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Biocoagulation Activity of *Moringa oleifera* Seeds for Water Treatment

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--ABSTRACT--
In this study, proximate analysis and jar test were carried out, to evaluate the characteristics of locally available Moringa oleifera seeds and its dose effect on selected water quality parameters of raw water from Kwalkwalawa River and wastewater from Dankure Market of Sokoto metropolis. Result of this study revealed that the seeds powder is rich in lipids (33.33%), and has carbohydrate and protein content of 31.64% and 25.70% respectively. Defatting had no significant effect (p>0.05) on the biocoagulant activity of the seeds powder, lowest turbidity values were obtained at dose levels of 0.1 g/L and 1.0 g/L, for raw water and wastewater respectively. pH, conductivity and total dissolved solids of M. oleifera treated water were not significantly increased (p>0.05) at the tested dose levels (0.1-5.0 g/L). There was progressive decrease in coliform count as seeds dose increases. Partially purified M. oleifera seeds proteins exhibited biocoagulant activity, this confirms earlier reports that the activity was due to the presence of short cationic polypeptides in the seed. These findings suggest that M. oleifera seeds powder can be used as a source of oil, while the cake is being employed as biocoagulant for point-of-use water treatment in developing countries.

Keywords: *Biocoagulation, M. oleifera, Proximate Analysis, Wastewater, Water Treatment.*

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I. INTRODUCTION

Surface water from rivers, streams and ponds are the most common sources of raw water, but are usually of low quality. Conventional methods such as chemical precipitation and filtration, disinfection, softening, pH regulation, oxidation and reduction, electrochemical treatment, reverse osmosis and ion exchange are used to improve water quality. However, these methods are economically non-viable due to high cost of chemicals, plant maintenance and requirement for expertise [1-3]. Low cost synthetic coagulants of aluminum, ferric salts and soda ash are widely used for water treatment, but the safety of these substances and their efficiency is increasingly becoming questionable. Some findings clearly raise strong doubt against the use of such coagulant in water treatment, due to health related issues such as Alzheimer's disease [4]. In rural areas, the problem tends to be compounded due to high level of illiteracy, lack of access roads and other social amenities [5]. Such communities depend on low quality water from rivers, ponds and/ or streams for their daily life, thereby making them prone to water borne diseases [6-10]. The search for alternative, low cost, effective and safe methods of water treatment is therefore timely.

The use of *M. oleifera* seeds for water purification is part of African indigenous knowledge and has been reported to have coagulant property after observing women in Sudan use the seeds to clarify the turbid Nile water [11]. The seeds act as a flocculant that attract and aggregate particles held in water suspension, which then precipitate out of the water as flakes, leaving clearer water. *M. oleifera* seeds also have the potentials to remove a wide range of Gram-positive and Gram-negative bacteria, algae, organic pollutants and pesticides from contaminated water and may produce less sludge than chemical coagulants [12-15]. However, adequate scientific and technical information is necessary if the full potentials of this renewable biocoagulant is to be fully exploited.

In this study, locally available seeds of *M. oleifera* were collected. Proximate analysis and jar test were carried out, to evaluate the characteristics of the seeds and its coagulant activity in promotion of selected water quality parameters in comparison to alum.

II. MATERIALS AND METHODS

2.1 Preparation of *M. oleifera* Seeds Powder

Dried seeds of *M. oleifera* were collected from Sokoto Central Market and authenticated at the Botany Unit of Biological Science Department, Usmanu Danfodiyo University Sokoto. The seeds of *M. oleifera* were shelled manually, the kernel was crushed using pestle and mortar, and then sieved using 200-250 µm aperture Laboratory Test Sieve [16].

2.2 Preparation of Defatted *M. oleifera* Seeds Powder

The method described by Abaliwano *et al.* [8] was adopted for defatting *M. oleifera* seeds powder. Briefly, 95% of Hexane was added to the powder (5%w/v) and stirred at 40 rpm for 30 minute. The resultant solution was centrifuged at 3000 rpm for 15 minutes, the supernatant was decanted and the residue dried at room temperature. The dried residue was termed defatted *M. oleifera* seeds powder.

2.3 Extraction of Proteins from *M. oleifera* Seeds

Extraction and partial purification of proteins were done according to the method described by Abaliwano *et al.* [8]. Ammonium acetate (10 mM, pH 6.8) was added to the dried defatted *M. oleifera* seeds powder (10% w/v) and stirred continuously for 30 minutes, followed by centrifugation at 3000 rpm for 15 minutes. The filtrate was termed crude extract. CM sepharose cation exchange (Sigma-Aldrich, USA) with bead size of 45-165 µm (equilibrated with ammonium acetate buffer) was added to the crude extract, followed by continuous stirring for 10 minutes. This was allowed to settle for 1 hour after which the supernatant was carefully decanted. The matrix was washed three times with ammonium acetate buffer to remove impurities and unbounded particles. This was followed by elution of the absorbed proteins with 0.6M NaCl solution. The eluted solution was allowed to settle for 1 hour and the supernatant containing extracted proteins was collected using a pipette. The supernatant obtained was used for this experiment.

2.4 Proximate Analysis

Proximate analysis was carried out to determine ash content [17], crude fat [18], crude protein [18], moisture content [17] and total carbohydrate [17].

2.5 Collection of Water Samples

Ten litre jerry cans were used to collect raw water and wastewater samples from Kwalkwalawa River and Dankure Market respectively, in February, 2014. The water samples were used as recommended by APHA [19].

2.6 Jar Test

To labeled Erlenmeyer flasks, *M. oleifera* seeds powder at doses of 0.0, 0.1, 0.5, 1.0, 3.0 and 5.0 g/L were added. Water samples were then added, and slowly agitated at 40 rpm for 20 minute. The solution was allowed to settle for 1 hour, after which the supernatant was collected. Selected water quality parameters were determined from the supernatant [8]. Comparative analysis using defatted *M. oleifera* seed powder and alum was carried out adopting the same procedure. Also, extracted *M. oleifera* seeds proteins (at different concentration) were employed for jar test.

2.7 Determination of Water Quality Parameters

pH was measured using AD14 pH meter (ADWA, Mauritius), Turbidity was measured using 2100P portable turbidity meter (HACH Company, USA), Conductivity was measured using Sension1 portable conductivity meter (HACH Company, USA), Total dissolved solids (TDS) was measured using TDS meter (Eutech Instrument Pte Ltd, Singapore), while Coliform count was estimated using Most Probable Number Method [20].

2.8 Data Analysis

Data obtained was expressed as Mean ± Standard deviation. All the parameters were analysed statistically at $P<0.05$ by one-way analysis of variance (ANOVA) or student t-test, using GraphPad InStat Software, Version 3.05 (San Diego, USA).

III. RESULT AND DISCUSSION

3.1 Proximate Composition of *M. oleifera* Seeds

The proximate composition of *M. oleifera* seeds and defatted *M. oleifera* seeds powder is given in Table 1. High lipid content (33.33%) was observed in the dried seeds powder, which was decreased significantly ($p<0.05$) after defatting. Also, crude protein content was significantly decreased ($p<0.05$), while ash and carbohydrate contents were significantly increased ($p<0.05$) after defatting. Moisture content was not significantly affected ($p>0.05$).

Table 1: Proximate composition of *M. oleifera* seeds powder

Parameter (%)	*M. oleifera*	Defatted *M. oleifera*
Moisture	6.00±0.866 [a]	5.33±0.577 [a]
Ash	3.33±0.289 [a]	4.33±0.289 [b]
Lipid	33.33±0.764 [a]	13.33±0.764 [b]
Crude protein	25.70±0.182 [a]	23.60±0.133 [b]
Carbohydrate	31.64±0.207 [a]	53.40±1.381 [b]

Data were expressed as Mean ± Standard deviation of triplicate values
Mean values with different superscript in a row indicate significant difference ($p<0.05$)

3.2 Effects of *M. oleifera* Seeds Powder on Water Quality Parameters

The pH of both *M. oleifera* and defatted *M. oleifera* treated water were within the WHO and Nigerian Industrial Standards (NIS) recommendation of 6.5-8.5 [21]. In contrast, alum reduces the pH to 5.70 (31.24%) for raw water and 7.73 (6.76%) for wastewater at a dose of 0.5g/L (Fig. 1).

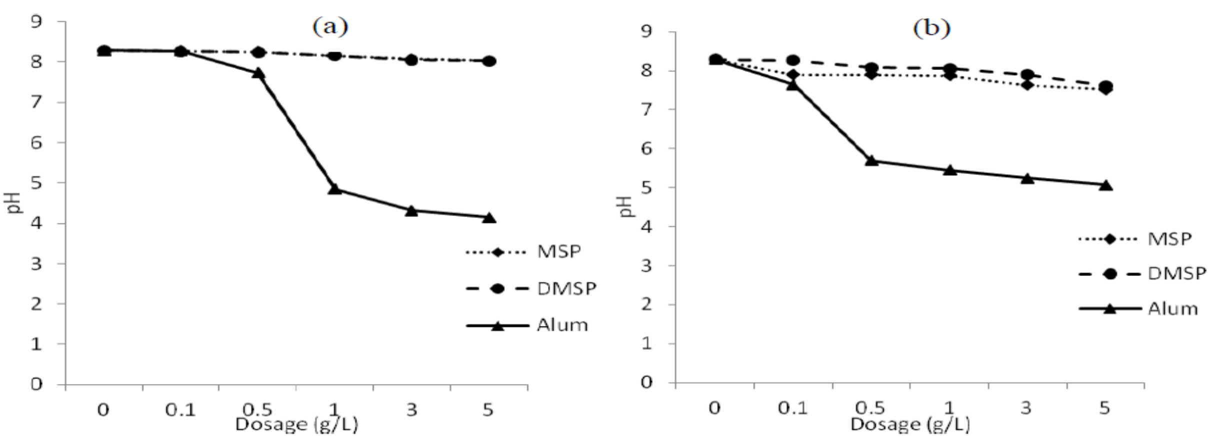

Figure 1: pH of water samples treated with *M. oleifera* seeds powder (MSP), defatted *M. oleifera* seeds powder (DMSP) and Alum. (a) Raw Water from Kwalkwalawa River, (b) Wastewater from Dankure Market.

Increased acidity by alum may lead to dissolution of metal ions in storage tanks and plumbing, and consequently cause corrosion [22]. In this regard, *M. oleifera* will be advantageous over alum, particularly in terms of reducing the cost of plant maintenance and pH regulation.

Lowest turbidity values for raw water were achieved at a dose of 0.1 g/L and are in order of alum (36.27 NTU) < defatted *M. oleifera* (38.93 NTU) < *M. oleifera* (47.43 NTU). For wastewater, the lowest turbidity values were achieved at a dose of 1.0 g/L, in the order of defatted *M. oleifera* (78.83 NTU) < *M. oleifera* (85.53 NTU) < alum (89.07 NTU) (Fig. 2).

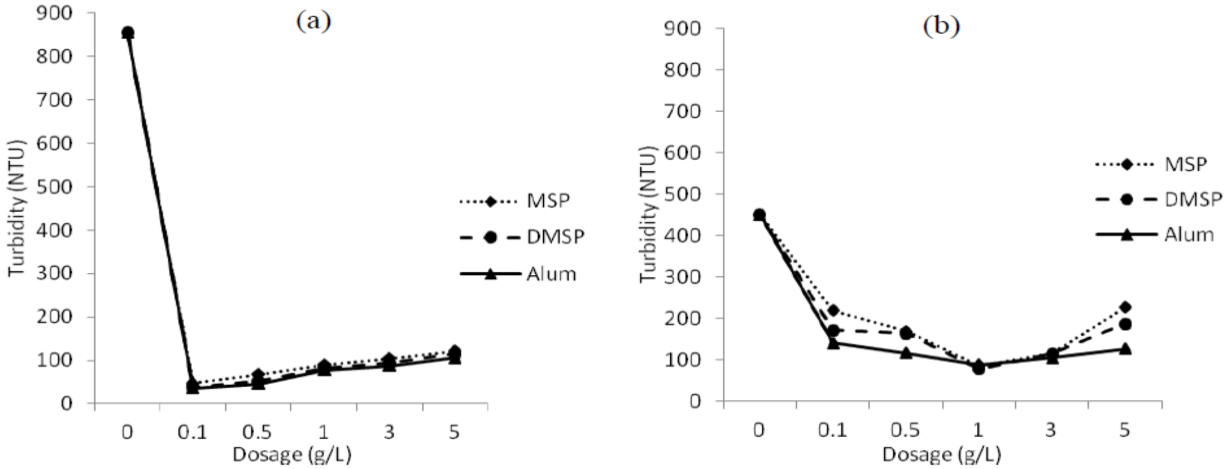

Figure 2: Turbidity (NTU) of water samples treated with *M. oleifera* seeds powder (MSP), defatted *M. oleifera* seeds powder (DMSP) and Alum. (a) Raw Water from Kwalkwalawa River, (b) Wastewater from Dankure Market.

Though the turbidity of treated water was above Nigerian Industrial Standard (NIS) maximum permitted level of 5 NTU, defatted *M. oleifera* gave good turbidity reduction of 75.67 % for raw water. When coupled with other water treatment processes (such as aeration, sedimentation and/ or filtration), the turbidity removal action of *M. oleifera* may be more pronounced.

There was progressive increase in conductivity and total dissolved solids (TDS) with increase in dosage for all the treatments (Fig. 3 and 4).

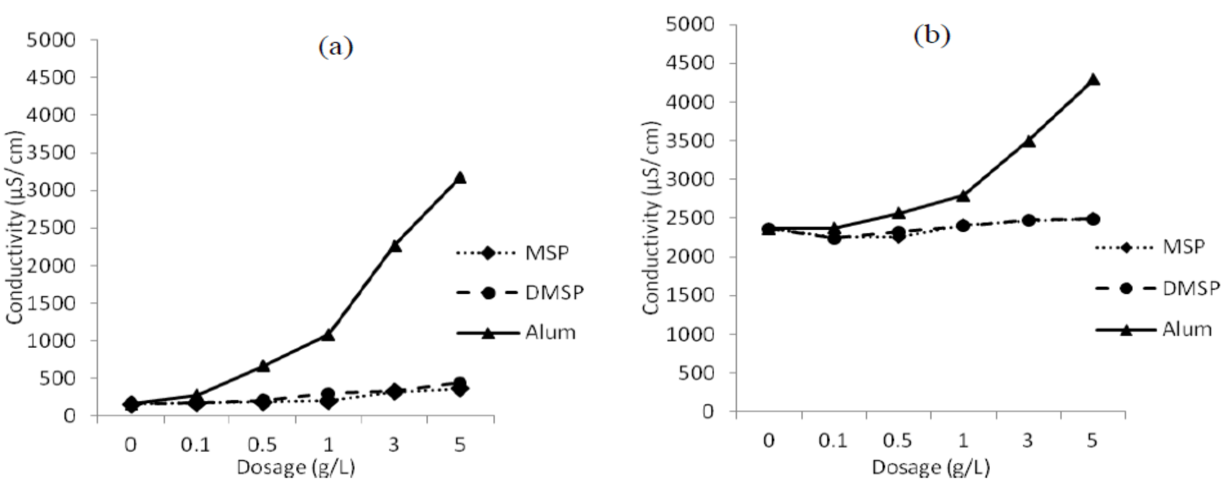

Figure 3: Conductivity (µS/ cm) of water samples treated with *M. oleifera* seeds powder (MSP), defatted *M. oleifera* seeds powder (DMSP) and Alum. (a) Raw Water from Kwalkwalawa River, (b) Wastewater from Dankure Market.

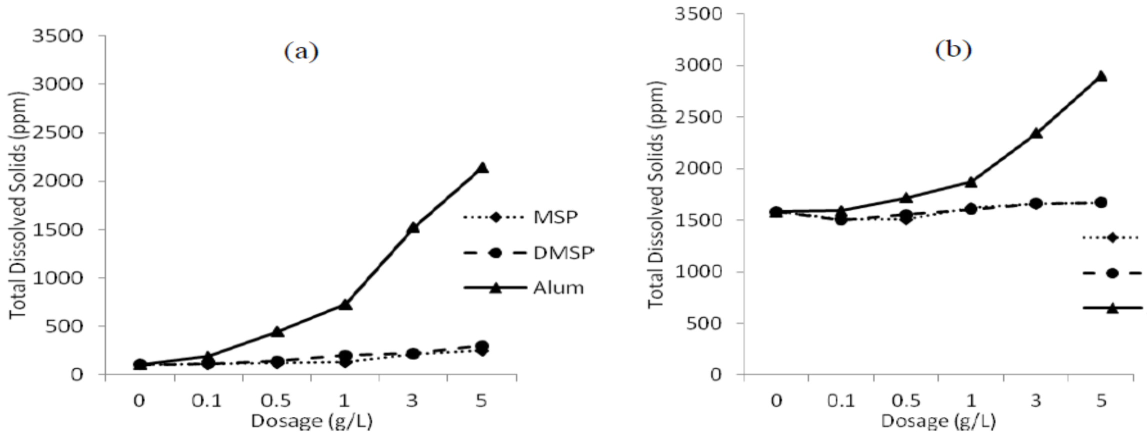

Figure 4: Total Dissolved Solids (ppm) of water samples treated with *M. oleifera* seeds powder (MSP), defatted *M. oleifera* seeds powder (DMSP) and Alum. (a) Raw Water from Kwalkwalawa River, (b) Wastewater from Dankure Market.

Increase in conductivity and TDS by *M. oleifera* and defatted *M. oleifera* may be due to the presence of mineral elements, charged macromolecules and other ionic compounds that dissolved or dissociated into the treated water. Since alum is a double salt of aluminum sulfate, it is expected to cause high increase in conductivity and TDS as they are positively correlated (R=0.999).

The antimicrobial activity of *M. oleifera* seeds powder is depicted in Fig. 5. Several researchers have reported the antimicrobial activity of *M. oleifera* seeds [8, 12-14, 23].

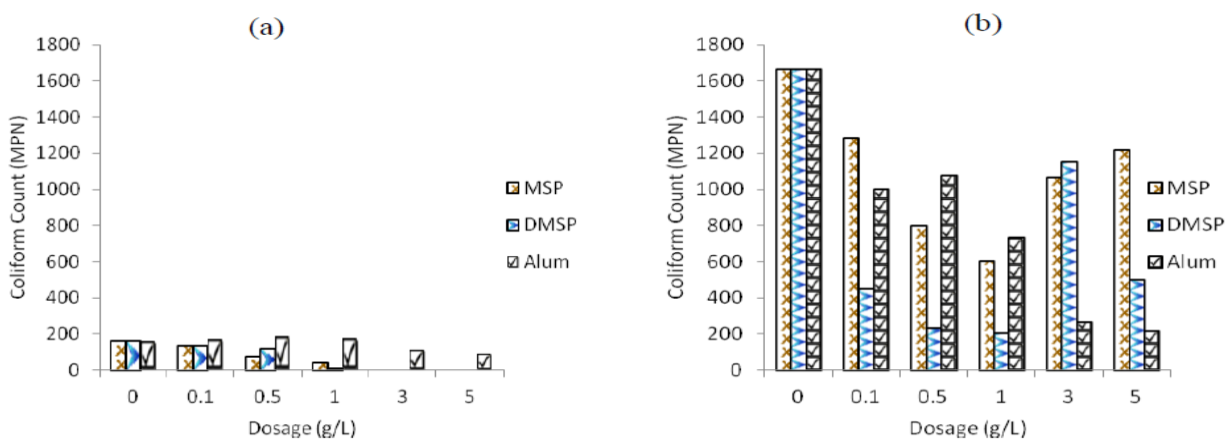

Figure 5: Coliform Count (MPN) of water samples treated with *M. oleifera* seeds powder (MSP), defatted *M. oleifera* seeds powder (DMSP) and Alum. (a) Raw Water from Kwalkwalawa River, (b) Wastewater from Dankure Market.

There was progressive decrease in coliform count for raw water, with increase dose for both *M. oleifera* seeds powder and defatted *M. oleifera* seeds powder. However, an increase in coliform count was observed beyond the dose of 1.0 g/L *M. oleifera* seeds powder and defatted *M. oleifera* seeds powder, this may be due to restabilisation of flocs/ decrease in the ability of *M. oleifera* to attract and neutralise charged ion and microbial cells in the wastewater. The antimicrobial activity of *M. oleifera* seeds is believed to be due to the ability of its cationic proteins to attract and neutralise charged molecules, thus, resulting in formation of flocs [24]. The reduction in coliform count by alum at high dose of 3.0 g/L and 5.0 g/L may be due to osmotic stress on microbial cells, caused by high salts presence in treated water. Also, low pH can have resultant negative effect on microbial growth [25, 26].

3.3 Effect of Extracted Proteins of *M. oleifera* Seeds Powder on Water Quality Parameters

The pH, turbidity and coliform count of raw water decrease with increase in dosage of extracted seeds proteins (Fig. 6, 7, and 8). The pH of the treated water was within the NIS recommended level. Turbidity decreases linearly with increase in dosage of extracted seeds protein. Though the turbidity obtained at dose level of 100ml/L was higher than NIS maximum permitted value, a 96.67% and 88.03% turbidity reduction was achieved for raw water and wastewater respectively. There was an increase in conductivity and TDS when the dose of extracted *M. oleifera* seeds protein was increased (Fig. 9 and 10), probably due to presence of dissolved salt in the protein eluent.

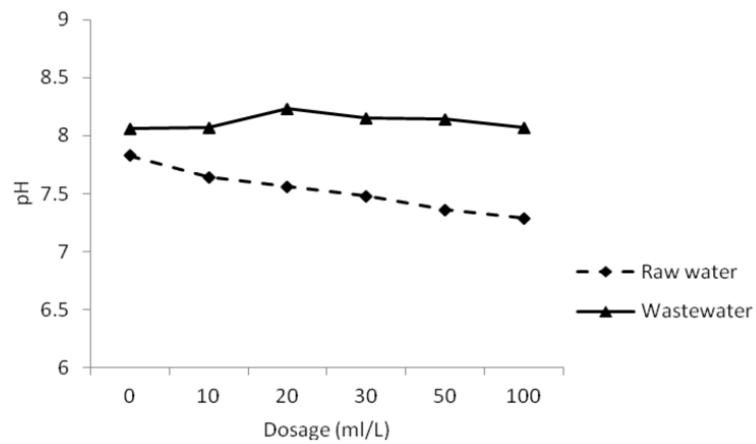

Figure 6: pH of water samples treated with *M. oleifera* seeds protein extract.

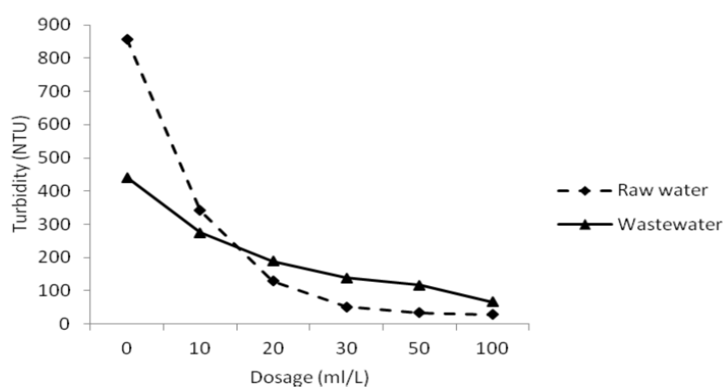

Figure 7: Turbidity (NTU) of water samples treated with *M. oleifera* seeds protein extract.

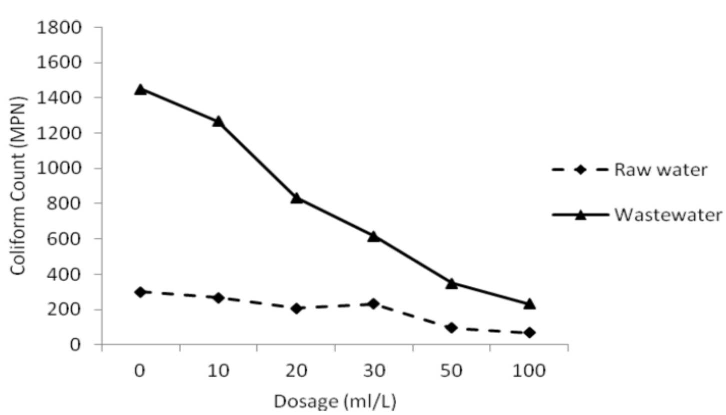

Figure 8: Coliform count (MPN) of water samples treated with *M. oleifera* seeds protein extract.

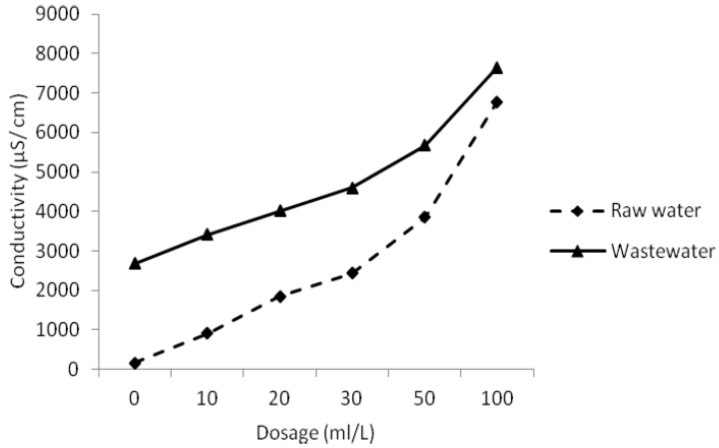

Figure 9: Conductivity (µS/cm) of water samples treated with *M. oleifera* seeds protein extract.

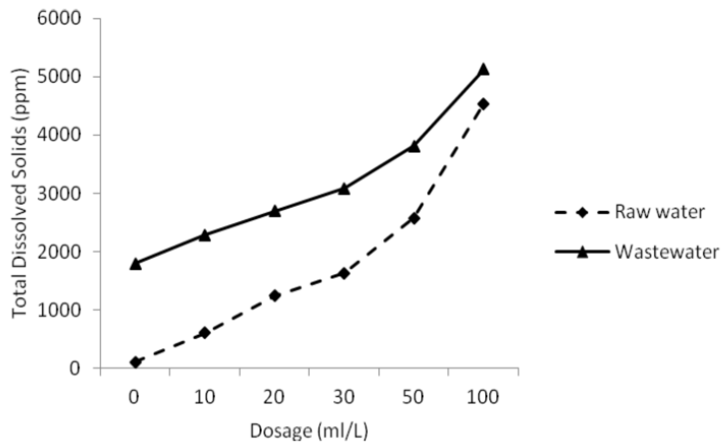

Figure 10: Total Dissolved Solids (ppm) of water samples treated with extracted *M. oleifera* seeds protein.

IV. CONCLUSION

M. oleifera seed was shown to be a potential biocoagulant, for treatment of both raw river water and contaminated wastewater from commercial activities. The effective biocoagulant activity of the defatted seed powder is an added advantage, as the oil of the seeds can be extracted while the cake is used as biocoagulant.

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