Moringa oleifera extract as an inhibitor of heavy metal phytotoxicity on lettuce germination

Extrato de Moringa oleifera como inibidor de fitotoxicidade de metais pesados na germinação de alface

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ABSTRACT  
The study aimed to analyze the influence of heavy metals of Moringa oleifera extract on the germination process and development of lettuce seedlings, as well as to describe the reduction of toxic effects of heavy metals due to the interaction with the extract. From June to October 2018, four tests were carried out in a completely randomized design, two of which were toxicity tests, with concentrations in mg L\(^{-1}\) of the metals, Arsenic, Cadmium and Lead; one of extract phytotoxicity, using six concentrations in g L\(^{-1}\) of Moringa oleifera seed powder; and another on the interaction between heavy metal solutions and Moringa oleifera extract. In all tests, four repetitions of each treatment were performed. The inhibitory effect of heavy metals and Moringa oleifera extract on lettuce germination was verified. In the interaction between the heavy metal solutions with the Moringa oleifera extract, the concentration of 0.25 g L\(^{-1}\) inhibited the toxicity of the metals Arsenic and Cadmium in the germination process of lettuce seedlings.

Keywords: phytoremediation, whitewattle, lactuca sativa
RESUMO

O estudo teve como objetivo analisar a influência dos metais pesados do extrato de Moringa oleifera no processo de germinação e desenvolvimento de mudas de alface, bem como descrever a redução dos efeitos tóxicos dos metais pesados devido à interação com o extrato. De junho a outubro de 2018, foram realizados quatro testes em um projeto completamente aleatório, dois dos quais foram testes de toxicidade, com concentrações em mg L⁻¹ dos metais, Arsênio, Cádmio e Chumbo; um de fitotoxicidade de extrato, usando seis concentrações em g L⁻¹ de pó de sementes de Moringa oleifera; e outro sobre a interação entre soluções de metais pesados e extrato de Moringa oleifera. Em todos os testes foram realizadas quatro repetições de cada tratamento. Verificou-se o efeito inibitório dos metais pesados e do extrato de Moringa oleifera na germinação da alface. Na interação entre as soluções de metais pesados e o extrato de Moringa oleifera, a concentração de 0,25 g L⁻¹ inibiu a toxicidade dos metais Arsênio e Cádmio no processo de germinação de mudas de alface.

Palavras-chave: fitorremediação, água branca, lactuca sativa.

1 INTRODUCTION

The environmental impacts caused by the development of human activities are a concern of humanity, especially contamination by heavy metals. With systemic and bioaccumulative effects, heavy metals promote serious irreversible diseases in biotic beings, in addition to resulting in permanent soil contamination, as described by Essien et al. (2019) when studying this contamination in Nigeria.

Yang et al. (2018) researched contamination by heavy metals in China and associated more serious risks with contamination by Arsenic, Cadmium and Lead in industrial and agricultural environments.

Among the different techniques for decontamination of the environment, phytoremediation by Moringa oleifera can be used. According to Pritchard (2010), this plant has a protein found in the leaves, fruits and seeds, with adsorptive property, able to aggregate suspended solids and promote the effective treatment of turbid water.

Several studies with similar results using Moringa oleifera have proven its decontaminating power in solutions with heavy metals, with the pH variable being directly related to the adsorption phenomenon (Melo et al., 2021; Junior et al., 2013; Meneghel, 2012; Souza et al., 2021).
In this context, the present study evaluated the influence of the Moringa oleifera extract of heavy metals on the germination process and development of lettuce seedlings, as well as sought to describe the reduction of toxic effects of heavy metals due to the interaction with the extract.

2 MATERIAL AND METHODS

The experiment was conducted from June to October 2018 at the Laboratory of Technology and Environmental Risks of the Cerrado Research Center (NUPEC), at the Federal University of Rondonópolis, located at latitude 16° 27' 56.23" S and longitude 54° 34' 52.08" O.

All tests were carried out in a completely randomized design in a factorial scheme, with four replications of each treatment, using the variables percentage of germination, fresh mass, radicle size, epicotyl and seedlings as explanatory parameters. After detecting a significant effect in the F test, Tukey’s test was continued at 5% probability for heavy metals and the regression test for concentrations.

Four germination tests were evaluated, two for the toxicity of heavy metals Arsenic, Cadmium and Lead, one for phytotoxicity of Moringa oleifera extract with six concentrations and one for inhibition of heavy metal toxicity by Moringa oleifera extract.

The germination tests were carried out in Petri dishes with three layers of Germitest paper and 10 butter lettuce seeds were placed on the second layer. Two and a half times the weight of the paper in mL of the solutions was added, the plates were closed with plastic film and conditioned in a dark environment with controlled temperature at 25 ºC, and data collection was performed on the fourth day.

In the verification of toxicity of heavy metals, for Arsenic, Cadmium and Lead, concentrations of 0; 0.033; 0.1; 0.3; 0.9 and 2.7 mg L⁻¹ in the first and increasing the analyzed variables as well as the equidistance of heavy metal concentrations to 0; 15; 35; 55; 75 mg L⁻¹ in the second germination test of lettuce seedlings.

In the third germination test, the extract of Moringa oleifera was used, which was prepared from peeled seeds, crushed in a multiprocessor, sieved in an 8 mm mesh, and the flour was macerated with a mortar.
This macerated flour was transferred to a 500 mL volumetric flask and completed the volume with distilled water, thus constituting six concentrations, 0.25; 0.50; 1.0; 1.5; 2.0 and 2.5 g L\(^{-1}\) of Moringa oleifera extract, which were characterized by means of pH determination.

The interaction of the solutions on the germination and development of lettuce seedlings was evaluated using a concentration of 75 mg L\(^{-1}\) for the three heavy metals and 0, 0.25 and 0.50 g L\(^{-1}\) of Moringa oleifera extract. The mixture of the solutions was made in the proportion of 30% of the extract to 70% of the solution with heavy metals.

3 RESULTADOS E DISCUSSÃO

3.1 HEAVY METAL TOXICITY

In the first test, there was a significant effect isolated from the concentrations of heavy metals in the Arsenic, Cadmium and Lead solution for the variable fresh mass of lettuce seedlings.

Lead had greater interference in the development of seedlings, and for each gram of this metal in the solution, there was a reduction of 0.0382 mg in weight and that about 75% of this reduction occurred due to the increase in concentration in the solution, obtaining the lowest mass of 0.050 mg with a lead concentration of 1.73 mg L\(^{-1}\) (Figure 1).

Figure 1. Fresh mass of lettuce seedlings as a function of concentration in heavy metal solutions

Source: The authors (2023)
For the metal Arsenic there was a significant effect at 1% of probability, being for each mg increased of the metal, it reduced 0.0242 mg in the weight of the lettuce seedlings, and 57.18% of this reduction was due to the increase in the concentration, obtaining the lowest value of fresh mass with 0.058 mg in the concentration of 2.25 mg L\(^{-1}\) of arsenic in the solution.

In the second germination test, the significant effect on the interaction regarding the unfolding of heavy metals in the concentrations, so for metals the Tukey test was applied at 5% probability and for the concentration the polynomial regression test was applied.

The results of germination of lettuce seedlings showed that the concentration of 35 mg L\(^{-1}\) of Lead in the solution resulted in a lower germination rate compared to Arsenic and Cadmium (Table 1).

<table>
<thead>
<tr>
<th>Concentration (mg L(^{-1}))</th>
<th>Arsenic</th>
<th>Cadmium</th>
<th>Lead</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95.0 A</td>
<td>95.0 A</td>
<td>95.0 A</td>
</tr>
<tr>
<td>15</td>
<td>90.0 A</td>
<td>95.0 A</td>
<td>97.5 A</td>
</tr>
<tr>
<td>35</td>
<td>87.5 A</td>
<td>92.5 A</td>
<td>70.0 B</td>
</tr>
<tr>
<td>55</td>
<td>77.5 A</td>
<td>80.0 A</td>
<td>77.5 A</td>
</tr>
<tr>
<td>75</td>
<td>67.5 A</td>
<td>75.0 A</td>
<td>70.0 A</td>
</tr>
</tbody>
</table>

Equal letters on the same line do not differ from each other by Tukey's test at 5% probability

Source: The authors (2023)

It was observed that the germination process of lettuce seedlings depends directly on the quality of the solution in which the seed is soaked, as well as on the high concentration of heavy metals in the solution, which reduced the germination rate.

Gordin et al. (2012) corroborated this by concluding that there was stress in the germination process of seeds subjected to different concentrations of salts in the soaking solution, as well as Silva et al. (2017) reported the toxic effects of lower concentrations of lead metal on the germination process of Schinus terebinthifolius raddi seeds.
The effects of heavy metal concentrations were adjusted to the linear regression models, showing the effect of reducing the germination rate of lettuce seedlings as the concentration of heavy metals in the solution increased.

For Cadmium, each mg increase in concentration resulted in a reduction of 0.2935% in lettuce germination, concluding that 89% of this reduction was due to the increase in the concentration of heavy metal in the solution. For lead, 68% of the reduction in the germination process occurred due to the increase in concentration, resulting in a reduction of 0.3688% for each mg of metal in the solution (Figure 2).

Figure 2. Lettuce seedling germination rate as a function of concentrations of solutions contaminated with heavy metals

For each 10 mg of heavy metals Lead and Arsenic, a reduction of 3.6 and 3.7% was observed in lettuce seedling germination, respectively, with 68 and 95% of this reduction occurring due to heavy metal contamination.

Compared to the first test, the increase in the equidistance of heavy metal concentrations showed greater expression in the germination variable, which is the only one that showed interaction between heavy metals and concentrations.

Seedling and radicle size had the greatest toxicological interference, with a significant isolated effect of heavy metals at each concentration. The lowest averages

\[
y = -0.2935x + 98.066 \\
R^2 = 0.891
\]

\[
y = -0.3688x + 95.276 \\
R^2 = 0.6862
\]

\[
y = -0.357x + 96.354 \\
R^2 = 0.9564
\]
were obtained with the 75 mg L-1 concentration, which is the dosage chosen to constitute the interaction test of heavy metals with the Moringa oleifera extract (Table 2).

Table 2. Influence of heavy metals according to concentrations for the variables radicle and seedling size (mm)

<table>
<thead>
<tr>
<th>Concentration (mg L⁻¹)</th>
<th>Arsenic</th>
<th>Cadmium</th>
<th>Lead</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Radicle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10.70 A</td>
<td>10.70 A</td>
<td>10.72 A</td>
</tr>
<tr>
<td>15</td>
<td>10.80 A</td>
<td>9.75 A</td>
<td>10.57 A</td>
</tr>
<tr>
<td>35</td>
<td>12.02 A</td>
<td>10.72 A</td>
<td>14.00 A</td>
</tr>
<tr>
<td>55</td>
<td>11.06 A</td>
<td>9.87 A</td>
<td>11.37 A</td>
</tr>
<tr>
<td>75</td>
<td>10.72 AB</td>
<td>8.75 A</td>
<td>12.67 B</td>
</tr>
<tr>
<td></td>
<td>Seedling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>26.75 A</td>
<td>26.75 A</td>
<td>26.75 A</td>
</tr>
<tr>
<td>15</td>
<td>25.60 A</td>
<td>24.37 A</td>
<td>27.37 A</td>
</tr>
<tr>
<td>35</td>
<td>35.97 A</td>
<td>25.32 A</td>
<td>29.57 A</td>
</tr>
<tr>
<td>55</td>
<td>26.86 A</td>
<td>25.12 A</td>
<td>27.92 A</td>
</tr>
<tr>
<td>75</td>
<td>23.85 AB</td>
<td>21.05 A</td>
<td>29.57 B</td>
</tr>
</tbody>
</table>

Equal letters on the same line do not differ from each other by Tukey’s test at 5% probability

Source: The authors (2023)

3.2 PHYTOTOXICITY OF MORINGA OLEIFERA EXTRACT

There were reductions in the germination rate of lettuce seedlings and in the pH of the Moringa oleifera extract as the concentration of seed powder in the solution increased (Figure 3A; Figure 3B).

Figure 3. Germination of lettuce seedlings under different extract concentrations (A) and pH values (B) of Moringa oleifera extract

Source: The authors (2023)
The germination variable that presented 90% of the reduction in function of the increase of the concentration proved to be significant. The result indicated that for each mg of extract in the solution there is a real reduction of 4.0785% in germination (Figure 3A).

It was verified that there was no difference in relation to the Control for concentrations below 0.5 g L⁻¹. Oliveira et al. (2018) reported similar results when testing the allelopathic effects of the extract with Moringa oleifera leaves. The authors found inhibitory effects on the lettuce germination process from a concentration of 50%.

Knowing the pH is fundamental for understanding the results of the studied variables, since the alteration of this parameter indicates that heavy metals have undergone hydrolysis (Silva, 2010).

Alkaline solutions favor the formation of insoluble hydroxide (Lima and Merson, 2011). The concentrations of 0.25 and 0.5 g L⁻¹ of Moringa oleifera extract showed pH 6.5 and 6.3, respectively, not statistically different from the Control. For each g L⁻¹ of Moringa oleifera there was a reduction of 0.9215 in the pH index, with 96% of this reduction due to the increase in the concentration of Moringa oleifera seed powder in the solution (Figure 3B).

The pH of the solutions directly influenced the germination and effluent treatment processes with the Moringa oleifera extract (Costa et al. 2013), as well as Matos et al. (2007) verified a greater action of the Moringa oleifera extract in the pH range between 4.0 and 5.0.

All analyzed variables had a reduction, which is expressed in the linearity or polynomial equations, for the development of seedlings that reduced 9.7061 mm for each g in the increase in the concentration of Moringa oleifera extract in the solution, with 88% of the reduction as the increase the concentration of seed powder in the solution (Figure 4).
The size of the epicotyl was reduced by 81% as the concentration increased, and for each g increase in concentration there was a reduction of 2.80 mm.

For the radicle size variable, there was a significant effect at 5% probability in the regression analysis, reducing 2.59 mm at each mg increase in extract concentration.

As in the heavy metals toxicology test, radicle size was the variable that most interfered with the development of lettuce seedlings, and may be related to two hypotheses: the first on the inhibition of the auxin phytohormone responsible for root development and the second on the presence of abscisic acid in the solutions, causing direct inhibition of seedling development.

3.3 TOXICOLOGICAL INHIBITION OF HEAVY METALS

Two concentrations of Moringa oleifera extract were used, 0.25 and 0.5 g L^{-1}, due to the absence of germination inhibiting effects and the interaction with the heavy metal.
solutions Arsenic, Cadmium and Lead at a concentration of 75 mg L⁻¹, because this dosage presents the greatest interference in the germination rate of lettuce seedlings.

There was a significant difference for all variables studied where the heavy metal Lead had the best performance in terms of growth of lettuce seedlings, statistically differing from the heavy metals Arsenic and Cadmium (Table 3).

Table 3. Effect of Moringa oleifera extract on heavy metal toxicology inhibition

<table>
<thead>
<tr>
<th>Metal</th>
<th>Germination (%)</th>
<th>Radicle (mm)</th>
<th>Epicotyl (mm)</th>
<th>Seedling (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>32.50 A</td>
<td>3.91 A</td>
<td>5.91 A</td>
<td>9.83 A</td>
</tr>
<tr>
<td>Lead</td>
<td>70.83 B</td>
<td>9.59 B</td>
<td>15.66 C</td>
<td>25.25 C</td>
</tr>
<tr>
<td>Cadmium</td>
<td>75.83 B</td>
<td>3.70 A</td>
<td>10.46 B</td>
<td>14.16 B</td>
</tr>
</tbody>
</table>

Equal letters in the same column do not differ from each other by Tukey’s test at 5% probability
Source: The authors (2023)

Using the regression method, it was observed that the development of radicle, epicotyl and lettuce seedlings was influenced by Moringa oleifera extract and solutions of heavy metals Cadmium, Arsenic and Lead (Figure 5).
Figure 5. Seedling development in the interaction of Moringa oleifera extract with heavy metals at 75 mg L⁻¹: Cadmium (A), Arsenic (B) and Lead (C)

There was a difference in the development of seedlings regarding the interaction between the heavy metal solution and the Moringa oleifera extract, showing a quadratic behavior for the three analyzed variables, where the dosage of 0.25 g L⁻¹ obtained a seedling size of 16.99 mm, radicle of 4.9 mm and epicotyl of 13.53 mm. These results
were different from the Control and the concentration of 0.5 g L\(^{-1}\) of the Moringa oleifera extract, which presented values below 13.1 mm (Figure 5A).

There was a quadratic difference for radicle development in the dosage of Moringa oleifera extract at 0.25 g L\(^{-1}\), with 4.75 mm in length. It was found that for each mL of Moringa oleifera extract there was a reduction of 2.5 mm in the development of seedlings and epicotyl, with 75% depending on the increase in concentration (Figure 5B).

There was a quadratic difference in the development of seedlings for the metal Lead, which presented an inverse effect for the dosage 0.25 g L\(^{-1}\), being this the dosage that presented the lowest development for the three analyzed variables (Figure 5C).

Corroborating this study, Varela (2017) concluded that Moringa oleifera seed can be used to remove heavy metals copper, zinc, due to the potential for electrostatic adsorption, complexation and precipitation mechanisms, pointing to a direct relationship between the influence of pH in facilitating adsorption mechanisms.

**4 CONCLUSIONS**

Moringa oleifera extract with a concentration greater than 0.5 mg L\(^{-1}\) showed phytotoxicity in the germination process of lettuce seedlings.

The heavy metal Arsenic showed the greatest interference in the germination and development of lettuce seedlings with the lowest means compared to Lead and Cadmium.

The concentration of 0.25 mg L\(^{-1}\) of Moringa oleifera extract showed greater efficiency in reducing the toxicity of metals Arsenic and Cadmium.
REFERENCES


