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5 **Trace elements and nitrogen in naturally growing moss *Hypnum cupressiforme* in urban and peri-urban**
6 **forests**

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23
24 **Abstract**

25 We monitored trace metals and nitrogen using naturally growing moss *Hypnum cupressiforme* Hedw. in urban and
26 peri-urban forests of the City Municipality of Ljubljana. The aim of this study was to explore the differences in
27 atmospheric deposition of trace metals and nitrogen between urban and peri-urban forests. Samples were collected at
28 a total of 44 sites in urban forests (forests within the motorway ring road) and peri-urban forests (forests outside the
29 motorway ring road). Mosses collected in urban forests showed increased trace metal concentrations compared to
30 samples collected from peri-urban forests. Higher values were significant for As, Cr, Cu, Hg, Mo, Ni, Pb, Sb, Tl and
31 V. Within the motorway ring road, the notable differences in element concentrations between the two urban forests
32 were significant for Cr, Ni and Mo. Factor analysis showed three groups of elements, highlighting the contribution
33 of traffic emissions, individual heating appliances and the resuspension of contaminated soils and dust as the main
34 sources of trace elements in urban forests.

35 Key words: heavy metals, biomonitoring, Ljubljana, ICP-MS, elemental analysis, factor analysis, traffic emissions

36

37 **1 Introduction**

38 The atmosphere is constantly affected by pollutants that originate from increased anthropogenic activities, which are
39 predominant in urban and industrial areas. Among different pollutants, trace metals are recognized to have toxic
40 effects when they accumulate in different environmental compartments (Hart 1982, He et al. 2005, Malea et al.
41 1994, Rainbow and Phillips 1993, Senesil et al. 1999). Because of the significant input of pollutants in the
42 atmosphere and their adverse effects on biota and human health, monitoring airborne pollutants is an essential part
43 of environmental planning and control programmes (Lee et al. 2005). As an integral part of the urban environment,
44 green spaces provide environmental, economic and social benefits (Tyrväinen et al. 2005). Airborne pollutants are
45 absorbed onto the leaves of trees and other vegetation more effectively than on other surfaces (Escobedo and Nowak
46 2009, Fantozzi et al. 2013), and this contributes to the removal of air pollutants from the atmosphere. On the other
47 hand, plants can be used as indicators in pollutant monitoring (Markert et al. 2003). Information on the presence and
48 type of pollutant can be obtained either from monitoring the changes in the composition and structure of plants or
49 measuring the content of the pollutants in their tissues (Wolterbeek 2002). Among different biomonitors used for
50 assessing air pollution, lichens and mosses are the most common due to their biological and physiological features
51 (Puckett 1988). Owing to their large surface/weight ratio, a lack of epidermis and cuticle and their high cation
52 exchange capacity, mosses can accumulate high concentrations of trace metals (Markert et al. 1999). In addition,
53 because mosses offer a cheap and simple sampling procedure, a large number of sites can be included in a pollution
54 monitoring survey (Szczepaniak and Biziuk 2003, Tyler 1990). As a method for monitoring air quality,
55 biomonitoring with mosses was first introduced in Scandinavia during the 1970s (Rühling and Tyler 1970), and
56 today it is part of many national and regional surveys, including the repeated 5-year survey coordinated by the
57 United Nations Economic Commission for Europe ICP-Vegetation programme (Harmens et al. 2004, Herpin et al.
58 1996, Schilling and Lehman 2002).

59 Major sources of airborne trace elements in urban areas are energy production, industry and traffic emissions
60 (Pacyna and Pacyna 2001). Even though the concentration of Pb has decreased with the introduction of unleaded
61 gasoline, other potentially toxic elements originating from exhaust and non-exhaust sources are significant
62 contributors to airborne trace element pollution. Metals, such as Pb, Cd, Cu, Cr, Ni, Zn, Sb and those from the
63 platinum group, are released from motor vehicles and deposited on the roads and plants close to the road (Ho and
64 Tai 1988, Legret and Pagotto 2006, Zechmeister et al. 2006). Many studies have shown that urban soils and plants
65 receive a considerable amount of trace metals mostly from motor vehicles (Biasioli et al. 2006, Naszradi et al. 2007,
66 Oliva and Espinosa 2007). Vehicle exhausts are also considered to be a major source of atmospheric nitrogen (N)
67 pollution in the form of nitrogen oxide (NO_x) emissions (Pearson et al. 2000). Apart from the above-mentioned
68 sources, the resuspension of particles from road dust is another source of trace metals that cannot be neglected (Abu-
69 Allaban et al. 2003).

70 The city municipality of Ljubljana is known for its green infrastructure, with many park–forest complexes including
71 two urban forests. Urban forests, having dense canopies, act as a natural filter for air pollutants (Nowak et al. 2014).
72 Air quality, especially sulphur dioxide (SO₂) pollution, has improved over the 45 years of continuous monitoring

73 (Ogrin et al. 2016) with the introduction of district heating and gasification infrastructure and applying requirements
74 from Integrated Pollution Prevention and Control legislation (IPPC Directive) (OECD 2012). However, as evident
75 from the Slovenian Environment Agency report (Cegnar et al. 2015), particle emissions (PM_{10}) and NO_x , mainly
76 originating from traffic, is still of major concern in Ljubljana. Monitoring of air quality (SO_2 , O_3 , NO_x , PM_{10}) in the
77 city is done at two monitoring stations by the Environmental Agency within the regular national monitoring
78 network; however, heavy metals (Pb, Cd, Ni and As) in precipitation and particles are measured at only one
79 sampling location. Additionally, air quality in urban forests is monitored at one monitoring station by the Slovenian
80 Forestry Institute (Skudnik et al. 2014, Vilhar et al. 2014).

81 The aim of this study was i) to evaluate the trace element and N deposition in urban and peri-urban forests of the
82 City Municipality of Ljubljana using naturally growing cypress-leaved moss *Hypnum cupressiforme* and ii) to
83 identify the possible sources of atmospheric deposition of trace elements and N in urban and peri-urban areas by
84 using factor analysis.

85

86 2 Materials and methods

87 2.1 Study area and sampling of moss material

88 The investigation took place in the City Municipality of Ljubljana (hereafter Municipality of Ljubljana), which is
89 one of eleven city municipalities in Slovenia. Its centre is Ljubljana, the largest city and the capital of Slovenia (**Fig.**
90 **1**). The municipality spans across 275 km² and has a population of approximately 280,000 citizens. The municipality
91 is situated in the central part of Slovenia (46°03'20"N 14°30'30"E) at an average altitude of 298 m above sea level
92 (a.s.l.). Forests cover about 42% of the municipality area and stretch to the city centre (Urbančič et al. 2010). The
93 climate of the city is continental (Köppen climate classification), with a prevailing wind direction from the
94 southwest at an annual frequency of 23.2% and from the west at an annual frequency of 19.1% (**Fig. 1**). The
95 characteristics of the Ljubljana basin include frequent temperature inversions, sometimes with more than 300-m
96 thick inversion layers, and low local air circulation.

97 The industrial activity of the city is small scale, with the major industrial sources being a central heating and power
98 plant and pharmaceutical and food-processing-related plants. There are more than 170,000 vehicles registered just in
99 Ljubljana (SURs 2014), but since the municipality is positioned at the crossroads of pan-European transport
100 corridors "V" and "X", it is exposed to additional transit traffic. There is a motorway ring road around the city of
101 Ljubljana (**Fig. 1**), and this forms the main hub of the Slovenian motorway network and connects to the A1 and A2
102 motorways. The ring road consists of four bypass sections: northern, southern, eastern and western, with the average
103 daily traffic (AADT) at more than 70,000 vehicles on the northern sections; this is also the highest level of traffic in
104 Slovenia.

105 The moss material *H. cupressiforme* Hedw. was collected in August 2013 at 44 sites within the Municipality of
106 Ljubljana. The locations were divided into two categories as follows:

- 107 i. urban forests—forests inside the ring road comprising the Rožnik and Šišenski hrib forests (hereinafter, Rožnik)
108 in the western part of the city at an elevation of 429 m a.s.l. and Golovec in the eastern part of the city at 450 m
109 a.s.l. at the highest point (22 sampling points) and
110 ii. peri-urban forests located outside the ring road (22 sampling points) (**Fig. 1**).

111 Sampling was carried according to the guidelines of the European moss survey protocol (ICP Vegetation
112 Coordination Centre 2010), except that the samples were collected at least 1 m away from the tree canopy and not 3
113 m as specified by the protocol to avoid canopy drip. We chose a shorter distance because of the absence of large
114 forest clearings and because confidence intervals for the N values at 3 m from the canopy and at 1 m from the
115 canopy overlapped (Skudnik et al. 2014). To avoid the direct influence of local emitters, the samples were collected
116 at least 50 m from main roads and industries. Each sample was composed of five to seven subsamples collected
117 within an area of 50 × 50 m.

118

119 2.2 Sample preparation and chemical analysis

120 In the laboratory, moss samples were cleaned of dead material and substrate, dried at room temperature, lyophilized
121 and homogenized with the addition of liquid N. For analysis, only live green segments from the uppermost part of
122 the plant were used. Portions of about 0.16 g moss were digested with a mixture of 4 mL concentrated Suprapur
123 HNO₃ and 1 mL Suprapur H₂O₂ in a microwave oven (Milestone). After the digestion, samples were filtered and
124 diluted with pure water (MiliQ) to a volume of 20 mL. Concentrations of the following elements were analysed
125 using the Agilent 7500ce ICP-MS: As, Ca, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Mo, Ni, Pb, Sb, Se, Sr, Ti, Tl, V and
126 Zn. Concentration of mercury (Hg) was determined using a direct mercury analyser (DMA-80; Milestone). The
127 analyses of trace elements were performed at the Jožef Stefan Institute (Ljubljana, Slovenia). N concentrations were
128 determined using the vario Pyro cube elemental analyser at the Slovenian Forestry Institute (Ljubljana, Slovenia).
129 Quality control of the analytical procedures for determining both, trace elements and N, was carried out by analysing
130 reference moss material M2 and M3 (Steinnes et al. 1997).

131

132 2.3 Statistical analysis

133 Concentrations of trace elements and N were not normally distributed. The differences in element concentrations in
134 moss between urban and peri-urban forests were tested with the non-parametric Mann–Whitney U test using log-
135 transformed data. Factor analysis was employed to identify how the elements grouped together at the urban and peri-
136 urban sampling sites. A correlation matrix was created from the log-transformed element concentrations in the
137 mosses. The ‘fa’ function of the R package ‘psych’ was utilised for factor analysis with orthogonal (Varimax)

138 rotation and the maximum likelihood (ml) factoring method. The factor analysis was run using the correlation
139 matrix, and therefore variables were standardized (each has a variance of 1). The number of factors was set to three
140 based on the examination of a scree plot and examination of resulting factors. All statistical analyses were
141 performed with R 3.2 (R Development Core Team 2016). Factor scores were projected on the GIS map of Ljubljana,
142 using ESRI ArcMap software (ESRI 2015).

143

144 **3 Results**

145 3.1 Element concentrations in mosses in the Municipality of Ljubljana

146 Summary statistics of elemental levels in *H. cupressiforme* collected in forests of the Municipality of Ljubljana,
147 together with median values for urban and peri-urban forests are presented in Table 1. A comparison of
148 concentrations obtained in this survey with median levels from a Slovenian national survey performed in 2010 at
149 102 locations (Harmens et al. 2013) in forests throughout the country is also presented in Table 1. Differences in
150 element concentrations between urban and peri-urban forests, expressed as median values, were statistically
151 significant ($p < 0.01$) for As, Cr, Cu, Hg, Mo, Ni, Pb, Sb, Tl, V and N (Table 1), with higher values found in the
152 urban forests. The median concentrations of Cr, Cu, Pb and Sb from the urban forests were also higher than the
153 median concentrations recorded from the Slovenian 2010 moss survey.

154 A comparison of element concentration between the two urban forests (Rožnik and Golovec) is presented in **Fig. 2**.
155 Concentrations of Cr, Cu, Mo and Ni were significantly higher ($p < 0.01$) from Rožnik compared to Golovec.
156 Concentrations of all other elements were similar in both forests, with somewhat higher concentrations found at
157 Golovec.

158 Results obtained in our investigation are in agreement with reported data (Table 2) of in-situ mosses collected in the
159 transect of Oslo (Reimann et al. 2006) and the Wienerwald biosphere reserve located near the city of Vienna
160 (Krommer et al. 2007) but lower than those reported for transplanted mosses in Belgrade (Vuković et al. 2015) and
161 Naples (Adamo et al. 2011).

162

163 3.2 Source apportionment of trace metals

164 Using factor analysis, three factors were extracted that accounted for 62% of the total variance of the whole data set
165 of 44 sampling points (Table 3). Factors were identified by comparing elements with significant factor loadings.

166 Factor 1 (F1) comprised elements As, Fe, Hg, Pb, Ti, Tl and V and represented 27% of the total variance. The
167 highest loading of this factor (**Fig. 3**) was found in urban forests of Rožnik and Golovec, with a decline in loadings
168 moving away from the urban centre. This demonstrates the influence of the city centre as a source of air pollution.

169 Factor 2 (F2) comprised elements Cr, Cu, Mo, Ni, Sb, Se, Zn and N and explained 21% of the variance. The highest
170 loadings of this factor were found in the urban forest of Rožnik, while some moderate loadings were also found at
171 certain locations in the urban forest of Golovec and in the western part of the Municipality of Ljubljana (**Fig. 4**).

172 Factor 3 (F3) elements were Ca, Co and Mg. High loadings of this factor were present mostly in the peri-urban
173 forests; however, Golovec forest also showed some higher loadings of this factor (**Fig. 5**).

174

175 **4 Discussion**

176 4.1 Element concentration in mosses in the Municipality of Ljubljana

177 Biomonitoring of trace elements using moss *H. cupressiforme* is the first pollution survey performed in the forests in
178 the Municipality of Ljubljana. As expected, concentrations of most trace elements, especially those resulting from
179 anthropogenic activities, in *H. cupressiforme* were higher in the urban forests compared to the peri-urban forests
180 (Table 1). The most exposed locations were those close to the centre of the city or close to the busiest streets or coal-
181 fired power plant (**Fig. 1**). The highest (maximum) concentrations of Hg (0.12 mg kg⁻¹), As (0.61 mg kg⁻¹) and Tl
182 (0.11 mg kg⁻¹) were found at point G11 located in the Golovec forest at the Castle above the city centre; the highest
183 concentrations of Cr (7.83 mg kg⁻¹), Mo (1.30 mg kg⁻¹) and Ni (4.30 mg kg⁻¹) were found at sampling point R05,
184 located in the northern part of Rožnik forest; and the maximum concentrations of Cu (4.58 mg kg⁻¹), Pb (13.12 mg
185 kg⁻¹) and V (5.22 mg kg⁻¹) were found at sampling point G14 (Golovec) also in close proximity to one of the major
186 streets in the city and close to the coal-fired power plant.

187 On the other hand, concentrations of macroelements Ca, Fe, Mg and Mn and additionally Se and Sr were higher in
188 the peri-urban forests, although not significantly. Since peri-urban forests in Ljubljana are not highly influenced by
189 anthropogenic activities and/or intensive agriculture, we assume that these elements were related to the
190 environmental conditions at the sites and were very likely supplied from the substrate. An additional investigation is
191 needed to confirm this. Økland et al. (1999) found that concentrations of K, Ca, Mg and Cd in tissues of
192 *Hylocomium splendens* were highest at sites with high soil pH and nutrient content. Other authors have also
193 emphasized the substrate as a potential nutrient source for bryophytes (Brown and Bates 1990) as well as upward
194 movements of inorganic ions in bryophyte carpets (Bates and Farmer 1990, Wells and Brown 1996). On the other
195 hand, Reimann et al. (2006) found that plant nutrients (Ca, K, Mg, Mn, P, S) in Norway did not show any spatial
196 dependency. They suspected that concentrations of plant nutrients in mosses are possibly so high that an additional
197 input from either anthropogenic or geogenic sources would not be sufficient to cause spatial patterns.

198 An interesting finding of our survey was that the Rožnik forest is more polluted than Golovec, especially with Cr,
199 Cu, Mo and Ni. We ascribe this to the forest's position between the busiest part of the motorway ring road and the
200 industrial zone of the city. Additionally, households located in the southern part of Ljubljana are not connected to
201 the district heating system (**Fig. 3-5**) and instead use traditional heating utilities, making this urban forest more

202 susceptible to emissions from different sources within the city. Also of note is that Rožnik lies in the western part of
203 the city and is therefore more exposed to north–east winds and the long range transport of contaminants.

204 From an overall comparison of median values between the Slovenian moss survey of 2010 (Harmens et al. 2013)
205 and this survey, higher median concentrations in urban forests were observed for Cr, Cu, Pb and Sb, showing an
206 anthropogenic influence on the urban forests. Compared to the national median values from 2010, a notable decrease
207 in concentrations in our study was observed for Cd, Fe, Hg, Se, Sr, Ti and V, suggesting improved emission
208 controls.

209 The results from our study were close to those from the Oslo transect and Wienerwald biosphere reserve (Krommer
210 et al. 2007) located near the city of Vienna, with few exceptions: concentrations of Sr, Ti, V and Zn were lower in
211 our study, and this difference can be attributed to the different intensity of anthropogenic activities in these areas and
212 perhaps different lithologies.

213 Greater differences in concentrations of trace metals were observed between Ljubljana and Naples (Adamo et al.
214 2011) and Belgrade (Vuković et al. 2015), especially for Cu, Pb, Fe, Ti, V and Zn. Naples is influenced by
215 Mediterranean xeric climatic conditions, which serve as a sink and source of trace metals originating from the
216 resuspension of contaminated soil (which is the case for Ti and Pb) (Adamo et al. 2011). In Naples the highest
217 concentrations of trace metals were observed in locations near coastal urban districts with high traffic flows.

218

219 4.2 Source apportionment of trace metals

220 Attributing elements to certain sources of pollution using factor analysis is a complex task. Often, factor analysis
221 cannot discriminate between two sources with similar emission profiles. As a consequence, elements can be
222 statistically ‘picked up’ and assigned to the most similar identified source, overstating its contribution (Thurston et
223 al. 2011). We observed that two of the factors (F1, F2) identified from our survey originated from two sources with
224 similar emission profiles.

225 The properties of F1 suggest that its origin was either from the mixing of crustal elements with anthropogenic
226 emissions or quite possibly the resuspension of already contaminated soil dust. Ti, Fe and Tl are typical crustal
227 elements (Reimann and de Caritat 1998), but the presence of As, Hg, Pb and V in F1 indicates some anthropogenic
228 influence. The association of Pb in this group can be attributed to the resuspension of dust particles already
229 contaminated with Pb that was most likely deposited on the surface of roads from combustion of leaded gasoline
230 (Miguel et al. 1997). The use of unleaded gasoline in Slovenia began in 2001, and emissions of Pb from traffic have
231 been decreasing since then. As, Hg and V are volatile elements that are usually emitted to the atmosphere from
232 combustion sources (Meij and te Winkel 2007). According to the Slovenian Environmental Agency report (Cegnar
233 et al. 2015), besides traffic and industry, small individual heating devices using out-of-date technology and
234 “unclean” fuels considerably contribute to pollution with particles. The city heating network supplies the heat to

235 almost 74% of the households in the Municipality; the remaining homes use traditional heating sources, such as
236 coal, biomass and oil.

237 Most of the elements present in F2 can be attributed to traffic emissions, which was further confirmed by the high
238 loadings (**Fig. 4**) present at locations where traffic intensity is greater (Schauer et al. 2006, Thorpe and Harrison
239 2008). In particular, the west wing of the ring road is not entrenched and has an AADT of 70,000 vehicles, among
240 which 11,000 are heavy duty vehicles (Slovenian Infrastructure Agency 2014). Additionally, in the southern part of
241 the urban forest of Rožnik, a road through the forest that connects to the northern part of the ring road is the busiest
242 street of the city with many traffic lights and where braking is frequent. Cu is among the most important components
243 in brake pads together with Sb (correlation between Cu and Sb, $r = 0.79$), which is added to reduce the vibrations
244 and to improve friction stability of vehicles (von Uexküll et al. 2005). Zn particles originate from tire wear and are
245 released more during urban driving due to increased acceleration, braking and cornering in cities (Stalnaker et al.
246 1996, Wik and Dave 2009). The association of N in this factor further supports traffic as the possible origin; vehicle
247 exhaust is a main contributor to N pollution in the form of NO_x pollution (Bermejo-Orduna et al. 2014, Pearson et al.
248 2000, Skudnik et al. 2015).

249 The elements Cr, Mo, Ni and Se, however, usually have another source of emission in addition to traffic. From the
250 analysis of particles in PM_{10} (Koleša and Planinšek 2013), a factor grouping elements Cr, Ni and Mo was obtained
251 but represented only 3% of the PM_{10} results. The correlation coefficient for elements Cr and Ni ($r = 0.86$), Cr and
252 Mo ($r = 0.76$) and Mo and Ni ($r = 0.74$) indicates a common source, which could be traffic, combustion and/or
253 industry (Dongarrà et al. 2007, Johansson et al. 2009). Element Se has the highest correlation with Mo ($r = 0.57$) and
254 Cu ($r = 0.57$) and is probably related to traffic emissions (Weckwerth 2001).

255 Mg and Ca are macronutrients (Glime 2006). The highest loadings of F3 were present in the peri-urban forests and
256 to some extent in the urban forest of Golovec (**Fig. 5**); these loadings may be a result of possible uptake of elements
257 from the substrate. Some elements (e.g. Ca, Mg, K) depend on uptake from the substrate, especially those mosses
258 growing in the form of turfs, cushions or cover (Zechmeister et al. 2003), which could also be the case for *H.*
259 *cupressiforme*. The lowest loadings of this factor were present in Rožnik. We assume that here low loadings of F3
260 are due to the replacement of abundant cations Ca^{2+} and Mg^{2+} with other cations (Bates 1992) that were highly
261 represented in F1 and F2.

262

263 **5 Conclusions**

264 The results from this survey confirmed our hypothesis that the concentrations of trace elements in moss collected
265 from urban forests were higher compared to moss collected from peri-urban and rural forests. The main sources of
266 the trace elements identified with factor analysis emissions were traffic, individual heating appliances and the
267 resuspension of contaminated soil. In particular, the Rožnik forest was the most exposed to pollution as determined
268 by factor analysis. Since the official monitoring of air quality in the Municipality of Ljubljana is performed only at

269 one location for measuring As, Cd, Ni and Pb in particulate matter, this study provides a better insight on the spatial
270 distribution of trace elements within the city and urban forests.

271

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278 **7 References**

- 279 Abu-Allaban M, Gillies JA, Gertler AW, Clayton R, Proffitt D (2003): Tailpipe, resuspended road dust, and brake-
280 wear emission factors from on-road vehicles. *Atmospheric Environment* 37, 5283-5293
- 281 Adamo P, Giordano S, Sforza A, Bargagli R (2011): Implementation of airborne trace element monitoring with
282 devitalised transplants of *Hypnum cupressiforme* Hedw.: assessment of temporal trends and element
283 contribution by vehicular traffic in Naples city. *Environ Pollut* 159, 1620-8
- 284 ARSO (2016): Wind Rose for the City of Ljubljana
- 285 Bates JW, Farmer AM (1990): An Experimental Study of Calcium Acquisition and its Effects on the Calcifuge
286 Moss *Pleurozium schreberi*. *Annals of Botany* 65, 87-96
- 287 Bates JW (1992): Mineral nutrient acquisition and retention by bryophytes. *Journal of Bryology* 17, 223-240
- 288 Bermejo-Orduna R, McBride JR, Shiraishi K, Elustondo D, Lasheras E, Santamaría JM (2014): Biomonitoring of
289 traffic-related nitrogen pollution using *Letharia vulpina* (L.) Hue in the Sierra Nevada, California. *Science*
290 *of The Total Environment* 490, 205-212
- 291 Biasioli M, Barberis R, Ajmone-Marsan F (2006): The influence of a large city on some soil properties and metals
292 content. *Science of The Total Environment* 356, 154-164
- 293 Brown DH, Bates JW (1990): Bryophytes and nutrient cycling. *Botanical Journal of the Linnean Society* 104, 129-
294 147
- 295 Cegnar T, Gjerek M, Koleša T, Logar M, Murovec M, Planinšek A, Paradiž B, Faganeli Pucer J, Rode B, Rus M,
296 Turšič J, Žabkar R 2015: *Kakovost zraka v Sloveniji v letu 2014*, Ministrstvo za okolje in prostor, Agencija
297 RS za okolje, Ljubljana (in Slovenian)
- 298 Dongarrà G, Manno E, Varrica D, Vultaggio M (2007): Mass levels, crustal component and trace elements in PM10
299 in Palermo, Italy. *Atmospheric Environment* 41, 7977-7986
- 300 Escobedo FJ, Nowak DJ (2009): Spatial heterogeneity and air pollution removal by an urban forest. *Landscape and*
301 *Urban Planning* 90, 102-110
- 302 ESRI (2015): *ArcGIS Desktop: Release 10.3.1*. In: Redlands (Hrsg.). CA: Environmental Systems Research Institute
- 303 Fantozzi F, Monaci F, Blanus T, Bargagli R (2013): Holm Oak (*Quercus ilex* L.) canopy as interceptor of airborne
304 trace elements and their accumulation in the litter and topsoil. *Environmental Pollution* 183, 89-95
- 305 Glime JM (2006): *Bryophyte Ecology*. Michigan Technological University and the International Association of
306 Bryologists, pp. 631
- 307 Harmens H, Buse A, Bükler P, Norris D, Mills G, Williams B, Reynolds B, Ashenden TW, Rühling Å, Steinnes E
308 (2004): Heavy Metal Concentrations in European Mosses: 2000/2001 Survey. *Journal of Atmospheric*
309 *Chemistry* 49, 425-436
- 310 Harmens H, Norris D, Mills G, and the participants of the moss survey 2013: Heavy metals and nitrogen in mosses:
311 spatial patterns in 2010/2011 and long-term temporal trends in Europe, ICP Vegetation Programme
312 Coordination Centre, Bangor UK
- 313 Hart BT (1982): Uptake of trace metals by sediments and suspended particulates: a review. *Hydrobiologia* 91, 299-
314 313

- 315 He ZL, Yang XE, Stoffella PJ (2005): Trace elements in agroecosystems and impacts on the environment. *Journal of*
316 *Trace Elements in Medicine and Biology* 19, 125-140
- 317 Herpin U, Berlekamp J, Markert B, Wolterbeek B, Grodzinska K, Siewers U, Lieth H, Weckert V (1996): The
318 distribution of heavy metals in a transect of the three states the Netherlands, Germany and Poland,
319 determined with the aid of moss monitoring. *Science of The Total Environment* 187, 185-198
- 320 Ho YB, Tai KM (1988): Elevated levels of lead and other metals in roadside soil and grass and their use to monitor
321 aerial metal depositions in Hong Kong. *Environmental Pollution* 49, 37-51
- 322 ICP Vegetation Coordination Centre 2010: Monitoring of atmospheric heavy metal and nitrogen deposition in
323 Europe using Bryophytes - Monitoring manual, ICP Vegetation Coordination Centre, Gwynedd
- 324 Johansson C, Norman M, Burman L (2009): Road traffic emission factors for heavy metals. *Atmospheric*
325 *Environment* 43, 4681-4688
- 326 Koleča T, Planinšek A 2013: *Opredelitev virov delcev PM 10 v Ljubljani*, Slovenian Environment Agency (in
327 Slovenian)
- 328 Krommer V, Zechmeister HG, Roder I, Scharf S, Hanus-Illnar A (2007): Monitoring atmospheric pollutants in the
329 biosphere reserve Wienerwald by a combined approach of biomonitoring methods and technical
330 measurements. *Chemosphere* 67, 1956-66
- 331 Lee CSL, Li X, Zhang G, Peng X, Zhang L (2005): Biomonitoring of trace metals in the atmosphere using moss
332 (*Hypnum plumaeforme*) in the Nanling Mountains and the Pearl River Delta, Southern China. *Atmospheric*
333 *Environment* 39, 397-407
- 334 Legret M, Pagotto C (2006): Heavy Metal Deposition and Soil Pollution Along Two Major Rural Highways.
335 *Environmental Technology* 27, 247-254
- 336 Malea P, Haritonidis S, Kevrekidis T (1994): Seasonal and local variations of metal concentrations in the seagrass
337 *Posidonia oceanica* (L.) Delile in the Antikyra Gulf, Greece. *Science of The Total Environment* 153, 225-
338 235
- 339 Markert B, Wappelhorst O, Weckert V, Herpin U, Siewers U, Friese K, Breulmann G (1999): The use of
340 bioindicators for monitoring the heavy-metal status of the environment. *Journal of Radioanalytical and*
341 *Nuclear Chemistry* 240, 425-429
- 342 Markert BA, Breure AM, Zechmeister HG (2003): Definitions, strategies and principles for
343 bioindication/biomonitoring of the environment. In: B.A. Markert AMB , Zechmeister HG (Editors), *Trace*
344 *Metals and other Contaminants in the Environment*. Elsevier, pp. 3-39
- 345 Meij R, te Winkel H (2007): The emissions of heavy metals and persistent organic pollutants from modern coal-
346 fired power stations. *Atmospheric Environment* 41, 9262-9272
- 347 Miguel Ed, Llamas JF, Chacón E, Berg T, Larssen S, Røyset O, Vadset M (1997): Origin and patterns of distribution
348 of trace elements in street dust: Unleaded petrol and urban lead. *Atmospheric Environment* 31, 2733-2740
- 349 Naszradi T, Badacsonyi A, Keresztényi I, Podar D, Csintalan Z, Tuba Z (2007): Comparison of two metal surveys
350 by moss *Tortula ruralis* in Budapest, Hungary. *Environmental Monitoring and Assessment* 134, 279-285
- 351 Nowak DJ, Hirabayashi S, Bodine A, Greenfield E (2014): Tree and forest effects on air quality and human health in
352 the United States. *Environmental Pollution* 193, 119-129
- 353 OECD (2012): *OECD Environmental Performance Reviews: Slovenia 2012*. In: OECD (Hrsg.), Paris

- 354 Ogrin M, Vintar Mally K, Planinšek A, Gregorič A, Drinovec L, Močnik G (2016): Nitrogen dioxide and black
355 carbon concentrations in Ljubljana. Faculty of Arts - Department of geography
- 356 Økland T, Økland RH, Steinnes E (1999): Element concentrations in the boreal forest moss *Hylocomium splendens*:
357 variation related to gradients in vegetation and local environmental factors. *Plant and Soil* 209, 71-83
- 358 Oliva SR, Espinosa AJF (2007): Monitoring of heavy metals in topsoils, atmospheric particles and plant leaves to
359 identify possible contamination sources. *Microchemical Journal* 86, 131-139
- 360 Pacyna JM, Pacyna EG (2001): An assessment of global and regional emissions of trace metals to the atmosphere
361 from anthropogenic sources worldwide. *Environmental Reviews* 9, 269-298
- 362 Pearson J, Wells DM, Seller KJ, Bennet A, Soares A, Woodland J, Ingrouille MJ (2000): Traffic exposure increases
363 natural $\delta^{15}\text{N}$ and heavy metal concentrations in mosses. *New Phytologist* 147, 317-326
- 364 Puckett KJ (1988): Bryophytes and Lichens as Monitors of Metal Deposition, 30. *Bibliotheca Lichenologica*, 231 pp
- 365 R Development Core Team (2016): R: A language and environment for statistical computing. R Foundation for
366 Statistical Computing, Vienna, Austria
- 367 Rainbow PS, Phillips DJH (1993): Cosmopolitan biomonitors of trace metals. *Marine Pollution Bulletin* 26, 593-601
- 368 Reimann C, de Caritat P (1998): Chemical Elements in the Environment - Factsheets for the geochemist and
369 environmental scientist. Springer-Verlag, 387 pp
- 370 Reimann C, Arnoldussen A, Boyd R, Finne TE, Nordgulen O, Volden T, Englmaier P (2006): The influence of a
371 city on element contents of a terrestrial moss (*Hylocomium splendens*). *Sci Total Environ* 369, 419-32
- 372 Rühling Å, Tyler G (1970): Sorption and Retention of Heavy Metals in the Woodland Moss *Hylocomium splendens*
373 (Hedw.) Br. et Sch. *Oikos* 21, 92-97
- 374 Schauer JJ, Lough GC, Shafer MM, Christensen WF, Arndt MF, DeMinter JT, Park J-S (2006): Characterization of
375 metals emitted from motor vehicles. Research report (Health Effects Institute), 1-76; discussion 77
- 376 Schilling JS, Lehman ME (2002): Bioindication of atmospheric heavy metal deposition in the Southeastern US
377 using the moss *Thuidium delicatulum*. *Atmospheric Environment* 36, 1611-1618
- 378 Senesil GS, Baldassarre G, Senesi N, Radina B (1999): Matter and Energy Fluxes in the Anthropocentric
379 Environment Trace element inputs into soils by anthropogenic activities and implications for human health.
380 *Chemosphere* 39, 343-377
- 381 Skudnik M, Jeran Z, Batič F, Simončič P, Lojen S, Kastelec D (2014): Influence of canopy drip on the indicative N,
382 S and $\delta^{15}\text{N}$ content in moss *Hypnum cupressiforme*. *Environmental Pollution* 190, 27-35
- 383 Skudnik M, Jeran Z, Batič F, Simončič P, Kastelec D (2015): Potential environmental factors that influence the
384 nitrogen concentration and $\delta^{15}\text{N}$ values in the moss *Hypnum cupressiforme* collected inside and outside
385 canopy drip lines. *Environmental Pollution* 198, 78-85
- 386 Slovenian Infrastructure Agency (2014): Traffic Loads in Slovenia
- 387 Stalnaker D, Turner J, Parekh D, Whittle B, Norton R (1996): Indoor Simulation of Tire Wear: Some Case Studies.
388 *Tire Science and Technology* 24, 94-118
- 389 Steinnes E, Rühling Å, Lippo H, Mäkinen A (1997): Reference materials for large-scale metal deposition surveys.
390 *Accred Qual Assur* 2, 243-249

- 391 SURS (2014): Statistical Yearbook 2013. Ljubljana: City of Ljubljana, Ljubljana (in Slovenian)
- 392 Szczepaniak K, Biziuk M (2003): Aspects of the biomonitoring studies using mosses and lichens as indicators of
393 metal pollution. *Environmental Research* 93, 221-230
- 394 Thorpe A, Harrison RM (2008): Sources and properties of non-exhaust particulate matter from road traffic: A
395 review. *Science of The Total Environment* 400, 270-282
- 396 Thurston GD, Ito K, Lall R (2011): A source apportionment of U.S. fine particulate matter air pollution.
397 *Atmospheric Environment* 45, 3924-3936
- 398 Tyler G (1990): Bryophytes and heavy metals: a literature review. *Botanical Journal of the Linnean Society* 104,
399 231-253
- 400 Tyrväinen L, Pauleit S, Seeland K, de Vries S (2005): Benefits and Uses of Urban Forests and Trees. In:
401 Konijnendijk C, Nilsson K, Randrup T, Schipperijn J (Editors), *Urban Forests and Trees: A Reference*
402 *Book*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 81-114
- 403 Urbančič M, Kobal M, Ferreira A, Simončič P (2010): Forest Soil in the Municipality of Ljubljana. *Gozdarski*
404 *Vestnik* 68, 292-300 (in Slovenian)
- 405 Vilhar U, Žlindra D, Ruper M, Simončič P (2014): Monitoring of ambient air quality in forests. *Vetrnica*, 109-119
406 (in Slovenian)
- 407 von Uexküll O, Skerfving S, Doyle R, Braungart M (2005): Antimony in brake pads-a carcinogenic component?
408 *Journal of Cleaner Production* 13, 19-31
- 409 Vuković G, Urosević MA, Goryainova Z, Pergal M, Skrivanj S, Samson R, Popović A (2015): Active moss
410 biomonitoring for extensive screening of urban air pollution: Magnetic and chemical analyses. *Sci Total*
411 *Environ* 521-522, 200-10
- 412 Weckwerth G (2001): Verification of traffic emitted aerosol components in the ambient air of Cologne (Germany).
413 *Atmospheric Environment* 35, 5525-5536
- 414 Wells JM, Brown DH (1996): Mineral nutrient recycling within shoots of the moss *Rhytidiadelphus squarrosus* in
415 relation to growth. *Journal of Bryology* 19, 1-17
- 416 Wik A, Dave G (2009): Occurrence and effects of tire wear particles in the environment – A critical review and an
417 initial risk assessment. *Environmental Pollution* 157, 1-11
- 418 Wolterbeek B (2002): Biomonitoring of trace element air pollution: principles, possibilities and perspectives.
419 *Environmental Pollution* 120, 11-21
- 420 Zechmeister HG, Hohenwallner D, Riss A, Hanus-Illnar A (2003): Variations in heavy metal concentrations in the
421 moss species *Abietinella abietina* (Hedw.) Fleisch. according to sampling time, within site variability and
422 increase in biomass. *The Science of the total environment* 301, 55-65
- 423 Zechmeister HG, Dullinger S, Hohenwallner D, Riss A, Hanus-Illnar A, Scharf S (2006): Pilot study on road traffic
424 emissions (PAHs, heavy metals) measured by using mosses in a tunnel experiment in Vienna, Austria.
425 *Environmental Science and Pollution Research* 13, 398-405
- 426
- 427

428 Table 1 Descriptive statistics of element concentrations (mg kg^{-1}) and comparison of median concentration in urban
 429 and peri-urban forests and the Slovenian 2010 survey (Harmens et al. 2013). Differences between concentrations in
 430 mosses collected in the peri-urban and urban forests were determined the Mann–Whitney U test (statistically
 431 significant differences are in bold letters)

ID	Minimum	Maximum	Standard deviation	Peri-urban median (n=22)	Urban median (n=22)	SI 2010 median (n=102)	Mann–Whitney U test	
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	W	p value
As	0.06	0.67	0.14	0.09	0.14	0.26	67	0.001
Ca	2162	6841	1065	4002	3744	3976	277	0.7865
Cd	0.08	0.84	0.12	0.15	0.16	0.27	256	0.870
Co	0.15	1.94	0.29	0.30	0.36	0.39	221	0.353
Cr	0.59	9.64	2.08	1.24	3.16	1.56	108	<0.001
Cu	2.44	9.43	1.30	4.97	5.94	5.42	134	0.004
Fe	222	3129	634	391	395	548	231	0.479
Hg	0.02	0.13	0.02	0.03	0.04	0.05	123	0.002
Mg	729	3278	424	1102	1043	1523	287	0.624
Mn	26	592	139	227	200	234	326	0.178
Mo	0.004	1.30	0.22	0.21	0.30	0.34	172	0.043
Ni	0.76	4.30	0.83	1.32	2.08	2.12	72	<0.001
Pb	1.54	13.12	2.88	2.67	5.96	5.01	91	<0.001
Sb	0.05	0.22	0.04	0.09	0.14	0.12	117	<0.001
Se	0.06	0.16	0.02	0.11	0.11	0.22	278	0.769
Sr	4.34	26.30	4.14	6.72	6.41	14.69	299	0.452
Ti	5.61	49.87	8.67	8.74	9.41	27.10	180	0.066
Tl	0.01	0.22	0.04	0.02	0.03	0.04	124	0.002
V	0.56	5.22	1.11	0.87	1.23	2.30	97	<0.001
Zn	10.89	45.98	8.11	24.95	25.47	29	258	0.905
N	8.50	17.50	2.07	10.78	12.85	1.29	120	0.002

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433

434 Table 2 A comparison of trace metal concentrations from the Municipality of Ljubljana with other similar
 435 investigations (Oslo, Belgrade, Naples, Wienerwald)

Element	Ljubljana (mg kg ⁻¹)	Oslo (mg kg ⁻¹)	Belgrade (mg kg ⁻¹)	Naples (mg kg ⁻¹)	Wienerwald (mg kg ⁻¹)
	median, n=44 <i>H. cupresiforme</i>	(Reimann et al. 2006) median, n=40 <i>H. splendens</i>	(Vuković et al. 2015) median, n=153 <i>H. cupresiforme</i>	(Adamo et al. 2011) mean, n=24 <i>H. cupresiforme</i>	(Krommer et al. 2007) mean, n=10 <i>S. purum</i> <i>H. cupressiforme</i> <i>A. abientina</i>
As	0.17	-	-	0.61	0.15
Ca	3777	2600	-	7580.25	-
Cd	0.16	0.17	0.44	0.29	0.24
Co	0.31	0.18	0.34	0.65	0.27
Cr	1.64	1.50	1.69	5.5	0.75
Cu	5.26	5.90	10.70	40.11	8
Fe	403	230	819	1280	503.07
Hg	0.03	0.04	-	0.07	0.04
Mg	1086	1170	-	992.87	-
Mn	214	542	-	50.8	-
Mo	0.23	0.20	-	1.30	0.21
Ni	1.50	1.60	3.06	2.61	1.23
Pb	3.68	3.46	6.18	22.77	4.53
Sb	0.10	0.13	-	-	0.15
Se	0.11	-	-	-	-
Sr	6.30	10.00	13.99	-	-
Ti	8.74	12.00	-	78.91	-
Tl	0.01	-	-	-	-
V	0.89	-	1.85	6.21	1.14
Zn	25.44	41	87	69.79	33.23

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437

438 Table 3 Factor loadings and variances for obtained factors. The elements with the highest loadings for each factor
 439 are presented in bold letters

Element	Factor 1	Factor 2	Factor 3	Communality
As	0.96	0.06	0.17	1.1
Ca	0.01	0.13	0.32	1.3
Cd	0.12	-0.01	0.03	1.1
Co	0.59	0.03	0.66	2.0
Cr	0.51	0.76	0.00	1.8
Cu	0.01	0.76	0.20	1.1
Fe	0.78	-0.01	0.39	1.5
Hg	0.71	0.27	0.63	2.3
Mg	0.19	-0.28	0.61	1.6
Mn	-0.08	-0.09	0.08	2.9
Mo	0.06	0.87	-0.10	1.0
Ni	0.30	0.81	-0.04	1.3
Pb	0.66	0.38	0.18	1.8
Sb	-0.03	0.68	0.30	1.4
Se	-0.18	0.65	-0.17	1.3
Sr	-0.26	-0.03	0.04	1.1
Ti	0.98	0.00	0.06	1.0
Tl	0.37	-0.09	0.21	1.7
V	0.97	0.11	0.220	1.1
Zn	-0.24	0.40	-0.06	1.7
N	0.43	0.60	0.29	2.3
Proportion of variance (%)	27	21	9	
Cumulative variance (%)	27	47	56	

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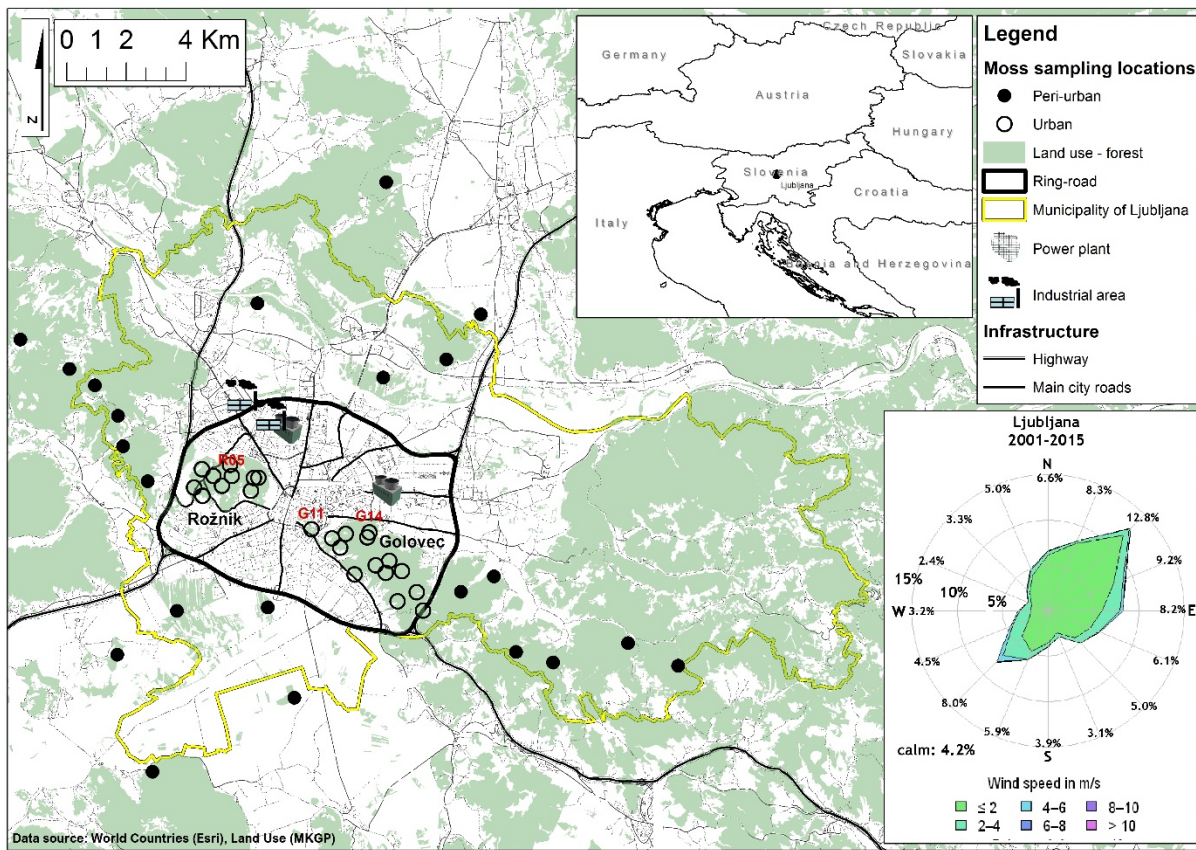


Fig. 1 Map of the study area with sampling points and a wind rose (ARSO 2016) for the Municipality of Ljubljana

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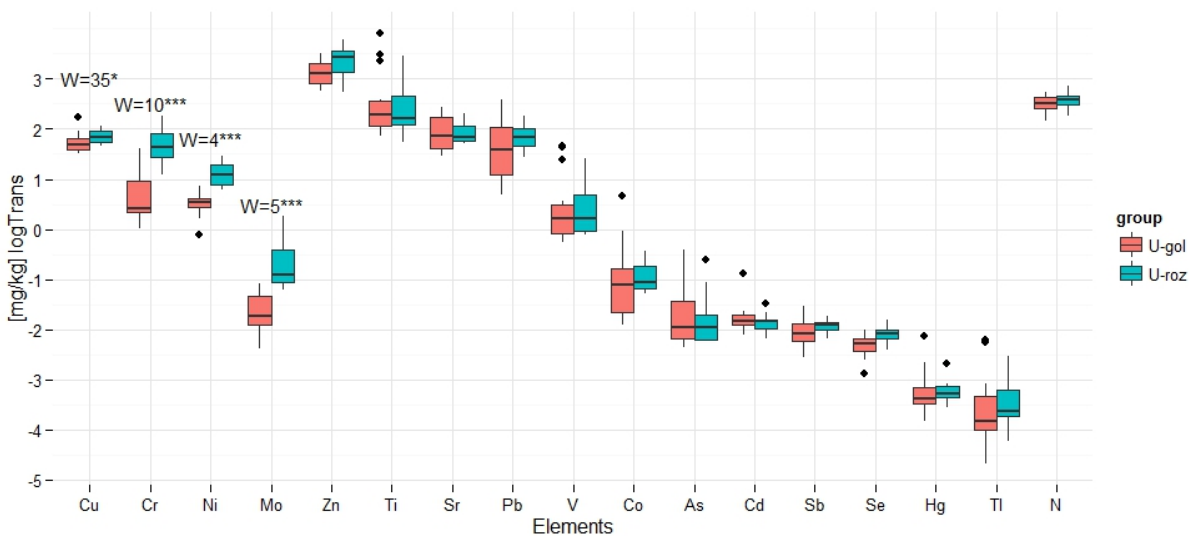
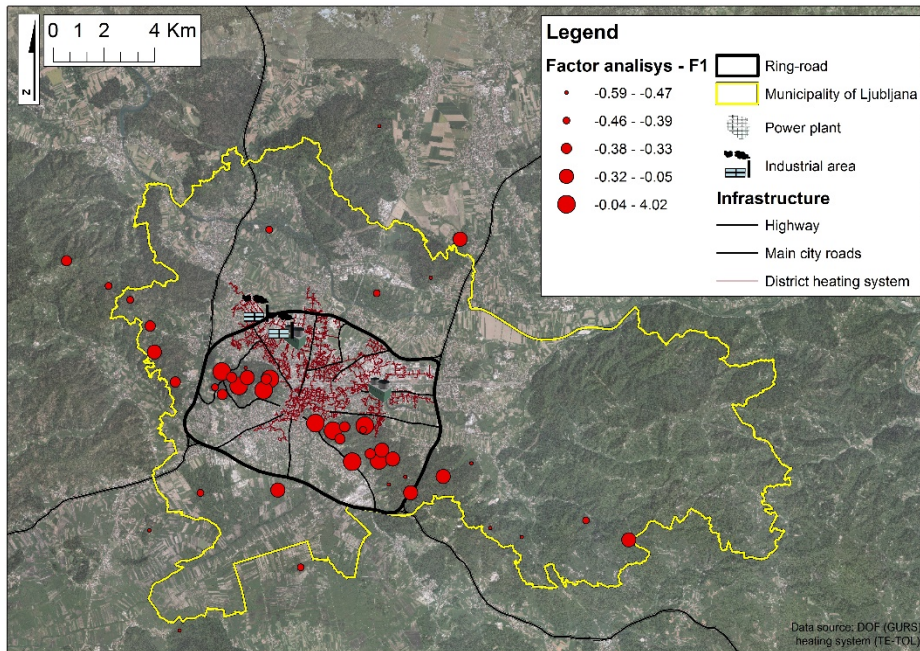


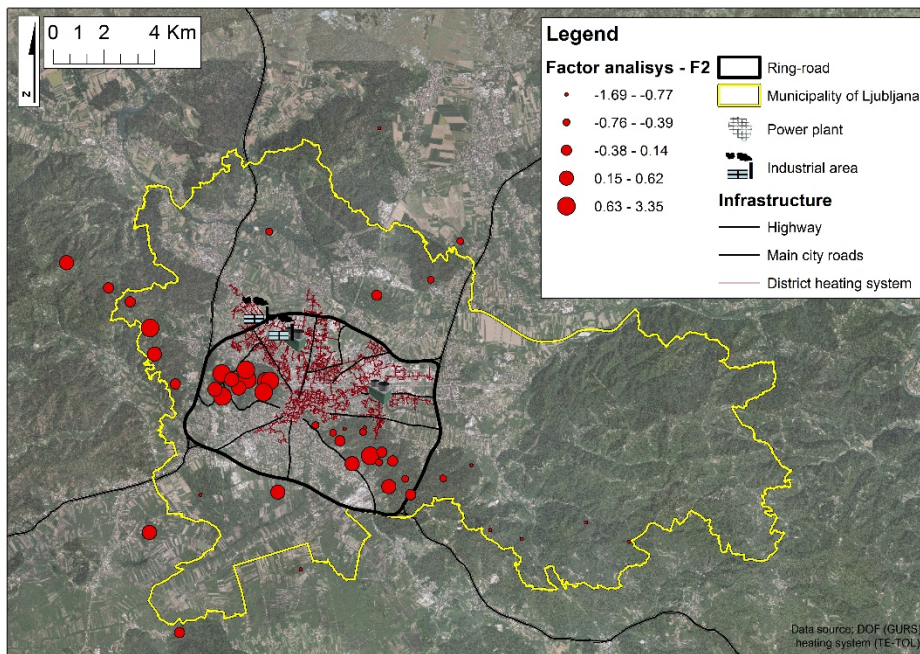
Fig. 2 Box plots showing the differences between element concentrations in mosses collected in urban forest Golovec (U-gol) and Rožnik (U-roz). (* 0.1, ** 0.01, *** 0.001)

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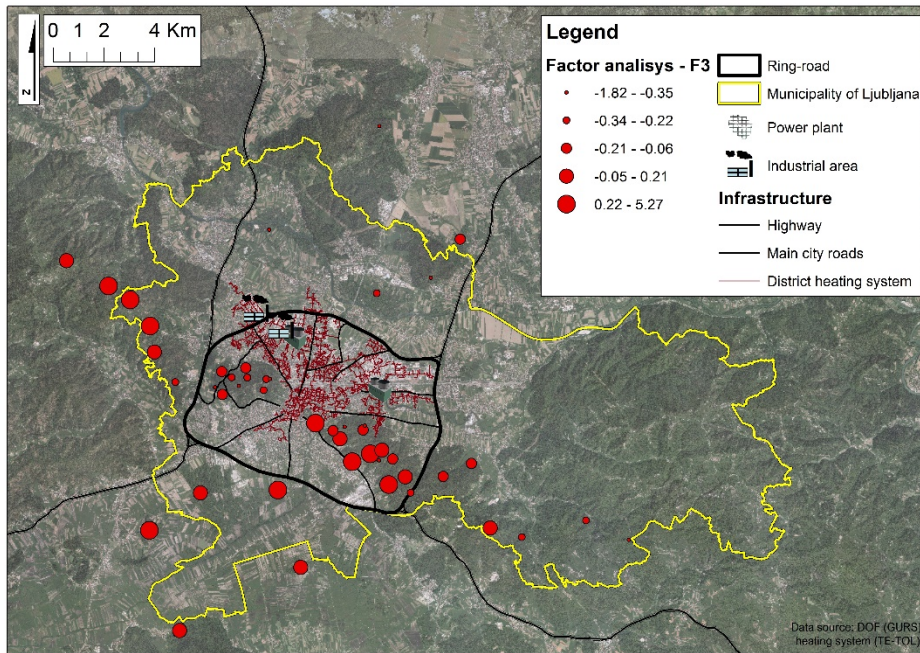
450 Fig. 3 Spatial distribution of Factor 1 (As, Fe, Hg, Pb, Ti, Tl, V)



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452 Fig. 4 Spatial distribution of Factor 2 (Cr, Cu, Mo, Ni, Sb, Se, Zn, N)

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455 Fig. 5 Spatial distribution of Factor 3 (Ca, Co, Mg)

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