

Evaluation of the impact of the leached and solubilized extracts on the germination of cabbage seeds (*Brassica oleracea* var. *capitata*)

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Abstract

The germination and seedling root development trials have been used to evaluate and quantify the toxicity of water-soluble compounds as well as mixtures of complex substances, leached, among others. The objective was to evaluate the impact of leached and solubilized extracts of coffee waste on the germination and development of cabbage seedlings (*B. oleracea* var. *Capitata*), as well as to evaluate the physical and chemical properties of these extracts. The extracts were prepared according to the Brazilian Association of Technical Standards 10.005 and 10.006, respectively. The experimental design had 7 treatments for the test group (leached), 7 treatments for the test group (solubilized) and one control group. The control group was composed of 4 mL distilled water. It was verified that from the lowest dosage administered (0.1 mL) there was an inhibition in the germination of 30% for both extracts. Starting the dose with 0.7 mL of leached, no root growth was observed. The determination of LOEC was established in the treatments with 0.1 mL for both extracts.

Keywords: *Leached, Solubilized, Coffee Waste, Germination.*

1. Introduction

The continuous changes in lifestyle, industrial and commercial growth in many countries of the world in recent decades have been accompanied by rapid increases in the production of urban and industrial waste. This evolution represented an increase in income and quality of life, however, culminating with a growing increase of toxic elements and components, which directly interfere with the quality and potential of natural resources (RENOU et al., 2008).

The residual biomasses of coffee and other beverages generated in coffee shops, restaurants, residences, bars among others do not have registers due to the difficulty of quantifying this type of waste in different places (FREITAS, 2000; VEGRO e CARVALHO, 2006; RAMALAKSHMI et al., 2009; PANUSA et al., 2013).

The chemical and physical characterization of these elements points out its contaminating potential, however, it is fundamental to evaluate its biological implications and possible interactions. While the chemical analyzes identify and quantify the concentrations of toxic substances, toxicity tests assess the effect of these substances on biological systems (COSTA et al., 2008; KALCIKOVA et al., 2011).

The main objectives the ecotoxicological are to identify the risks associated with a substance and to determine under what conditions of exposure these risks are induced. Toxicology as a science serves both to protect living beings and the environment from harmful effects caused by toxic substances and to facilitate the evaluation of more selective harmful chemical agents such as clinical drugs and pesticides. About eleven million chemicals are known and only a small percentage is well studied in relation to their effects and dynamics in the environment (ZAKRZEWSKI, 1994; MANNING and TIENDMANN, 1995; HODGSON, 2004).

To analyze the toxic effects for substance or mixed substances improperly discarded should be performed toxicity tests that aim to predict the potential impact of a xenobiotic (toxic agent) on environment (FLOHR et al., 2005). For such tests, certain organisms are used and they are called test organisms.

In this context, germination and seedling root development trials have been used to evaluate and quantify the toxicity of water-soluble compounds as well as mixtures of complex substances, leachate, sediments among others (BOWERS, et al., 1997). These substances are submitted to acute phytotoxicity tests, aiming at evaluating both lethal effects through inhibition of seed germination and sublethal effects via root development (USEPA, 1996; OECD, 2003). The objective of this work was to evaluate the impact of leached and solubilized extracts of coffee waste on the germination and development of cabbage seedlings (*B. oleracea var. capitata*), as well as to evaluate the physical and chemical properties of these extracts.

2. Materials and methods

2.1 Preparation of extracts

The leached and solubilized extracts were prepared according to the Brazilian Association of Technical Standards 10.005 and 10.006, respectively (Brazilian Association of Technical Standards, 2004a, 2004b). To prepare the leached extract, 50.0 g samples of traditional Pilão® coffee waste (about 70% Arabica and 30% Robusta, grain size <9.5 mm) were transferred to polyethylene bottles and treated with 1.0 L of the extraction solution (5.7 mL of glacial acetic acid, 64.3 mL of 1.0 mol/L NaOH and 930 mL of Milli-Q water). The bottles were closed and shaken for about 18 h using a rotary shaker at 100 rpm (Nova Etica® model 430) at room temperature, and then filtered through a 0.45 µm membrane.

To prepare the solubilized extract, 250 g samples of Pilão® coffee waste were transferred to polyethylene bottles and treated with 1 L of ultra-pure water. The bottles were closed, shaken for 5 min using a rotary shaker at room temperature and then left standing for 7 days before filtering through a 0.45 µm membrane.

2.1.1 Physicochemical analysis

The physicochemical parameters such as pH, conductivity, TDS (total dissolved solids) and salinity were determined in the leached and solubilized extracts using a multi-parameter analyzer (COMBO 5 – AKSO).

2.2 Seed sampling

To perform the tests were used cabbage seeds (*B. oleracea* var. *capitata*). The seeds were from ISLA PAK® with 99.7% purity, 85% germination and validity until July / 18, directly, without any previous procedure.

2.2.1 Phytotoxicity test

Toxicity tests about radicle germination and development using cabbage seeds (*B. oleracea* var. *capitata*) were performed to evaluate both, the lethal inhibition effects on seed germination and the sub lethal effects on radicle development (SOBRERO and RONCO, 2004 and OECD, 2003).

The experimental design had 7 treatments for the test group (leached), 7 treatments for the test group (solubilized) and one control group. The control group was composed of 4 mL deionized water. The treatments for the test group were: (a) 0,1 mL extract leached + 3,99 mL deionized water, (b) 0,4 mL extract leached + 3,6 mL deionized water, (c) 0,7 mL mL extract leached + 3,3 mL deionized water, (d) 1 mL extract leached + 3 mL deionized water, (e) 2 mL extract leached + 2 mL deionized water, (f) 3 mL extract leached + 1 mL deionized water and (g) 10 mL only extract leached (without added water). The same procedure was used for the solubilized extract (extract + water). For all treatments, had three replicates. Additionally, twenty seeds of cabbage were placed in a Petri dish of 9.5 cm in diameter and moistened with the solution prepared according to established treatments, having as substrate a qualitative filter paper (porosity 14 μm). The Petri dishes were conditioned in a BOD incubator incubator with photoperiod (CIENLAB and model CE-300/350-F) at a temperature of $25 \pm 2^\circ\text{C}$ for 12 h light / 12 h dark.

2.2.2 Phytomass assesement

At the end of 120 h (5 days), the following parameters were evaluated: number of germinated seeds and length of rootlets.

In order to determine the sublethal effects, it was sought to identify the concentration of unobserved effect (NOEC), which corresponds to the highest concentration / dose of toxic agent that does not cause deleterious effect statistically significant in the organisms in the time and in the conditions of the test; and the observed effect concentration (LOEC), which corresponds to the lowest concentration / dose of toxic agent that causes statistically significant deleterious effects on organisms at the time of exposure and the test conditions (RONCO, BAEZ and GRANADOS, 2004). The determination of NOEC and LOEC were carried out from the comparison between the means of the root length.

2.3 Statistical analysis

The germination tests measurements and the length of rootlets were analyzed using a graduated ruler and a digital caliper. After these measurements, the parameters averages were calculated and Tukey's test used to evaluate the significance at 0.05 (5% probability) among them (COSTA NETO, 1977; MILLER; MILLER, 1993).

All calculations and graphs presented in this work were performed by BioEstat 5.0 and SigmaPlot 12.5 software.

3. Results and Discussion

The table 1 shows the physicochemical properties of the leached and solubilized extracts. It can be seen that leached extract showed higher conductivity, total dissolved solids (TDS) and salinity than the solubilized extract. In addition, was observed a very acid pH. The extract solubilized although prepared in aqueous medium also presented acidic characteristics.

In addition, according to Technical Standard NBR 10.004 Solid waste - Classification (ABNT, 2004c), this waste is classified as: Class II A, Not Hazardous – Not Inert.

Table 1. Physicochemical properties of the leached and solubilized extracts.

Coffee Waste	pH	Conductivity ($\mu\text{S}/\text{cm}$)	TDS (ppm)	Salinity (ppm)
Leached	4.9	530	345	272
Solubilized	6.0	450	289	220

The average values of the germination percentage as a function of the different treatments with the leached and solubilized extracts are shown in Figure 1. The control group presented 76% germination. It was verified that from the lowest dosage administered (0.1 mL) there was an inhibition in the germination of 30% for both extracts. For the leached, the percentage of inhibition for treatment with 0.4 mL was 66% and from 0.7 mL the germination rate was zero. The treatments with 0.4 and 0.7 mL of solubilized presented results lower than 50% (46%). To 1 mL of the solubilized the inhibition on germination was 45%. The same inhibitory behavior was observed for the other treatments (2 mL, 61%, 3 mL, 74%, 10 mL, 87%). The p-value found for the treatments was 0.0003. Significant differences were observed between the control group and the Tukey test ($p < 0.05$). The quadratic regression describes a decreasing behavior for the germination response in relation to the different treatments with the leached and solubilized extracts.

According to Beweley and Black (1994) and Shafaei et al. (2014) the germination process depends on the presence of water to the seed matrix, the oxygen content and some external environmental factors such as light and temperature. Therefore, these results indicate that the different dosages of leached and solubilized extracts had an influence on the percentage of germination of cabbage seeds (*B. oleracea var. capitata*).

Santos et al. (1992) shows that, in addition to water, the salinity of the local significantly influences the germinative response of the species because it causes a reduction of the water potential of the local. In this sense, it hinders the kinetics of water absorption, but also facilitates the entry of toxic ions into imbibed seeds. As the leached is a saline extract, the deleterious effects found in this study may be due to the increased concentration of the salts which should impair the germination and initial development of the seedling.

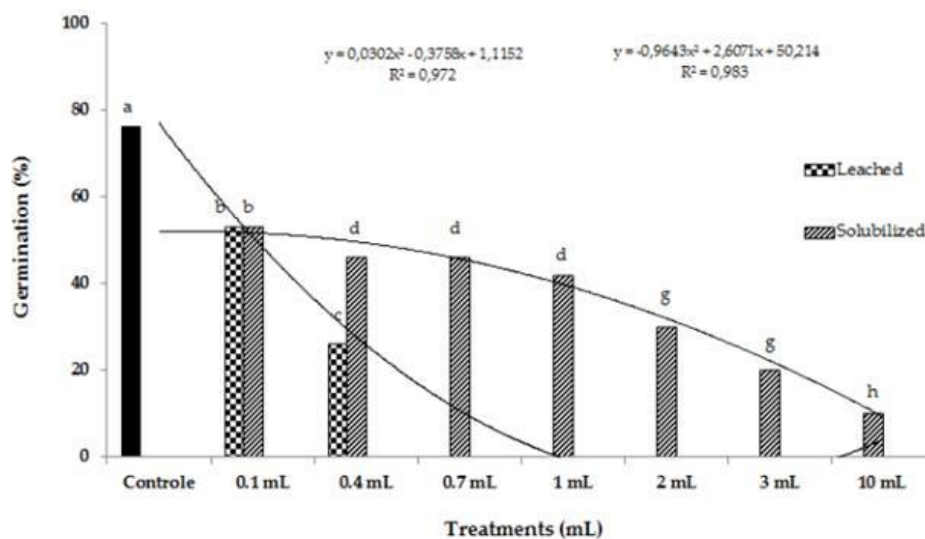


Figure 1. Evaluation of the percentage of germination of cabbage seeds (*B. oleracea var. Capitata*) contaminated with different dosages of the leached extract and solubilized from the coffee grounds. Means followed by the same lowercase letter do not differ statistically from each other by Tukey test ($p < 0.05$).

The medium values of root structure growth in function of the different treatments with the leached and solubilized extracts are shown in graph 2. The control group showed a growth of 47 mm. From the lower dosage (0.1 mL) there was an inhibition in the radicle growth of 47% for the leached and 17% for the solubilized extracts. For the dose with 0.4 mL of leached the percent inhibition was 66%. From 0.7 ml of leachade the growth of the radicle was zero. Treatments with 0.4 and 0.7 mL of the solubilized presented growth inhibition lower than 45% (38% and 42%, respectively). From 1 mL of the solubilized extract, an inhibition greater than 50% was found. Treatment with 10 mL showed a 79% inhibition of root growth. The p-value found for the treatments was 0.0010. Significant differences were observed between the control group and the Tukey test ($p < 0.05$). The quadratic regression describes a decreasing behavior for the length of rootlets growth response as a function of the different treatments with the leached and solubilized extracts. It was not possible to identify NOEC because the lowest dose was able to promote subtly perceptible effects by inhibiting the development of the radicle. The determination of LOEC was established in the treatments with 0.1 mL for both extracts.

It was verified that the acidic character of the extract and that the salinity present in the leached of the coffee grounds influenced significantly the germination process of the cabbage. Bernardes et al. (2015) evaluating the germination of cabbage seeds have identified that the salt as for example the KCl can provoke more aggressive results on the germination and the development of the seedlings than the NaCl. This statement corroborates with the results found in the present study from the variables evaluated.

The evaluation of the effect on the elongation of the radicle of the seedlings may reflect the toxicity of the soluble compounds present at such low concentration levels that they are not sufficient to inhibit germination but may delay or inhibit the root elongation process depending on the mode and site of action of the compounds. Thus, inhibition of root elongation shows a sensitive sublethal indicator for evaluating biological effects on plants (TAMADA et al., 2012).

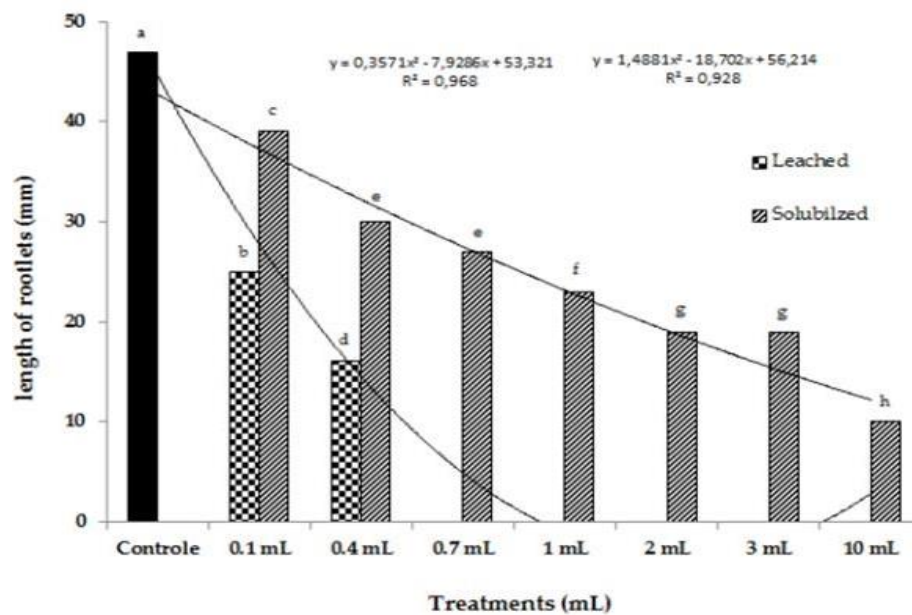


Figure 2. Radicle length of rootlets on the germination of cabbage seeds (*B. oleracea var. capitata*) contaminated with different dosages of the leached extract and solubilized from the coffee grounds. Means followed by the same lowercase letter do not differ statistically from each other by Tukey test ($p < 0.05$).

Guedes et al. (2011) verified that the root length was affected as the salinity levels increased in *Chorisia glaziovii* seeds. Thus, the higher the dose of the pollutant (leached and solubilized) introduced, the lower the root growth, mainly due to the high salinity of the effluent.

The accumulation of salts in the root zone at high concentrations are sufficient to restrict water absorption by the plant. This can lead to a water deficiency state, and cause symptoms very similar to those caused by lack of water (AYERS & WESTCOT, 1999).

None of the extracts studied presented morphological benefits to the seedlings (Figure 1 and 2). The leached extract had a more acidic and saline character than the solubilized one. The length of the radicle underwent a strict reduction, with visible growth inhibition occurring with increasing the dose of the leached (Figure 3).

The ability of plants to obtain water and mineral nutrients from soil was related to their ability to develop an extensive root system (TAIZ et al., 2017). It is assumed, therefore, that it can severely compromise the vegetative development of this plant, since, with the root system compromised, the plant is not able to absorb the essential elements to complete its cycle.

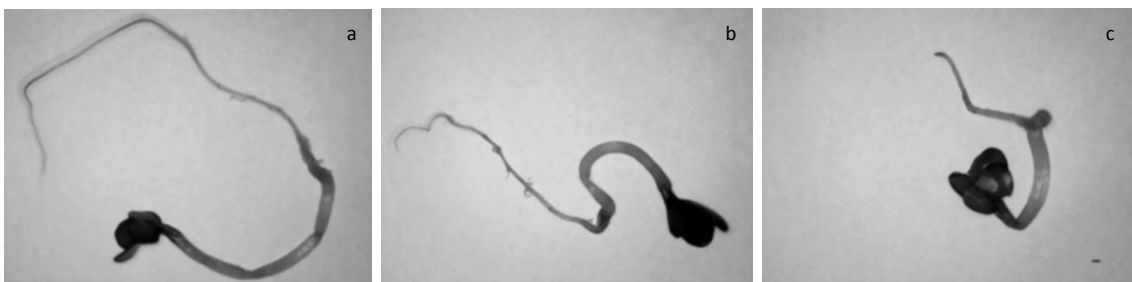


Figure 3. Radicle length of rootlets on the germination of cabbage (*B. oleracea var. capitata*) seeds contaminated with different dosages of the leach extract from the coffee grounds. (a) control; (b) 0.1 ml of leached; (c) 0.4 ml of leached. All the figures were evaluated with the same scale (1 mm).

The same inhibitory behavior was observed for the solubilized extract, but with less severe effect; Being this gradual (Figure 4). It is evidenced that with the increase of the solubilized dose, the morphological anomaly in the root system is also accentuated. Thus, it can be seen that these seedlings could not survive in field conditions, since the ability to absorb water and nutrients is greatly impaired.

Thus, the presence of a less developed root system in a plant leads a lower production of cytokines, due to the lower number of root ends, consequently the growth rate was reduced and survival was also compromised in adverse environments, specially of marked water deficiency (REIS et al., 2006).

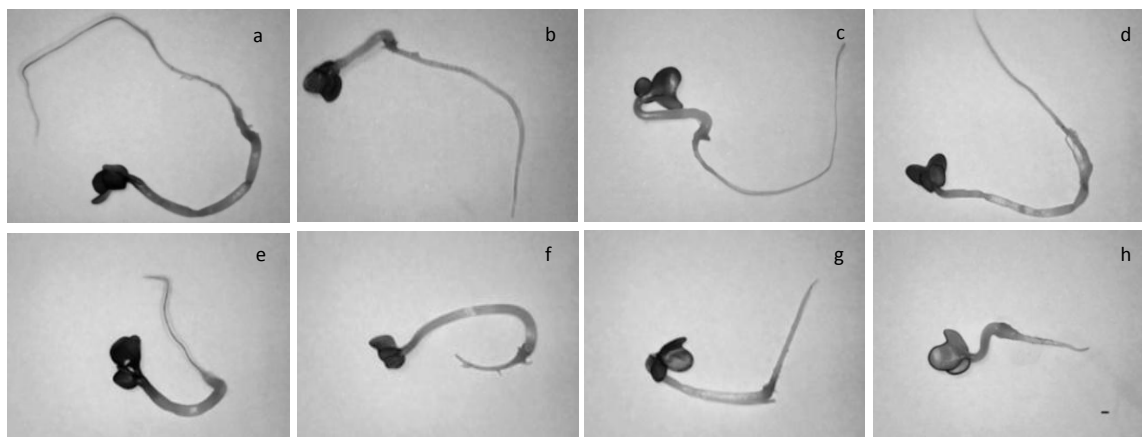


Figure 4. Radicle length of rootlets on the germination of cabbage (*B. oleracea var. capitata*) seeds contaminated with different dosages of the solubilized extract from the coffee waste. (a) control; (b) 0,1 mL de solubilized; (c) 0,4 mL de solubilized; (d) 0,7 mL de solubilized; (e) 1,0 mL solubilized; (f) 2,0 mL de solubilized; (g) 3,0 mL de solubilized e (h) 10,0 mL de solubilized. All the figures was evaluated with the same scale (1 mm).

4. Conclusion

The leached and solubilized coffee waste are classified as a not hazardous, not inert residue due to their solubilizing ability. Therefore, its final destination should employ some special measures of collection and protection of the environment. There was a negative effect on the germination rate and root growth for both extracts under study, due to the increase of the doses tested. It was verified that from the lowest dosage administered (0.1 mL) there was an inhibition in the germination of 30% for both extracts. Starting the dose with 0.7 mL of leached, no root growth was observed. The determination of LOEC was established in the treatments with 0.1 mL for both extracts.

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