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Biomonitoring of Airborne Heavy Metal Contamination

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1. Introduction

During the last few decades, heavy metal contamination of biotic component of environment has attracted the attention of many investigators. The main reason of these researches based on the heavy metal concentration may have a potential hazard in our food chain after a long period of procrastination. Using biological materials in the determination of environmental pollution as indicators is a cheap and reliable method. Various types of plant such as lichens, mosses, bark and leaves of higher plants, have been used to detect the deposition, accumulation and distribution of metal pollution (Akosy, 2008), Because of plants greatly affected by physical and chemical environmental conditions. If conditions become altered, the exposed plant community can accurately reflect these changes (Nash, 1988).

This chapter discusses the possibility of various types of plant usage as biomonitors for detection atmospheric heavy metal pollution in different conditions and factors that affect their accumulative potential.

1.1 Heavy metals

Over the past two decades, "heavy metals" has been used increasingly in various publications and in legislation related to chemical hazards and the safe use of chemicals. It is often used as a group name for metals and semimetals (metalloids) that have been associated with contamination and potential toxicity or ecotoxicity (Duffus, 2002).

Now, it is known that heavy metals represent a large group of chemical elements (> 40) with atomic mass > 50 carbon units (Fig 1.) Most of heavy metals may be important trace elements in the nutrition of plants, animals or humans (e.g. Zn, Cu, Mn, Cr, Ni, V), while others are not known to have positive nutritional effects (e.g. Pb, Cd, Hg). However all of these may cause toxic effects (some of them at a very low content level) if they occur excessively (Spiegel, 2002). The toxicity of heavy metal depends a great deal on their chemical form, concentration, residence time, etc (Mielke & Reagan, 1988). Because of these elements do not decay with time, their emission to the environment is a serious problem which is increasing worldwide due to the rapid growth of population, increasing combustion of fossil fuels, and the expansion of industrial activities (Smodis & Bleise, 2000).

1 H 1.007																	2 He 4.00
3 Li 6.941	4 Be 9.012											5 B 10.81	6 C 12.01	7 N 14.00	8 O 15.99	9 F 18.99	10 Ne 20.18
11 Na 22.99	12 Mg 24.30											13 Al 26.98	14 Si 28.08	15 P 30.97	16 S 32.06	17 Cl 34.45	18 Ar 39.94
19 K 39.09	20 Ca 40.08	21 Sc 44.95	22 Ti 47.8	23 V 50.94	24 Cr 51.99	25 Mn 54.93	26 Fe 55.84	27 Co 58.93	28 Ni 58.69	29 Cu 63.54	30 Zn 65.41	31 Ga 69.72	32 Ge 72.64	33 As 74.92	34 Se 78.96	35 Br 79.90	36 Kr 83.79
37 Rb 85.47	38 Sr 87.62	39 Y 88.90	40 Zr 91.22	41 Nb 92.91	42 Mo 95.94	43 Tc (98)	44 Ru 101.07	45 Rh 102.90	46 Pd 106.42	47 Ag 107.86	48 Cd 112.4	49 In 114.82	50 Sn 118.71	51 Sb 121.76	52 Te 127.60	53 I 126.90	54 Xe 131.29
55 Cs 132.9	56 Ba 137.33	57 La 138.9	72 Hf 178.49	73 Ta 180.95	74 W 183.84	75 Re 186.21	76 Os 190.23	77 Ir 191.22	78 Pt 195.08	79 Au 196.96	80 Hg 200.59	81 Tl 204.38	82 Pb 207.2	83 Bi 208.98	84 Po (209)	85 At (210)	86 Rn 222
87 Fr (223)	88 Ra (226)	89 Ac (227)	104 Rf (261)	105 Db (262)	106 Sg (266)	107 Bh (264)	108 Hs (271)	109 Mt (268)	110 Ds (271)	111 Rg (272)							
Lanthanides		58 Ce 140.11	59 Pr 140.90	60 Nd 144.24	61 Pm (145)	62 Sm 150.36	63 Eu 151.96	64 Gd 157.25	65 Tb 158.93	66 Dy 162.5	67 Ho 164.93	68 Er 167.26	69 Tm 168.93	70 Yb 173.04	71 Lu 174.96		
Actinides		90 Th 232.04	91 Pa 231.03	92 U 238.03	93 Np (237)	94 Pu (244)	95 Am (243)	96 Cm (247)	97 Bk (247)	98 Cf (251)	99 Es (252)	100 Fm (257)	101 Md (258)	102 No (259)	103 Lr (262)		

Fig. 1. Heavy metal position in Periodic Table.

1.1.1 Origin of heavy metals in the environment

There are two different sources for heavy metals in the environment. These sources can be both of natural or anthropogenic origin.

1.1.1.1 Natural

The principal natural source of heavy metals in the environment is from crustal material that is either weathered on (dissolved) and eroded from (particulate) the Earth’s surface or injected into the Earth’s atmosphere by volcanic activity. These two sources account for 80% of all the natural sources; forest fires and biogenic sources, account for 10% each. Particles released by erosion appear in the atmosphere as windblown dust. In addition, some particles are released by vegetation. The natural emissions of the some heavy metals are 12,000 (Pb); 45,000 (Zn); 1,400 (Cd); 43,000 (Cr); 28,000 (Cu); and 29,000 (Ni) metric tons per year, respectively (Nriagu, 1990).

1.1.1.2 Anthropogenic

There are a multitude of anthropogenic emissions in the environment. Generally heavy metals enter into the environment mainly via three routes: (I) deposition of atmospheric particulates (e.g. Mining, Smelting, Fossil fuel combustion, municipal waste incineration, cement production and phosphate mining). (II) disposal of metal enriched sewage sludge and sewage effluents, commercial Fertilizers and pesticides and animal waste specially to the terrestrial and aquatic environment (III) by-product from metal mining processes (Shrivastav, 2001; Smodis & Bleise, 2000). Among them, the major source of metals is from mining and smelting. Mining releases metals to the fluvial environment as tailings and to the atmosphere as metal-enriched dust whereas smelting releases metals to the atmosphere as a result of high-temperature refining processes (Adriano, 1986). The estimation of metal input into environment from the two latter sources (II & III) is relatively easy to measure, but atmospheric input is difficult to quantify accurately because of after emission, the

pollutants are subjected to physical, chemical and photochemical transformations, which ultimately decide their fate depending upon the atmospheric concentrations (Shrivastav, 2001; Smodis & Bleise, 2000). Air pollutants do not remain confined near the source of emission, but spread over distances, transcending natural and political boundaries depending upon topography and meteorological conditions, especially wind direction, wind speed and vertical and horizontal thermal gradients (Smodis & Bleise, 2000). Among the various species present in the particulate matter, a great attention has been devoted since many years to the study of the elements with elevated toxicity and great diffusion in the environment (As, Cd, Cr, Hg, Ni, Pb, etc.) because of both anthropogenic and natural pollutant emissions (Beijer & Jernelöv, 1986). The contributions of natural and anthropogenic sources in atmospheric heavy metal pollution are shown in table 1.

Element	Fluxes from crustal origin	Fluxes from volcanic origin	Fluxes in gas of volcanic origin	PM emission of industrial origin	Element flux from fossil fuel	Total Emission	Atmospheric Alteration Factor
Ag	0.5	0.1	0.0006	40	16	50	8333
Al	356,500	132,750	8.4	40,000	32,000	72,000	15
As	25	3	0.1	620	160	780	2786
Cd	2.5	0.4	0.001	40	15	55	1897
Co	40	30	0.04	24	20	44	63
Cr	500	84	0.005	650	290	940	161
Cu	100	93	0.012	2200	430	2630	1363
Fe	190,000	87,750	3.7	75,000	32,000	107,000	39
Hg	0.3	0.1	0.001	50	60	110	27,500
Mn	4250	1800	2.1	3000	160	3160	52
Mo	10	1.4	0.02	100	410	510	4474
Ni	200	83	0.0009	600	380	980	348
Pb	30	8.7	0.012	16,000	4300	20,300	34583
Sb	9.5	0.3	0.013	200	180	380	3878
Se	3	1	0.13	50	90	140	3390
Sm	32	9	-	7	5	12	29
Sn	50	2.4	0.005	400	30	430	821
Ti	23000	12,000	-	3600	1600	5200	15
V	500	150	0.05	1000	1100	2100	323
Zn	250	108	0.14	7000	1400	8400	2346

Table 1. Natural and anthropogenic element fluxes (value $\times 10^8 \text{ g y}^{-1}$)

Atmospheric Alteration Factor (%) = Total Emission/ (Fluxes from crustal origin+Fluxes from volcanic origin) $\times 100$ (from Avino et al., 2008).

1.1.2 Fate and transport of atmospheric heavy metals in the environment

The environmental and human health effects of heavy metals depend on the mobility of each metal through environmental compartments and the pathways by which metals reach humans and the environment. The free ion is generally the most bioavailable form of a metal, and the free ion concentration is often the best indicator of toxicity. However, there are exceptions, such as the well known case of mercury, where the organic form, (methyl

mercury) is more toxic than the inorganic ion. Metals exert toxic effects if they enter into biochemical reactions in the organism and typical responses are inhibition of growth, suppression of oxygen consumption and impairment of reproduction and tissue repair (Long et al., 1995). The World Health Organization (WHO) estimates that more than 2 million premature deaths occur annually worldwide, and these can be attributed to the effects of outdoor and indoor air pollution (WHO, 2002). The main outdoor air pollutants are as follows: particulate matter (PM), carbon monoxide, nitrogen oxides, sulfur oxides, ozone, and volatile organic compounds. Most of the mortality/morbidity caused by atmospheric pollution in urban areas is caused by PM with aerodynamic diameters less than 10 μm , known as the inhalable fraction (MacNee & Donaldson, 2000). Heavy metals occur, in atmosphere, basically in particulate form. PM-containing heavy metals may induce the oxidative stress mediated by reactive oxygen, with potential mutagenic effects as a strong correlation between the high elemental concentrations in aerosol particles and high mortality and morbidity has been found in several epidemiological studies (Manoli et al., 2002).

1.1.2.1 Mechanisms of heavy metal deposition

The aerosols, which have a very small falling velocity, are easily transferred by the wind and it is possible to be deposited through the rain at long distances from the point of their emission (Smirnioudi et al., 1998). Therefore, it is expected that chemical components in the rainwater (acid components, anions, cations and heavy metals) damage significantly the environment (surface waters, plants, animals, human beings). These metals even if deposited constantly in small rates over long periods of time, accumulate in the environment and will probably pose an increasing major environmental and human health hazard in future.

The transfer of airborne particles to land or water surfaces by dry, wet and occult deposition constitutes the first stage of accumulation of atmospheric heavy metals. The predominant path depends upon the type of chemical species and upon meteorological factors such as the intensity and distribution of rain fall.

1.1.2.1.1 Dry deposition

Dry deposition involves four distinct processes: gravitational settling, impaction, turbulent transfer and transfer by Brownian motion. The relative importance of these processes depends primarily on the size of particles (Shrivastav, 2001). Dry deposition is more likely to remove particulate forms. Two major categories of particulate matter are fine particles and coarse particles. Fine particulate matter (FPM) comprises particles with aerodynamic diameters of 2.5 μm or less. They are emitted from fossil fuel combustion, motor vehicle exhausts (including diesel) and wood burning. Several toxic metals, including arsenic, cadmium, lead, zinc, antimony and their compounds are associated with FPM in ambient air. This is important from a public health perspective since these fine particles are respirable and can be transported over very long distances. Coarse particulate matter or PM_{10} is the mass concentration of particulate matter having aerodynamic diameters less than 10 μm (USEPA, 1996). Anthropogenically added particulates and aerosols in atmosphere show a broad size distribution from 0.001 μm to 50 μm and are strongly influenced by atmospheric transport processes. In rural regions anthropogenic particles > 5 μm are absent (Ward et al., 1975) and in Polar Regions heavy metals are mostly associated with small aggregated particles in the range of 0.1-10 μm . Dry deposition of heavy metals in rural and remote regions is therefore, through impaction and turbulent transfer (Barrie &

Schemenauer, 1986). Dry deposition represents a major removal pathway for many pollutants from the atmosphere, and it is especially important in arid and semiarid regions where removal by wet deposition (i.e., rainfall scavenging) is greatly diminished because of limited precipitation (Seinfeld & Pandis 1998).

1.1.2.1.2 Wet deposition

In wet deposition, there are always some atmospheric hydrometeors which scavenge aerosol particles. This means that wet deposition is gravitational, Brownian and/or turbulent coagulation with water droplets. Wet deposition involves two major processes: nucleation and within and below cloud scavenging, whereby the wet aerosol particles are collected by falling raindrops (Barrie & Schemenauer, 1986). Wet deposition (via rain and other types of precipitation) is most efficient at removing soluble form of heavy metals from the air. A great percentage of metals fall through the rain at the place of their production (Nurnberg et al., 1984).

For example, dry deposition can remove gaseous mercury forms (particulate forms), while wet deposition is responsible for removing divalent mercury form (soluble form) (Nurnberg et al., 1984).

1.1.2.1.3 Occult deposition

Generally, this route is considered as one of the wet deposition processes. In occult deposition, wetted particles (fog and mist) are deposited by impaction or turbulent transfer (Barrie & Schemenauer, 1986).

However, heavy metals are principally dispersed by atmospheric transportation (Dobrovolsky, 1980), but it is necessary to consider that most atmospherically dispersed heavy metals enter the soil surface and the aboveground plant tissues very rapidly. In the soil, they are sorbed by the absorption complex and redistributed through the soil profile. A proportion of heavy metals is taken up by plants and leached by surface and subterranean runoff. The need to control atmospheric contamination of soils by heavy metals and take preventive and remediation measures is dictated by the serious ecological consequences of this contamination, as observed in different regions of the world (Galiulin et al., 2002).

2. Urgency of monitoring air contamination

The degree and extent of environmental changes over the last decades has given a new urgency and relevance to the detection and understanding of environmental change, due to human activities, which have altered global biogeochemical cycling of heavy metals and other pollutants. Approximately 5 million chemicals are presently known and 80,000 in use; 500 - 1,000 are added per year resulting in a progressive increase in the flux of bioavailable chemical forms to the atmosphere. Therefore air pollution has been one of the major threats to human health and the environment since the last century (Obbard et al., 2005; Batzias & Siontorou, 2006; Dmuchowski & Bytnerowicz, 2009).

2.1 Approaches for monitoring air contamination

There are two conceptual approaches for collecting samples relevant to air and atmospheric deposition related pollution studies. The first approach involves the direct collection of

airborne particulate matter, precipitation and total deposit whereas the second approach uses air pollution biomonitors. The first approach is aimed at quantitative surveys at local, short-range, medium-range or global transport of pollutants, including health-related studies when collecting size fractionated airborne particulate matter. It requires continuous sampling on a long-term basis at a large number of sites, in order to ensure the temporal and spatial representativeness of measurements. The application of such direct measurements on a large scale is extremely costly and person-power intensive. Furthermore, it is not possible, due to logistic problems, to install instrumental equipment at all needed locations. Therefore, the second approach is considered as a non-expensive, yet reliable means of air quality status assessment in a country or a region. Certain types of biological organisms provide a measure of integrated exposure over a certain amount of time and enrich the substance to be determined so that the analytical accessibility is improved and the measurement uncertainty reduced. Sampling is relatively simple (even in remote areas and areas with difficult access) because of no expensive technical equipment is needed (Bagla et al., 2009). Furthermore, the sample treatment and analysis steps in the laboratory are facilitated (Wolterbeek, 2002). Also, this method avoids the need for deploying large numbers of precipitation collectors with an associated long-term program of routine sample collection and analysis (Harmens et al., 2008). Therefore, a much higher sampling density can be achieved than with conventional precipitation analysis and increases the possibility of monitoring many sites simultaneously (Wannaz et al., 2006). Also Bioindicators may be very useful due to their high sensitivity towards a broad spectrum of substances or because of their tolerance to high levels of a substance, accumulated in their tissues over an extended period of time or to integrate its influence in an area of known and relevant size (De Temmerman, 2004). Biomonitoring data are being compared with precipitation, particulate matter fractionation, and speciation data and also with medical statistics to evaluate the correlation between the amount of pollutants in the atmosphere and the human reaction to it (Szczepaniak & Biziuk, 2003).

2.2 Definition of biomonitoring and related terms

The term bioindicator/biomonitor is used to refer to an organism, or a part of it, that depicts the occurrence of pollutants on the basis of specific symptoms, reactions, morphological changes or concentrations (Markert et al., 1997). There is considerable variation in the use of the terms bioindicator and biomonitor: A bioindicator is an organism (or part of an organism or a community of organisms) that contains information on the quality of the environment (or a part of the environment). A biomonitor, on the other hand, is an organism (or part of an organism or a community of organisms) that contains information on the quantitative aspects of the quality of the environment. The clear differentiation between bioindication and biomonitoring using the qualitative/quantitative approach makes it comparable to instrumental measuring systems (Markert, 2007). Therefore biomonitoring, in the general sense, may be defined as the use of organisms and biomaterials to obtain information on certain characteristics of the biosphere. With proper selection of organisms, the general advantage of the biomonitoring approach is related primarily to the permanent and common occurrence of the organism in the field, even in remote areas, the ease of sampling, and the absence of any necessary expensive technical equipment (Wolterbeek, 2002).

2.3 Classification of biomonitors

Organisms can be classified according to the way in which the reaction is manifested: (1) reaction indicators, which have a sensitive reaction to air pollutants and which are used especially in studying the effects of pollutants on species composition, and on physiological and ecological functioning, and (2) accumulation indicators that readily accumulate a range of pollutants and are therefore used especially when monitoring the amount of pollutants and their distribution (Markert et al., 1997). Bioaccumulation monitoring methods can be divided into two groups: (Fig 2.)

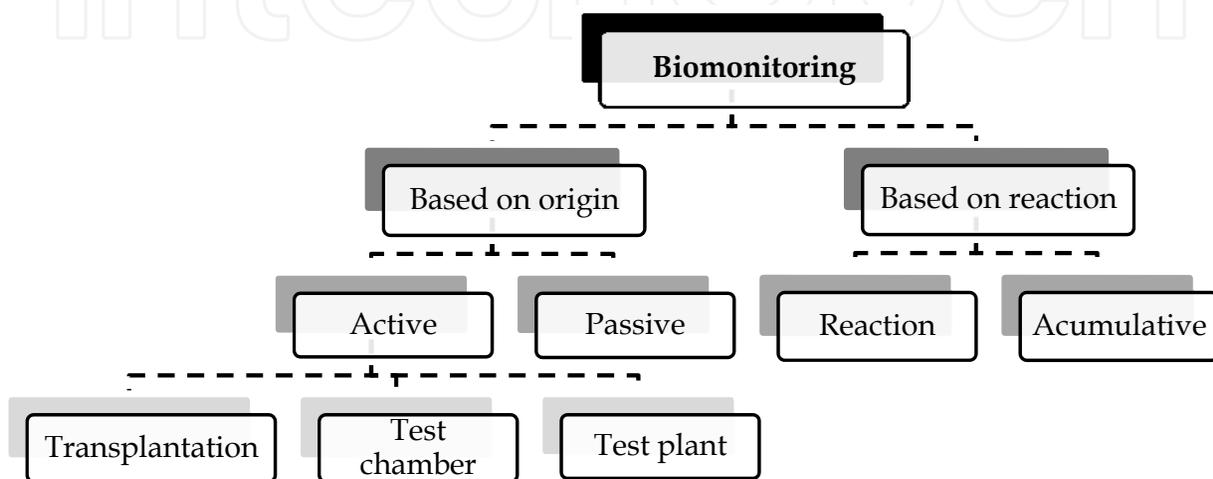


Fig. 2. General scheme of heavy metal biomonitoring using different materials and sampling techniques.

2.3.1 Active biomonitoring

Active biomonitoring includes the exposure of well-defined species under controlled conditions and can be divided into transplantation, test plant, and test chamber methods (C̃ eburnis & Valiulis, 1999). In the transplantation procedure, suitable organisms (mainly mosses and lichens) are transplanted from unpolluted areas to the polluted site under consideration. The exposure time thus is well defined, but the change in uptake efficiency due to climatic change is usually applied to testing either synergistic or single species effects of pollutants on sensitive biomonitors. Transplant techniques appear to be useful, in particular at relatively high pollutant levels. One distinct advantage, compared to the use of indigenous species, is that of well-defined exposure time, but the reproducibility of this technique appears not to be very satisfactory for parameters such as air concentration and deposition rate (C̃ eburnis and Valiulis, 1999).

2.3.2 Passive biomonitoring

Passive biomonitoring refers to the observation or chemical analysis of indigenous plants. In general, the passive biomonitoring method has one major disadvantage; all processes and all sources act at the same time and there is no possibility of separating them and looking for a particular one (C̃ eburnis and Valiulis, 1999). Natural variabilities in ambient macro and microclimate conditions, such as acidity, temperature, humidity, light, and altitude (Gerdol

et al., 2002), or ambient elemental (nutritional) occurrences may cause the biomonitors to exhibit variable behavior. Part of this variance is local (Wolterbeek & Verburg, 2002), but it may be clear that this variable behavior becomes a problem when it seriously affects the biomonitors in its accumulative exposure (Wolterbeek, 2002).

2.4 Principles of bioaccumulation monitoring

Biomonitoring is regarded as a means to assess trace element concentrations in aerosols and deposition. This implies that the monitor should concentrate the element of interest and quantitatively reflect its ambient conditions (Wolterbeek, 2002). In general, a good accumulation indicator of air pollutants should:

1. Accumulate pollutants from the air in the same way and to the same degree under different conditions (Wittig, 1993; Conti & Cecchetti, 2001).
2. The pollutants should be easily measured and the measurements should provide information about the level of pollutant deposition.
3. It should also indicate the risk limits caused by increasing levels of pollutants.
4. The species of organism used should be common enough and be available for collection throughout the year in the same area.
5. Its use should be based on standard sampling and analysis methods.
6. In order to determine the state of the ecosystem in relation to the pollutant under study, the state of the ecosystem in the background area should also be known (Seaward 1995).

The background level is usually considered to be the “natural” level at which emissions have as small an effect as possible (Conti & Cecchetti, 2001). The background level of different pollutants varies between plant species.

2.5 Type of biomonitors

As plants are immobile and more sensitive in terms of physiological reaction to the most prevalent air pollutants than humans and animals, they better reflect local conditions (Nali and Lorenzini 2007). For these reasons, plants are the most common used bioindicators in air quality biomonitoring studies (Balasooriya et al. 2009). Types of plant and their different parts have been applied in trace element air monitoring programs, such as lichens, mosses, ferns, grass, tree bark, tree rings, tree leaves and pine needles (Szczepaniak & Biziuk, 2003; Morselli et al., 2004). For all biomonitors used, the mechanisms of trace element uptake and retention are still not sufficiently known. For grass, tree rings, and ferns, substantial element contributions from other sources than atmospheric, such as the soil or the tree bole, have to be taken into account (Szczepaniak & Biziuk, 2003). Rossini Oliva and Mingorance (2006) reported that the accumulation pattern in the different parts of *Pinus pinea* L. and *Nerium oleander* L. was the following: wood < bark ≤ leaves, because of metals are taken up by wood stem from the soil and soil water, while the outer part (leaves and bark) intercept metals also by deposition from the atmosphere (Rossini Oliva & Mingorance, 2006).

It is important to note that a unique species that can be a suitable bioindicator for biomonitoring of toxic metal pollution all over the world has not been found yet. But lower and higher plants use as suitable biomonitors world-wide.

Plants accumulate metals due to many factors, such as element availability, the characteristics of the plants (such as species, age), state of health, and type of reproduction, temperature, available moisture, substratum characteristics, etc (Conti & Cecchetti, 2001)

2.5.1 Biomonitoring by lower plants

Lower plants are resistant to many substances which are highly toxic for other plants species. They are able to survive in such diverse and often extreme environment; these sedentary organisms possess an equally diverse set of physiological adaptations (Cenci et al., 2003; Fernandez et al., 2006; Dragovič & Mihailovič, 2009). Of all biological species used in biomonitoring, lichens and mosses have the most common occurrence. The morphology of lichens and mosses does not vary with seasons; thus accumulation can occur throughout the year. Lichens and mosses usually have considerable longevity, which led to their use as long-term integrators of atmospheric deposition (Szczepaniak & Biziuk, 2003).

2.5.1.1 Mosses as biomonitor

Botanically, mosses are bryophytes¹. About 15,000 species of mosses are known world-wide (Richardson, 1981), most of which are abundantly distributed amongst the different ecosystems, growing on a variety of substrates (Onianwa, 2000). Two Swedish scientists Åke Rühling and Germund Tyler (1960) have discovered that mosses are good bioindicator of heavy metal pollution in the atmosphere, after this successful discovery many European countries have used mosses in national and multinational surveys of atmospheric-metal deposition. Mosses are cryptogams that thrive in a humid climate. Ectohydric mosses have been used as biomonitors – in most cases terricolous bryophytes. They possess many properties that make them suitable for monitoring air pollutants (Onianwa, 2000). These species obtain nutrients from wet and dry deposition because of they do not have real roots so there is no uptake of mineral substrates from other sources than the atmosphere. Nutrient uptake from the atmosphere is promoted by their weakly developed cuticle (so metal ions easily penetrate the cell wall), large surface to weight ratio, and their habit of growing in groups. Other suitable properties include a slow growth rate, small size and easy to handle, undeveloped vascular bundles (so transport of minerals between segments is limited), minimal morphological changes during the mosses' lifetime, perenniality, wide distribution (even in industrial and urban areas), an ability to survive in highly polluted environment, ease of sampling and the possibility to determine concentrations in the annual growth segments (Poikolainen, 2004; Dragovič and Mihailovič, 2009).

Air pollutants are deposited on mosses in aqueous solution, in gaseous form or attached to particles. The accumulation of pollutants in mosses occurs through a number of different mechanisms:

- As layers of particles or entrapment on the surface of the cells.
- Incorporation into the outer walls of the cells through ion exchange processes
- Metabolically controlled passage into the cells (Poikolaonen, 2004).

The attachment of particles is affected e.g., by the size of the particles and the surface structure of the mosses.

¹Contains Mosses and Liverworts

Ion exchange is a fast physiological-chemical process that is affected e.g., by the number and type of free cation exchange sites, the age of the cells and their reaction to desiccation, growing conditions, temperature, precipitation pH, composition of the pollutants and leaching (Brown & Brûmelis 1996). In the ion exchange process, cations and anions become attached to functional organic groups in the cell walls among other things through chelation (Rao, 1984).

The cell wall has a high polyuronic acid content which makes moss a very good natural ion exchanger. The cell walls of bryophytes possess many negatively charged anionic sites to which cations are bound in exchangeable form. Studies of electron microscope have shown that the sorbed metal may be held either in the extracellular region outside of the cytoplasm, bound to the cell wall, and due to the highly reduced presence or absence of cuticle in the moss, ions have a direct access to the cell wall, mosses surfaces and rhizoids do not perform any active heavy metal ion discrimination (Shakya et al., 2008; Reimann et al., 2006; Onianwa, 2000).

Results of previous studies have shown that the degree of metal uptake efficiency retention proved to decrease in the order $Cu > Pb > Ni > Co > Cd > Zn, Mn$. Lead is very strongly fixed in the moss, and for this reason the correlation between concentration in moss and bulk deposition is particularly high (Szczepaniak & Biziuk, 2003; Rosman, et al., 1998).

Mosses have been used for monitoring atmospheric heavy metal levels in various forms, these include indigenous naturally-occurring epiphytic forms, moss transplants, moss-bags, and Sphagnum mosses. The majority of investigations have utilised epiphytic mosses growing naturally at a given location. Such mosses may be found growing on rocks, tree barks, walls or forest floors, and may be of the acrocarpous or pleurocarpous types. Many species of mosses have been so used, but perhaps the most commonly reported in the literature are the species *Hypnum cupressiforme*, *Hylocomium splendens*, and *Pleurozium schreberi*, particularly in parts of Europe where they are largely abundant (Onianwa, 2000). The comparisons between indigenous and transplanted mosses are summarized in table 2.

Factors affecting on uptake efficiency by mosses

The chemical composition of deposition has a large effect on the accumulation of pollutants, because the uptake efficiency of mosses for individual elements varies considerably (Berg et al., 1995). A high proportion of the pollutant load accumulates in mosses through wet deposition. The amount, duration and intensity of precipitation affect accumulation and leaching (Berg et al. 1995). Ross (1990) and Berg et al (1995) found the best correlation between the concentrations in mosses and in wet deposition for elements such as Pb, Cd, Co and Cu that have high uptake efficiency from wet deposition. The contribution of dry deposition increases on moving from humid to arid climates (Couto et al., 2004). Uptake efficiency is also affected by competition for free cation exchange sites; for instance, the presence of sea salts and acidic deposition has been found to have an effect on the absorption of metals by mosses (Gjengedal & Steinnes, 1990). The types of vegetation and soil dust have also been reported to cause regional differences in uptake efficiency (Szczepaniak & Biziuk, 2003). Other factors affecting the concentrations include stand throughfall and leaching from vegetation layers located above the mosses (Steinnes, 1993), the nutrient status of the site (Pakarinen & Rinne 1979; Økland et al., 1999) and snowmelt water (Ford et al. 1995). The altitude may also have an effect (Gerdol et al. 2002), due e.g., to changes in the amount of precipitation, dust or biomass production. The sampling and

measuring methods employed can also have a considerable influence on the analytical results in biomonitoring studies (Steinnes et al. 1993; Wolterbeek & Bode 1999).

Indigenous	Transplants
Results of pollution patterns can be obtained within a few days.	A survey period of a year is required to allow for effects of seasonal variation.
Results demonstrate pollution in previous years.	Results illustrate pollution only over the sampling period.
Accumulation levels are usually above detectable levels due to longer exposure time.	Concentrations of accumulated levels may be undetectable over shorter sampling period.
Minimal supervision and risk from vandalism.	Potential risk from vandalism.
Costs acquired from transport to sites and chemical analyses.	Additional costs from materials, increased transport and more sampler preparation.
Potential shortage of indigenous samples.	Density of sampling sites, samplers and their position under the control of investigators.
Pollution deposition rates difficult to estimate.	Deposition rates calculated from controlled exposure time.
Metal concentrations reflect influences from other factors such as age of plant, metal content of substrate and local contamination.	Pollutant concentrations in plants can be more directly related to airborne pollution.
Plants may be stressed or undergo morphological/physiological changes, which affect uptake, by long-term exposure to certain pollutants.	

Table 2. Comparisons of indigenous samplers and transplants in heavy metal deposition monitoring (from Gailey and Lloyd, 1993).

It must be noted that the high concentrations of atmospheric pollutants such as sulphur dioxide and heavy metal particulates are harmful to the full development of mosses, and mosses do not thrive well around locations of such very high pollution levels (Onianwa, 2000).

2.5.1.2 Lichens as biomonitor

Lichens are the most studied biomonitors of air quality, since in 1866 a study was published on use of epiphytic lichens as bioindicators. They have been defined as “permanent control systems” for air pollution assessment (Conti & Cecchetti, 2001). Because of their high sensitivity toward specific pollutants and ability to store contaminants in their biological tissues, lichens are defined as bioindicators and/or bioaccumulators, respectively. Lichen form conspicuous gray, green, orange, or red patches on trees or rockes (Blasco et al., 2008).

Lichens are perennial cryptogams. They live on different types of substrate, usually on dry or nutrient-poor sites in boreal and sub-arctic regions (Nash 1996). The lichen species best suited as biomonitors are foliose² and fruticose³ epiphytic lichens. Lichens consist of a symbiotic association of two organisms: the fungal component is usually an Ascomycetes

2. Some lichens have the aspect of leaves
 3. Some lichens adopt shrubby forms

fungus (mycobiont), and the algal component (photobiont), a green alga (Chlorophyceae) and/or blue-green alga (Cyanobacteriae). The fungal component is responsible for taking up water and minerals, and the algal component, which grows amidst the fungal mycelia, for photosynthesis. Most lichen species obtain their nutrients from wet and dry deposition (Martin & Coughtrey 1982; Garty 1993). The lichen surface, structure, and roughness facilitate the interception and retention particles (Szczepaniak & Biziuk, 2003). They possess many of the same properties as mosses that make them suitable for monitoring purposes: the cuticle and vascular bundles are weakly developed, (means that the different pollutants are absorbed over the entire thallus surface of the organism), they do not have any real roots, they are slow-growing and long-lived, and they have an extremely broad distribution (Wolterbeek et al., 2003).

In general, three mechanisms have been reported with regard to the absorption of metals in lichens:

- Intercellular absorption through an exchange process
- Intercellular accumulation
- Entrapment of particles that contain metals (Szczepaniak & Biziuk, 2003).

Lichens may be used as bioindicators and/or biomonitors in two different ways: (1) By mapping all species present in a specific area; entitled "mapping lichen diversity" that consists recording changes in arboreal species diversity in fixed plots over time, either through the use of grid or transects samples, using a standardized method developed by lichenologists and (2) Through the individual sampling of lichen species and measurement of the pollutants that accumulate in the thallus, or by transplanting lichens from an uncontaminated area to a contaminated one and then measuring the morphological changes in the lichen thallus and/or evaluating the physiological parameters and/or evaluating the bioaccumulation of the pollutants (Conti & Cecchetti, 2001).

It was found that concentrations of Pb, Fe, Cu, Cr and Zn in *Hypogymnia physodes* correlated strongly with annual average atmospheric deposition (Sloof, 1995).

In the context of pollution monitoring which uses naturally occurring lichens at monitoring sites as well as lichen transplants, changes in lichen morphology, cytology, metabolism and physiological processes, all provide environmental insight. Normal *Hypogymnia physodes* (L.) Nyl., for example, has a smooth, grey thallus with large lobes but when exposed to air pollution, the surface becomes cracked and the coloration brown or black (Richardson & Nieboer, 1981)

Factors affecting on uptake efficiency by lichen

There are a considerable number of factors, associated with the site where lichens are growing, which may change the concentrations of pollutants in lichens (Brown, 1991; Garty, 2000). These factors are, in most cases, the same as those affecting mosses including quality of the deposition (form of occurrence, composition, pH), climate (composition of precipitation, temperature, wind, drought, length of the growing period) and local environmental factors (vegetation, quality of the substrate, stand throughfall and stemflow, dust derived from soil, altitude of area). On the other hand, throughfall and stemflow, which vary according to the type of canopy cover, have a greater effect on epiphytic lichens than on terricolous mosses (Poikolainen, 2004; Rasmussen, 1978).

Most species are especially sensitive to SO₂, nitrogen and fluoride compounds and to ozone. These compounds affect the condition of lichens and thus reduce the capacity of lichens to accumulate and absorb elements from the atmosphere (Poikolainen, 2004).

Air pollutants have a different effect on the fungal and on the algal partner. The algal partner has been reported to react more sensitively e.g., to acidic deposition and heavy metals, and to show varying accumulation of metals depending on the acidity of precipitation. Sporadic desiccation of lichens may also have an effect on the accumulation and absorption of elements (Poikolainen, 2004). After a dry period, rainfall may result in appreciable washing off particles and the exchange of cations bound on negatively charged exchange sites on the cell walls and plasma membranes of the cells (Bargagli, 1998).

Results of different studies about heavy metal concentrations in various lichen species are summarized in table 3. There are clear differences in the accumulation of elements between different lichen species (even in the same studies) as a result of morphological and physiological differences.

Lichens (Bowen, 1979)	<i>Pseudevernia furfuracea</i> (Adamo, 2003)	Foliose (e.g. <i>Parmelia</i> , <i>Hypogymnia</i>) (Adamo, 2003)	<i>Evernia prunastri</i> (Conti, 2004)	<i>Dirinaria picta</i> (NG et al., 2006)	<i>C.rangiformis</i> (Cayir et al., 2007)	<i>Pyxine cocoas</i> (Rajesh et al., 2011)	<i>Phaeophyscia hispidula</i> (Rajesh et al., 2011)
Cd	-	0.46	(<0.1-0.3)	(0.05-0.09)	(0.14-0.28)	(0.14-0.69)	(0.9-6.3)
Cr	(0.6-7.3)	2.23	(1-4)	(1.04-2.81)	-	(2.24-13.0)	(0.8-26.2)
Cu	(9-24)	5.42	(4-10)	(1.94-4.45)	(11.75-45.13)	(1.06-5.29)	-
Pb	(1-78)	23	(1-8)	(1.05-3.62)	(2.83-16.59)	(1.35-33.8)	(0.1-13.3)
Zn	(20-60)	99	(20-90)	(20.3-53.2)	(44.17-83.15)	(9.15-47.6)	(57.3-194.4)

Table 3. Heavy metal concentrations in lichen species from different studies (mg/kg), (ranges in brackets).

2.5.1.3 Moss to lichens comparison

As above mentioned mosses and lichens do not have root systems like higher plants therefore their contaminant content depend on surface absorption. These organisms have been shown to concentrate particulates and dissolve chemical species from dry and wet deposition. Differences in substrata result in differences in lichen metal content. For mosses, relatively high contribution of crustal elements such as Al, Sc, La and lanthanides is observed. The increase in cation exchange capacity from moss apex to base is apart of its natural balance of elements, which in turn is affected by the proximity of the soil.

Lichen identification and collecting turns out to be very complicated, whereas for mosses it is much easier. The annual growth increment is easier to distinguish for mosses than for lichens; therefore they are considered superior to lichens, if any time resolution in the measurement is required. Older parts of lichens carry fruiting bodies rich in metals. Mosses build carpets during a period of 3–5 years, and their metal content is generally considered to reflect the atmospheric deposition during that period (Szczeplaniak & Biziuk, 2003). Loppi and Bonini showed that lichens and mosses can be used indifferently as accumulators of As, Cd, Cu, Mo, and S. Differences in concentrations between lichens and mosses were statistically significant ($P < 0.05$) for Al, B, Fe, Hg, Pb, Sb and Zn, with mosses retaining higher values than lichens except for Hg and Zn. The elements found in higher concentrations in mosses were associated with particulate matter (Szczeplaniak & Biziuk, 2003). Bargagli et al. found higher

concentrations of lithophile elements (Al, Cr, Fe, Mn, Ni, and Ti) in moss and atmophile elements (Hg, Cd, Pb, Cu, V, and Zn) in lichen (Bargagli et al., 2002). According to studies of Kansanen and Venetvaara (1991) mosses and lichens as the most effective indicators for low and moderate level of metal deposition in polluted areas.

2.5.2 Biomonitoring by higher plants

However mosses and lichens most frequently used for monitoring metal pollution, but these lower plants are characterized by irregular and patchy distribution and their sampling should be done by specialists who can differentiate between similar-looking species. These limitations become more pronounced in industrial and densely populated areas, where severe anthropogenic pressure may cause scarcity or even lack of indicator species at some sampling points. For instance lichens are characterized by slow regeneration and relatively weak tolerance to the complex influence of mycophytotoxic pollutants, Therefore intensive sampling may lead to their reduced availability and even disappearance (Berlizov et al., 2007).

The use of higher plants, especially different parts of trees (leaves and barks), for air monitoring purposes is becoming more and more widespread. The main advantages are greater availability of the biological material, simplicity of species identification, sampling and treatment, harmless sampling and ubiquity of some genera, which makes it possible to cover large areas. Higher plants also exhibit greater tolerance to environmental changes which is especially important for monitoring areas with elevated anthropogenic influence (Berlizov et al., 2007), therefore higher plants have appeal as indicators in air pollution monitoring in highly polluted areas where lichens and mosses are often absent.

Higher plants not only intercept pollutants from atmospheric deposition but also accumulate aerial metals from the soil. Aerial heavy metal depositions are taken up from the soil by plants via their root system and translocated them to other parts of the plant (Mulgrew & Williams, 2000). An otherwise in the industrial and urban areas, higher plants can give better quantifications for pollutant concentrations and atmospheric deposition than non-biological samples (Markert, 1993).

Some plant species are sensitive to single pollutants or to mixtures of pollutants. Those species or cultivars are likely to be used in order to monitor the effects of air pollutants as bioindicator plants (DeTemmerman et al., 2005).

In general, it can be assumed two separated groups of higher plants for biomonitoring purposes including herbs/grasses and trees/shrubs on both groups, aboveground plant tissues (leaf and bark) contribute in airborne heavy metal accumulation.

2.5.2.1 Herbs and grasses as biomonitor

Kovács (1992a) recommended the use of ruderal plants as bioaccumulative indicators due to their ability to accumulate metals in high quantities without visible injury (Kovács, 1992a).

Some plant species may be more efficient in retaining atmospheric metal particles than others. A measure of this efficiency can be resolved by calculating air accumulation factors (AAF) according to the following equation:

$$AAF (m^3.g^{-1}) = PAC (mg.g^{-1} \text{ dry weight}) / CA (mg.m^{-3}) \quad (1)$$

P_{Ac} = atmospheric contribution of the metal in plants and CA = concentration of the metal in the atmosphere (Mulgrew & Williams, 2000).

2.5.2.2 Trees and shrubs as biomonitor

Both coniferous and deciduous trees can be used in the detection of aerial heavy metal pollution, but Coniferous trees indicate pollution over a longer time period such as, *Pinus eldarica*, *Cupressus arizonica* (Ataabadi et al., 2010a), *Pinus brutia* (Baslar et al., 2009), *Cupressus semeruirens* (El-Hasan et al., 2002). Broad-leaved tree species regarded as sensitive to metal contamination include *Betula pendula*, *Fraxinus excelsior*, *Sorbus aucuparia*, *Tilia cordata* and *Malus domestica* (Mulgrew & Williams, 2000). Numerous bioaccumulative indicators exist; some tree examples include *Ailanthus glandulosa*, *Celtis occidentalis*, *Salix alba*, *Tilia tomentosa*, *Sambucus nigra*, *Quercus robur*, *Fagus silvatica* (Mulgrew & Williams, 2000) and *Elaeagnus angustifolia* (Akosy and Sahin, 1999). Also results of studies indicate that *Robinia pseudoacacia* (black locust tree) is appropriate species because of this tree is genetically homogeneous, easily identifiable and ubiquitously distributed (Kovács, 1992b).

However, there are limited studies about biomonitoring by shrub species, but it could numbered some shrub species such as *Nerium oleander* (Ataabadi et al., 2010a; Rossini Oliva & Mingorance, 2006), *Lantana Camara* (Fernandez Espinoza & Rossini Oliva, 2005) *Ligustrum vulgare*, *Photinia serrulata*, *Berberis vulgaris* and *Thuja orientalis* (Ataabadi et al., 2010a,b; Ataabadi et al., 2011).

For comparative studies, it is important that sampling is undertaken at the same time of the year to reduce variability. Chemical composition of foliage varies with season and rainfall (Taylor et al., 1990). For most deciduous species, suitable time is period of year when metal content in leaves will be highest. For instance standard sampling of heavy metal accumulation in *Populus nigra* in central Europe is carried out in August.

Metal content will vary depending on which part of the plant is sampled. The extent of accumulation in different plant parts will vary with species and the nature of the element.

It should be noted that using transplantation exercises are not common for higher plants (Mulgrew & Williams, 2000).

2.5.2.2.1 Plant leaves as biomonitor

Leaves of higher plants have been used for heavy metals biomonitoring since 1950s (Al-Shayeb et al., 1995). The use of leaves as bioindicators of environmental pollution has been studied, more and more, to assess their suitability, to assess effect of a specific pollution source, to differentiate between background (unpolluted) and polluted sites and to monitor or assess the level of pollution in an area (Turan et al., 2011).

Rossini Oliva & Mingorance (2006) and Ataabadi et al (2010a), reported that pine needles can be considered suitable biomonitor for atmospheric heavy metal contamination (e.g. Fe, Al, Pb).

Factors that affect efficiency of heavy metal accumulation on leaf surface

Particle (containing heavy metals) deposition on leaf surfaces may be affected by two different factors including plant-dependent factors such as morphological and structural properties of leaves contain orientation and size (Mulgrew & Williams, 2000) of leaves,

cuticle thickness, cork existence, roughness, existence of surface waxy layer, specific leaf area (SLA), stomatal density (SD) and stomatal pore surface (SPS) (Ataabadi et al., 2011; Rossini Oliva & Mingorance, 2006; Balasooriya et al. 2008) and plant-free factors such as particle size and wind velocity (Mulgrew & Williams, 2000). Also the accumulation of heavy metals by higher plants depends on the binding and solubility of particles deposited on leaf surfaces (Mankovska et al., 2004).

The deposited particles may be washed by rain into the soil, resuspended or retained on plant foliage. The degree of retention is influenced by weather conditions, nature of pollutant, plant surface characteristics and particle size (Harrison and Chirgawi, 1989).

Leaves of evergreen species are considered to be better traps because of higher accumulation on a longer period of time (Turan et al., 2011).

2.5.2.2.2 Plant bark as biomonitor

The physiological function of bark is to protect the tree from mechanical injury, damaging agents and excessive evaporation. Bark quality varies considerably in different tree species and at different stages in the lifetime of a tree species. The outer bark of trees consists of the inner layer (phloem), the cork-forming layer (phellogen), and the outer layer (rhytidom or phellem) composed of dead cork cells (Prance et al., 1993). This dead cork layer has usually been employed in biomonitor studies. The chemical composition of the bark is specific to each tree species. For instance, the pH, electrical conductivity and ash content of the bark of coniferous trees are usually lower than those of broad-leaved species (Barkman, 1958). When bark is exposed to air pollutants either directly from the atmosphere or from the rainwater running down the stem, the chemical composition of the surface layers of the bark changes, such changes can be utilised in investigating the extent of the area subjected to air pollutants. In this respect tree bark is a good bioindicator because it remains in place for an extended period of time, it is easily accessible and sampling does not damage the tree (Berlizov et al., 2007). Retention of suspended particles is promoted by a moist, porous, rough, or electrically charged surface, making bark a highly effective collector (Panichev et al., 2004). Therefore bark has been widely employed as a passive monitor for airborne metal contaminations. The accumulation of atmospheric pollutants in bark is purely a physiological-chemical process. The pollutants either accumulate passively on the bark surface or become absorbed through ion exchange processes in the outer parts of the dead cork layer (Poikolainen, 2004). Although a number of air pollution biomonitoring studies have been performed using bark of different tree species include oak, elm, willow, poplar, pine, olive, cedar, eucalyptus etc. (Berlizov et al., 2007).

Metal accumulation in bark and plant foliage in urban and industrial areas can be considerable, with the greatest amount of the heavy metal burden located in the bark (Cećburnis & Steinnes, 2000; Watmough & Hutchinson, 2003).

Factors that affect efficiency of heavy metal accumulation on bark surface

Factors, in addition to atmospheric pollutants, that affect the chemical composition of tree bark are mainly the same as those for mosses and lichen, although the chemical reactions that occur in bark are somewhat different because bark is a non-living plant material. The concentrations in bark are mainly affected by bark quality, stand throughfall and stem flow. The concentrations are highest in the surface layers of the outer bark, and decrease rapidly on moving towards the inner layers. Many different factors have an effect on the collecting

of heavy metals in bark surface, such as heavy metal quantities in air, physiological and chemical properties of the bark, through fall, soil factors, contamination of other plants, climate factors, etc. A coarse and rough surface more readily accumulates atmospheric pollutants than a smooth surface (Poikolainen, 2004), As Barnes et al., (1976) showed that rough barks accumulate metals more than smooth barks. Other factors include the bark texture and thickness (Poikolainen, 2004), the presence of epiphytic organisms, the time of exposure to the atmosphere and the depth of sampling (Bellis et al., 2001). Also bark acidity has an effect on the concentrations of some heavy metals. For instance, Bates and Brown (1981) found a clear negative correlation between bark pH and the Fe concentration in a study on the occurrence of epiphytic lichens on oak and ash. They concluded that this is due to the increased mobility of Fe with decreasing bark pH. There is no significant migration of elements from the bark surface through the cork tissue into the underlying wood, or vice versa. The migration of heavy metals from the soil via the roots into the bark as it is being formed is also usually insignificant. On the other hand, heavy metals and other compounds may be carried by the wind from the soil to the bark surface (Poikolainen, 2004).

The study carried out by Szopa et al (1973) on lead concentrations along highways in the US indicated that the lead concentration in bark reacts rapidly to marked changes in lead concentrations in the atmosphere (Poikolainen, 2004).

2.6 Analytical method

Finally, the choice of analytical method for heavy metal detection in all plant materials depends on the purpose of the respective survey. Some analytical methods are non-destructive (e.g. Neutron Activation Analysis: N.A.A.) and are useful for repetitive surveys such as baseline studies. Samples can also be archived and used at a later date for additional analysis. Destructive techniques include atomic absorption spectrometry (AAS) and inductively coupled plasma (ICP) analysis (Mulgrew & Williams, 2000).

On consideration of the methods available for moss monitoring in Norway, Steinnes et al. (1993) concluded that ICP-ES works well for Fe, Zn, Pb and Cu, to a lesser extent for V and Ni and but is not satisfactory for Cr, Cd and As. ICP-MS analysis proves a good method for all of the above except As and Cd where less satisfactory results were observed (Steinnes, 1993).

2.7 Some atmospheric heavy metal contamination indices

Several integrated environmental indices reflecting the quality of environment and life have begun to appear in recent years. On the other hand, quantitative information can be obtained by calculating different indices to estimate air quality (Calvelo & Liberatore, 2004).

2.7.1 Index of Atmospheric Purity (IAP)

DeSloover(1964) and DeSloover and LeBlanc (1968) suggested the IAP method for mapping air pollution on the basis of epiphytic lichen and bryophyte sensitivity for mapping aerial pollution (Moore, 1974). This method is based on the fact that the epiphytic lichen and mosses diversity is impaired by air pollution and environmental stress. Therefore the frequency of occurrence of species on a defined portion of a tree trunk is used as a parameter to estimate the degree of environmental stress. This index gives an evaluation of

the level of atmospheric pollution, which is based on the number, frequency, and tolerance of the epiphytic species present in an area.

$$IAP = \sum_{i=1}^n (Q_i \times f_i) \quad (2)$$

n , the number of epiphytic species per site; Q_i , the resistance factor or ecological index of each species, thus representing the sensibility of a species against pollutants; f_i , the frequency or coverage score of each species per site (Max. 10; $1 \leq f_i \leq 10$). The IAP-index was calculated separately for each sampling site (Blasco et al., 2008; Zechmeister & Hohenwallner, 2006).

The frequency method makes it possible to predict pollution levels with a certainty of over 97% (Conti & Cecchetti, 2001). After calculation of IAP, pollution intensity will be determined by IAP classification is shown in Table 4.

Level A	$0 \leq IAP \leq 12.5$	Very high level of pollution
Level B	$12.5 \leq IAP \leq 25$	High level of pollution
Level C	$25 \leq IAP \leq 37.5$	Moderate level of pollution
Level D	$37.5 \leq IAP \leq 50$	Low level of pollution
Level E	$IAP > 50$	Very low level of pollution

Table 4. Quality levels of index of atmospheric purity (IAP) (from Szczepaniak & Biziuk, 2003).

Quality levels may somewhat differ in various studies. As Dymytrova (2009) studied a total of 1730 trees and 272 sampling plots in different parts of Kyiv (Ukraine) including industrial areas, residential areas, roads and inner parks and investigated epiphytic bryophytes and lichens on isolated trees. Results showed the highest and lowest epiphytic richness in the inner parks and industrial area respectively. Consequently based on IAP, four zones with different air pollution were distinguished: highly polluted (0-8.5), moderately polluted (8.6-20.2), slightly polluted (20.3-31.9) and unpolluted (32-88.8). In these studies, heavy metal concentrations in epiphytic vegetation and quality levels of IAP are correlated.

2.7.2 Enrichment factor

One approach used to characterize airborne particulate matter in terms of chemical composition is to calculate so called 'enrichment factors' (EF), relating the concentration of an anthropogenic 'pollutant' element (X), such as Pb, to that of a crustal element (typically Al, Ti, Sc or Fe) in air, normalized to the ratio of these elements in the average continental crust:

$$EF = (X_{\text{air}} / Al_{\text{air}}) / (X_{\text{crust}} / Al_{\text{crust}}) \quad (3)$$

Enrichment factors close to unity thus indicate that windblown dusts are the dominant airborne source, whilst values in excess of unity indicate that analyte concentrations have been elevated as a result of anthropogenic inputs. The calculation assumes that the anthropogenic contribution of the normalizing element (i.e. Al) is insignificant (Zoller et al., 1974; Puxbaum, 1991).

Pacheco et al. (2002) evaluated olive-tree bark for the biological monitoring of airborne trace elements and found significant enrichment of Cu in olive bark relative to *Parmelia* spp. lichen was reported (Pacheco et al., 2002).

3. Conclusion

Application of lower and higher plants as biomonitors seems to be a good way to monitor airborne heavy metal contamination, but the choice of proper phytomonitor for environmental studies depends on many factors such as availability of the biological material, contamination extent, study scale and etc. However lichen and moss due to have superior ability to accumulate elements and indicate them without interference with soil are reported the best bioindicators for the atmospheric heavy metal contamination, but in the urban and industrial areas, using aboveground parts of indigenous higher plants is recommended. Simplicity of species identification, sampling and treatment and ubiquity of some genera makes it possible to cover large areas. For the comprehensive conclusion, both of lower and higher plants should be studied simultaneously in the specific area and manifested with respect to their limitations and advantages.

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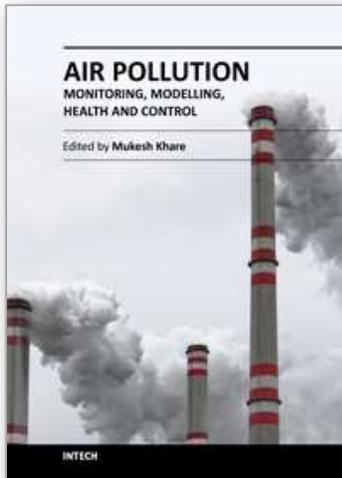
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