Bioaccumulation of heavy metals in aquatic and terrestrial vegetation tissues evaluated in areas where there are mining environmental liabilities in Peru

Lizardo Cahuana[1]; Osvaldo Aduvire[2,]

[1]*Agronomist Consultant for the Environment and Mine Closure Plans, SRK Consulting (Peru) S.A.*

[2]*PhD. Mining Engineer. Principal Consultant and Practice Leader the Environmental and Mine Closure Plans, SRK Consulting (Peru) S.A.*

ABSTRACT

Considering that mining environmental liabilities are those installations, effluents, emissions, remains or deposits of waste produced by old abandoned mining operations that constitute a permanent and potential risk to the health of the population, the surrounding ecosystem and property. Therefore, they constitute one of the main sources of contamination by toxic elements, among them, the mobilization of metallic load in the acid drainages produced by the oxidation of sulfides. These discharges with contributions of heavy metals represent a potential risk of accumulation in the tissues of the surrounding plant species in areas of location of mining environmental liabilities.

The present work focuses on evaluating the bioaccumulation of heavy metals in the tissues of aquatic vegetation (macrophytes), mainly in species such as: *Anomobryum prostratum*, *Marchantia Polymorpha* L. and *Bryophyta sp*., and terrestrial vegetation in species such as: *Stipa mucronata*, *Festuca dolichophylla* and *Cortaderia sp*., in areas surrounding old abandoned mining facilities.

The actions of remediation of Mining Environmental Liabilities in Peru, allows to undertake studies to evaluate the effect of the bioaccumulation of metals in macrophyte species induced by the different concentrations of heavy metals present in the water column, in the case of terrestrial vegetation is evaluated the bioaccumulative effect of these due to the effect of the metals present in the soil.

INTRODUCTION

Plants can adopt different strategies against the presence of metals in their environment (Baker, 1981, Barceló et al., 2003). Many species tolerate the high concentrations of metals in the soil because they restrict their absorption and / or translocation to the leaves (exclusion strategy). However, others actively absorb and accumulate them in their aerial biomass (accumulation strategy), which requires a highly specialized physiology (Baker, 1981).

Macrophytes are macroscopic forms of aquatic vegetation, such as: macroalgae, pteridophytes (mosses, ferns) adapted to aquatic life and angiosperms, present adaptations to this type of life such as: thin cuticle, non-functional stomata, and slightly lignified structures. The natural concentrations of metals in aquatic ecosystems depend on their distribution, weathering and leaching in the area to be studied. The presence of metals in aquatic environments is the result of natural activity, such as the erosion of rocks containing minerals, dust moved by the wind, volcanic activity, forest fires not caused by man and the evaporation of water produced by the same vegetables; Likewise, human activity in urban and industrialized centers contributes significantly to the accumulation of metals in bodies of water (Fernández-Leborans and Olalla 2000) with high potential for transfer to vegetation.

Plant species, including some crops, have the ability to accumulate metals in their tissues. Plants capable of absorbing and accumulating metals above what is established as normal for other species in the same soils are called hyperaccumulators and are found mainly in soils that are rich in metals by natural geochemical conditions or anthropogenic contamination. The hyperaccumulating plants, generally, have little biomass because they use more energy in the necessary mechanisms to adapt to the high concentrations of metal in their tissues (Kabata - Pendias, 2000). Plants have developed highly specific mechanisms to absorb, translocate and accumulate nutrients (Lasat, 2000); However, some metals and non-essential metalloids for vegetables are absorbed, translocated and accumulated in the plant due to their electrochemical behavior similar to the required nutrients.

In the present work, the aim is to evaluate the probable bioaccumulation of heavy metals present in mining passive zones by vegetation present in aquatic ecosystems (macrophytes), as well as in terrestrial environments (terrestrial plants).

METHODOLOGY

Sampling method

The development of the sampling of Macrophytes was carried out in accordance with the provisions of the Protocol for monitoring the sanitary quality of surface water resources, approved by Directorial Resolution No. 2254-2007-DIGESA / SA of Peru, as well as in the Protocols of sampling and analysis for Macrophytes, published by the Ministry of the Environment of Spain (2005).

The evaluation included stretches of 100 m in length, in each station. Samples were taken from the entire width of the river, zigzag from one bank to the other. The macrophytes were recorded, noting their abundance, considering a scale of 1 to 5, as well as the percentage of coverage.

The samples picking was of *Anomobryum prostratum*, *Marchantia Polymorpha* L. and *Bryophyta sp*. because they were the only species of macrophytes recorded in the study area. The collection included samples of 500 grams each for the analysis of heavy metals in the accredited laboratories of SGS, its conservation and transfer was in coolers with refrigerated gel.

Table 1 Guidelines for the quantification of macrophytes

Scale	Abundance of each species Descriptor	Percentage of coverage (%) Clase
1	Weird	Isolated individuals
2	Occasional	1-10%
3	Frequent	10-50%
4	Abundant	50-70%
5	Very abundant	> 70%

Image 1 Macrophytes sampling (Station MAC-03)

In the case of the sampling of the terrestrial vegetation, when we worked with small grasses less than 50 cm high, the whole plant was collected with the root, from which the excess soil was removed. When dealing with larger grasses or small shrubs the terminal part of the stem was cut. In this case, we proceeded to collect samples of the most abundant species in the area, mainly Poaceae Families. The collection included samples of 500 grams for the analysis of metals in the SGS accredited laboratories, their conservation and transfer was in coolers with refrigerated gel. The sampling procedure was carried out following the Guide for the collection and preparation of botanical samples published by the National Herbarium of the National Museum of Costa Rica (2008).

Image 2 Sampling of Vegetable Tissue

Sampling in terrestrial vegetation and macrophytes

The evaluation of metals in the plant tissue was carried out in 05 stations of macrophytes and 05 stations of terrestrial vegetation, located in mining passive zones in the District of Hualgayoc, in the Province of the same name and department of Cajamarca in the Peru.

The purpose of evaluating macrophytes is to determine the probable bioaccumulative effect of these against the different concentrations of heavy metals present in the water column. In the case of terrestrial vegetation, the aim is to evaluate the bioaccumulative effect of these, compared to the metals present in the soil. Next, the location of the 10 stations for the evaluation of metals in plant tissue is indicated.

Table 2 Units of evaluation of metals in vegetal tissue

	SAMPLING STATION	DESCRIPTION
Macrophytes	MAC-1	Ravine Tres Ríos. Upstream Of the Mining Environmental Liabilities, a low flow rate was observed, water of transparent type, without turbidity.
	MAC-2	Ravine Tres Ríos. Adjacent to the cultivation areas, upstream before the confluence with the stream S / N 1.
	MAC-3	Ravine S / N 1. Downstream Ravine Tres ríos. Considered as an impact zone, it would receive any alteration caused upstream.
	MAC-4	At 400 meters, downstream of the Hualgayoc Bridge considered an impact zone downstream of the confluence of the discharge of the effluents from the Mining Environmental Liabilities. I present sparse macrophyte rocky type bottom.
	MAC-5	Sampling station located upstream Hualgayoc River, at the confluence with the discharge waters of the effluents of the Mining Environmental Liabilities, quite rugged relief, mountain slope pronounced approximately 30%, rocky outcrop.
Terrestrial Plants	PT-1	Assessment point located in the upper part of the study area on the 3517 masl of the area of direct influence of the environmental liabilities 14471, 14474, 14469, 4157, 14469, 7289, 7304, among others. It has a rocky surface.
	PT-2	Located on the intermediate side of the study area located approximately between 3 300 meters. Near to the environmental liabilities 7300, 7303, 7305, 7209, 7310 presents drained soils pending moderate agricultural land.
	PT-3	Point located in the high part of the unit of pajonal vegetation on the 3500 msnm next to the PAM, 14466, 7294, located high intermediate side of the study area, the vegetal cover is abundant of medium height to high, terrain relief varies from moderate a pronounced.
	PT-4	Point located in the high part of the unit of pajonal vegetation on the 3500 msnm very next to the passive miners, 14466,7294, located high intermediate side of the study area, the vegetal cover is abundant of medium height to high, relief of the land It varies from moderate to pronounced.
	PT-5	Point is located in the lower part of the project area presents a rugged geography very steep slopes, which borders the bed of the Hualgayoc River, is located near the PAM 14465, 7312, 7302, 7308, 7311.

RESULTS AND DISCUSSION

Results of the scale of abundance of Macrophytes

In the present evaluation, only *Anomobryum prostratum*, *Marchantia Polymorpha* L. and *Bryofita sp*. they are aquatic plants whose habitat is the banks of permanent watercourses, springs, flooded lands with outcrops of water.

Table 3 Results of the scale of abundance of Macrophytes

CLASS		Bryopsida	Bryopsida	Marchantiopsida
ORDER		Bryales	Bryales	Marchantiales
FAMILY		Bryaceae	Bryaceae	Marchantiaceae
SPECIES		*Bryofita sp.*	*Anomobryum prostratum*	*Marchantia Polymorpha* L.
REGISTERED (STATION)		MAC-4	MAC-2, MAC-3 y MAC-5	MAC-1
MAC-1	**% Channel coverage**	-	-	1
	% Shore Coverage	-	-	3
	Scale of Abundance	-	-	2
MAC-2	**% Channel coverage**	-	2	-
	% Shore Coverage	-	5	-
	Scale of Abundance	-	2	-
MAC-3	**% Channel coverage**	-	1	-
	% Shore Coverage	-	4	-
	Scale of Abundance	-	3	-
MAC-4	**% Channel coverage**	1	-	-
	% Shore Coverage	5	-	-
	Scale of Abundance	2	-	-
MAC-5	**% Channel coverage**	-	1	-
	% Shore Coverage	-	5	-
	Scale of Abundance	-	2	-

The abundance scale of *Anomobryum prostratum* "moss" was "occasional" scale (2) for the MAC-2 and MAC-5 stations, for the MAC-3 station it was the "frequent" abundance scale (3); while, in the MAC-4 station, the abundance scale of the species *Bryophyta sp*. was "occasional" scale (2), finally, with respect to the MAC-1 station was recorded the species *Marchantia Polymorpha* L. "moss" which reflected an "occasional" scale of abundance (2).

Results of the concentration of heavy metals in Macrophytes

The results of the concentration of heavy metals in the tissue of the aquatic vegetation of the species *Anomobryum prostratum*, *Marchantia Polymorpha* L. and *Bryophyta sp*. according to Test Report No. AG1622656 / SGS (Method SGS-PO-ME-101: 2016, Determination of metals in organic samples by mass spectrometry with inductively coupled plasma - ICP-MS).

Table 4 Concentration of metals (mg / Kg) in aquatic plant tissue

Bioaccumulation of heavy metals in macrophytes			09:00:00 Vegetal Tissue	12:20:00 Vegetal Tissue	13:30:00 Vegetal Tissue	11:40:00 Vegetal Tissue	12:20:00 Vegetal Tissue
Parámetro	**Unidad**	**LD**	**MAC-01**	**MAC-02**	**MAC-03**	**MAC-04**	**MAC-05**
Mercury (Hg)	mg/kg	0.05	ND	0.2	< 0.10	2.2	--
Arsenic (As)	mg/kg	0.05	3.3	6.9	2.7	9.7	--
Cadmium (Cd)	mg/kg	0.05	0.2	4.1	2.9	< 0.10	--
Copper (Cu)	mg/kg	0.05	2.3	3.8	85	15	--
Chrome (Cr)	mg/kg	0.05	5.2	5.5	1.8	1.2	--
Tin (Sn)	mg/kg	0.05	0.1	0.2	0.2	0.4	--
Manganese (Mn)	mg/kg	0.05	>166.67	>166.67	>166.67	44	--
Nickel (Ni)	mg/kg	0.05	1.9	3.8	2.1	0.3	--
Plumbum (Pb)	mg/kg	0.05	5.8	19	58	64	--
Selenium (Se)	mg/kg	0.05	ND	ND	0.1	ND	--
Zinc (Zn)	mg/kg	0.05	17	>166.67	>166.67	32	--

From the results of Table 4 it can be concluded with respect to the macrophytes, that the following elements are absent or in trace concentrations in the plant tissue:

• Mercury (Hg) absent and not detectable in the case of the MAC-01 and MAC-05 stations, while in the MAC-02 station, MAC-03 and MAC-04 presents values above the Detection Limit (LD). Of the analytical method used by the laboratory.

• Cadmium (Cd) with trace values or absent in the MAC-05 station.

• Selenium (Se) not detectable in stations MAC-01, MAC-02, MAC-04 and MAC-05 while MAC-03 station with value of 0.1 mg / kg, all other parameters above the limit of detection.

The simple linear regression coefficient of the concentration of metals in plant tissue in macrophytes and the concentration of metals in aquatic sediment are shown below:

Table 5 Linear regression coefficient macrophytes - aquatic sediments

ELEMENT	R^2
Mercury	75.02
Arsenic	57.42
Cadmium	72,19
Copper	63,64
Chrome	51.06
Plumbum	38.59
Zinc	89.74

According to the results there is a linear and statistically significant relationship between Zinc (Zn) concentrations of sediments and macrophytes (R^2 = 89.74%), since a higher concentration of zinc in sediments will bring with it a higher concentration Zinc in plant tissue, it should be mentioned that high concentrations of zinc were recorded in sediments for all monitoring stations greater than 500 mg / kg. The second element with the highest R^2 was Mercury with 75.02%, the mercury widely used in mining works. It is followed by Cadmium (Cd) with R^2 = 72.19%, Copper (Cu) with R^2 = 63.64%, Arsenic (As) with R^2 = 57.42%, Chromium (Cr) with R^2 = 51.06% and finally Lead (Pb) with R^2= 38.59%.

The rest of the elements did not present statistically significant regressions that indicate the existence of relationships between the metal concentrations of both samples (sediments and vegetation).

The results previously exposed would demonstrate that the metals of greater availability for the aquatic vegetation (macrophytes) are Zinc, Cadmium and Mercury, which would be bioaccumulated in the vegetal tissue of the Macrophytes present in the study area. However, it is important to indicate that the results should be taken as a reference since there are no preliminary studies that demonstrate the bioaccumulative power of said species.

Results of the concentration of heavy metals in terrestrial plants

The purpose of evaluating terrestrial vegetation is to evaluate the bioaccumulative effect of these against the metals present in the soil.

The results of the concentration of heavy metals in the tissue of the terrestrial vegetation of the species *Stipa mucronate*, *Festuca dolichophylla* "iru ichu" and *Cortaderia sp.* according to the Test Report No. AG1622656 made by SGS del Peru S.A.C.

Table 6 Concentration of metals (mg / kg) in terrestrial plant tissue

Bioaccumulation of Heavy Metals in Terrestrial Plants			**09:00:00 Vegetal Tissue**	**12:20:00 Vegetal Tissue**	**13:30:00 Vegetal Tissue**	**11:40:00 Vegetal Tissue**	**12:20:00 Vegetal Tissue**
Parámetro	**Unidad**	**LD**	**PT-01**	**PT-02**	**PT-03**	**PT-04**	**PT-05**
Mercury (Hg)	mg/kg	0.05	ND	0.1	<0.10	ND	0.1
Arsenic (As)	mg/kg	0.05	0.6	6.5	0.8	0.3	7.5
Cadmium (Cd)	mg/kg	0.05	0.2	0.1	0.7	0.5	0.6
Copper (Cu)	mg/kg	0.05	2.3	6.5	1.6	1.9	18
Chrome (Cr)	mg/kg	0.05	3.8	3	1.1	1.2	3.5
Tin (Sn)	mg/kg	0.05	0.1	0.3	<0.10	ND	0.4
Manganese (Mn)	mg/kg	0.05	104	>166.67	48	116	>166.67
Nickel (Ni)	mg/kg	0.05	1.5	1.1	0.6	0.6	2
Plumbum (Pb)	mg/kg	0.05	7.5	27	22	5.2	53
Selenium (Se)	mg/kg	0.05	ND	ND	ND	ND	ND
Zinc (Zn)	mg/kg	0.05	31	19	71	48	78

From the results it can be concluded that the following elements are absent in the plant tissue of the evaluated terrestrial plants: Mercury (Hg), absent and not detectable, only in stations PT-01, PT-03 and PT-04 while in the station PT-02 and PT-05 record values of 0.1 mg / kg. On the other hand, Tin (Sn), absent in stations PT-03, PT-04 and present with values above detection limit in stations PT-01, PT-02 and PT-05.

The concentrations of selenium (Se) in samples of vegetal tissue obtained in all of the sampling stations in the area of influence of the Mining Environmental Liabilities yield trace values or not detectable (ND) in all the evaluation stations. All other station parameters presented values above the detection limit.

It should be noted that the average concentration of mercury (Hg) for the world's soils, rarely exceeds 400 μg kg-1, associated mainly with organic soils, soils with mercury values (Hg) greater than 500 μg kg-1 can be considered contaminated.

Below is the Simple Linear Regression coefficient of the concentration of metals in plant tissue in terrestrial plants and the concentration of metals in soil:

Table 7 Coefficient of linear regression terrestrial plants - soil

ELEMENT	R^2
Mercury	85.49
Arsenic	26.14
Cadmium	36.57
Chrome	89.16
Plumbum	0.93

According to the results there is a linear and statistically significant relationship between the concentrations of Chromium (Cr) in soils and terrestrial plants (R^2 = 89.16%), since a higher concentration of Chromium in soils will bring about a higher Chromium concentration in the plant tissue of terrestrial plants. The second element with the highest R^2 was Mercury with 85.49%, the mercury very used in the mining works. It is followed by Cadmium (Cd) with R^2 = 36.57%, Arsenic (As) with R^2 = 26.14%, and finally Lead (Pb) with R^2 = 0.93%.

It should be noted that these elements are present in high concentrations in the sediments evaluated, in such a way that their availability and capacity to accumulate are increased.

Finally, the rest of the elements evaluated did not present statistically significant regressions that indicate the existence of relationships between the metal concentrations of both samples.

Comparison criteria

Due to the fact that in Peru there are no national environmental quality standards for metals in plant tissues, which establish the maximum permitted values of contaminants in the mining passive environment; For this reason, in addition to the Linear Regression for terrestrial plants, comparisons have been made based on what was established by the Association of American Feed Control Officials (AAFCO, 1996), as well as that established by the European Union (1988) for 3 plant species (PT -1: *Stipa mucronata*, PT-2: *Festuca dolichophylla* and PT-4: *Cortaderia sp*) which sets maximum values for heavy metals in animal feed.

The American AAFCO (1996) classifies metals as highly toxic, toxic, moderately toxic and slightly toxic and gives maximum recommended values (Table 8), which are generally quite high.

Table 8 Maximum recommended levels of heavy metals in animal feed (AAFCO, 1996)

Category	Metal	Maximum Level (mg/kg)
Highly Toxic	– Cadmium – Mercury – Selenium	10
Toxic	– Barium – Cobalt – Copper – Plumbum – Molybdenum – Tungsten – Vanadium	40
Moderately Toxic	– Antimonium – Arsenic – Iodo – Níckel	400
Slightly Toxic	– Aluminum – Boron – Bromine – Bismuth – Chrome – Manganese – Zinc	1 000

And the European Union there is a legislation that sets maximum values for heavy metals in animal feed. In Spain it is included in Royal Decree 747/2001 of June 29, and whose summary values for complete feed are shown in Table 9. For raw materials and complementary feed the values vary slightly.

Table 9 Maximum levels of heavy metals in complete feeds (Order 11 October 1988)

Metal	Maximum Content (mg/Kg)
Arsenic	2
Lead	5
Fluorine	100 - 350
Mercury	0.1
Cadmium	0.5 – 1.0

Faced with this situation, it was decided to make these comparisons using the international quality standard for species considered as palatable for livestock, therefore, of the 5 stations of evaluation for terrestrial plants, 3 stations (PT-1, PT-2 and PT - 4) species considered as palatable were registered.

On the other hand, are indicated the results of the concentrations of six (6) heavy metals traditionally considered for their toxic effects in plant tissue: (selenium, arsenic, cadmium, copper, lead and mercury), in samples obtained in the areas of influence of Mining Environmental Liabilities, and compare their results with the maximum limits established by the AAFCO (1996). And the European Union (1998).

Results of the evaluation

The results of the concentration of heavy metals in the tissue of the terrestrial vegetation of the species are shown below: *Stipa mucronate*, *Festuca dolichophylla* "iruichu" and *Cortaderia sp*. According to the Test Report No. AG1622656 made by SGS del Peru S.A.C.

Table 10 Concentration of metals (mg / kg) in terrestrial plant tissue.

Bioaccumulation of Heavy Metals in Terrestrial Plants			03/09/2016 09:00:00 Vegetal Tissue *Stipa mucronata*	03/09/2016 12:20:00 Vegetal Tissue *Festuca dolichophylla*	03/09/2016 11:40:00 Vegetal Tissue *Cortaderia sp.*
Parameters	**Unidad**	**LD**	**PT-01**	**PT-02**	**PT-04**
Mercury (Hg)	mg/kg	0.05	ND	0.1	ND
Arsenic (As)	mg/kg	0.05	0.6	6.5	0.3
Cadmium (Cd)	mg/kg	0.05	0.2	0.1	0.5
Copper (Cu)	mg/kg	0.05	2.3	6.5	1.9
Chrome (Cr)	mg/kg	0.05	3.8	3	1.2
Manganese (Mn)	mg/kg	0.05	104	>166.67	116
Nickel (Ni)	mg/kg	0.05	1.5	1.1	0.6
Plumbum (Pb)	mg/kg	0.05	7.5	27	5.2
Selenium (Se)	mg/kg	0.05	ND	ND	ND
Zinc (Zn)	mg/kg	0.05	31	19	48

Arsenic (AS).

The concentrations of arsenic (As) in samples of vegetal tissue obtained in some of the sampling stations in the area of influence of the Mining Environmental Liabilities, show values of concentration higher than those established in the limits of detection (LD = 0.05) of the analytical method used by the laboratory, as observed in Table 10, However, these values are below the maximum concentration allowed by the AAFCO (400 mg / kg).

This result shows that the concentrations of (As) found in the palatable species (*Stipa mucronata*, *Festuca dolichophylla* and *Cortaderia sp*.) registered in the station (PT-1, PT-2 and PT-4) do not constitute a danger or toxic risk for the livestock or local wild species when these concentrations are within the limits allowed by the AAFCO (1996). However, these values compared with the European Union legislation (Royal Decree 747/2001, Spain) show that the concentrations of (As) found in the PT-2 station with (6.5 mg / kg) are above the content maximum allowed (2 mg / kg) this increase in concentration is possibly due to the presence of Mining Environmental Liabilities with codes (7300, 7303, 7305, 7209, 7310), however this value is much less than the máximum concentration of the AAFCO (400 mg / kg). As seen in Table 10.

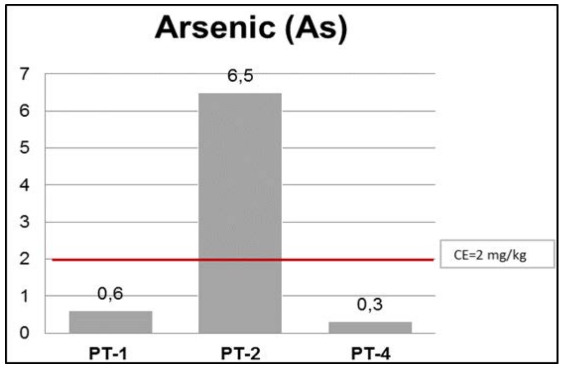

Figure 1 Arsenic concentration in the stations evaluated

Cadmium (Cd)

The concentrations of cadmium (Cd) in samples of plant tissue obtained in all the sampling stations in the area of influence of the Mining Environmental Liabilities, show values below the maximum concentrations established by the European Union (0.5 to 1.5 mg / kg Royal Decree 747/2001, Spain), also obtained values below the maximum concentration established, as well as for the maximum limits of the AAFCO (10 mg / kg) (1996), all stations were below the maximum concentration. This would indicate that cadmium concentrations would not cause biological adverse effects to terrestrial biota. As seen in the following figure.

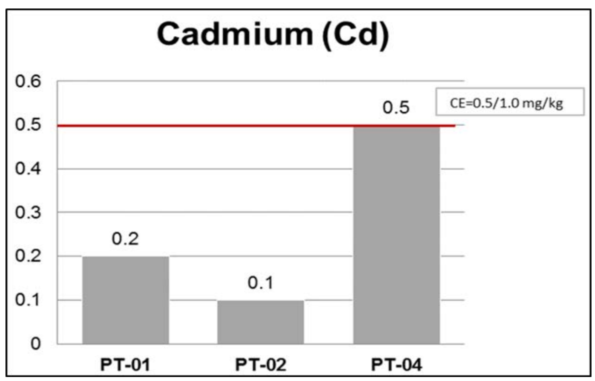

Figure 2 Cadmium concentration in the evaluated stations

Copper (Cu)

The concentrations of Copper (Cu) in samples of plant tissue obtained in the stations (PT-1, PT-2 and PT-4) of sampling in the area ofinfluence of the Mining Environmental Liabilities, give values of concentration higher than those established in the limits of detection (LD = 0.05) of the analytical method used by the laboratory, as shown in Table 10. However, these values are below the maximum concentration allowed by the AAFCO (40 mg / kg).

This result shows that the concentrations of (Cu) found in the palatable species (*Stipa mucronata*, *Festuca dolichophylla* and *Cortaderia sp*.) registered in the station (PT-1, PT-2 and PT-4) do not constitute a danger or toxic risk for the livestock or local wild species, since these concentrations are well below what is allowed by the AAFCO (1996). However, the legislation of the European Union (Royal Decree 747/2001, Spain) does not establish maximum concentration limits related to this metal.

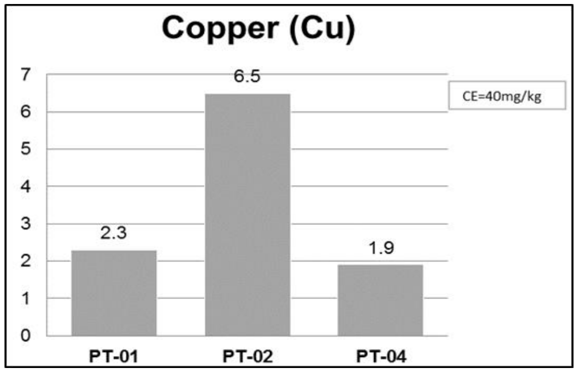

Figure 3 Copper concentration in the evaluated stations

Mercury (Hg)

The concentrations of mercury (Hg) in samples of vegetal tissue obtained in all the sampling stations in the area of influence of the Mining Environmental Liabilities, show values below the maximum concentrations established by the European Union (0.1 mg / kg Royal Decree 747/2001, Spain), as well as the maximum limits of the AAFCO (10 mg / kg) (1996). Therefore, these concentrations probably would not cause adverse biological effects to terrestrial biota. See the following figure:

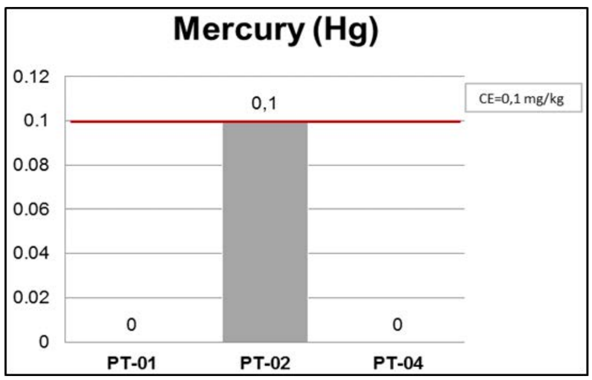

Figure 4 Mercury concentration in the evaluated stations

Plumbum (Pb)

In this regard, the concentrations of lead (Pb) in samples of vegetal tissue obtained in the sampling stations in the area of influence of the Mining Environmental Liabilities, (PT-1, PT-2 and PT-4) show values above the Maximum concentrations established by the European Union (5.0 mg / kg, Royal Decree 747/2001, Spain), there is an increase in concentration from the PT-1 point to the PT-2, in (7.5 to 27 mg / kg) this possibly due to the fact that the land use is of mining type and according to the geological conditions of the area would be formed by sedimentary rocks and igneous acid rocks, which may contain Cu, Pb Zn and Ag, however this concentration of lead (Pb) decreases again at point PT-4 where finally the concentration of this parameter reaches (5.2 mg / kg). However, none of the evaluated stations exceeded the maximum limits established by the AAFCO (40.0 mg / kg) (1996). As shown in Table 10, it is important to indicate these values are referential, so we can assure if there is adverse biological effect to terrestrial biota in the area, so it is suggested to follow up and research.

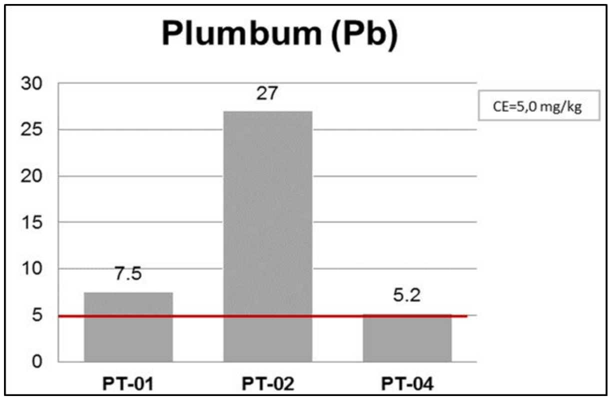

Figure 5 Plumbum concentration in the stations evaluated

Zinc (Zn)

The concentrations of Zinc (Zn) in samples of plant tissue obtained in the stations (PT-1, PT-2 and PT-4) of sampling in the area of influence of the Mining Environmental Liabilities, show values of concentration higher than those established in the limits of detection (LD = 0.05) of the analytical method used by the laboratory, as shown in Table 10, however, these values are below the maximum concentration allowed by the AAFCO (1000 mg / kg),

This result shows that the concentrations of (Zn) found in the palatable species (*Stipa mucronata*, *Festuca dolichophylla* and *Cortaderia sp*.) Registered in the station (PT-1, PT-2 and PT-4) do not constitute danger or toxic risk for the cattle or local wild species, since these concentrations are much lower than what is allowed by the AAFCO (1996). However, the legislation of the European Union (Royal Decree 747/2001, Spain) does not establish maximum concentration limits related to this metal.

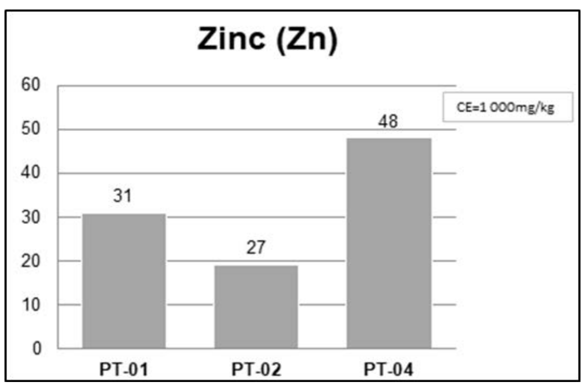

Figure 6 Zinc concentration in the evaluated stations

It can be seen that stations PT-01, PT-2 and PT-4 present Zinc concentrations at low levels, which means that these concentrations would not cause adverse effects to aquatic or terrestrial biota, specifically to the palatable species consumed by the won.

CONCLUSION

- Macrophytes were represented by three species; *Anomobryum prostratum*, *Marchantia Polymorpha* L. and *Bryophyta sp*. distributed in 2 Classes (Bryopsida and Marchantiopsida), 2 Orders (Bryales, Marchantiales) and 2 Families (Bryaceae and Marchantiaceae). The "moss" *Anomobryum prostratum* was the most frequent being present at the MAC-2, MAC-3 and MAC-5 points. Unlike the MAC-1 evaluation point, only the species *Marchantia Polymorpha* L. "Moss" was recorded, while the species *Bryophyta sp*. Was present at the MAC-4 point.
- *Anomobryum prostratum* presented an abundance that varied in scale from 2 to 3 (MAC-2, MAC-3 and MAC-5), while *Bryophyta sp.* presented an abundance in scale of 2, Finally *Marchantia Polymorpha* L. was registered only in the point (MAC-4). where he presented an abundance in scale of 2.
- About the accumulation of heavy metals in the vegetal (aquatic) tissue, the element Mercury (Hg) was absent and not detectable in the case of the MAC-01 and MAC-05 stations, in comparison with the stations MAC-02, MAC-03 and MAC-04 present values above the (LD). Of the analytical method used by the laboratory. The mercury concentrations and all other parameters were absent in the MAC-05 station. While Selenium (Se) was absent and not detectable in stations MAC-01, MAC-02, MAC-04 and MAC-05, while MAC-03 station with value of 0.1 mg / kg, being above the limit detection.
- Of the three species of macrophytes Anomobryum prostratum "moss", it has a greater capacity for the accumulation of metals since it registered in the majority of the cases the highest concentrations of elements (Arsenic, Copper, Chromium, Manganese, and Zinc) in comparison with the rest of the species.
- Macrophytes easily accumulate the following zinc metals (Zn) with R^2 = 89.74%, followed by Mercury (Hg) with R^2 = 75.02%. and Cadmium (Cd) R^2 = 72.19%. Finally, the lowest regression coefficient was presented by Lead (Pb) with R^2 = 38.59%.
- In the case of aquatic plant tissue or macrophytes, strong relationships were found with the concentrations of aquatic sediment metals. These results could be due to several factors, it must be taken into account that the toxicity of metals does not depend only on their total concentration.
- In the case of terrestrial plant tissue, strong relationships were found with the concentrations of soil metals. These results could be due to many factors, so it must be taken into account that the toxicity of metals does not depend only on their total concentration.
- About the concentration of metals in terrestrial plant tissue (terrestrial plants), it could be seen that there is a strong relationship between chromium concentrations (R^2 = 86.16%) of terrestrial plants with respect to concentrations in sediment. Followed by Mercury (R^2 = 85.49%). The rest of the metals did not present statistically significant relationships. In this way we can conclude if the concentration of metals present in the matrix (sediment, have a significant relationship with the concentration of metals in the species under study).
- The analysis of heavy metals made in plant tissue of terrestrial plants in the monitoring stations of the area of influence of the Mining Environmental Liabilities, show bioaccumulation of heavy metals (arsenic and lead) surpassing in some stations, the maximum concentrations established by the Union European (Royal Decree 747/2001, Spain) and by the AAFCO (1996) Association of American Feed Control Officials. It is thus,

that PT-2 stations with 6.5 mg / kg according to European Union legislation (Royal Decree 747/2001, Spain), Arsenic are above the maximum allowed content (2 mg / kg), while the high values for Lead in the stations PT-1, PT-2 and PT-4 would have characteristics of the soil components that would be determining high values, taking into account that the land use is of the mining type.

- The concentrations of metals are associated with the Mining Environmental Liabilities, since these are at a certain distance from them. Distant stations also showed a certain concentration of metals because in some cases pollution can occur due to weather conditions (dragging of mineral by run-off, by wind, among others).

NOMENCLATURE

mg/Kg	Milligrams per kilogram
LD	Limit of Detection
ND	No Detectable
R^2	Determination Coefficient
$\mu g\ kg^{-1}$	Microgram per kilogram

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