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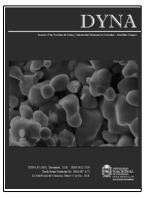

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Quantification of lead using atomic absorption spectrometry in thermoformed and biodegradable flexible films made from cassava *(Manihot esculenta crantz)*

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Abstract
Recently developed biopolymers can contain lead due to contamination from the origin of the material used to make them. A method for determining the presence of lead is proposed, using GF-AAS in thermoformed and biodegradable flexible films and flour and starch samples from Cassava. Acid digestion with reflux was optimized and the statistical quality parameters were standardized. The graphite furnace heating program was adjusted through pyrolysis and atomization curves. The working range was from 2.0 to 7.0 µg/L, with limits of detection and quantification of 0.618 and 1.853 µg/L, respectively. The precision was evaluated using intermediate precision and repeatability of the method, which showed standard deviations of less than 4.70% and 4.36%, respectively. The percentage of recovery ranged from 94.8% to 106.5%. The results obtained support the suitability of the method for determining the presence of lead. Lead concentrations were below 1 mg/Kg, indicating that these polymers can be used for food containers.

Keywords: biodegradable polymers; cassava *(Manihot esculenta crantz)*; grafite furnace; lead.

Cuantificación de plomo por espectrometría de absorción atómica en termoformados y películas flexibles biodegradables elaboradas a partir de yuca *(Manihot esculenta crantz)*

Resumen
Los recientemente desarrollados biopolímeros podrían contener plomo, debido a la contaminación desde el origen del material usado en su elaboración. Proponemos un método para la determinación de plomo por EAA-HG en termoformados y películas flexibles biodegradables, harinas y almidones elaboradas a partir de yuca. Se optimizó la digestión ácida con reflujo y se estandarizaron los parámetros de calidad estadísticos. El programa de calentamiento del horno de grafito fue ajustado mediante las curvas de calcinación y atomización. El rango de trabajo fue de 2.0 a 7.0 µg/L, con límites de detección y cuantificación de 0.618 y 1.853 µg/L, respectivamente. La precisión se evaluó por la precisión intermedia y repetibilidad del método, que mostraron desviaciones estándar menores a 4.70% y 4.36%, respectivamente. El porcentaje de recuperación varió desde 94.8% a 106.5%. Los resultados obtenidos soportan la idoneidad del método para la determinación de plomo. Las concentraciones de plomo fueron inferiores a 1 mg/Kg, indicando que estos polímeros pueden usarse como contenedores de alimentos.

Palabras clave: polímeros biodegradables; yuca *(Manihot esculenta crantz)*; horno de grafito; plomo

1. Introduction

Biodegradable thermoformed and flexible films can be made from cassava flours and starches by adding substances such as: fique fiber, gelatin, poly(butylene adipate-co-terephthalate), polylactic acid, glycerol, plasticizer, cellulose, pullulan, and natural extracts [1-12]. Nowadays, industries are trying to improve their food container products made from natural compounds [13], such as thermoformed and

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flexible films obtained from renewable sources (cassava). These constitute a new and environmentally friendly industrial alternative due to their fast and easy degradation and main purpose which is to replace the synthetic plastic that people use regularly [2,4,6,7,10,14,15]. These characteristics greatly benefit different environmental ecosystems.

A typical method for thermoformed manufacture is the compression molding technique, in which the material is placed into an open mold to which pressure and heat are applied. Single screw extrusion is used for the manufacture of biodegradable flexible films, and by turning the screw and applying heat, the material is pushed along and melted [5,12,16].

In developing countries, the accelerated process of industrialization combined with the use of intensive agricultural techniques and the inappropriate management of waste have led to an increase in the levels of substances considered harmful or toxic to living beings, such as heavy metals. These metals can cause serious damage and can enter the human body from emissions in the environment, through contact with industrial fallout as well as agricultural activities such as preparing pesticides, contamination from chemical fertilizers and irrigating with inadequate quality water [17-19]. Materials in contact with foods can cause heavy metal migration, this being a negative interaction between packaging and food. Additionally, heavy metals are not biodegradable, they remain in the environment and accumulate in living organisms over time because they are not metabolized [17,20-23].

Contamination with heavy metals may be produced during the manufacture of biodegradable polymers due to contamination of the raw material used for their processing (cassava flour and starch, fique fiber, polylactic acid, glycerine, *etc.*) or cross-contamination during manufacture, for example, during petroleum and non-petroleum activities, from pots used for cooking and storage, during drying techniques for moisture content reduction processes, and from utensils or contaminated water [18,24-26]. For this reason, a tracking process during manufacturing is necessary to determine the presence of toxic heavy metals in the film, for the purpose of safeguarding health by limiting exposure [20,27]. Among these metals, lead (Pb) is considered an unsafe and toxic heavy metal that can cause serious health hazards, affecting the cardiovascular, nervous and genitourinary systems, the biosynthesis of hemoglobin and long-term exposure may cause anemia [17,18] and the pathological change of organs. Lead accumulates not only in individual organisms, but also through the use of biodegradable polymers in the packing of dry foods and other degradable products, as it enters the food chain. Excessive Pb accumulation in humans may even cause cancer because lead is considered a "possible human carcinogen" [18,26,28]. Additionally, certain plants can accumulate heavy metals in their tissues and this increases in plants that are grown in zones with soil contamination [22].

Presently, there are no studies reporting the presence of Pb in thermoformed and biodegradable flexible films; there are, however, reports on the presence of Pb in the cortex of cassava tubers being higher than the values recorded in soils [25].

The safety of the materials that come into contact with food is evaluated by the quantity of substances that migrate from the biopolymer into food and fulfil the requirements in the legislation on foods. In the current regulation NTC 4096 [29], a maximum level of Pb of 1 mg/Kg is allowed for plasticizers. Additionally, the World Health Organization (WHO), estimates that the total lead intake from air and water in adults is in the range of 4.0 - 10 µg/day, respectively [26].

Numerous techniques have been used to determine the level of metals in different samples, such as flame atomic absorption spectrometry (F-AAS), and continuous flow micro-extraction combined with graphite furnace-atomic absorption spectrometry (GF-AAS). GF-AAS is a good alternative for the determination of trace elements such as lead due to its high sensitivity, with limits of detection in the order of µg/L [28,30-32]. In this study, sample preparation involving acid digestion was employed.

The goal of the present study is the quantification of lead in samples of thermoformed and biodegradable flexible films and their raw materials, using the standardized technique of GF-AAS after a sample acid digestion treatment. The reason for this is that the thermoformed and flexible films can be used as food containers, and so quality and safety must be ensured in their processing and manufacturing as well as handling and storage, as this can contribute to the intake of lead by the consumer.

2. Materials and methods

2.1. Samples

Thermoformed (MBRA-383, MPER-183, CM 523-7, CM 7951-5, CM 4574-7, NATAIMA 31, HMC 1) and biodegradable flexible films (SM 707-17, SM 1498-4 and CM 7138-7, all pristine and hydrolyzed samples), were elaborated from cassava flour and starch, respectively.

Prior to the analysis, all samples were cut manually and thermoformed samples were macerated down to a particle size smaller than 1.135 mm (Sieve Newark USA Standard Series No. 18). Then, the water content was eliminated from thermoformed and biodegradable flexible films by drying at 70ºC in a furnace (Fisher) for 4.5 h and 4 h, respectively.

2.2. Acid digestion with reflux

The process of digestion was performed using a mass of 1.0 g of the sample dissolved in 20 mL of a mixture containing HNO_3 (65%, Merck):$HClO_4$ (48%, Merck) prepared in a 3:1 ratio. The solution was heated at 70ºC for 3 h for the thermoformed and 45 minutes for the biodegradable flexible films. After cooling off, they were filtered through a Gooch crucible (Schott Duran glass porosity 2), stored in polyethylene containers at 4ºC, and finally analyzed using GF-AAS (Thermo AA S4) [23,33].

2.3. Standardization of GF-AAS

The following statistical quality parameters were determined in order to carry out the standardization of the analytical method GF-AAS for the quantification of lead in

thermoformed and biodegradable flexible films [34,35]:

The linear range was evaluated by preparing a calibration curve of lead concentration from 2.0 to 12.0 µg/L. Dilutions were prepared from a stock solution of lead, 1000 mg/L ($Pb(NO_3)_2$ in HNO_3, Merck).

The precision was evaluated at two levels: a) for intermediate precision, eight calibration curves of Pb (2.0 to 7.0 µg/L) were analyzed over eight days; b) for repeatability, five calibration curves of lead (2.0 to 7.0 µg Pb/L) were prepared and analyzed on the same day. The concentration of lead was determined using GF-AAS.

The sensitivity of the method was established by comparing the slopes of the calibration curves used to verify the precision.

For the limit of detection (LOD) and limit of quantification (LOQ), three calibration curves with lead concentration ranging from 1.5 to 4.0 µg/L were prepared to obtain the standard deviation of the intercept and average of the slopes [36,37].

The accuracy of the method was determined in terms of percent recovery. Known amounts (50, 60 and 70 µL) of a stock solution of lead (1000 µg/L) were added to thermoformed (MPER 183) and pristine flexible film (CM 7138-7) before the digestion treatment followed by GF-AAS analysis.

In addition to this standardization, stability was another parameter taken into account in order to optimize the method for the quantification of lead in polymers using GF-AAS. Samples of a standard solution of lead (3.0 µg/L), a thermoformed (CM 4574-7) and a pristine flexible film (CM 7138-7) were analyzed using GF-AAS over seven consecutive days.

2.4. Quantification of lead (Pb)

The quantification of lead was carried out using atomic absorption spectrometry (Thermo AA S4) with a graphite furnace (GFS-97). 20 µL of the concentrated sample, obtained from the acid digestion with reflux, was injected into the graphite cell. Previously, the optimal pyrolysis (500 to 800ºC) and atomization (1000 to 1700ºC) temperatures were determined and programmed into the graphite furnace. Drying (100ºC) and clean (2500ºC) temperatures were programmed according to instructions in previous reports (30,31,38). The technique used a normal electrographite cell (Thermo Elemental Solaar) with argon flowing at a rate of 0.2 L/min and 0.5 nm slit.

The concentration of lead was determined by building a calibration curve with concentrations of lead ranging from 2.0 to 7.0 µg/L and reading the maximum absorbance at a wavelength of 216.9 nm. These dilutions were prepared from a stock solution of lead (1000 µg/L) in 0.2% HNO_3 in a 5 mL volumetric flask. All samples were measured in triplicate and the mean values were expressed as µgPb/Kg.

2.5. Statistics

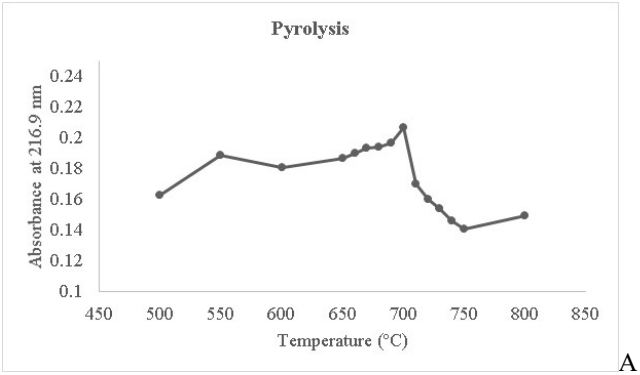
The statistical SPSS analysis, using version 11.5 Windows and Microsoft Office Excel 2007, support the results of the standardization and implementation. Initially, the Shapiro-Wilk test and the test of equality of variances using Levene were applied. Finally, each calibration curve was statistically evaluated by applying one-way ANOVA, *Pearson correlation*, the coefficient of determination and analysis of the relation [39].

3. Results and discussion

Thermoformed samples showed moisture values (after drying at 70ºC in a furnace for 4.5 h) of between 3.71 to 5.80% (RSD lower than 3.4%). Biodegradable flexible films presented higher values of moisture (after drying at 70ºC in a furnace for 4 h) at 7.81-10.35%, with RSD lower than 1.96%, these levels being similar to those reported by Cha *et al.*, 2001 [40].

The graphite furnace heating program was optimized through pyrolysis and atomization curves. Fig. 1A shows the change in absorbance at several pyrolysis temperatures and exhibits a maximum absorbance when the pyrolysis temperature was 700ºC, with an acceptable coefficient of variation of 1.16%. Fig. 1B illustrates absorbance against different temperatures at which atomization was performed; the coefficients of variation ranged from 4.44 to 22.95%. Maximum absorbance was observed when the atomization temperature was 1500°C (Fig. 1B), with an acceptable coefficient of variation of 3.88%.

In order to establish the performance of the method [35] for the accurate quantification of lead in thermoformed and biodegradable flexible films using GF-AAS, the following statistical quality parameters were determined: linear range, precision, sensitivity, LOD and LOQ and accuracy.



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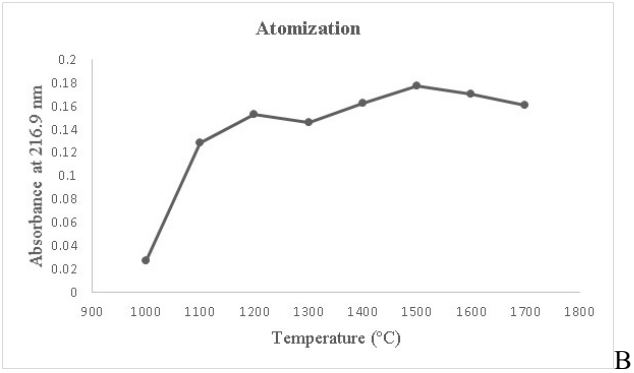

B

Figure 1. Optimization of graphite furnace temperature for Pb: (A) Pyrolysis; (B) Atomization
Source: The authors

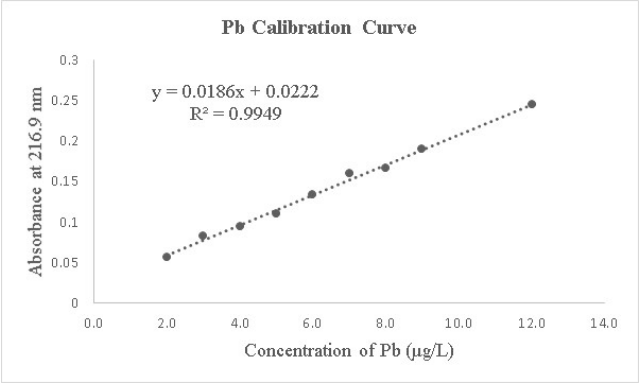

Figure 2. Calibration curve of lead obtained for the linear range (N= 4).
Source: The authors

To verify the linearity, a calibration curve was built for aqueous standard solutions of lead with concentrations of 2.0-9.0 and 12.0 µg/L. Fig. 2 shows values for the maximum absorbance at 216.9 nm vs concentration of lead. This calibration curve exhibits a linear behavior for different concentration solutions of lead ranging from 2.0 to 12.0 µg/L with relative standard deviations of 1.41-3.71%; the Pearson correlation coefficient was determined to be 0.995.

Table 1 represents the concentration (in µg/L) of lead for each standard solution prepared and analyzed for intermediate precision and repeatability as follows: a) The calibration curves were analyzed to determine the intermediate precision and were statistically evaluated using the Shapiro-Wilk and Levene tests. They did not show significant differences among them and the dispersion of the data was less than 4.7% (coefficient of variation, CV). This indicates that the proposed method for the determination of lead using GF-AAS provides a good intermediate precision. The average linear equation was y=0.028 (±0.000) x + 0.006 (±0.001) with r^2 of 0.9989. The Pearson correlation coefficient was determined to be 0.9997.

Table 1.
Average absorbance (± standard deviation) of calibration curve for precision: Intermediate precision and Repeatability

Concentration of Pb (µg/L)	Intermediate Precision		Repeatability	
	Statistic		Statistic	
	X (µg/L) ± SD	CV (%)	X (µg/L) ± SD	CV (%)
2.0	0.0638 ± 0.0030	4.70	0.0788 ± 0.0030	3.81
2.5	0.0758 ± 0.0026	3.43	0.0936 ± 0.0038	4.06
3.0	0.0920 ± 0.0038	4.13	0.1102 ± 0.0048	4.36
3.5	0.1043 ± 0.0034	3.30	0.1253 ± 0.0042	3.35
4.0	0.1196 ± 0.0040	3.34	0.1388 ± 0.0041	2.95
4.5	0.1339 ± 0.0035	2.61	0.1564 ± 0.0060	3.84
5.0	0.1481 ± 0.0044	2.97	0.1724 ± 0.0056	3.24

Note: Intermediate precision (8 calibration curves) and Repeatability (5 calibration curves
Source: The authors

In all cases, the standard deviation was less than 0.005 µg/L of lead. b) The Shapiro-Wilk and Levene tests showed that each concentration of lead in the five calibration curves was from the same population, presenting a dispersion with a variation less than 4.4%. This indicates that this method has a good repeatability, with an average linear equation of y=0.030 (±0.000) x + 0.019 (±0.002) with r^2 of 0.9989. The Pearson correlation coefficient was determined to be 0.0095. The precision is adequate according to the acceptance criteria set by INMETRO [41].

The sensitivity of the method was calculated from the calibration curve's steepest slope with concentrations of lead ranging from 2.0 to 7.0 µg/L. These values correspond to 0.028 and 0.031 for intermediate precision and repeatability, respectively. This method provides an increased sensitivity when the analysis is carried out on the same day, indicating that the proposed method for sample preparation is adequate. Therefore, the sensitivity of the method was 0.030.

The limit of detection (LOD) was 0.618 µg/L (12.4 µg/Kg) and corresponds to the minimum amount of lead derived from the lowest analytical signal that can be detected with reasonable certainly. The limit of quantification (LOQ) was 1.853 µg/L (37.1 µg/Kg) and represents the minimum concentration that can be measured with precision and accuracy. The LOD and LOQ are adequate for the quality control of bio-polymers.

Certified bio-polymers were unavailable, so the validity of the method was evaluated by addition-recovery tests. The accuracy of the method (Table 2) takes into account sample preparation, digestion treatment and analysis of samples using GF-AAS. The percent recovery of lead ranged from 94.79 to 98.52% for the MPER 183 thermoformed material and from 102.75 to 106.50% for the CM 7138-7 pristine flexible film. Comparing percent recoveries of lead in the thermoformed and flexible films with 100% recovery (using a *t student* test), it was found that some significant differences can be attributed to random errors such as preparation of solutions and analyte loss during the digestion treatment. These values (94.8 and 106.5%) can be accepted as good percent recovery according to the criteria set by INMETRO [41] and the European Commission [42]; therefore, this

Table 2.
Percentages of lead recovery (µg/L) (N=4) in the analysis of thermoformed (MPER 183) and biodegradable flexible films (CM 7138-7). Volume 20 mL.

Sample	Amount			Recovery (%) ± SD	CV (%)
	In the sample (µg/L)	Added (µL)	Detected (µg/L)		
MPER 183	1.989 (± 0.086)	50	4.271 (± 0.064)	94.79 (± 0.25)	0.26
		60	4.510 (± 0.058)	96.01 (± 1.60)	1.67
		70	5.157 (± 0.017)	98.52 (± 1.27)	1.29
CM 7138-7	1.451 (± 0.097)	50	4.161 (± 0.033)	102.75 (± 0.62)	0.60
		60	4.501 (± 0.114)	106.50 (± 2.49)	2.34
		70	4.921 (± 0.033)	106.23 (± 2.30)	2.16

Source: The authors

method exhibits excellent accuracy for the determination of lead in these samples. The values of the standardization parameters studied demonstrated quality assurance when using GF-AAS for the determination of lead in thermoformed and flexible films.

The statistical analysis of a solution of 3.0 µg/L of lead, a thermoformed (CM 4574-7) and a pristine flexible film (CM 7138-7), showed high stability over seven consecutive days. For the standard solution, the average concentration was 2.76 µg/L ± 0.06, while the coefficients of variation for the thermoformed and flexible film were less than 4.97%.

Once the analytical technique of GF-AAS was standardized for the determination of lead in bio-polymers, the implementation was performed. Table 3 shows the concentrations of lead in µg/Kg that were quantified in seven thermoformed samples. The concentrations typically ranged from 39.70 to 63.99 µg/Kg. In NATAIMA 31 and HMC 1 samples, traces of lead were found to be under the LOQ (1.853 µg/L), with concentrations of less than 37.2 µg/Kg. This variation in lead may be attributed to the quality of the raw materials used in the elaboration of bio-polymers.

Other authors found mercury, arsenic and selenium present in these thermoformed samples. Del Castillo *et al.*, 2012 [43] found mercury ranging from undetectable to 1343.4 µg/Kg, Alvira *et al.*, 2012 [44] found arsenic with concentrations of less than the LOQ (39.2 µg/Kg) and Rada-Mendoza *et al.*, 2014 [45] detected selenium ranging from undetectable to 1240.0 µg/Kg.

As shown in Table 4, for all flexible films in the pristine and hydrolyzed forms, the detected amounts of lead were below the LOQ. This corresponds to a concentration of a trace level of lead of between 37.14 and 37.18 µg/Kg. In these flexible films, mercury was reported to be undetectable to 127.3 µg/Kg [43], arsenic was undetected [44] and selenium was reported to be undetectable to 122.0 µg/Kg [45]. In thermoformed and biodegradable flexible films, the values of heavy metals accumulated were higher for mercury than for Se, Pb and As, suggesting that absorption and bioaccumulation depend upon the availability of metals.

In Europe, Asia, the US and Brazil, there is no legislation for regulating the content of heavy metals in bio-polymers. The Colombian regulation NTC-4096 [29] establishes 1.0 mg/Kg as the maximum level of lead allowed in DOA and DOP plasticizers used in the manufacture of plastics that are in contact with food which indicates that lead concentrations in the range of 39.70 to 63.99 µg/Kg found in the thermoformed material(Table 3), and in lower concentrations than the LOQ found in flexible films (Table 4), are all below the limit established by the regulation. Therefore, it is expected that there is no incidence of this metal in the final product.

However, it is necessary to investigate the source of the lead in these samples. In this case, lead was detected in raw materials (cassava flour and starch and fique fiber) used in both the thermoformed and flexible films. Table 5 shows the concentration in µg/Kg of lead in different samples of the raw materials used in the production of biodegradable thermoformed film. Of these, MBRA 383, CM 523-7 and fique fiber had concentrations in the range of 39.56 to 46.21 µg/Kg of lead, with the fique fiber having the highest concentration of lead. From this, it is evident that this source contributes the most lead to the thermoformed film.

Lead found in the flexible films made from cassava starch (Table 6), the raw material used for producing this bio-polymer, was below the LOQ.

With the results shown in Tables 3 and 4, it can be inferred that lead found in thermoformed material comes principally from cassava flour and fique fiber, and for flexible films, this heavy metal derives from the cassava starch. The conclusion here is that traces of lead detected in

Table 3.
Average concentration (± standard deviation) of lead (N = 3) in samples of biodegradable thermoformed material

Thermoformed	Concentration (µg/Kg)	CV (%)
MBRA-383	52.83 (± 1.89)	3.58
MPER-183	39.70 (± 1.71)	4.30
CM 4574-7	44.25 (± 1.90)	4.29
CM 523-7	63.99 (± 2.42)	3.78
CM 7951-5	49.10 (± 1.92)	3.91
NATAIMA 31	37.15	----
HMC 1	37.13	----

Source: The authors

Table 4.
Average concentration (± standard deviation) of lead (N = 3) in samples of biodegradable flexible films

Flexible films	Concentration (µg/Kg)	CV (%)
SM 707-17 Pristine	37.17	----
SM 707-17 Hydrolyzed	37.18	----
CM 7138-7 Hydrolyzed	37.14	----
CM 7138-7 Pristine	37.15	----
SM 1498-4 Hydrolyzed	37.16	----
SM 1498-4 Pristine	37.18	----

Source: The authors

Table 5.
Average concentration (± standard deviation) of lead (N = 3) in samples of raw materials used in the production of biodegradable thermoformed material

Sample (flour)	Concentration (µg/kg)	CV (%)
MBRA-383	39.59 (± 1.33)	3.37
MPER-183	37.18	----
CM 4574-7	37.17	----
CM 523-7	46.14 (± 2.05)	4.43
CM 7951-5	37.17	----
NATAIMA 31	37.14	----
HMC 1	37.14	----

The concentration of lead in Fique Fiber was 46.21 µg/kg (± 1.94)
Source: The authors

Table 6.
Average concentration (± standard deviation) of lead (N = 3) in samples of raw materials used in the production of biodegradable flexible films.

Sample (starch)	Concentration (µg/kg)	CV (%)
SM 707-17 Pristine	N.D.	----
SM 707-17 Hydrolyzed	N.D.	----
CM 7138-7 Hydrolyzed	37.12	----
CM 7138-7 Pristine	37.17	----
SM 1498-4 Hydrolyzed	37.15	----
SM 1498-4 Pristine	37.14	----

Source: The authors

the studied bio-polymers were low, indicating non absorption and contamination from anthropogenic sources, meaning that these materials are not toxic and may be employed as food wrappers and containers, conserving food [1,2,46] and extending its shelf-life, without potential health risks to the consumer.

Lead uptake from soil is highest in plants that are grown in areas with high clay content [22]. It is very important to take precautions when obtaining and handling raw materials, as was mentioned above, because lead is released into the environment and can contaminate the flour, starch and fique fiber through exposure to car fumes, emissions from industrial processes, soils containing heavy metals, and the irrigation of vegetables with contaminated water [21,22,25,38].

Additionally, is important to note that the best way to reduce lead contamination is through the control of raw materials and a thorough understanding of the manufacturing process.

4. Conclusions

This study demonstrates the immense potential of using bio/polymers in packaging and food conservation, and how these materials add value to agricultural activity and help to reduce non/biodegradable plastics in the environment. It is inferred that lead found in these samples comes from the flour, starch and fique fiber at cultivation and shows that lead contamination may be not the result of the manufacturing process.

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References

[1] Bilck, A.P., Ruffo, S., Eiras, M.V. and Yamashita, F., Efficacy of some biodegradable films as pre-harvest covering material for guava. Scientia Horticulturae, 130(1), pp. 341-343, 2011. DOI: 10.1016/j.scienta.2011.06.011

[2] Vicentino, S.L., Floriano, P.A., Dragunski, D.C. and Caetano, J., Films of starch cassava to coat and conservation of grapes. Química Nova, 34(8), pp. 1309-1314, 2011. DOI: 10.1590/S0100-40422011000800003

[3] Machado, B.A.S., Nunes, I.L., Pereira, F.V. and Druzian, J.I., Development and evaluation of the effectiveness of biodegradable films of cassava starch with nanocellulose as reinforcement and yerba mate extract as an additive antioxidant. Ciencia Rural, 42(11), pp. 2085-2091, 2012. DOI: 10.1590/S0103-84782012001100028

[4] Ezeoha, S.L. and Ezenwanne, J.N., Production of biodegradable plastic packaging film from cassava starch. IOSR Journal of Engineering (IOSRJEN), 3(10). pp. 14-20, 2013. DOI: 10.9790/3021-031051420

[5] Villada-Castillo, H.S., Navia, D.P. and Castañeda, J.P., PATENT INFORMATION, WO 2013042083 A1. 2013-03-28.

[6] Santos, R.A.L., Muller, C.M.O., Grossmann, M.V.E., Mali, S. and Yamashita, F., Starch/poly (butylene adipate-co-terephthalate)/montmorillonite films produced by blow extrusion. Química Nova, 37(6), pp. 937-942, 2014. DOI: 10.5935/0100-4042.20140170

[7] Costa, S.S., Druzian, J.I., Machado, B.A.S., de Souza, C.O. and Guimaraes, A.G., bi-functional biobased packing of the cassava starch, glycerol, licuri nanocellulose and red propolis. Plos One, 9(11), pp. e112554, 2014. DOI: 10.1371/journal.pone.0112554

[8] Arrieta, A., Jaramillo, A. and Palencia, M., Conductive films from cassava starch as material for an electrochemical accumulator (battery). Revista De La Sociedad Química Del Perú [Online], 81(4), pp. 328-338, 2015. [date of reference: February 28th of 2018]. Available at: http://www.scielo.org.pe/scielo.php?pid=S1810-634X2015000400005&script=sci_abstract&tlng=en

[9] Pagno, C.H., de Farias, Y.B., Costa, T.M.H., Rios, A. de O. and Flôres, S.H., Synthesis of biodegradable films with antioxidant properties based on cassava starch containing bixin nanocapsules. Journal of Food Science and Technology, 53(8), pp. 3197-3205, 2016. DOI: 10.1007/s13197-016-2294-9

[10] Sueiro, A.C., Faria-tischer, P.C.S., Lonni, A.A.S.G. and Mali, S., Biodegradable films of cassava starch, pullulan and bacterial cellulose. Química Nova, 39(9), pp. 1059-1064, 2016. DOI: 10.5935/0100-4042.20160118

[11] Medina-Jaramillo, C., Ochoa-Yepes, O., Bernal, C. and Fama, L., Active and smart biodegradable packaging based on starch and natural extracts. Carbohydrate Polymers, 176, pp. 187-194, 2017. DOI: 10.1016/j.carbpol.2017.08.079

[12] Liu, Y., Cassava starch degradable film, and preparation method thereof, Faming Zhuanli Shenqing, CN 106565998 A 20170419, 2017.

[13] Adebowale, A.A., Olatunde, O.O., Adegunwa, M.O., Asiru, W.B. and Sanni, L.O., Mechanical and sensorial characteristics of cassava and yam composite starch films. Journal of Food Processing and Preservation, 38(4), pp. 1994-1998, 2014. http://dx.doi.org/10.1111/jfpp.12175.

[14] Shimao, M., Biodegradation of plastics, Current Opinion In Biotechnology, 12, pp. 242-247, 2001. DOI: 10.1016/S0958-1669(00)00206-8

[15] Ruíz, G., Montoya, C. and Paniagua, M., Degradabilidad de un polímero de almidón de yuca. Revista EIA [En línea], 12, pp. 67-78, 2009. [fecha de referencia: Marzo 15 de 2018]. Disponible en: http://www.scielo.org.co/pdf/eia/n12/n12a06.pdf

[16] Funke, U., Bergthaller, W. and Lindhauer, M.G., Processing and characterization of biodegradable products based on starch. Polymer Degradation and Stability, 59(1-3), pp. 293-296, 1998. DOI: 10.1016/S0141-3910(97)00163-8

[17] Graeme, K.A. and Pollack, C.V., Heavy metal toxicity, part II: lead and metal fume fever. The Journal of Emergency Medicine, 16(2), pp. 171-177, 1998. http:// doi.org/10.1016/S0736-4679(97)00283-7

[18] Järup, L., Hazards of heavy metal contamination. British Medical Bulletin [Online], 68, pp. 167-182, 2003. [date of reference March 15th of 2018]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/14757716

[19] Rodrigues, A. and Nascentes, C.C., Development of a simple method for the determination of lead in lipstick using alcaline solubilization and grafite furnace atomic absorption spectrometry. Talanta, 105, pp. 272-277, 2013. DOI: 10.1016/j.talanta.2012.09.021

[20] Vílchez-Vargas, R., Eliminación de metales pesados de aguas subterráneas mediante sistemas de lechos sumergidos: Estudio microbiológico de las biopelículas, Ph.D. Thesis, University of Granada, Granada, España, 2005..

[21] Núñez, A., Martínez, S., Moreno, S., Cárdenas, M.L., García, G., Hernández, J.L., Rodríguez, A. and Castillo, I., Determinación de metales pesados (aluminio, plomo, cadmio y níquel) en rábano (Raphanus sativus L.), brócoli (Brassica oleracea L. var. italica) y calabacín (Cucurbita pepo L. var. italica), Asignatura (Laboratorio Química Analítica), Autonomous University of Nuevo León, México, 2008.

[22] LeCoultre, D.A., Meta-analysis and risk assessment of heavy metal uptake in common graden vegetables. Master of Science in Environmental Health Thesis [Online], East Tennessee State University, USA, 2001. [date of reference January 25th of 2018]. Available at: https://dc.etsu.edu/etd/113.

[23] Bakkali, K., Ramos, N., Souhail, B. and Ballesteros, E., Characterization of trace metals in vegetables by graphite furnace atomic absorption spectrometry after closed vessel microwave digestion. Food Chemistry, 116, pp. 590-594, 2009. DOI: 10.1016/j.foodchem.2009.03.010

[24] Obanijesu, E.O. and Olajide, J.O., Trace metal pollution study on cassava flour's roadside drying technique in Nigeria. In: Yanful E.K. (eds.), Appropriate technologies for environmental protection in the developing world, 1, pp. 333-339, 2009.. DOI: 10.1007/978-1-4020-9139-1_32

[25] Idodo-Umeh, G. and Ogbeibu, A.E., Bioaccumulation of the heavy metals in cassava tubers and plantain fruits grown in soils impacted with petroleum and non-petroleum activities. Research Journal of Environmental Sciences, 4(1), pp. 33-41, 2010. DOI: 10.3923/rjes.2010.33.41
[26] WHO, World Health Organization., Lead in drinking-water, World Health Organization, Geneva, 2011, 26 P.
[27] Weber, C.J., Biobased packaging materials for the food industry. The Royal Veterinary and Agricultural University, Dinamarca, pp. 11-136, 2000.
[28] Shrivas, K. and Patel, D.K., Separation and preconcentration of trace level of lead in one drop of blood sample by using graphite furnace atomic absorption spectrometry. Journal of Hazardous Material, 176, pp. 414-417, 2010. DOI: 10.1016/j.jhazmat.2009.11.045
[29] ICONTEC. Norma Técnica Colombiana 4096. Instituto Colombiano de Normas Técnicas., Plásticos. Plastificantes DOP y DOA grado alimento, Santafé de Bogotá D.C., 2006, P. 3.
[30] Cao, J., Liang, P. and Liu, R., Determination of trace lead in water samples by continuous flow microextraction combined with graphite furnace atomic absorption spectrometry. Journal of Hazardous Materials, 152(3), pp. 910-914, 2008. DOI: 10.1016/j.jhazmat.2007.07.064
[31] Valdebenito-Zenteno, G.A., Desarrollo de un método para la determinación directa de Pb mediante espectrometría de absorción atómica electrotérmica (ETAAS) en suspensiones de pelo y uña "slurries" como biomarcadores de exposición. Chemistry Thesis [En línea], Chile University, Santiago de Chile, Chile, 2008. [consulta: 1/12 de enero de 2018]. Disponible en: http://repositorio.uchile.cl/tesis/uchile/2008/valdebenito_g/sources/valdebenito_g.pdf
[32] Damin, I.C.F., Dessuy, M.B., Castilhos, T.S., Silva, M.M., Vale, M.G.R., Welz, B. and Katskov, D.A., Comparison of direct sampling and emulsion analysis using a filter furnace for the determination of lead in crude oil by graphite furnace atomic absorption spectrometry. Spectrochimica Acta Part B: Atomic Spectroscopy, 64(6), pp. 530-536, 2009. DOI: 10.1016/j.sab.2009.03.002
[33] Nemati, K., Abu, N.K., Bin, M.R., Sobhanzadeh, E. and Low, K.H., Comparative study on open system digestion and microwave assisted digestion methods for metal determination in shrimp sludge compost. Journal of Hazardous Materials, 182(1-3), pp. 453-459, 2010. DOI:
[34] Coy, G.A., Protocolo: Estandarización de métodos analíticos. Institute of Hydrology, Meteorology and Environmental Studies. Ministerio de Ambiente, Vivienda y Desarrollo Territorial. Código TP0131. Versión 02. Colombia, 2006, 12 P.
[35] EURACHEM, The Fitness for purpose of analytical methods: A laboratory guide to method validation and related topics, LGC, Teddington, UK, 2nd edition, 2014, 62 P.
[36] IUPAC. Nomenclature in evaluation of analytical methods including detection and quantification capabilities. In: Pure and applied chemistry, 67(10), pp. 1699-1723, 1995.
[37] Mocák, J., Janiga, I. and Rábarová, E., Evaluation of IUPAC limit of detection and iso minimum detectable value-Electrochemical determination of lead. Nova Biotechnologica [Online], 9(1), pp. 91-100, 2009. [date of reference February 8th of 2018]. Available at: http://www.nbc-journal.fpv.ucm.sk/archive/revue_nova_biotechnologica_9_1/Mocak2009_1.pdf
[38] Santos, M.C., Nóbrega, J.A., Baccan, N. and Cadore, S., Determination of toxic elements in plastics from waste electrical and electronic equipment by slurry sampling electrothermal atomic absorption spectrometry. Talanta, 81(4-5), pp. 1781-1787, 2010. DOI: 10.1016/j.talanta.2010.03.038
[39] Guisande, C., Barreiro, A., Maneiro, I., Riveiro, I., Vergara, A. and Vaamonde, A., Tratamiento de datos. Madrid: Ed. Díaz de Santos, 2006.
[40] Cha, J.Y., Chung, D.S., Seib, P.A., Flores, R.A. and Hanna, M.A., Physical properties of starch-based foams as affected by extrusion temperature and moisture content. Industrial Crops And Products, 14(1), pp. 23-30, 2001. DOI: 10.1016/S0926-6690(00)00085-6
[41] INMETRO. Instituto Nacional de Metrologia., Normalização, e qualidade industrial. orientação sobre validação de métodos de ensaios químicos DOQ-CGCRE-008 [Online], 2007, 24 P. [date of reference January 1 /14th of 2018]. Available at: http://www.inmetro.gov.br/Sidoq/Arquivos/CGCRE/DOQ/DOQ-CGCRE-8_02.pdf
[42] European Commission., Commission decision 2002/657/EC of 12 August 2002. Implementing Council Directive 96/23/EC concerning performance of analytical methods and the interpretation of results. Official Journal of the European Communities [Online]. L 221/8, 2002, 29 P. [date of reference January 1/13th of 2018]. Available at: http://extwprlegs1.fao.org/docs/pdf/eur49615.pdf
[43] Del Castillo, R., Rada-Mendoza, M., Hoyos, O.L. and Villada, H.S., Quantification of Hg in thermoformed and flexible films biodegradable made from cassava (Manihot esculenta crantz) by atomic absorption spectrometry. Biotecnología En El Sector Agropecuario y Agroindustrial [Online], 10(2), pp. 227-235, 2012. [date of reference January 1/13th of 2018]. Available at: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S1692-35612012000200026
[44] Alvira, L.F., Rada-Mendoza, M., Hoyos, O.L. and Villada, H.S., Quantification of As by atomic absorption spectrometry in flexible thermoformed and biodegradable films. Biotecnología En El Sector Agropecuario y Agroindustrial [Online], 10(1), pp. 157-165, 2012. [date of reference January 27th of 2018]. Available at: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S1692-35612012000100018
[45] Rada-Mendoza, M., Claros, L.M. and Hoyos, O.L., Quantification of selenium by atomic absorption spectrometry in flexible thermoformed and biodegradable films. Revista Facultad Nacional de Agronomía, 67(2), pp. 548-550, 2014.
[46] Chiumarelli, M., Ferrari, C.C., Sarantópoulos, C.I.G.L. and Hubinger, M.D., Fresh cut ´Tommy Atkins´ mango pre-treated with citric acid and coated with Cassava (Manihot esculenta Crantz) starch or sodium alginate. Innovative food science & emerging technologies, 12(3), pp. 381-387, 2011. DOI: 10.1016/j.ifset.2011.02.006

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