Lichen *Candelaria concolor* (Dicks.) Stein as bioindicator of air pollution in Podgorica (Montenegro)

Nataša Sekulić
Faculty of Natural Sciences and Mathematics, University of Montenegro, Montenegro
Slađana Krivokapić
Faculty of Natural Sciences and Mathematics, University of Montenegro, Montenegro
Branka Knežević
Capital city of Podgorica, Secretariat of Spatial Planning and Environment, Montenegro
Snežana Dragićević
Natural History Museum of Montenegro, Montenegro
Dijana Đurović
Institute for Public Health, Montenegro

Abstract

Lichen *Candelaria concolor* (Dicks.) Stein, which was collected on the barks of deciduous trees, has been used as bioindicator of atmospheric pollution in Podgorica. Method of atomic absorption spectrophotometry was applied on selected lichen species in order to determine heavy metals content: lead (Pb), iron (Fe), cupper (Cu), zinc (Zn) and chromium (Cr). Values of lead (Pb) content ranged from 0.017 to 0.099 mg/kg, of iron (Fe) from 12.959 to 25.780 mg/kg, of cupper (Cu) from 0.168 to 3.100 mg/kg, of zinc (Zn) from 0.239 to 1.151 mg/kg and of chromium (Cr) from 0.023 to 0.078 mg/kg of dry matter. Results of this research indicated the highest concentration of heavy metals on localities close to city centre, with car traffic as dominant source of air pollution.

Keywords: *Candelaria concolor,* haevy metals, bioindicator, Podgorica.

Introduction

Podgorica is the capital city of Montenegro. It's located on attitude of 44,5 meters ASL (Šehić & Šehić, 2005). On climatic conditions in Podgorica big influence is from Adriatic Sea. That influence is coming upstream river Bojana and across Skadarsko lake. By influence of Dinars mountains, Mediterranean climatic is a bit changed and Podgorica now has modified Mediterranean climatic, which is characteristic in hot and dry summers and mild and rainy winters. Thanks to thermal influence of Adriatic sea, Podgorica has high winter temperatures. Average temperature in Podgorica is 16.4°C, maximal was 40.7°C and minimal was 4.6°C. Summers in Podgorica are very hot and long. It's about 135 days in summer when the temperature is above the 25°C mark. Average annual precipitation is 1544mm, and relative humidity is 59.6%. Lot of sunny days and heat, relatively small number of rainy days, adequate humidity and good airiness are main characteristics of climate in Podgorica, what places this city in areas with comfortable climatic (http://www.podgorica.me/klima).

Based on presence or absence of certain plants and other species of vegetative life in ecosystem we can bring important conclusions about health of environment (Šaulović & Mujić, 2009). In biomonitoring of air quality mostly used indicators are lichen, because of their big sensitivity on air pollution (Stamenković, 2013). Lichen are recognized as sensitive indicators of state of environment for longer periods of time (Nayaka *et al.,* 2003), as well as indicators of metals air pollution (Rizzo *et al.*, 2001). First study on thematic epiphytic lichens as bioindicatros was published in mid XIX century (Nylander, 1866). Lichens are most examined bioindicators of air quality (Ferry *et al.*, 1973). They are defined as "permanent control system" for evaluation of air quality (Nimis *et al.*, 1989).

Lichens are organisms which are capable to accumulate lot of different substances in their body (talus). Lichens are great indicators of industry air pollution, because they don't have capability of excretion of accumulated substances; and when the amount of harmful substances crosses their tolerance, lichens are dying (Vasilev, 2012). Lichens integrally maintain their influence on environment and they are one of the best tools to estimate contamination with heavy metals (Van Doven & Ter Braak, 1999; Shukla & Upreti, 2007, 2008; Bajpai *et al.*, 2004, 2010; Rani *et al.*, 2011). Some authors during their examinations had interesting results, like Garty (1993) allegate that different metals as lead (Pb), zinc (Zn), copper (Cu), mercury (Hg) and chrome (Cr), considered as toxic for many other living organisms, can be accumulated at the same time into one lichen, which looks intact in many cases.

Because of their physical, chemical and biological characteristics lichens are very sensitive on air changes, especially on an increase of sulfur dioxide (SO_2). Some of characteristics because of which epiphytic lichens are good air pollution indicators are:

- absence of surface protection structure, like a cuticle, and because of that pollutants can easily penetrate into inside of talus and impact negative on their cells.
- lichens almost all substances adopt from the air and precipitation, that means, by the good substances they adopt the harmful ones too
- lichens don't have ability to throw away part of body, so they can get free of harmful substances which are accumulated in those body parts (Vasilev, 2012).

In Podgorica, and Montenegro global, so far weren't done any researches of this kind, in spite of globally recognized part of lichens as bioindicators . So we can say that this research is one of pioneer steps of its kind in that way, and a basis for further researches.

Materials and metods

Sampling lichen *Candelaria concolor* (Dicks.) Stein, was made on 6 locations in Podgorica area, in the end of June 2016. Names on locations and GPS coordinates are shown it table 1. During choosing locations on which we made sampling, we take care that locations are out of areas with pollution sources (traffic, industry…)

Lichens are carefully detached from the tree bark with knife, and then put into paper bags with field data. During selection of trees for sampling, age of trees was not considered. After sampling, material was cleaned from impurities and then dried on room temperature. Material was identified (determined) and then herbalized in laboratory of Museum of Natural History of Montenegro. Determination of species was done by Wirth (1995).

Preparing samples for analysis: Dried material at first chopped, then with use of analytic beam scale, we take 0.5gram of sample and put into a 250ml glass. Next we add 15ml HNO3 and 5ml H2O2. Next step is warming up inside of sandy bath up to 100-150°C for about 1 hour to evaporate hydrogen peroxide H2O2, and then more intense on about 200-250°C until the moment that we get dry sample which we dissolve in distilled water and then percolate. Filtrate is moved to standard 50 ml utensil, and the rest is filled up with distilled water.

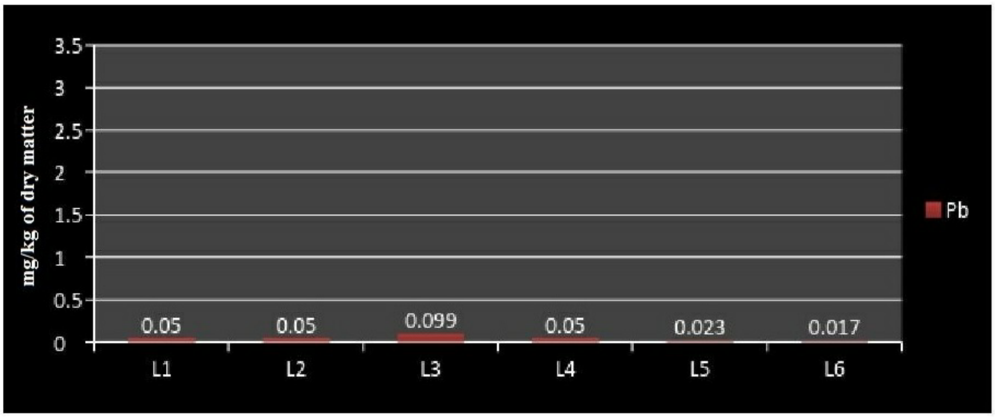
Determination of amount of heavy metals: lead (Pb), iron (Fe), Zinc (Zn), copper (Cu), and chrome (Cr) in lichen *Candelaria concolor* (Dicks.) Stein was did by atomic absorption spectrophotometry in Institute for public health of Montenegro.

Table 1: Locations and GPS coordinates of locations.

Sample	Location	GPS
L1	Mareza	N 42°28´17.75´´ E 19°11´10.87´´
L2	Tološka šuma	N 42°26´44.27´´ E 19°14´23.63´´
L3	Kraljev park	N 42°26´18.90´´ E 19°15´44.28´´
L4	Avenue at roundabout next to Delta City	N 42°26´19.04´´ E 19°14´16.52´´
L5	KAP - Kombinat aliminuma Podgorica	N 42°23´22.38´´ E 19°14´17.00´´
L6	Park šuma Gorica	N 42°26´46.04´´ E 19°15´55.94´´

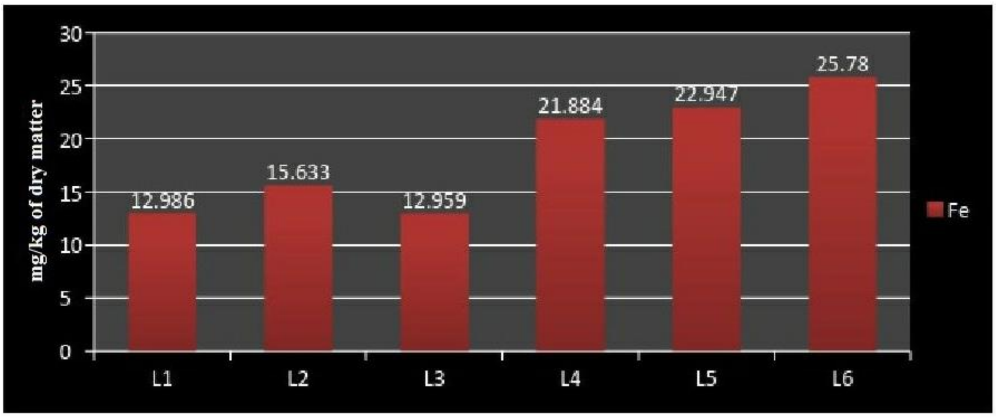
Results

Values of amount of lead (Pb) were in range from 0.017 to 0.099 mg/kg of dry matter (Graph 1).



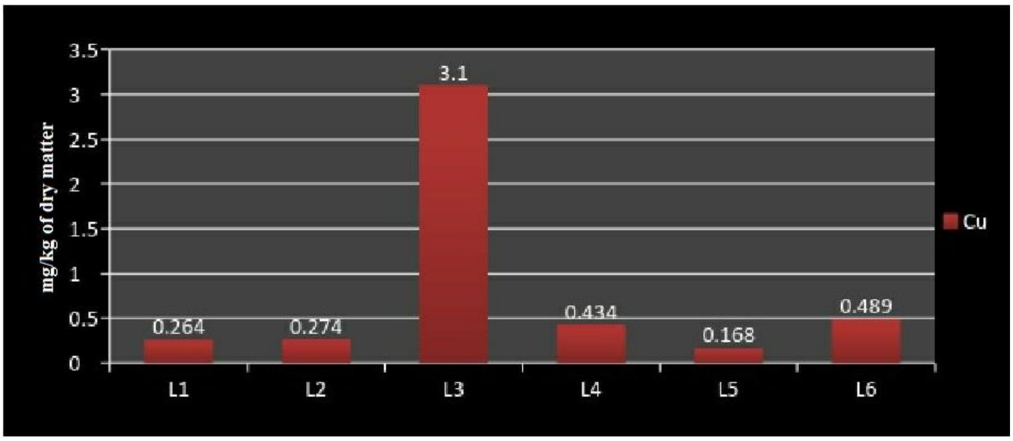
Graph 1: Lead levels in species *Candelaria concolor* (Dicks.) Stein

Values of iron (Fe) were in range of 12.959 to 25.780 mg/kg of dry matter (Graph 2).



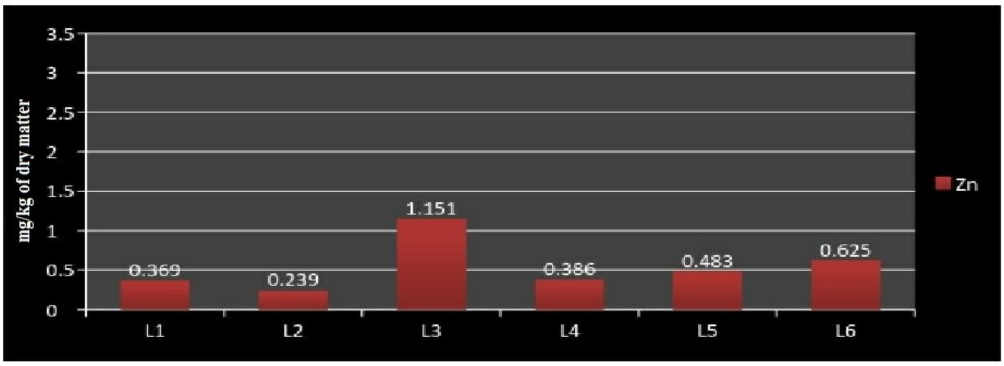
Graph 2: Iron levels in species *Candelaria concolor* (Dicks.) Stein

Values of cooper (Cu) were in range from 0.168 to 3.100mg/kg of dry matter (Graph 3).



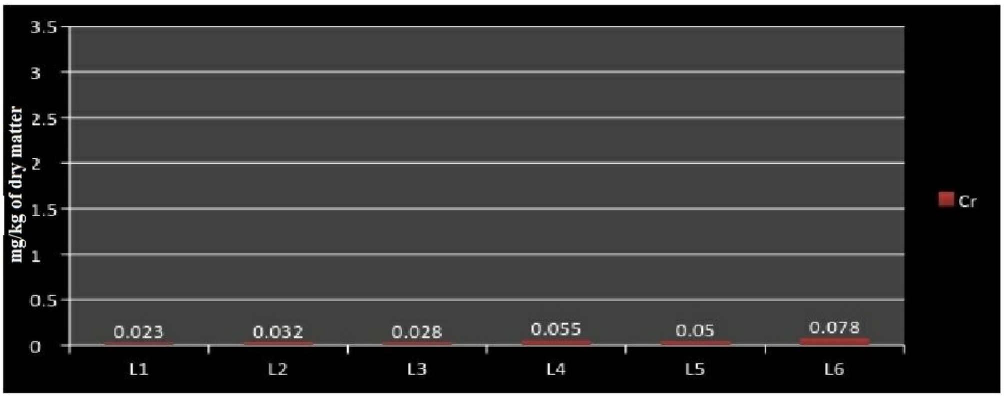
Graph 3: Cooper levels in species *Candelaria concolor* (Dicks.) Stein

Values of zinc (Zn) are in range from 0.239 to 1.151 mg/kg of dry matter (Graph 4).



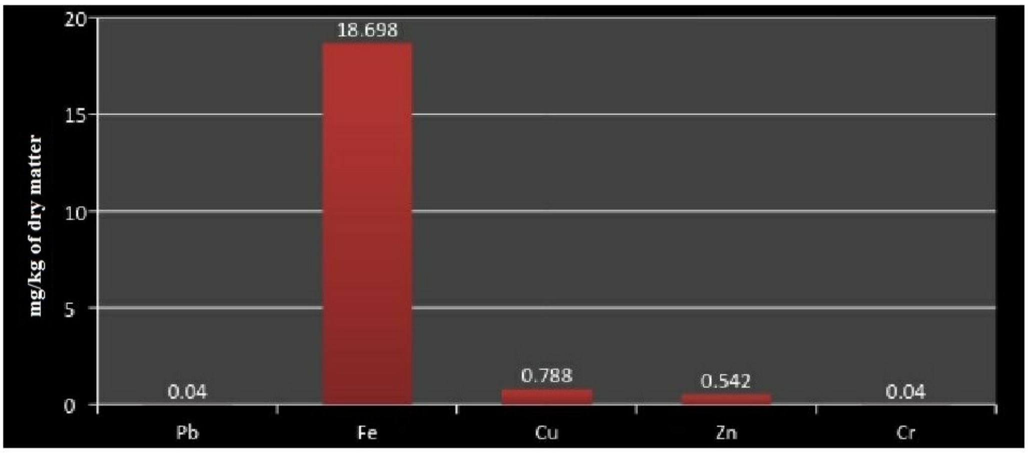
Graph 4: Zinc levels in species *Candelaria concolor* (Dicks.) Stein

Values of chrome (Cr) were in range from 0.023 to 0.078 mg/kg of dry matter. (Graph 5).



Graph 5: Chrome levels in species *Candelaria concolor* (Dicks.) Stein.

Comparing average values of the content of heavy metals: lead (Pb), iron (Fe), Zinc (Zn), copper (Cu), and chrome (Cr) we can see that iron has biggest average value 18.698mg/kg of dry matter, and on the other side are lead and chrome with lowest average values of 0.4mg/kg of dry matter (Graph 6).



Graph 6: Comparison of average values of researched metals in species *Candelaria concolor* (Dicks.) Stein

Discussion

Maximum amount of lead was measured on location 3 (Kraljev park), and minimal amount on location 6 (Park šuma Gorica). Location 3 is at the same time closest place to the city center, what implies direct exposure to effects from high frequency of traffic. This kind of correlation between lead concentration and close range to city center was made also in town Obrenovac using moss as bioindicator (Savovljević *et al.*, 2009). Lead is element which is used a lot in modern industry. Its added as an additive to oil derivates and gasoline. With burning of them lead is released into atmosphere. Second biggest pollution source with this heavy metal are lead car batteries (Treub, 1996). In Montenegro from 1-st January 2011 is forbidden to use lead gasoline in traffic, as well like gasoline which contains higher values of sulfur, than prescribed in EU standards (EURO5). In Italy, different studies of biomonitnoring which are preformed with lichens, showed up that lead is still widespread, in spite of introduction lead-free gasoline (Cardarelli *et al.*, 1993; Deruelle, 1996; Monaci *et al.*, 1997). This shows up that high concentrations of this metal are accumulated in lichens talus.

Maximum amount of iron was measured on location 6 (Park šuma Gorica), and minimal amount on location 3 (Kraljev park).Iron shows values which are significantly higher than all others elements, on all locations of sampling, what matches with researches of El Rhzaoui *et al.,* (2015), what explains specific affinity of lichens to the iron. Close by location 6 was located oldest gas station in city. Gas station was moved to another location in October 2011. Considering age of trees in this location (Park šuma Gorica), and a fact that lichens live very long, it's possible to assume that certain amount of this metal is coming from that source (gas station). High concentration of iron were found also on locations 4 (Avenue at roundabout next to Delta City) and 5 (KAP). Those locations are under influence of industry (Tobacco factory Podgorica and Aluminum compound Podgorica). Iron in the air can show as consequence of industry activity, by burning coal, traffic, Earth activities (Ötvös *et al.*, 2003).

Maximal amount of cooper was measured on location 3 (Kraljev park) and minimal amount was measured at location 5 (KAP). Cooper in nature we can find as product of anthropogenic activity, trough processes of mining, melting ore, industrial production, agriculture and technologies and waste disposal.

Maximal concentration of zinc was measured on location 3 (Kraljev park) and minimal concentration was measured on location 2 (Tološka šuma). Main source of zinc pollution are: mines, using waste sludge in agriculture, pesticides and fertilizers.

Cooper and zinc are metals with concentrations who are in correlation in urban areas (Pascale & Pasquini, 2010). Po Pascale & Pasquini (2010), construction activities like processing, grinding and welding of metals, can be sources of cooper and zinc in urban and agricultural areas. Second possibility is that source of this metals is industry, and that they are brought by air streams. Results of this research are in correlation with research of Pascale & Pasquini (2010), since a year before sampling the location (Kraljev park) was reconstructed (inter alia it was made a new metal fence).

Maximal concentration of chromium was measured on location 6 (Park šuma Gorica) and minimal concentration was measured on location 1 (Mareza). In explanation of this results we have to consider age of trees on location 6 (Park šuma Gorica), and the influence of the oldest gas station in city. In research which was made by Anđić *et al., (*2015), on same location was found maximal concentration of chrome in species *Bryum argentum i Hypnum cupressiforme*, what matches maximal concentrations of chrome in this research on species *Candelaria concolor*. Chrome is used in industry for making stainless steel, many alloys in which chrome improves their capabilities, used to catalyze, as well for tanning and impregnation of wood. In air comes from industry, especially from ferro - chromic compounds, processing of ore, production of cement, burning fossil fuels.

Considering that for needs of realization of this project, samples were collected on locations which mainly gravitate in urban area, and that they are under big influence of anthropogenic activities, recommendation is that next researches cover also isolated locations (city areas which are less urbanized, or peripheral areas where as winter fuels for heating is used wood). This way will provide basics for to compare results, and additional component which will point to level in which is environment under human activities pressure.

During future researches in this way, it seems worthwhile to collect lichens and from other substrates like rocks, walls, rocky and other fences, as well as considering age of substrates, in order to fulfill picture about all factors which impacts on a stage that lichens accumulate certain elements from the air.

Obtained results are indicative and they can be used for comparison with researches of same species of lichens sampled from other area, and just then eventually will be possible to give conclusions about relation of heavy metals origin, especially if the measurements are performed at "state zero", which means measurements of value of heavy metals in all three mentioned mediums (air, soil, water) in areas of not polluted environment.

References

Anđic, B., Dragićević, S., Stešević, D., Jančić, D. & Krivokapić, S. (2015): Comparative analysis of trace elements in the mosses- Bryum argenteum Hedw. and Hypnum cupressiforme Hedw. in Podgorica (Montenegro). J.Mater. Enveron. Sci. 6 (2) 333-342.

Bajpai, R., Upreti, D.K. & Mishra, S.K. (2004): Pollution monitoring with the help of lichen transplant technique at some residential sites of Lucknow. J. Environ. Biol. 25 (2): 191-195.

Bajpai, R., Upreti, D.K. & Mishra, S.K. (2010): Passive monitoring of atmospheric heavy metals in a historical city of central India by Lepraria lobificans Nyl. Environ. Mon. Assess. 166: 477-484.

Cardarelli, E., Achilli, M., Campanella, C. & Bartoli, A. (1993): Monitoraggioo dell´inquinamento da metallic pesanti mediante l´uso di licheni nella citta di Roma. Inquinamento 6: 56-63 .

Deruelle, S. (1996): The reliability of lichens as biomonitors of lead pollution. Ecologie (Brunoy) 27 (4): 285-290.

Garty, J. (1993): Plants as Bioindicators (ed. Marker, B.), VCH Verlagsgesellschaft mbH, Germany, 193-263.

Ferry, B.W., Baddeley, M.S., & Hawksworth, D.L. (1973): Air Pollution and Lichens. The Athlone Press, London.

Monaci, F., Bargagli, R. & Gasparo, D. (1997): Air pollution monitoring by lichens in a small medieval town of central Italy. Acta Botanica Neerlandica 46 (4): 403-412.

Nayaka, S., Upreti, DK., Gadgil, M. & Pandey, V. (2003): Distribution Patern ans Heavy Metal Accumulation in Lichen of Bangalore City with Special Reference to Lalbagh Garden. Current Science. 84 (5): 674-680.

Nimis, P.L., Ciccarelli, A., Lazzarin, G., Barbagli, R., Benedet, A., Castello, M., Gasparo, D., Lausi, D., Olivieri, S. & Tretiach, M. (1989): I licheni come bioindicatori di inquinamento atmosferico nell area di Schio-Thiene-Breganze (VI). In Bolletino del Museo Civido di Storia Naturale di Verona, 16. CO.GE.V. s.r.l., Verona, Ecothema s.r.l., Trieste.

Nylander, W. (1866): Less lichens du Jardin du Luxembourg.Bull. Soc. Bot. Fr. 13: 341-372.

Ötvös, E., Pázmándi, T. & Tuba, Z. (2003): First national survey of atmospheric heavy metal deposition in Hungary by the analysis of mosses. The Science of the Total Environment 309: 151-160.

Pascale, N. & Pasquini, F. (2010): Differentiation of copper pollution origin: agricultural and urban sources, Novatech 2010.

Rani, M., Shukla, V. & Upreti, D.K. (2011): Periodical monitoring with lichen Phaeophyscia hispidula (Ach.) Moberg in Dehradun city, Uttarakhand, India. Environ. 31: 376-381.
Rizzo, E., Bergamaschi, L., Valcuvia, MG., Profumo, A. & Gallorini, M. (2001): Determination of Cadmium, Copper, Iron, Manganese, Lead and Zinc in Lichens and botanic.
Sabovljević, M., Vukojević, V., Sabovljević, A., Mihajlović, N., Dražić, G. & Vučinić, Z. (2009): Determination of heavy metals deposition in country of Obrenovac (Serbia) using mosses as bioindicators. IV. Manganese (Mn), Molybdenum (Mo), and Nickel (Ni), Arch. Biol. Sci., Belgrade, 61 (4): 835-845.
Shukla, V. & Upreti, D.K. (2008): Effect of metallic pollutants on the physiology of lichen Pyxine subcinerea in Garhwal Himalayas. Environ. Monit. Assess. 141: 237-243.
Shukla, V. & Upreti, D.K. (2007): Heavy metal accumulation in Phaeophyscia hispidula en route to Badrinath, Uttaranchal, India. Environ. Monit. Assess. 131: 365-369.
Stamenkovic, O. (2013): Korišćenje lišaja kao bioloških indikatora kvaliteta vazduha urbanog područja Sokobanje. Master rad. Prirodno- matematički fakultet, departman za biologiju i ekologiju, Univerzitet u Nišu, Niš, 61 pp.
Šaulovic, Đ. & Mujić, Dz. (2009): Prirodni bioindikatori u zivotnoj sredini. 1st International Conference Ecological Environment, Banja Luka, 26-27. Juny 2009.
Šehić, D. & Šehić, D. (2005): Nacionalni atlas Crne Gore. Daily Press, Podgorica.
Treub, L.F. (ed.) (1996): Die chemischen Elemente. S. Hirzel Verlag, Stuttgard, Leipzig. 1-416.
Vasilev, I. (2012): Lišajska indikacija kvaliteta vazduha na ruralnom području grada Blaca. Master rad. Prirodno matematički fakultet, departman za biologiju i ekologiju, Univerzitet u Nišu. Niš, 60 pp.
Van Doven, H.F. & Ter-Braak, C.J.F. (1999): Ranking of epiphytic lichen sensitivity to air pollution to air pollution using survey data: A comparison of indicator scales. Lichenologist 31: 27-39.
Wirth, W. (1995): The Lichens Baden Wurttemberg, Volume 1 2, edition 1995.
http://www.podgorica.me/klima (25.08.2016).