



IMPACT ON THE INCORPORATION OF METALS IN PHYSICOCHEMICAL AND ANTIMICROBIAL PROPERTIES IN FILMS BASED ON ARROWROOT STARCH

IMPACTO EN LA INCORPORACIÓN DE METALES EN LAS PROPIEDADES FISICOQUÍMICAS Y ANTIMICROBIANAS EN PELÍCULAS A BASE DE ALMIDÓN DE ARRURRUZ

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Abstract

New biodegradable packaging has been developed from renewable sources, mainly of vegetable origin. Arrowroot starch has been recently used to produce high-quality biodegradable films capable of behaving well when incorporating oils, extracts, metal, and metal nanocomposites. The study aimed to verify the impact of incorporating metals in the sulfate and chloride forms in a biopolymeric matrix from arrowroot starch in terms of biodegradability, physicochemical and microbiological parameters. Different arrowroot films were produced to incorporate solutions with a concentration of 1 Mol L⁻¹ of sulfate and chloride metals. The action of biodegradability in soil, UV transmittance, and visible light were observed in UV-Vis spectrophotometry and antimicrobial action on *Escherichia coli*, *Staphylococcus aureus*, *Salmonella serovar Typhimurium*, and *Salmonella serovar Enteritidis*. Good results were obtained, such as biodegradability time between 81.70 to 100% (30 days), a low transmission rate of UV radiation and visible light between 250 to 890 nm, high capacity for bacterial inhibition between 22.08 to 10.05 mm for *E. coli*, among 25.59 to 11.10 mm for *S. aureus*, between 22.14 to 11.66 mm for *S. serovar Typhimurium* and between 21.11 to 8.26 mm for *S. serovar Enteritidis*. It is concluded that the biodegradable films of arrowroot starch incorporated with metals showed potential in all the evaluated tests, thus characterizing possible new products for different uses, such as low time available in the environment, preservation of the characteristics of special products, and antimicrobial capacity.

Keywords: *Escherichia coli*, *Salmonella*, *Enterococcus*, Biodegradable packaging, Antimicrobial activity.

Resumen

Se han desarrollado nuevos embalajes biodegradables de fuentes renovables principalmente de origen vegetal. El almidón de arrurruz se ha usado en la producción de películas biodegradables de alta calidad, capaces de tener un buen comportamiento al incorporar aceites, extractos, metales y nanocompuestos metálicos. El estudio tiene como objetivo verificar el impacto de la incorporación de metales en las formas de sulfato y cloruro en una matriz biopolimérica de almidón de arrurruz en términos de biodegradabilidad, parámetros fisicoquímicos y microbiológicos. Se produjeron diferentes películas de arrurruz con soluciones a una concentración de 1 Mol L^{-1} de metales sulfato y cloruro. La acción de biodegradabilidad en el suelo, transmitancia UV y luz visible se observó en espectrofotometría UV-Vis, y acción antimicrobiana sobre *Escherichia coli*, *Staphylococcus aureus*, *Salmonella serovar Typhimurium* y *Salmonella serovar Enteritidis*. Se obtuvieron buenos resultados, como tiempo de biodegradabilidad entre 81,70 a 100 % (30 días), baja tasa de transmisión de radiación UV y luz visible entre 250 a 890 nm, alta capacidad de inhibición bacteriana entre 22,08 a 10,05 mm para *E. coli*, entre 25,59 a 11,10 mm para *S. aureus*, entre 22,14 a 11,66 mm para *S. serovar Typhimurium* y entre 21,11 a 8,26 mm para *S. serovar Enteritidis*. Se concluye que las películas biodegradables de almidón de arrurruz incorporadas con metales mostraron potencial en todas las pruebas evaluadas, caracterizando así posibles nuevos productos para diferentes usos, tales como bajo tiempo disponible en el ambiente, preservación de las características de productos especiales y capacidad antimicrobiana.

Palabras clave: *Escherichia coli*, *Salmonella*, *Enterococcus*, Embalaje biodegradable, Actividad antibacteriana.

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1 Introduction

The first packages on the market were produced from synthetic polymers of the processed oil. Currently, there is a wide variety of packaging for different purposes, although its use is to preserve the product during transport, during storage and protection against mechanical shocks that may reduce or render the use of the product unfeasible by the consumer (Kubowicz and Booth, 2017; Santos et al., 2021).

The indiscriminate use of plastics with prolonged degradation time, which can reach 500 years, presents serious problems, mainly environmental and human health. Plastic polymers undergo abrasion over time, forming microplastics absorbed during feeding by mainly marine animals and humans (Sobral et al., 2011; Olivatto et al., 2018; Haider et al., 2019).

Countless countries have been promoting the development of means capable of altering this catastrophic scenario caused by the excess of plastic packaging. Several researchers from the green line of research have been developing new biodegradable polymers from naturally renewable sources such as starch, fats, chitosan, and methylcellulose (Brito et al., 2011). According to Cheviron et al. (2014) y Farias et al. (2016), renewable sources of polymers offer an alternative for maintaining the sustainable development of economically and ecologically attractive technologies. Natural polymers have biological, physicochemical, mechanical, and morphological characteristics comparable to synthetic polymers (Pitt et al., 2011; Akter et al., 2012).

A branch of natural polymers focuses on incorporating metals, nanocomposites, plant extracts, fixed and essential oils capable of promoting numerous active and intelligent functions to these biodegradable polymers. Several studies evaluate the structural biodegradability of the natural polymer with incorporated materials, verifying its harmonic capacity to promote a resistant product capable of being used in different production chain processes (Gómez-Estaca et al., 2010; Nor Adilah et al., 2018; Youssef et al., 2019).

Several biodegradable packages of starch from

different plant sources such as arrowroot have been tested with surprising results. *Maranta arundinacea* (arrowroot) belonging to the Marantaceae family, which has rhizomes rich in starch (>85%), has specific characteristics that are different from other sources of natural starches and can also be used for therapeutic purposes (Madineni et al., 2012). Several studies have evaluated this new source of natural starch capable of promoting the development of high-quality biodegradable films, in addition to providing stability of interaction support, for example, with metal solutions (Yin et al., 2009; Cruz et al., 2020; Nogueira et al., 2018; Valadares et al., 2020).

Various metals as sulfates, chlorides, or nanocomposites have structural characteristics with potential activity as an antibacterial agent, in itself, the biopolymer from arrowroot starch has no action capable of inhibiting the development of bacteria, due to being a source of carbohydrates, but when incorporated with metals that present this action, the films promote an active action in the preservation of the product, such as food (Cruz et al., 2020; Shafiei Shafiei et al., 2021).

Thus, this study aimed to produce biodegradable films from arrowroot starch incorporated with metals (sulfates and chlorides), verifying the impact of these metals on some characteristics such as biodegradability time, the transmission of UV radiation and visible light, and aptitude as an antibacterial source.

2 Materials and Methods

2.1 Production of biodegradable films incorporated with metals

Biodegradable films incorporated with metals were obtained using the Casting technique described by Issa et al. (2017), but modified. To produce all films, 5 g of commercial arrowroot starch dissolved in 100 mL of distilled water was used. The film-forming solution was then moderately stirred at an ambient temperature of 25 °C for 5 min.

Then, the solution was heated to 70 °C, with constant mechanical stirring for 30 min. After the gelatinization of the starch, glycerol was added as a plasticizer 30% (w/w), and the emulsion was again

stirred for another 10 min. When filmogenic emulsions reached 30 °C, they were incorporated with different metal solutions as described in (Table 1).

All filmogenic solutions were poured on polystyrene slabs and dried in an air circulation oven at 35 °C for about 48 h.

Table 1. Formulas (films) of arrowroot starch in different solutions of incorporated metals.

Films	Metals
1 control	5 g starch + 1.5 g glycerol
2	5 g starch + 1.5 g glycerol + 1000 μ L Iron sulfate II 1 Mol L ⁻¹
3	5 g starch + 1.5 g glycerol + 1000 μ L Nickel sulfate 1 Mol L ⁻¹
4	5 g starch + 1.5 g glycerol + 1000 μ L Manganese sulfate 1 Mol L ⁻¹
5	5 g starch + 1.5 glycerol + 1000 μ L Cobalt chloride 1 Mol L ⁻¹
6	5 g starch + 1.5 g glycerol + 1000 μ L Iron chloride II 1 Mol L ⁻¹
7	5 g starch + 1.5 g glycerol + 1000 μ L Cooper chloride II 1 Mol L ⁻¹

Source: Authors, 2021.

2.2 Determination of biodegradability time and light transmission

The biodegradability was carried out by the methodology described by Martucci and Ruseckaite (2009), with modifications. Film samples (2 x 2 cm²) were dried up to constant weight (*M_i*). Samples were then placed in open polyethylene packaging to enable microorganisms and moisture to gain access to the 40%. After that, they were buried in natural soil, at constant moisture, and room temperature, and natural luminosity. Three, ten, fifteen and thirty days after the experiment installment, the artificial packaging with the samples was removed from the soil, washed with distilled water, and dried up to constant weight (*M_f*). The time of biodegradability was calculated using equation [1]. Ultraviolet-visible (UV-Vis) light transmission of the film samples was placed in a quartz cuvette and transmittance was measured at wavelengths that ranged from 900-200 nm, in agreement with Santos et al. (2021).

$$\text{Bio}(\%) = (M_f - M_i) / M_i * 100 \quad (1)$$

2.3 Antibacterial activity

Antibacterial assay was evaluated *in vitro* against four bacteria: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Salmonella serovar* Thyphimurium (ATCC 14028), and *Salmonella serovar* Enteritidis (ATCC 13076) commercially acquired and maintained from the bacteriological bank of the first author. Briefly, 150 μ L of bacterial culture

1x10⁴ cells mL⁻¹ were grown on *Petri* dishes with dextrose tryptone agar (DTA) and 150 μ L of spore suspension 1x10⁸ CFU mL⁻¹ on *Petri* dishes with plant count agar (PCA). Films (7 mm diam.) were then placed on the surface agar and incubated at 36 °C for 36 h. The diameter of the inhibition zone was measured with a digital caliper. As a positive control, Azithromycin (15 μ g disc) and Cephalixin (30 μ g disc) discs were used and opposing control starch metals films disc. The minimum acceptable diameter was 5 mm. The assay was performed in triplicate by described Valadares et al. (2020) modified.

2.4 Statistical analysis

The statistical program used was PAST 3 (version 2019). The data were subjected to analysis of variance (ANOVA), and the averages were compared using the Tukey's test at a 5% significance level.

3 Results

Biodegradable films from biopolymers such as arrowroot showed a high biodegradability rate *in vitro* soil, as seen in (Figure 1). The films incorporated compared to the standard presented statistical difference except for the films incorporated with Iron sulfate II = 94%, Manganese sulfate = 94%, Iron chloride II = 93%, and Copper chloride II = 91%.

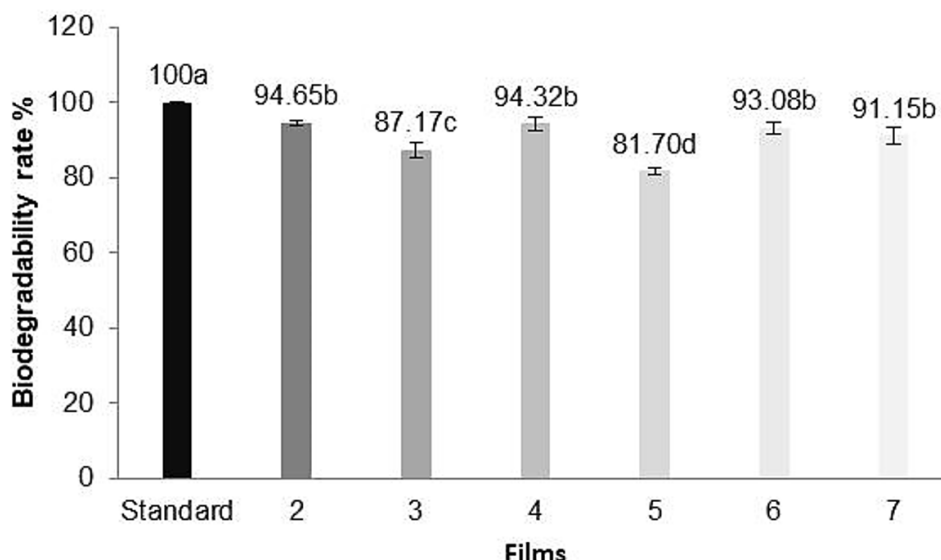


Figure 1. Biodegradability time of arrowroot starch films incorporated with metals. Standard, 2. Iron sulfate II, 3. Nickel sulfate, 4. Manganese sulfate, 5. Cobalt chloride, 6. Iron chloride II, 7. Copper chloride II. Equal letters between the averages for each biodegradable film incorporated with metals do not show a significant difference by the Tukey test 5%. Source: Authors, 2021.

Figure 2 shows seven photographic images of films produced with arrowroot starch incorporated with metals: (A) Standard film, (B) Iron sulfate II, (C) Nickel sulfate film, (D) Manganese sulfate film, (E) Cobalt chloride film, (F) Iron chloride II film, and (G) Copper chloride II.

Concerning colors of films under development, the results show a decrease in light transmittance rates of films incorporating different metals in the visible region (from 250 to 890 nm). Maximum light transmission rate for Standard film 69.36 T%, Iron sulfate II film 68.22 T%, Nickel sulfate film 59.10 T%, Manganese sulfate, 30.85 T%, Cobalt chloride film 65.68 and 17.45 T%, Iron chloride II film 78.82 T%, and Copper chloride II film 62.94 T% (Figure 3).

The antibacterial activity demonstrated potential inhibitory activity for all films incorporated with metal compared to the reference antibiotics Azithromycin and Cephalexin (Table 1). *E. coli* statistical difference was observed according to Tukey test 5% for all metallic films, except for Nickel sulfate film and Cobalt chloride, both with inhibition zone = 22 mm, and Manganese sulfate = 10 mm, and Iron chloride II = 13 mm. *S. aureus* a difference was observed only for the films Cobalt chloride = 25 mm, and Iron chloride II = 13 mm according to

Tukey test 5%. *S. serovar* Typhimurium showed statistical difference according to Tukey test 5% for the metallic films incorporated with Manganese sulfate = 14 mm, and Cobalt chloride = 22 mm, and for *S. serovar* Enteritidis significant difference by Tukey test 5% for the films incorporated with Manganese sulfate = 8 mm, Iron chloride II = 15 mm, and Copper chloride II = 19 mm. The control films did not show any inhibitory activity for the bacterial strains evaluated.

4 Discussion

In general, the incorporation of metals in a biodegradable polymeric matrix presents satisfactory results, both mechanical, thermal, biodegradability, and visual and ultrastructural morphological aspects. Arrowroot starch in this study demonstrated good aptitude during the incorporation of metals in the form of sulfates and chlorides and plasticity with glycerol. A harmonious interaction between the components in the matrix is observed, promoting resistant films and specific intermolecular interaction. Although this is a pioneering study different from that observed that includes metal nanocomposites, other tests need to evaluate this interaction.

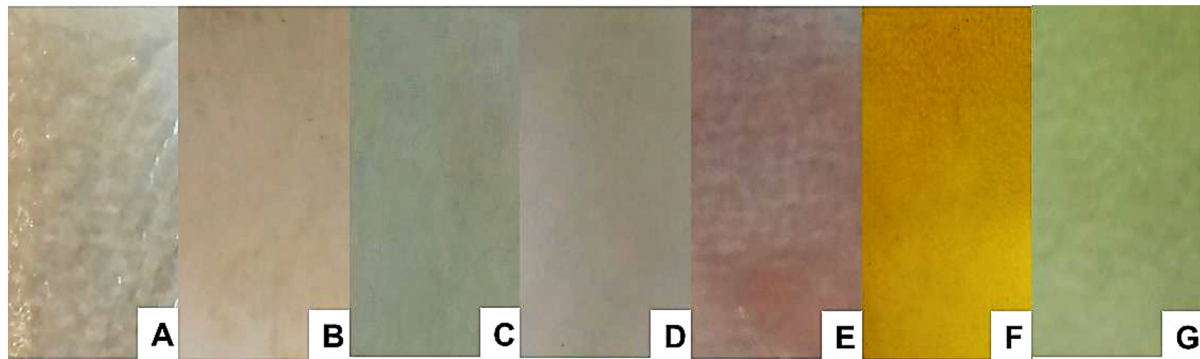


Figure 2. (A) Standard film, (B) Iron sulfate II film, (C) Nickel sulfate film, (D) Manganese sulfate film, (E) Cobalt chloride film, (F) Iron chloride II film and (G) Copper chloride II. Source: Authors, 2021.

Table 2. Antibacterial activity of biodegradable arrowroot films incorporated with different metals. Source: Authors, 2021.

Films	Inhibition zone (mm)			
	<i>E. coli</i>	<i>S. aureus</i>	<i>S. serovar Typhimurium</i>	<i>S. serovar Enteritidis</i>
1 Control	0.00±0.00f	0.00±0.00e	0.00±0.00f	0.00±0.00g
2	19.35±0.012c	20.11±0.19b	17.03±0.21c	21.11±0.06b
3	22.06±0.09b	20.35±0.10b	18.12±0.33c	13.67±0.17ed
4	10.05±0.21e	11.10±0.30dc	14.82±0.05d	8.26±0.66f
5	22.08±1.12b	25.59±0.08a	22.14±0.94b	21.02±1.66b
6	13.07±0.08e	13.90±1.14c	11.66±0.05de	15.09±0.96d
7	17.33±0.67d	20.15±1.02b	16.55±0.18c	19.13±0.27c
Antibacterial references^{a/b}	27.60±0.17^ba	23.68±0.06^ab	28.12±0.11^aa	28.36±0.83^aa

1. Standard film, 2. Iron sulfate II film, 3. Nickel sulfate film, 4. Manganese sulfate film, 5. Cobalt chloride film, 6. Iron chloride II film and 7. Copper chloride II.

^aCephalexin and ^bAzithromycin.

Same lowercase letters in the same column do not differ statistically by the Tukey test ($p < 0,05$); equal capital letters on the same line do not differ statistically by the Tukey test 5%.

The assays evaluated in this study demonstrated that arrowroot starch is a prosperous biodegradable polymer capable of being compared to polymers that have been studied for a long time, as observed below in the discussion of the results obtained in this study for biodegradability, UV and visible light transmission, and antibacterial activity.

Biodegradability is one of the main factors when choosing a biopolymer, where the less it stays in the environment, the more attractive its production source. In this study, it was observed that most arrowroot films incorporated with metals had a high biodegradation rate (90%) in the soil. All metals, except Nickel sulfate and Cobalt chloride, did not interfere negatively during natural microbial activity. Although it was not the objective of this study

to evaluate the behavior of the metal regarding its toxicity in the microbiota, the visual organoleptic analysis was satisfactory.

Thus, this evaluation provides subsidies for further studies evaluating all metals, especially Cobalt, in the bioaccumulation of organisms living in that soil. Some metals are part of developing various forms of life in the soil, supplying the needs throughout the development process. However, some metals have a toxicity degree in some groups of microorganisms. Saral Sarojini et al. (2019) obtained variable time during the biodegradability test of chitosan-based films with Zinc oxide nanoparticle (Zn-NP), although all films had a good degradability rate of 28 days with a maximum median of 95%.

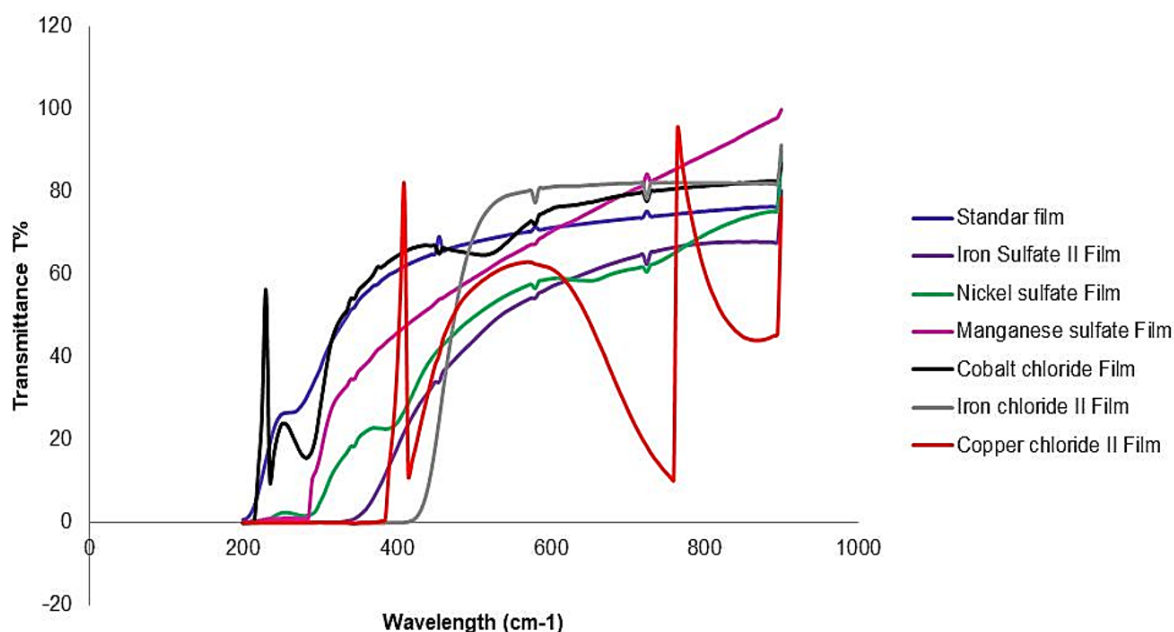


Figure 3. UV-Vis light transmittance rate in arrowroot films incorporating different metals. Source: Authors, 2021.

The transmission of ultraviolet rays and visible light negatively influences products, especially in foods and photosensitive solutions. The action caused by visible and UV light can cause lipid oxidation in foods with high-fat content, such as meat (Hosseini et al., 2015; Fathi et al., 2018). Biodegradable packaging incorporated with metals has a low rate of transmission or absorption of UV and visible light, thus promoting the maintenance of the product to be stored without changing its characteristics. The films incorporated with metals presented in this study demonstrated to be viable alternatives for the maintenance of various products, observing the desired metal and, of course, its concentration for a determined use. All embedded films were shown to promote the low transmission of both UV and visible light.

Several studies using biopolymers incorporated with metals, as in Pagno et al. (2015), evaluated the transmission rate of UV radiation in biodegradable quinoa starch films incorporated with Gold (Au) nanocomposites (NPs), obtaining a high radiation absorption rate. The same was observed by Hashe-ninya et al. (2018) using kefir-carboxymethyl cellulose with NPs of Copper oxide (CuO). High concentrations of CuO NPs resulted in a signifi-

cant decrease in light transmission between the UV (200-400 nm) and visible (400-800 nm) ranges. Similar results were also observed by Arfat et al. (2017) using fish skin gelatin incorporated with NPs Ag-Cu, where the higher the concentration of NPs, the lower the transmission rate of UV and visible light. Semolina films incorporated with ZnO also showed a low UV and visible light transmission in the study by Jafarzadeh et al. (2017).

The metals incorporated in the arrowroot biopolymer matrix demonstrated potential in the capacity of bacterial inhibition against gram-positive and gram-negative bacteria, demonstrating that they are new options for active biodegradable films with antimicrobial capacity. There are several studies with metals, their cations, anions, or oxides incorporated in polymeric matrices (gelatin fibers/Ce³⁺) showing bacterial inhibition activity for *S. aureus* and *E. coli* (Yin et al., 2009), a film produced from TiO₂ incorporated with CeO₂ with 95% inhibition on sulfate-reducing bacteria (Wang et al., 2010).

Films based on carboxymethyl cellulose with modified zeolite incorporating particles of silver (Ag⁺) and gold (Au³⁺) exhibited potential bacte-

riostatic (*S. aureus* and *E. coli*) and fungistatic (*Candida albicans* and *Aspergillus niger*) effects in the study by Youssef et al. (2019). Cruz et al. (2020) found for two Cobalt I/II complexes a high inhibition activity for *E. coli* of 22.66 and 20.66 mm, respectively. These results are similar to that observed in this study for the biodegradable film containing Cobalt chloride on *E. coli*.

5 Conclusions

This study presented a proposal for the development of six new biodegradable packaging's (films) incorporated with different metals in the form of sulfates or chlorides, directly and mainly impacting on biodegradability, the transmission of UV rays and visible light and as an effective antibacterial agent from the biopolymer considered relatively new is arrowroot starch, which has characteristics that make it possible to incorporate materials without losing its capacity to form an active resistant film.

The results obtained demonstrate that arrowroot films incorporated with metals proved to be excellent options aiming at a complex ecological system that aligns biodegradability, with physical-chemical characteristics in decreasing the transmission rate of UV rays or visible light, and also microbiological with characteristic active use of products capable of acting with antibacterial and/or bacteriostatic action.

Finally, this work allows new *in vitro* assays to be produced evaluating both the biopolymer incorporated with metals, having its evaluation in cytotoxic terms the microbial flora of the soil to evaluate possible bioaccumulation of these metals in different types of tissues, organs or even absorption those with development effect and biotransformation.

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Author contribution

A.C.P.M.F.: Conceptualization, data processing, writing of studies, translation, final corrections, correspondence; M.V.A.V.: Data analysis, writing, translation corrections.

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