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Evaluation of extracts of *Moringa oleifera* Lam seeds obtained with NaCl and their effects on water treatment

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ABSTRACT. Several natural coagulants have been studied for use in water treatment. The seed of *Moringa oleifera* Lam, for example, is a natural coagulant whose extract has been mentioned as effective not only for removing color, turbidity, and compounds with absorption at UV-254 nm, but also for significantly reducing the amount of sludge and bacteria in wastewaters. Therefore, the present study (1) evaluated the molecular weight of the extract of *Moringa oleifera* seed utilizing electrophoresis, and (2) compared the efficiency of different extracts obtained, using solutions of NaCl (0.01 M, 0.1 M and 1 M), distilled water, and *Moringa oleifera* Lam seed, acting as a natural coagulant in order to obtain drinking water. The tests were performed in Jar Test, and the effectiveness of the process was assessed regarding the removal of color, turbidity and UV-254 nm. It was observed that the molecular weight found in this study is consistent with literature data. Moreover, the highest removal efficiency of color, turbidity, and UV-254 nm occurred with 1M NaCl solution, with coagulant concentration between 100 and 300 mg L^{-1}. The results obtained evidenced that the seed of *Moringa oleifera* Lam is a great alternative for use as a coagulant in drinking water treatment systems.

Keywords: *Moringa oleifera* Lam, drinking water, coagulation/flocculation.

Avaliação de extratos obtidos da semente de *Moringa oleifer*a Lam com NaCl e seus efeitos no tratamento para obtenção de água potável

RESUMO. Alguns coagulantes naturais estão sendo estudados no tratamento para obtenção de água potável. Um exemplo de coagulante natural é a semente de *Moringa oleifera* Lam, cujo extrato tem sido mencionado por ser eficiente na remoção de cor, turbidez e compostos com absorção em UV-254 nm e ainda por diminuir drasticamente o lodo e a quantidade de bactérias em águas residuárias. Portanto, o objetivo do presente trabalho foi (1) avaliar o peso molecular do extrato de sementes de *Moringa oleifera* utilizando o processo de eletroforese e (2) comparar a eficiência de diferentes extratos obtidos utilizando soluções de NaCl (0,01 M, 0,1 M e 1 M), água destilada e sementes de *Moringa oleifera* Lam, agindo como coagulante natural no processo de obtenção de água potável. Os ensaios foram realizados em "Jar Test" e a eficiência do processo foi avaliada em função da remoção de cor, turbidez e UV-254 nm. Observou-se que o peso molecular encontrado neste trabalho está de acordo com dados encontrados na literatura e que a solução de NaCl 1M, com concentrações do coagulante entre 100 e 300 mg L^{-1}, foi a combinação de melhor eficiência na remoção de cor, turbidez e UV-254 nm. Os resultados obtidos neste trabalho mostram que a semente de *Moringa oleifera* Lam é uma ótima alternativa para ser utilizada como coagulante na obtenção de água potável.

Palavras-chave: *Moringa oleifera* Lam, água potável, coagulação/floculação.

Introduction

The monitoring of several indicators is required for controlling water quality. The concern about the contamination of aquatic environments increases especially when the water is used for human consumption. Thus, there is great importance in developing more sophisticated treatment techniques or even in improving of existing ones, for the removal of color, turbidity, and compounds with absorption at UV – 254 nm in the treatment of surface water to obtain drinking water.

The aluminum sulfate stands out as the most used chemical coagulant in Brazil, due to its good effectiveness and low cost. Nevertheless, as the aluminum is not biodegradable, high concentrations of this compound can harm human health, including the acceleration of the degenerative process of Alzheimer's disease (CARDOSO et al., 2008). Natural coagulants/flocculants have shown advantages in relation to the chemical ones, specifically in relation to biodegradability, low toxicity and low production of waste sludge. The sodium aluminate, considered a basic coagulant is used in special cases or as an additive for secondary coagulation of highly colored surface waters. The poly-aluminum-chloride (PCA) also known as aluminum hydroxide is widely used due its high efficiency and low toxicity.

For these reasons, some countries, like Japan, Chine, Indian and United States have adopted the use of natural polymers in the treatment of surface water to produce drinking water by the major advantages over chemical coagulants/flocculants (KAWAMURA, 1991).

In some tropical developing countries, the clarification of turbid river waters is an ancient practice, based on the use of natural materials that act as primary coagulants. One of these is the seed of the tropical tree *Moringa oleifera* Lam, which contains active agents with excellent coagulant activity. The extract of the seed has been mentioned by drastically reduce the sludge and amount of bacteria in wastewater (MUYIBI; EVISON, 1995).

According to Ghebremichael et al. (2005), the proteins are the main active components in extracts *Moringa oleifera* seeds. Coelho et al. (2009) assert that the protein found in *Moringa oleifera* is the lectin, responsible for coagulation/flocculation of the water.

Moreover, it is reported the presence of a cationic dimeric protein with high molecular weight in *Moringa oleifera*, which destabilizes the particles contained in the water and flocculates the colloids through a process of neutralization and adsorption followed by sedimentation (NDABIGENGESERE et al., 1995).

In this context, this study evaluated the molecular weight of the protein in the *Moringa oleifera* seed by means of electrophoresis, and examined the efficiency of different extracts obtained from seeds of *Moringa oleifera* Lam as natural coagulant in processes to obtain drinking water.

Material and methods

Electrophoresis and protein content in the extracts

In order to prepare the solution of *Moringa oleifera* Lam, first of all, 1 g of seed pulp was pulverized in the blender, along with 100 mL of distilled water. Then, the solution was stirred for 30 minutes and vacuum-filtered.

Two different seed samples were used, one from Aracaju city, Sergipe State (Sample A) and another from Maringá city, Paraná State (Sample B), harvested in the same period and stored under refrigeration.

The molecular weight of the protein was estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (1970). The gels were stained with Coomassie Brilliant Blue for proteins (LAEMMLI, 1970). It was used the standard of the brand Bio Rad (161-0324).

The Lowry method (LOWRY et al., 1951) was adopted to determine the amount of total proteins extracted in the preparation of the *Moringa oleifera* Lam solution, and to verify the ratio between the protein dosage and coagulation efficiency, the essays were performed in triplicate.

Characterization of the raw water and coagulation/flocculation

Four different extracts were used as coagulant solution, obtained by means of extraction with solution of NaCl (0.01 M, 0.1 M and 1 M) and distilled water. In literature are found data citing the NaCl as extractor agent (OKUDA et al., 1999; NKURUNZIZA et al., 2009), but in all these cases the authors use only solutions with one molar, thus, in the present study, we chose to investigate other molarities applying the same salt.

The coagulation solution of *Moringa oleifera* Lam was always prepared at the day of analysis; firstly 1 g of seed pulp was pulverized in the blender with 100 mL of the given solution. Then, the solution was stirred for 30 minutes and vacuum-filtered.

In the coagulation/flocculation process, it was used concentrations of the extract of *Moringa oleifera* Lam of 25 to 300 mg L^{-1}. The raw water used was characterized according to procedure recommended by Standard Methods (APHA, 1995).

Tests of coagulation/flocculation/sedimentation were accomplished using simple Jar Test, Milan – Model JT101/6 of six proofs with rotation regulator of the mixing rods. The working conditions used for the process of coagulation/flocculation/ sedimentation were used according to Cardoso (2008); with rapid mixing speed (100 rpm), coagulation time (3 min.), slow mixing speed (15 rpm), flocculation time (15 min.) and settling time (60 min.).

The effectiveness of the process was evaluated by removal of color, turbidity and compounds with absorption at UV – 254 nm measured in spectrophotometer HACH DR/2010, following recommendations of Standard Methods (APHA, 1995).

Results and discussion

Electrophoresis and protein content in the extracts

After the extraction with water of the active compound of two different samples of *Moringa oleifera* seed, the characterization was performed through electrophoresis (Figure 1), in order to evaluate which was the molecular weight (MW) of the active compound found in the extracts.

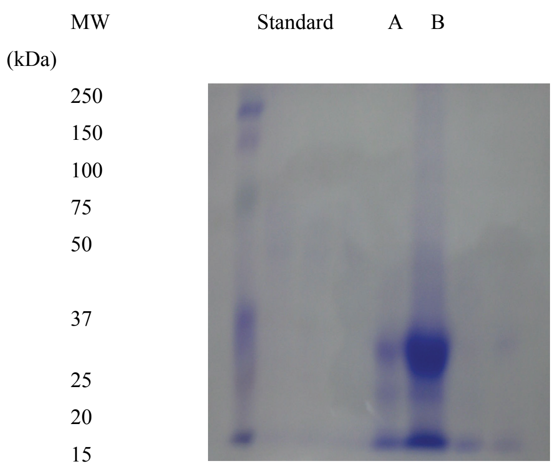

Figure 1. Polyacrylamide gel electrophoresis (12%) of SSMoL (100 μg), treated with β-mercaptoethanol.

According to Santos et al. (2005), the protein found in the aqueous extract obtained from the seed of *Moringa oleifera* Lam has molecular mass of approximately 30 kDa. The Figure 1 presented the result of the electrophoresis analysis in the aqueous extract obtained from two different samples of *Moringa*, one from Aracaju city, Sergipe State (Sample A) and another from Maringá city, Paraná State (Sample B). As can be observed, the same molecular mass found by Santos et al. (2005) was recorded for the protein of the two evaluated samples, and the sample B probably has higher concentration of this protein.

Comparing the data obtained with the literature, the coagulant proteins were previously reported in seeds of *Moringa oleifera* extracted with water. Ndabigengesere et al. (1995) described a cationic dimeric protein with molecular mass of 12-14 kDa and isoelectric point (Ip) between 10 and 11.

Ghebremichael et al. (2005) studied a cationic protein with Ip higher than 9.6 and molecular mass lower than 6.5 kDa; Gassenschmidt et al. (1995) purified a cationic protein with Ip higher than 10 and molecular mass around 6.5 kDa. The coagulant activity of cationic derivatives with high molecular weight is explained by the bond formation model; coagulation of negatively charged particles derives from bonds via Coulomb forces; positively charged proteins bind to surface of negatively charged particles (GASSENSCHMIDT et al., 1995).

In general, the results found in literature present different molecular weights for the protein from the seed of *Moringa oleifera*, ranging from 6.5 kDa to 30 kDa.

The values indicative of proteins present in solutions of *Moringa* with water and NaCl at different molarities (0.01 M, 0.1 M and 1 M) are listed in Table 1; as previously mentioned in methodology the analyses were made in triplicate.

Table 1. Amount of protein for the different extracts evaluated.

Type of extracted solution	Protein content (mg L^{-1})
Extraction with water	1,832.60
Extraction with NaCl 0.01 M	1,290.86
Extraction with NaCl 0.1 M	4,388.01
Extraction with NaCl 1 M	4,499.19

The Table 1 shows the value of the protein as a function of the extraction made. An increase in the amount of proteins is observed by the increased concentration of NaCl. This is an important fact, once it is known that the protein in the seeds of *Moringa oleifera* is the most important compound for the process of water clarification. In literature, there are evidences that the seed of *Moringa oleifera* has a cationic dimeric protein with high molecular weight, which destabilizes the particles contained in the water through a process of neutralization and adsorption, flocculation of colloids, followed by sedimentation (VIEIRA et al., 2009). Noteworthy, the lowest value of protein concentration was obtained with extraction with 0.01 M of NaCl.

Characterization of the raw water

Although several studies have used synthetic water in experiments, this study used raw water collected directly from the Pirapó river – Maringá, Paraná State. It is important to consider that the natural compounds can cause variations in the composition, which can interfere with the treatment process. All these factors are taken into account when assessing the obtained results.

The characteristics of the surface water used in this study are found in Table 2.

Table 2. Characterization of the raw water.

Parameters	Unit	Value
Apparent color	uH(1)	1,030
Turbidity	NTU	450
UV- 254 nm	cm^{-1}	0.539

(1) Hanzen unit = (mg Pt-Co L^{-1}).

The water used in the experiments has apparent color, turbidity and an amount of compounds with relatively high absorption at UV-254 nm. Nkurunziza et al. (2009) concluded that *Moringa oleifera* is not a good coagulant in waters with low turbidity and color.

Coagulation/flocculation

The Figures 2 to 4 present the percentage of removal of color, turbidity and compounds with absorption at 254 nm, respectively, for the different extracts used in the process of coagulation/flocculation at the different concentrations of the extracts used as coagulant solution.

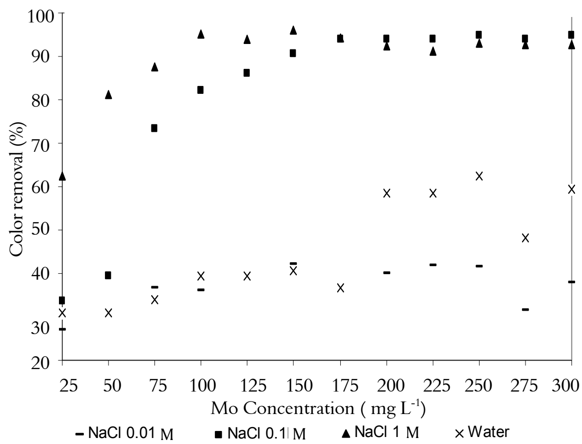

Figure 2. Influence of the concentration of the different extracts of *Moringa oleifera* used on the percentage of color removal.

The best percentages of color removal were observed in the range between 150 and 300 mg L^{-1} of coagulant solution (Figure 2). The solution extracted with NaCl 1 M presented removal of 97% of color, followed by the solution extracted with NaCl 0.1 M, water and NaCl at 0.01 M.

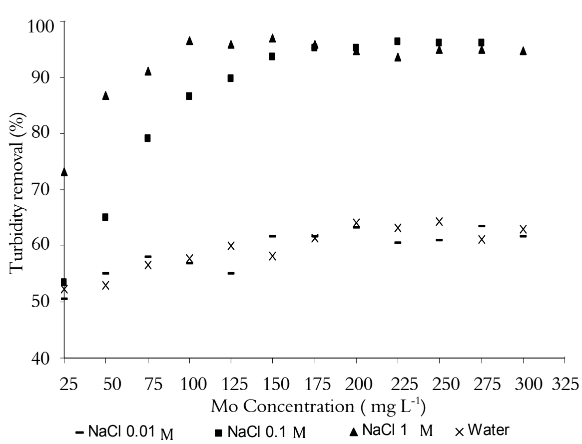

Figure 3. Influence of the concentration of the different extracts of *Moringa oleifera* used on the percentage of turbidity removal.

For the turbidity removal (Figure 3), the best results were verified within the range between 100 and 300 mg L^{-1}, with the highest salt concentration, i.e. NaCl 1 M, with removal degree of approximately 99.8%. For NaCl at 0.1 M, the values were between 55 and 97%, 50% for NaCl at 0.01 M, and only 52% for the extraction with water. Therefore the decline in salt concentration causes decrease in percentages of turbidity removal. This can be related to the amount of protein found in the three different salt solutions (Table 1). For higher molarities, with increasing protein level, the greater is the coagulation activity. Consequently, the better is the efficiency in turbidity removal.

Okuda et al. (1999) studied the coagulation process in water solutions using coagulant obtained from *Moringa oleifera*, and extracted the active compound with NaCl at 1 M, which allowed a turbidity removal of 95%. In this case, the water sample had an initial turbidity of 50 NTU. For the extraction with water, it was required a solution with 32 mg L^{-1} to remove 78% of turbidity.

Nkurunziza et al. (2009) employed a solution of 3% of MO, extracted with a solution of NaCl at 1 M, and have found a removal rate of turbidity of 83.2% (water initial turbidity of 50 NTU) and 99.8% (water initial turbidity of 450 NTU). It is observed then that the seed of MO is more effective for the treatment of waters with high turbidity.

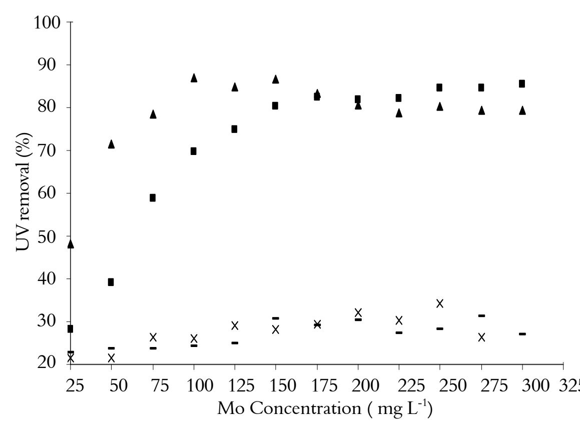

Figure 4. Influence of the concentration of the different extracts of *Moringa oleifera* used on the percentage of removal of compounds with absorption at UV-254 nm.

For the removal of compounds with absorption at UV-254 nm, it was registered a similar trend to turbidity removal; the concentration range with best removal was between 100 and 300 mg L^{-1}, the best extraction with NaCl 1 M, and the worst, with water.

In cases of low removal efficiency, Amagloh and Benang (2009) recommend the combination of *Moringa* with other coagulant to obtain promising results.

Conclusion

In summary, our results allow concluding that:

- the molecular weight found for the active compound is in accordance with the literature data. The molecular weight registered in this study for the seed active compound was the same for seeds cultivated in the two different regions evaluated;

- the best removals of the parameters studied (color, turbidity and UV- 254 nm) occurred with the extraction using solution of NaCl at 1 M, in the concentration range between 100 and 300 mg L^{-1} of coagulant solution;

- the employment of the seed of *Moringa oleifera* Lam showed to be advantageous and promising at the stage of coagulation/flocculation/sedimentation for obtaining drinking water.

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