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# DETERMINATION OF BIOSORPTION MECHANISM IN BIOMASS OF AGAVE, USING SPECTROSCOPIC AND MICROSCOPIC TECHNIQUES FOR THE PURIFICATION OF CONTAMINATED WATER

# DETERMINACIÓN DE MECANISMOS DE BIOSORCIÓN EN BIOMASA DE AGAVE UTILIZANDO TÉCNICAS ESPECTROSCÓPICAS Y MICROSCÓPICAS PARA DEPURACIÓN DE AGUA CONTAMINADA

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## Abstract

Lead ($Pb^{2+}$) and copper ($Cu^{2+}$) are polluting metals due to their toxicity; however, the extraction of these metals is essential for economic development, so it is important to look for efficient and low-cost alternatives that can remove heavy metals from the various bodies of water. One of the alternatives used in this work is biosorption, for which an Agro-industrial waste (epidermis from *Agave atrovirens*) was used to evaluate the affinity of removal of lead and copper in aqueous solutions; in addition, spectroscopy and microscopy techniques were used to elucidate and corroborate the removal and affinity capacity of the *A. atrovirens* epidermis for both metals studied. The optimal pH value for the removal of both metals was 3. The adsorption isotherms yielded a $q_{\max}$ of 25.7 and 8.6 mg/g for lead and copper, respectively. Adjusting to the Langmuir-Freundlich model, the adsorption kinetics were pseudo-second order, and it was found that the equilibrium time was at 140 min. The spectroscopy and microscopy analyses corroborated the affinity between metals and functional groups of the agave, as well as with the elemental analysis, which reported 17.38% of lead and 4.25% of copper.

*Keywords*: Agave epidermis waste, metals, biosorption, microscopy, spectroscopy.

## Resumen

El plomo ($Pb^{2+}$) y el cobre ($Cu^{2+}$) son metales contaminantes debido a su toxicidad; sin embargo, la extracción de estos metales es indispensable para el desarrollo económico, por lo que es importante buscar alternativas eficientes y de bajo costo que puedan remover metales pesados de los diversos cuerpos de agua. Una de las alternativas utilizadas en este trabajo es la biosorción, para la cual se utilizó un residuo agroindustrial (epidermis de *Agave atrovirens*), para evaluar la afinidad de remoción del plomo y cobre en soluciones acuosas; adicionalmente, se emplearon técnicas de espesctroscopía y microscopía que permitieron elucidar y corroborar la capacidad de remoción y afinidad que tuvo la epidermis de *A. atrovirens* para ambos metales estudiados. El valor óptimo de pH para la remoción de ambos metales fue 3. Las isotermas de adsorción arrojaron una $q_{\max}$ de 25.7 y 8.6 mg/g para el plomo y cobre, respectivamente. Ajustando al modelo de Langmuir-Freundlich, las cinéticas de adsorción resultaron de pseudo-segundo orden, se encontró que el tiempo de equilibrio es a los 140 min. El análisis espectroscópico y microscópico, corroboró la afinidad entre metales y grupos funcionales del agave, así como con el análisis elemental, el cual reportó 17.38% de plomo y 4.25% de cobre.

*Palabras clave*: residuo de epidermis de agave, metales, biosorción, microscopia, espectroscopia.

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# 1 Introduction

In Mexico, the mining-metallurgical sector contributes with 4% of the national gross domestic product, according to data from the Ministry of Economy, it is among the top 10 producers of 16 different minerals. This activity generates around 352 thousand direct jobs and more than 1.6 million indirect jobs. Within the 16 minerals are lead ($Pb^{2+}$) and copper ($Cu^{2+}$); however, these metals are considered contaminants due to their toxicity and abundance (Covarrubias and Peña, 2017; Secretaría de Economía, 2018). The extraction of these metals is increasing and, consequently, also the contamination and the environmental impact. For this reason it´s important to treat the contamination caused by heavy metals. One of the alternatives that helps to solve this problem is the use of Agro-industrial residues that serve as bio-adsorbents for the removal of heavy metals and other contaminating compounds, which has resulted in an efficient and low-cost technique.

An important specie to consider as an agro-industrial waste is the Agave, since from this plant various beverages such as tequila, mezcal and pulque are obtained, all of them nationally and internationally demanded but generating a large amount of waste. This waste generated from agave fibers has been studied and nowadays presents several crafts or national uses. Studies by Hernández-Botello *et al*., 2014, showed that Agave's epidermis is susceptible to capture lead without giving expensive treatments to the biomaterial. The present work proved that the epidermis of Agave with a smaller particle size, is a biosorbent material for two metals (lead and copper) in order to give an added value to this biomaterial and change its image from an agro-industrial waste to an industrial raw material able to be used in biosorption process. Therefore, it is important to study this technique deeply in order to elucidate the mechanism of adsorption that occurs between the biosorbent and the contaminant.

There are several microscopy techniques that have been little used in the area of biosorption and that could help to complement the information obtained from the kinetics and isotherms of adsorption, such as scanning electron microscopy and characteristic X-ray scattering spectroscopy, confocal laser scanning microscopy (MCBL), X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FTIR). Therefore, this work focused on the study of the biosorption process, using the residue of the *A. atrovirens* epidermis for the batch removal of lead and copper, and the use of microscopy and spectroscopy techniques to elucidate the mechanism of biosorption.

# 2 Materials and methods

## 2.1 Biomass Sample preparation

The biomaterial *A. atrovirens* epidermis was obtained from waste of agave leaves remnant of handcraft elaboration of a local beverage ("pulque"). Agave leaves were collected from 5-7 years old plants (Mexico City). Agave leaves were washed with distilled water and the cuticle was removed manually. The pre-treatment of the samples was carried out as previously reported by Hernandez-Botello *et al*., (2014), afterwards samples were triturated using an analytical mill (IKA A10, WerkeGmbH & Co. KG, Staufen, Germany) and sieved, selecting a particular size ranging 0.5-1 mm, were packed in hermetic bottles and stored at room temperature for further use.

## 2.2 Influence of pH on biosorption

The dependence of metal uptake on pH was studied by using a concentration of 100 mg/L of lead and 50 mg/L of copper in the pH range of 1-4. Amounts of 0.1 ± 0.01 g of the biosorbent were placed into conical flasks and $HNO_3$ or NaOH 0.1 M solutions were used for pH adjustment. The reagents used in this work were from Merck® (Germany) and the solutions were prepared with deionised water (Moreno-Rivas *et al*., 2016). The determination of metals concentrations was carried out by atomic absorption spectroscopy (SpectAA 55B, Varian)

## 2.3 Adsorption isotherms

$Pb^{2+}$ and $Cu^{2+}$ adsorption isotherms were performed for separated by adding 0.1±0.01g of the *A. atrovirens* epidermis into 100 mL conical flasks, to which 40 mL of solution were added at various concentrations (from 5 to 200 mg/L), were stirred in a rotary shaker at 175 rpm for 24 h until equilibrium concentration was reached, afterwards the biomass was filtered through a 0.45 $\mu$m pore size cellulose nitrate membrane filter and the filtrate was analysed. To adjust the pH to the desired value, $HNO_3$ or NaOH solutions were added (Megat *et al*., 2012; Zhang *et al*., 2013; Flores-Alamo *et al*., 2015). The metal uptake per gram of biosorbent material ($q_e$), was determined by means of mass balance analysis (Eq. 1), where $C_i$ and $C_e$ are the initial and equilibrium metal concentrations (mg/L), $V$ is the volume of the solution (mL), and $m$ is the mass of *A. atrovirens* epidermis used (g).

$$q_e = \frac{V(C_i - C_e)}{1000m} \tag{1}$$

The distribution of adsorbate between the liquid phase and the solid phase when adsorption process reaches equilibrium is given by the adsorption isotherms. The adsorption isotherms were analysed using three models Langmuir, Freundlich and Langmuir-Freundlich (Ec. 2, 3 and 4, respectively).

The Langmuir model is described by the following equation:

$$q_e = \frac{q_{\max} b C_e}{1 + bC_e} \tag{2}$$

The Freundlich model equation is given as:

$$q_e = kf(C_e)^{1/n} \tag{3}$$

The Langmuir-Freundlich model is described by the following equation:

$$q_e = \frac{(b * C_e)^{1/n}}{(1 + (b * C_e)^{1/n}} \tag{4}$$

For the three mathematical models $q_e$ is the amount of adsorbate retained by the unit of mass of adsorbent (mg/g), $C_e$ is the equilibrium concentration of the adsorbate in the liquid phase (mg/L). For the Langmuir model two constants $q_{\max}$ and b are obtained, which are the Langmuir constants related to the maximum sorption capacity for a complete monolayer (mg/g), and with the affinity between the adsorbent and the adsorbate (L/mg), respectively; whereas for the Freundlich model, $k_f$ is the Freundlich constant and is related to the adsorption capacity of the biosorbent; n is a dimensionless constant related to the affinity between the adsorbent and the adsorbate. For the Langmuir-Freundlich model, the constants are b and n, which represent the affinity in the biosorption process (Velázquez- Jiménez *et al*., 2013).

## 2.4 Kinetic studies

Kinetic experiments were performed by adding 0.25 g of the *A. atrovirens* epidermis in 100 mL of a solution of $Pb^{2+}$ (100 mg/L), $Cu^{2+}$ (50 mg/L) using a thermostated glass cell at 25.0 ± 0.1 °C, which was continuously bubbled with nitrogen to remove dissolved $O_2$ and $CO_2$. Aliquots of 1 mL at different time intervals were collected during 24 h. The temperature was kept constant at 25 °C (Corral-Escárcega *et al*., 2017; Andrade *et al*., 2018). The kinetic parameter to express the amount of metal adsorbed at time t, $q_t$ (mg/g), was calculated by means of the following equation:

$$q_t = \frac{(C_o - C_t)V}{1000m} \tag{5}$$

Where $C_o$ is the initial concentration of the metal (mg/L), $C_t$ is the concentration at time t of the metal (mg / L), V is the volume in which the metal is distributed (mL) and m is the amount of biomaterial of agave epidermis used (g).

Models of the pseudo first-order, equation (6), and pseudo second-order kinetic, equation (7), were used to fit the kinetic data:

$$q_t = q_e \left[1 - \exp(-k_1 t)\right] \tag{6}$$

Where $q_e$ (mg/g) and $q_t$ (mg/g) are the amount of metal adsorbed at equilibrium and at time t (min), respectively; $k_1$ (1/min) is the velocity constant of the pseudo-first order model, while $k_2$ (g / (mg*min)) is the velocity constant of the pseudo-second order model.

## 2.5 Microscopy and spectroscopy techniques

### 2.5.1 Atomic absorption spectroscopy

Atomic absorption spectroscopy was performed by using the equipment SpectAA 55B (Varian) set with hollow cathode lamps suitable for the determination of $Pb^{2+}$ and $Cu^{2+}$. The gas stream was composed of acetylene used as a fuel and air as a carrier (Lasheen *et al*., 2012, Medellin-Castillo *et al*., 2017).

### 2.5.2 FTIR spectroscopy

In order to estimate the structural composition of the *A. atrovirens* epidermis, and how it was transformed after the metal biosorption process, analyses were performed by Fourier transform infrared spectroscopy (FTIR). Infrared spectra were captured on a LabRAM HR 800 (Horiba Jobin Yvon; Miyanohigashi, Kyoto, Japan) computer with a module coupled with ATR-FTIR, the scan was performed in the range of 400 to 4000 $cm^{-1}$, the scans were performed in triplicate to identify functional groups present in the epidermis

(Lasheen *et al*., 2012; Velázquez- Jimenez *et al*., 2013; Romero-Cano *et al*., 2016; Baby and Beeregowda., 2018).

#### *2.5.3 Confocal laser scanning microscopy*

Confocal laser scanning microscopy (CLSM), was conducted to the biomaterial before and after being exposed to biosorption, and after dried at 60 °C. This was carried out to obtain spectra of the auto fluorescence (Perea-Flores *et al*., 2011). Biomaterial was mounted on a glass slide and observed by CLSM (710, Carl Zeiss, Germany). Samples were excited using laser wavelength at 405, 488, 561 and 633 nm with a working model of spectral channels for the detection of autofluorescence signals of the major components of *A. atrovirens* epidermis. The fluorescence intensity was detected by LSM software ZEN 710.

#### *2.5.4 Characterisation by scanning electron microscopy and EDX*

The characterisation of *A. atrovirens* epidermis before and after the biosorption process was carried out with an environmental scanning electron microscope (ESEM, MIRA3LMU in Low Vacuum Secondary Electron TESCAN Detector mode, TESCA Company, BSE detectors for quantification the chemical composition). At least, 5 view fields were determined for each sample at 15 kV with magnifications 100, 200 and 500 $\mu$m (Orozco-Guareño *et al*., 2010; Megat *et al*., 2012; Oliveira *et al*., 2014). Additionally, elemental chemical microanalysis was performed to identify and observe the presence of the metals in the sample. This analysis was carried out using the EDX detector (EDAX, model New XL-30, Mahwah, NJ, USA, and an activation area of 10 $mm^2$) which was coupled to the ESEM XL-30 (Philips Electronics, Holland). At a voltage acceleration of 25 kV, 5 observation fields were analysed with a retention time of 60 s and a count rate of 1000 to 3000 cps (Perea-Flores *et al*., 2011; Moreno-Rivas *et al*., 2016).

#### *2.5.5 Photoelectron X-ray Spectroscopy*

Biosorbent XPS analysis before and after contact with $Pb^{2+}$ and $Cu^{2+}$ was carried out using a K-$\alpha$ spectrometer from Thermo Scientific. Employing an Al K$\alpha$ source (1486 eV) monochromatised to determine the elements C, N, O, Pb and Cu present in the surface of the biosorbent. The conditions for analysis were step = 1 eV, fixed time = 100 ms, pass energy = 50 eV (Cruz-Olivares *et al*., 2010; Oliveira *et al*., 2014).

## 3 Results and discussion

### *3.1 Influence of pH on biosorption*

The pH of the solution is an important parameter that can affect the process of biosorption of heavy metals in the aqueous phase. Fig. 1 shows the effect of pH of the equilibrium solution on the adsorption of metals by *A. atrovirens* epidermis. The biosorption of metals reached the highest elimination of lead and copper at pH 3.0, the capacity of *A. atrovirens* epidermis biosorption depended strongly on the pH of the solution in equilibrium. Therefore, if the groups responsible for adsorption of the metals are weak acids or bases (Velazquez-Jimenez *et al*., 2013), the availability of free sites depended on the pH. Hence, the characterisation of the pH effect on equilibrium studies is necessary to accurately assess the biosorption parameters. (Hamissa *et al*., 2010; Auta and Hameed., 2011; Megat *et al*., 2012). It is also important that the pH value is less than 4.5, because the values above 4.5 can be precipitate, and the focus of this research is the process of biosorption, not the precipitation process (Certucha-Barragan *et al*., 2010).

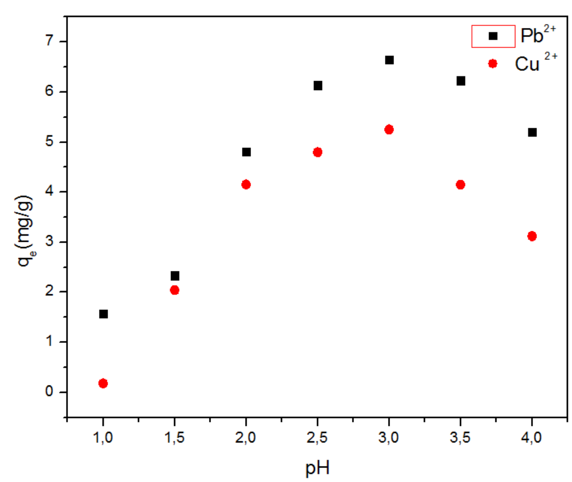


Fig. 1. Effect of pH on $Pb^{2+}$ and $Cu^{2+}$ biosorption onto agave epidermis waste.

Table 1. Langmuir, Freundlich and Langmuir-Freundlich isotherm model parameters for the adsorption of $Pb^{2+}$ and $Cu^{2+}$ onto agave epidermis waste.

| Langmuir isotherm | $q_{max}$ (mg/g) | b(L/mg) | | $R^2$ |
|---|---|---|---|---|
| $Pb^{2+}$ | 23.4±1.68 | 0.05±0.01 | | 0.95 |
| $Cu^{2+}$ | 5.2±0.35 | 0.03±0.00 | | 0.95 |
| **Freundlich isotherm** | **Kf (mg/g)** | **n** | | **$R^2$** |
| $Pb^{2+}$ | 3.4±0.48 | 2.58±0.22 | | 0.98 |
| $Cu^{2+}$ | 0.6±0.08 | 2.57±0.20 | | 0.97 |
| **Langmuir-Freundlich isotherm** | **$q_{max}$ (mg/g)** | **b(L/mg)** | **n** | **$R^2$** |
| $Pb^{2+}$ | 25.7±2.03 | 0.04±0.01 | 1.16±0.00 | 0.96 |
| $Cu^{2+}$ | 8.6±4.11 | 0.01±0.01 | 1.66±0.43 | 0.97 |

## 3.2 *Isotherms of biosorption*

The adsorption isotherms, as mentioned in the methodology, were adjusted to three mathematical models. The mathematical model that best fitted the experimentally obtained data was the Langmuir-Freundlich model, which reported a $q_{max}$ of 25.7 y 8.6 mg/g, for lead and copper, respectively,. The values corresponding to each model are summarised in Table 1.

As mentioned above, the term b, is the affinity constant related to the adsorption energy, n is the constant relative to the heterogeneity of the biosorbent surface, this constant shows values between 1.16 and 1.66, which suggests that the adsorption is favourable and that the surface of the biosorbent is heterogeneous. This mathematical adjustment at low concentrations of adsorbate is simplified to the Freundlich isotherm, where the adsorption has different affinities, first occupying the sites of higher affinity, and later occupying the lower affinity sites, forming a multilayer. Therefore, it may be assumed that an adsorption of physical type is occurring. While at high concentrations it was similar to Langmuir type adsorption, which suggests a monolayer adsorption of chemical type. Medillin-Castillo *et al*. (2017), used residues of natural fibers of *Agave lechuguilla Torr* from the ixtlera industry and reported that ten runs 9 were modelled better by the Langmuir model and 8 by the Freundlich model. It should be noted that in that work only two mathematical models were adjusted. In the present work, the data obtained was better explained with the Langmuir-Freundlich model. In the work of Hamidpour *et al*. (2018) the same mathematical adjustment was obtained to the Langmuir-Freundlich isotherm but in that case it corresponds to the Sips isotherm, in which as the concentration of the metal increases the biosorption capacity increases, in that work pistachio residues show the biosorption of cadmium and lead, having greater affinity for the removal of lead (similar to what was found in the present work). The biosorption process studied in the present work showed that the biomaterial at high concentrations reported a higher removal capacity, namely 25.7 and 8.6 mg / g for lead and copper, respectively.

## 3.3 *Biosorption kinetics*

Adsorption kinetic studies of $Pb^{2+}$ and $Cu^{2+}$ by *A. atrovirens* epidermis waste were developed in order to determine the minimum time necessary to achieve the sorption equilibrium. The best fitting for metals was obtained with the pseudo-second order model, where all the determination coefficients ($R^2$) were greater than 0.98 for $Pb^{2+}$, and 0.97 for $Cu^{2+}$. Cruz-Olivarez *et al*., (2011) reported similar results to those obtained in the present work, corresponding to the process of adsorption of lead by *Pimenta dioica*; when performing the adsorption kinetics, they reported an adjustment to the mathematical model of pseudo-second-order, which suggests a chemisorption process between the adsorbent and the adsorbate. Table 2 summarises the pseudo-second order kinetic models that were used to fit the experimental data of the adsorption kinetics for $Pb^{2+}$ and $Cu^{2+}$, whose equilibrium time was 140 min (Auta and Hameed., 2011; Cruz-Olivares *et al*., 2011 Zhang *et al*., 2013; Corral- Escárcega *et al*., 2017; Andrade *et al*., 2018; Ramirez-Rodriguez *et al*., 2018).

As well, the mathematical adjustment showed that the *A. atrovirens* epidermis had greater affinity for lead biosorption ($q_t$ = 0.65 mg/g), with respect to copper biosorption ($q_t$ = 0.17 mg/g).

Table 2. Pseudo-second-order kinetic analysis for $Pb^{2+}$ and $Cu^{2+}$ adsorption onto *A. atrovirens* epidermis waste.

| Pseudo-second-order kinetic | qt, exp(mg/g) | $K_2$ | $q_e$ | $R^2$ |
|---|---|---|---|---|
| $Pb^{2+}$ | 0.65 | 0.09±0.01 | 0.70±0.00 | 0.98 |
| $Cu^{2+}$ | 0.17 | 1.01±0.13 | 0.16±0.00 | 0.97 |

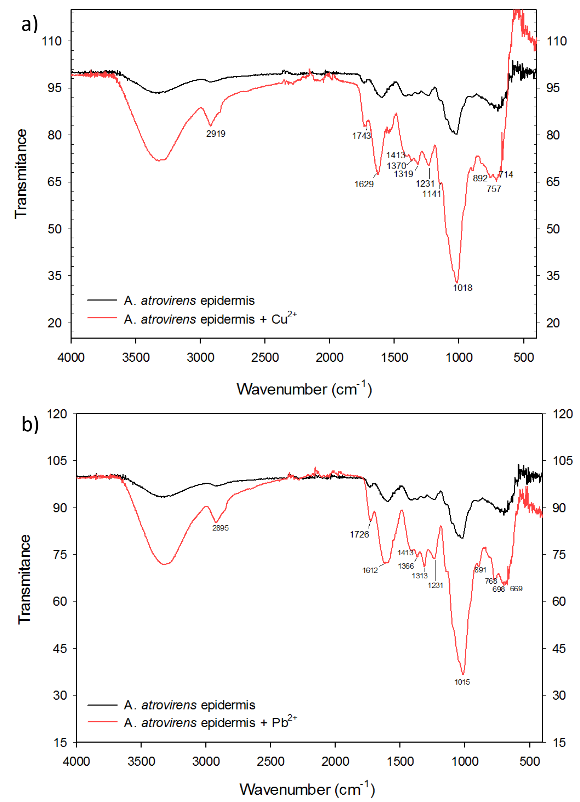


Fig. 2. FTIR spectra of agave epidermis after and before of process biosorption with $Pb^{2+}$ and $Cu^{2+}$.

These results corroborate the values obtained in the adsorption isotherms, that is, removal of a greater amount of lead than that of copper was observed. Furthermore, the occurrence of physisorption and chemisorption processes forming monolayers and multilayers was observed. Additionally, the work reported by Hamidpour *et al*. (2018) obtained the same mathematical adjustment to the pseudo second-order model, in which the maximum removal capacities were at 120 min.

## *3.4 Microscopy and Spectroscopy studies*

### *3.4.1 FTIR of Agave atrovirens epidermis waste*

Figure 2(a-b) shows the FTIR spectrum of the biosorbent before and after the biosorption of $Pb^{2+}$ and $Cu^{2+}$on *A. atrovirens* epidermis, as observed, there are different peaks that allow to identify functional groups such as: hydroxide, carboxyl, sulfhydryl, nitriles, etc., have been proposed as responsible for the metal binding capability of agave waste (Velasquez-Jimenez *et al*., 2013)., however, according to the cited authors, no enough information is available on the agave epidermis. Different reports confirm the presence of these chemical groups and the use of such information to know the number of binding sites, accessibility, chemical state or affinity between binding sites and metals (Wase and Forster 1997; Velázquez-Jimenez *et al*., 2013).

The groups in *A. atrovirens* epidermis before the biosorption process three bands were found: a strong symmetric one at 1018 $cm^{-1}$, a weaker asymmetric at 1231 $cm^{-1}$ and 1590 $cm^{-1}$ corresponding to cellulose, lignin and C-O, C=O, respectively. After the biosorption process appeared the peak in the frequency 3326 $cm^{-1}$ is due to stretching and vibrations of several hydroxyl bonds C-OH, which indicate the presence of absorbed water, aliphatic primary and secondary alcohols found in molecules such as cellulose, hemicellulose and lignin. The principal compounds of the *A. atrovirens* epidermis associated with the process of biosorption for affinity they: Cellulose, Lignin and Hemicellulose, the signals apparent in both figures showed that this peaks corresponding this compounds, the signals in 2919 $cm^{-1}$ , 2920 $cm^{-1}$ , 1413 $cm^{-1}$, 1370 $cm^{-1}$, 1366 $cm^{-1}$, 1018 $cm^{-1}$, 1015 $cm^{-1}$, 892 $cm^{-1}$ and 891 $cm^{-1}$ can be attributed to the cellulose groups correspond to the stretch of methyl groups, $-CH_2$ and -CH, present especially in aliphatic cellulose fragments. The signals at 1743 $cm^{-1}$, 1724 $cm^{-1}$ correspond to the Hemicellulose. The bands in 1629-1612 $cm^{-1}$, 1319 $cm^{-1}$,1313 $cm^{-1}$, 1141 $cm^{-1}$, 1231 $cm^{-1}$, 768 $cm^{-1}$, 669 $cm^{-1}$ can be ascribed to the vibration by the presence of Lignin. Those peaks can be attributed to the vibration of C=C bonds present in lignin and C=O of carbonyl groups and the presence of N-H bonds and C = S.The identified adsorption bands in the spectra are similar to agave bagasse (Hernandez-Hernandez *et al*., 2016; Cruz-Olivares *et al*., 2010; Velazquez-Jimenez *et al*., 2013; Flores-Alamo *et al*., 2015; Romero-Cano *et al*., 2016).

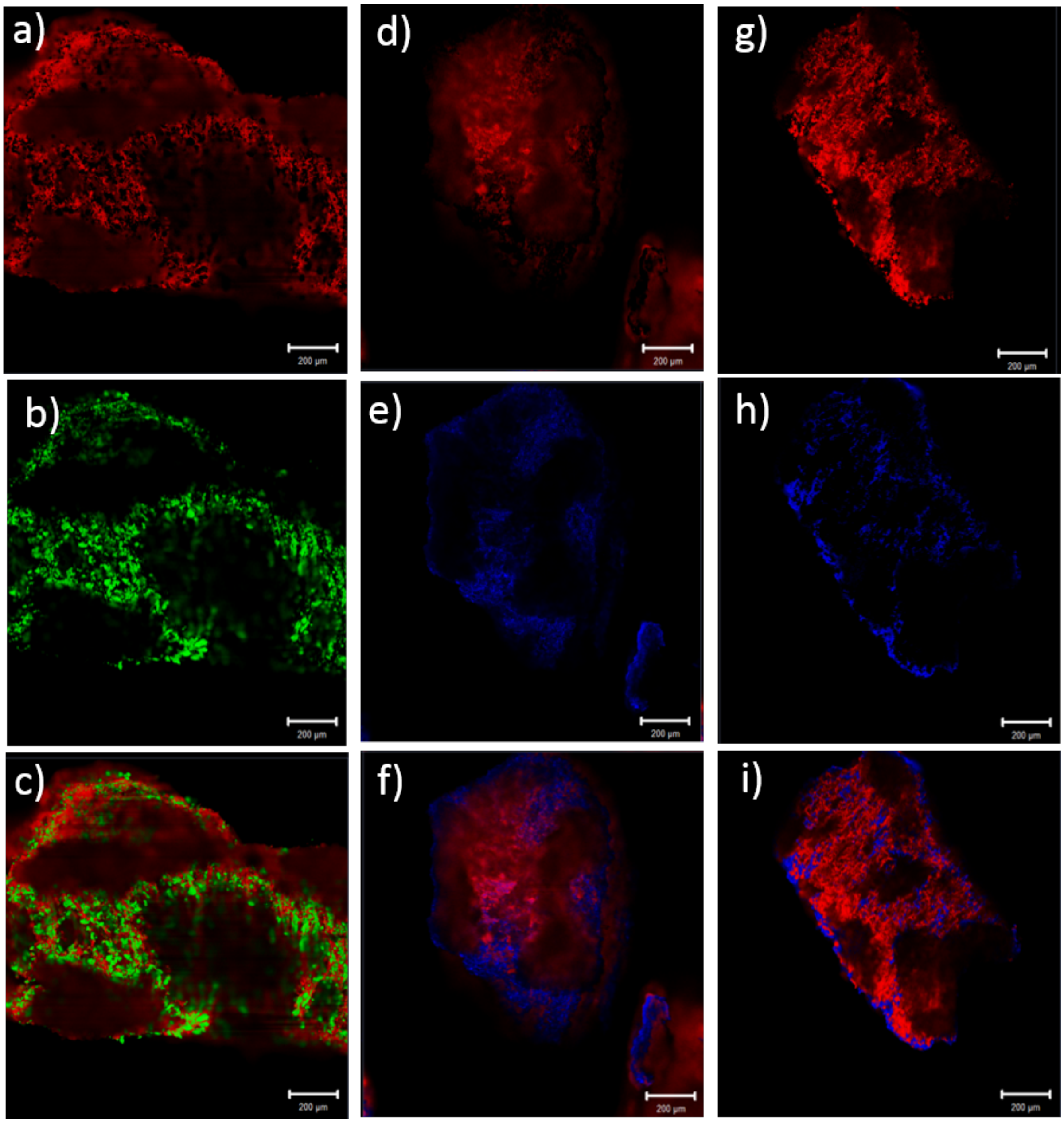


Fig. 3. 3D images of confocal microscopy of laser scanning of agave epidermis before and after the biosorption process.3a-c fluorescence of lignin, chlorophyll and epidermis, respectively, before the biosorption process. 3d-f fluorescence of lignin, metal and epidermis after the process of biosorption with Lead, 3g-i, fluorescence of lignin, metal and epidermis after the process of biosorption with copper.

Each of these groups is involved in the processes of adsorption according to what is reported in literature. The signal displacement, the intensity decrease, as well as the disappearance and appearance of frequency peaks indicate that there are interactions between the biosorbent and the metals, thus causing the attraction between components, which allows the adsorption of the lead and copper studied in this work

### *3.4.2 Confocal scanning laser microscopy*

The samples before and after the biosorption process were analysed. Fig. 3 a-c shows the fluorescence of the *A. atrovirens* epidermis before the biosorption process. In Fig. 3a, the fluorescence of the lignin present in the epidermis is observed, while in Fig. 3b the fluorescence of chlorophyll is shown, and in 3c, the fluorescence of both materials (lignin + chlorophyll) appears. In Fig. 3d-f the agave epidermis is observed after the biosorption process with lead, and in Fig. 3g-i the biosorption process with copper is observed. As it is possible to observe in Fig. 3f and 3i, the Langmuir model adjusted to the isotherm is corroborated, by means of which it is inferred that the surface of the agave epidermis is heterogeneous, as well as a greater affinity for the adsorption of lead, showing in this case a greater fluorescence of lead. Apparently, the interaction that is taking place in this process can be attributed to lignocelluloses groups and to the chlorophyll present in the epidermis, since their corresponding spectra are attenuated when the biosorption process is carried out (Cruz-Olivares *et al*., 2011; Dos Santos *et al*., 2014).

### *3.4.3 Scanning electron microscopy (SEM) analysis*

As shown in Table 3 and the Fig. 4 (a-c), the data obtained by SEM, agree with the data obtained from the isotherms, adsorption kinetics, CLSM, as well as with the FTIR, in which it is shown that the agave epidermis has greater affinity to lead, having a greater biosorption capacity. Also, in this case the disappearance of Mg is observed. The molecules responsible for the biosorption can be lignin, chlorophyll, cellulose, and hemicellulose, which have groups and metals that can participate in the biosorption process, mainly the carboxyl, hydroxyl and metals groups of the porphyrin ring of chlorophyll (Hernandez-Hernandez *et al*., 2016; Velazquez-Jimenez *et al*., 2013).

### *3.4.4 X-ray photoelectron spectroscopy (XPS)*

The X-ray photoelectron spectroscopy (XPS) is a technique used to identify the interaction of a metal ion with the chemical groups on the surface of an adsorbent. This study was performed to confirm the presence of lead and copper on the *A. atrovirens* epidermis (Fig. 5). XPS spectra showed in Fig. 5a, provided evidence of the presence of all elements (C, N, O) associated with those chemical groups and showed that, by creating a chemical bond between

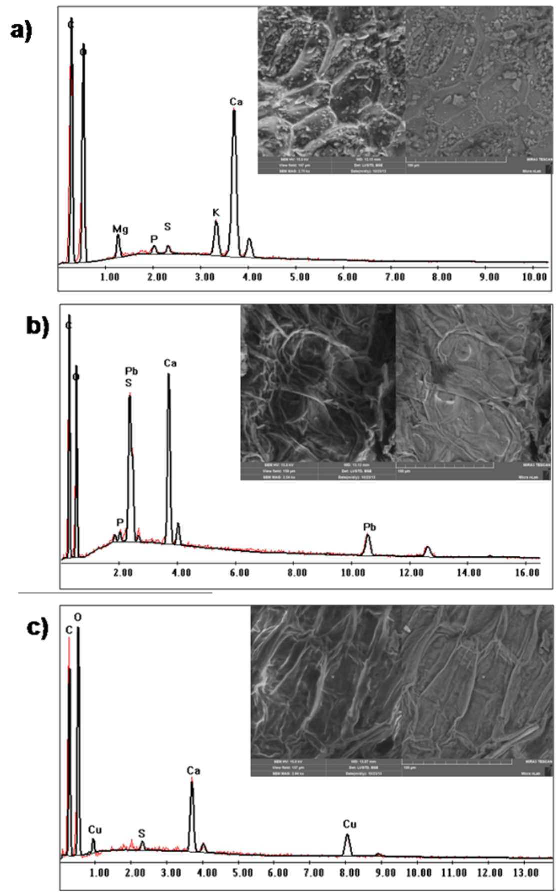


Fig. 4. (a-c) images of scanning electron microscopy and spectrum EDX of *A. atrovirens* epidermis before and after the biosorption process with lead and cupper respectively.

the metal ion and an atom on the surface of the adsorbent, this changed the distribution of electrons around the atoms (Oliveira *et al*., 2014). Electron-donating components may decrease the binding energy (BE) of electrons located at levels close to the atomic nucleus, while electron-withdrawing components may increase its binding energy (BE). Subsequently, the individual spectra of lead (144.78 and 139.88 eV) and copper (953.38 and 933.58 eV) were obtained, which proved the presence the lead and copper on agave epidermis (Cruz- Olivares *et al*., 2010; Orozco-Guareño *et al*., 2010).

Also in Fig. 4, it is confirmed, together with the other techniques used in the present investigation that the agave epidermis showed greater affinity with lead, obtaining a greater signal in the XPS spectrum, with respect to that obtained with copper.

Table 3. Elemental analysis of agave epidermis before and after the biosorption process.

| Element | Weight Agave epidermis (%) | Weight Agave epidermis + $Pb^{2+}$ (%) | Weight Agave epidermis + $Cu^{2+}$ (%) |
|---|---|---|---|
| *C* | 48.02 | 45.23 | 49.32 |
| *O* | 43.31 | 30.64 | 43.2 |
| *Mg* | 1 | 0 | 0 |
| *P* | 0.25 | 0.23 | 0 |
| *S* | 0.24 | 0.72 | 0.28 |
| *K* | 1.21 | 0 | 0 |
| *Ca* | 5.96 | 5.8 | 2.94 |
| *Cu* | 0 | 0 | 4.25 |
| *Pb* | 0 | 17.38 | 0 |

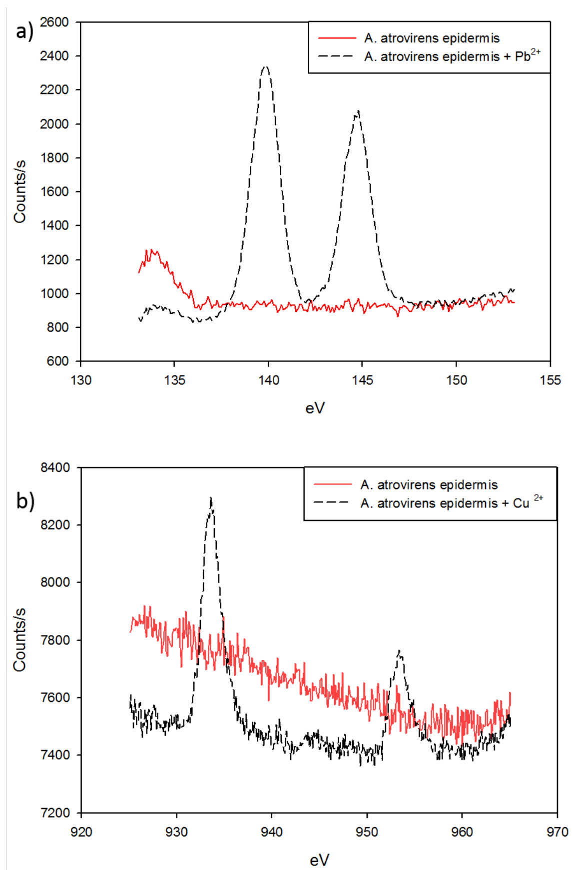


Fig. 5. XPS spectra for the agave epidermis after the biosorption process for lead (a) and copper (b).

## Conclusions

The use of agro-industrial waste is a friendly alternative to the environment, in this case the use of the agave epidermis, which is usually left as waste, showed properties that make it capable to biosorb lead and copper from a contaminated effluent. This allows us to conclude that it is an efficient and inexpensive biosorbent material. Likewise it is demonstrated that the techniques of Environmental Scanning Electron Microscopy (E-SEM), Confocal Laser Scanning Microscopy, Scanning Electron Microscopy (SEM), Infrared Spectroscopy with Fourier Transform (FTIR) and Atomic Absorption Spectroscopy, are novel techniques that are implemented in the characterization of biosorbent materials with the aim of elucidating its biosorption process.

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### *Nomenclature*

| | |
|---|---|
| ATR | Attenuated Total Reflectance |
| b | fit coefficient Langmuir model, L/mg |
| *b* | fit coefficient Langmuir-Freundlich model, L/mg |
| BSE | detectors for quantification the chemical composition |
| $C_e$ | equilibrium metal concentration, mg/L |
| $C_i$ | initial metal concentration, mg/L |
| CLSM | Confocal Laser Scanning Microscopy |

| | |
|---|---|
| $C_o$ | the initial concentration of the metal, mg/L |
| $C_t$ | concentration at time t of the metal, mg / L |
| ESEM | Environmental Scanning Electron Microscope |
| FTIR | Fourier Transform Infrared Spectroscopy |
| $k_1$ | velocity constant of the pseudo-first order model, 1/min |
| $k_2$ | velocity constant of the pseudo-second order model, g/mg*min |
| $k_f$ | fit coefficient Freundlich model, mg/g |
| $m$ | mass of agave epidermis used, g |
| n | fit coefficient Freundlich model, - |
| $n$ | fit coefficient Langmuir-Freundlich model, - |
| $q_e$ | the metal uptake per gram of biosorbent material, mg/g |
| $q_e$ | amount of metal adsorbed at equilibrium, mg/g |
| $q_{\max}$ | the maximum sorption capacity, Langmuir constants, mg/g |
| $q_t$ | amount of metal adsorbed at time t, mg/g |
| $R^2$ | coefficient of determination |
| t | time, min |
| $V$ | volume of the solution, mL |
| XPS | X-ray Photoelectron Spectroscopy |

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