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Trace elements in soil and mosses (*Dicranoweisia crispula* (Hredw.) Milde) by the road to Stelvio pass, Northern Italy Apls: A case of small scale pollution biomonitoring

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Abstract. The moss species *Dicranoweisia crispula* for bioindication were collected together with soil along road to the second highest paved mountain pass (Passo dello Stelvio) in the Alps. The trace elements such as P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Rb, Sr, Zr, Mo, Sn, Sb, Ba and Pb were determined using the hand-held XRF spectrometer DELTA CLASSIC (USA). From principal component analysis we determined four main phenomena, which were then evaluated in the context of localisation by the road. Several phenomena were described which could be ubiquitous in mountain regions, such as transboundary emission and traffic pollution related to touristic pressure.

Key words: bioindication, mosses, heavy metals, X-ray spectrometry, Italy, Stelvio paas

Introduction

The use of mosses as bioindicators of environmental pollution gained broadly attention, as a successful model for studying spatial pollution of environment from various sources, such as from minerals in geological parent material and inputs from wide range of possible antropogenic sources. The whole idea use bryophytes as bioindicators arose in seventies (Clymo 1963; Rühling and Tyler 1970) and immediately has spread across all Europe and the USA (Gordon *et al.* 1971; Briggs 1972; Grözinger 1974; Ratcliffe 1975; Groet 1976; Pilegaard *et al.* 1979; Gydesen and Rasmussen 1981; Sumerling 1984; Zoltai 1988; Burton 1990; Šoltés 1992; Zechmeister 1994). This simple model is based on the fact that, bryophytes are incapable of avoiding heavy metal uptake from deposition due great exchange capacity, absence of a cuticle and a simple organisation of the tissue (Tyler 1990; Sabovljević *et al.* 2005). From nutritional point of view, the bryophytes are independent of the soil (Ratcliffe 1975; Tyler 1990; Burton 1990). The cation exchange capacity is related to the concentration of peptic substances in moss tissue (Šoltés and Gregušková 2013). Mosses do not have real roots, epidermis or continuous cuticle layer, and they absorb water and dissolved elements directly across their surface (Salemaa *et al.* 2004). Therefore, moss species can be used as biological samplers for dry, wet, and occult deposition over a long period of time (Meyer *et al.* 2015; Gonzales and Pokrovsky 2014) and furthermore, they are rather resistant to toxic elements such as heavy metals (Berg and Steinnes 1997; Basile *et al.* 2013). Currently, surveys of the atmospheric deposition of trace element through mosses, using the moss biomonitoring technique are regularly performed in several European countries every 5 years since 1990 (Harmens *et al.* 2015; Schröder *et al.* 2016) And the most important guideline for the application of the moss technique is "Monitoring of atmospheric deposition of heavy metals, nitrogen and POPs in Europe using bryophytes" published by the UNECE ICP Vegetation 2014 (ICP Vegetation 2014; Frontasyeva *et al.* 2014). During the European moss survey 2010, moss was sampled at 4.499 sites in 25 countries and 14 elements were determined (Schröder *et al.* 2016). The results revealed that in general, mosses from countries in Northern Europe had the lowest HM concentrations, whereas countries in Eastern and South-eastern Europe had the highest (Harmens *et al.* 2013, 2015). Undoubted, the passive biomonitoring with terrestrial mosses, constitutes a useful tool for the study of air quality and the atmospheric deposition of heavy metals, even though there exist some limitations (Aboal *et al.* 2010; Fernández *et al.* 2015). Some problem such as number and design of sampling sites, (Wolterbeek and Bode 1995; Aboal *et al.* 2006; Fernández *et al.* 2005; Amblard-Gross *et al.* 2004; Pesch *et al.* 2008) timing of surveys and moss species are important factors influencing the concentration of elements in moss tissues.

The following aims are addressed in this paper: (1) determination of trace elements concentrations in collected samples in altitudinal transect, (2) identify main phenomena based on correlations of trace elements concentrations and (3) describe interaction of main phenomena in context of altitude and the occurrence of road.

Material and Methods

The samples of *Dicranoweisia crispula* (Hredw.) Milde, were collected together with soil along road to The Stelvio Pass (Passo dello Stelvio) from Bormio

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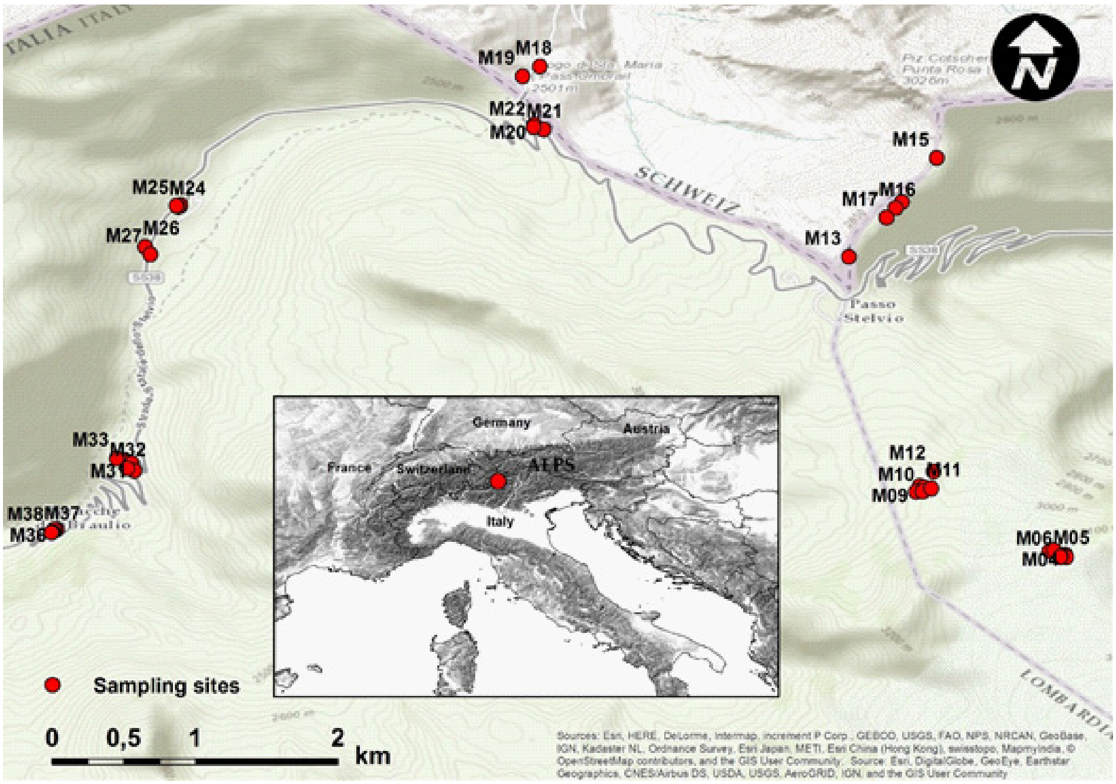

Fig. 1. Study area of the sampling sites where mosses *Dicranoweisia crispula* (Hredw.) Milde were collected together with soil (The Stelvio Pass, road from Bormio, northern Italy).

in northern Italy (Fig. 1) in altitudes from 2000 to 3171 m a.s.l., in 20th August 2015. The Stelvio Pass is the second highest paved mountain pass in the Alps. Localization data are described in Table 1. After the return to the laboratory, moss samples were gently cleaned from obvious soil particles, dried at 45° C for two days. During the drying process, the moisture of samples was analysed using laboratory scales. Each sample was carefully divided into two parts, the moss sample and sample of soil. Moss sample consisted only from green shoots and the soil sample was extracted from appropriates moss. After then moss and soil samples were homogenized in CryoMill (Retsch GmbH 2015) and analysed by X-ray fluorescence (Stephens and Calder 2004) using the hand-held XRF spectrometer DELTA CLASSIC (USA). The following elements were determined: P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Rb, Sr, Zr, Mo, Sn, Sb, Ba, Pb. Measured data was joined to single data matrix of locality attributes and log-transformed.

For statistical analysis, the Statistica 12 (StatSoft, USA) was used. Nonparametric analysis (Mann-Whitney U test) and principal component analysis (PCA) was used to extract the potential relationships between the variables. First four principal components were tested with ANOVA to reveal the effect of the presence or absence of roads.

Results

By means of Shapiro-Wilk normality test all data do not have normal distribution except K, Cl in mosses and K, Ti in soil. A Mann-Whitney U test was used to compare the medians of the trace elements in areas without road (n=16) and areas by the road (n=22). Significant differences ($p<0.05$) between the median values of variables for two groups of data were in altitude, distance from road, concentration of K, Ti, Mn, Zn, Mo, Pb (p=0,003) in mosses and concentration of Mn, Ni, Mo, Hg in soil (Fig. 2).

Through nonparametric statistic for comparing two dependent samples we analysed concentrations of trace elements in mosses with concentrations in soil samples. Significant relations we observed in concentration of P in soil and Ni, Sn, As in mosses. Then concentration of Cl in soil with Ni, Zn in mosses, concentration of Pb in soil with Pb, Sn, Zn in mosses, concentration of S in soil with Sn in mosses, and concentration of Sn in soil was related with Cl, Ni, Zn, Sn, Pb in mosses.

From principal component analysis we determined four main phenomena. First of them, phenomenon PC1 (Table 2) represents the interaction of elements from background (soil) with elements stored in mosses in the context of altitude and localisation by the road (Fig. 3). Generally speaking, the level of trace elements concentration such as K Ti, Cr, Fe, Cu, Zn, As, Zr, Sn, Ba, and Pb increases with altitude in soil more than in mosses, except Fe and Cu.

Second phenomenon PC2 (Table 2, Fig. 4) describes situation of trace elements concentration in relation to road distance. Close to road are significant concentrations of Mn, As, Rb, Mo in mosses and P, K, As, Rb, Sn in soil. But further away from road are significant concentrations of S, Cl, Ca, Zn, Sr in mosses and Ca, Sr, Hg in soil.

Third phenomenon PC3 is a phenomenon of traffic pollution in lower altitude and by the roadside (Fig. 5) where trace elements such as Ti, Cr, Fe Zn, Sb together with Pb are deposited in soil more than in mosses.

Sample	Coordinates N	Coordinates E	Altitude [m a.s.l.]	Road distance [m]
M01	46 30.773	10 27.968	3170	2176
M02	46 30.779	10 27.986	3169	2178
M03	46 30.760	10 28.028	3171	2236
M04	46 30.753	10 28.033	3169	2249
M05	46 30.755	10 28.013	3169	2232
M06	46 30.753	10 28.009	3169	2234
M07	46 31.019	10 27.479	3023	1485
M08	46 31.014	10 27.496	3025	1502
M09	46 30.998	10 27.465	3022	1519
M10	46 30.998	10 27.488	3024	1527
M11	46 31.009	10 27.522	3024	1520
M12	46 31.073	10 27.531	3010	1413
M13	46 31.879	10 27.211	2830	240
M14	46 32.086	10 27.411	2849	712
M15	46 32.250	10 27.542	2873	1040
M16	46 32.062	10 27.387	2845	640
M17	46 32.027	10 27.352	2837	565
M18	46 32.593	10 26.043	2506	1
M19	46 32.557	10 25.978	2507	1
M20	46 32.376	10 26.021	2496	1
M21	46 32.357	10 26.057	2499	2
M22	46 32.365	10 26.019	2495	8
M23	46 32.073	10 24.687	2349	5
M24	46 32.067	10 24.677	2349	4
M25	46 32.071	10 24.671	2352	15
M26	46 31.917	10 24.552	2329	15
M27	46 31.888	10 24.571	2326	10
M28	46 31.115	10 24.474	2180	30
M29	46 31.108	10 24.489	2181	42
M30	46 31.104	10 24.503	2182	32
M31	46 31.075	10 24.512	2183	40
M32	46 31.085	10 24.485	2179	19
M33	46 31.123	10 24.445	2179	5
M34	46 30.856	10 24.217	2004	4
M35	46 30.856	10 24.211	2004	10
M36	46 30.856	10 24.209	2001	13
M37	46 30.846	10 24.201	2001	6
M38	46 30.844	10 24.199	2000	3

Table 1. Samples and characteristics of data collection.

Fourth phenomenon PC4 describes effect probably caused by cross-border transmission of trace elements. Concentration of lead is significantly higher in higher altitudes and further away from road (Fig. 6) mainly in soil. Conversely, concentrations of S, Ni, Cu, Mo in mosses and S in soil are higher in lower altitude.

Discussion

According the ICP-Vegetation manual (ICP Vegetation 2014), extensive surveys and sampling should be carried at a distance of least 300 m from main roads or urban and industrial areas, and at least 100 m from smaller roads and isolated houses (Frontasyeva *et al.* 2014). However, small scale biomonitoring based on the use of terrestrial mosses must be possible to verify differences in trace elements concentrations from areas

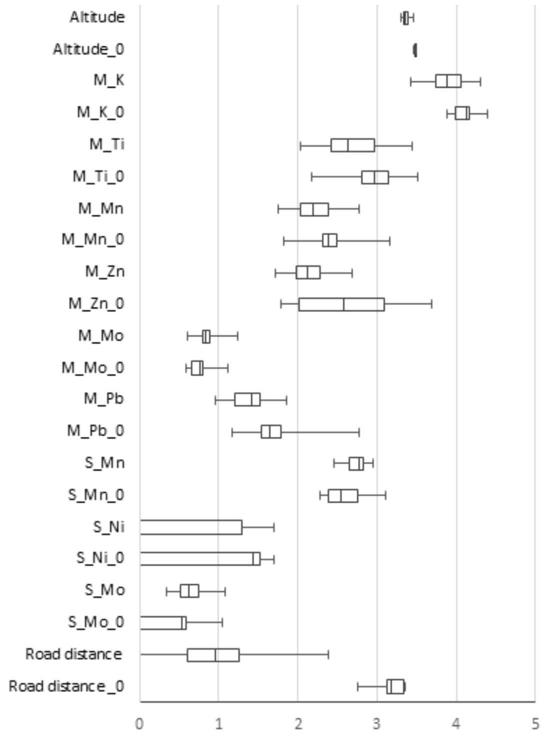

Fig. 2. Box plots of trace elements with significant differences (Mann-Whitney U test, $p<0.05$) between the median values (log transformed) of variables [Code: type of sample_ trace element_type of area; S - soil samples; M - mosses samples; areas without road - 0, (ex. Altitude_0)].

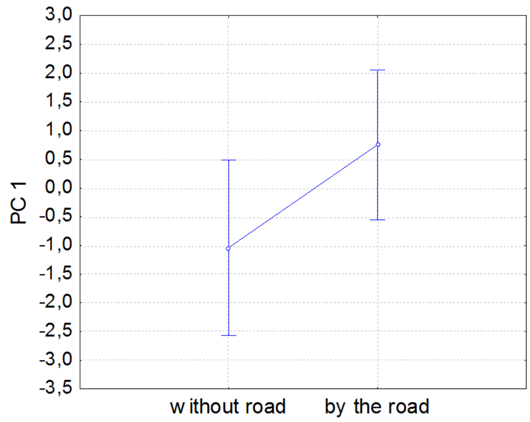

Fig. 3. The interaction of elements from soil with elements stored in mosses in the context of localisation by the road. ANOVA (from factor coordinates of cases based on correlations) Wilks lambda = 0.22436, $F(4, 33) = 28.522$, $p = 0.00000$.

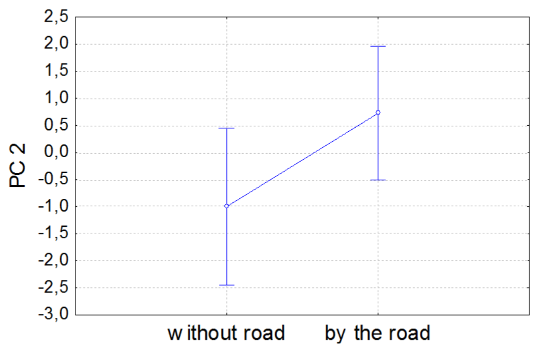

Fig. 4. The relation of trace elements stored in soil and mosses in the context of localisation by the road which are probably affected by acidification in higher altitude. ANOVA (from factor coordinates of cases based on correlations) Wilks lambda = 0.22436, $F(4, 33) = 28.522$, $p = 0.00000$.

Variables	PC1	PC2	PC3	PC4
Altitude [m a.s.l.]	-0.478	-0.297	-0.421	0.525
Road distance [m]	-0.334	-0.569	-0.426	0.483
M_S	-0.369	-0.457	0.038	-0.449
M_Cl	-0.347	-0.628	-0.037	-0.378
M_K	-0.658	0.204	-0.365	0.050
M_Ca	-0.035	-0.670	-0.451	-0.038
M_Ti	-0.772	-0.187	-0.295	-0.151
M_Cr	-0.552	0.342	-0.495	-0.266
M_Mn	-0.271	0.437	-0.129	0.145
M_Fe	-0.818	0.292	-0.328	-0.087
M_Ni	-0.251	-0.165	-0.291	-0.482
M_Cu	-0.643	0.090	-0.113	-0.548
M_Zn	-0.554	-0.429	0.353	0.367
M_As	-0.426	0.685	-0.290	0.288
M_Rb	-0.207	0.606	-0.358	0.306
M_Sr	-0.366	-0.547	-0.177	-0.084
M_Zr	-0.775	0.338	-0.387	-0.096
M_Mo	-0.033	0.555	-0.218	-0.430
M_Sn	-0.574	0.377	-0.033	0.051
M_Sb	0.069	-0.197	0.142	0.349
M_Ba	-0.708	-0.317	-0.231	-0.006
M_Pb	-0.439	-0.205	0.087	0.269
S_P	-0.411	0.444	0.071	0.011
S_S	-0.057	-0.197	0.495	-0.441
S_Cl	-0.207	-0.204	-0.022	0.254
S_K	-0.376	0.764	-0.101	0.251
S_Ca	0.088	-0.779	-0.498	0.075
S_Ti	-0.786	-0.070	0.418	-0.247
S_Cr	-0.710	-0.137	0.540	-0.039
S_Mn	-0.322	0.154	0.762	0.081
S_Fe	-0.607	0.299	0.461	-0.243
S_Ni	-0.714	-0.284	-0.033	0.041
S_Cu	-0.544	-0.180	0.165	0.132
S_Zn	-0.436	-0.345	0.568	0.363
S_As	-0.040	0.834	0.125	0.142
S_Se	0.085	-0.210	-0.238	0.144
S_Rb	-0.002	0.828	-0.139	0.316
S_Sr	-0.154	-0.649	-0.365	0.019
S_Zr	-0.663	0.226	0.219	-0.116
S_Mo	0.286	0.397	0.257	-0.179
S_Sn	-0.272	0.643	-0.297	0.158
S_Sb	-0.300	-0.222	0.438	0.368
S_Ba	-0.640	-0.181	0.220	-0.116
S_Hg	0.097	-0.418	-0.543	0.121
S_Pb	-0.346	-0.342	0.524	0.452
Eigenvalue	9.660	8.712	5.256	3.406
% Total variance	21.466	19.360	11.681	7.568
Cum. Eigenvalue	9.660	18.372	23.628	27.034
Cumulative %	21.466	40.826	52.506	60.075

Table 2. Principal component (PC) vectors (loadings), which indicate mutual interaction of trace elements in soil (S) and mosses (M). (Factor coordinates of the variables, based on correlations).

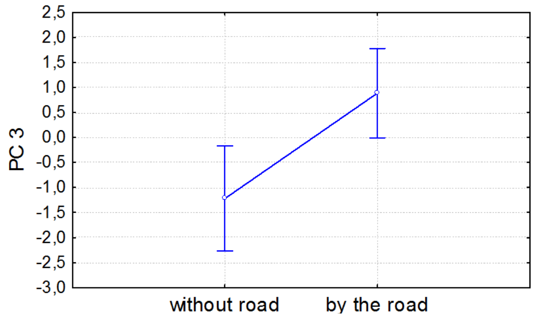

Fig. 5. The phenomenon of traffic pollution in lower altitude in the context of localisation by the road. ANOVA (from factor coordinates of cases based on correlations) Wilks lambda = 0.22436, F (4, 33) = 28.522, p = 0.00000.

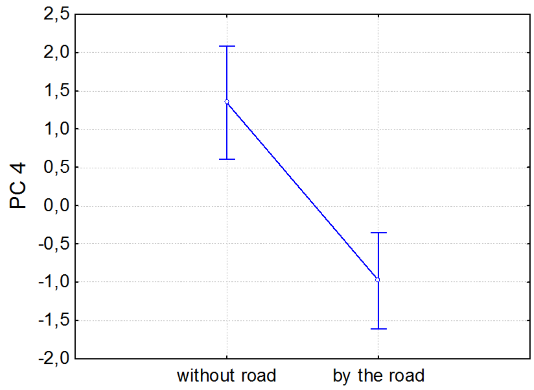

Fig. 6. The phenomenon of cross-border transmission of lead in higher altitudes in the context of localisation by the road. ANOVA (from factor coordinates of cases based on correlations) Wilks lambda = 0.22436, F (4, 33) = 28.522, p = 0.00000.

considered to be clean, and areas the surroundings of the focal point of the pollution (Fernández *et al.* 2007). Therefore, our data were localised in the places without road and in the places by the road. Samples from the places without road were at higher altitude due to elimination possible effect of traffic pollution. We selected only one moss species for this purpose. *Dicranoweisia crispula* (Mountain Pincushion) is an extremely variable species (Flowers 1956). It is a plant of montane rocks widespread mainly at higher elevations (Schofield 2007). It is bipolar species (Putzke and Pereira 2001) and one of the most dominant species of snow beds (Elvebakk 1984). *Dicranoweisia crispula* grows directly on rock or on damp gravel (Sean 2012), but also on burnt logs or downed wood in mid to advanced stages of decay (Clark 2012). We consider that this species could by a good species for indication of pollution in mountain regions. The process of trace elements accumulation to mosses depends on the nature of the contaminant and form in which it is emitted, then on physicochemical and biological processes and finally, all of these variables will depend on environmental factors such as precipitation, pH, salinity, temperature (Boquete *et al.* 2011). We suppose that the spatial variability of the trace elements concentration is very high due to heterogeneity of mountain condition and distances from anthropogenic sources of emission. Our results and detected higher concentrations of Pb in soil and mosses at higher altitude are consistent with the study of Zechmeister (1995) who analysed correlation between altitude and heavy metals deposition in the Alps. Our observed phenomena pointed to two sources of Pb in the environment. One is from transboundary emission and second is related to vehicular traffic. For possible sources of transboundary emission in Europe see e.g. Rühling (1994). Zechmeister *et al.* (2005) noted that the elements such as Sb, Mo, Cr Cu, As, Zn, and V can be regarded as indicators for traffic, Cd seems to be connected to other sources and elements like Ca or Al are predominantly an indicator for soil dust, as Ca is a frequent component of grit used on many roads during winter. The phenomenon of traffic pollution was significant in soil samples and closely connected except Pb also with other elements such as Ti, Cr, Mn, Fe, Zn, Sb. The phenomenon most likely related to touristic pressure as mentioned in study of Nascimbene *et al.* (2014). Schröder *et al.* (2010) found moderate to high correlations between cadmium and lead concentrations in mosses and modelled atmospheric deposition of these metals. In case of wet deposition some authors analysed trace element in Alpine snow. In general, mean trace elements concentrations found on the Eastern Alps (Gabrielli *et al.* 2006) were higher than those snow samples taken in the French western Alps (Veysseyre *et al.* 2001). In lower parts of Alps were concentrations found in the two studies are in good agreement. In medium altitudes concentrations found by Veysseyre *et al.* (2001) can be comparable to values determined in remote Greenland snow samples taken by Barbante *et al.* (2003). Dry deposition during dry weeks affecting the concentration in moss much more than the wet deposition (Berg and Steinnes 1997, Couto *et al.* 2004). If we have accepted some critical comments Reimann *et al.* (2001) and Aboal *et al.* (2010) about short-time concentration variability and Brown and Brumelis (1996) laboratory experiments, that the frequently presented interpretation that mosses progressively accumulate elements throughout their life is false. We have to accepting that mosses might then rather reflect the last rainfall instead of several years-accumulated depositions (Reimann *et al.* 1999). Which is in agreement with the results of Boquete *et al.* (2011) that the concentrations of the elements in the moss varied greatly within very short periods, and that the error associated with the temporal variability in the results was high. Notwithstanding the abundance of trace elements concentration, our data provide valuable information for improving the management and protection of mountain ecosystems in popular tourist areas.

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