature (^{o}C)	-	3000	2600
Time (s)	-	3	3

sample collected from the site closer to highway due to vehicular emissions. Similarly the concentration of elements related to steel refineries and marble factories are significantly high in the industrial area as compared to rural site [18]. The elemental concentration in moss samples collected at three different sites in Lahore city and its vicinity are illustrated in Table 5. The variation of typical anthropogenic elements is prominent. High APM values pattern has also been identified in Fig. 1 based on the data obtained from the samples collected with the Gent Air sampler [19]. This pattern is prominent, particularly in dry seasons of June and December and are further being analysed for their elemental contents for confirmation.

5.   Conclusions

Results obtained in this work indicate the feasibility of using Broussonetia Papyrifera leaves and moss, in particular leaves because they are available all over the county in all seasons as bioindicators for environmental pollution monitoring. They can be inexpensive alternate for extensive countrywide programs as compared to direct APM collection, which require enormous infrastructure with extensive labour. Furthermore bioindicators provide a measure of integrated exposure over a certain period of time with ease of sampling. Precision and accuracy of the results has also confirmed that the combined use of INAA and AAS is one of the most adequate methods for environmental studies.

Table 3:   Elemental concentration ($\mu g\ g^{-1}$) in certified reference materials (CRM).

Elements	Citrus Leaves (NBS-SRM-1572)			IAEA Soil (S-7)		
	Our value ($C_{det.}$)	Certified value ($C_{cert.}$)	$C_{det.}/C_{cert.}$	Our value ($C_{det.}$)	Certified value ($C_{cert.}$)	$C_{det.}/C_{cert}$
Al	94.6±4.6	92±14.7	1.03	46000±2300	(47000)	0.98
As	3.27±0.23	3.1±0.3	1.05	12.9±0.9	13.4±0.84	0.96
Ba	22.2±2.2	21±2.9	1.06	146±14.6	(159)	0.92
Br	8.04±0.8	(8.2)	0.98	7.2±0.72	(7)	1.03
Ca	30400±2432	31500±1008	0.97	159000±15900	(163000)	0.98
Cd	0.028±0.002	0.03±0.0099	0.93	1.2±0.09	(1.3)	0.92
Ce	0.32±0.02	(0.28)	1.14	59±2.36	61±6.71	0.97
Co	0.022±0.002	(0.02)	1.10	8.2±0.67	8.9±0.85	0.92
Cr	0.84±0.03	0.8±0.20	1.05	62±3.1	60±12.6	1.03
Cs	0.096±0.004	(0.098)	0.98	5.1±0.31	5.4±0.7	0.94
Cu	15.35±1.23	16.5±1.0	0.93	11.2±0.6	11±1.98	1.02
Eu	0.0095±0.0005	(0.01)	0.95	0.98±0.05	1±0.2	0.98
Fe	96.5±4.8	90±9.9	1.07	26000±1170	(25700)	1.01
Hf	-	-	-	5.3±0.27	5.1±0.4	1.04
K	18000±900	18200±600.6	0.99	12000±360	(12100)	0.99
La	0.175±0.016	(0.19)	0.92	28.2±1.41	28±1	1.01
Lu	-	-	-	0.28±0.022	(0.3)	0.93
Mg	5684±568	5800±301.6	0.98	11000±1045	(11300)	0.97
Mn	22.71±0.91	23±2	0.99	629±25.2	631±22.72	1.00
Na	160.4±7.22	160±20.8	1.00	2300±120	(2400)	0.96
Nd	-	-	-	31±3.1	30±6.0	1.03
Pb	12.5±0.75	13.3±2.39	0.94	57±3.42	60±7.8	0.95
Rb	4.75±0.24	4.84±0.06	0.98	53±2.65	51±4.5	1.04
Sb	0.042±0.004	(0.04)	1.05	1.82±0.18	1.7±0.2	1.07
Sc	0.011±0.0005	(0.01)	1.10	8.3±0.17	8.3±0.1	1.00
Se	0.026±0.003	(0.025)	1.04	0.45±0.05	(0.4)	1.13
Sm	0.057±0.003	(0.052)	1.10	5.3±0.21	5.1±0.4	1.04
Sn	0.265±0.026	(0.24)	1.10	-	-	
Ta	-	-	-	0.87±0.13	0.8±0.2	1.09
Tb	-	-	-	0.56±0.06	0.6±0.2	0.93
Th	-	-	-	8.2±0.2	8.2±1	1.00
Ti	-	-	-	2967±445	3000	0.99
V	-	-	-	63±3.15	66±7.3	0.95
Zn	29.91±1.50	29±2	1.03	98±6.86	104±6	0.75

Concentrations in ( ) are information values

Table 4.   Elemental contents (μg g^{-1}) of Broussonetia Pepyrifera leaves along with soils (Islamabad).

Element	Site 1-Leaves	Site 1 Soil	Site 5 Leaves	Site5 Soil	Site 9 Leaves	Site 9 Soil
Al	2040	39000	8570	49400	5990	50800
As	0.52	9.02	2.45	7.61	4.68	7.55
Ba	22.53	298.49	103	265.3	105.35	297.09
Br	4.82	4.32	228	5.5	14.54	9.53
Ca	48100	152800	88900	94000	62800	125300
Cd	0.03	12.17	0.08	8.91	0.21	9.1
Ce	0.61	80.76	13.58	86.74	6.04	89.98
Co	0.21	14.76	3.33	33.63	4.66	44
Cr	0.99	118.62	20.65	95.30	17	105.56
Cs	0.07	5.77	1.24	5.78	0.75	6.22
Cu	1.40	22.25	11.49	18.93	12.25	23.2
Eu	0.01	1.25	0.20	1.31	0.08	1.51
Fe	500	29350	11700	31500	10950	33400
Hf	0.04	8.50	1.49	7.1	0.54	7.62
K	6190	13700	15700	15400	16800	15300
La	1.75	34.1	6.61	35.62	3.61	28.67
Lu	0.01	0.43	0.07	0.39	0.19	0.36
Mg	3503	7620	11600	12200	12000	10900
Mn	21.22	551.2	119.53	540.4	73.33	543.2
Na	306.19	7240	1221	7990	1446	9266
Nd	0.54	36.91	2.77	34.09	2.51	35.51
Pb	0.57	16.09	45.77	13.60	19.4	26.58
Rb	2.08	64.23	27.71	84.46	17.94	108.84
Sb	0.04	0.79	0.5	0.82	0.61	0.86
Sc	0.07	10.11	7.13	10.96	0.71	11.28
Se	0.04	0.77	0.60	0.5	0.55	0.64
Sm	0.06	6.05	1.13	6.75	0.72	7.41
Sn	1.05	20.48	9.33	22.2	13.75	17.65
Ta	0.04	1.27	0.18	1.48	0.16	1.43
Tb	0.02	1.18	0.15	0.98	0.16	1.11
Th	0.04	15.12	2.17	15.5	1.08	14.86
Ti	158.9	6261	776.50	8011	776.3	4197
V	0.83	74.35	16.96	83.48	9.69	79.68
Zn	15.97	63.36	74.33	25.73	335	78.58

Table 5.   Comparison of trace elements concentration (μg/g) in soil, moss and grass samples (Lahore).

Element	Range Mean	Moss (Semibarbula Orientalis)	Soil	Grass
Ca*	Range	0.56-2.68	0.47-1.52	0.21-0.45
	Mean	1.83	0.82	0.35
K*	Range	0.23-1.22	1.02-1.21	1.18-2.01
	Mean	0.61	1.08	1.78
Ti	Range	143-908	4327-6721	305-934
	Mean	783	5409	403
Mn	Range	78-289	512-598	38-84
	Mean	156	548	58
F*	Range	0.08-0.63	2.13-3.98	0.04-0.21
	Mean	0.31	2.89	0.18
Cu	Range	8.9-76.3	13.59-33.84	18-43
	Mean	48.9	22.32	34
Zn	Range	40-632	22.87-86.15	23.2-87
	Mean	301	65.48	81
Br	Range	5.1-128	4.14-9.53	3.1-67
	Mean	24.8	7.35	31
Rb	Range	2.3-29.3	54.62-108.3	12-28
	Mean	18.2	84.21	16.1
Sr	Range	23-76	87.2-198	18-26
	Mean	41	148	22
Pb	Range	3.5-89.8	10.34-50.54	12-46
	Mean	63	34.98	17

* = %

References:

[1]   J. O. Nariagu and J. M. Pacyna, Nature, **333** (1988) 134.

[2]   E. Steinnes, Chemosphere, **35** (1992) 735.

[3]   A. Ruhling. Nord, **9** (1994) 53.

[4]   D. L. Fox, Air Pollution, Anal. Chem. **69** (1997) 1R.

[5]   E. Cortes and L. Reina, J. Radioanal. Nucl. Chem., **262** (2004) 269.

[6]   M. V. Frontasyeva, T. Ye. Galinskaya, S. S. Pavlov, E. A. Povtoreyko, M. Krmar, M. Matavuly, D. Radnovic and E. Steinnes, J. Radioanal. Nucl. Chem., **259** (2004) 141.

[7]   E. V. Ermakova, M. V. Frontasyeva and E. Steinnes, J. Radioanal. Nucl. Chem., **259** (2004) 51.

[8]   J. H. Buchmann, J. E. De Souza Sarkis and C. Rodrigues, Sci. Total Environ., **263** (2000) 221.

[9]   Z. H. Zhang, Z. F. Chai and X. Y. Mao, J. Radioanal. Nucl. Chem., **259** (2004) 75.

[10] M. V. Rao and P. S. Dubey, Sci. Total Environ., **126** (1992) 1.

[11] N. Bangfa, T. Weizi, N. Huining, W. Pingsheng and H. Gaokui, Bio. Trace Elem. Res., **71-72** (1999) 267.

[12] B. Smodis and R. M. Parr, Bio. Trace Elem. Res. **71-72** (1999) 257.

[13] M. C. Freitas, S. M. Almeida, M. A. Reis and O. R. Oliveira, Nucl. Inst. Methd. Phys. Res. A. **505** (1-2) (2003) 430.

[14] S. Waheed, A. Rahman, N. Khalid and S. Ahmad, Radiochim. Acta **94** (2006) 161.

[15] S. Ahmad, A. Mannan and I. H. Qureshi, J. Radioanal. Chem. **78** (1983) 375.

[16] J. Gardea-Torresdey, S. Landsberger, D. O'Kelly, K. J. Tiemann, K. J. and J. G.

Parsons, J. Radioanal. Nucl. Chem. **250**(3) (2001) 583.

[17] S. Ahmad, S. Waheed, A. Mannan and I. H. Qureshi, JAOAC **77** (1994) 11.

[18] S. Waheed, S. Ahmad, J. H. Zaidi, A. Rahman, I. H. Qureshi and M. Saleem, Toxicol. Environ. Chem. **83** (2001) 13.

[19] P. K. Hopke, Y. Xie, T. Raunemaa, S. Biegalski, S. Landsberger, W. Maenhaut, P. Artaxo and D. Cohen, Aerosol Sci. Technol. **27** (1997) 726.