GREEN CLAY FARM COMPANY SAMPLE REPORT FOR COSMETIC USE

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TECHNICAL/SCIENTIFIC TEAM

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SUMMARY

Green clay is a mineral that has specific properties and characteristics, such as very fine particles, high charge and surface area, high cation exchange capacity is a raw material with wide applicability in the cosmetic industry. In the present report, the objective was to characterize the Green Clay of the company Green Clay Farm LTDA, referring to the area of the process ANM 820.123/2018 to evaluate its compliance with the legislation of ANVISA RDC No. 48/2006 and the European Union regulation EC No. 1223/2009. For the study of the metals and semimetals of the clay, the sample was submitted to acid digestion for release and quantification of trace elements in the Inductively Coupled Plasma Optical Emission Spectrometry - ICP-OES. Analyses of pH, density, solubility, and moisture content were performed. Sterilization tests were performed via dry heat in an oven at 120 °C for 24 hours, followed by microbiological analyses to validate the disinfection protocol. The presence of elements, such as aluminum, magnesium, iron, zinc, cobalt, titanium, manganese, copper, barium, arsenic, mercury, cadmium, lead, and vanadium, reveal a diversity of properties and functions of the metals and semimetals present in clay, highlighting their influence on health and skin care. The discussion on potential toxicities highlights the importance of complying with legislation and regulatory standards, especially regarding elements such as barium, chromium, arsenic, lead, nickel, vanadium, and mercury. These are in accordance with ANVISA's RDC No. 44/2012, but EC No. 1223/2009 of the European Union contains in its annex the prohibition of these metals in cosmetic products. Therefore, complementary tests of cosmetic products are suggested to verify the perfusion of these metals into the skin. No bacterial growth was observed in the sample submitted to decontamination, thus validating the disinfection process. pH 5.46 is slightly acidic and suggests skin compatibility. The deeper understanding of the physical and chemical properties of Green Clay Farm's green clay ensures the stability and effectiveness of the cosmetic products that contain it.

Keywords: green clay, regulations, cosmetic products.

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LIST OF ABBREVIATIONS, ACRONYMS, AND UNITS

ANVISA - National Health Surveillance Agency

Al – Aluminum

The - Arsenic

Ba - Barium

Cd - Cadmium

Co-Cobalt

Cr - Chrome

Cu - Copper

Fe - Iron -

ICP-OES - Inductively Coupled Plasma Optical Emission Spectrometry

Hg - Mercury

K – Kelvin

MERCOSUR - Southern Common Market

Mg - Magnesium

Mn - Manganese

Ni - Nickel

Pb – Lead

pH - hydrogen potential

PPM- Part Per Million

RDC - Resolution of the Collegiate Board of Directors

Ti - Titanium

V - Vanadium

Zn - Zinc

1. INTRODUCTION

Clays are objects of study in various areas such as chemistry, agronomy, mineralogy, and materials technology, as they are a naturally occurring and abundant raw material, covering a wide spectrum of products. They are used as adsorbents in bleaching processes in the textile and food industries, as carriers of medicines and excipients in the pharmaceutical industry, or even in soil remediation processes (BERGAYA, THENG, LAGALY, 2006; TEIXEIRA-NETO, E; TEIXEIRA-NETO, A, 2009)

Present in various types of soils, clays are made up of extremely small crystalline particles of a restricted number of minerals known as clay minerals. In addition, they may contain organic matter, impurities in the form of salts and waste minerals, as well as amorphous minerals. Clays are widely used in aesthetic treatments because they have characteristic functions such as absorption of impurities, moisturizer, tensioner, among many others (SANTOS, 1989; SILVA, 2011; TEIXEIRA-NETO, E; TEIXEIRA-NETO, A; 2009).

They have numerous therapeutic purposes and have been used since the dawn of civilization to treat wounds, inhibit hemorrhages, animal bites and in aesthetic treatments. Clay face masks are the oldest cosmetic preparations used for beauty treatments (MATTIOLI et. al, 2016; SILVA, 2011).

Clays used for cosmetic and pharmaceutical purposes must comply with a series of chemical safety requirements (purity, stability, chemical inertness), physical (particle size, texture) and toxicological (controlled heavy metal content). To offer products with a high degree of quality (BERGAYA, THENG, LAGALY, 2006; MATTIOLI et. al, 2016, LOPÉZ-GALINDO; VÍSERAS, 2004).

Green Clays are known as a mineral derived from the decomposition of feldspathic rocks, formed by a mixture of various substances, such as iron oxide, magnesium, calcium, potassium, manganese, phosphorus, zinc, silicon, copper, selenium, and cobalt. In general, its composition depends on the source of extraction and is subject to variations that imply its adherence to the current standards for use in cosmetics.

Thus, the present study seeks to characterize a sample of green clay aiming at its use in cosmetics, evaluating its chemical composition, in addition to establishing a protocol for decontamination of the clay sample validated by microbiological analyses, the results of the experiments were those required in the cosmetic regulations of the legislation of ANVISA RDC No. 48/2006 and of the European Union regulation EC No. 1223/2009.

2 THEORETICAL FRAMEWORK

2.1 National and International Standards for Cosmetic Products

Resolution No. 79 of August 25, 2000 of the National Health Surveillance Agency ANVISA, establishes standards and procedures for the registration of Personal Hygiene Products, Cosmetics and Perfumes. It adopts the definition of Cosmetic Product. Article 2 of this resolution refers to the lists of substances allowed in its annexes, such as preservatives, dyes, UV filters, which must be duly updated.

The current updated resolution that deals with heavy metals, present in dyes in permitted concentrations for personal care products, cosmetics and perfumes throughout Mercosur, is RDC No. 44, of August 2012. In its annex and in the clarification paragraph in topic 2, it deals with the impurities in the maximum permitted concentrations for artificial organic colors, as shown in table 1. In addition, this resolution determines that insoluble chips of Barium (Ba), Strontium (Sr) and Zirconium (Zr), as well as salts and pigments derived from these dyes, can be authorized as long as their insolubility is proven by means of an appropriate test (ANVISA, 2012).

Table 1: Acceptable limit of elements contained in cosmetic products.

Element	Quantity in PPM
Hydrochloric acid soluble barium 0.001N (expressed as barium chloride)	500 ppm
Arsenic (expressed as As2O3)	3 ppm
Lead (expressed in Pb)	20 ppm
Other Heavy Metals	100 ppm

Source: ANVISA (2012)

The microbiological control parameters for personal care products, cosmetics, and perfumes and their acceptable limits are described in table 2 and are established by Resolution RDC No. 630, of March 10, 2022, and internalize GMC MERCOSUR Resolution No. 51/1998, and according to its article 1 classifies products as type I: products for children's use; eye products;

and products that come into contact with mucous membranes and type II other cosmetic products susceptible to microbiological contamination.

Table 2: Parameters for microbiological control of cosmetic products.

	Area of application and age range	**Limits of acceptability**
TYPE-1	Products for children's use Eye Products Products that meet mucous membranes	a) Count of total aerobic mesophilic microorganisms, not more than 102 CFU/g or ml b) Absence of pseudomonas aeruginosas in 1g or 1ml; c) Absence of Staphylococcuis aureus in 1g or 1ml; d) Absence of total and fecal coliforms in 1g or 1ml; e) Absence of sulfite reducing clostrides in 1g (exclusively for talc).
TYPE-II	Other cosmetic products susceptible to microbiological contamination	f) Count of total aerobic mesophilic microorganisms, not more than 10^3 CFU/g or ml; g) Absence of pseudomonas aeruginosas in 1g or 1ml; h) Absence of staphylococcus aureus in 1g or 1ml; i) Absence of total and fecal coliforms in 1g or 1 ml; j) Absence of sulfite reducing clostrides in 1g (exclusively for talc).

Source: ANVISA (2022)

Currently, RDC Resolution No. 83, issued in June 2016, revokes certain provisions of RDC Resolution No. 48 (ANVISA, 2006, 2016). This updated resolution lists 1373 banned substances in personal care products, cosmetics, and perfumes, aligning with the list of banned substances set out in the European Union document. Elements such as: Cl, Ni, As, Be, Cd, Cr, I, P, Pb, Hg, Se, Zr, Co, Te, Tl and radioactive substances are among the items mentioned in the list (ANVISA, 2016; EUROPEAN UNION, 2009).

Directive 76/768/EEC of the European Council is an important piece of legislation dealing with the regulation of cosmetic products in the European Union. However, it is important to note that this directive was replaced by Regulation (EC) No. 1223/2009 of the European Parliament and of the Council, which entered into force on 11 July 2013. Regulation (EC) No 1223/2009 replaces Directive 76/768/EEC and established stricter and more detailed requirements for the safety of cosmetic products. This new regulation more specifically addresses the presence of hazardous substances, including heavy metals, by imposing more precise limits and requiring more comprehensive testing to ensure that products comply with these limits. Therefore, while Directive 76/768/EEC was important in introducing some restrictions for heavy metals in cosmetics, Regulation (EC) No. 1223/2009 is the most recent and comprehensive legislation currently governing the safety of cosmetic products in the European Union, including the provisions related to heavy metals.

2.2 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

ICP-OES has the ability to reach very high temperatures and has an inert atmosphere, in addition to enabling the determination of up to 70 elements simultaneously. When high volumes of sample are available, it is possible to reach detection limits considered low (reaching ppb to be determined), depending on the application and also on the analyte, for example, for the analysis of some metals in clay it is possible to achieve detection limits much lower than the values determined by legislation, which makes it a highly recommended technique (HOU, JONES, 2000; OLESIK, 1991).

ICP-OES is a technique based on the emission of photons from ions excited by plasma, which is generated by a radiofrequency coil (HOU, JONES, 2000). After the sample is inserted into the equipment, it is converted into an aerosol that is directed to the plasma, where the atoms are converted into ions and then raised to an excited level. These species, when they return to the ground state, emit photons, which are characteristic of each chemical species, and the amount of photons is proportional to the concentration of the analyte. A portion of these photons are collected with a lens or concave mirrors, which form an image at the aperture of a wavelength selection equipment. These wavelengths are converted into electrical signals by a photodetector, and in a computer the signal is amplified and processed, the operation of the equipment is shown in figure 1 (HOU, JONES, 2000; BAIRD, 2002).

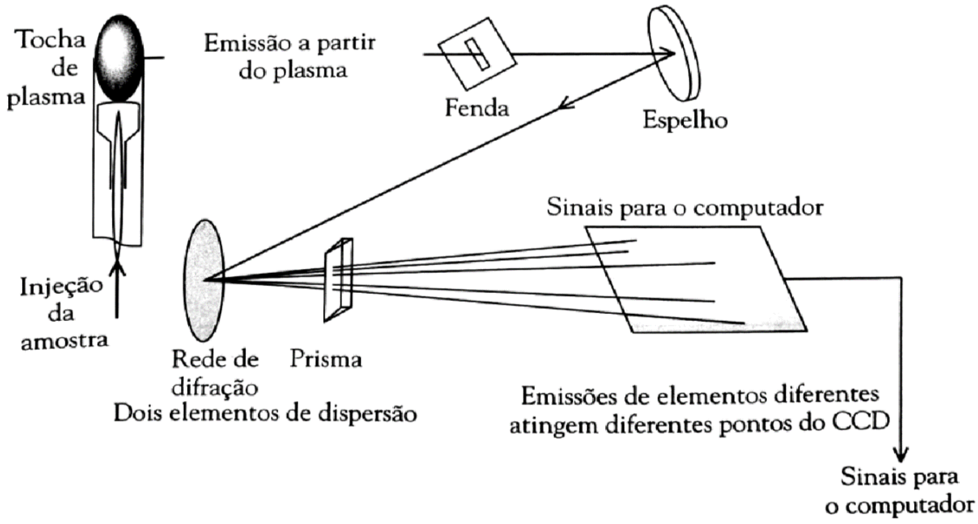


Figure 1: Schematic of the operation of an ICP-OES (BAIRD, 2002).

ICP-OES operates with emission of electromagnetic radiation in the visible and ultraviolet regions of the spectrum, and the analysis technique is based on the excitation of the analyte by argon plasma. The plasma temperatures reach very high values (7000 to 15000 K), which provides a low interference in the analysis of the chemical elements, since the plasma reaches much higher temperatures and due to the presence of the stable and chemically inert environment of the argon atmosphere, when compared to the analyses that make use of flames. whose environment is violent and highly reactive (SKOOG et al., 2002). The analysis of the elements is done through an established relationship with a reference standard and the intensity of energy detected at the indicated wavelength, it is possible to determine the concentration of the analyte in the sample. This relationship is constructed through analytical curves (CIENFUEGOS, VAITSMAN, 2000).

This technique is widely used for the determination of metals in different matrices because it has many advantages when compared to other spectrometric techniques, such as efficient ionization or excitation of the elements (given by the high temperatures reached in the plasma), for the possibility of performing multielement analyses, for presenting high sensitivity and enabling the analysis of a wide range of concentrations. because of its characteristic linearity.

2.3 Metals and semimetals found in green clay

The following is a description of the properties of some metals and semimetals analyzed in this study.

2.3.1 Aluminium

Aluminum is the predominant metal and the third most abundant chemical element in the Earth's crust, after oxygen and silicon. Human beings are constantly exposed to aluminum, in the form of dust and particles dispersed in the environment (NASCIMENTO, 2004). It is always found combined with other elements like oxygen, silicon, and fluorine.

Aluminum metal is lightweight and has a silvery-white appearance. It is poorly absorbed after oral or inhalation exposure and is essentially not absorbed dermally and has astringent properties and its effect tends to shrink or contract the tissues of the body, usually at the site of application of a topical or cosmetic drug. The astringents applied externally cause slight coagulation of the skin proteins, dries out, hardens and protects the skin. People suffering from acne and oily skin are often advised to use products with astringent action (PUBCHEM, 2023).

2.3.2 Iron

Iron is a vital mineral for cellular homeostasis. Its ability to accept and donate electrons makes it essential for various biological reactions (GROTTO, 2010). Metallic element, in almost all soils and mineral waters. It is an essential constituent of hemoglobin, cytochrome, and other components of the respiratory enzyme systems. Its main functions are in the transport of oxygen to the tissues (hemoglobin) and in the mechanisms of cellular oxidation (PUBCHEM, 2023).

The trace element iron plays a crucial role in cellular respiration and electron transfer. Recognized as a remineralizer, it is responsible for healthy skin tone, being an essential nutrient for oxygen metabolism and performing a vital function in the skin's mitochondria. Its importance in cutaneous homeostasis is reflected in its ability to repair damage and its participation in intracellular oxidation-reduction processes. In addition to regulating mitochondrial DNA during the synthesis of metabolically active basal cells of the epidermis. It also stimulates collagen production and contributes to the healing process in the dermis. The deficiency of this element in the skin is manifested by a thin, dry epidermis with a loss of elasticity. When present in the bioavailable form, it promotes a healthy and balanced appearance for the skin as a whole (COSTA, 2012).

2.3.3 Magnesium

Magnesium is classified as an alkaline earth metal and has 2 layers of hydration. The element can be found in abundance in the hydrosphere (PUBCHEM, 2023). According to Costa

(2012), it is particularly indicated for the biological reactions of the dermis, facilitating the transfer of the 3-phosphoadenosine-5-phosphosulfate sulfate (PAPS) sulfate ion, which represents the biologically active form of this ion.

The role of the magnesium ion is interconnected with that of calcium and performs several functions, such as: It contributes to the production of specific genetically encoded proteins, helping to stabilize the structure of the DNA double helix, it is an essential element in the synthesis of several compounds with high energy bonds, such as the phosphoric anhydride bond found mainly in ATP or ITP, in addition to actively participating as a cofactor in approximately 300 enzymes essential for the metabolism of carbohydrates, nucleic acids, proteins and lipids.

This brief explanation of magnesium highlights its utmost importance in biology. When in its bioavailable form through biotechnological technology, it energizes and tones the skin, working in tandem with zinc to promote natural revitalization (revitalizing properties). In partnership with vitamin C in topical applications, magnesium inhibits tyrosinase activity, stimulates collagen synthesis and exhibits antioxidant activity against free radicals (COSTA, 2012).

2.3.4 Titanium

Classified as a transition metal, titanium, in the form of titanium dioxide (TiO2), is widely employed in the pharmaceutical and cosmetic industries for the production of a variety of makeup items, including pressed powders, blushes, eyeshadows, and nail polishes, as well as playing a key role in sunscreens. It is often combined with different pigments to create pearlescent and satin effects, leaving the skin dull. Playing a crucial role as a physical sunscreen, titanium dioxide is free of irritative effects found in other chemicals (COSTA, 2012).

2.3.5 Zinc

Zinc has been identified as a cofactor for more than 70 different enzymes, including alkaline phosphatase, lactic dehydrogenase, and RNA and DNA polymerase. Zinc facilitates wound healing, helps maintain normal growth rates, normal skin hydration, and senses of taste and smell (PUBCHEM, 2023).

According to Costa (2012), zinc in its bioavailable form helps to improve the healthy appearance of the skin, minimizing fine lines caused by environmental stress, normalizing the surface of the skin Zinc supplements are useful in the treatment of skin problems, such as ulcers of the lower limbs, but only in cases where initial zinc levels are reduced. Ointments based on this mineral, applied directly to the skin, seem to be more effective than supplements, in reducing infections and stimulating wound healing, except in cases of serum deficiency of this metal, as occurs in acrodermatitis enteropathica.

2.3.6 Cobalt

Cobalt plays an essential role in the synthesis of vitamin B12, also known as cyanocobalamin. This vitamin is a complex organic molecule, whose active nucleus is the cobalt atom, presenting a structure similar to hemoglobin. Vitamin B12 is crucial for the functioning of several enzyme systems involved in energy utilization.

Vitamin B12 deficiency can result in a lack of hemoglobin formation, leading to a range of damage to the central nervous system, as noted in previous studies (LEHNINGER, 1985). In ruminant animals, the absence of this vitamin significantly affects vital metabolic processes such as gluconeogenesis and hematopoiesis. In addition, it influences the metabolism of carbohydrates, lipids, and nucleic acids, as highlighted by (GRAHAM, 1991).

2.3.7 Manganese

Manganese is a naturally occurring metal found in many types of rocks. Pure manganese is silver in color, but it does not occur naturally. It combines with other substances such as oxygen, or sulfur or can be found in various foods, including grains and cereals, and is found in high amounts in other foods, such as tea. Manganese is an essential and necessary trace element for good health (ATSDR, 2021).

2.3.8 Copper

Copper, in its natural form as Cu^{2+} ions, offers several benefits for health and dermatological care. Since the 1970s, studies have shown that copper peptides, such as GHK Cu, aid in wound repair and increase the expression of important molecules in the extracellular matrix. The use of copper was noted in wound healing creams and later incorporated into cosmetic moisturizers for wrinkle reduction, as it stimulates collagen production. In addition, it has been shown to be effective in improving facial skin through copper oxide pillows, in post-treatment with CO_2 laser, and in reducing the risk of infections in diabetics through copper-impregnated socks. Copper exhibits algaecidal, fungicidal, nematicide, molluscocide, antibacterial, antiviral, and anti-inflammatory properties. Recently, it has been discovered that water-absorbing copper ions can stimulate melanin production, offering a possible natural cure for vitiligo, either through topical application or by consuming stored or cooked water in copper containers, increasing oral absorption of the metal. Although copper penetrates the skin with difficulty, it binds to proteins to exert biological activity. It is involved in more than 100 enzymes, including cytochrome C-oxidase, superoxide dismutase, tyrosinase, lysyl-oxidase, dopamine beta-hydroxylase, and factor V, promoting thrombin formation. Systemic copper deficiency can affect collagen synthesis and decrease melanin production. In specific areas, such as the anogenital region, copper can be used in repairing creams for localized vitiligo and in local washes with water containing copper ions, with antibacterial and antiviral properties (COSTA, 2012).

2.3.9 Cadmium

It is a metal found in the earth's crust, with a silvery white color and used as a pigment, among other applications. Cadmium is a toxic element that is likely to cause cancer in humans (ATSDR, 2012). Despite this, regulations from ANVISA (2016) and the European Union (2009) prohibit the use of this element in cosmetic formulations. However, analyses conducted by Grosser, Davidowski and Thompson (2011) demonstrated the presence of 111Cd at concentrations ranging from 0.0110 μg/g to 0.0832 μg/g.

2.3.10 Barium

Barium is a silvery-white metal that exists in nature only in ores containing mixtures of elements. It combines with other chemicals, such as sulfur or carbon and oxygen, to form barium compounds. Barium compounds are used by the oil and gas industries to make drilling muds. Drilling muds make it easier to drill into the rock while keeping the drill bit lubricated. They are also used to make paints, bricks, ceramics, glass, and rubber. Barium sulfate is sometimes used by doctors to perform medical tests and take X-rays of the gastrointestinal tract (ATSDR, 2021).

2.3.11 Chrome

Chromium is used in a variety of chemical, industrial, and manufacturing applications, such as wood preservation and metalworking. The uses of chromium compounds depend on the valence of chromium, where trivalent Cr(III) compounds are used for dietary Cr supplementation and hexavalent Cr(VI) compounds are used as corrosion inhibitors in commercial settings and are known to be human carcinogens (DRUGBANK, 2023).

2.3.12 Arsenic

It is a semimetal found in significant amounts in the earth's crust. Inorganic arsenic, in contact with the skin, can cause irritation and contact dermatitis. Effects can range from mild to severe, and may cease when exposure is stopped. Mild symptoms include redness and swelling, while severe symptoms can result in papules, blisters, or necrotic lesions (ATSDR, 2007).

Arsenic is recognized for its carcinogenicity in humans, being associated with cutaneous, pulmonary and bladder neoplasms, and possibly kidney, liver and prostate cancers (LIU, GAYER and WAALKES, 2012). In cosmetic clays, arsenic concentrations ranging from 0.6 to 8.9 µg/g have been detected (Silva et al., 2011).

2.3.13 Lead

It is one of the metals that most cause poisoning in humans and that most pollutes the environment. Modern man has 500 to 1,000 times more lead in his body than his prehistoric ancestors. Considered a neurotoxin, it affects all the noble tissues of the body and promotes the displacement of essential minerals for its proper functioning, such as calcium, iron, copper and zinc. In addition, it blocks and inactivates enzymes and increases cell membrane permeability. In large doses of contamination, it seriously affects the central nervous system and causes damage to the liver, kidneys and reproductive organs and in the gastrointestinal region (COSTA, 2012).

2.3.14 Nickel

Nickel is a metal with a silvery appearance. It is employed in painting ceramics, making certain types of batteries, and is found in coins and jewelry. Exposure to nickel is often associated with allergic reactions, affecting about 10% to 20% of the population sensitive to this metal. Some nickel compounds have been identified as carcinogenic to humans (ATSDR, 2005; LIU, GAYER and WAALKES, 2012).

2.3.15 Vanadium

Vanadium is a compound that occurs in nature as a white to gray metal and is often found in the form of crystals. Pure vanadium has no smell. It usually combines with other elements such as oxygen, sodium, sulfur, or chloride. Vanadium and vanadium compounds can be found in the Earth's crust and in rocks, some iron ores, and crude oil deposits. Vanadium is mostly combined with other metals to form special metal mixtures called alloys. Vanadium in the form of vanadium oxide is a component of special types of steel used in automotive parts, springs, and ball bearings. Most of the vanadium used in the United States is used to make steel. Vanadium oxide is a yellow-orange powder, dark gray flakes, or yellow crystals. Vanadium is also mixed with iron to make important parts for aircraft engines. Small amounts of vanadium are used in the manufacture of rubber, plastics, ceramics, and other chemicals (ATSDR, 2021).

2.3.16 Mercury

This element can be metallic (elemental mercury), organic, and inorganic. Metallic mercury is silvery white in color (ATSDR, 1999).

Mercury has a propensity to accumulate mainly in the kidneys and in the bones, liver, spleen, brain, and fatty tissue of the human body. What is not expelled through urine or feces remains in the body, interfering with protein synthesis. In addition, its harmful action extends to the central nervous system, increasing the release of various neurotransmitters. A strong association of this substance with cases of multiple sclerosis has been observed (COSTA, 2012).

Table 3: Main properties of metals in cosmeceutical use.

Copper	It acts on the development of connective tissue and melanin synthesis**.**
Zinc	Maintenance of physiological cutaneous pH, synthesis of collagen and elastin, essential for cellular oxygenation and reconstitution of the cell membrane. It protects nucleic acids (RNA-DNA) and ensures the molecular and cellular integrity of skin and hair. It inhibits the action of the enzyme 5a-reductase. Sebum-regulating and antimicrobial action.

Magnesium	Anti-aging Improves electron transport and protein production. It has the power to fix potassium and calcium ions and participates in the synthesis of collagen responsible for muscle tone. Helps fight chronological aging of the skin.
Iron	Hydration It plays an important role in cellular respiration and electron transfer. In the skin, deficiencies of this element are manifested by a thin, dry epidermis with a lack of elasticity.
Aluminium	Antiperspirant agent, melanin adsorbent, thickener, pigment.
Titanium	Pigment of varied use, physical sunblock.

Source: Costa (2012)

3 GOALS

3.1 GENERAL OBJECTIVE

To characterize the Green Clay of the company Green Clay Farm LTDA, referring to the area of the process ANM 820.123/2018 to assess its compliance with the legislation of ANVISA RDC No. 48/2006 and the European Union regulation EC No. 1223/2009.

3.2 SPECIFIC OBJECTIVES

- To determine the chemical composition of Green Clay Farm's green clay by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).
- To estimate the physicochemical profile of the green clay sample from the Green Clay Farm.
- Perform an assessment of the microbial activity of the clay sample from Green Clay Farm.
- Verify that the study clay complies with the legislation of ANVISA RDC No. 48/2006, 2006 and the European Union regulation EC No. 1223/2009 on the presence of metals harmful to health.

4 METHODOLOGY

The research work of Argila Verde was restricted to the area of the process ANM 820.123/2018, proposed in an area of 33.09 hectares, where MRP Participações Eireli, established a new company Green Clay Brazil Ltda, the deposit is located at Fazenda Trevo Branco, located in the municipality of Quadra, state of São Paulo, Brazil.

The clay underwent mineralogical characterization tests, where two sets of analyses were carried out, one of which was completed by the Lito Jr Company – Junior Geology Company of Unesp/Rio Claro. The other battery of analyses was carried out at CETEM – Center for Mineral Technology, headquartered in Rio de Janeiro. The report concluded that the samples collected at different points of the deposit had a great similarity, which is quite interesting, aiming at the quality control of the product. The basic mineralogy identified encompasses kaolinite, illite, quartz, and muscovite.

The clay samples arrived in the municipality of Porto Velho - RO, on September 30, 2023, packed in a cardboard box, which contained three different samples packed in a plastic bag, as can be seen in figure 2.

Legend: (a) box received; (b) samples of clays received

Figure 2: Receipt of samples.

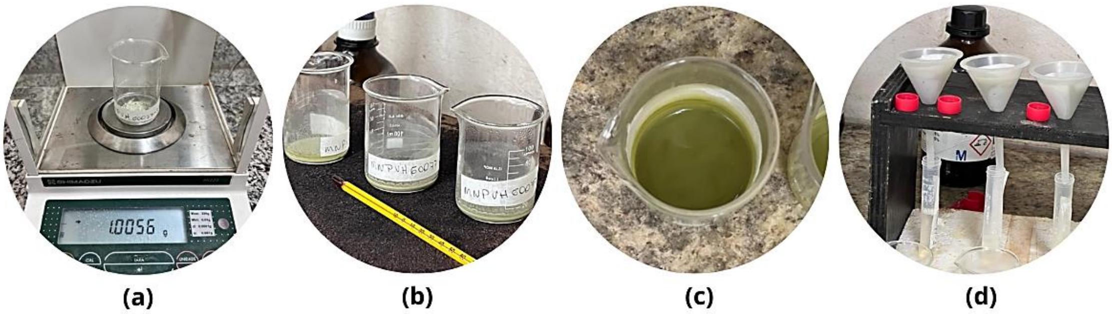
We received 3 samples of Green Clay from the company Green Clay Farm, one in its natural state and two more samples pre-processed, through drying, crushing and screening:

1. 3.5 kg, raw clay.
2. 0.9 kg, screened at 270 mesh, powder particle size at 53 micrometer
3. 0.5 kg, screened in 325 mesh, particle size at 44 micrometer.

4.1 Sample preparation and analysis

4.1.1 Acid digestion of the sample

For the quantification of trace elements in Green Clay Farm, a 325 mesh sample was used, weighing about 1.0 gram of sample in triplicate, in a 100 mL beaker for chemical solubilization. For this process, about 8.0 mL of HNO3 (nitric acid, Merck, Germany) 65% (m.m-1) was added to each beaker and evaporated under heating in a heating plate (Q313F, Quimis – Brazil) at a temperature of 120°C. Then, about 8.0 mL of aqua regia solution (HCl:HNO3 [3:1]) was added to each beaker and evaporation of the solution was repeated. After evaporation, the samples were resuspended with 0.1N HCl and stored in Falcon tubes for a final volume of 15 mL. Some steps of the process are illustrated in figure 3.



Legend: (a) weighing of the sample; (b) evaporation of acid in the heating plate; (c) clay after evaporation of acid; (d) Samples were re-suspended.

Figure 3: Process of sample preparation through acid digestion.

After preparation, the trace elements present in the samples were quantified by the ICP-OES technique.

4.1.2 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

Analytical quality control aims to provide greater reliability in the results obtained at the end of the entire analytical procedure. Among the available devices, we can mention the use of white samples, the use of triplicates and certified reference materials (MRC). The latter are made available by recognized entities, and provide known values of certain analytes, as well as the associated uncertainties. These values are nothing more than consensual results from accredited laboratories and cannot be considered as true values, but those that are closest to what is considered as such (JARDIM and SODRÉ, 2009).

To calculate the Analytical Technique Detection Limits (LDT) of the green clay samples for the sixteen trace elements evaluated, the following equation was used:

$$\text{LDT} = [(\text{average of whites. final volume}) \,/\, \text{average of masses}]$$

The blanks were performed through the entire analytical procedure, but only with the chemical reagents used, i.e., without the presence of a sample, multiplying by the final volume (15 mL) and dividing by the mean of the masses of all the samples analyzed (BASTOS et al., 1998).

Analytical quality control was performed through triplicate analysis and adoption of duplicates in each sample, blank and control, to verify possible contaminants present in the reagents used throughout the process. In addition, samples of the SS2 certified reference (SSP-SCIENCE) were used, whose function is to determine the accuracy of the results obtained by the analysis of the ICP-OES equipment shown in figure 4.

Figure 4: Perkin Elmer ICP-OES equipment, model Optima 8300 with autosampler model S10 used for trace element determinations.

The following analysis conditions were adopted in ICP-OES for operation on the equipment using Perkin Elmer's WinLab 32 for ICP software version 5.5.0.0174. The main parameters can be checked in detail in table 4:

Table 4: Values used for ICP-OES parameters

Parameter	Established value
Plasma Flow	8.0 L.min-1
Auxiliary Flow	0.2 L.min-1
Nebulizer Flow	0.70 L.min-1
Radio Frequency Power	1500 W

Plasma Vision	Axial
Integration time	5 sec
Stabilization time	11 s
Sample Flow	1.00 mL.min-1
Sample aspiration time	15 s
Rinse time	15 s
Numbers of replicates	2

4.2 Decontamination of the clay sample

Currently, for clay to be incorporated into cosmetic formulations, it is essential to validate a decontamination process. This procedure aims to ensure the effective removal of unwanted microorganisms. Initially, temperature decontamination was tested by subjecting the sample to dry heat in an oven, keeping it at 120 °C for 24 hours.

4.3 Microbiological analysis

With the collaboration of FIOCRUZ-RO, the microbiological analysis can be carried out, for the validation of the decontamination process of the Green Clay Farm sample, 50 mL Falcon tubes, called AV 270 and AV 270 DEFINFEC, were sent to the care of Dr. Naja Benevides Matos, in order to evaluate the microbiological purity of these samples. The experiment was based on a careful scientific approach, such as triplicate analyses and reanalyses to confirm the results.

The materials used in this study were sterile and disposable 10μL plastic handles, test tubes containing 5 mL of Luria Bertani (LB) and Brain Heart Infusion (BHI) culture media, Petri dishes with Blood agar, MacConkey agar, Chromagar and Mannitol agar, glass slides and the kit of reagents for Gram staining. in addition to the microscopy equipment and the bacteriological oven at a temperature of 37°C

The clay samples were inoculated in the test tubes with LB medium and BHI medium. The dissemination of the samples in the Petri dishes, with different culture media, provides a diverse environment for microbial growth.

The incubation of Petri dishes in the bacteriological oven at 37°C for 24 to 48 hours is essential for microbial development and allows the observation of bacterial colony formation, which is followed by microscopic analysis, with the preparation of slides stained by the Gram Staining Kit, for microscopic observation.

4.4 Complementary physicochemical tests

The green clay sample from Green Clay Farm underwent physicochemical experiments to obtain complementary data for the technical sheet, an important document for the quality control of pharmaceutical raw materials.

4.4.1 Determination of pH

Figure 5: Gehaka PG3000 Digital Tabletop Foot Gauge.

To estimate the pH of the Green Clay, 1 gram of the clay sample and a 100 mL Becker were weighed and distilled water was added until the volume of 100 mL was completed. The preparation was analyzed with the aid of the GEHAKA caliper, and the results of pH and temperature were collected. The experiment was performed in triplicate and the mean of the results were calculated.

4.4.2 Density

Actual density represents the mass per unit volume of a substance, considering its actual volume without including voids or porosity. The determination of clay density was segmented into two distinct phases: uncompacted density and compacted density. To conduct this experiment, clay samples, a high-precision Uni Bloc analytical balance and a 50 mL beaker were required.

Legend: (a) compacted clay; (b) uncompacted clay

Figure 6: Density analysis using glass beaker.

Initially, the beaker was weighed and its value was zeroed, then the beaker was filled with a volume of 13 mL of clay, resulting in a measured mass of 10.3 grams. Subsequently, clay was compacted, reducing its volume to 11 mL. The values were calculated using the formula:

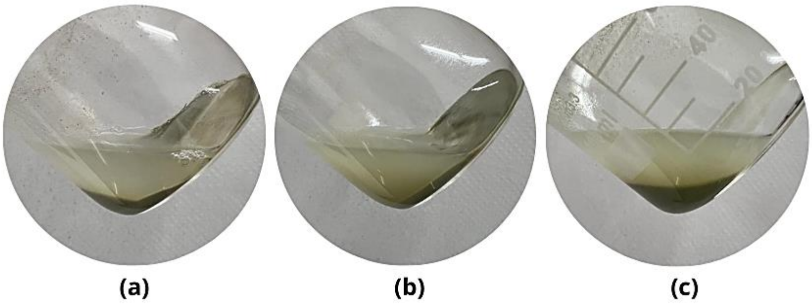
$$D = \frac{m}{v}$$

Where: (D) Density; (*m*) Mass; (*v*) Volume

The two-step segmentation, first evaluating the uncompacted density and then the compacted density, allowed the understanding of the variations in the density of the clay under different compaction conditions.

4.4.3 Solubility

According to the Brazilian Pharmacopoeia, the solubility test is performed to evaluate the suitability of a substance for pharmaceutical use, considering its dissolution in different solvents. The experiment was conducted using three distinct samples and three assorted solvents, distilled water, Mega chemical alcohol and an organic solvent acetone PA Exodus scientific, as shown in figure 7.



Legend: (a) Distilled water; (b) Absolute Alcohol; (c) Acetone PA.

Figure 7: Solubility analysis of Green Clay from Green Clay Farm.

For the experiment, three 100 mL glass beakers were used, in which 1 gram of green clay from the green clay farm were weighed on a Uni Bloc analytical balance. The solvents were added at a temperature of 25 ºC. The expression *"parts* " refers to the number of milliliters of solvent per gram of solid to be dissolved. The approximate solubilities established in the monographs are designated in descriptive terms, the meanings of which are listed in Table 4:

Table 5: Descriptive terms of solubility and their meanings.

Descriptive term	Approximate volumes of solvent in milliliters per gram of substance
Very soluble	Less than 1 piece
Easily soluble	From 1 to 10 parts
Soluble	From 10 to 30 pieces
Moderately soluble	From 30 to 100 pieces
Poorly soluble	From 100 to 1000 pieces
Very little soluble	From 1000 to 10000 pieces
Virtually insoluble or insoluble	More than 10000 pieces

Source: Brazilian Pharmacopoeia, 6th edition

For each clay sample, 10 parts of each solvent were added. The indicated solubility should not be considered strictly as a physical constant, but as a complement to the other tests.

4.4.4 Moisture Content by Greenhouse Method

To date, this is the most accurate method of determining the moisture content of soils, being applied in laboratories. This methodology has an advantage over the others, because it presents reliable results, but it has as a drawback, the excessive time to obtain this physical index.

Legend: (a) Weighing of raw clay; (b) Samples cooling in the desiccant; (c) Clay processed after kilning; (d) Raw clay after kilning.

Figure 8: Moisture content test by greenhouse.

The empty crucible was weighed where the mass varied from 37.2 g to 45.7 g, then 10 g of green clay was placed, the test was carried out in triplicate for the raw clay in its natural state, as well as the processed one. The crucibles with the samples were placed in the preheated oven at a specific temperature of 105°C to 120°C, for 32 hours, during which time two monitoring weighing took place. After the drying time, the crucibles with the samples were removed from the oven and it was used in a desiccator to cool and prevent the absorption of atmospheric moisture. With the crucibles with the sample cooled and dried, the final weighing of the experiment took place. The formula below was used to calculate the moisture content and the result was expressed as a percentage.

Moisture content (UM) can be calculated using the formula:

$$UM(\%) = \left(\frac{m2 - m3}{m2 - m1}\right) X\ 100$$

Where: *m1* - is the mass of the empty crucible; *m2* - is the mass of the crucible with the wet sample; *m3* - is the mass of the crucible with the dry sample.

This method provides a reliable estimate of the moisture content in the sample, which is essential for ensuring quality and compliance in a variety of industrial sectors.

5 RESULTS AND DISCUSSION

The analysis of the metals and semimetals mentioned in the study shows the diversity of properties and functions performed by these elements in the cosmetic and dermatological spheres. Each of these elements exhibits unique characteristics that exert a significant influence on both health maintenance and skin care, being able to be applied in cosmetic products, medicines and, on the other hand, can pose risks when present in disproportionate concentrations, table 6 and graph 1 illustrate the parameters present in the study of Green *Clay Farm*' s green clay.

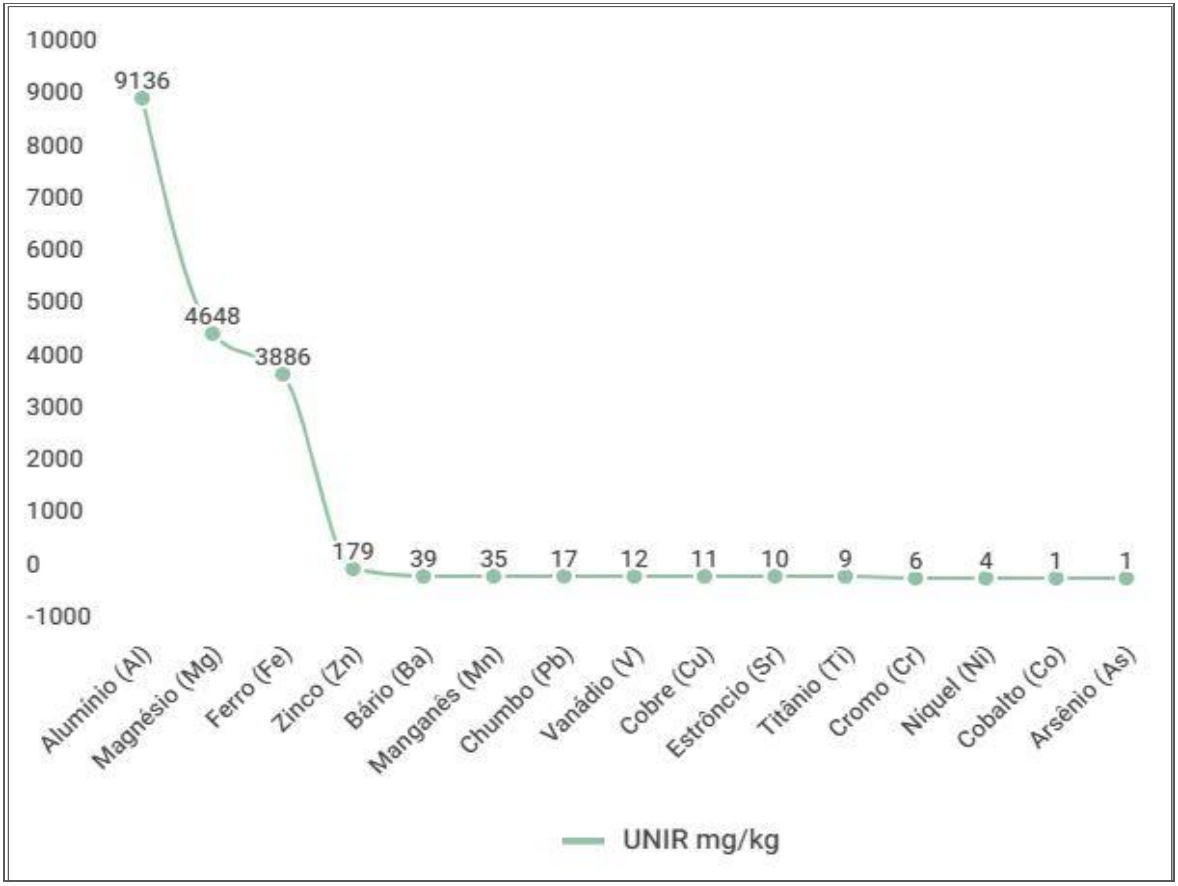


Figure 9: Inductively Coupled Plasma Optical Emission (ICP-OES) sample analysis. Source: author own

When looking at the graph it is evident that the chemical elements with the highest concentrations are Aluminum, Magnesium, Iron and Zinc, these metals were detected through Inductively Coupled Plasma Optical Emission Spectrometry and the list of substances found in the Green Clay Farm sample and their respective amounts are expressed in table 6.

Table 6: Parameters of metals found in clay by Inductively Coupled Plasma Optical Emission Spectrometry

Parameter *Ppm*	**Join** *mg/kg*
Aluminum (Al)	9.136,10
Magnesium (mg)	4.648,06
Titanium (Ti)	9,22
Iron (Fe)	3.886,78
Zinc (Zn)	179,48
Cobalt (Co)	1,60
Manganese (Mn)	35,63
Copper (Cu)	11,16
Cadmium (Cd)	0
Barium (Ba)	39,83
Chromium (Cr)	6,43
Arsenic (AS)	1,55
Lead (Pb)	17,40
Nickel (Ni)	4,54
Vanadium (V)	12,27
Mercury (Hg)	0,079

Source: author own

From the results of the analyses performed through the ICP-OES, expressed in Table 6, the chemical elements verified in the green clay sample were expressed by the unit of measurement, parts per million (ppm), milligrams per kilogram (mg/kg). The results reveal a diverse chemical composition, highlighting some elements in particular.

The element with the highest concentration is Aluminum (Al) of 9,136.10 ppm, a remarkable presence, which is justified according to Gardolinsk (2001) due to the presence of the clay mineral Kaolinite ($Al_{2Si2O5}(OH)_4$) which is a natural aluminosilicate, this result is intrinsically linked to the nature of green clay.

Also noteworthy are the concentrations of Magnesium (Mg), Iron (Fe) and Zinc (Zn), in the respective concentrations of 4,648.06 ppm, 3,886.78 ppm and 179.48 ppm. These elements are characteristic of the qualitative mineralogical composition of green clay, which corresponds to a mixture of quartz, smectite, illite and kaolinite. It is essentially formed of iron oxide, magnesium, silicon and zinc, offering astringent and purifying activity. Therefore, it is effective in regulating oiliness in the skin (AMORIM; PIAZZA, 2012; BUENO; RODRIGUES, 2018; GOPINATH; CROSS; FREIRE, 2003; SANTANA et al., 2021).

The presence of the elements, Cobalt (Co), Titanium (Ti), Manganese (Mn) and Copper (Cu) may contribute to the ability of green clay to nourish the skin and promote cell regeneration, as well as play a role in the antimicrobial properties of Co and Ti show potential in inhibiting the growth of *staphylococcus aureus*, a bacterium responsible for causing skin infections. studies report that, although little researched, Mn can act to inhibit bacteria of the *miycrobacterium family* , which also causes skin infections (ASSIS, 2008; VALLEJO, 2016; QUIAN, 2022; COSTA, 2012).

A concentration of 39.83 ppm of barium (Ba), an alkaline earth metal naturally occurring in the soil, was detected in the analyzed sample. It is a constituent of carbonates, sulfates, and silicates and occurs in minerals as an isomorphic substitute for elements such as Ca and K (VAZQUEZ & ANTA, 2009). When available at high soil levels, it can cause toxicity to plants and invertebrates (KUPERMAN et al., 2006; COSCIONE & BERTON, 2009). However, a study carried out in France by Labarthe et al. (2023), Analyse the diffusion of barium to the organism. The experiment used a clay with one of the highest levels of Ba available in France for medical therapy and volunteers with a higher characteristic for transcutaneous diffusion (young individuals with low fat mass). Therapeutic pastes derived from clay are applied to the skin for the treatment of some rheumatologic conditions. Twenty-four healthy young volunteers were surveyed, blood and urine samples were collected at pre- and post-treatment and analyzed with inductively coupled plasma mass spectrometry, and no significant systematic increase in plasma or urinary Ba levels was evidenced, so the study concluded unequivocally that there is no risk of Ba overexposure in patients receiving clay therapy according to the procedure used in French medical spas.

The low concentration of Arsenic (As), 1.55 ppm and traces of Mercury (Hg) suggests an acceptable level of safety, according to current legislation cited in the text below. The element Cadmium (Cd), on the other hand, was tested and was considered absent and the absence is a positive aspect, indicating a product free of potentially toxic elements. Cadmium is classified as a toxic substance that is considered carcinogenic to humans. In contact with the body, it accumulates for a long time, especially in the kidneys and liver, when it is found its biological half-life is around 10 years and can reach 40 years in the body (FERNANDES, 2014).

On the other hand, Lead (Pb) and Vanadium (V) were found at concentrations of 17.40 ppm and 12.27 ppm, respectively, in a study carried out by Whiteside and collaborators in 2020, they tested the total concentrations of lead in 3 different brands of Green Clay of French origin and found average concentrations of lead that ranged from 21.457 to 54.754 ppm, in the same study, arsenic (As) was measured and revealed high concentrations, averaging from 8.483 to 31.607 ppm. Another relevant data of this study is that rats with dermatitis were treated with these clays to evaluate the biological harmful effects caused by these metals, but observed that cutaneous absorption was not a significant route of absorption, the study analyzed skin samples from mice treated with saline solution, compared with those treated with clay and saw no differences in the total concentrations of arsenic and lead between rats treated with saline solution and groups treated with clay.

These interwoven results provide a comprehensive understanding of the composition of green clay, grounding its application in a variety of contexts, from aesthetic procedures to specific therapies. The analysis of these elements is supported by the legislation in force, especially by the Resolution of the Collegiate Board (RDC) No. 44, of August 2012, of the National Health Surveillance Agency (ANVISA).

On the other hand, it is crucial to highlight that element such as Cd, Ba, Cr, As, Pb, Ni, V, and Hg, when present in inappropriate concentrations or toxic forms, can pose risks to dermal health. In this sense, the presence of Cd and Hg, known for their toxicity, as well as other elements such as As, Pb and Ni, requires caution in their use in cosmetic products.

It is important to note that, despite their potential toxicities, the concentrations of these metals in green clay are minimal and within the standards established by ANVISA, as provided for in RDC No. 44/2012. In general, in their most recent editions, both the Brazilian and European

Pharmacopoeia (EP), within the legislation of the current regulatory bodies, highlight the need for limit tests for heavy metals in various raw materials and finished products. These tests are virtually present in all other pharmacopoeias. Some of these tests are directed to specific elements, such as Pb, Hg, Ni, As, among others (BARIN, 2007).

Establishing quality control is essential in the pharmaceutical industry to ensure that the drugs and cosmetics marketed have safety, efficacy and proven quality. The procedures carried out in order to ensure the adequate standard of these products must be in accordance with specifications pre-established in the Brazilian Pharmacopoeia and international compendiums recognized by ANVISA are released for commercialization after satisfactory results for their use.

The raw materials must have documentation containing tests made of some properties, such as organoleptic characteristics, pH, average weight, density, volume, moisture content, solubility, microbiological purity, among others, so that the same related tests are carried out to compare and ensure that there has been no alteration or violation of the products.

Table 7: Physicochemical tests

Physicochemical Tests	Parameters
Ph	5.46 at 24.5 °C
Compacted density	0.93 g/ml
Uncompressed density	0.79 g/ml
Solubility	Not soluble in water, alcohol and acetone
Moisture content of crude clay	37%
Moisture content of processed clay	2,51%
Colour	Mint Green
Brilliance	Opaque glossy
Odour	Soft characteristic of clay
Sense of touch	Smooth

Source: author own

pH plays a crucial role in cosmetic product formulations, especially to ensure their compatibility with human skin. The pH measured was 5.46, characterized as slightly acidic. This suggests that the pH of clay can maintain the skin's natural balance, being in line with the pH range of human skin, which ranges from 4.7 to 5.75. This similarity can contribute to healthier, more protected skin. Knowledge of pH is essential to guide the development of specific cosmetic formulations.

Another important point is the analysis of Compacted and Uncompacted Density, revealing structural differences that affect the physical properties of cosmetic products, such as texture, absorption, and application to the skin. Compared to a previous study on clay density (DIAS, 2019), values ranging from 3.285 g/ml to 0.808 g/ml were found. In this study, densities of 0.93 g/ml were revealed for compacted clay and 0.79 g/ml for non-compacted clay. This variation suggests differences in the physical structure of the clay, indicating greater porosity or less compaction in its original form, with considerable densification after the compaction process.

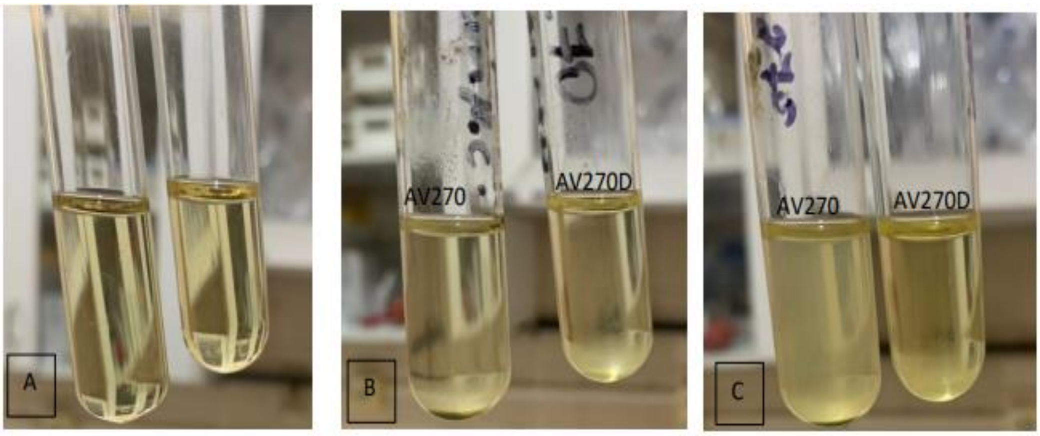
Clay has been shown to be insoluble in water, alcohol, and acetone, an advantageous characteristic for its application in cosmetic formulations, as it does not degrade quickly as water-soluble substances, maintaining a longer durability (ROCHA, 2022). This allows for the stable incorporation of clay into products, without dissolution or loss of its desirable properties.

The moisture content of raw clay is 37% and processed clay is 2.51%, this reduction in water concentration indicates an effective drying or purification process from natural clay to refined clay. The significant reduction in moisture content is crucial for the stability of the product, ensuring durability and preventing the unwanted growth of microorganisms. This reduction contributes to the consistency and effectiveness of the cosmetic products in which clay is used. According to Souza Santos (1975), clays do not eliminate interspersed, coordinated, and adsorbed water at temperatures below 250°C. Therefore, below these temperatures, the values are unlikely to remain constant.

In the organoleptic analysis, the sensory characteristics are described, as well as the color, brightness and odor. The indicated color is mint green, which according to Branco (2014) is due to iron compounds in reduced form, present in minerals such as chlorite, montmorillonite and glauconite. In some cases, the color may occur due to copper or olivine minerals. The opaque gloss may suggest a luminous surface, but not reflective, its appearance is influenced by drying and crushing processing. The mild odor characteristic of clay generates a relevant factor for the acceptance of the product by consumers and can influence the sensory perception during use. The tactile sensation described as smooth proposes a texture that is soft to the touch, this aspect is crucial as it affects the user's experience when applying cosmetic products containing clay. These organoleptic characteristics provide an initial and subjective view of the properties of clay and enhance sensory perceptions that can influence the acceptance and efficacy of cosmetic products containing it.

The microbiological analysis process aims to confirm the absence of some microorganisms or to verify the maximum limit allowed by law for the following agents: Total Bacteria, Total Coliforms, Fecal and Sulfite Reducing Clostridia (exclusively for talc).

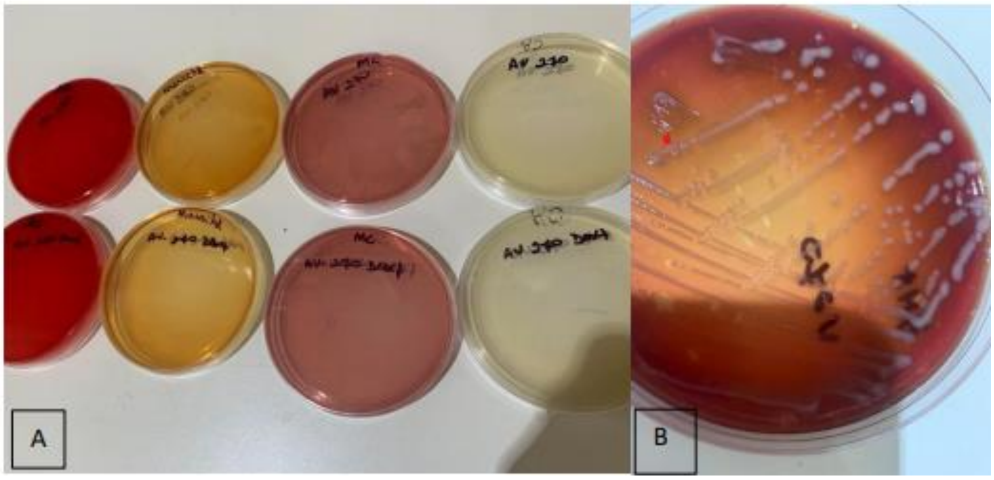
The AV 270 sample is the one that did not go through the disinfection process, while the AV 270 DESINFEC sample (AV 270D) had the dry heat decontamination process, the results of the microbiological analysis obtained can be seen in figures 7, 8 and 9.



Legend: A) Half LB and stereo BHI. B) LB medium with AV 270 and AV 270D after incubation. C) BHI medium with AV 270 and AV 270D after incubation.

Figure 10: Bacterial growth observed in the AV270 sample (BHI) after the 24-hour incubation period at 35ºC.

Figure 7A shows the LB and BHI stereo media. In Figure 7B, no bacterial growth was observed in the LB medium containing the samples AV 270 and AV 270D. When the BHI medium was analyzed, bacterial growth was observed related to the AV 270 sample, and no growth was observed in the culture corresponding to the AV 270D sample (Figure C).



Legend: A) Blood, Mannitol, MacConkey and Chromagar agar petri dishes. B) Blood agar culture from the AV 270 sample.

Figure 11: Results of bacterial culture seeded on agar culture media after 24 hours of incubation at 35ºC.

To confirm the results, as seen in figure 8, all cultures were seeded on Blood agar, mannitol agar, MacConkey agar, and Chromagar culture media. And there was no microbiological growth in the plates corresponding to the cultures of the LB medium and neither in the BHI medium in the cultures corresponding to the AV 270D sample. However, we observed bacterial growth in the blood agar culture medium of the AV 270 sample, as can be seen in Figure 8B.

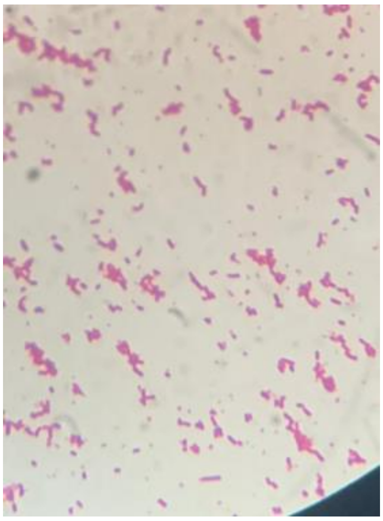


Figure 12: Gram staining result of AV 270 sample seeded on blood agar

The bacterial growth of the AV 270 sample observed on the blood agar was subjected to gram staining. In the analysis, it was observed that it consists of a Gram-negative bacillus bacterium, as noted in Figure 9.

Silva (2011) conducted a survey with some clay samples and the results of the microbiological evaluations indicated that there are microorganisms present. Therefore, the samples were sterilized, and new microbiological evaluations were performed. The absence of microorganisms in the second microbiological evaluation shows the importance of decontamination in this process, since after sterilization the samples showed negative results. This corroborates the results presented in this study.

5. FINAL THOUGHTS

The results and discussions reveal the diversity of properties and functions of metals and semimetals present in clay, highlighting their influence on health and skin care. Detailed analysis of the elements, such as aluminum, magnesium, iron, zinc, cobalt, titanium, manganese, copper, barium, arsenic, mercury, cadmium, lead, and vanadium, provides a comprehensive understanding of the composition of Green Clay Farm's green clay.

The significant concentration of aluminum, associated with the presence of kaolinite in the clay, stands out, along with the benefits of magnesium, iron and zinc in regulating skin oiliness. The presence of elements such as cobalt, titanium, manganese, and copper suggests nourishing and regenerative properties, contributing to skin health and inhibiting bacterial growth.

The controlled presence of barium reinforces the safety of the use of this substance. The low concentration of arsenic, the absence of cadmium and the acceptable levels of mercury indicate the compliance of the clay with current legislation, such as RDC No. 44/2012 of ANVISA.

On the other hand, the presence of lead and vanadium, although within regulatory limits, underscores the need for caution, because in high concentrations it can cause toxicity as mentioned in other articles. The integrated analysis of the results provides a solid basis for the application of green clay in various cosmetic contexts, from aesthetic procedures to specific therapies.

The discussion on potential toxicities highlights the importance of complying with legislation and regulatory standards, especially with regard to elements such as barium, chromium, arsenic, lead, nickel, vanadium and mercury. These are in accordance with ANVISA's RDC No. 44/2012, but EC No. 1223/2009 of the European Union in its annex contains the prohibition of these metals in cosmetic products, therefore, it is suggested complementary tests of cosmetic products to verify the perfusion of these metals in the skin.

No bacterial growth of the AV 270D sample was observed in any condition submitted in this analysis. Bacterial growth of the AV 270 sample was observed in the BHI medium and in the blood agar. BHI (Brain Heart Infusion Broth) medium is a rich culture medium suitable for the development of demanding microorganisms. Therefore, the method used to decontaminate the sample was efficient, since the dry heat method causes cell death because the penetration of heat oxidizes the bacterial cells, resulting in the sterilization of the sample

Analysis of pH compacted and uncompacted density, solubility, and moisture content contributes to a deeper understanding of the physical properties of clay. The slightly acidic pH suggests compatibility with the skin, while the limited solubility and reduction in moisture content after processing ensure the stability and effectiveness of cosmetic products containing it.

In summary, the green clay from the Green Clay Farm company has a composition rich in beneficial elements for the skin, with results that support its safe and effective application in cosmetic products.

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Attachments

Certificates of SCP-SCIENCE.pdf SS-2 Soil Contaminant Reference Samples

Appendix Table comparativa.pdf

MICROBIOLOGICAL RELATORIO_ANALISE[1].pdf

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