



A Preliminary Study on *Moringa Oleifera* Seed Cake Extract as Biocoagulant and Biosorbent in Tannery Waste Water Treatment

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ABSTRACT

Moringa oleifera seeds have for a long time been used in water treatment. However, there is scarce research on its use for treatment of highly turbid industrial wastewaters such as tannery waste water. Effluent streams from tanneries are laden with metals that can be toxic when above standard threshold limits. The effect of accumulation of metals over a period of time can also be harmful. In this study, 0.1M sodium chloride solution was prepared and used to extract protein from the defatted Moringa oleifera seeds for active coagulation. High Performance Liquid Chromatography (HPLC) was employed to analyse the proteins in the extract. The proteinous extract was used as coagulant in treating tannery wastewater from one of the tanneries in Kano, Nigeria. The pH was varied between 3 and 11 while coagulant dosages from 10 to 50 ml/L were investigated. The maximum turbidity removal of 68 % was achieved at pH of 9 and coagulant dosage of 10 ml/L. Treated effluent at optimum coagulant dosage was further studied for the biosorption of selected heavy and nutritive metals - chromium, iron, magnesium, calcium, sodium and iron. Moringa oleifera plant extracts were found to be effective in physical and chemical treatment of tannery waste water.

INTRODUCTION

Tanning industry is one of the oldest industries which is highly complex and is characterized by high BOD, COD, suspended solids, settleable solids, sulphide, chloride and chromium in its effluent [1]. Untreated tannery effluents when discharged directly into the water bodies or into the open lands cause irreversible damage to the environment.

Coagulation–flocculation is one of the most important physicochemical treatment steps employed in industrial wastewater treatment to reduce the suspended and colloidal materials responsible for turbidity of the wastewater [2]. Conventional chemical-based coagulants, namely, alum (AICI₃), ferric chloride (FeCI₃) and

poly aluminium chloride (PAC) have major drawbacks such as detrimental effects on human health, ineffectiveness in low-temperature water, relatively high procurement costs, and production of large sludge volumes and resultant pH change of treated water [3]. This has sparked the recent interest in the use of natural coagulants which are healthy, environmentally safe, relatively cheap, sustainable and readily available. Moringa oleifera is a pan African tree whose seeds have been for a long time used in treating turbid waters. Plate 1.0 shows a pictorial view of its mature seeds. Moringa oleifera seeds have been studied by Ndbigengesere et al., [4] to be more effective than alum in treating water and it produces four times less volume of sludge which

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is environmentally benign. So far, there is scarce literature on the use of natural coagulants in the

treatment of specifically tannery wastewater.



Plate 1.0: Moringa oleifera seed pods

In their study, Broin *et al.*, [5] described *Moringa oleifera* seed extract as a watersoluble protein very rich in amino acids with a net positive charge. They also proposed that the coagulation mechanism is mainly a net charge mechanism same as reported by Muthuraman and Sikala [6].The Figure 1.0 shows a pictorial view of the charge neutralization mechanism.



Figure 1.0: Coagulation via charge neutralization and flocculation mechanism of the colloidal particles present in water [7].

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This mechanism is proposed for the coagulating action of *Moringa oleifera* as for most natural proteinous coagulants. As explained by Abderrezzaq *et al.*, [8] the mechanism of coagulant extraction using salt solution as employed in this study, is that of solubilizing and dissolving of the active coagulating agent in the solution, which is then extracted.

Heavy metals are one of the most harmful of all chemical pollutants because of their toxicity, bioaccumulation and biomagnifications along the food chain [9]. They are produced from a variety of natural and anthropogenic sources. Traditional methods of metal removal from wastewater including ion exchange, chemical oxidation/ reduction, chemical precipitation, ultrafiltration and reverse osmosis have major drawbacks like high cost, prolonged period with less efficiency, production of other waste / harmful products [10]. Therefore, bio-coagulants are currently been researched as environmentally safe alternatives. Bio-coagulants are not only water clarifying agents, but they also have antimicrobial and heavy metal removal properties (biosorption) in some instances [7].

Biosorption is defined as the cleaving of heavy metal ions to surface functional groups like amino groups. This technique is an inexpensive way of treating polluted waste waters [11]. According to the study by Ravikumar and Sheeja [9], Moringa oleifera seeds were able to remove 95% Copper, 93% Lead, 77 % Cadmium and 70% Chromium from wastewater. Hossein et al., [12] reported that Moringa oleifera seed is a potential adsorbent for Manganese, Nickel, Chromium, Copper, Iron, and Zinc removal and optimum pH for heavy metal removal was 7.0. Matar et al., [13] also reported Moringa oleifera seed powder to be very effective in the removal of heavy metals such as Iron, Copper, Arsenic and Lead.

According to Basra *et al.*, [14], the aqueous extract from these plant seed powder has been more effective than the seed powder in the removal of Lead and Chromium from sewage waters via the amino acids present in them. Plant protein extracted using 1.0 M sodium chloride

solution has also been reported to perform better in extracting active coagulants than fresh or distilled water [18]. Other advantages of using the plant seed cake extract as a natural coagulant other than efficiency include relative low cost, lesser volume of sludge, sustainability. There is scarce research focused on the direct use of protein extracts from *Moringa oleifera* seed cake in reducing metal concentration from tannery wastewaters. In this study, proteinous extract from *Moringa oleifera* (Drumstick) seeds were used to treat tannery waste water via coagulation and reduction in metal concentration.

High Performance Liquid Chromatography was used for protein separation analysis. The technique uses a liquid mobile phase responsible for separating the sample components that will flow through a column and provide data on the separation of the compounds [16]. The various types of amino acids such as proline. arginine. cvsteine and their concentrations were revealed via this analysis. While, Atomic Absorption Spectrometry was used to determine the concentration of selected metals before and after protein coagulation.

MATERIALS AND METHODOLOGY Sample collection and preparation

Wastewater was collected from a tannery at Kano, Nigeria within a 24- hour period. Jatropha curcas seeds were obtained from a local market in Hunkuyi, Kudan Local Government Area, Kaduna State, Nigeria. Moringa oleifera seeds were procured from a local market within SabonGari Local Government Area of Zaria, Kaduna state, Nigeria. From a selection of good quality seeds, they were sundried and dehulled manually. The kernels were ground to fine powder with a domestic mortar and pestle then sieved to get particle sizes between 1 to 5 mm. The oils from the seeds were extracted using n-hexane solvent to obtain the seed cake. The active coagulants were then extracted from the seed cake using 1 M solution of sodium chloride [17]. The tannery wastewater was then analysed before treatment for physicochemical properties as shown in Table

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3.1 and for initial metal concentrations which were reported as control in the metal uptake Figures. The proteins in the filtrate were analysed using High Performance Liquid Chromatography (HPLC) and thereafter used for coagulation and sedimentation of dissolved solids. The HPLC results are tabulated on Table 3.2. After the sedimentation, the supernatant was decanted and taken for metal analysis via Atomic Absorption Spectrometry.

Coagulation Studies

A batch test was used in the experiments to coagulate the Tannery waste water collected, involving rapid mixing, slow mixing and sedimentation. Initial physicochemical analysis of pH, conductivity and total dissolved solids were taken and recorded. Measurements of conductivity and total dissolved solids readings were taken at reference temperature and pH. The stirring was conducted in different rotating speeds, which consist of rapid mixing (100 rpm) for 2 minutes and slow mixing (30rpm) for 10 minutes. 100 ml of the Tannery wastewater samples was put in to each 500 ml beaker, stirred and the sedimentation rates observed. Extracted solutions of the coagulant were also introduced in varying doses of the waste water and subjected to the coagulation procedure. After the agitation being stopped, the suspensions were allowed to settle for the sedimentation studies from 30 mins to 24 hrs at recorded intervals. The sedimentation rates were observed and recorded. Finally, a sample was withdrawn using a pipette from the middle of supernatant for physicochemical measurements, so that the effect of coagulant dose on coagulation at fixed pH could be studied. The pH was also varied at fixed coagulant dosages and studied.

Optimization of Coagulant Dosage and pH

The original pH of the untreated tannery wastewater was 7.4. The study was conducted to evaluate optimum dosage by varying the dosage as 1, 2, 3, 4, and 5 ml/L at original pH of tannery wastewater. Using the optimum dosage, optimum pH was determined by varying the pH of Tannery wastewater at 3, 5, 9 and 11.

Sample Digestion

Heavy or trace metals were determined after digestion of the samples. Tannery waste water sample digestion was carried out by taking 10 ml of the sample and adding 4 ml Perchloric acid, 20 ml concentrated nitric acid and 2 ml concentrated tetraoxosulphate (VI) acid. This was digested using Aluminium block digester 110. The mixture was heated until white fumes evolved and clear solution obtained. After digestion, the samples were allowed to cool and then transferred to 100 ml volumetric flask. This was made up to 100 ml with distilled water and thoroughly mixed. The samples were allowed to stand overnight to separate insoluble materials. Filtration was done through 0.45 µm Millipore type filter. Iron (Fe), Chromium (Cr), Calcium (Ca), Sodium (Na) and Magnesium (Mg) were determined using Unican 929 Atomic Absorption Spectrometry. The analysed metals were selected based on the metals eminent in the tanning process streams. The pH was left unadjusted at 7.3. The results before coagulation and after were compared with each other as discussed hereafter in Figures 3.1 to Figure 3.5.

RESULTS AND DISCUSSIONS

Preliminary physicochemical analysis

Preliminary physicochemical analysis was taken on the untreated tannery wastewater as shown in Table 3.1.

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Physicochemical Analysis	Initial Values
Color	Brownish – Green
pH	7.3
Conductivity	230 µScm-1 at 26ºC
Total Dissolved Solids	150 mg/L at 26ºC
Turbidity	408 NTU

Amino acid analysis

The amino acid analysis was shown in Table 3.2 for *Moringa oleifera* seed cake extract (MO) via HPLC.

Table 3.2: Amino acid analysis of Moringa oleifera plant extract

Amino Acids	MO
Proline	93.17959
Phenylalanine	40.61529
Cysteine	45.25055
Asparagines	13.18196
TOTAL	192.2274

Generally, these amino acids provide more functional groups and strong affinity for heavy metal ions over a wide pH range [18]. The pH of the solution was decreased from 7.2 to 6.5 when *Moringa oleifera* was introduced in a solution containing heavy metals attributed to their two protons exchanged against the metal ions as a metal chelator [19].

Proline functions as an osmoticum that detoxifies the heavy metal stress effects on cytoplasm of plants [20]. It does this by forming complexes with metal species thereby reducing their toxic effect on plant species. Several authors have studied this ameliorating effect of proline with several metals [21], [20].

Cysteine is a α -amino acid the thiol side chain in cysteine often participates in enzymatic reactions. Cysteine has been established to be a heavy metal chelator as reported by various authors who studied its use in Mercury reduction from plants and contaminated soils [22], and in Chromium reduction [23].

Phenylalanine is an amino acid that plays an important role in plant defense against abiotic and biotic stressors. It was reported to alleviate

the harmful impacts of salinity and acts as an antioxidant due to its metal chelating properties [24]. All of these amino acids were employed in the coagulation and metal uptake studies.

Effect of Ph and Coagulant Dosage on Turbidity, TDS and Conductivity

Natural coagulants would perform differently when tested over a wide range of pH, and this governs the need to select the ideal pH at which maximum turbidity reduction can be attained. As pH plays a vital role in determining the solubility and dominant surface charges of the active coagulation agents, a balance between the extent of pH adjustments needed and the resultant turbidity removal efficiencies should be obtained [25]. The effect of pH on turbidity reduction, TDS and conductivity was studied by adjusting the tannery wastewater pH values at 3, 5, 7, 9 and the coagulation test was performed at room temperature using coagulant dosages of 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L and 50 mg/L. The pH of the wastewater was adjusted using prepared 0.1 M NaOH and 1 M HCl solutions. Figure 2.0 shows the results for turbidity reduction.

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Figure 2.0: Variation of turbidity with pH

From Figure 2.0, turbidity reduction was favoured at pH 3 and pH 9, with best results of turbidity reduction of 68 % at pH 9.

For studying the effect of coagulant dosage, the tannery wastewater pH was fixed at several values where the maximum TDS and conductivity was obtained while coagulant dosage was varied at 10 ml/L, 20 ml/L, 30 ml/L, 40 ml/L and 50 ml/L. Larger flocs were seen to form with increasing coagulant dosage, and the

final wastewater after treatment was more clear. Turbidity reduction was favoured at low coagulant dosages of 10 ml/L and 20 ml/L, higher dosages yielded a decrease in turbidity reduction. The declared turbidity removal for safe drinking water is 5 NTU. The lowest turbidity value achieved was 132 NTU which is still above acceptable drinking water limits. Nevertheless, it can still be reused for other industrial applications within the leather industry.



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Figure 4: Variation of conductivity with coagulant concentration and pH.

From Figures 3.0 and 4.0, TDS and conductivity were observed to increase as coagulant dosage increased. This led to maximum TDS and conductivity readings of 1010 mg/L and 1724 µScm⁻¹respectively at highest coagulant dosage of 50 ml/L. This is attributed to the concentration of salt solution used in the protein extraction, increasing its volume (coagulant dosage) will mean increasing the amount of dissolved salts in the solution. This gives rise to increased total dissolved solids and conductivity at increasing coagulant dosages. Maximum TDS and conductivity of 1010 mg/L and 1724µScm⁻¹ were achieved at pH of 5. Higher residual turbidity compared to the control sample was observed at pH 5. 7 and 11. This can be attributed to the presence of ions with greater surface charge during the pH stabilization. This dominates the coagulation process, affects the neutralization of these

colloidal particles and results in increased residual turbidity [7].

Unacceptable limits of TDS in water is greater than 1200 mg/L which when compared to the maximum value of 1010 mg/L obtained from this study makes the treated water acceptable. Conductivity values on the other hand between 800 and 2800 μ Scm⁻¹are fair, above which is not acceptable by W.H.O. The value of 1724 μ Scm⁻¹obtained from this study is thus within acceptable limits. These limits for turbidity, TDS and conductivity are stipulated by W.H.O.

Metals Reduction

The supernatant from the treated wastewater was further taken for metal analysis via AAS. The results are presented in Figures 5.0 and 6.0. The selected metals were based on the predominant metals used during the tanning process.

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Figure 5.0: Reduction in concentration of metals



Figure 6.0: Concentration of nutritive elements before and after treatment

Using *Moringa oleifera* seed cake extract (MO), a reduction in Chromium concentration of 57.4%, a reduction in concentration of 50.8%, a reduction in Calcium concentration of 6%, a reduction in Magnesium concentration of 4.6% were reported.

From these results, *Moringa oleifera* proteins were observed to function as an active natural metal biosorbent suitable for treatment of water containing undesirable heavy metals like chromium in substantial concentrations as supported in studies by Gallegos Tintoré *et al.*, [27] and Zaroual *et al.*, [19].

CONCLUSION

From this study, it can be concluded that *Moringa oleifera* seedcake protein extract has a good potential as a natural coagulant and is suitable for treatment of tannery wastewater.

Maximum reduction in turbidity of 68 % was obtained at pH of 9 and coagulant dosage of 10 ml/L. Lowest values of TDS and conductivity were observed at a coagulant dosage of 10 ml/L. Generally, the aalkaline pH range most favoured the coagulation process of Moringa oleifera. Thus, the pH of raw waste water from the tanning industry must be adjusted in order to achieve effective treatment using Moringa oleifera. Moringa oleifera seedcake protein extract also exhibits good potentials in reducing metals eminent in the process streams of tanning processes in line with acceptable limits by FEPA. The use of these seeds, represent an ecofriendly, economical wastewater treatment technology. Moringa oleifera seedcake protein extract was able to reduce metals like Chromium and Iron by 57% and 51% respectively. All values of metal concentration in treated wastewater

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were lower than standards by NESREA [28] and WHO [29]. Further improvement in the efficiency of this technology in tannery effluent treatment should be investigated.

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