

## Assessment of air pollution by toxic elements on petrol stations using moss and lichen bag technique

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### ABSTRACT

Demková L., Baranová B., Oboňa J., Árvay J., Lošák T. (2017): Assessment of air pollution by toxic elements on petrol stations using moss and lichen bag technique. *Plant Soil Environ.*, 63: 355–361.

Three moss (*Pleurozium* spp., *Polytrichum* spp., *Rhytidiadelphus* spp.) and two lichen taxa (*Hypogymnia physodes* L., *Pseudevernia furfuracea* L.), were exposed for four weeks in six petrol stations, two consecutive years (2015–2016), in urban area of the Prešov city (Slovakia), to assess accumulation of selected airborne elements Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb and Zn. Significantly highest ( $P < 0.01$ ) ability to accumulate Zn, Ni, Co and Fe was found in *Pleurozium* spp.; *Pseudevernia furfuracea* was determined the best accumulator of Hg, whereas *Rhytidiadelphus* spp. was found as the least suitable for this purpose. No significant differences in heavy metal accumulation between moss and lichen taxonomic group were found. Samples of conifer (used as a moss/lichen bag holder) showed significantly lower content of heavy metals compared to mosses and lichens. Major content of heavy metals trapped in the air around petrol stations, did not originate from the petrol combustion, but predominantly from the car body, which is mechanically disrupted during fuelling.

**Keywords:** road dust; roadside environment; emission; bio-monitoring; pollutant; coniferous tree

The rapid progress of modern society has significantly increased the level of urbanization followed by transport sector development. An increasing number of vehicles has recently directed research on air pollution originating from traffic rather than from industry (Salo and Mäkinen 2014). A lot of authors studied roadside environment to demonstrate that the traffic contributes significantly to heavy metal pollution (Rossi et al. 2015, Motuzas

et al. 2016). It has been reported repeatedly that heavy metal concentrations in soils, waters and air have increased with increasing traffic (Lenkeppa et al. 2015).

The petrol stations represent places, where the numbers of cars pass through – stop and start again. Starting a vehicle and the first few minutes of driving generate higher emissions because the emissions-control equipment has not yet reached

Supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic, Grant No. VEGA 1/0147/17, and by the Grand Agency of Prešov University, Grant No. GaPu 23/2017.

doi: 10.17221/297/2017-PSE

its optimal operating temperature (Ludykar et al. 1999).

Air quality is usually measured by sampling stations but additionally, some organisms and biomaterials could be used as a part of bio-monitoring methods for air quality (Markert 2007).

The moss and lichen bag technique introduced by Goodman and Roberts (1971) has been developing over the last decades, and it was found an effective method for bio-monitoring of hazardous air pollutants such as heavy metals, non-metals and PAHs (polycyclic aromatic hydrocarbons) (Lodenius 2013, Salo and Mäkinen 2014, Demková et al. 2016). This method provides advantages for the study of industrial and urban environments where the presence of native mosses is limited or absent.

The advantage of moss and lichen bag techniques is the possibility of long-term exposure compared to the instrumentation measurements, which are restricted to a short period. While instrumentation measurements present the results of one day pollution concentrations, long-term sampling is a prerequisite for the assessment of cumulative exposure (Vuković et al. 2016). The aim of the 2-year study was: (i) assess the level of selected heavy metals (Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb, Zn) at six selected petrol stations situated in urban areas of Prešov city (Slovakia) using the moss and lichen (M/L) bag technique; (ii) compare the accumulation capacities of different moss and lichen taxa; (iii) to compare values of heavy metals in moss/lichen samples and samples of coniferous tree (growing next to the petrol stations) which were used as M/L bags holder.

## MATERIAL AND METHODS

**Study area.** The research was conducted in the urban area of the Prešov city (70.43 km<sup>2</sup>; 90 187 inhabitants) (49°00'16.94"N; 21°14'28.46"E), Eastern Slovakia. Selected petrol stations, were situated next to busy roads, heading out of the city. The research was conducted in 2015 and 2016, from September 17 to October 17. During the research period, the average daily temperature was 17.5°C, the average air humidity was 65% and the average precipitation was 44.5 mm per month. Comparing long-term average values, September and October average air temperature, precipitation, and humidity in the Prešov city are 14.3°C, 40.3 mm, 61% and 8.9°C, 65% and 123.3 mm, respectively.

**Mosses and lichens sampling.** Three moss (*Pleurozium* spp., *Polytrichum* spp., *Rhytidiadelphus* spp.) and two lichen (*Hypogymnia physodes* L., *Pseudevernia furfuracea* L.) taxa samples were collected the same day from a Čergov mountain forest, more than 500 m from the forest road. The M/L samples (ca. 500 g for each taxa) were transported to the laboratory in plastic bags, mixed, carefully cleaned from debris and dead or senescent parts. Samples were washed three times, lasting 20, 15 and 10 min, with 10 L of distilled water per 100 g moss and lichen dry weight. After washing, M/L samples were hand squeezed and dried out (60°C for 24 h) according to the procedures described in Adamo et al. (2007). The preparation process was the same in both years of research.

**Bag preparation and exposure conditions.** Moss and lichen bags were prepared as follows: nylon mosquito net, with a mesh of approx. 2 mm, was cut in pieces of a 12 × 12 cm, where 5 g of M/L material (model organisms) was inserted and closed with a nylon thread. At each exposure site, the bags of each moss and lichen taxa (in two replicates) were exposed approximately 2 m above the ground for 4 weeks, in both years. In total, 60 samples (5 taxa in two replicates at 6 petrol stations) were exposed each year (120 samples for both years). Coniferous trees, which were the part of the petrol stations (growing along one side of the petrol stations) were used as the M/L bags holders. After exposure, M/L bags were stored in plastic bags in low temperatures (–18°C). Two samples of coniferous tree branches (standard organism), were also collected and analysed for heavy metal content.

**Heavy metal analyses.** M/L samples were air dried, homogenized and mineralized using Mars X-Press 5 (CEM Corp., Matthews, USA) in a mixture of 5 mL 67% HNO<sub>3</sub> and 5 mL deionized water. The content of heavy metals was determined using the flame atomic absorption spectrophotometry (FAAS): Zn, Cr, Ni, Mn and Fe on the SpectrAA 240FS (Varian Inc., Mulgrave, Australia) and electrothermal atomic absorption spectrophotometry (GF-AAS): Cd and Pb with Zeeman background correction on the SpectrAA 240Z (Varian Inc., Mulgrave, Australia) (Árvay et al. 2015). The total mercury content was determined by selective mercury analyser AMA-254 (Altec, spol. s r.o., Prague, Czech Republic). The total content of heavy metals was determined in M/L samples before (the values were used as a reference) and after exposure (the

values measured before exposure were deducted from final concentration).

**Data analysis.** One-way ANOVA test, followed by the Tukey's multiple comparison test were used to find out the differences in heavy metal concentrations among taxa at the  $P < 0.05$  and  $P < 0.01$  level. Cluster analysis, Ward's method, was used to highlight similarity/distinction between taxa based on their ability to accumulate heavy metals. Spearman's correlation coefficient was used to confirm similar origins of pollution. All statistical analyses were performed in PAST 2.17c software (Hammer et al. 2001). Data are listed as average values of both evaluated years.

## RESULTS AND DISCUSSION

The average values of 2-year data, representing the amounts of elements (post-exposure minus pre-exposure concentration) in moss and lichen taxa after the 4-week exposure are reported in

Table 1. The element concentrations in the exposed M/L bags were higher than the initial element concentrations (Table 2). Pb, Cd, Co and Zn are the major metal pollutants of the roadside environment and are released from the burning of petrol, wearing out of tyres, leakage of oils, and corrosion of batteries and metallic parts etc. (Ikenaka et al. 2010). The concentrations of Pb in model organisms reached very high values, exceeding several times the permitted limits (Table 2). Especially lead poisoning from vehicle pollution has been addressed internationally by removal of leaded petrol (Maher et al. 2008). Metals such as Fe, Cu, Zn are essential components of many alloys, pipe, wire and tyres in motor vehicles and are released into the roadside environment as the result of mechanical abrasion and normal wear and tear (Huber et al. 2016).

*Pleurosium* spp. was found to be the best accumulator of almost all evaluated heavy metals, except of Hg (Figure 1), which was proven by statistical results at a significance level  $P < 0.01$  (Table 2).

Table 1. Contents of selected heavy metals (mg/kg) in three moss and two lichen taxa exposed for 4 weeks at petrol stations in the Prešov city (Slovakia)

		<i>Hypogymnia physodes</i> L.	<i>Pseudevernia furfuracea</i> L.	<i>Pleurosium</i> spp.	<i>Polytrichum</i> spp.	<i>Rhytidiadelphus</i> spp.
Cd	min–max	1.57–2.09	nd – 0.83	0.91–6.10	0.12–0.65	0.22–0.69
	mean $\pm$ SD	1.89 $\pm$ 0.28	0.54 $\pm$ 0.46	2.83 $\pm$ 2.62	0.42 $\pm$ 0.27	0.46 $\pm$ 0.24
Pb	min–max	17.4–26.6	15.4–21.5	20.2–25.8	9.96–16.5	11.1–17.1
	mean $\pm$ SD	22.1 $\pm$ 4.59	17.9 $\pm$ 3.17	22.9 $\pm$ 2.80	13.9 $\pm$ 3.43	13.6 $\pm$ 3.16
Zn	min–max	64.6–82.5	36.9–46.0	144–229	58.1–61.3	25.6–38.3
	means	72.0 $\pm$ 9.33	42.1 $\pm$ 4.66	172 $\pm$ 43.4	59.9 $\pm$ 1.63	31.5 $\pm$ 6.42
Cu	min–max	5.64–6.38	4.67–6.97	9.61–22.1	9.91–11.4	3.01–5.06
	means	5.98 $\pm$ 0.37	6.0 $\pm$ 1.19	14.7 $\pm$ 6.25	10.8 $\pm$ 0.81	4.38 $\pm$ 1.18
Cr	min–max	nd – 0.18	nd – 0.21	1.05–5.41	0.42–0.98	nd – nd
	means	0.06–0.10	0.07–0.12	2.52 $\pm$ 2.22	0.64 $\pm$ 0.30	nd $\pm$ nd
Ni	min–max	6.11–8.09	6.16–6.5	10.8–18.1	5.21–6.96	5.06–6.88
	means	6.80 $\pm$ 1.12	6.36 $\pm$ 0.18	13.8 $\pm$ 3.66	6.13 $\pm$ 0.88	5.91 $\pm$ 0.92
Co	min–max	2.96–3.92	2.57–2.76	5.41–5.58	2.61–3.16	2.72–3.62
	means	3.30 $\pm$ 0.54	2.66 $\pm$ 0.10	5.51 $\pm$ 0.08	2.87 $\pm$ 0.28	3.19 $\pm$ 0.45
Mn	min–max	115–162	35.2–36.6	124–163	64–69.2	21.9–33.5
	mean $\pm$ SD	134 $\pm$ 24.4	36.0 $\pm$ 0.72	141 $\pm$ 19.3	67.3 $\pm$ 2.89	26.3 $\pm$ 6.23
Fe	min–max	425–474	625–739	1251–2155	556–670	468–481
	mean $\pm$ SD	458 $\pm$ 28.1	665 $\pm$ 64.1	1578 $\pm$ 457	625 $\pm$ 60.6	472 $\pm$ 8.04
Hg	min–max	0.16–0.16	0.14–0.19	0.10–0.14	0.11–0.12	0.05–0.10
	mean $\pm$ SD	0.16 $\pm$ 0.002	0.17 $\pm$ 0.03	0.11 $\pm$ 0.02	0.12 $\pm$ 0.003	0.07 $\pm$ 0.02

nd – not detected ( $\leq$  LOD – limit of detection); SD – standard deviation

doi: 10.17221/297/2017-PSE

Table 2. Mean contents of selected airborne heavy metals (mg/kg DW) determined in model organisms (mosses/lichens) and standard organisms (coniferous tree) and the results of one-way ANOVA indicating the significant differences (\* $P < 0.01$ ) in metal concentration among taxa (model organisms)

	Cd	Pb*	Zn*	Cu*	Cr	Ni*	Co*	Mn*	Fe*	Hg*
Model organisms	1.23	18.1	75.6	8.39	1.24	7.81	3.51	81.2	760	0.13
Standard	0.57	4.71	30.7	3.83	0.40	4.88	2.64	40.4	158	0.02
Limit value <sup>#</sup>	0.05	1.0	50.0	10.0	1.5	1.5	0.2	200	150	0.1

<sup>#</sup>Markert 1991; DW – dry weight

The lowest mean contents of Cu, Hg, Mn, Ni, Pb, Zn were found in *Rhytidiadelphus* spp. The lowest values of Cd, Co and Fe were determined in *Polytrichum* spp., *P. furfuracea*, and *H. physodes*, respectively. In the samples of *H. physodes* L., *P. furfuracea* L. and *Rhytidiadelphus* spp. the content of chromium was under the detection limit.

The results of one-way ANOVA confirmed significant differences ( $P < 0.01$ ) between taxa in accumulation ability of all evaluated heavy metals except Cd and Cr (Table 2). Based on the results of Tukey's post hoc test, *Pleurosium* spp. was found as the best accumulator of Zn, Ni, Co and Fe at a significance level 0.01. Significantly highest

( $P < 0.01$ ) ability to accumulate Hg was found for *P. furfuracea*. The ability to accumulate Mn was significantly highest ( $P < 0.01$ ) in *H. physodes* and *P. furfuracea* compared to other taxa.

**The heavy metal content in standard organisms.** The mean values of Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb, Zn accumulated in model organisms were 2.15; 1.32; 2.19; 4.81; 6.5; 2.0; 1.6; 3.84 and 2.46 times higher comparing to standard organisms, respectively (Table 2). Despite its long-term exposure in petrol stations, the pine trees were found as the worst heavy metal accumulator in comparison with mosses and lichens. Based on the results of numerous studies, the bioaccumula-

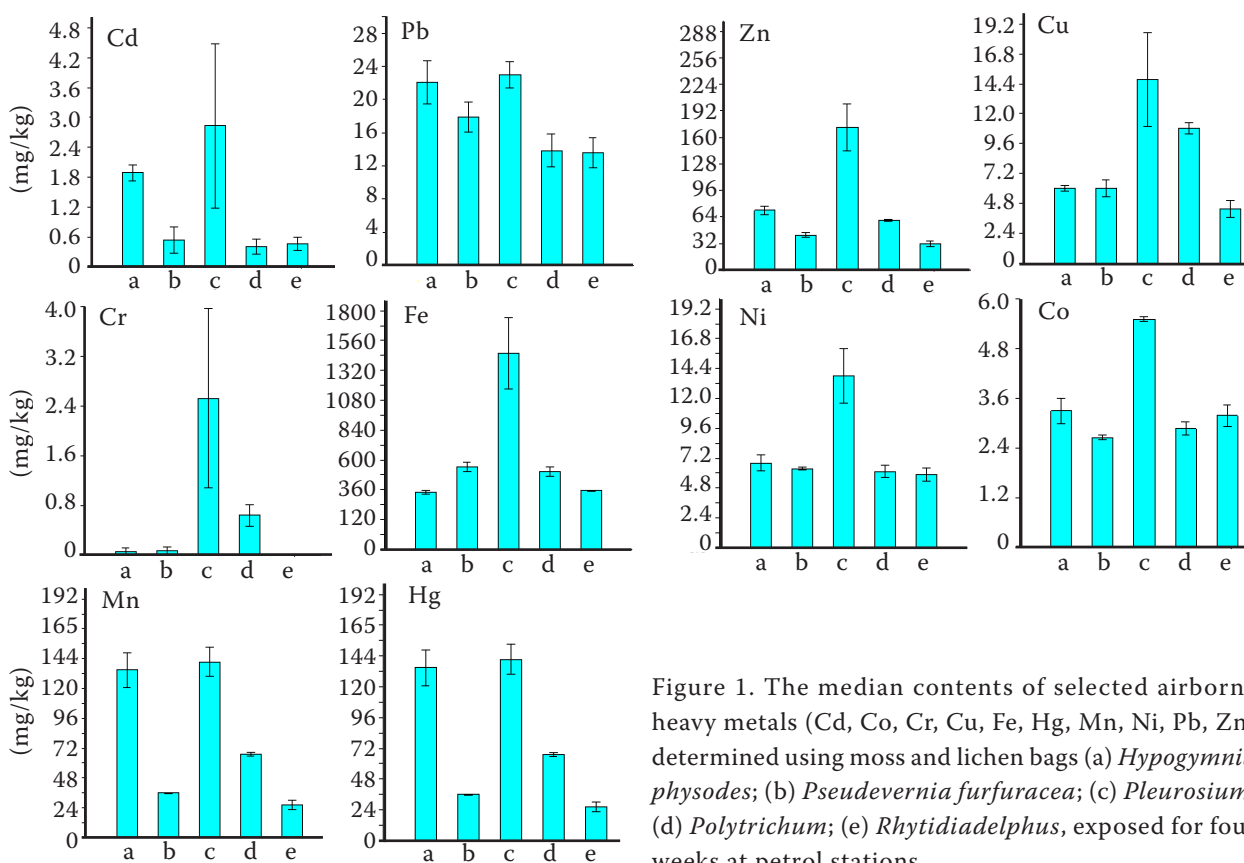


Figure 1. The median contents of selected airborne heavy metals (Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn) determined using moss and lichen bags (a) *Hypogymnia physodes*; (b) *Pseudevernia furfuracea*; (c) *Pleurosium*; (d) *Polytrichum*; (e) *Rhytidiadelphus*, exposed for four weeks at petrol stations

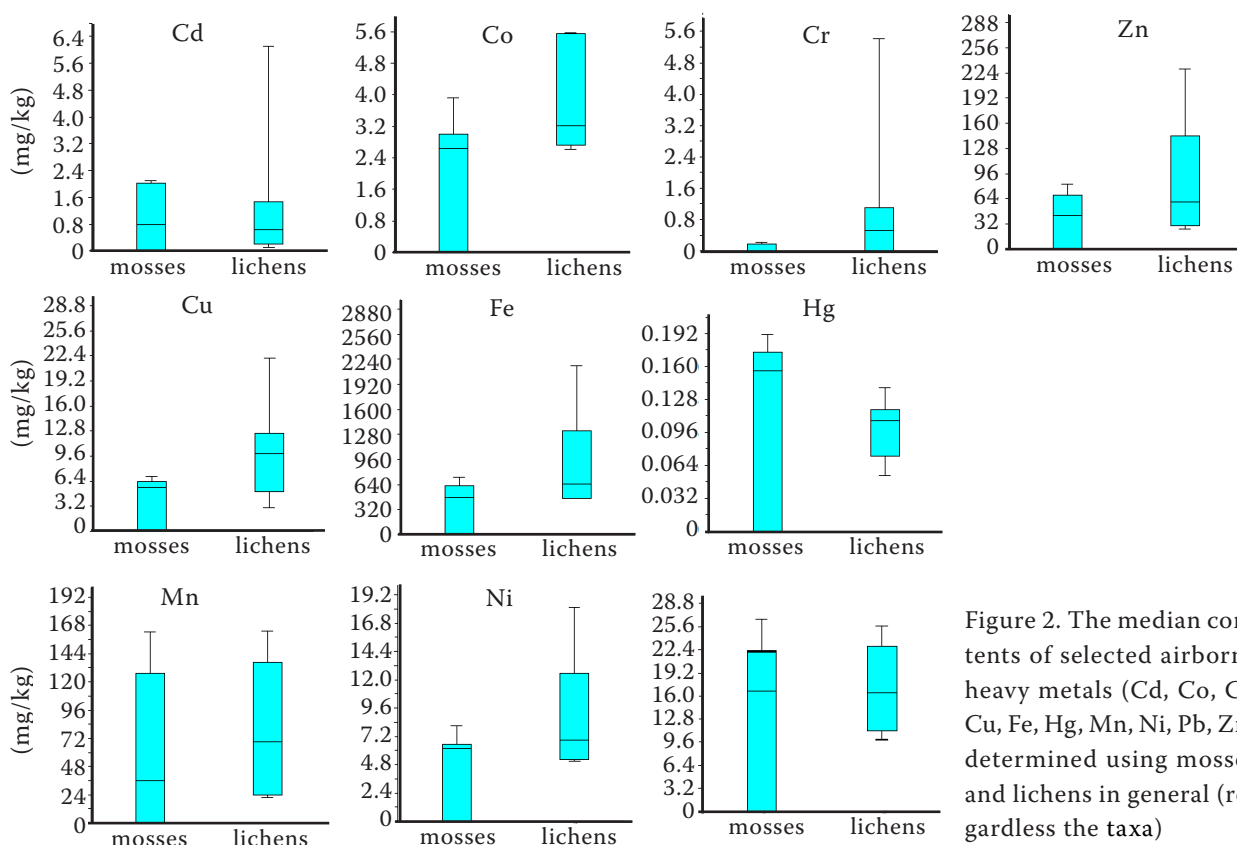


Figure 2. The median contents of selected airborne heavy metals (Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn) determined using mosses and lichens in general (regardless the taxa)

tion ability of coniferous trees is much lower in comparison to deciduous trees or shrubs (Weaver and Mogensen 1919). Coniferous trees could be more appropriate for assessing soil contamination when toxic substances are accumulated through the root system (Kalinovic et al. 2016). It was assumed that the differences could originate from different structure and composition of coniferous tissue resulting in the different measures of heavy metals accumulation. Long-term exposure could lead to a certain resistance of the exposed organism and thus to lower accumulation.

**Comparing mosses and lichens accumulation ability.** Mosses were observed to accumulate higher amounts of heavy metals in comparison to lichens (Figure 2). Probably it is because of *Pleurozium*, while the rest of mosses, *Polytrichum* and *Rhytidiadelphus* accumulated less amount of heavy metals in comparison to lichens in general. Numerous studies have found, that lichens proved better resistance to environmental stress, preserving or recovering their vitality during bio-monitoring (Spaquuolo et al. 2011). It was also found that lichens and mosses can be used indifferently as accumulators of different heavy metals (Loppi

and Bonini 2000). In our study, mean contents of Cd, Pb, Mn and Hg were higher in lichen taxa, and the mean contents of Cu, Cr, Ni, Co and Fe were 1.7; 16.7; 1.3; 1.5 and 2.0 times higher in moss taxa compared to lichens, respectively. The results of one-way ANOVA did not confirm significant differences in heavy metal content between moss and lichen taxonomic groups (Table 2).

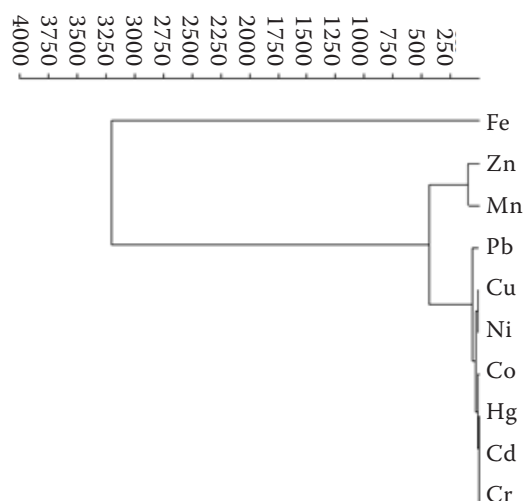


Figure 3. Cluster analysis of selected airborne heavy metals assessed using moss and lichen bags



doi: 10.17221/297/2017-PSE

Table 3. Correlation relationship between selected airborne heavy metals determined using moss and lichen (M/L) bags regardless the taxa

	Pb	Zn	Cu	Cr	Ni	Co	Mn	Fe	Hg
Cd	0.87**	0.72**	0.28	0.22	0.42	0.47	0.70**	0.01	0.26
Pb	–	0.69**	0.29	0.24	0.36	0.28	0.72**	0.21	0.27
Zn	–	–	0.74**	0.68*	0.51*	0.55*	0.94**	0.47	0.125
Cu	–	–	–	0.87**	0.35	0.21	0.62*	0.67*	0.03
Cr	–	–	–	–	0.45	0.40	0.61*	0.73**	0.14
Ni	–	–	–	–	–	0.67**	0.45	0.49	0.10
Co	–	–	–	–	–	–	0.43	0.18	0.3
Mn	–	–	–	–	–	–	–	0.40	0.27
Hg	–	–	–	–	–	–	–	–	0.01

\*\* $P < 0.01$ ; \* $P < 0.05$ 

**Cluster analyses.** According to cluster analysis based on the overall heavy metal content (mg/kg), three metal groups were observed (Figure 3). Elements belonging to the same cluster had strong correlations among themselves and may originate from a common source (Yıldırım and Tokaloğlu 2016). The results indicate three clusters: (1) Fe; (2) Zn-Mn; (3) Pb-Cu-Ni-Co-Hg-Cd-Cr. Zn and Mn are extensively used in automobile industry and could predominantly originate from the wearing of the vehicle body not the combustion of fuel, which explains their close relationship confirmed by the cluster analysis the same as the correlation analyses. Iron, according to the cluster analyses, stands alone. Compared to other metals, iron reached the highest values in moss and lichen insoles. Iron could be released during manipulation with fuel tank during fuelling or can originate directly from the car bodywork.

**Correlation between heavy metals.** No significant correlations were found between mercury and other heavy metals (Table 3). Iron content was significantly positively correlated with Cr ( $P < 0.01$ ) and Cu ( $P < 0.05$ ). The presence of iron in dust particles around petrol stations are predominantly caused by car bodies' corrosion and has no connection to the combustion of petrol. Chromium and copper are also used for the surface treatment of materials and subject to corrosion (Maher et al. 2008). Yıldırım and Tokaloğlu (2016) who evaluated the heavy metal content in street dust have found a significant positive correlation between the pairs: Ni-Co, Zn-Cu and Cd-Pb. The same correlations were confirmed in our study. Zinc significantly positively correlated with all evaluated heavy metals except Fe and Hg

and manganese significantly positively correlated with Cd, Pb, Zn ( $P < 0.01$ ) and Cu, Cr ( $P < 0.05$ ). Manganese is used in automobile industry as part of fuel additives as well as the part of car bodies, which explains the relation with petrol, the same as car body-related heavy metals (Kabata-Pedias 2010).

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Received on May 12, 2017

Accepted on July 25, 2017

Published online on August 15, 2017