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QUANTIFICATION OF HEAVY METALS IN LICHENS FROM THE UPPER BASIN OF THE RIVER BOGOTÁ

Rodríguez , A.O.E.[1*]; Andrade, B. W.[1]; Díaz, L.F.[1]; Celis, C.[2]; Ortiz-Ardila A.[2]

[1]Engineering Faculty, Environmental Engineering Program, Universidad el Bosque, Bogotá.
[2]Faculty of Sciences, Chemistry Department, Pontificia Universidad Javeriana, Bogotá.

[*]rodriguezoscare@unbosque.edu.co

Abstract

Lichenic associations are used as bio-indicators because lichens are long-lived, known to be sensitive to changes in the habitat and environment, and obtain their nutrients from the atmosphere. In this study we quantified the Pb, Cr, As, Co and Cd metals absorbed by the *Ramalina celastri, Usnea sp., Flavopunctelia flaventor, Teloschiste sexilis, Punctelia subrudecta, Parmotrema simulans, Ramalina complanata, Parmotrema bangii, Everniastrum columbiense, Parmotrema praesorediosum, Parmotrema reticulatum,* and *Heterodermial eucomela* lichens gathered on the west bank of the river Bogotá, Colombia, in its upper basin near the town of Villapinzón, where the air and the health of the community on its banks have been affected for decades by the emissions of particulates into the air and shedding of waste waters into the river by the local tanning industry. The study found high levels of the bio-accumulation of Cd, Co, As and Cr in the lichens, with Pb values which range from 4.1 to 25.8 ppm, with the highest levels in *Parmotrema reticulatum.* The levels of Cd ranged from 0.8 to 45.7 ppm, with *Parmotrema simulans* showing the highest level. The levels of *Co* ranged from 0.8 to 6.3 ppm, with *Heterodermia leucomela* showing the highest bio-accumulation. The levels of As ranged from 9.8 to 76 ppm, with *Heterodermia leucomela* showing the highest level. And the levels of Cr ranged from 0.1 to 141.0 ppm, with the highest levels in *Teloschiste sexilis*. The results show a high accumulation of heavy metals in the lichens we studied, which were mainly derived from particulate material and indicate an impact on the community which lives in the area.

Key words: Lichens, heavy metals, bio-indicators, bio-monitors

Introduction

Lichens are probably the least known and least appreciated organisms in the biological world [1]. Linnaeus called them "the poor trash" of vegetation. This underestimation of the lichens is due to the fact that they are found everywhere in a disorderly manner, they do not resemble plants like mosses and thus far do not resemble the known species of fungi [2]. According to Filho, lichens are complex vegetal species which originate from an association between higher or lower fungi and algae which are nearly always unicellular [3], in a manner which leads both to form new morphological units, which are often completely different from their components, or rather, lichens are stable associations between a photobiont and a mycobiont, capable of sustaining themselves [4]. In the words of Ahmadjlan: "Lichens are photosynthetic associations consisting of tightly coiled populations of green algae or Cyanophyta which possess a unique combination of characteristics, since they are primarily fungal but they are also algal" [2]. Lichens have been used in popular medicine due to their antibiotic properties, above all, those lichens which produce Usnic acid [5-6], which has pharmacological or medicinal uses, [7-9]. The *Cetraria islandica, Umbilicaria sp.* And *Lecanora esculenta* are also used as foodstuffs and some believe they are the Biblical Manna. Others have industrial uses, as the ingredients of colorants and perfumes, or they are used for decorative purposes. At the current time they are utilized as indicators of environmental contamination [10-11], since in places with a lot of pollution, they are the first organisms which disappear. They are highly vulnerable to pollution and rapidly show variations in the physical and chemical characteristics of the environment. There are only a few families which can survive in contaminated places. Lichens accumulate a high concentration of metabolites which they can store in their thallus, many of which are involved in their anti-microbial activity [12]. Among the lichenic metabolites in these organisms we find amino acids, sugars, fatty acids, macrocyclic lactones, monocyclic aromatics, quinones, chromones, xanthones, terpenoids, steroids and carotenoids. They also contain: depsides, depsidones, depsones, dibenzofurans and usnic acids. The known pharmacological activities of lichenic substances may be classified as antibiotic, anti-tumoral-mutagenic, analgesic and antipyretic and they also act as an inhibitor of the human immunodeficiency virus (HIV) and an enzymatic inhibitor. Finally, they have an analgesic and antipyretic activity [12]. lichens also absorb heavy metals, due to the fact that they mainly take their nutrients from the atmosphere and turn them into effective bio-indicators of environmental contamination [13]. These contaminants which are absorbed from the atmosphere remain in the interior of the lichens because they are not able to excrete them and thus they accumulate in their tissues. In that way, detecting high levels of contaminants in lichens indicates that these contaminants are habitually found in the environment in which they live and may result from a constant shedding of wastes into waters and a significant contamination of the air. Lichens accumulate metals by trapping particles with their fungal hyphae, which, as they grow, surround and incorporate all types of particles into their thallus. Once they are trapped, the metals may be incorporated into their walls and cells through an ionic exchange of soluble particles, which would thus explain the affinity between the heavy metals and the COOH radical in the bio-compounds of the lichen. The binding of these metals varies in accordance with the particular metal and the amount of it in the atmosphere. Many species of lichens are used as bio-indicators of the contamination of heavy metals, due to their capacity to accumulate them. For example *Cladonia rangiferina* is an indicator of U, Fe, Pb and Ti; *Parmelia caperata* of Cr; and *Hypogymnia physodes* of Fe, Zn, Ti and V.

The percentages of the accumulation of particles of heavy metals in lichens may be correlated with those absorbed by the inhabitants who live around the place where they are found. The difference between humans and lichens is that the lichens accumulate the heavy metals, while, depending on the concentrations, humans may eliminate them or when their bodies cannot adequately do that, they show such symptoms as premature aging, pains in the limbs and the weakening of vision, memory and concentration. Generally speaking, the heavy metals displace nutrients and they interrupt metabolic processes, activate the production of free radicals and cause the destruction of the cellular membranes and their components: when that happens, the tissues lose their elasticity and water, which can reduce the vitality of the victim and his or her organs. Even in low concentrations, heavy metals accumulate in the cardiovascular system. Like any group of chemical agents, these metals may produce an acute pathology, which rapidly develops after contact with a heavy dose, or a chronic pathology when the victim is exposed to a low dose over a long period of time [14]. Lead may cause fatigue or insomnia, a loss of appetite, headaches, a weakening of memory or concentration, a loss of coordination, irritability, muscular pains, pains in the bones, stomach pains, gout, anemia, infections, hypertension, constipation, hyperactivity, depression, muscular spasms, dizziness or a weak sense of balance, addiction to sweets, a feeling of tiredness, excessive sweating or cold sweats and kidney cancer, among others [15]. Exposure to cadmium is cumulative and it is highly toxic for humans and animals. 90% of the ingested Cd accumulates in diverse tissues which are associated with the SH-groups proteins and form the "metallothionein" complex, which is seven times more toxic than Cd on its own [16] and may cause hypertension, vascular diseases, bronchitis, emphysema, infertility, cancer of the prostate, pains in the bones and muscles, anemia, lumbar pain, arteriosclerosis, an irritated tongue, nausea, loss of appetite and kidney problems: and ailments associated with the loss of minerals, amino acids and proteins in the urine [17]. Arsenic causes a general malaise; bronchitis; cancer of the esophagus, larynx, lungs and bladder; vascular diseases; muscular weakness; eczema; dermatitis; cataracts in the eyes; hypertension and frequent infections [18]. Exposure to chrome may come from natural or industrial sources. Emissions of chrome into the air are mostly of trivalent chromium, in the form of small particles or aerosols.

The biggest industrial sources of chrome in the atmosphere are those related to the production of ferrochrome, among them: the processing or refining of minerals, chemical products, cement, tanning, steel manufacturing, chrome plating, the manufacture of colorants and pigments and the preservation of wood. The respiratory tract is the biggest target for chrome. Studies of humans have clearly shown that inhaled chrome VI is carcinogenic, resulting in a higher risk of lung cancer. Other effects of the exposure through an acute inhalation of high concentrations of chrome VI which should be noted are secondary gastrointestinal ones (including abdominal pains, vomiting and hemorrhages) and neurological ones. It may cause skin eruptions, stomach and ulcer complaints, respiratory problems, a weakening of the immune system, damages to the kidneys and liver, an alteration of genetic material, lung cancer and death [19-20]. It may also cause dermatitis. Animals exposed to high levels of cobalt also register effects in the liver and the lungs. An exposure to high levels of the radioactivity emitted by cobalt may harm the cells of your body, problems with blood pressure and the heart, and damage to the thyroid [21]. We can control all of these impacts on health caused by heavy metals by using bio-monitors, like lichens, in industrial areas. For many years now, the main economic activity of the town of Villapinzón, Colombia, has been its tanneries and they produce a variety of particulate material and waste waters, among them, ones which contain lead, chrome, arsenic, cadmium and cobalt. This study undertook a quantification of the metals accumulated in the *Ramalina celastri, Usnea sp, Flavopunctelia flaventor, Teloschiste sexilis, Punctelia subrudecta, Parmotrema simulans, Ramalina complanata, Parmotrema bangii, Everniastrum columbiense, Parmotrema praesorediosum, Parmotrema reticulatum* and *Heterodermia leucomela* species, all of which grow on the monopodal trees located on the banks of the river Bogotá, near the tanneries, and by nature are bio-accumulative species. In order to analyze the heavy metals in the lichens we utilized the atomic absorption spectrometry technique, which has provided us with quantitative data on the presence of heavy metals in the lichens under study: species for which there were no previous reports on the bio-accumulation of heavy metals.

Methods

Origin of the botanical material, The lichens were collected in the upper basin of the river Bogotá, on its western bank (N 05° 07' 23.4" W 73° 39' 10.5"), from monopodal trees with a ground height ranging from one to two meters. A specimen of each one was taken to the Cryptogams section of the Curatorship of the UDBC Forestry Herbarium of the Colombian Lichenology Group (GCOL) at the Francisco José de Caldas District University in Bogotá, where Dr. Bibiana Mondada, Ph.D., identified them as: *Ramalina celastri, Usnea sp, Flavopunctelia flaventor, Teloschiste sexilis, Punctelia subrudecta, Parmotrema simulans, Ramalina complanata, Parmotrema bangii, Everniastrum columbiense, Parmotremapra esorediosum, Parmotrema reticulatum* and *Heterodermia leucomela.*

Obtainment of the Extracts: The material was dried at room temperature for a week and pulverized in a grinder until a particle size suitable for the extraction processes was obtained. 2 grams of lichens were subjected to digestion with HCl:HNO_3 1:1; then evaluated with 25 ml of type-1 water. The contents of Pb, Cr, As, Co and Cd were determined by atomic absorption in a Varian SPECTRA A 240 FS Atomic Absorption *Spectrophotometer.*

Results

The evaluation, by atomic absorption, of the presence *of Pb, Co, Cd, As and Cr in the lichens collected in the upper basin of the river Bogotá, which consisted of Ramalina celastri, Usnea sp. , Flavopunctelia flaventor, Teloschiste sexilis, Punctelia subrudecta , Parmotrema simulans, Ramalina complanata, Parmotrema bangii, Everniastrum columbiense, Parmotrema praesorediosum, Parmotrema reticulatum,* and *Heterodermia leucomela* found alarming levels of contamination, with: a) Pb levels which varied between 4.1 and 25.8 ppm, with *Parmotrema reticulatum* showing the highest level; b) Cd levels between 0.8 and 45.7 ppm, with *Parmotrema simulans* showing the highest level; c) Co levels ranging from 0.8 to 6.3 ppm, with *Heterodermia leucomela* showing the highest bio-accumulation; d) As levels ranging from 9.8 to 76 ppm, with *Heterodermia leucomela* showing the highest level; and e) Cr levels ranging from 0.1 to 141.0 ppm, with*Teloschiste sexilis* showing the highest level (Table 1, Figure 1).

Analysis

The metals which are emitted into the natural environment by industries are found in the form of volatile particles at relatively high levels, which include lead (Pb), cadmium (Cd), zinc (Zn), copper (Cu), manganese (Mn), silver (Ag), mercury (Hg) and chrome (Cr), among others, depending on the kind of industry [22]. In Villapinzón, the main industrial source are the tanneries, which have been producing particulate materials for decades which include heavy metals and the shedding of waste waters from them have contaminated the river Bogotá, its tributaries and the surrounding region [23] [24]. Consulting the few studies which have been made of the bio-accumulation of heavy metals in lichens, we compared the figures obtained by Boscar [25] in Portugal and Saxeta [26] in India. The former found high levels of chrome and lead in *Lecanor sp, Caloplaca sp* and *Candelariella sp.* lichens growing in the surroundings of the church of Santos Juanes de Valencia, Portugal (Table 2) and the latter of chrome in the *Pyxine coces, Phaeophyscia orbicularis, Lecanora leprosa, Arthopyrenia nidulans, Sphinctrina anglica* and *Bacida submedialis* lichens in India (Table 3).

Our study found concentrations of Pb in most of the species of lichens collected in Villapinzón ranging from 4.1 to 17.9 ppm, which are low compared to those in Portugal, which showed levels ranging from 69.6 to 90.9 ppm. The levels of cadmium in our study ranged from 0.8 to 45.7 ppm, which are high compared to those absorbed by the species in Portugal (0.4 and 0.6 ppm) and India (0.3 ppm). The levels of cobalt found in Villapinzón were between 0.3 and 3.2 ppm, compared to 2.7 and 3.7 ppm in Portugal, while those of arsenic (9.7 and 76.0 ppm) were very high compared to those in the lichens of Portugal (0.3 and 0.4 ppm). The levels of chrome in Villapinzón were 0.07 and 141.0 ppm, which were high compared to those of Portugal (23.9 and 39.1 ppm) and similar to those in India (50.7 and 137.0 ppm). These high levels of contamination have affected the population of Villapinzón for decades of [27]. After using atomic absorption to determine the presence of Pb, Co, Cd, As and Cr in the following lichens collected in Villapinzón, in the upper basin of the river Bogotá, *Ramalina celastri, Usnea sp. , Flavopunctelia flaventor, Teloschiste sexilis, Punctelia subrudecta , Parmotrema simulans, Ramalina complanata, Parmotrema bangii, Everniastrum columbiense, Parmotrema praesorediosum, Parmotrema reticulatum,* and *Heterodermia leucomela,* we found alarming levels of contamination. Pb values ranged from 4.1 and 25.8 ppm, with *Parmotrema reticulatum* showing the highest level. Cd values ranged from 0.8 and 45.7 ppm, with *Parmotrema simulans* showing the highest level. Co values ranged from 0.8 to 6.3 ppm, with *Heterodermia leucomela* showing the highest bio-accumulation. As ranged from 9.8 to 76 ppm, with *Heterodermia leucomela* showing the highest bio-accumulation. And Cr ranged from 0.1 to 141.0 ppm, with *Teloschiste sexilis* showing the highest levels. The above shows the need to control the shedding of contaminants into the water and air in industrial zones, especially those with tanneries.

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Conflicts of interest

The authors are not subject to any conflict of interest in this article

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Table 1. accumulation of metals per mg/Kg of lichen in the upper basin of the river Bogotá

SPECIES	Pb (ppm)	Cd (ppm)	Co (ppm)	As (ppm)	Cr (ppm)
Ramalina celastri	6.7	0.8	0.3	9.8	6.9
Usnea sp	4.1	2.1	0.9	39.0	7.0
Flavopunctelia flaventor	8.6	3.4	1.4	14.0	8.5
Teloschiste sexilis	13.5	11.4	0.8	24.4	141.0
Punctelia subrudecta	8.8	28.6	3.2	14.8	0.1
Parmotrema simulans	6.5	45.7	1.3	35.0	0.8
Ramalina complanata	6.5	11.1	3.3	35.0	0.0
Parmotrema bangii	18.0	27.7	2.1	56.0	2.8
Everniastrum columbiense	13.5	44.3	5.1	56.0	0.3
Parmotrema praesorediosum	12.2	11.0	1.6	47.5	1.5
Parmotrema reticulatum	25.8	27.5	4.0	19.0	1.2
Heterodermia leucomela	4.7	44.1	6.3	76.0	0.0

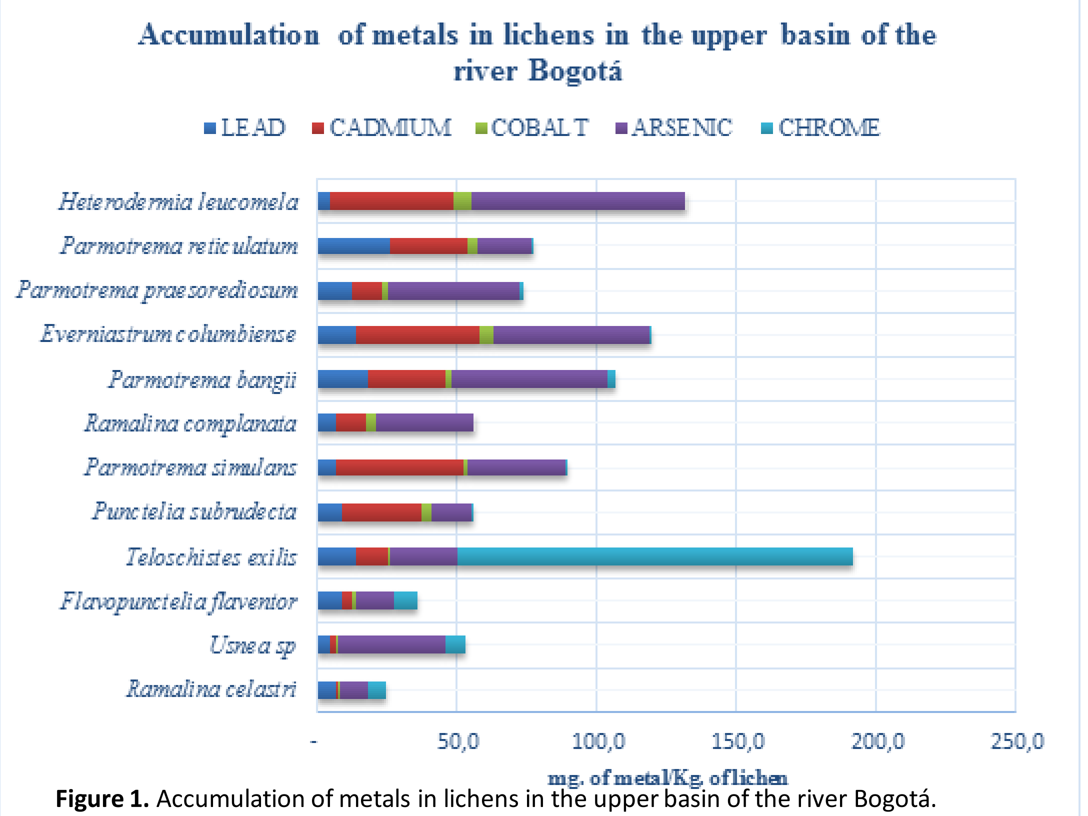

Figure 1. Accumulation of metals in lichens in the upper basin of the river Bogotá.

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Table 2. Quantification of metals in lichens in the *Santos Juanes de Valencia Church (Portugal).*

Lichen	Cr (ppm)	Co (ppm)	As (ppm)	Cd (ppm)	Pb (ppm)
Lecanor sp	24.75	3.19	0.44	0.43	68.97
Caloplaca sp	39.19	3.70	0.39	0.61	90.29
Candelariella sp	23.96	2.79	0.33	0.49	69.60

Table 3. Quantification of metals found in the lichens in India.

Lichen	Pb (ppm)	Cr (ppm)	Cd (ppm)
Pyxine coces	10.6	-	-
Phaeophyscia orbicularis	4.8	62.2	-
Lecanor aleprosa	12.8	-	0.3
Arthopyrenia nidulans	15.6	137.5	-
Sphinctrina anglica	3.1	50.7	-
Bacida submedialis	5.9	127.4	-