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Combination of *Moringa oleifera* seeds and *Acanthus ilicifolius* leaves as a natural coagulant for groundwater treatment

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ABSTRACT

The need for clean water is increasing along with the rising population. Water use in developing countries still relies on surface water, such as groundwater, which is very easily contaminated by the surrounding environment. The coagulation method for water purification is usually used to remove colloidal residues and assist the coagulation-flocculation process using aluminum sulfate, which can be harmful to health if used for a long time. The coagulation method utilizing natural coagulants, such as proteins and tannins, is better because it is cost-effective and relatively safer for health if used for a long time. Tests on the tannin and protein content in the natural coagulant, as well as characterization using fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM) on the water sediment after the coagulant is added, were conducted. The groundwater sample that did not meet the physical, chemical, and biological criteria was chosen as the water sample. *Moringa oleifera* seed powder and *Acanthus ilicifolius* leaves were added to the water sample at doses of 0.025 g/L and 0.05 g/L, respectively, and then allowed to settle for 30 minutes. Subsequently, tests were conducted to determine the optimum pH, COD, BOD, TSS, TDS, and the number of *E. coli* bacterial colonies was determined. The natural coagulants used were proven to reduce COD by 38%, BOD by 52%, TSS by 78%, and TDS by 62%, and to remove 92% of the total *E. coli* bacteria. FTIR analysis showed that the natural coagulants used contained -OH and C=O functional groups, indicating the presence of natural coagulant molecules.

Keywords: *Moringa oleifera, Acanthus ilicifolius*, natural coagulant, groundwater.

INTRODUCTION

Water is one of the primary necessities for human life; therefore, one of the goals of SDGs 2030 is to "create clean water and sanitation" to ensure the availability of clean water. The water supplies widely utilized by developing countries correspond to surface water, specifically groundwater. This is chosen by the community, because it is easily accessible (Aysen et al., 2024). However, groundwater is very easily contaminated by its surroundings, whether from household waste, human waste disposal sites, or when waste and sewage seep into the ground and contaminate the groundwater, causing it to smell bad, become murky, and unpleasant (Dong et al., 2024). This affects the physical, chemical, and biological conditions of the groundwater. Therefore, groundwater must receive special attention, because it can threaten public health (Eccles et al., 2017).

Several methods are used to address this issue, one of which is using coagulants. The addition of coagulants in water causes destabilization and helps colloidal particles to coagulate (Ferrari et al., 2020). Colloids, which are particles that do not naturally settle, generally originate from sand and soil, which can deteriorate the quality of groundwater. If a solution is added with a substance that has a different charge from the colloidal system, the colloidal system will attract the different charge and coagulation as well as sedimentation will occur (Anisa et al., 2024).

Therefore, in water treatment, the addition of coagulant substances is necessary to assist the coagulation process because the higher the concentration of differently charged ions, the faster the coagulation-flocculation process will occur (Gao et al., 2021).

Coagulants provide good results in the process of water purification and improvement (Ibrahim et al., 2021). However, the method of water purification using synthetic coagulants such as aluminum sulfate, which are commonly used, can pose serious health threats (Zaidi et al., 2022). To address this issue, the use of biocoagulants in assisting the coagulation-flocculation process is employed as an excellent alternative in improving water quality parameters. Also, it is relatively safe for health when used over a long period, and easy to process without requiring special. The utilization of biocoagulants can be categorized based on their active ingredients, namely proteins, polyphenols, and polysaccharides (Kristianto, 2017). The utilization of protein-based coagulants, such as *Moringa oleifera* seeds, was demonstrated in several studies to be effective in treating groundwater, river water, and even coal waste (Varkey, 2020). *Moringa oleifera* seeds can be used as natural biocoagulants, because they contain the active compound rhamnosyloxy-benzyl-isothiocyanate, which has a positive charge. This compound destabilizes colloids, attracting negatively charged suspended dirt particles, leading to coagulation, followed by flocculation and sedimentation to clarify the water (Ramavandi, 2014). The research conducted by Kapse & Samadder (2021) on the use of natural coagulants, specifically *Moringa oleifera* seed biocoagulants, for the treatment of coal beneficiation plant waste, can be one of the effective methods in coal waste treatment, reducing turbidity by 97.48% and total dissolved solids by 97%. It can also eliminate total coliforms in synthetic raw water (Asrafuzzaman et al., 2011).

The utilization of polyphenol groups, namely the active ingredient tannin which is used as a biocoagulant, tannin is utilized as a biocoagulant, because tannin is a polyphenol that can dissolve in water with a molecular weight between 500–3000 g/l and is rich in functional groups, such as hydroxyl and carboxyl (Ibrahim et al., 2021). The tannin content in plants has recently attracted attention for use as a natural coagulant in the water purification process and can also serve as a natural antimicrobial agent in water purification. The research conducted by (Thakur and Choubey, 2014) utilized tannin as a natural coagulant derived from the powder of acacia catechu bark, which was able to reduce the turbidity of surface water samples by 91% and remove TDS by 57.3%. Additionally, it achieved a color removal of up to 92% and a COD reduction of 82% (Chaibakhsh et al., 2014). Additionally, the utilization of pine trees, eucalyptus, and quebracho wood are examples of plants the tannin extracts of which are used as natural coagulants and have also been widely commercialized under brand names such as tanfloc (Hameed et al., 2018). One of the plants that can be utilized in water purification due to the content of tannin compounds, is *Acanthus iliicifolius*. The leaves contain tannin, as per the research (Latief et al., 2022), which found flavonoid, saponin, and tannin compounds in the phytochemical screening of the ethanol extract of *A. ilicifolius* leaves.

The tannins contained in the leaves of *Acanthus ilicifolius* are polyphenols that have the potential to be used as biocoagulants because these compounds have many hydroxyl and carboxyl groups, allowing them to form complexes with colloids. Essentially, colloidal particles have a similar charge, which is negative (Camacho et al., 2017), while proteins and tannins that determine the effectiveness of coagulation contain positive ions. The utilization of tannins in A. *ilicifolius* leaves can also be used to inhibit the growth of *E. coli* bacteria in water samples. The research conducted by (Pringgenies et al., 2020) showed that *A. ilicifolius* leaves have activity in inhibiting the growth of Klebsiella and Escherichia coli bacteria, thus potentially serving as biocoagulants to improve the quality of water parameters, such as pH, COD, BOD, TSS, TDS, and *E. coli.*

MATERIAL AND METHODS

The employed materials included sodium bicarbonate ($NaHCO_3$), manganese sulfate ($MnSO_4$) 0.4ml, azide alkaline iodide 0.4 ml as an oxidizer, sulfuric acid (H_2SO_4) 0.4 ml, sodium thiosulfate ($Na_2S_2O_3$) 0.025ml, potassium dichromate ($K_2Cr_2O_3$) 0.4, 50% methanol alcohol, and Compact Dry EC. Six chemical glasses were used as containers for groundwater, each containing 1 liter of groundwater, with two repetitions using natural coagulants: *Moringa oleifera* seeds =P1, *Acanthus ilicifolius* leaves =P2, and a combination of

Moringa oleifera seeds and *Acanthus ilicifolius* leaves = P3, as well as a control without biocoagulant treatment = P0 with dose variations of 0.025 grams and 0.05 grams. Each sample was stirred using a magnetic stirrer, with stirring at a speed of 150 rpm for 2 minutes to dissolve the coagulant compounds, followed by slow stirring at 30 rpm for 15 minutes to maintain the flocculant (Khalid Salem et al., 2023). The stirring aimed to increase the chances of biocoagulant and particle reaction, as well as to quickly precipitate and agglomerate the particles. Sedimentation was carried out for 30 minutes, followed by further observations, including water quality measurements such as pH using a pH meter, chemical oxygen demand (COD) with the measurement standards SNI 6989.02:2019 using the closed reflux method, biological oxygen demand (BOD) using the SNI 6989.72:2009 standard, with the Winkler method, total suspended solid (TSS) using the gravimetric method, total dissolved solids (TDS) using the gravimetric method, and calculating the total colony count of *E. coli* bacteria using the Compact Dry EC method. AOAC Performance Tested Certificate No. 110402, Microval Certificate No. 2008LR04/05 (Pichel et al., 2023).

Preparation of biocoagulants

Mature and brown *Moringa oleifera* seeds were washed and then soaked in a 0.5% $NaHCO_3$ solution with a 1:3 ratio for 24 hours to remove bitterness (Gunawan et al., 2020). Afterwards, the *Moringa oleifera* seeds were placed in an oven at 105 °C for 2 hours, then ground and sieved to a 100 mesh size to obtain a homogeneous size. The *Acanthus ilicifolius* leaves are washed and cleaned, then the thorns at the leaf tips are removed, and dried at a temperature of 30–37 °C for 4 days, then ground until a powder is obtained, and subsequently sieved with a 100 mesh sieve. The grinding of *Moringa oleifera* seeds and *Acanthus ilicifolius* leaves aims to increase the surface area of the biocoagulant because by reducing the size, the active substances of the biocoagulant will be more abundant due to the larger surface area of the *Moringa oleifera* seeds, making the use of the biocoagulant more effective.

Characteristics of tannin content in A*canthus Ilicifolius* leaves

A qualitative test was conducted to determine the tannins present in *Acanthus ilicifolius*. The test results showed that the *Acanthus ilicifolius* leaves containing tannins would exhibit a color change. The extract of the *Acanthus ilicifolius* leaves turned dark green or dark blue when the $FeCl_3$ 1% reagent was added. In the $FeCl_3$ 1% reaction, the tannin structure, which is a polyphenolic compound, will interact with $FeCl_3$ 1%, forming a dark blue or dark green complex due to the presence of polyphenolic groups.

Characteristics of total protein content in *Moringa oleifera* seeds

This study uses the Lowry method to determine protein content. To determine the protein content in a sample, a straight-line equation can be used, which will be obtained from the standard solution graph. Therefore, the standard solutions used are made in various concentrations, using concentrations of 0.01 mg/mL, 0.02 mg/mL, 0.04 mg/mL, and 0.16 mg/mL. To find the maximum lambda length, a wavelength range of 600–800 nm is first established.

Characteristics of the biocoagulant of *Moringa oleifera* seeds and *Acanthus ilicifolius* leaves using FTIR

Functional group analysis with FTIR is a method used to observe molecular interactions with electromagnetic radiation, aimed at identifying the components of the produced polymer. The basic principle of infrared spectroscopy is the interaction between the vibrations of atoms within a molecule and the adsorption of infrared electromagnetic wave radiation, which causes higher vibrational excitation.

Characterization of morphological of coagulant and sediment results with SEM

The SEM test was conducted to determine the microstructural characteristics of the samples after the treatment with the addition of biocoagulants, which resulted in images of flocs formed during the coagulation and flocculation processes in three dimensional form. The amount of absorbed frequencies is measured as a percentage of transmittance (Al Moharbi et al., 2020). Thus, infrared measurements can be used to identify the presence of functional groups in a molecule.

Analysis data

Chemical oxygen demand (COD) analysis

$$COD = \frac{(A-B) \times N\,FAS \times 1000 \times BeO_2 \times P}{V\ sample} \quad (1)$$

where: A – mL of blank titrant, B – mL of sample titration, N – normality FAS, BeO_2 – S, P – dilution.

Biological oxygen analysis (BOD) analysis

$$BOD_5 = \frac{(A_1-A_2)-\left(\frac{B_1-B_2}{V_B}\right)V_C}{P} \quad (2)$$

where: BOD_5 – is the value of BOD_5 of the test sample (mg/L), A_1 – is the dissolved oxygen content of the test sample before incubation (0 days) (mg/L), A_2 – is the dissolved oxygen content of the test sample before incubation 5 days (mg/L), B_1 – is the dissolved oxygen content of the blank before incubation (0 days) (mg/L), B_2 – is the dissolved oxygen content of the blank before incubation 5 days (mg/L), V_B – is the volume of microbial suspension (mL) in the blank DO bottle, V_C – is the volume of microbial suspension in the test sample bottle (mL), P – is the ratio of the volume of the test sample (V_1) to the total volume (V_2).

Total suspended solid (TSS) analysis

$$TSS(\text{mg/l}) = \frac{(W_1-W_0) \times 1000}{V} \quad (3)$$

where: W_0 – weight of the balance containing the initial filter medium (mg), W_1 – weight of the balance containing the filter medium and dry residue (mg) V – test sample volume (mL).

Total dissolved solids (TDS) analysis

$$TDS = 1000/V \times (\text{weight of the sample evaporating dish} - \text{weight of the evaporating dish}) \times 100 \quad (4)$$

Calculating the percentage reduction

The percentage removal efficiency of BOD, COD, TDS, and TSS values is calculated based on the initial concentration and the final concentration after the treatment with biocoagulants. The formula:

$$\frac{(A-B)}{A} \times 100\% \quad (5)$$

where: A – initial concentration, B – final concentration.

Quantitative descriptive

The data in this study were analyzed using the quantitative descriptive analysis method. Through a literature review, this method seeks to explain the facts under study, bolstering the analytic findings to support a research conclusion. The supplied data and the mathematical calculation of the research variable indicators yield the research findings.

RESULT AND DISCUSSION

Characteristics of tannin content in *Acanthus ilicifolius* leaves

On the basis of the results of the tests conducted, it can be seen that after the addition of the 1% $FeCl_3$ reagent, a significant color change occurred, transforming from a yellowish-brown to a dark greenish-black in the test tube containing the *Acanthus ilicifolius* leaf extract. This indicates the presence of tannins in the *Acanthus ilicifolius* leaves. The change of the leaf extract to a dark greenish-black color signifies that the *Acanthus ilicifolius* leaves positively contain condensed tannins (Mailoa et al., 2013). The formation of a dark greenish or dark blue color in the leaf extract indicates the presence of phenolic groups, which can be detected by adding 1% $FeCl_3$ to the leaf extract. This is because the Fe^{3+} ion in $FeCl_3$ acts as an O atom with a free electron pair that can coordinate as a ligand (Hamzah et al., 2020). Another study mentions that research has been conducted on the Ethnobotany, Phytochemistry, and Pharmacology of *Acanthus ilicifolius*, where the tannin test results showed a color change of the solution to a deep black, indicating that the leaves of *Acanthus ilicifolius* are positive for tannins (Zakaria et al., 2024).

Characteristics of *Moringa oleifera* seed protein

After determining the wavelength range of 600–800 nm, a maximum wavelength of $\lambda = 742$ nm was obtained, resulting in the graph shown in Figure 1.

On the basis of the measurement results of the total dissolved protein content in *Moringa oleifera* seeds, as shown in Figure 1, the values

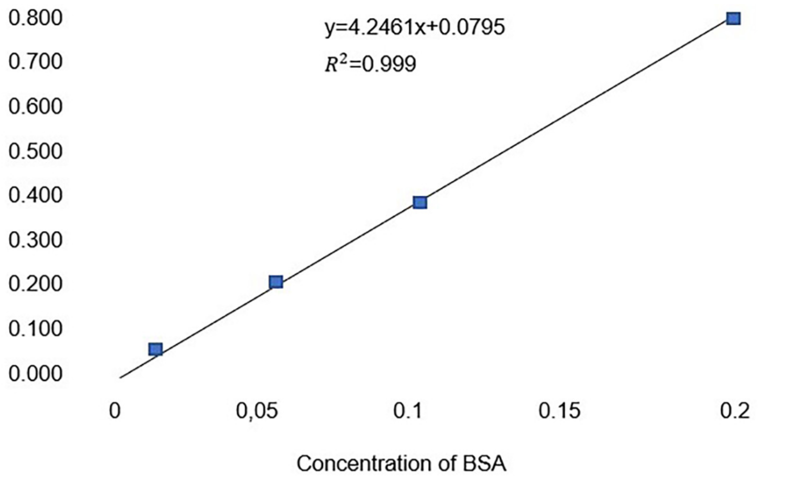

Figure 1. Standard curve of BSA observation for determining the total protein content in *Moringa oleifera* seeds

obtained from the measurements are represented by the linear equation y = ax + b (y = 4.2461x + 0.0795), where Y is absorbance and X is concentration, to determine the protein content, the concentration value obtained is multiplied by the dilution factor; in this study, the dilution factor is 10 times. The final result of the protein calculation on the *Moringa oleifera* seed sample yielded a value of 1.012 mg/ml. This is consistent with the research conducted by Hidayat, (2009) who measured the protein content in *Moringa oleifera* seeds using the same method, where the highest protein concentration was found in the peeled *Moringa oleifera* seeds, approximately 147.280 ppm/gram. This indicates that the potential of *Moringa oleifera* to be used as biocoagulants is very high due to the relatively high protein content in *Moringa oleifera* seeds.

Characteristics of coagulant of *Moringa oleifera* seeds and *Acanthus ilicifolius* leaves used FTIR

The characteristics of the *Moringa oleifera* seed and *Acanthus ilicifolius* leaf biocoagulants using FTIR (Shimadzu FTIR 8400) can be seen in Figure 2. The biocoagulant samples were prepared from powdered *Moringa oleifera* seeds and *Acanthus ilicifolius* leaves that had been finely ground. The spectrum was recorded between 400–500 cm^{-1}.

On the basis of the FTIR interpretation results, as seen in figure (a), the FTIR spectrum of *Moringa oleifera* seeds shows a broad and strong absorption peak at 3342.64 cm^{-1}, where this absorption peak indicates the -OH vibration of the carboxylic acid group, reinforced by an absorption at 1745.58 cm^{-1} indicating the C=O vibration of the carboxylic acid. Additionally, the absorption at 2926.01 cm^{-1} indicates the stretching vibration of the C-H alkane, followed by an absorption at 1543.05 cm^{-1} indicating the N-H vibration of secondary amines. Meanwhile, the interpretation results of the coagulant from the *Acanthus ilicifolius* leaves show the presence of -OH groups from carboxylic acids, indicated by an absorption peak at 3439.08 cm^{-1}, reinforced by the stretching vibration of the C=O group from carboxylic acids at 1651.07 cm^{-1}. The absorption at 2924 cm^{-1} indicates the stretching vibration of the C-H group from alkanes. Additionally, the absorption at 1053.13 cm^{-1} is due to the stretching vibration of C-O and the N-H group from secondary amines. -OH and C=O indicate the presence of carboxyl groups, while N-H indicates the presence of amine groups. Amino acids have two functional groups, namely $-NH_2$ and -COOH. Therefore, it can be concluded based on FTIR characterization that the polymer found in the *Moringa oleifera* seed coagulant is protein, while in the *Acanthus ilicifolius* leaf, it is tannin.

Initial characterization of groundwater

Initial characterization is conducted on the water sample (Table 1) to determine whether the collected sample meets the desired sampling criteria. The initial measurements conducted included pH, total suspended solids, total dissolved solids, odor, color. On the basis of the results of the initial characterization test on the

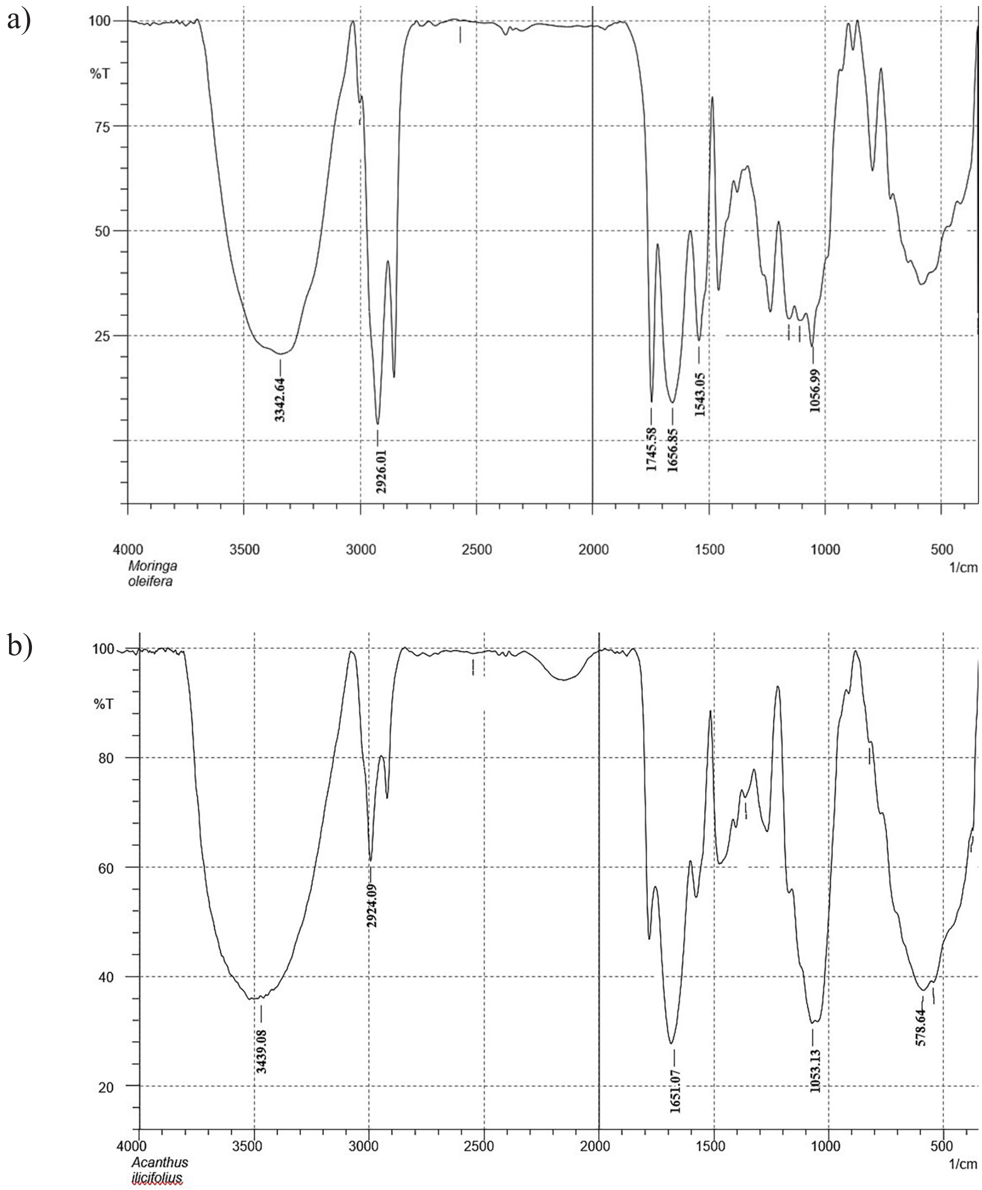

Figure 2. FTIR spectra of biocoagulant: a – *Moringa oleifera* seeds, b –*Acanthus ilicifolius* leaves

groundwater samples taken around Tamalanrea Indah, as shown in Table 1, it can be seen that the sampled water is less suitable for daily use due to its brown color, which can be seen in Figure 1. It contains total suspended solids of 41 mg/l and total dissolved solids of 420 mg/l. The pH value is 6.8, with a metallic and slightly rancid smell, and no taste. For the *E. coli* content, there are about 130 colonies formed on the compact dry, marked by the presence of blue spots on the agar medium. The condition of this groundwater is also oily due to bacterial activity that is suspected to increase the levels of COD and BOD in the water. This can occur because the well is located near a disposal site used by the local community.

Tabel 1. Initial characterization of groundwater

No	Parameter	Value	Unit
1	pH	6.8	-
2	TSS	41	mg/l
3	TDS	420	mg/l
4	Odor	-	-
5	*E. coli*	130	CFU/mL
6	Color	Brown	-
7	Taste	Nothing	-

Measurement of pH

One of the main factors in determining the quality of clean water is by looking at the pH value (degree of acidity) to determine the acid/base content in the water. The pH measurement in this study was conducted using a pH meter. The pH is one of the factors that can influence the processes of coagulation and flocculation. If the coagulation process is carried out at a non-optimal pH, it will result in low water quality, even leading to the failure of the floc formation process (Aboulhassan et al., 2016), because certain coagulants cannot work optimally in acidic or basic environments. The pH measurement in this study was conducted to determine the pH range before and after the addition of biocoagulants to the samples, as shown in Figure 2.

Figure 3 shows that the pH measurement results obtained a pH value of the groundwater sample before treatment, which was 6.8, and after treatment, the groundwater sample showed a value ranging from 6.23 to 7.5. This indicates that the coagulant used has an optimum pH of 6–7, where amino acids will undergo ionization, producing carboxylate ions and protons. The proton charge attracts electrons (colloids), forming neutral groups and then producing flocs. This indicates that the groundwater sample has a neutral pH range. Adding biocoagulant doses of 0.025 g/L and 0.05 g/L with a sedimentation time of 30 minutes resulted in a fairly stable pH change, which is by the water quality standards set by the Ministry of Health. The addition of *Moringa oleifera* seed biocoagulant at the 0-hour sedimentation point showed the highest pH change in this study. The pH value of water can increase due to the content of the coagulant itself, such as *Moringa oleifera* seeds, which contain cationic proteins that are basic because of the proton acceptance of water by amino acids that will release hydroxyl groups, making the solution basic (Amagloh & Benang, 2009). The tannin content also plays a role in the process of pH change in the sample water.

Measurement of chemical oxygen demand

In this study, the measurement of COD levels was conducted using the SNI 6989.02:2019 standard with the closed reflux method. The application of *Moringa oleifera* seed biocoagulant and *Acanthus ilicifolius* leaf on groundwater samples was conducted to determine the ability of the biocoagulants used in reducing the pollutant load of the water samples, where one of the parameters that can determine whether the water is suitable for use and whether it is polluted or not is by measuring the COD value. The results of the COD concentration reduction measurements in this study are shown in Figure 4. The biocoagulants used in this study included 4 types of treatments: control without biocoagulant addition = P0, *Moringa oleifera* = P1, *Acanthus ilicifolius* leaves = P2, and a combination of *Moringa oleifera* and *Acanthus ilicifolius* leaves =P3, with dosage variations of 0.025 grams and 0.05 grams during a 30-minute sedimentation period.

On the basis of the research results of the biocoagulant application on COD values as shown in Figure 6, it is indicated that before the treatment of adding *Moringa oleifera* seed coagulant and *Acanthus ilicifolius* leaf, the COD value was at 17.47 mg/L. After the biocoagulant treatment was applied, the COD value decreased from 13.85 mg/L to 10.8 mg/L. According to Figure 4, the measurement results show that in treatment P1 with a dose of 0.025, the COD value decreased from the initial condition of 17.85 to 12.65 mg/L, while at a dose of 0.05 gram/L, the COD value became 13.85 mg/L, resulting in a 27% reduction in COD value in treatment P1. In treatment P2, the COD value at a dose of 0.025 gram showed

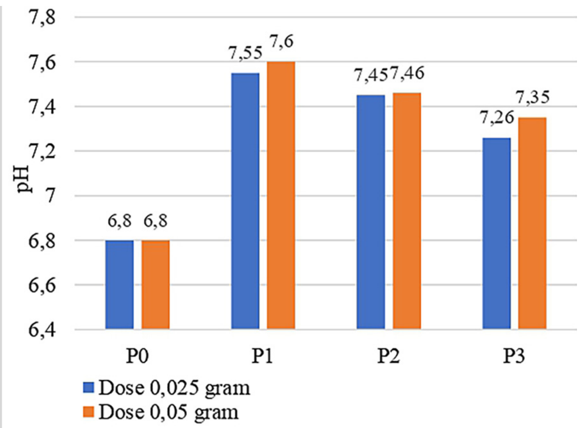

Figure 3. Variation in types of coagulants and coagulant doses on pH values

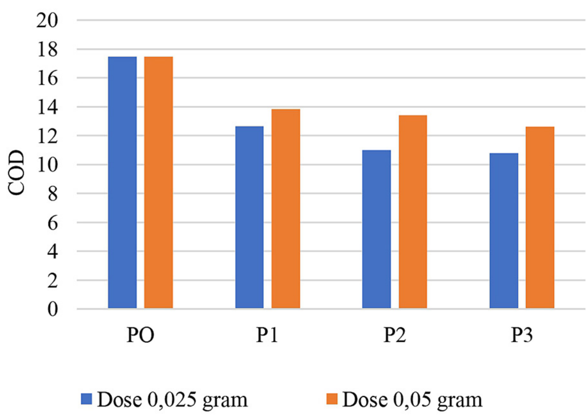

Figure 4. Variation in types of coagulants and coagulant doses on COD values

a decrease from the initial condition of 17.85 to 10.99 mg/L, while at a dose of 0.05 gram/L, the COD value was at 13.4, with a 37% reduction in COD value in treatment P2. In treatment P3, the COD value at a dose of 0.025 gram showed a value of 10.8 mg/L, and at a dose of 0.05 gram/L, the COD value became 12.61 mg/L, resulting in a 38% reduction in COD value in treatment P3.

The ability to reduce COD levels in this study is due to the biocoagulant used, which has a positive charge that can neutralize colloidal particles. This can help pollutants based on organic and inorganic materials to settle quickly and reduce the organic compound content in water or waste. Therefore, this can result in a decrease in the total oxygen demand needed to oxidize organic and inorganic materials (Tanjung et al., 2019). Moreover, rapid and slow stirring are important stages in the coagulation-flocculation process, as they help distribute the biocoagulant throughout the groundwater, allowing the cationic proteins contained in the biocoagulant to interact well with the water containing colloidal particles or organic compounds, which will form microflocs (Das et al., 2021).

However, there is a decrease in the optimum ability to remove COD values at a dosage of 0.05 grams/L, with treatment P1 showing the lowest COD removal rate. This occurs because the increasing amount of biocoagulant used leads to an imbalance between the added biocoagulant and the available colloids, resulting in the sample water and the coagulant reaching a saturated condition due to the colloid restabilization process

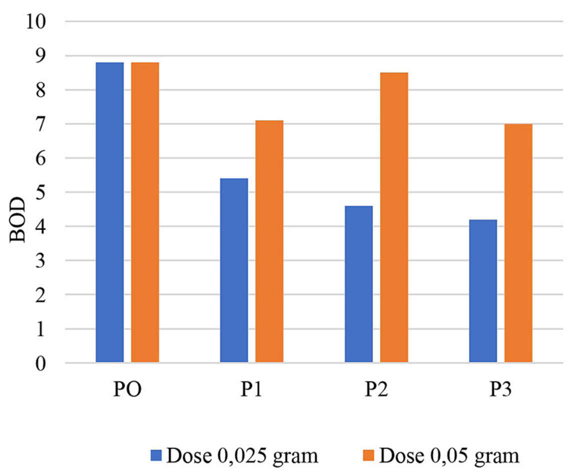

Figure 5. Variation in types of coagulants and coagulant doses on BOD values, treatment: P0 = control, P1 = *Moringa* seeds, P2 = *A. ilicifolius* leaves, P3 = combination of *Moringa* seeds and *A. illcifolius* leaves

(Fitriani et al., 2024). Consequently, the water and coagulant do not interact to form flocs, and more colloids remain suspended, causing the colloidal particles and coagulant not to bond with each other. Therefore, the optimum dose for COD removal in groundwater is 0.025 grams/L, as it can reduce the COD value by 38%. Research has reported that the use of banana peels as a natural coagulant in domestic wastewater at its optimum dose can lessen the COD value by 79.89% (Zaidi et al., 2022). An even better result in reduction efficiency was reported by (Kumar et al., 2017), who found that using *Moringa oleifera* seeds could reduce the COD value by 83%.

Measurement of biological oxygen demand

In this study, the measurement of BOD levels was conducted using the SNI 6989.72:2009 standard with the Winkler method. The application of *Moringa oleifera* seed and *Acanthus ilicifolius* leaf biocoagulants on water samples was conducted to determine the ability of the biocoagulants to reduce the pollutant load of the water samples. One of the parameters that can determine whether water is polluted is by measuring the environmental parameter BOD. The results of the biocoagulant measurements on the BOD values can be seen in Figure 5. The biocoagulants used in this study included four treatments: control without biocoagulant treatment =P0, *Moringa oleifera* seed =P1, *Acanthus ilicifolius* leaf = P2, and a combination of *Moringa oleifera* and *Acanthus ilicifolius Moringa oleifera* and *Acanthus ilicifolius* leaves = P3, with dosage variations of 0.025 grams and 0.05 grams during a 30-minute sedimentation period.

On the basis of the research results of the biocoagulant application on BOD values as shown in Figure 5, it is indicated that before the treatment with the addition of *Moringa oleifera* seed coagulant and *Acanthus ilicifolius*, the BOD value was at 8.8 mg/L. After the biocoagulant treatment was applied, the BOD value decreased from 8.5 mg/L to 4.2 mg/L. According to Figure 5, the measurement results show that in treatment P1 with a dose of 0.025, the BOD value decreased from the initial condition of 8.5 to 5.4 mg/L, while at a dose of 0.05 grams/L, the BOD value became 7.1 mg/L, resulting in a BOD reduction of 39% in treatment P1. In treatment P2, the BOD value at a dose of 0.025 grams was 4.6 mg/L, while at a dose of 0.05 grams/L, the BOD value was 8.5 mg/L, with a BOD reduction percentage of 48%

in treatment P2. In treatment P3, the BOD value at a dose of 0.025 grams was 4.2 mg/L, and at a dose of 0.05 grams/L, the BOD reduction was 7 mg/L, with a BOD reduction percentage of 52% in treatment P3.

The decrease in BOD levels occurs due to the presence of cationic proteins in the *Moringa oleifera* seed biocoagulant used, which plays a role in the biocoagulant. Additionally, the tannin content found in the leaves of *Acanthus ilicifolius* also plays a significant role in the coagulation-flocculation process in the groundwater sample, leading to a reduction in BOD levels. The tannin compounds contained in the *Acanthus ilicifolius* leaves are believed to be able to form complexes, accelerate the sedimentation process, and bind other macromolecules Another reason is that tannins have a high molecular weight and contain long chain structures (Benalia et al., 2024), thus providing sufficient active hydrogen for the coagulation-flocculation process and absorption on colloids. Therefore, tannin-based coagulants are very effective in colloidal adsorption, which likely involve substitution reactions and do not have electrophilic groups. Adsorption and neutralization occur due to the absorption of two ions with positive and negative charges between particles when the polysaccharide coagulant chain absorbs particles. According to Bolto & Gregory (2007), to bridge flocculation, there must be sufficient empty surface area so that there is a place for the adsorbed polymer chain segments or colloids to adhere to the coagulum surface, thereby showing statistically significant results in removing colloids, total solids, and turbidity from wastewater samples.

Figure 4 shows that Treatment P3, a combination of *Moringa oleifera* and *Acanthus ilicifolius* leaves, is the treatment with the highest reduction efficiency, with a reduction percentage of 52%. Therefore, the optimum dose for BOD removal is at a dose of 0.025 grams/L in Treatment P3. There is a decrease in BOD removal efficiency at the dose of 0.05 grams/L used. This occurs because the more coagulant used, the more the sample water and the coagulant become saturated (Fitriani et al., 2024). This condition arises because the water and coagulant do not interact to form flocs, and more particles will remain suspended, causing the colloidal particles and coagulant not to bond with each other. Thus, it can no longer form floc aggregates, and this can cause the floc to be unable to maintain its floc aggregate concentration. Similar results were found in the study (Dela Justina et al., 2018), on milk waste, where the BOD removal efficiency of tannin-based biocoagulants was optimal at a dose of 300mg/L. Therefore, if the dose is increased, the BOD removal efficiency will decrease as the coagulant dose increases.

Measurement of the total suspended solids parameter

The application of *Moringa oleifera* seed and *Acanthus ilicifolius* leaf biocoagulants on groundwater samples was conducted to determine the ability of the biocoagulants to reduce the pollutant load of the water samples, where one of the parameters that can determine whether water is polluted is by measuring the environmental parameter known as TSS. The results of the biocoagulant measurements on the TSS values can be seen in Figure 6. The biocoagulants used in this study included four treatments: control without biocoagulant treatment = P0, *Moringa oleifera* seeds = P1, *Acanthus ilicifolius* leaves = P2, and a combination of *Moringa oleifera* and *Acanthus ilicifolius* = P3, with dose variations of 0.025 grams and 0.05 grams during a 30-minute sedimentation period.

Figure 6 shows that before the treatment of adding *Moringa oleifera* seed coagulant and *Acanthus ilicifolius* leaf, the TSS value was 41 mg/L. After the addition of biocoagulant treatment, the TSS value decreased differently based on the type of coagulant and the dosage used,

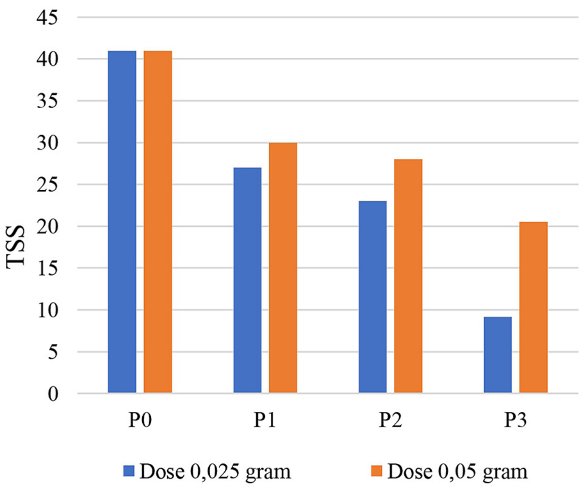

Figure 6. Variation in types of coagulants and coagulant doses on TSS values, treatment: P0 = control, P1 = *Moringa* seeds, P2 = *A. ilicifolius* leaves, P3 = combination of *Moringa* seeds and *A. illcifolius* leaves

with TSS values ranging from 30 mg/L to 20.5 mg/L. On the basis of Figure 6, the measurement results show that in treatment P1 with a dosage of 0.025, the TSS value decreased from the initial condition of 41 to 27 mg/L, while at a dosage of 0.05 gram/L, the TSS value became 30 mg/L, resulting in a 34% reduction in TSS value in treatment P1. In treatment P2, the TSS value at a dosage of 0.025 gram showed a decrease from the initial condition of 41 to 23 mg/L, while at a dosage of 0.05 gram/L, the TSS value became 28 mg/L, with a 44% reduction in TSS value in treatment P2. In treatment, P3, the TSS value at a dosage of 0.025 gram showed an increase to 180 mg/L, and at a dosage of 0.05 gram/L, the TSS value increased to 412 mg/L, with a 78% reduction in TSS value in treatment P3.

Moringa oleifera and *Acanthus ilicifolius* leaf biocoagulants are quite effective in reducing TSS pollutant load. This is because the coagulation process aids sedimentation and can collect total suspended solids along with the biocoagulant used due to the interaction between colloids and particles. The protein content containing positive ions in *Moringa oleifera* seeds and *Acanthus ilicifolius* leaves can interact with negatively charged particles, assisted by the jar test mechanism. Before using the biocoagulant, the TSS pollutant load was 41 mg/L, and after adding the coagulant, it decreased to 30-6.41 mg/L. The combination biocoagulant of *Moringa oleifera* and *Acanthus ilicifolius* leaves in treatment P3 at a dose of 0.025 grams is the most optimal treatment, because it can reduce the highest TSS value by 78%.

The decrease in TSS levels is caused by the properties of *Moringa oleifera* and *Acanthus ilicifolius* leaves, which contain proteins and tannins that can dissolve in water. When dissolved, these biocoagulants will generate a large amount of positive charge. Therefore, the biocoagulants will react to become natural polymeric biocoagulants with a positive charge. Thus, when rapid and slow stirring is performed, the cationic proteins and tannins that play a crucial role in the coagulation-flocculation process will be distributed throughout the water containing negatively charged colloidal particles, leading to interactions between the colloids and the natural coagulant (Camacho et al., 2017). The success of the coagulation and flocculation processes in water purification is greatly influenced by the sedimentation time, type of coagulant, and coagulant dosage. The research conducted by Precious Sibiya et al., (2021), using a combination of conventional coagulants and natural coagulants found that the use of natural coagulants from eggshells effectively reduced TDS levels by up to 80% in water and wastewater treatment, indicating the potential of natural coagulants for the future.

However, the addition of a 0.05 gram/L dose shows an increase in the amount of TSS compared to the addition of a 0.025 g/L dose in water. This can occur because the excessive addition of biocoagulants will increase the flocs that remain floating due to the coagulation-flocculation process not working optimally. This is caused by the lack of space to connect between the coagulant and the particles. According to the research conducted by Ahmad et al., (2021), the results showed a decrease in TSS levels at a dose of 0.1 g/L with a reduction of 70%. However, when the mass ratio was increased from 100 to 1000 mg TSS per mg of coagulant, the ability to reduce and eliminate TSS did not show a significant difference in TSS removal, indicating that the ability to reduce TSS reached its optimal condition. Therefore, if the dose is increased, it will cause the flocs to break (Adelodun et al., 2020), resulting in the remaining natural coagulant contaminating the solution because it does not interact with the colloids. Higher coagulant doses increase TSS levels, making the water more turbid, as not all particles interact with the colloidal particles to form flocs in the water.

Measurement of the total dissolved solids parameter

One of the parameters of clean water that need to be considered is total dissolved solids (TDS), the TDS value is a physical parameter that can determine whether the water is contaminated or not and whether it is suitable for use. In this study, the measurement of Total Suspended Solids was conducted using the gravimetric method. The application of *Moringa oleifera* seed and *Acanthus ilicifolius* leaf biocoagulants on water samples was conducted to determine the ability of the biocoagulants to reduce the pollutant load of the water samples, both organic and inorganic substances. The change in TDS values in the water samples before and after treatment can be seen in Figure 7. The biocoagulants used in this study included four treatments: control without biocoagulant treatment = P0, *Moringa oleifera* = P1, *Acanthus ilicifolius* leaves = P2, as well as a

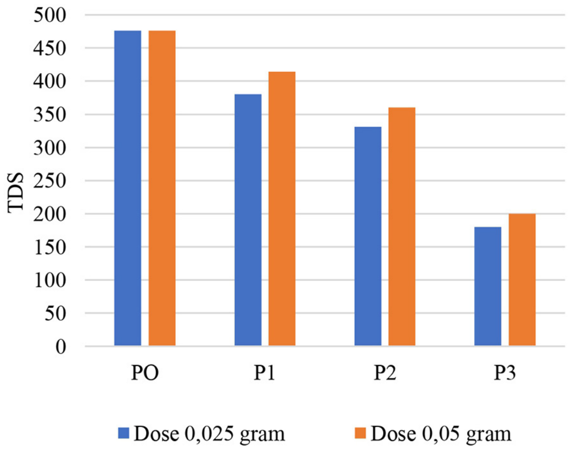

Figure 7. Variation in types of coagulants and coagulant doses on TDS values, treatment: P0 = control, P1 = *Moringa* seeds, P2 = *A. ilicifolius* leaves, P3 = combination of *Moringa* seeds and *A. illcifolius* leaves

combination of *Moringa oleifera* seeds and *Acanthus ilicifolius* leaves =P3, with dosage variations of 0.025 grams and 0.05 grams during a 30-minute sedimentation period.

After the addition of biocoagulant treatment, the TDS value decreased from 457 mg/L to 313 mg/L. The measurements in Figure 7 show that the P1 treatment with a dose of 0.025 grams lowered the TDS value from 476 mg/L to 380 mg/L. At a dose of 0.05 grams/L, the TDS value rose to 414 mg/L, which means that the P1 treatment lowered the TDS value by 20%. The P2 treatment with a dose of 0.025 grams showed a TDS value from the initial condition of 476 mg/L to 331 mg/L, while at a dose of 0.05 grams/L, the TDS value became 360 mg/L, with a TDS reduction capability percentage of 30% in P2. Meanwhile, the P3 treatment with a dose of 0.025 grams showed a TDS value of 180 mg/L, and at a dose of 0.05 grams/L, the TDS value reduction became 412 mg/L, with a TDS reduction capability percentage of 62% in P3.

The ability of *Moringa oleifera* seeds and *Acanthus ilicifolius* leaves as natural coagulants in the clarification process and the obtained TDS reduction value indicates that the optimum TDS reduction is at a coagulant dose of 0.025 grams/L with a total reduction of 62%, making treatment P3 the treatment with the highest TDS reduction value. The removal of TDS at that dosage is optimal, because the total dissolved solids in the well water sample and the used coagulant dosage are appropriate. This allows the coagulation-flocculation and sedimentation processes of colloidal particles that cause high TDS values to proceed maximally. This is due to the cationic proteins present in *Moringa oleifera* seeds and the active tannin compounds contained in *Acanthus ilicifolius* leaves, which are generally positively charged and opposite to the negatively charged colloidal particles, thus capable of attracting the colloidal particles that cause TDS (Hak et al., 2019).

The measurement of TDS values based on the graph in Figure 7 shows that the coagulant dose affects the reduction of TDS values. TDS values are greatly influenced by macromolecular-sized mineral substances and other ionic compounds that can generally be dissolved into a solution. The addition of coagulant at a dose of 0.05 grams, as shown in Figure 7, increased TDS values. The more coagulants used, the higher the TDS values can increase. This is because there are residual coagulants that do not bind the colloids, reducing the coagulant's ability to lower TDS values. Additionally, the increase in TDS values is due to the higher content of dissolved minerals and ionic compounds in the water, which also decreases during treatment. TDS is greatly influenced by the content of minerals, charged macromolecules, and other ionic compounds that can be dissolved or dissociated into a solution. (Kristianto, 2017). A similar finding was obtained by (Thakur and Choubey, 2014), in their research, where the natural coagulant activity of acacia catechu plant extract, utilizing tannin extract as a biocoagulant, was able to reduce the TDS value by 57.4% in pond water from 390 mg/L to 170 mg/L at a dosage of 4 ml/L. However, there was a deviation in the TDS value when the coagulant dosage was increased to 5 ml/L, rising to 210 mg/L.

Measurement of the *E. coli* bacteria parameter

One of the important parameters to know is the biological parameter marked by the presence of bacteria in groundwater, such as *Escherichia coli*, which originate from human or animal feces or activities around the groundwater source. The biocoagulants used in this study included four treatments: control without biocoagulant treatment = P0, *Moringa oleifera* seeds = P1, *Acanthus ilicifolius* leaves = P2, and a combination of *Moringa oleifera* and *Acanthus ilicifolius* leaves = P3, with dosage variations of 0.025 grams and 0.05 grams during a 30-minute sedimentation period. The activity of the biocoagulants in reducing *Escherichia coli* bacteria can be seen in Figure 8.

Figure 8 shows that based on the research results, the application of biocoagulants on *E. coli* bacterial content indicates that the initial condition before the treatment with the addition of *Moringa oleifera* seed coagulant and *Acanthus ilicifolius* leaf showed an *E. coli* bacterial content of 109 ml. After the treatment with the addition of biocoagulants, the *E. coli* bacterial content decreased from 35 ml to 8 mg/L. On the basis of Figure 8, in treatment P1 with a dose of 0.025, the *E. coli* content decreased from the initial condition of 109 ml to 35 mg/L, while at a dose of 0.05 gram/L, the E. coli content became 11 mg/L, resulting in a 67% reduction in *E. coli* content in treatment P1. In treatment P2, the *E. coli* content at a dose of 0.025 grams became 33 mg/L, while at a dose of 0.05 gram/L, the *E. coli* content became 9 mg/L, with a 68% reduction in *E. coli* content in treatment P2. In treatment, P3, the *E. coli* content at a dose of 0.025 gram was 10 mg/L, and at a dose of 0.05 gram/L, the *E. coli* content became 8 mg/L, with a 92% reduction in *E. coli* content in treatment P3.

E. coli is a bacterium found in the digestive tracts of animals and humans. *E. coli* is also present in surface water due to water distribution from rivers, and seas, contamination from animal and human waste, or water sources near human pollution sources (Khan et al., 2021). This is what causes the presence of *E. coli* bacteria in the groundwater samples used. The removal of *E. coli* in this study is because *Moringa oleifera* seeds have bactericidal activity. This was demonstrated in a study utilizing *Moringa oleifera* plant water extract, which can inhibit the growth of pathogens and bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* at certain doses (Dede, 2020). This indicates that *Moringa oleifera* seeds have bactericidal capabilities, along with the presence of rhamnosyloxy-benzyl-isothiocyanate, which can act as an antibacterial agent. According to research by (Kacem et al., 2024), The research that uses moringa seeds (M. *oleifera*) as a natural antibacterial coagulant in wastewater purification, provides excellent results with an efficiency of 98% reduction in total coliform bacteria, 98% reduction in fecal coliform, and 100% reduction in Escherichia coli and streptococcus at a dose of 20 g·L.

Not only the *Moringa oleifera* seed content, but also the tannin content found in the leaves of *Acanthus ilicifolius* can act as a bacterial growth inhibitor. Tannins can inhibit bacterial growth, because they have a toxic effect (Grehs et al., 2019). The poisonous effect of tannins will cause the bacterial cell membrane to shrink, leading to the shrinkage of the cytoplasmic membrane and resulting in changes in cell permeability. In cells, the cytoplasmic membrane is the site for the entry and exit of nutrients and maintaining the integrity of cell components. Therefore, if the bacterial cell membrane is damaged, the bacteria will die (Czerkas et al., 2024). According to the research conducted by (Latief et al., 2022), the extract of *Acanthus ilicifolius* leaves has antibacterial activity against Klebsiella, CN, *Escherichia coli*, Entero 5, Entero 10, and *Pseudomonas* sp.

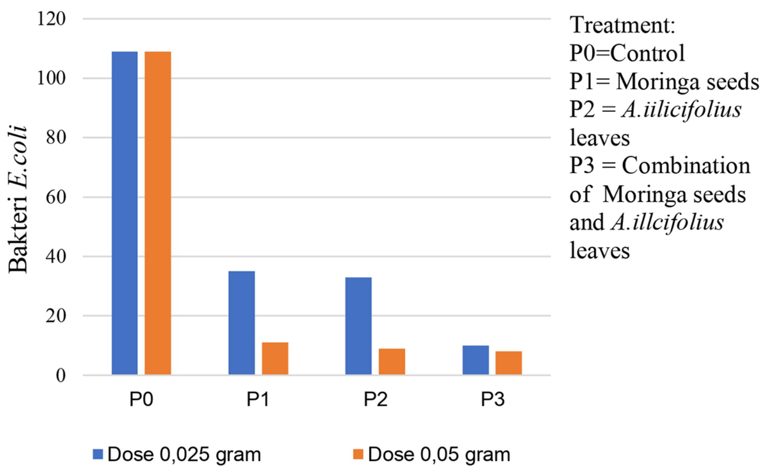

Figure 8. Variation in types of coagulants and coagulant doses on *E. coli* values, treatment: P0 = control, P1= *Moringa* seeds, P2 = *A. ilicifolius* leaves, P3 = combination of *Moringa* seeds and *A. illcifolius* leaves

Characterization of a coagulant and sediment used scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS)

Characterization of the surface morphology of water deposits as well as *Moringa oleifera* and *Acanthus ilicifolius* biocoagulants was conducted using scanning electron microscopy (SEM) imaging techniques. SED PC-std can be seen in Figure 9 with 4 types of scales: 100 µm scale with 300 kx magnification and landing energy capacity at 15kV, 50 µm scale with 500 kx magnification and landing energy capacity at 15kV, 20 µm scale with 1000 kx magnification and landing energy capacity at 15kV, and 10 µm scale with 200 kx magnification and landing energy capacity at 15kV. This allows for observation of the surface morphology of the deposits resulting from the coagulation and flocculation processes that have been carried out.

Figure 9 shows how the surface of the precipitate between the coagulant and the colloidal particles merges and clumps together with the coagulant from *Moringa oleifera* seeds and *Acanthus ilicifolius* leaves. *Moringa oleifera* seeds have a molecular weight of 6000–16000 daltons, which are long-chain carbon compounds (polymers), thus possessing active groups as adsorbents. Colloidal particles will adhere to the active groups, forming flocs. The merging of one floc with another forms larger and stronger flocs, which then precipitate (Camacho et al., 2017). The coagulation mechanism of this type is called interparticle bridging. Cationic proteins with a positive charge will combine with colloids that have a negative charge, which over time will form flocs and become large clumps capable of precipitating the colloids causing water turbidity. The cationic protein content in *Moringa oleifera* seeds and the tannin content in *Acanthus ilicifolius* leaves, which contain amine groups, can be used for water treatment, indicating that proteins and tannins are cationic due to their tertiary monomer amine groups. The effectiveness of tannins as natural coagulants in water treatment is influenced by the chemical structure of tannins. The presence of phenolic groups in tannins indicates that tannins possess cationic properties, because they are good

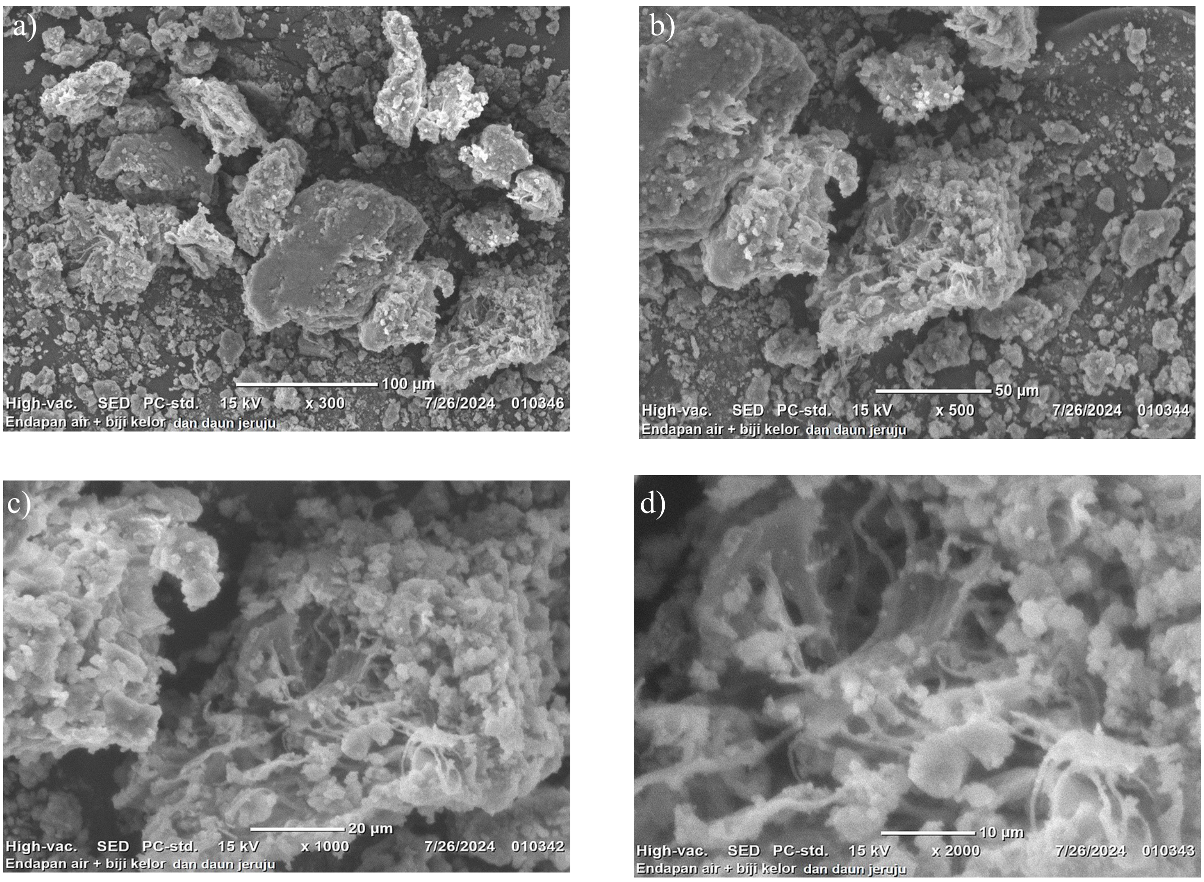

Figure 9. Characterization of colloidal precipitates and natural coagulants: (a) 300x, (b) 500x, (c) 1000x, (d) 2000x zoom

hydrogen donors capable of destabilizing colloids present in water (Tetteh and Rathilal, 2021).

CONCLUSIONS

The combination of *Moringa oleifera* seed biocoagulant and *Acanthus ilicifolius* leaf to improve the quality of groundwater can be utilized. At the optimum dose and type of coagulant used, the percentage reduction in each parameter tested on groundwater including pH with the optimum pH known to be 7 was as follows: COD with a reduction percentage of 38%, BOD with a reduction percentage of 52%, TSS with a reduction percentage of 78%, TDS with a reduction percentage of 62%, and *E. coli* bacteria with a reduction percentage of 92%. The optimum dose used in this study was 0.025 grams/L, while increasing the dose further yielded suboptimal results in several parameters. Future research using the same type of coagulant with different parameters and varying sedimentation times is hoped to be conducted.

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