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INVESTIGATION OF THE ENVIRONMENTAL TOXICITY OF CALCIUM PHOSPHATE TOOTHPASTE PRODUCED BY A STARTUP IN JOINVILLE/SC

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Abstract: Objective: To carry out a study on environmental toxicity by means of ecotoxicological tests of toothpastes containing hydroxyapatite, in order to verify whether there are harmful effects caused by the interaction of the contaminating chemical agent with organisms. Methodology: The HA toothpastes used in this study contain similar formulas, the difference being that cream A was calcined at 900ºC and contains 10% HA, cream B at 600ºC and contains 10% HA and cream C was not calcined and contains only 5% HA. Statistical analysis of variance was used to check the influence of the concentrations on the organisms *Daphnia magna* and *Euruca sativa.* Relevance: there is little research on pollutants present in toothpastes and the work provides subsidies for public managers to seek compliance with the New Legal Framework for Sanitation of 2020. Results: with the help of ANOVA analysis, it was found that in the acute tests with *Euruca sativa* there were abnormal effects on the test organism and it was found that what affects root growth is the type of toothpaste and not the concentration of HA. In the acute test with *Daphnia magna,* it was found that what affects mortality is the concentration and not the type of toothpaste. As for the chronic toxicity on the organisms, a reduction of around 68.18% in the average fecundity in relation to the control can be observed for the FGC 900-10% cream, 67.08% for the FGC 600-10% cream and 71.65% for the FGH - 5% cream. The organism *Daphnia magna* proved to be more sensitive than the organism *Euruca sativa.* Social contributions: the work shows the impact of toothpaste on water quality parameters, so it is hoped that a large part of the population will soon be served by sewage networks and treatment plants with state-of-the-art technologies, so that all chemical compounds can be treated before they are discharged, reducing the harmful effects on the environment and so that we can have healthier bodies of water.

Keywords: Contaminant, *Daphnia magna*, Ecotoxicological, *Euruca sativa*, Hydroxyapatite.

INTRODUCTION

Joinville is located in Santa Catarina, the third most populous city in the southern region of Brazil, located at geographical coordinates 26º18'14"S. and 48º50'45"O., covering an area of 1,130.87 square kilometers, 4 meters above sea level, 180 km from the capital Florianópolis, is an industrial city. According to Joinville Cidade em Dados (2021), by the end of 2020 116 active *startups* had been identified in the city, employing and involving more than 1,600 people, with an estimated revenue budget of around R$153 million.

A *startup* is a company that uses technology as one of the pillars to standardize and scale delivery and uses innovation as another pillar, always thinking of creative solutions and allowing decisions made quickly to be corrected quickly if they don't achieve the expected result (VILENKY, 2021).

In this context, the Joinville and Region Technological Innovation Park (Inovaparq) was created to create an environment conducive to innovative practice. The project is maintained by the Joinville Regional Education Foundation (FURJ) and managed by the University of the Joinville Region (UNIVILLE). The partnership aims to solidify a collaborative network for the advancement of sustainable technological development in Joinville and the region, bringing together academia, companies and the government to stimulate and support innovative entrepreneurship (INOVAPARQ, 2022).

One of the *startups* located within Inovaparq, which can greatly contribute to the city's economic and social development, produces ceramic/biomaterials that are designed to restore the biological function of some part of the body. The same *startup* also produces toothpaste that contains hydroxyapatite in its formulation, which is a mineral composed of calcium, phosphate and hydroxyl, structured in the form of hexagonal crystals. The crystal structure of hydroxyapatite is complex and the hydroxyapatite crystal is made up of thousands of units of the chemical formula $Ca_{(10)}(PO_{4})_{(6)}(OH)_{(2)}$ (MALTZ *et al*., 2016).

According to Bonan *et al.* (2014), hydroxyapatite is the largest component of human hard tissue, constituting up to 69% of natural bone mass, in addition to different proportions of dental tissues, i.e. 96% of enamel, 45% of dentin in the form of nanocrystalline carbonated apatite and 65% of cementum. Hydroxyapatite can be obtained from natural sources, such as bovine bones, eggshells, shells and corals, or through chemical methods, which can be classified into dry chemical methods, such as solid-state reaction and combustion synthesis, and wet chemical methods, such as hydrolysis, hydrothermal processes, precipitation, sol-gel synthesis, among others.

Fluoride is a substance contained in many toothpastes but is toxic if ingested in large quantities. Toothpastes containing hydroxyapatite (HA; $Ca_{(5)}(PO_{4})_{(3)}(OH)$) are fluoride-free alternatives that have recently been shown to be effective as anti-caries agents. The predominant ingredient in HA toothpaste is calcium and phosphate, in the form of HA, which can be used in micro or nanocrystalline form. At the nano and micro level, HA particles resemble naturally occurring enamel apatite crystals. Hydroxyapatite particles have been shown to bind to the damaged enamel surface and fill in porous surface irregularities to restore surface integrity (O'HAGAN-WONG *et al*., 2021).

However, because it is leachable and water-soluble, phosphate in the form of apatites (calcium phosphate) goes directly into rivers. When phosphates increase the concentration of nutrients in rivers and lakes, they can cause excessive eutrophication. Nutrients stimulate the growth of algae and plants, which generates excessive oxygen consumption and kills fish (KLEIN and AGNE, 2013).

Nanoparticles can pollute the environment and cause damage that is very difficult and laborious to reverse, such as contaminating river water. It is essential to note that this type of material can intoxicate animal species in a process called bioaccumulation. According to Huber (2012), bioaccumulation is the sum of bioconcentration, which is the process that causes the concentration of a chemical substance in an aquatic organism to increase in relation to its concentration in the water due to incorporation, through absorption solely by the water, with biomagnification, which is the accumulation of a substance in the biota throughout the food chain through feeding.

As toothpaste enters the market, there may be an increase in the concentration of phosphates in the region's water bodies. However, the advantage of using the toothpaste in question is that it does not contain fluoride. Excess fluoride is toxic and the ideal amount of this substance to prevent cavities is added to the water after the treatment process at the city's water treatment plant, so there is no need to use it in toothpaste.

Laboratory studies show that it accumulates in regions of the brain involved in learning and memory and *alters* proteins and neurotransmitters in the central nervous system (GREEN *et al*., 2019). The Ministry of Health's drinking water ordinance 888/21 establishes that the ideal amount of fluoride for drinking water is 1.5 mg/L. If this amount is exceeded in rivers, there can be environmental imbalances and also contamination of people, ichthyofauna and animals in general.

Precisely to prevent certain types of environmental problems and lack of drinking water, Brazil has laws aimed at protecting natural resources, such as Federal Law No. 9.433/97, which establishes the National Water Resources Policy, Law No. 9.748 of November 30, 1994, which provides for the State Water Resources Policy and CONAMA Resolution No. 430 of March 17, 2005, which provides for effluent discharge standards. Phosphates are an environmental concern, so CONAMA Resolution 357/05, which provides for the classification of bodies of water, established the maximum amount of phosphate allowed in water, which is 0.1 mg/L for class 2 fresh water, 0.062 mg/L for class 1 saline waters and 0.124 mg/L for class 1 brackish waters.

In order to make sure that the hydroxyapatite contained in toothpaste is no longer a polluting/harmful factor for the environment, the aim of this work was to carry out a study on its toxicity by means of ecotoxicological tests, to assess whether there are harmful effects caused by the interaction of the contaminating chemical agent with the organisms *Daphnia magna* and *Euruca sativa* (rocket seeds).

METHODOLOGY

The methodological sequence adopted in this work is described below:

1. Obtaining the toothpaste (the startup provided the toothpaste samples for the tests);
2. Obtaining the solubilized extract (ABNT NBR 10006);
3. Phytotoxicity analysis with Euruca Sativa (relative seed germination, root elongation and germination index);
4. Toxicity analysis with Daphnia magna (acute and chronic tests);
5. Analysis of results.

Obtaining the toothpaste sample

The toothpastes were provided by a *startup* in Joinville, SC, in the amount of 250g of each cream, for the ecotoxicity tests. The composition of the creams is shown in Table 1.

Composition	A - FGC 900-10% (%)	B - FGC 600-10% (%)	C - FGH - 5% (%)
Aqua	47,8	47,8	52,81
Calcium carbonate	21	21	21
Sorbitol	17	17	17
Hydroxyapatite	10	10	5
Sodium lauryl sulfate	4	4	4
Silica	0,15	0,15	0,15
Saccharin	0,02	0,02	0,02
Methyl paraben	0,015	0,015	0,015

Table 1: Composition of toothpastes.

Source: Own elaboration

Granulated Phosphate toothpaste calcined at 900°C with 10% hydroxyapatite is represented by the acronym FGC 900-10%; Granulated Phosphate toothpaste calcined at 600°C with 10% hydroxyapatite has the acronym FGC 600-10%; and Hydrated Granulated Phosphate toothpaste with 5% hydroxyapatite is represented by the acronym FGH- 5%.

Obtaining the solubilized extract

When we brush our teeth, the cream is diluted due to contact with saliva, but we also use water in the process, which must be taken into account. To do this, the ABNT NBR 10.006 Standard - Procedure for obtaining solubilized extract from solid waste was used as a reference for carrying out the solubilization. A representative sample of 250 g of toothpaste was placed in a 1 500 mL flask, 750 mL of distilled water was added and the flask was shaken slowly by hand for 5 minutes, then the flask was covered with PVC film and left for 7 days at a temperature of 25°C.

Gravity filtration was carried out on a filter with a pore size of 0.45 µm and then the pH, temperature, dissolved oxygen, conductivity and dissolved solids of the solubilized extract were measured using a Hanna HI98194 Multiparameter device. A Hach 2100P Turbidimeter was used to measure turbidity.

Phytotoxicity analysis with *Euruca sativa*

The following analyses were carried out for each experiment: relative seed germination, root elongation and the germination index of *Euruca sativa* (rocket) seeds. The tests with *Euruca sativa* do not have an ABNT standard, so they were repeated with five dilutions (0.02%, 0.01%, 0.005%, 0.0025%, 0.00125% of solubilized extract sample) and five repetitions per dilution. these percentages were tested for toothpastes with concentrations of FGC 900 - 10%, FGC 600 -10% and FGH 5%. These dilutions were chosen because *Euruca sativa* is very sensitive and it was from these values that the species grew.

The seeds were placed in Petri dishes containing two filter paper disks soaked in the test solution. Following the guidelines of the Rules for Seed Analysis of the Ministry of Agriculture, Livestock and Supply (2009), ten seeds were placed in each Petri dish at a uniform spacing of 1.5-5.0 times their width or diameter, thus being sufficient to minimize competition and contamination between the seeds and developing seedlings.

Distilled water was used to make the extract solubilized with the toothpaste. The tests were kept at 20/+-2°C and light cycles of 8 hours (the light being well distributed over the entire surface of the substrate) and 16 hours of darkness. After four days, the first checks were made to ensure that the seeds were developing well and on the seventh day, the roots were measured to finalize the phytotoxicity analysis with rocket (*Euruca sativa*).

Relative seed germination (%G)

According to the handout Rules for Seed Analysis from the Ministry of Agriculture, Livestock and Supply (2009), seed germination in a laboratory test is the emergence and development of the essential structures of the embryo, demonstrating its ability to produce a normal plant under favorable conditions.

The relative germination of the seeds was determined using Equation 1 below, which relates the seeds germinated in the experiment with the solubilized toothpaste to the seeds germinated in the control tests. Germinated seeds are those that measure 2 mm or more; values below this are considered non-germinated.

$$\%G = \left(\frac{SGa}{SGc}\right) x\ 100 \qquad \text{Eq. (1)}$$

%G: Relative seed germination percentage
SGa: Total number of seeds germinated in the sample
SGc: Total number of germinated seeds in the control

Root elongation (%R)

To determine root elongation, a calculation is made based on the average root size of the germinated seeds in relation to the average root size of the germinated seeds in the control, as shown in Equation 2.

$$\%R = \left(\frac{MRaM}{Rc}\right) x\ 100 \qquad \text{Eq. (2)}$$

%R: Root elongation percentage;
MRa: Average root elongation of germinated seeds in the sample;
MRc: Average root elongation of germinated seeds in the control.

Germination index (GI)

The germination index is given by combining Equations 1 and 2 in relation to the relative germination of the seeds in the control with the root elongation in the control, as shown in Equation 3.

$$\%IG = \left(\frac{\%G}{\%R}\right) x\ 100 \quad \text{Eq. (3)}$$

Daphnia magna test

According to ISO 10706:2000 - Water quality - Determination of the long-term toxicity of substances to *Daphnia magna straus* (*Cladocera, Crustacea*), *Daphnia magna* is obtained by acyclic parthenogenesis during at least three generations under specified culture conditions. *Daphnia magna* is a bioindicator and the sensitivity of this organism to toxic substances can be affected by the origin of the culture. Crustacean stress should also be avoided.

Acute test with *Daphnia magna*

To carry out the acute test, which aims to check whether adverse effects occur in a short period of time, young organisms were used, with an average age of 2 to 25 hours, obtained from the fourth spawning. The *Daphnias magna* were exposed to the sample for 48 hours. There were four replicates for each sample, each containing 5 *Daphnias magna* in 20 mL test tubes.

The method used to carry out the acute test was based on NBR 12713/2004 Aquatic Ecotoxicology - Acute Toxicity, which provides guidelines for the test method with *Daphnia spp* (Crustacea, Cladocera).

To determine the CL50, which according to Queiroz (2015) is the lethal concentration of a chemical or substance that leads to the death of 50% of individuals in a pre-established time, Toxstat software was used.

Chronic test with *Daphnia magna*

The chronic test lasted 21 days, as the aim is to study repeated and prolonged exposure to a substance and follows the specifications of ISO 10.706 which describes the method for determining the long-term toxicity to *Daphnia magna straus* of: a) chemical substances, which are soluble under the test conditions, or which can be maintained as stable suspensions or dispersions under the test conditions, b) industrial or sewage effluents, treated or untreated, after decantation, filtration or centrifugation, c) surface water or groundwater.

For the chronic test, young organisms were used, with an average age of 2 to 25 hours, and were exposed to the solubilized extract for a period of 21 days. The dilution used was 0.00125% of toothpaste A, B and C and 1 organism was used per plastic cup, in sterilized 50 mL plastic cups. Each cup contained 25 mL of the test solution. After preparing the samples, the *Daphnias magna* were exposed to 16 hours of light and 8 hours of darkness, at a temperature of between 20º and 22ºC. The test organisms received daily feedings of the chlorophycean algae *Scenedesmus subspicatus*, at concentration of 106 cells.mL^{-1}. Live organisms were counted twice a week.

Fecundity was assessed by counting the neonates produced by the females in all the plastic cups over a 21-day period. The parameter used was the average number of offspring generated per female, taking into account the number of spawnings throughout the test. This average was obtained using Equation 4.

$$Fecundidade = \left(\frac{nº\ total\ de\ filhotes}{nº\ de\ mães\ X\ nº\ de\ posturas}\right) x\ 100 \quad \text{Eq. (4)}$$

RESULTS AND DISCUSSION

CHARACTERIZATION OF THE SOLUBILIZED EXTRACT

In order to check that the solubilized extract presented the ideal conditions for the toxicity tests, it was characterized before the tests were carried out and the parameters analyzed were temperature, dissolved oxygen, conductivity, dissolved solids, pH and turbidity. It was observed that the pH measured was slightly above the ideal range (7.6 to 8.0) for the survival of the microcrustacean *Daphnia magna*, but as ABNT NBR 12713/2016 points out, only pH values above 9 can influence the test results, so no corrections were made. The temperature was within the ideal range for the organisms used, which according to the aforementioned standard is 18 to 22°C.

The toothpaste used should be sent to the sewage collection networks, which send the effluent to a treatment plant so that it can undergo a treatment process and all the parameters can be adjusted to comply with the release standard established by CONAMA 430/11. However, according to data from SINIS (2020), the three southern states of Brazil have only 47.4% treated sewage, meaning that most of the effluent generated in homes is connected to the rainwater drainage network, which flows directly into the region's rivers.

The standard for discharging effluents into water bodies is a tool that, together with the classification of water bodies, aims to preserve and conserve drinking water sources. Therefore, comparisons were made between the parameters of solubilized toothpaste, effluent discharge parameters according to CONAMA 430/11 and class 1 and class 2 saline and brackish freshwater parameters, according to CONAMA 357/05.

In this study, a comparison was made with class 1 and 2 waters, since article 42 of CONAMA 357/05 states that until the respective frameworks have been approved, fresh waters will be considered class 2 and saline and brackish waters class 1, unless current quality conditions are better, which will determine the application of the corresponding stricter class.

Table 2 below shows the dissolved oxygen values.

The minimum value of dissolved oxygen (DO) for the preservation of aquatic life established by CONAMA Resolution 357/05 for fresh and brackish waters is 5.0 mg/L. For saline class 1, this Resolution defines a minimum of 6 mg/L (CETESB, 2022).

The amount of dissolved oxygen is a primary indicator of quality, so the concentrations of DO in the solubilized toothpaste extracts, which reached a minimum value of 4.45 mg/L and a maximum of 5.27 mg/L, probably do not interfere negatively with the ecosystem, as these values are very close to those established by CONAMA 357/05. According to ABNT NBR 12713, which deals with the *Daphnia magna* test method, dissolved oxygen values of less than 1.0 mg/L can interfere with the test results. The results of the solubilized extracts were higher than 1 mg/L, so there was no negative interference.

In studies with lettuce, Conesa *et al.* (2015) recorded changes in DO concentrations depending on the time of year and the intensity of oxygenation of the solution. In the fall, the DO values were 5.2, 6.2 and 8.1 mg/L in solution without aeration, with low aeration and high aeration, respectively. In summer, the average DO values were 3.3, 5.4 and 6.9 mg/L, respectively. These values confirm that the amount of DO in the solubilized toothpaste was adequate for carrying out the acute toxicity tests with *Euruca sativa*.

The conductivity results of the solubilized extracts are shown in Table 3.

There is no conductivity standard in Brazilian legislation, but natural waters have conductivity levels in the range of 10 to 100 µS/cm and in environments polluted by domestic or industrial sewage the values can reach up to 1000 µS /cm (VON SPERLING, 2007).

This shows that the characteristic of the solubilized toothpaste extracts in relation to conductivity is that of a polluted body of water, as they exceed the value of 100 µS/cm.

In the work by Frack and Bednarczyk (2021) on the mortality of organisms in aquatic ecosystems with *Daphnia magna*, the solu-

Concentration	OD (mg/L) cream A	OD (mg/L) cream B	OD (mg/L) cream C	CONAMA 430/2011	CONAMA 357/2005 Class 2 sweet	CONAMA 357/2005 Class 1 saline	CONAMA 357/2005 Class 1 brackish
0,02%	4,96	5,35	5,19	x	>5mg/L	>6mg/L	>5mg/L
0,01%	5,00	5,09	4,83	x	>5mg/L	>6mg/L	>5mg/L
0,005%	5,10	5,27	4,87	x	>5mg/L	>6mg/L	>5mg/L
0,0025%	5,17	5,14	4,45	x	>5mg/L	>6mg/L	>5mg/L
0,00125%	5,06	4,83	4,63	x	>5mg/L	>6mg/L	>5mg/L

Table 2: Dissolved oxygen of the solubilized extracts

Source: Own elaboration

Concentration	Cond (µS /cm) cream A	Cond (µS /cm) cream B	Cond (µS /cm) cream C	CONAMA 430/2011	CONAMA 357/2005 Class 2 sweet	CONAMA 357/2005 Class 1 saline	CONAMA 357/2005 Class 1 brackish
0,02%	399	397	490	x	x	x	x
0,01%	394	400	368	x	x	x	x
0,005%	401	390	320	x	x	x	x
0,0025%	389	394	406	x	x	x	x
0,00125%	392	384	378	x	x	x	x

Table 3: Conductivity of solubilized extracts

Source: Own elaboration

Concentration	Dissolved Solids (ppm) cream A	Solids Dissol. (ppm) cream B	Solids Dissol. (ppm) cream C	CONAMA 430/2011	CONAMA 357/2005 Class 2 sweet	CONAMA 357/2005 Class 1 saline	CONAMA 357/2005 Class 1 brackish
0,02%	200	199	240	x	500 ppm	x	x
0,01%	197	200	184	x	500 ppm	x	x
0,005%	201	195	150	x	500 ppm	x	x
0,0025%	195	197	204	x	500 ppm	x	x
0,00125%	196	192	189	x	500 ppm	x	x

Table 4: Dissolved solids of the solubilized extracts

Source: Own elaboration

Concentration	pH cream A	pH cream B	pH cream C	CONAMA 430/2011	CONAMA 357/2005 Class 2 sweet	CONAMA 357/2005 Class 1 saline	CONAMA 357/2005 Class 1 brackish
0,02%	8,24	8,23	8,15	5 a 9	6 a 9	6,5 a 8,5	6,5 a 8,5
0,01%	8,21	8,2	8,24	5 a 9	6 a 9	6,5 a 8,5	6,5 a 8,5
0,005%	8,16	8,18	8,86	5 a 9	6 a 9	6,5 a 8,5	6,5 a 8,5
0,0025%	8,13	8,12	7,81	5 a 9	6 a 9	6,5 a 8,5	6,5 a 8,5
0,00125%	8	8,13	7,98	5 a 9	6 a 9	6,5 a 8,5	6,5 a 8,5

Table 5: pH of the solubilized extracts

Source: Own elaboration

Concentration	Turbidity (NTU) cream A	Turbidity (NTU) cream B	Turbidity (NTU) cream C	CONAMA 430/2011	CONAMA 357/2005 Class 2 sweet	CONAMA 357/2005 Class 1 saline	CONAMA 357/2005 Class 1 brackish
0,02%	80	92	23	x	≤100 NTU	x	x
0,01%	42	47	10	x	≤100 NTU	x	x
0,005%	16	17	2	x	≤100 NTU	x	x
0,0025%	7	8	3	x	≤100 NTU	x	x
0,00125%	3	4	2	x	≤100 NTU	x	x

Table 6: Turbidity of the solubilized extracts

Source: Own elaboration

tions were prepared with distilled water and natural water, with a conductivity of 390 µS/cm. In the present study on toothpaste, the minimum conductivity was 320 µS/cm and the maximum was 490 µS/cm, and the average conductivity was 393.46 µS/cm, so the values are in line with other studies carried out and are the ideal conductivity condition for *Daphnia magna*.

In relation to *Euruca sativa*, one study found that both nutrient deficiency (very low conductivity) and osmotic stress (very high conductivity) can lead to a reduction in chlorophyll content, yield and cause an imbalance between shoot and root growth, leading to typical symptoms of nutritional disorders (YANG, 2021).

Table 4 shows the results obtained for the dissolved solids of the solubilized toothpaste extracts.

The high concentrations of dissolved solids are due to the difficulty for toothpaste to fully dilute in the water, which makes the water unacceptable for human consumption. Liquid water is in its pure state when it has no suspended or dissolved solids, thus having low reflectance (BIAS, BARBOSA and BRITES, 2013).

All the solubilized extract values comply with CONAMA Resolution 357/05, so it is unlikely that the amount of dissolved solids present in the samples will cause major disturbances to water bodies.

For the organism *Daphnia magna*, it was found that dissolved solids may have influenced the life cycle because they are directly related to the turbidity of the water, since light is essential for the proper development of this organism. With this amount of dissolved solids, luminosity was reduced.

As for *Euruca sativa*, it was observed that the presence of dissolved solids from toothpaste hinders the proper development of the seeds, as it decreases the absorption of nutrients. These results illustrate that dissolved solids in water can cause abnormal behavioral responses in *Daphnia magna and Euruca sativa.*

The pH values obtained for the solubilized extracts are shown in Table 5. Chemical reactions can develop differently according to pH, so this is an important parameter for understanding the results of the test with *Euruca sativa* and *Daphnia magna.*

pH is a measure of the degree of acidity or alkalinity of water, with 7 being the neutral pH. Values above 7 (up to 14) indicate an increase in the degree of alkalinity and below 7 (down to 0) an increase in the degree of acidity of the environment. For example, as the pH of the water decreases (through the dumping of chemical substances), the fish will have a higher respiratory rate and will begin to gulp air at the surface; at extremely low pH, they die immediately. As the pH rises, calcium oxide is formed, which causes corrosion of the gill epithelium and fins, leading the fish to die (CETESB, 2022).

The pH of the solubilized extracts complies with the limits established by CONAMA 430/11 for release parameters and CONAMA 357/05 for fresh, saline and brackish bodies of water.

For *Daphnias magna*, Chein *et al*. (2012) found that acid stress on motility strength was significant mainly in the pH range between 5.0 and 5.5. At pH 8.0 to 9.0, few negative effects on motility could be detected with the Biological Early Warning System, but not with the naked eye. But in general, with the pH value of the water around 8.0 there were no obvious and significant abnormal behavioral changes. Therefore, the pH of the solubilized extract of toothpastes A, B and C did not negatively influence the test.

In relation to *Euruca sativa*, in the study by Almeida *et al*., (2011) the absorption of nutrients by the root was affected by the pH or acidity of the medium, which, whether very low or very high, has a negative influence on plant growth and the best performance of the rocket plant occurred at pH 6.0 and 6.5. The pHs of the toothpaste samples were slightly above this favorable range for seed growth, which may have influenced the non-germination of some less healthy seeds.

The turbidity results of the solubilized extract are shown in Table 6. Turbidity represents the optical property of light absorption and reflection.

According to CONAMA Resolution 357/05, the ideal turbidity condition in class 2 river water corresponds to a maximum value of 100 NTU, so the toothpaste samples comply with this Resolution.

The study by Chein *et al.* (2012) showed the effects of turbidity on the behavioral responses of *Daphnia magna*. Behavioral strength decreased significantly when turbidity increased to 50 NTU or 100 NTU. These results demonstrate that water turbidity can cause abnormal behavioral responses of *Daphnia magna.* This may justify the mortality of all the organisms in the 0.02% and 0.01% concentrations which had the highest turbidity.

Turbidity limits the penetration of sunlight, restricting photosynthesis, which in turn reduces oxygen replenishment, leading to imbalances in the food chain (FLECK, 2012).

In the tests with the solubilized toothpaste, it was observed that turbidity reduced the penetration of ambient light into the water, damaging the photosynthesis *of Euruca sativa* and compromising the development of the more fragile seeds.

Acute test with *Euruca Sativa*

The toxicity tests with rocket seeds (*Eruca sativa*) were carried out by exposing the seeds to toothpaste at the same concentrations used in the acute test with *Daphnia magna*. After 7 days, the growth of the plant roots was observed for each Petri dish, as shown in Table 7.

A two-way repeated measures analysis of variance (ANOVA) was carried out to check the influence of concentration and type of cream on the size and development of the *Euruca sativa* root. The p-value for concentration was 0.070021213, for cream was 6.06978E-07 and Concentration x cream was 0.077618633.

ANOVA tests whether the effect of one factor on the response variable depends on the level of a second factor (interaction).

The ANOVA data showed that there was a significant difference ($p \leq 0.05$) for the type of toothpaste and so it was found that what affects the root growth of *Euruca sativa* is the type of cream and not the type of concentration, as it can be seen that the value of 6.06978E-07 is less than 0.05, so concentration and type of toothpaste act independently.

Seed germination was also observed and, using equations 1, 2 and 3, the germination percentage (G), root elongation percentage (R) and germination index (GI) were calculated, as shown in Table 8, for 5 (five) different dilutions. For cream A and B, the *Euruca sativa* had the same behavior in terms of the number of germinated seeds, root elongation and germination index.

With regard to root elongation, it can be seen in Table 12 that at the highest concentration of toothpaste A and B, the lowest growth was obtained, showing a possible toxic effect, which may be due to the substances sorbitol, sodium lauryl sulfate, saccharin and methyl paraben present in the toothpaste. When the concentration of toothpaste decreases, there is greater root elongation, which may be due to the hydroxyapatite present. At these lower concentrations, the effects of the toxic substances are less and then the hydroxyapatite, which is a great growth stimulator, can act to stimulate root growth.

CREAM A - FGC 900-10%

Concentration	Plate 1 (cm)	Plate 2 (cm)	Plate 3 (cm)	Plate 4 (cm)	Plate 5 (cm)	Median (cm)
Control	4,30	4,00	5,05	4,50	4,15	4,30
0,02%	4,75	2,80	4,20	3,30	5,05	4,20
0,01%	5,70	5,20	4,90	4,45	4,55	4,90
0,005%	5,50	5,10	5,50	4,50	5,35	5,35
0,0025%	4,40	4,90	5,30	6,80	4,70	4,90
0,00125%	5,90	5,15	5,30	6,15	5,50	5,50

CREAM B - FGC 600-10%

Concentration	Plate 1 (cm)	Plate 2 (cm)	Plate 3 (cm)	Plate 4 (cm)	Plate 5 (cm)	Median (cm)
Control	4,30	4,00	5,05	4,50	4,15	4,30
0,02%	3,20	2,90	2,20	3,60	4,45	3,20
0,01%	4,10	4,25	2,90	4,25	3,75	4,10
0,005%	4,25	3,70	4,00	3,70	3,45	3,70
0,0025%	4,30	4,10	3,85	4,60	4,50	4,30
0,00125%	4,35	3,85	5,80	3,55	4,20	4,20

C CREAM - FGH - 5%

Concentration	Plate 1 (cm)	Plate 2 (cm)	Plate 3 (cm)	Plate 4 (cm)	Plate 5 (cm)	Median (cm)
Control	4,30	4,00	5,05	4,50	4,15	4,30
0,02%	4,80	4,10	3,30	4,50	4,50	4,50
0,01%	3,60	2,75	4,05	2,25	4,60	3,60
0,005%	3,55	2,80	5,05	2,25	4,30	3,55
0,0025%	2,50	4,00	3,10	1,60	3,90	3,10
0,00125%	3,65	3,00	4,60	6,50	3,20	3,65

Table 7: Median size of plant roots.

Source: Own elaboration

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	Concentrations	Number of germinated seeds (pcs)	G (%)	R (%)	GI (%)
CREAM A- FGC 900-10%	Control	48	96	-	-
	0,02%	43	89,58	91,36	81,85
	0,01%	43	89,57	112,73	100,98
	0,005%	46	95,83	117,95	113,04
	0,0025%	44	91,67	118,64	108,75
	0,00125%	42	87,50	127,27	111,36
CREAM B - FGC 600-10%	0,02%	45	93,75	74,32	69,67
	0,01%	45	93,75	87,50	82,03
	0,005%	43	89,58	89,58	77,77
	0,0025%	43	89,58	97,05	86,94
	0,00125%	45	93,75	98,86	92,68
C CREAM - FGH - 5%	0,02%	44	91,67	96,36	88,33
	0,01%	43	89,58	78,41	70,24
	0,005%	41	85,42	81,59	69,69
	0,0025%	45	93,75	71,23	66,77
	0,00125%	46	95,83	95,23	91,26

Table 8 : Relative seed germination (%G), root elongation (%R) and germination index of creams A, B and C

Source: Own elaboration

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CREAM A - FGC 900-10%

Concentrations	Total number of individuals	Mortality (un)	Mortality (un)	Mortality (un)	Mortality (un)
Control	5	0	0	0	0
0,02%	5	5	5	5	5
0,01%	5	5	5	5	5
0,005%	5	5	3	2	4
0,0025%	5	1	3	2	2
0,00125%	5	0	1	3	2

CREAM B - FGC 600-10%

Concentrations	Total number of individuals	Mortality (un)	Mortality (un)	Mortality (un)	Mortality (un)
Control	5	0	0	0	0
0,02%	5	5	5	5	5
0,01%	5	5	5	5	5
0,005%	5	4	3	2	2
0,0025%	5	1	3	1	1
0,00125%	5	0	1	0	1

C CREAM - FGH - 5%

Concentrations	Total number of individuals	Mortality (un)	Mortality (un)	Mortality (un)	Mortality (un)
Control	5	0	0	0	0
0,02%	5	5	5	5	5
0,01%	5	5	5	5	5
0,005%	5	5	4	3	2
0,0025%	5	1	4	3	1
0,00125%	5	0	2	3	1

Table 9: Results of the acute toxicity test

Source: Own elaboration

Samples	Sum of puppies	No. of postures	Average fertility
Control	248	4	6,075
A - FGC 900-10%	58	3	1,93
B - FGC 600-10%	54	3	2,00
C - FGH - 5%	47	3	1,74

Table 10: Chronic toxicity test results

Source: Own elaboration

There was no pattern of behavior in the results of cream C, however, possibly because the concentration of hydroxyapatite was lower, there was less growth stimulation compared to the other toothpastes that have a higher percentage of hydroxyapatite in the formulation.

ANOVA analysis showed that the type of toothpaste (A, B or C) influences root size. Creams A and B contain 10% hydroxyapatite and cream C only 5%. It was observed that root growth was lower in the tests with cream C, which may be related to the amount of $CaCO_3$present in each cream, since calcium, in the form of calcium carbonate ($CaCO_3$) and in the form of hydroxyapatite, is essential for healthy plant growth.

Calcium promotes adequate elongation of plant cells, participates in enzymatic and hormonal processes and plays an important role in the absorption of other nutrients (IPNI, 2022).

Toxicity experiments allow us to determine a cause-effect relationship, but they are not realistic enough, as the results obtained are only valid under the environmental conditions used in the laboratory and do not allow us to extend the conclusions to other species (PERIN, 2005).

The germination test is conducted under favorable conditions of temperature, humidity and light, allowing the expression of the maximum potential to produce normal seedlings and for the seeds to germinate.

Acute test with *Daphnia magna*

The results of the acute toxicity test with *Daphnia magna* are shown in Table 9. It can be seen that there was no mortality in the control solution and for this reason the test was validated.

The tests were carried out in quadruplicate, each tube containing 5 organisms. In order to determine the concentration that causes half of the organisms to die $C(L_{)(50)}$, the results obtained in the test were entered into the Toxstat - TSK software, resulting in a $C(L_{)(50)}$equal to 0.003348% for cream A; 0.004058% for cream B; and 0.004244% for cream C.

A two-factor analysis of variance with repetition was carried out to check the influence of concentration and type of toothpaste on *Daphnia magna* mortality. This analysis showed that what affects *Daphnia magna* mortality is concentration ($p = 1.94351E-15$) and not the type of toothpaste ($p = 0.158057427$), so concentration and toothpaste act independently ($p = 0.933790932$).

The effect of the concentration of toothpastes on *Daphnias magna* is possibly due to the components of the formulas.

According to Brasil (2014), water hardness is expressed in mg/L of calcium carbonate equivalent ($CaCO_3$) and can be classified as soft: < 50 mg/L of $CaCO_3$; moderate hardness: between 50 mg/L and 150 mg/L of $CaCO_3$; hard: between 150 mg/L and 300 mg/L of $CaCO_3$; and very hard: >300 mg/L of $CaCO_{(3)}$. The possible effects of excess calcium carbonate in water (hard water) on the health of organisms, animals and humans are positive, since hard water does not interfere with potability and can even prevent cardiovascular diseases.

As mentioned by Abdalla *et al.* (2010), the leaching of alkaline soil and sedimentary rocks can also cause the phenomenon of hard water. Therefore, $CaCO_3$was probably not responsible for the death of the organisms in the tests.

On the other hand, sorbitol is a toxic substance. In a study carried out by Vieira, *et al.* (2012), to verify the effect of sorbitol *on the in vitro* conservation of cassava, the development of the plants with 5 g/L sorbitol was much slower than when sucrose was added without sorbitol, indicating that sorbitol is efficient in conservation. In the toothpaste tests in question, the substance may have prevented the *Daphnias magna* from continuing and maintaining life.

With regard to hydroxyapatite, *in a* study by Sepúlveda (2000), *in* the (*in vitro*) cytotoxicity test, the sintered hydroxyapatite bodies indicated that the processed material, *in* addition to its high degree of purity, is non-cytotoxic.

Sodium lauryl sulphate is the most commonly used surfactant, both in industry and in the home. It is an anionic surfactant, because when dissolved in water it produces the sulfate ion in its structure. It is the main active ingredient in detergents and dishwashing products (BAIN, COMPANY, 2014).

In the work carried out by Siqueira and Mendes (2019), it was found that sodium lauryl sulfate causes major problems, especially in water resources, as its micellar action causes encapsulation of nutrients for microfauna, thus affecting the entire food chain, in addition to the formation of foam and contamination of water bodies. It is likely that this substance has caused the encapsulation of nutrients, causing the death of the organisms used in the test with the toothpaste solubilizers.

Dissolved silica concentrations are higher in rivers (the source of material from rock weathering) than in seawater. The higher the salinity, the lower the concentrations. The main source of this element in nature is silica minerals found in igneous, metamorphic or sedimentary rocks. Silica concentrations are little influenced by human activities (ZHANG, 2006).

Given that it is an abundant element in nature, the chance that this was the substance that caused the death of *Daphnia magna* and *Euruca sativa* is small, as it has been found that land use and land cover (agriculture, forests, deforestation, urban areas) can influence silica concentrations in the environment, including in Brazil (SOUZA *et al.*, 2003).

Saccharin, on the other hand, can be very harmful to the human body, as well as to other organisms. Its consumption can result in mutagenic damage, bladder cancer and significant damage to the stomach, according to studies in rodents. In addition, in 1994, cases of hepatoxicity were reported in individuals who had ingested medicines containing saccharin. As saccharin has a potential carcinogenic effect, its commercialization was banned in Canada and the United States in 1991 (RIBEIRO; PIROLLA; NASCIMENTO, 2020).

In the toxicity study of methylparaben with *Daphnia similis*, it was found that this substance is toxic, although it is less potent than propylparaben, because the longer the chain of the substituent radical in paraben, the greater the toxicity. The aggravating factor is that methylparaben is an endocrine disruptor, which is not found in the environment separately; it interacts with other substances and causes additive or synergistic effects, making it very difficult to predict what the effect will be (MOREIRA *et al.*, 2015).

Chronic test with *Daphnia magna*

In order to assess whether even small concentrations of toothpaste would have a toxic effect on the *Daphnia magna* organism over a longer period of time (21 days), it was decided to carry out chronic tests on samples with concentrations of 0.00125% for cream A, B and C, since they showed low mortality at the end of the acute test. Table 10 shows the results obtained for the concentration tested and the control sample. The average fecundity was calculated using Equation 4 presented above. The same solubilizer was used as in the acute test, so the parameters are the same (see Tables 2, 3, 4, 5, 6 and 7).

The results are in line with the life cycle of *Daphnia magna*, since the expected number of *Daphnia magna* spawns is between three and five (EBERT, 2005).

As for the chronic toxicity on the organisms, we can see a reduction of around 68.18% in the average overall fecundity in relation to the control for cream A, 67.08% for cream B and 71.65% for cream C, which is possibly due to the presence of toxic substances such as sorbitol, sodium lauryl sulphate, saccharin and methyl paraben (according to the beneficial and harmful effects of these substances described in section 3.3).

The organisms were all female, so the most acceptable explanation for this sudden decrease in fecundity is that the ovaries of the exposed *Daphnias magna* did not develop, so they did not reach sexual maturity and could not reproduce (BRENTANO, 2006).

CONCLUSION

The toxicity tests showed that toothpastes A, B and C influence the behavior of these organisms, which is possibly due to the presence of toxic substances such as sorbitol, sodium lauryl sulfate, saccharin and methyl paraben that are part of the composition of toothpastes, as well as factors such as dissolved solids and turbidity that reduce the luminosity of the solubilized extract.

However, the use of the toothpastes tested in this study is of great advantage, given that other toothpastes available on the market contain fluorine, which can be toxic if ingested in excess, as well as causing severe environmental damage. With the universalization of the New Legal Framework for Sanitation in 2020, it is hoped that a large part of the population will soon be served by sewage networks and treatment plants with state-of-the-art technologies, so that all chemical compounds can be treated before being discharged, reducing the harmful effects on the environment and thus allowing us to have healthier bodies of water.

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