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Impact of *Moringa oleifera* Seed-Derived Coagulants Processing Steps on Physicochemical, Residual Organic, and Cytotoxicity Properties of Treated Water

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Abstract: This study explored the application of whole and defatted *Moringa oleifera* seed-derived coagulants in powder (P-MOS and DP-MOS), aqueous extraction (AEP-MOS and AEDP-MOS), and saline extraction (SEP-MOS and SEDP-MOS) in the treatment of a synthetic turbid water by coagulation, flocculation, and sedimentation in a jar test apparatus. The performance of *M. oleifera* seed-derived coagulants was quantified and compared with alum in terms of the ability to neutralize and restabilize the suspension charge, turbidity removal, effect on pH and electrical conductivity, residual organic matter, as well as cytotoxicity in the treated water. All evaluated forms of *M. oleifera* seed-derived coagulants were able to neutralize and restabilize (in overdose) the particles charges in the suspension. Saline extractions obtained the best turbidity removal results (90%) between the *M. oleifera* seed-derived coagulants, while alum removed 98% of turbidity. Differently from alum, increased dosage of *M. oleifera* seed-derived coagulants did not change pH value. Saline extractions and, to a lesser extent, alum increased the electrical conductivity with increasing coagulant dosage. *M. oleifera* seed-derived coagulants increased residual organic matter (DOC), unlike alum, which did not change this property with increasing dosage. Saline extractions at high dosages enhanced the cytotoxicity to mammalian cells. On the other hand, defatted seeds reduced water cytotoxicity when compared to whole seeds. Despite not being able to reduce the residual organic matter, the previous oil extraction proved to be an important step in the processing of *M. oleifera* seed-derived coagulants, not changing the turbidity removal capacity and reducing the cytotoxicity of the treated water in addition to generating a significant by-product (Ben oil). Although saline extractions have shown the best turbidity removal results, they should be used with caution due to increased electrical conductivity and cytotoxicity of the treated water at high dosages.

Keywords: natural coagulants; coagulation; extraction; defatted; electrical conductivity; dissolved organic carbon

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1. Introduction

Recently, the seeds of *Moringa oleifera* have received attention among plant-based coagulants for the treatment of drinking water and wastewater towards a green economy and cleaner production [1,2]. It is a plant native to South Asia but is widely cultivated worldwide across the tropics [3,4]. The active compound present in the seeds of *M. oleifera* responsible for the coagulant activity is formed by cationic proteins soluble in water with an isoelectric point at pH 10–11 and molecular weight ranging between 6.5–30 kDa. The dominant coagulation mechanism is believed to be consistent with adsorption and charge neutralization [5–7].

The cationic proteins present in *M. oleifera* seeds are not in a readily available form, and one or more processing steps are needed before use. The steps usually consist of primary (powder processing), secondary pre-treatment (oil extraction), secondary (cationic proteins extraction), and tertiary (purification) stages. *M. oleifera* seeds can be used as a coagulant during any of the mentioned stages. The more advanced the processing stage, the higher the costs and complexity involved, and different results in water treatment can be found. The primary, secondary pre-treatment, and secondary stages are the most studied and usually applied where specialized expertise and equipment are lacking. A tertiary stage of purification, such as lyophilization, ion exchange, or dialysis, is rarely applied in plant-based coagulants due to the complexity and high costs involved [1,8].

Despite the simplicity of processing, few studies have investigated the direct use of *M. oleifera* whole-seed powder (primary processing stage) as a coagulant because there is a concern that cationic proteins may not be efficiently extracted, reducing the coagulant efficiency. However, some specific studies have shown satisfactory results from the direct use of the powder [9,10]. According to Silva et al. [11], the direct use of *M. oleifera* seed powder was capable to remove 93% of the turbidity after sedimentation in the harvesting of microalgae cultivated in anaerobically digested blackwater (171 NTU).

The aqueous extraction of the seed powder (secondary stage) is the most frequently used method because the cationic proteins of interest are soluble in water in addition to the fact that this solvent is easily available and at low cost [8,12]. Ribeiro et al. [13] achieved turbidity removal of up to 98% via in-line filtration technique of a low-turbidity (25 NTU) synthetic water with aqueous extraction of the whole-seed powder. However, to enhance the coagulant activity of the *M. oleifera* seed, some studies have shown that the use of saline extraction (commonly NaCl but also $NaNO_3$ and KCl) instead of aqueous gives even better turbidity-removal results. Okuda et al. [14] evaluated the turbidity removal after sedimentation of a synthetic water (50 NTU) with aqueous extract and compared it with the saline extract (NaCl 1M), obtaining removals of 78% and 95%, respectively. Furthermore, the saline extract dosage was 7.4 times lower than that using aqueous extraction.

M. oleifera seeds contain a significant amount of an oil (up to 40% w/w) known as Ben oil, which has industrial, human consumption, and energy applications. Therefore, a previous extraction of this oil (secondary pre-treatment) is of commercial and environmental interest [15,16]. Recent studies have evaluated the effect on the coagulant activity of the defatted seed, and a consensus has not yet been reached in the literature. Skaf et al. [17] found similar results (88%) of turbidity removal from synthetic water (60 NTU) after sedimentation using an aqueous extraction from whole and defatted seed. On the other hand, Garcia-Fayos et al. [18] observed that the aqueous extract of the whole seed showed a 30% lower efficiency than the defatted seed for removing turbidity from a synthetic water (200 NTU) after sedimentation.

The use of *M. oleifera* seed as a coagulant has some challenges to be overcome, as the unfractionated seed also has other compounds that are released together with the cationic proteins of interest that can cause undesired results, such as residual organic carbon and cytotoxicity after water treatment [19,20]. *M. oleifera* seeds kernel contains approximately 36.7% protein, 34.6% lipids, and 5.0% carbohydrate (w/w) [5], and as an undesirable consequence, these compounds (organic matter) can facilitate regrowth of microorganisms and lead to the formation of disinfection by-products [21,22]. Vega Andrade et al. [23] reported an increase of 104% in BOD in the tertiary treatment of a domestic effluent using an aqueous extraction of the whole seed (600 mg/L). Although the literature indicates that the secondary pre-treatment (defatted seed) is a potential solution to reduce residual organic carbon in the treated water [18,24], no manuscript has been found that studied the different ways of using *M. oleifera* seed (whole and defatted) to confirm or refute this hypothesis, which is one of the objectives of this study.

Another issue to be better studied is related to the increase in cytotoxicity in water treated with *M. oleifera* seed-derived coagulants, which may be related to the presence of mustard oil glycosides in the seeds, which break down into isothiocyanates, which in turn

have cytotoxic activity [25,26]. Araújo et al. [27] reported that the aqueous extract of the whole seed showed cytotoxic activity towards peripheral blood mononuclear cells (PMBC). Therefore, further studies are needed to evaluate the effect of residual organic carbon and cytotoxicity as a function of the *M. oleifera* seed-derived processing steps to minimize the negative effects of its use for drinking water or wastewater treatments.

In this sense, the objective of this study was to evaluate the application of whole and defatted *M. oleifera* seed-derived coagulants in powder, aqueous extraction, and saline extraction for the treatment of a synthetic turbid water by coagulation, flocculation, and sedimentation in a jar test apparatus. The performance of *M. oleifera* seed-derived coagulants was quantified in terms of the ability to neutralize and restabilize the suspension charge (zeta potential), turbidity removal, effect on pH and electrical conductivity, residual organic matter, as well as cytotoxicity in the treated water. To provide a comparative basis, the treatments were also performed with alum.

2. Materials and Methods

2.1. Processing of M. oleifera Seed-Derived Coagulants

The *M. oleifera* seeds used in this study were harvested in the city of Araçatuba-SP/Brazil (20°56′19.72″ S, 50°40′6.17″ W), purchased from Arbocenter. Figure 1 shows the processing steps for the *M. oleifera* seed-derived coagulants preparation.

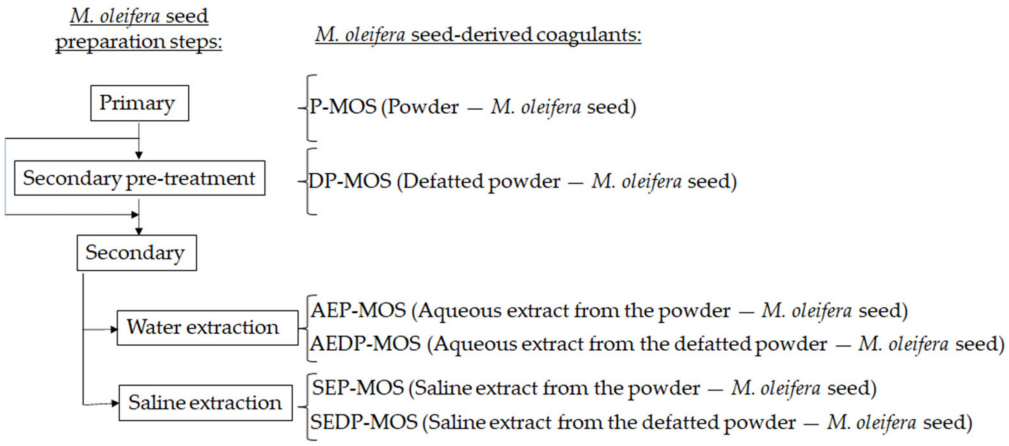

Figure 1. Processing steps for the production of *M. oleifera* seed-derived coagulants.

2.1.1. Primary Stage—Powder Processing

M. oleifera seeds were initially dried in a laboratory oven at 40 °C until constant mass was obtained (~2 h). Then, the dry seeds were manually shelled and selected, and the poor-quality seeds were discarded. The husks obtained by the shelling process were stored for later to study their use as an adsorbent [28]. The seed kernel was then ground in a domestic blender and sieved through a 30-mesh (600 µm) steel sieve. The powder obtained was stored in a closed container and kept at 4 °C to avoid deterioration of cationic proteins [29]. The powder, henceforth called P-MOS (Powder—*M. oleifera* seed), is the basis of the next steps of cationic proteins extraction, and it was also evaluated as a coagulant with direct use.

2.1.2. Secondary Pre-Treatment Stage—Oil Extraction

The oil extraction from the P-MOS was carried out via a Soxhlet extractor apparatus using hexane as a solvent with a solid/solvent ratio of 0.05 g/mL during a period of 3 h (20 cycles), which was used to perform a high oil-removal yield when compared to other

removal techniques (batch) or other solvents (ethanol, ethyl acetate, and acetone) [18,30]. The oil-removal yield was calculated according to Equation (1):

$$\text{Oil yield } (w/w\%) = \frac{\text{Quantity of oil extracted (mg)}}{\text{Quantity of P} - \text{MOS (mg)}} \times 100 \quad (1)$$

Three oil extractions were carried out, and the yield achieved was 38.4 ± 0.2%, which is in agreement with the values normally reported in the literature for these extraction conditions [18,30].

After the extraction, the defatted powder was dried in a laboratory oven at 40 °C until constant mass was obtained in order to remove any remnant solvent and then stored in a closed container and kept at 4 °C to avoid deterioration of cationic proteins [29]. The defatted powder, henceforth called DP-MOS (Defatted powder—*M. oleifera* seed), is the basis of the next steps of cationic proteins extraction, and it was also evaluated as a coagulant with direct use.

2.1.3. Secondary Stage—Cationic Protein Extraction

The extraction of cationic proteins was performed with both whole-seed powder (P-MOS) and defatted powder (DP-MOS). As extraction solvent, both demineralized water (aqueous extraction) and 1 M NaCl [14] solution (saline extraction) were used. For the aqueous extraction, a 0.5% (*w*/*w*) suspension was prepared, and for the saline extraction, a 5.0% (*w*/*w*) suspension was prepared to minimize the effect of dissolved solids from NaCl, which increases electrical conductivity (Figure S1—Supplementary Material). In all cases, extraction was stirred at 100 rpm for 1 min. According to Jung et al. [31], rotation speed does not affect the coagulant activity in the range of 100 to 800 rpm, so 100 rpm was used for energy saving. Furthermore, according to the same study [31], the extraction time strongly affects the coagulation activity, and the time of 1 min. was suggested to obtain an efficient extraction condition because as the extraction time is increased, the positive charge of the extract decreased (measured by the zeta potential) and, consequently, reduced the charge-neutralization capacity. The suspension was then filtered firstly through Whatman filter paper n° 1 (11 μm) and then through a glass fiber microfilter GF 52/C (1.2 μm) to remove seed fragments. Extracts were prepared and used on the same day to prevent deterioration of cationic proteins [32].

The aqueous extract filtrate was then named AEP-MOS (Aqueous extract from the powder—*M. oleifera* seed) and AEDP-MOS (Aqueous extract from the defatted powder—*M. oleifera* seed). The filtrate of the saline extract was then named SEP-MOS (Saline extract from the powder—*M. oleifera* seed) and SEDP-MOS (Saline extract from the defatted powder—*M. oleifera* seed).

Further, to compare the performance of *M. oleifera*-derived coagulants with a commercial coagulant, $Al_2(SO_4)_3 \times 18\ H_2O$ (alum) was also tested as a reference, using a 0.5% (*w*/*w*) stock solution under the same test conditions.

2.2. Jar Test Procedure

A Policontrol six-paddle 2 L square jar test apparatus (Model FlocControl III) was used according to the typical three step procedure of coagulation (20 s/1000 s^{-1}), tapered flocculation (5 min/60 s^{-1}, 5 min/40 s^{-1}, and 10 min/20 s^{-1}), and sedimentation (0.12 $cm.s^{-1}$ = 60 min of settling). After settling, samples of the treated water were collected from sampling points in each jar. The tests were carried out at room temperature (23.0 ± 0.4 °C) and in triplicate. These parameters were kept the same for jar tests with all tested coagulants. The tests were performed in the dosage range from 0 (control—turbid water without coagulant) to 60 mg/L of *M. oleifera* seed-derived or alum coagulants. Coagulation concentration of *M. oleifera* seed-derived coagulants was calculated considering all the seed powder added for extract processing or direct addition of seed powder. This is a simple method of measuring the concentration of applied dosages of *M. oleifera* seed-

derived coagulants, which is widely found in the literature for its easy reproducibility in low-income regions with a shortage of skilled labor [13,18,23].

Jar tests were performed with turbid synthetic water prepared by adding kaolin suspension (50 g/L) (Synth) to well water, with a turbidity target of 200 NTU. Table 1 shows the characteristics of the synthetic turbid water.

Table 1. Characteristics of tested turbid water.

Parameter	Value
Turbidity	200.8 ± 9.3 NTU
pH	7.10 ± 0.12
Electrical conductivity	49.1 ± 4.4 μS/cm
Alkalinity	25.50 ± 3.06 mg $CaCO_3$/L
Zeta potential	−21.62 ± 0.29 mV
DOC	1.23 ± 0.85 mg/L

The turbidity removal percentage was calculated according to Equation (2):

$$\text{Turbidity removal } (\%) = \frac{\text{Initial turbidity (NTU)} - \text{ Residual turbidity (NTU)}}{\text{Initial turbidity (NTU)}} \times 100 \quad (2)$$

2.3. Physicochemical Properties—Analytical Methods

Sample characterization before and after the treatment was conducted by the measurement of physicochemical parameters following the methodologies from the Standard Methods for the Examination of Water and Wastewater (SMWW) [33]. Turbidity was measured using a nephelometer (Policontrol AP2000), pH and alkalinity (potentiometric titration curve) were determined using a pH meter (Lucadema Luca 210), and electrical conductivity was measured using a conductivity meter (Tecnofon mCA150). The zeta potential was analyzed with Anton Paar's Litesizer 500 Particle Analyzer. To measure dissolved organic carbon (DOC), the collected sample was firstly adjusted with sulfuric acid to pH < 2.0, filtered through a 0.45 μm membrane, and then analyzed with a total carbon analyzer (Analytik Jena multi N/C 3100). Data obtained after treatment with *M. oleifera* seed-derived and alum coagulants were compared to control by one-way ANOVA and Tukey's test post hoc multiple comparison, and the level of significance was set at 5%.

2.4. Cytotoxicity Analysis

Cells of the Vero lineage, originating from *Cercopithecus aethiops* monkey kidney, were used in this study. These cells have been used for toxicological evaluation in water samples [34] and wastewater [23,35]. Cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS (Fetal Bovine Serum), penicillin (100 U/mL), and streptomycin (100 μg/mL) and kept incubated at 37 °C and 5% CO_2 until they reached the desired confluence. To evaluate cytotoxicity, 8×10^3 cells per well were plated in 96-well plates in two independent experiments (n = 12). After 24 h, the cells were washed with PBS (phosphate-buffered saline) and then exposed to the coagulants at different concentrations. To this end, 100 μL of *M. oleifera* seed-derived and alum coagulants in the concentration of optimal dosages and 10 times the optimal dosages (related to turbidity removal) were added to the wells, with 100 uL of culture medium. The extracts were previously filtered (PES membrane—polyether sulfone—0.22 μm). In the control group, the cells were exposed to 100 μL of sterile distilled water and the same amount of medium. After 24 h, the contents of the wells were removed, and MTT reagent (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-tetrazolium; Sigma, St. Louis, MI, USA) was added, which remained in contact with the cells for 60 min. Subsequently, the reagent was removed from the wells, to which DMSO (Dimethyl Sulfoxide; Sigma-Aldrich, St. Louis, MI, USA) was added, and the plates

were agitated for 10 min. The resulting optical density of the solution was measured in a spectrophotometer at 570 nm. Absorbance data were normalized by the control group (=100%). The cytotoxicological analysis was performed based on ISO 10993-5/2009 [36] and classified according to Table 2 [37]. Additionally, statistical analysis was performed by Mann-Whitney test ($p < 0.05$) to compare each condition regarding oil extraction and the different dosages. The data were evaluated in the Graphpad Prism program, version 7 (GraphPad Software, Inc., La Jolla, CA, USA).

Table 2. Cytotoxicity classification based on the cell viability percentage [37].

Cell Viability	Cytotoxicity
>90%	Non
90–60%	Low
59–30%	Moderate
29–0%	Severe

3. Results and Discussion

3.1. Coagulation Efficiency

Figure 2 presents the turbidity removals obtained with the use of *M. oleifera* seed-derived coagulants and alum, and Table 3 shows a summary of the optimal dosages for highest turbidity removal and the lowest coagulant concentration added. The control samples (coagulant dosage of zero) achieved 39 ± 4% of turbidity removal, and it is related to the removal by sedimentation of settleable particles from the turbid synthetic water, for which a coagulant was unnecessary. As can be seen, alum presented the highest turbidity removal, reaching 98% removal at the optimal dose of 20 mg/L, which is in agreement with what has been reported in the literature [38]. Dosages equal to or greater than 40 mg/L of alum resulted in a drastic reduction in turbidity removal efficiency, and it is related to the drop in suspension pH (pH < 6.0), which will be further discussed in another section. The higher standard deviation of turbidity removal using *M. oleifera* seed-derived coagulant compared with alum has various sources, including the size distribution of seed fragments after grinding and sieving, the variability in seed composition, and the efficiency of extraction. Among the *M. oleifera* seed-derived coagulants, the best efficiencies were found for saline extractions, both SEP-MOS (optimal dosage of 20 mg/L) and SEDP-MOS (optimal dosage of 5 mg/L), reaching 90% turbidity removal. Then, 1 M NaCl (a component of the extraction solution) was also tested as a coagulant against the same kaolin suspension, demonstrating that the 1 M NaCl solution alone has no coagulant activity (Figure S2—Supplementary Material). The improvement in turbidity removal with saline extraction is associated with the salting-in mechanism in proteins in which a salt increases protein-protein dissociations, leading to a greater protein solubility as the ionic strength of the salt also increases [14].

Table 3. Optimal dosages for the highest turbidity removal (%) and the lowest coagulant concentration (mg/L) dosage using *M. oleifera* seed-derived and alum coagulants. Initial turbidity was 200.8 ± 9.3 NTU.

Coagulant	Dosage (mg/L)	Turbidity Removal (%)
P-MOS	5.0	75.7 ± 7.5
DP-MOS	2.5	74.5 ± 3.1
AEP-MOS	20	72.1 ± 10.5
AEDP-MOS	20	73.0 ± 16.0
SEP-MOS	20	90.2 ± 0.5
SEDP-MOS	5	89.5 ± 7.0
Alum	20	98.0 ± 0.2

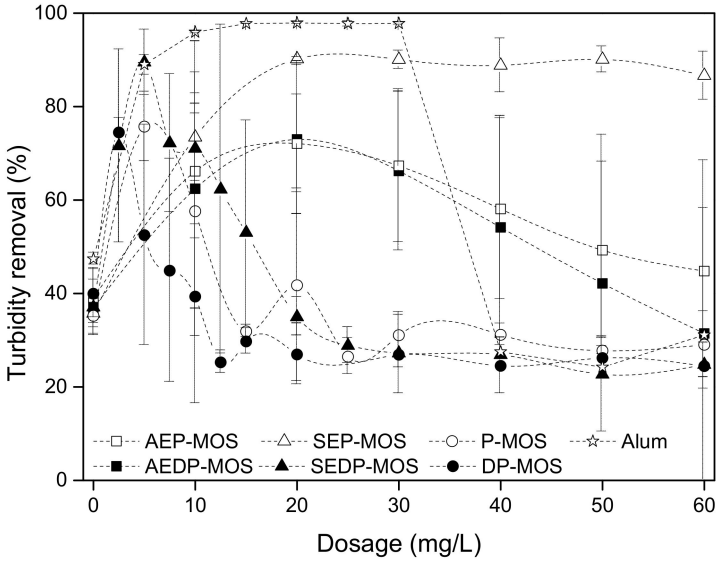

Figure 2. Turbidity removal (%) as a function of *Moringa oleifera* seed-derived and alum coagulants dosages in jar tests after coagulation, flocculation, and sedimentation. Initial turbidity was 200.8 ± 9.3 NTU.

The direct use of the powder (P-MOS—5 mg/L and DP-MOS—2.5 mg/L) or aqueous extract (AEP-MOS—20 mg/L and AEDP-MOS—20 mg/L) showed similar higher percentages of turbidity removals, ranging between 72% and 76% (Table 3), although the powder has showed a consumption up to eight times lower than the aqueous extract. The oil removal performed in the secondary pre-treatment did not present a significant variation in the turbidity removal. According to Nordmark et al. [6], fatty acids do not interfere with the adsorption of *M. oleifera* seed proteins to silica. However, a reduction in the dosages of defatted *M. oleifera* seed-derived coagulants was observed, which can be explained by the higher protein concentration in the extract obtained from the 5 mg defatted seed/L versus 5 mg whole seed/L since 38% of oil was extracted from the defatted seeds [17]. Therefore, the removal of the oil provides a better use of this component without affecting coagulant activity.

For *M. oleifera* seed-derived coagulants, an overdose may be observed, causing a drop in the turbidity removal efficiency (Figure 2). This drop in efficiency is related to the restabilization of the particles caused by the excess of positive charge from the *M. oleifera* seed-derived coagulants as can be seen by the variation of the zeta potential as a function of the dosage of coagulants (Figure 3). Therefore, regardless of the way in which *M. oleifera* seed-derived coagulants were used, they were able to neutralize and restabilize the particles present. This result agrees with the prediction that the adsorption and charge-neutralization mechanism would be the dominant mechanism attributed to the seed of *M. oleifera* [5–7].

3.2. Effect on pH

Figure 4 shows the pH variance due to the use of *M. oleifera* seed-derived and alum coagulants. The use of *M. oleifera* seed-derived coagulants did not show a statistically significant pH variation in the dose range studied (0–60 mg/L). This property brings a competitive advantage to *M. oleifera* seed-derived coagulants, as increasing alum dosage caused a considerable drop in water pH (Figure 4). Consequently, at dosages above 40 mg/L of alum, the removal of turbidity after sedimentation dropped dramatically (Figure 2), as the pH was not adjusted to the optimal range of sweep coagulation. This pH drop refers to the release of H^+ ions when alum is added to the water, consuming alkalinity and reducing the pH of water [39]. These results are in agreement with what Vega Andrade et al. [23] reported when they performed coagulation in tertiary domestic wastewater, increasing the concentrations of *M. oleifera* seed aqueous extract (0–600 mg/L) and alum (0–250 mg/L).

For all dosages tested with the natural coagulant, the pH remained stable at around 7.6, while the pH was reduced to 6.7 when using alum [23].

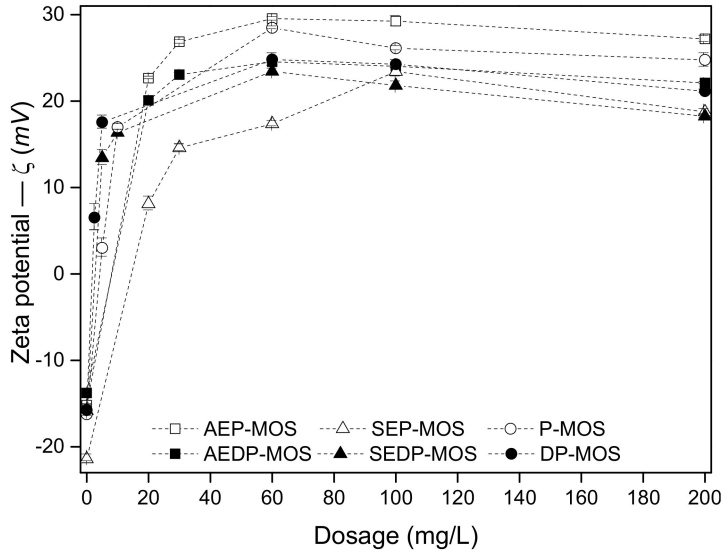

Figure 3. Zeta potential (mV) as a function of *Moringa oleifera* seed-derived coagulants dosages in jar tests.

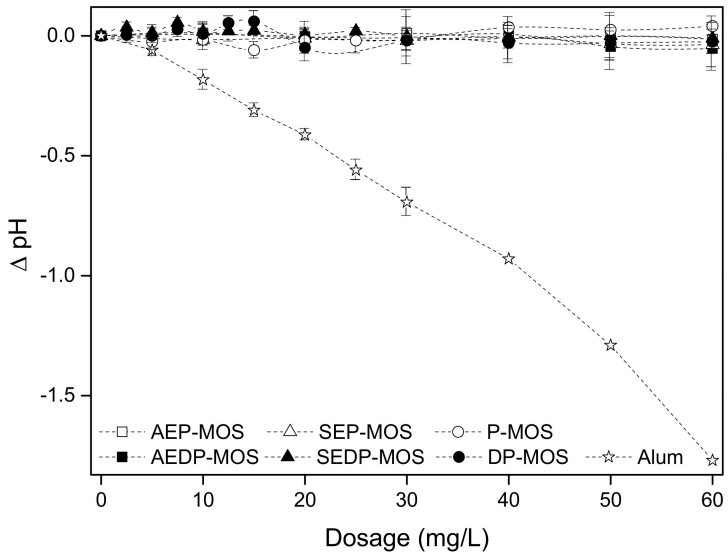

Figure 4. pH variation as a function of *Moringa oleifera* seed-derived and alum coagulants dosages in jar tests after coagulation, flocculation, and sedimentation. Initial pH was 7.10 ± 0.12.

3.3. Effect on Electrical Conductivity

The electrical conductivity variance after the use of *M. oleifera* seed-derived and alum coagulants are shown in Figure 5. Both powder (P-MOS and PD-MOS) and aqueous extracts (AEP-MOS and AEDP-MOS) did not show a statistically significant electrical conductivity variation in the dose range studied. Alum showed an increase in electrical conductivity with increasing dosage, which is related to the release of cations and anions into the aqueous medium when alum is added [39]. On the other hand, saline extracts (SEP-MOS and SEDP-MOS) showed the greatest increases in electrical conductivity with increasing dosage even though a 5% saline extraction was performed to minimize the impact. Further, 1 M NaCl (a component of the extraction solution) was also tested as a coagulant against the same kaolin suspension, showing that the 1 M NaCl solution alone increased statically

equally to SEP-MOS and SEDP-MOS (Figure S3—Supplementary Material). Thus, it can be inferred that the increase in electrical conductivity is related to the Na^+ and Cl^- ions present in the saline solution (1 M NaCl). Therefore, although saline extractions are proven to be more efficient in removing turbidity, the side effects of salt addition in higher dosages must be evaluated in the water treatment, such as drinking water palatability, excessive scaling in water pipes, heaters, boilers, and household appliances [40].

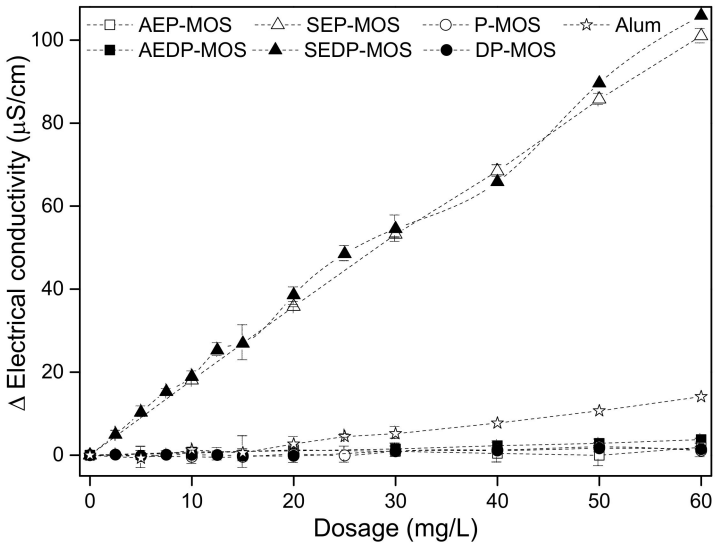

Figure 5. Electrical conductivity variation as a function of *Moringa oleifera* seed-derived and alum coagulants dosages in jar tests after coagulation, flocculation, and sedimentation. Initial conductivity was 49.1 ± 4.4 µS/cm.

3.4. Effect on Dissolved Organic Carbon (DOC)

Figure 6 shows the dissolved organic carbon (DOC) variance after the use of *M. oleifera* seed-derived and alum coagulants. The results refer to the optimal dosages for coagulation efficiency (according to Table 3) and an overdose of 10 times the optimal dosage to evaluate the cytotoxicity in case of greater coagulant demand. Considering only the optimal dosages, there was no statistically significant difference between the water treated by alum and *M. oleifera* seed-derived coagulants groups and the well water. The addition of alum, even in the overdose (200 mg/L), did not statistically change the DOC when compared to the well water, confirming that alum, as it is an inorganic coagulant, does not have a negative influence on the increase of residual organic carbon [23,41]. On the other hand, the DOC results found in well water treated with all tested *M. oleifera* seed-derived coagulants showed that this property was enhanced with an increasing dosage of these coagulants. This can be explained by the fact that *M. oleifera* seeds contain a variety of organic compounds, including coagulation-active and coagulation-inactive components, and at least 78% of the DOC of *M. oleifera* whole-seed extract is kept in treated water [38,42].

The literature usually suggests the secondary pre-treatment (defatted seed) as a potential solution to reduce residual organic carbon [18,38], but the results found in this work indicate that the oil extraction from the seed alone is not capable of decreasing residual organic carbon. In fact, the highest DOC results found (Figure 6) was with a defatted seed (AEDP-MOS: DOC = 3.9 mg/L and 25.8 mg/L, respectively, at 20 mg/L and 200 mg/L dosages), and it is related to the high applied dosage (20 mg/L) of a more protein-concentrated seed (38% of oil was removed). Oil extraction from the seed does not reduce the remaining organic matter of the treated water probably because *M. oleifera* seed oil, like oils (nonpolar molecule) in general, are considered insoluble (solubility < 100 ppm) in water (polar molecule) [43]. Therefore, according to the results found in this work, only the primary, secondary pre-treatment, and secondary processing stages of *M. oleifera* seeds-derived coagulants are not able to reduce the remaining organic matter caused by the

use of these coagulants in water treatment, and an advanced method of protein purification is recommended to achieve this goal [31,44]. To reduce the impact of dissolved organic matter in the treated water without an advanced purification processing stage, reduced dosages of *M. oleifera* seed-derived coagulants are recommended, combined with other treatment processes such as filtration, adsorption, and physical disinfection [38].

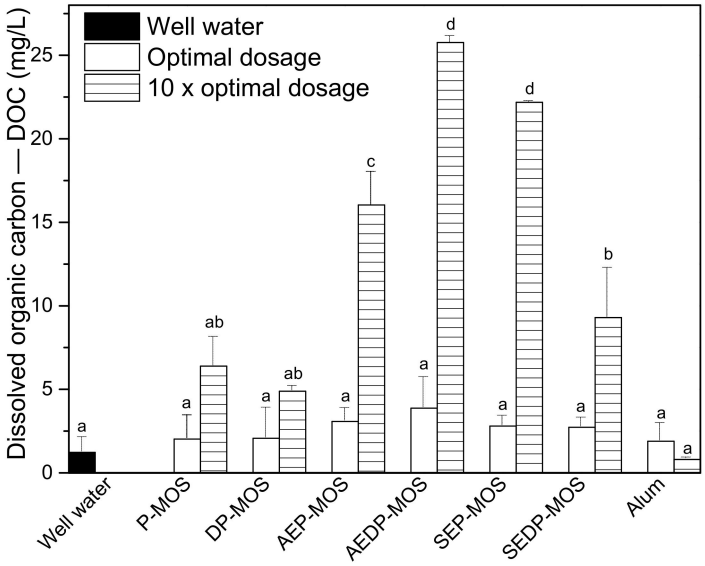

Figure 6. Dissolved organic carbon (DOC) variation as a function of *Moringa oleifera* seed-derived and alum coagulants dosages (optimal dosage and 10× optimal dosage) in jar tests after coagulation, flocculation, and sedimentation. Bars with the same letter represent means that did not differ significantly ($p \leq 0.05$) based on Tukey HSD post hoc test.

3.5. Effect on Cytotoxicity

Figure 7 shows the cell viability after contact with *M. oleifera* seed-derived and alum coagulants. The results refer to the optimal dosages for coagulation efficiency (according to Table 3) and an overdose of 10 times the optimal dosage to evaluate the cytotoxicity in case of greater coagulant demand. It is possible to observe that alum, both at the optimal dosage (20 mg/L) and at 10 times the optimal dosage (200 mg/L), showed low cytotoxicity, respectively, with 82% and 67% of cell viability when compared to the control group. Vega Andrade et al. [23] reported similar cytotoxicity values with the use of alum at a dosage of 200 mg/L, with 62% of cell viability, classified as low cytotoxicity. The low cytotoxicity for alum was already expected since it is a coagulant with wide use in the treatment of supply water and wastewater.

M. oleifera seed-derived coagulants showed cytotoxicity to mammalian cells (Figure 7). However, the results pointed out that different processing stages of the coagulants and the dosages can strongly influence the cytotoxicity. The increase in cytotoxicity in treated water may be related mainly to the presence of mustard oil glycosides in the seeds, which in turn break down into isothiocyanates, which are reported to have cytotoxic effects [25,45]. Vega Andrade et al. [23] reported a reduction in the cell viability with the increase of *M. oleifera* seed aqueous extract dosage from 30 mg/L to 750 mg/L. Barros et al. [46] also supported the hypothesis that the toxicity of the aqueous extract of *M. oleifera* seed is dependent on the dosage (0.78 mg/L to 200 mg/L).

Interestingly, our results pointed out that the oil extraction via secondary pre-treatment of *M. oleifera* seed powder increased the cell viability (Figure 7). This phenomenon was observed mainly in the powder groups and aqueous extraction condition ($p < 0.05$), e.g., extracting oil from *M. oleifera* seed powder at the optimal dosage increased cell viability from 43% (moderate cytotoxicity) in P-MOS to 84% (low cytotoxicity) in DP-MOS. Al-Anizi et al. [25] observed that the water-soluble fraction of *M. oleifera* seed has low toxicity, and the

dominant toxicity comes from insoluble fatty acidic components (mustard oil glycosides), which would remain in the supernatant. Therefore, the use of *M. oleifera* seed-derived coagulants with oil extraction via secondary pre-treatment may be an alternative to reduce toxicity in drinking water or wastewater effluents.

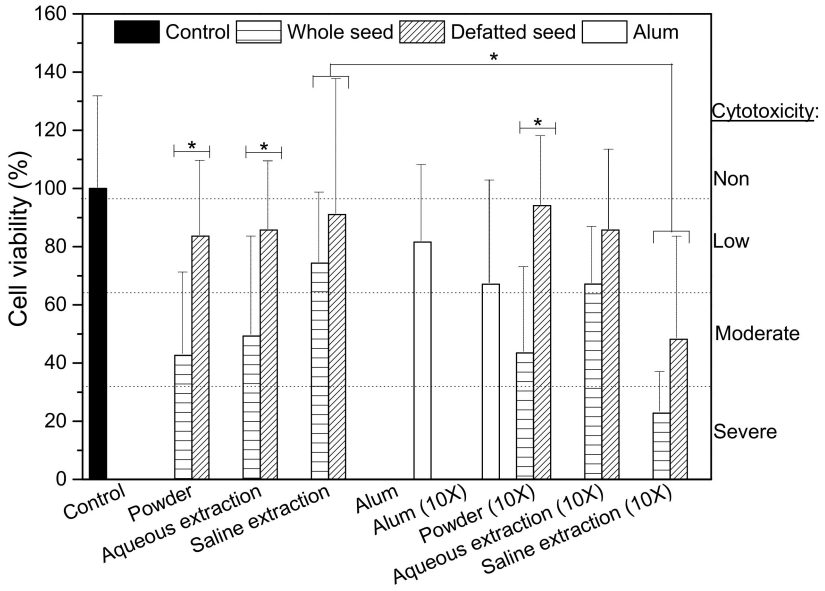

Figure 7. Viability (% in relation to control group) of Vero cells after 24 h of exposure to *Moringa oleifera* seed-derived and alum coagulants in deionized water—optimal dosage (Table 3) and 10× optimal dosage. * Indicates significant differences between the groups regarding oil extraction or dosage (Mann-Whitney test, $p < 0.05$).

On the other hand, an increase in cytotoxicity was detected in experiments with saline extracts tested at 10 times the optimal dosage (Figure 7). SEDP-MOS (defatted) was non-cytotoxic (91% cell viability) at the optimal dosage but showed moderate cytotoxicity (48% of cell viability) at 10 times higher dosage, with significant differences between these conditions ($p < 0.05$). Similarly, SEP-MOS (whole) induced low cytotoxicity (74% of cell viability) at the optimal dosage, while severe cytotoxicity (23% of cell viability) and significant differences ($p < 0.05$) were observed at 10 times higher dosage. The reduction in cell viability after exhibition to saline extraction of *M. oleifera* seed using NaCl may be related to the dehydration- or contraction-induced cell injury mechanism when exposed to a hypertonic solution, which is salt-concentration-dependent [47]. Therefore, the use of high doses of *M. oleifera* seed-derived coagulants with secondary saline extraction for the treatment of drinking or wastewater should be evaluated for cytotoxicity prior to use.

4. Conclusions

This study evaluated the use of whole and defatted *M. oleifera* seed-derived coagulants in powder (P-MOS and DP-MOS), aqueous extraction (AEP-MOS and AEDP-MOS), and saline extraction (SEP-MOS and SEDP-MOS) for the treatment of synthetic turbid water. All evaluated forms of *M. oleifera* seed-derived coagulants were able to neutralize and restabilize (in overdose) the charges of the particles in the suspension, characteristic of coagulation by adsorption and charge neutralization. In addition, all *M. oleifera* seed-derived coagulants did not change the pH of the treated water, unlike alum, which showed an undesired and expected reduction in pH. Saline extractions obtained the best turbidity removal results but showed an undesirable increase in electrical conductivity and cytotoxicity to mammalian cells with increasing dosage when compared to the other *M. oleifera* seed-derived coagulants. Therefore, high doses of saline extraction should be avoided, and further studies should be carried out to reduce the concentration of saline solution while maintaining high rates of turbidity removal. Defatted seeds did not significantly alter turbidity removal

or residual dissolved organic carbon despite the general indication that the oil extraction would be able to reduce residual DOC. On the other hand, defatted seeds reduced the toxicity of the coagulant in the treated water when compared to the whole seed, proving to be an important stage in the processing of *M. oleifera* seed-derived coagulants in addition to generating a by-product with added value.

Supplementary Materials: The following are available online at: https://www.mdpi.com/article/10.3390/w14132058/s1, Figure S1: Comparison of the effect on the electrical conductivity of treated water using saline extract (NaCl 1 M) in the suspension prepared at 5.0% (w/w) versus 0.5% (w/w), based on M. oleifera seed-derived coagulant SEP-MOS; Figure S2. Residual turbidity in jar tests using 1 M NaCl as coagulant against kaolin suspensions; Figure S3. Electrical conductivity variation as a function of M. oleifera seed-derived coagulants (SEP-MOS and SEDP-MOS) and NaCl 1M dosages in jar tests after coagulation, flocculation, and sedimentation. Initial conductivity was 49.1 ± 4.4 µS/cm.

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