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CHILEAN LICHEN AND MOSSES MODIFIED CARBON PASTE ELECTRODES FOR MONITORING DISSOLVED ECOTOXIC METAL IONS

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ABSTRACT

Lichens (a symbiotic association of fungi and algae) and mosses are capable of accumulating heavy metals in their tissues. The retention of ecotoxic metals may take place in the functional groups that contain oxygen and nitrogen atoms, most likely carboxylic groups that form part of the cell wall of the fungus, through an ion exchange process. In this work, the study of carbon-lichen paste electrodes and carbon-moss paste electrodes has been carried out. Eighteen species of lichens and two species of mosses were used as sensitive agents. Additionally, the response of each of them with nine metal ions such as Cu, As, Pb, Hg, Zn, Cd, Al, Mo and Cr was studied. Being the electrode that is most sensitive to the lichen *Protousnea Magallánica* and the mosses *Hypnum Cupresiforme* and *Ortotrichum Assimile*, it presents better signals with the lead (II), Copper (II) and Mercury (II) ion.

Keywords: Lichens, mosses, modified electrodes, Ecotoxic metals.

1. INTRODUCTION

Chemically modified electrodes (CMEs) have developed rapidly in recent years, especially in the electroanalytical field. They should allow the development of new voltammetric measurement schemes with enhanced selectivity and sensitivity [1-2]. One approach to using CMEs to increase the sensitivity and selectivity of a voltammetric procedure is to use a surface capable of preconcentrating the analyte from the solution. The most popular schemes used to trap analytes on the electrode surface are based on complexation [3-5], and electrostatic attractions [6-8] covalent attachment can also be used [9]. Another interest of modified electrodes is that the surfaces can be tailored using various potentially accumulating (incorporating) substances. In the most popular schemes, the preconcentration agent is commonly introduced into the surface as part of an appropriate polymeric film or by mixing with a carbon paste matrix [10-13].

Microorganisms such as bacteria, algae, fungi, mossses, and aquatic plants possess mechanisms to remove metal ions from solution through metabolic pathways and passive adsorption [14-17]. The latter are rapid and highly pH-dependent. The binding is thought to involve mainly carboxylate groups on cell wall surfaces [18]. Cellular material thus has the properties of an ion exchanger in which hydrogen ions compete with metal ions for binding sities.

Lichen, a symbiotic association of fungus and algae, can accumulate heavy metals in their tissue. Therefore, they are commonly used as environmental pollution biomonitoring [19-20]. As indicated by Dempsey *et al.*, [21], the retention of heavy metal ions seems to take place in the lichen functional groups containing oxygen and nitrogen donor atoms, most likely carboxilic, thiol groups, that form part of the fungal cell wall, through an ion-exchange process [22-23].

Ecologically and structurally, mosses are closer to lichens than to other plant kingdom members. Both mosses and lichens depend upon external moisture to transport nutrients. Because of this, they prefer damp places and have evolved particular methods of dealing with long dry periods. In contrast, higher plants have specialized organs for transporting fluids, allowing them to adapt to various habitats. Bryophytes used to be classified as three classes of a single phylum, *Bryophyta*. Modern texts, however, now assign each class to its phylum: Mosses (*Bryophyta*), Liverworts (*Hepatophyta*), and Hornworts (*Anthoceraphyta*). This variety reflects the current taxonomic wisdom that the Liverworts and Hornworts are more primitive and only distantly related to Mosses and other plants. In particular, because of their ability to collect metals from dilute solutions, mosses have been used as indicators of heavy metal deposition from the atmosphere. *Shaghnum* is known to act as an ion-exchanger, similar to certain resins and zeolites [24]. The electrostatic uptake is believed to involve unterrified polyuronic acids, particularly galactorunic acid [25]. Trace element determination as possible by combining the pre-concentration step of metal ions in the active sites of the biomasses tissue with their quantitative determination using different techniques, such as X-ray fluorescence spectrometry, emission spectrometry, atomic absorption spectrometry, neutron activation analysis or voltammetric [26-27]. Lichens and biomasses, for instance, mosses or algae, have been incorporated into carbon paste electrodes (CPEs), where they act as the sensing phase for the recognition of toxic cations [28-31] and encapsulate in the polysiloxane matrices for the application as electrochemical sensors for the determination of heavy metal ions [32]. Following the bioaccumulation process in the biomass-modified CPEs, cation was determined through anodic stripping combined with voltammetric techniques such as a cyclic, differential pulse of square wave voltammetry [33].

In this paper we study the response to 18 species of lichens: *Darbishirella Gracillima* (II, region, Paposo); *Roccellinastrum Spongioideum* (II region, Paposo); *Ingraderia Pulcherrima* (II region, Paposo); *Darbishirella Gracillina* (III region, pan de Azúcar); *Ingraderia Pulcherrina* (III region, pan de Azúcar); *Roccella Portentosa* (VIII region, Concepción); *Psoroma Pallidium* (IX region, Villarrica); *Pseudocyphellaria Hirsuta* (IX region, Conguillio); *Psoroma Reticulatum* (X region, Croshuenco); *Erioderma Leylanoii* (X region); *Pseudocyphellaria Exanthenatica* (Cordillera Pelada); *Protousnea Magallanica* (XI region, Cochane); *Pseudocyphellaria Hirsuta* (XI region, Cochane); *Pseudocyphellaria Coriifolia* (XI region, Cochane); *Stereoraulom Alpinum* (isla Robert, Antarctica); *Umbilicaria Antarctica* (isla Robert, Antarctica); *Himantormmia Cugubris* (Isla rey Jorge, Antarctica); *Pseudephebe Pubeseans* (Isla Rey Jorge, Antarctica) and two mosses, against to copper (II), Arsenic (III), Lead (II), mercury (II), zinc (II), Cadmium (II), Aluminum (III), molybdenum (V) and chromium (III).

2. MATERIAL AND METHODS

Reagents

All the reagents used in this investigation were of analytic grade, and the solutions were ready with high-purity water, coming from a team cleanser of water NANOpure Barstead ultrapure water. The powder of 1-2 µm graphite was obtained from Aldrich and the mineral oil of Fluka. For the production of the electrodes, tubes of glass 9 mm in diameter and 6 cm long and wire of copper was used.

The standard metal ion solution (titrisol of 1000 mg L^{-1}, Merck) was prepared to start from the standard solution, carrying out the appropriate dilutions, and stored in polyethylene bottles.

Solution tampon: Acetate of sodium - acetic acid (pH = 5.0). This solution was prepared starting from a fraction of acid acetic 0.10 mol L^{-1}, and of solid sodium acetate was added (Merck) until reaching pH=5.0.

The 18 species of lichens and two mosses, were ground in the lyophilizator and milled and separated with sizes of particles 300 < 150, 150 < 75 and smaller than 75 µm.

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Sensor preparation

The paste was prepared by mixing 0.4 g of graphite with 0.4 g of oil mineral and 0.2 g of the powdered biomasses modified. After obtaining this paste, the working electrode is manufactured, as shown in the diagram in Figure 1 (geometrical area 0.64 cm^2). A portion of the modified carbon paste was packed at the end of a glass tube (9 mm o.d.), and its inner end was connected to a copper wire, followed by measurement in a cell consisting of an Ag/AgCl reference electrode and another platinum coil which was an auxiliary electrode.

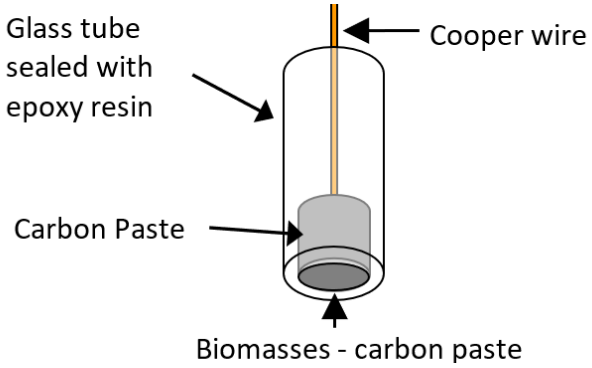

Figure 1. Scheme of the preparation of lichen- modified carbon paste electrodes.

Instrumentation

Reductive potential and scan voltammetry measurements were performed with VoltaLab-40 potentiostat (Radiometer), controlled with the Volta Master 4.0.

Electrochemical procedures

The determination of ecotoxic metals included three steps: (1) preconcentration was carried out in 1 mg L^{-1} of ecotoxic metals in 0.1 mol L^{-1} acetate buffer, pH 5.0, for 10 and 30 minutes at an open circuit for the lichen and the mosses respectively; (2) after thoroughly rinsing with deionized water, the sensor was transferred to the measurement cell containing acetate buffer, pH 5.0; first, a reductive potential of −1.0 V was applied for 40 seconds for the lichen and 180 seconds for the mosses and then the potential was swept between −1.0 and +0.6 V at a scan rate of 50 mV s^{-1} to induce the anodic stripping of the reduced ecotoxic metals; (3) a voltammogram (current v/s applied voltage) was recorded and the electrical conductivity of the electrode was identified by presence of current peaks for the oxidation of each metal. Finally, the sensor's immersion in HCl 0.5 mol L^{-1} for 5 minutes assures the surface renewal, with the biomasses' active sites available for new measurements. All experiments were carried out at room temperature (20°C).

Interferent

Solve of diverse ions to study the interferent effect; they were prepared by diluting each compound's calculated quantity so that the resulting solutions have the desired concentration of the respective species. All the solutions were stored in polyethylene containers previously treated with nitric acid.

Recover

The ranges of the recoveries of the methodologies were carried out with High purity standards of metal ion.

3. RESULTS AND DISCUSSION

The application of biomass-modified carbon paste electrodes to the determination of metal ion by cyclic voltammetry has been previously investigated [23], who optimized parameters such as pH, cleaning time, and surface renewal. The performance of electrodes modified biomasses was further assessed considering 18 species of Chilean lichen, and two mosses in which particles size, electrolysis time, analytical parameters, interferent study, and % recovery were studied.

The study of the answers of the biomasses with Cu (II), As (III), Pb (II), Hg (II), Zn (II), Cd (II), Al (III), Mo (V), and Cr (III). Table 1 shows that the electrode that has as sensitive agents the lichen *Protousnea Magallánica* and the mosses *Hypnum Cupresiforme* and Ortotrichum Assimile, displaying the best values with the lead (II), copper (II), and mercury (II) ions.

When comparing lichen *Pseudocyphellaria hirsuta* (IX region, Conguillio) and *Pseudocyphellaria hirsuta* (XI region, Cochane), although the same species, it has the same behavior as the lichen of the region of the Conguillio, shows signs of ions all concentrations studied however, that of Cochane lichen, shows signs of cans concentrations (10 mg L^{-1}), and no signal with the Hg (II), this behavior could be explained by the influence of the environment, as if exposed to a high amount of these ions, should produce more detoxifying and therefore show no sign with a large number of ions and increased sensitivity.

Table 1. The behavior of the biomass-carbon paste electrodes of the mosses and lichens, with the metal ion Cu, As, Pb, Hg, Zn, Cd, Al, Mo, and Cr (10 mg L^{-1}).

	Cu (II)	As (III)	Pb (II)	Hg (II)	Zn (II)	Cd (II)	Al (III)	Mo (V)	Cr (III)
Mosses									
Hypnum cupressiforme (IV, la Serena).	√	X	√	√	X	X	X	X	X
Ortotrichum assimile (IV, la Serena).	√	X	√	√	X	X	X	X	X
Lichens									
Protousnea Magallanica (XI, Cochane).	√	X	√	√	X	√	X	X	X
Pseudocyphellaria Coriifolia (XI, Cochane).	√	X	X	√	X	X	X	X	X
Roccella Portentosa (VIII, Concepción).	√	X	X	√	X	X	X	X	X
Pseudocyphellaria Hirsuta (IX, Conguillio).	√	X	X	√	X	X	X	X	X
Pseudocyphellaria Hirsuta (XI, Cochane).	√	X	X	X	X	X	X	X	X
Darbishirella Gracillima (II, Paposo).	√	X	X	X	X	X	X	X	X
Roccellinastrum Spongioideum (II, Paposo).	√	X	X	X	X	X	X	X	X
Ingraderia Pulcherrima (II, Paposo).	√	X	X	√	X	X	X	X	X
Darbishirella Gracillina (III, pan de Azúcar).	√	X	X	X	X	X	X	X	X
Ingraderia Pulcherrina (III, pan de Azúcar).	√	X	X	X	X	X	X	X	X
Psoroma Pallidium (IX, Villarrica).	√	X	X	X	X	X	X	X	X
Pseudocyphellaria Psoroma Reticulatum (X, Croshuenco).	X	X	X	X	X	X	X	X	X
Erioderma Leylanoii (X, Croshuenco).	X	X	X	√	X	X	X	X	X
Pseudocyphellaria Exanthenatica (X, Cordillera Pelada).	X	X	√	X	X	X	X	X	X
Stereoraulom Alpinum (isla Robert, Antarctica).	X	X	X	√	X	X	X	X	X
Umbilicaria Antartica (isla Robert, Antarctica).	X	X	X	X	X	X	X	X	X
Himantormmia Cugubris (Isla rey Jorge, Antarctica).	X	X	√	X	X	X	X	X	X
Pseudephebe Pubeseans (Isla Rey Jorge, Antarctica).	X	X	X	√	X	X	X	X	X

Study of lichen in the modified carbon paste electrode

The particle size study was carried out with particle size < 75 µm, among 75 - 150 µm and enter 150 - 300 µm. Figure 2 shows, what small particle size, because the intensity of the signal is bigger. A similar behavior presents the other types of lichens and mosses.

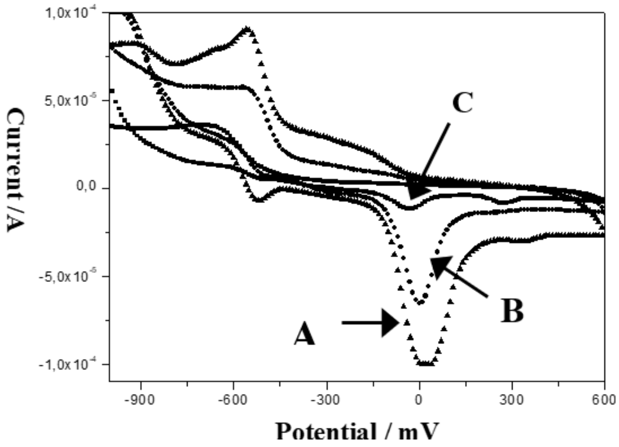

Figure 2. Voltammograms of the effect of particle size of the lichen *(Protousnea Magallanica)* with the mixture of metal ions of Cu, Hg, Pb, and Cd (5 mg L^{-1}), preconcentration time 10 min., pH = 5,0; electrolysis 40 s. A) < 75 µm; B) 150 < 75 µm; C) 300 < 150µm.

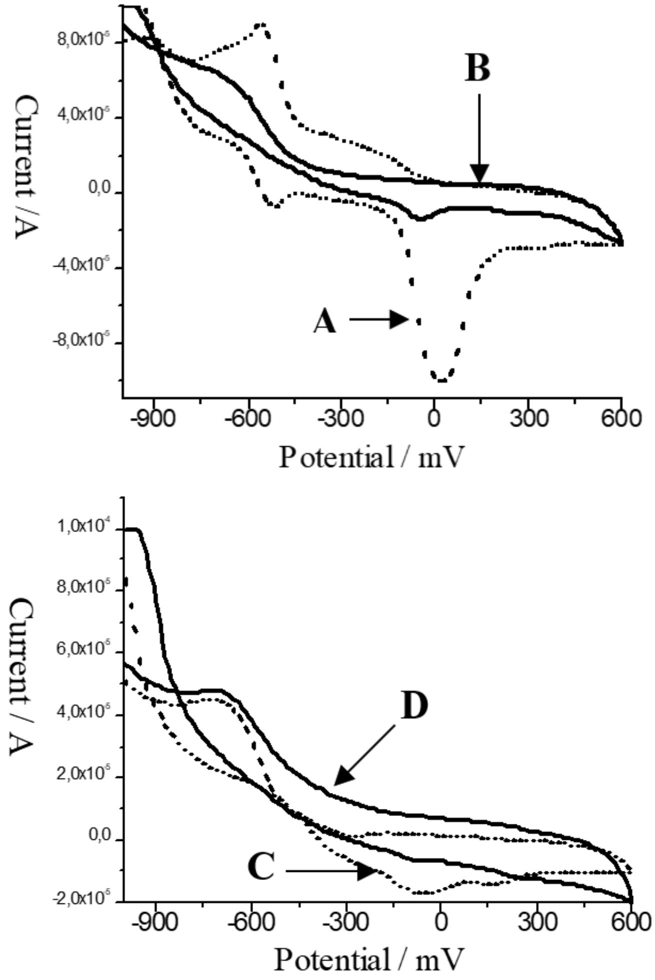

Figure 3. Voltammograms of the lichens-carbon paste electrodes with. A) *Protousnea Magallanica,* B) *Pseudoey Phellaria Carüfolia,* C) *Rosella Portentosa,* D) *Pseudoey Phellaria Hirsuta,* with the mixture of metal ions of Cu, Hg, Pb, and Cd (5 mg L^{-1}), in the optimum conditions of work.

The study of the voltammetric signals of the lichen-modified carbon paste electrodes with the best results (Figure 3) shows that the lichen *Protousnea Magallanica* has the best signal for detecting copper, mercury, and lead.

Figure 4 shows the behavior of the electrode modified with *Protousnea Magallanica*, showing lead, copper, and mercury signals at -490, 50, and 330 mV, respectively. When one studies the mercury, the lichen *Pseudoey Phellaria Carüfoliia* behavior similarly is observed that presents the lead. However, the best signal was present for the *Protousnea Magallanica*-modified electrode.

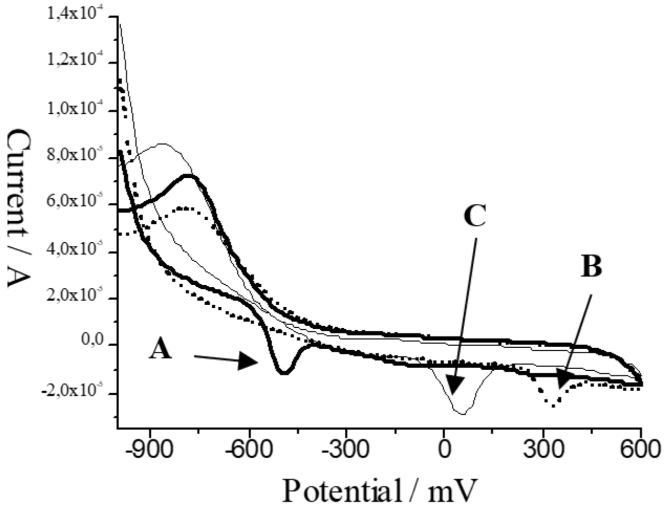

Figure 4. Voltammograms of the *Protousnea Magallanica*-carbon paste electrodes*,* with the mixture of metal ions (5 mg L^{-1}), in the optimum conditions of work: A) Pb (II); B) Hg (II) y C) Cu (II).

Figure 5 shows the study of the effect of the electrolysis time with the mixture of metals, having the highest current value at the same electrolysis time of 40 sec.

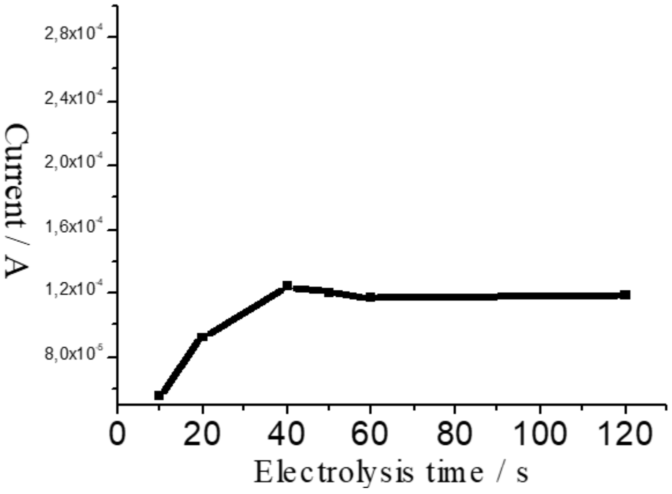

Figure 5. Effect of the electrolysis time over the signal, with the *Protousnea Magallanica*-carbon paste electrode*,* with the mixture of metal ions (5 mg L^{-1}), preconcentration time 10 min., pH = 5.0; and particle size < 75 µm., electrolysis time of the 10 a 120 second.

The analytical parameters, % recovery (Table 2), and the effects of interfering metal ions in the determination of the Pb (II), Cu (II), and Hg (II) with *Protousnea Magallanica*-carbon paste electrode could be seen in table 3.

Table 2. Analytical parameters and % Recovery of the modified electrode by *Protousnea Magallanica* with lead (II), copper (II), and Mercury (II).

	Lead		**Copper**		**Mercury**	
Detect. Limit (μmol L^{-1})	0.423		1.67		0.160	
Quant. Limit (μmol L^{-1})	1.41		5.57		0.534	
Rang. Deter. (μmol L^{-1})	1.41 to 96.50		5.57 to 78.60		0.530 to 74.80	
Standard Deviation	0.463		2.34		0.163	
Equation (C in mol L^{-1})	Y=–24.62+3.28x10^{6}C		Y=–16.78+4.20x10^{6}C		Y=–26.45+3.05x10^{6}C	
Correlation coefficient	0.994		0.997		0.994	
Recovery	C (μmol L^{-1})	% R	C (μmol L^{-1})	% R	C (μmol L^{-1})	% R
	9.65	106.9	15.70	103.2	4.98	105.8
	14.40	95.8	31.40	99.3	9.97	103.2
	19.30	98.4	47.20	97.6	14.90	95.3
	24.10	102.1	78.60	99.8	19.90	102.5

Calibration curves were constructed for Pb (II), Cu (II), and Hg (II) concentrations in the range 4.81– 24.10 μmol L^{-1}, 15.70 – 78.60 μmol L^{-1}, and 4.98 – 19.90 μmol L^{-1}, respectively. The ranges of the recoveries of Pb (II), Cu (II), and Hg (II) are between 106.9 to 95.8, from 103.2 to 97.6, and 105.8 to 95.3, respectively.

Table 3. Study of the maximum concentrations from the different ions to those that the signs of the respective voltammograms are not affected in the determination of lead 14.40 μmol L^{-1}, copper 47.50 μmol L^{-1}, and Mercury 14.90 μmol L^{-1} using lichen *Protousnea Magallanica* with solution of metallic and metalloid ecotoxic in μmol L^{-1}.

	Pb(II)	**Cu(II)**	**As(V)**	**Zn(II)**	**Mo(V)**	**Cr (III)**	**Al(II)**	**Cd(II)**	**Hg(II)**
Pb(II)	----	16.0	27.0	15.0	21.0	0.96	37.0	8.90	12.0
Cu(II)	19.0	-----	1.30	15.0	10.0	0.96	37.0	8.90	12.0
Hg(II)	19.0	16.0	1.30	1.50	10.0	1.90	37.0	8.90	-----

The effects of interfering metal ions, under optimum conditions of accumulation and measurement, were investigated by measuring the normal response for a 14.40 μmol L^{-1} for lead, 47.20 μmol L^{-1} for copper, and 14.90 μmol L^{-1} for mercury, on the addition of concentration of the metal ions. Table 3 shows the maximum concentrations of the different ions unaffected by the signals of the respective voltammograms. For example, arsenic does not interfere in determining mercury, lead, and copper at low concentrations of 1.30 mmol L^{-1} and zinc at low concentrations of 15.0 mmol L^{-1}.

An interesting point to highlight is the effect of Cr (III) on the response of Pb (II) and Hg (II), while Cr (III) affects the highest peak of Hg (II), Pb (II) and Cu (II) at 1.90 mmol L^{-1}; 0.96 mmol L^{-1} and 0.96 mmol L^{-1}, respectively.

Study of mosses in the modified carbon paste electrode

The mosses *Hypnum Cupresiforme* and *Ortotrichum Assimile* present only Pb, Cu, and Hg signals at -450, 10, and 330 mV under the previously studied experimental conditions.

Calibration curves were constructed for Pb (II), Cu (II), and Hg (II) concentrations in the range 4.81 to 24.10 μmol L^{-1}, 15.70 to 78.60 μmol L^{-1}, and 4.98 to 19.90 μmol L^{-1}, respectively for the modified electrodes with mosses.

Hypnum Cupresiforme-carbon paste electrode

The analytical parameters, % recovery (table 4), and the effects of interfering metal ions in the determination of the Pb (II), Cu (II), and Hg (II) with *Hypnum Cupresiforme*-carbon paste electrode, could be seeing in table 5. The ranges of the recoveries of Pb (II), Cu (II), and Hg (II) are from 105.4 to 97.8; from 104.3 to 96.5, and 104.3 to 101.1, respectively.

Table 4. Analytical parameters and % Recovery of modified electrode *Hypnum Cupresiforme* with lead (II), copper (II), and Mercury (II).

	Lead		**Copper**		**Mercury**	
Detect. Limit (μmol L^{-1})	0.022		0.238		0.046	
Quant. Limit (μmol L^{-1})	0.732		0.794		0.153	
Rang. Deter. (μmol L^{-1})	0.732 to 48.30		0.794 to 157.0		0.153 to 49.80	
Standard Deviation	0.183		0.427		0.103	
Equation (C in mol L^{-1})	Y= 17.01+2.50x10^{7}C		Y= –4.56 + 5.38x10^{6}C		Y= –3.39+6.69x10^{6}C	
Correlation coefficient	0.999		0.999		0.990	
Recovery	C (μmol L^{-1})	% R	C (μmol L^{-1})	% R	C (μmol L^{-1})	% R
	9.65	99.9	15.70	104.3	4.98	100.1
	14.40	97.8	31.40	99.7	9.97	103.8
	19.30	105.4	47.20	96.5	14.90	104.3
	24.10	102.8	78.60	100.3	19.90	101.5

Table 5. Study of the maximum concentrations from the different ions to those that the signs of the respective voltammograms are not affected in the determination of lead (II) 14.40 μmol L^{-1}, copper (II), 47.50 μmol L^{-1} and Mercury (II) 14.90 μmol L^{-1} using of modified electrode with the *Hypnum Cupresiforme* electrode with solution of metallic and metalloid ecotoxic in μmol L^{-1}.

	Pb (II)	**Cu (II)**	**As (V)**	**Zn (II)**	**Mo (V)**	**Cr (III)**	**Al (II)**	**Cd (II)**	**Hg (II)**
Pb (II)	----	16.0	27.0	15.0	21.0	0.96	37.0	8.90	12.0
Cu (II)	19.0	----	1.30	15.0	10.0	0.96	37.0	8.90	12.0
Hg (II)	19.0	16.0	1.30	1.50	10.0	1.90	37.0	8.90	----

Ortotrichum Assimile- carbon paste electrode

The analytical parameters, % recovery (table 6), and the effects of interfering metal ions in the determination of the Pb (II), Cu (II), and Hg (II) with moss-carbon paste electrode could be seen in table 7. A calibration curve was constructed for Pb (II), Cu (II), and Hg (II) concentrations in the range of 4.81 to 24.10 µmol L^{-1}, 15.70 to 78.60 µmol L^{-1} and 4.98 to 19.90 µmol L^{-1}, respectively. The ranges of the recoveries of Pb (II), Cu (II), and Hg (II) are from 103.9 to 94.9, from 99.5 to 95.6, and 105.1 to 99.3, respectively

Table 6. Analytical parameters and percentages of the Recovery of the modified electrode with the *Ortotrichum Assimile* by lead (II), copper (II), and Mercury (II).

	Lead		**Copper**		**Mercury**	
Detect. Limit (µmol L^{-1})	0.064		0.538		0.174	
Quant. Limit (µmol L^{-1})	0.214		1.790		0.581	
Rang. Deter. (µmol L^{-1})	0.214 to 48.30		1.79 to 157.0		0.58 to 48.30	
Standard Deviation	0.221		1.359		0.753	
Equation (C in mol L^{-1})	Y= –23.62 + 3.52x10^{7}C		Y= 0.40 + 1.26x10^{6}C		Y=–17.59 + 2.34x10^{7}C	
Correlation coefficient	0.999		0.998		0.999	
Recovery	C (µmol L^{-1})	% R	C (µmol L^{-1})	% R	C (µmol L^{-1})	% R
	9.65	103.2	15.70	99.5	4.98	101.8
	14.40	94.9	31.40	95.6	9.97	99.3
	19.30	101.4	47.20	99.1	14.90	102.5
	24.10	103.9	78.60	98.1	19.90	105.1

The effects of interfering metal ions in the determination of Pb (II), Cu (II), and Hg (II) using *Ortotrichum Assimile*-carbon paste electrode (table 7) under optimum conditions of accumulation and measurement were investigated by measuring the normal response for a 14.40 µmol L^{-1} for lead; 47.50 µmol L^{-1} for copper and 14.90 µmol L^{-1} for mercury, on addition of concentration of the metal ions. The concentration of ions at which the analytical signals are not affected can be seen in this table. For example, lead interferes with determining mercury at concentrations above 19.00 µmol L^{-1}.

Table 7. Study of the maximum concentrations from the different ions to those that the signs of the respective voltammograms are not affected in the determination of lead (II); 14.40 µmol L^{-1} copper (II); 47.50 µmol L^{-1} and Mercury (II) 14.90 µmol L^{-1} using of the modified electrode with the *Ortotrichum Assimile* electrode with a solution of metallic and metalloid ecotoxic in µmol L^{-1}.

	Pb (II)	**Cu (II)**	**As (V)**	**Zn (II)**	**Mo (V)**	**Cr (III)**	**Al (II)**	**Cd (II)**	**Hg (II)**
Pb (II)	----	16.0	27.0	15.0	21.0	0.96	37.0	8.90	12.0
Cu (II)	19.0	----	1.30	15.0	10.0	0.96	37.0	8.90	12.0
Hg (II)	19.0	16.0	1.30	1.50	10.0	1.90	37.0	8.90	----

CONCLUSIONS

This study is the result of using electrodes modified with lichens and mosses to determine 9 metal ions such as Cu, As, Pb, Hg, Zn, Cd, Al, Mo, and Cr. It was found that the electrode with the lichen Protousnea Magallánica and the mosses Hypnum Cupresiforme and Ortotrichum Assimile as sensitive agents presents the best signals with the lead (II), Copper (II) and Mercury (II) ions.

The preconcentration was carried out in order of 1 to 5 mg L^{-1} of ecotoxic metals in 0.1 mol L^{-1} acetate buffer (pH 5.0) for 10 and 30 minutes at an open circuit for the lichen and the mosses, respectively, after thoroughly rinsing with deionized water, a reductive potential of –1.0 V was applied for 40 seconds for the lichen and 180 seconds for the mosses and then the potential was swept between –1.0 and +0.6 V at a scan rate of 50 mV·s^{-1} to induce the anodic stripping of the reduced ecotoxic metals. Finally, the sensor's immersion in HCl 0.5 mol L^{-1} for 5 minutes assures the surface renewal, with the biomasses' active sites available for new measurements. All experiments were carried out at room temperature (20°C).

Studying the determination of the lead, copper, and mercury with the *Protousnea Magallanica*-carbon paste electrode presents a range of determination of 1.41 to 96.50 µmol L^{-1} for lead, 5.57 to 78.60 µmol L^{-1} for copper and 0.534 to 74.80 µmol L^{-1} for mercury. The recovery goes from the 106.9 to 95.8; 103.2 to 97.6 and 105.8 to 95.3 for Pb (II), Cu (II) and Hg (II), respectively.

When studying the determination of the lead, copper and mercury with the *Hypnum Cupresiforme* - carbon paste electrode, present a range of determination of the 0.732 to 48.30 µmol L^{-1} for lead; 0.794 to 157.0 µmol L^{-1} for copper and 0.153 to 49.80 µmol L^{-1} for mercury. The recovery goes from 105.4 to 97.8, 104.3 to 96.5, and from 104.3 to 101.1 for Pb (II), Cu (II), and Hg (II), respectively.

Studying the determination of the lead, copper, and mercury with the *Ortotrichum Assimile*-carbon paste electrode presents a range of determination of 0.732 to 48.30 µmol L^{-1} for lead, 0.794 to 157.0 µmol L^{-1} for copper, and 0.153 to 49.80 µmol L^{-1} for mercury. The recovery goes from 103.9 to 94.9, 99.5 to 95.6 and 105.1 to 99.3 for Pb (II), Cu (II), and Hg (II), respectively.

The effects of different interfering metal ions in the modified electrode with biomasses could be used to classify the nature of the functional groups involved in the binding based on the selectivity sequence. An interesting point to highlight is the effect of Cr (III) on the Pb (II), Cu (II), and Hg (II) response. Concentrations where Cr (III) is not interfering in the determination of Pb (II), Cu (II), and Hg (II) are 9.60 µmol L^{-1}, 9.60 µmol L^{-1}, and 1.90 µmol L^{-1}, respectively.

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CONFICT OF INTEREST

The authors declared that they have not conflict of interest.

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