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Moringa oleifera Seed Powder for Improvement of the Microbiological and Physicochemical Quality of Sullage and River Water

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Abstract:
Water security is becoming an increasingly more alarming global issue hence the need for more sustainable approaches to wastewater treatment and water remediation. This study explores the capacity of extracts of the powdered seed of *Moringa oleifera* to improve the physicochemical and microbiological qualities of water from Okporoama stream in Umuariaga, and sullage from the cafeteria of the Michael Okpara University of Agriculture, both in Umudike, Nigeria. Following the application of the powder extracts, the variations in selected physicochemical and microbiological parameters of the water samples were monitored using standard techniques. The results revealed that for sullage and river water samples respectively, turbidity reduced by 60% and 63.6%; total organic carbon by 62% and 68.1%; chemical oxygen demand (COD) by 68.5% and 63.7% and biochemical oxygen demand (BOD) by 82.2% and 85.7%. pH values dropped from relatively neutral levels of around 7.0 to about 6.0 for both sullage and river water. Correlation analysis showed a strong positive correlation between BOD_5 and COD for both river water and sullage. The observed biodegradability indices were 0.430 and 0.412 for sullage and river water respectively. The heterotrophic and coliform bacteria were completely removed by around day 5 of the study. The findings established the efficiency of *M. oleifera* seed powder as a coagulant in wastewater treatment and a biotreatment agent for the improvement of surface water quality.

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INTRODUCTION

Water security has been highlighted as one of the chief challenges facing mankind. It is a challenge that cuts across several spheres including health, ecology, and even human rights. Across the globe, the competition for limited water resources has become of paramount concern (Aeschbach-Hertig and Gleeson 2012). Water is a vital resource; its uses range from the basic, like drinking, domestic and sanitary activities and recreation, to the more complex, like transportation, tourism, electricity generation, and infrastructure. These anthropogenic activities strongly influence the quantity and quality of usable water resources in any region (Dunca, 2018). Industrial, agricultural and other anthropogenic activities consume considerable quantities of water and generate equally copious amounts of liquid waste (effluent); domestic wastewater also referred to as sullage falls into the category of liquid waste. This underscores the necessity for research into effective water management and recycling technologies.

Effluent will often follow natural or man-made drainage patterns into ground or surface water. Rivers have been described as one of the most ancient waterbodies in existence (Higler, 2012). The quality of river water is crucial as these surface water resources often serve as the sole source of drinking, domestic, agricultural and recreational water supplies to small communities in rural areas in the sub-Sahara (Higler, 2012; Dunca, 2018). In a lot of developing countries, drainage channels are usually constructed by evacuating the raw effluent directly into local rivers thereby tainting the freshwater and degrading the riverine ecosystems (Olorode *et al*, 2015). Jin *et al*. (2017) associated this practice with eutrophication and rapid deterioration in water quality in a lake in China. Effluent has equally been associated with water-borne diseases (Ayeni, 2014).

Improvement of clarity and removal of biological contaminants including microorganisms are the key goals for treatment of surface water and sullage (Idris *et al*., 2016). For effluent, it is important to bring these parameters to acceptable levels such that there is minimal impact on the environmental quality when discharged. Coagulation–Flocculation is an important part of the traditional water/ wastewater treatment process. The coagulants used in the process may be inorganic salts, synthetic organic polymers or natural coagulants (Gautam and Saini, 2020). The inorganic salts and synthetic compounds have been known to bioaccumulate and impact strongly on the pH levels in receiving water bodies. Furthermore, they are opined to trigger disease conditions; one such example is Alzheimer's disease which has been connected to residual aluminium in chemically treated water (Khalid *et al*., 2020). Biological coagulants are preferred as they are considered more environmentally friendly and therefore, sustainable. They are also relatively inexpensive and produce lower quantities of sludge that is more readily biodegradable compared to the sludge generated using non-biological agents.

Moringa oleifera (MO), the most popular species in the *Moringa* genus, is typically found in countries of the tropics and sub-tropics. Its long tap root endows it with drought resistance. MO seed extracts have been studied extensively for their bio-sorption and coagulation properties, a quality attributed to the presence of natural polyelectrolytes in the seed (Idris *et al*., 2016; Khalid *et al*., 2020). There is a strong push for the development of more sustainable approaches to wastewater treatment and water remediation. This study explores the capacity of extracts of the powdered seed of *Moringa oleifera* to improve the physicochemical and microbiological qualities of Okporoama stream in Umuariaga, and sullage from the cafeteria of the Michael Okpara University of Agriculture, both in Umudike, Nigeria.

MATERIALS AND METHODS

Sample Collection

Sullage samples were collected from the cafeteria of the Michael Okpara University of Agriculture in Umudike, Nigeria while surface water samples were collected from the

Okporoama stream in Umuariaga, Umudike, Nigeria.

The composite sampling technique was used. A total of five composite samples were collected over time from each location. The samples were collected in clean sterile plastic bottles. Analysis was done within 3 hours of sample collection.

Preparation of the *Moringa oleifera* (MO)–based Coagulant

The MO seeds were obtained in pods from the Michael Okpara University of Agriculture, Umudike where they were also authenticated and identified. The seeds were sorted out manually and then ground using a laboratory blender. The powered samples were sterilised, stored in labelled plastic bags and kept at room temperature in the dark until required.

The stock solution was obtained by mixing the powder with sterile distilled water to achieve a 2% suspension. The suspension was agitated using a magnetic stirrer at 100rpm for about 15 minutes to extract the active agents in the powder. The milky solution was then filtered using Whatman's No. 1 filter paper. This filtrate was used as the coagulant (Jahn, 1984).

Set-Up for Water Treatment

After addition of the MO-based filtrate to the wastewater, the mixture was stirred at 120 rpm for 1 minute then at 40 rpm for 20 minutes to facilitate the sedimentation process. The treated wastewater was covered and left to settle for about an hour before initial sampling (USEPA, 2012). The control experiments consisted of wastewater samples with no MO-based coagulant applied.

The effect of the MO-based coagulant on the physicochemical, biological and microbiological properties of the wastewater was observed. Sampling was done at 24-hour intervals over a 7-day period. Samples were collected without disturbance using a sterile pipette.

Determination of Physicochemical Properties of Wastewater

Determination of Turbidity

Based on the method recommended by ASTM (2012), the turbidity was determined using a turbidity meter (Jenway, UK) and the standard solutions.

Determination of pH

The pH values of the water samples were determined by a combined glass calomel electrode and a pH meter (Jenway, UK).

Determination of Total Organic Carbon (TOC) Content

The colorimetric method recommended by APHA (2005) was employed.

Biological Analysis of Water Samples

Determination of Chemical Oxygen Demand (COD) and Determination of Five Day Biological Oxygen Demand (BOD_5)

The COD and BOD_5 of the samples were determined using the method outlined by APHA (2005) and USEPA (2012). COD was determined by the dichromate closed reflux method. The BOD_5 was approximated by measuring the dissolved oxygen levels on days 1 and 5 and then calculating the difference between the two days.

Determination of Correlation between COD and BOD_5 and the Biodegradability Index (BI)

The biodegradability index is a measure of the relationship between chemical oxygen demand and five-day biochemical oxygen demand for the water samples. It is described as the BOD_5/COD ratio at different points. Plots of BOD_5 values against COD values were obtained and used in regression analysis to develop the corresponding correlation coefficients (Abdalla and Hamman, 2014).

Enumeration of Microorganisms

The media used were nutrient agar (NA) and Eosin Methylene blue (EMB) agar. All the media were prepared according to the manufacturers' instructions and sterilised by autoclaving (Cheesbrough, 2006).

The total viable bacterial count (TVC) was determined using the pour plate technique on nutrient agar. Following serial dilution, 1 ml aliquots of the water samples were plated unto the appropriate medium in triplicate with incubation at 35°C for 48 hours. Only plates with counts of 30 – 300 colonies were considered (Cheesbrough, 2006).

Determination of Total Coliform Count

The multiple tube fermentation technique as described by APHA and AWWA (2012) was employed in the enumeration of coliform bacteria in a known volume of treated wastewater sample. The coliform bacteria are detected and quantitated by their ability to grow and produce gas in lactose-containing liquid medium at 37°C for 48 hours. Following incubation, the abundance of coliforms was determined by noting the number of positive and negative tubes and comparing this to a standardised MPN table. The confirmatory test for the presumptive positive tubes in the total coliform enumeration was carried out to verify the presence of coliform and to detect any false positive results. The presence of coliforms was confirmed via isolation on EMB medium.

RESULTS AND DISCUSSION

The baseline characteristics of the wastewater samples are shown in Table 1 while Figure 1 illustrates the observed changes in turbidity, total organic carbon and pH of the water and wastewater samples tested.

Table 1: Baseline Characteristics of Wastewater

Parameters	Sullage	River Water
Colour	Cloudy	Colourless
Odour	Slightly Offensive	Odourless
Temperature (°C)	30.0	28.0
Turbidity (NTU)	45.0	4.4
pH	7.05	7.07
TOC (mg/L)	132.31	130.43
COD (mg/L)	310.24	306.40
BOD_5 (mg/L)	126.02	124.72
TVC (LogCFU/ml	4.92	4.83
TCC (LogCFU/ml	0.799	4.613

TCC – Total Coliform count; TVC – Total Viable Bacterial Count; TOC – Total Organic Carbon; BOD_5 – Five-day Biochemical Oxygen Demand; COD – Chemical Oxygen Demand

The pH levels were within the recommended upper limits of between 6.5 and 8.5 for the most part (WHO, 2017; NIS, 2007). Values had dropped below this by the end of the investigation. The TOC values were in the medium class at the onset of the study but fell to acceptable limits of below 80 mg/L based on the categorisation of Tchobanoglous and Burton (2003).

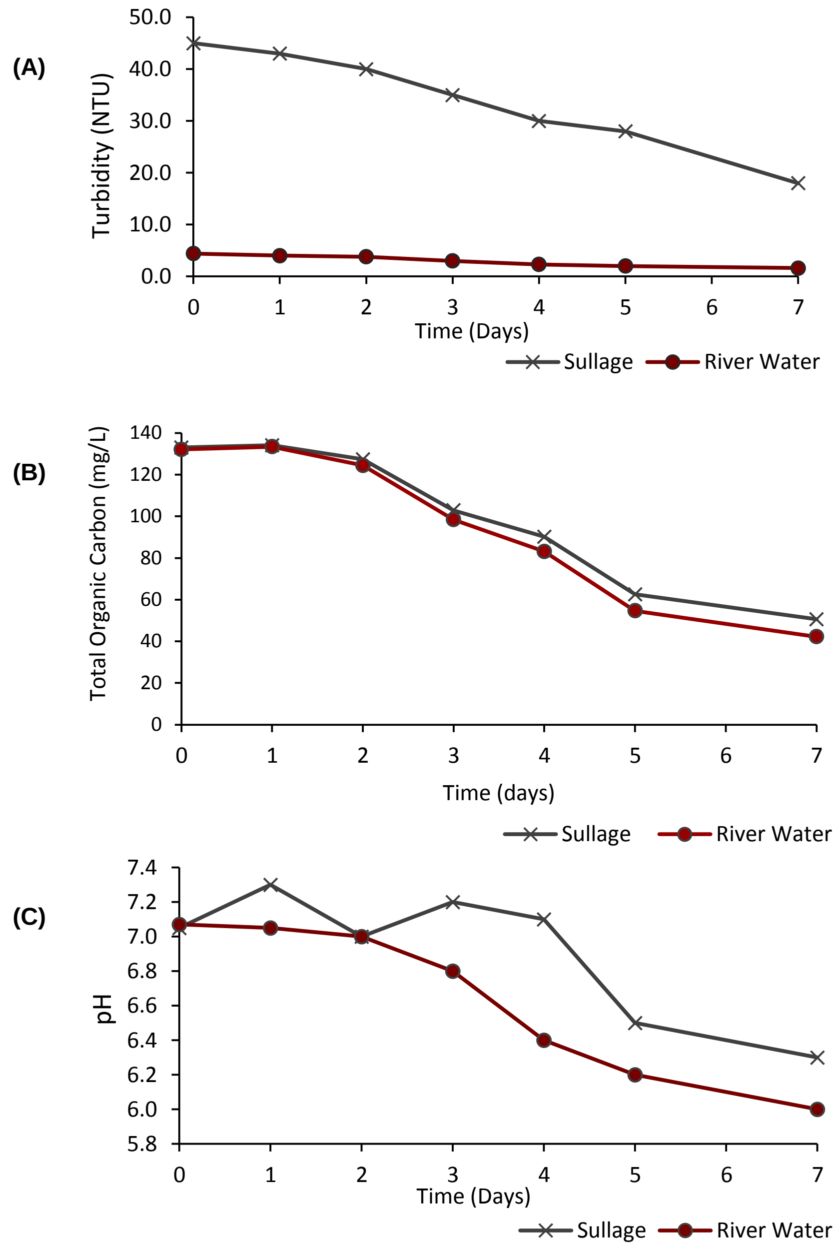


Fig. 1. Variations in Physicochemical Properties of Samples
(A) shows variations in mean turbidity while **(B)** and **(C)** show variations in mean total organic carbon and pH levels respectively

The COD values dropped from 312.2 mg/L to 98.2 mg/L and 309.32 mg/L to 112.3 mg/L for sullage and river water samples respectively while for BOD_5, levels decreased from 134.2 mg/L to 23.87 mg/L and 127.3 mg/L to 18.2 mg/L for sullage and river water samples respectively (Figures 2 and 3). Correlation analysis showed a strong positive correlation between BOD_5 and COD for both river water and sullage. The biodegradability indices based on mean values for the 5-day biochemical oxygen demand and the chemical oxygen demand for the wastewater samples were 0.430 and 0.412 for sullage and river water respectively showing the susceptibility of the water samples to biological treatment techniques.

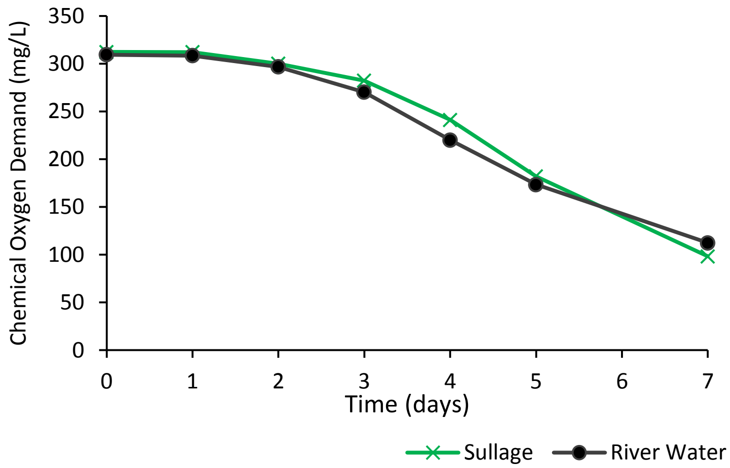
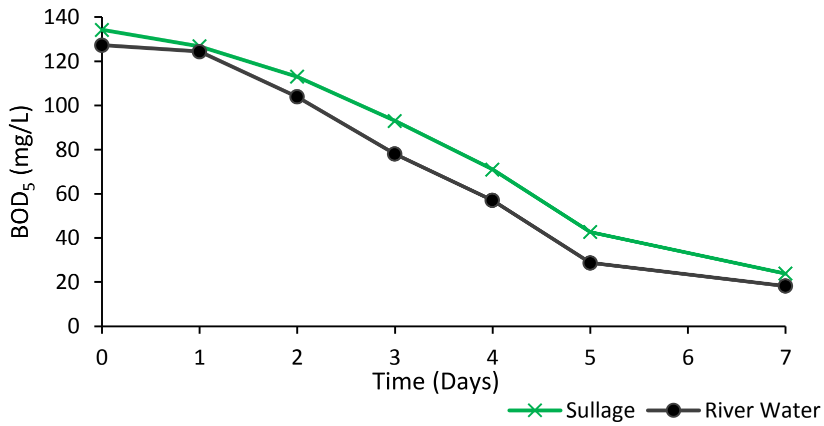


Fig. 2. Observed Variations in Mean Chemical Oxygen Demand

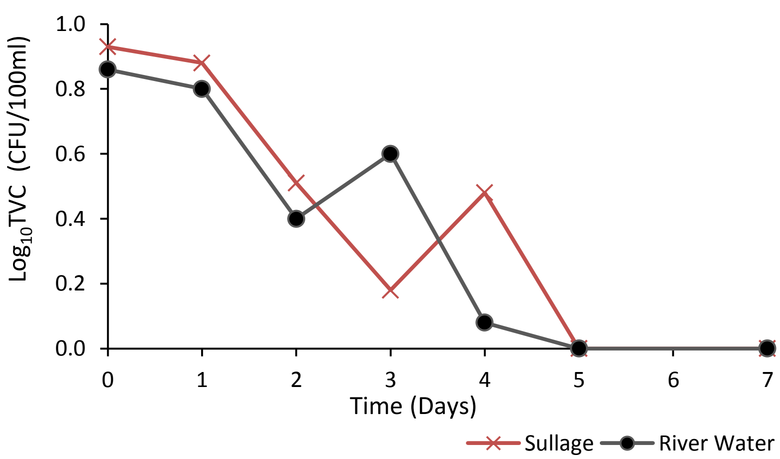


BOD_5 – Biochemical Oxygen Demand

Fig. 3. Observed Variations in Five-day Biochemical Oxygen Demand

The coliform bacteria and total viable heterotrophic bacteria present in the all samples were completely eliminated by Day 5 of the study. The variations in total viable bacterial counts and total coliform counts are depicted in Figure 4. The baseline river water samples had higher coliform counts than the sullage samples (Table 1) which implies the presence of faecal matter in the stream.

(A)



(B)

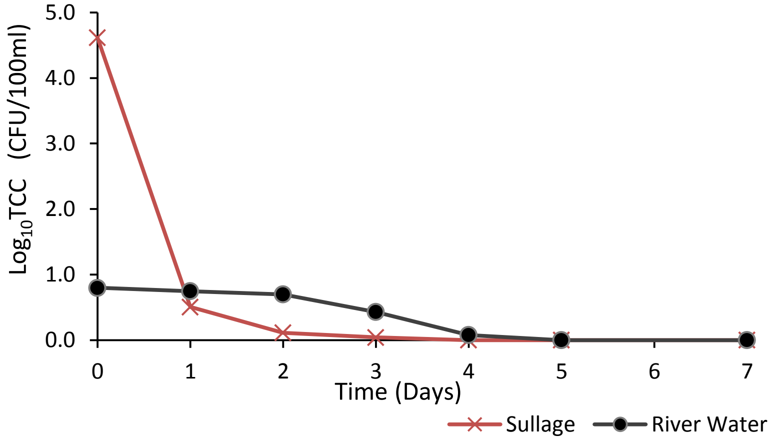
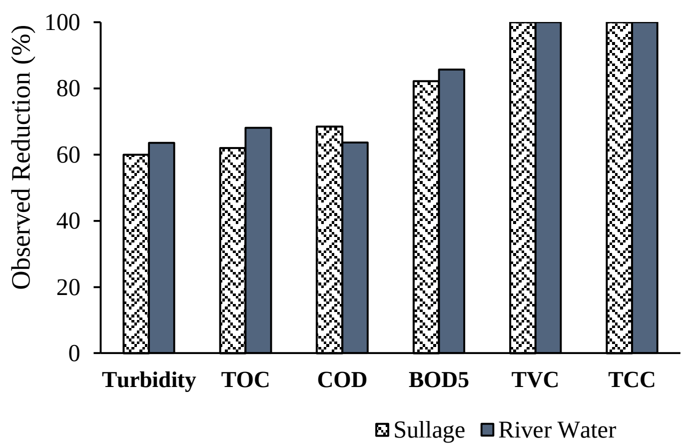


Fig. 4. Variations in Microbial Abundance in the Wastewater during the Study
(A) shows variations in mean total viable bacterial counts while (B) shows the variations in mean total coliform counts

The observed percentage reduction in all the parameters studied is shown in Figure 5. For sullage and river water samples respectively, turbidity reduced by 60% and 63.6%; total organic carbon by 62% and 68.1%; chemical oxygen demand by 68.5% and 63.7% and biochemical oxygen demand by 82.2% and 85.7%.



TCC – Total Coliform count; TVC – Total Viable Bacterial Count; TOC – Total Organic Carbon; BOD_5 – Five-day Biochemical Oxygen Demand; COD – Chemical Oxygen Demand

Fig. 5. Reduction Observed in Selected Parameters on Day 7

The observed decrease in turbidity is very likely due to the precipitation of the suspended solids as sludge. Although the level of reduction in turbidity recorded in the current study is similar to the value of 63.7% obtained by Dehghani and Alizadeh (2016), it is much lower than found in other comparable studies. In studies by Vieira *et al*. (2010), Ndabigengesere and Narasiah (1998) and Hendrawati et al. (2016), turbidity in water samples reduced by 98.0%, 89.2% and 97.5% – 98.6% respectively upon application of MO seed extracts. The concentration of the MO seed powder extract and the baseline turbidity level could be significant factors. Rodríguez-Núñez et al. (2012) also recorded 88.9% reduction in turbidity on water samples with an initial turbidity of 118 NTU, using a dosage of 2.5% compared to the 2% used in the current study. The higher turbidity reduction level recorded by Hendrawati et al. (2016) may be attributed to the higher concentration of MO-based coagulant being about five times more than used in the present research work. This conclusion is, however, not supported by Adelodun et al. (2020) and

Suhartini et al. (2013) who maintained that the concentration of the MO seed powder did not impact the efficacy of the coagulation process as there were no significant differences in turbidity outcomes using different concentrations of MO seed coagulant in their studies. MO extracts have been confirmed to have limited impact on turbidity removal at low turbidity levels (Prasad and Rao, 2013). The baseline turbidity in the current study for both river water and sullage were less than a third of baseline values in the compared studies (Vieira *et al*., 2010; Hendrawati *et al*., 2016; Rodríguez-Núñez *et al*., 2012).

The reported drop in pH levels in this study is greater than found in other studies. A study in Malaysia used MO seed powder to treat industrial wastewater with initial pH close to neutral. At 2% concentration, pH declined by only about 4% (Eman *et al*., 2014). This is somewhat negligible when compared to the drop of 10 – 15% seen in the current study across samples of sullage and river water. Most comparable studies record increases in pH and not decreases as in the current study. *M. oleifera* leaf extracts raised the pH value in wastewater from approximately 1.7 to 5.8 (70%) in a study by Khalid et al. (2020). High TOC in wastewater is indicative of a high environmental pollution potential and underlines the necessity for treatment prior to dumping (Penn *et al*., 2003). The observed decrease in the TOC levels in the present study by an average of 62.0% for sullage and 68.1% for riverwater conflicts with certain reports of increased TOC following the application of MO seed-based coagulant (Awad *et al*., 2013). Okoya et al. (2020), however, similar to the current study albeit higher, recorded an 88.9% reduction in TOC during the treatment of wastewater using activated MO seed powder.

The biodegradability index (BI) defines the potential success of biological treatment for any water sample. When the BI values are within the limits of 0.3 – 1.0, the water samples are considered biodegradable and can be effectively treated biologically. At values less than 0.3 or over 1.0, the sample is considered unsuitable for biological treatment (Rim-Rukeh, 2016). It is expected that BI values for untreated municipal wastewater will characteristically be between 0.4 and 0.8 but may get up to 10 for industrial wastewater suggesting that such wastewater could not be treated using biological means (Achoka, 2002). Rim-Rukeh (2016) treated wastewater from 6 different cassava mills in Nigeria and described mean biodegradability indices of 0.507 – 0.548 not far from values obtained in the current study. They further concluded that there was a strong positive correlation between BOD_5 and COD as also found in the present study. The strong positive correlation between BOD_5 and COD is buttressed by the report of Abdalla and Hammam (2014).

The preliminary BOD and COD values were far greater than the limits set by Federal Ministry of Environment, FMoE (Nigeria) (1995) but were successfully lowered to levels within the acceptable disposal limits of 20mg/L and 120 mg/L for BOD_5 and COD respectively (FMoE, 1995; DPR, 2002). Both the COD and BOD baseline levels in sullage and river water were classed as medium range, however, following treatment with the MO seed powder, the levels were brought down to low based on the classification of Tchobanoglous and Burton (2003). A study in Jakarta observed 11.7% and 18.0% drops in BOD_5 for wastewater and groundwater respectively following the application of 7% MO seed powder (Hendrawati *et al*., 2016). The elimination of bacteria in the samples is not unexpected as the antibacterial capabilities of *M. oleifera* have been reported by several researchers (Lürling and Beekman, 2010; Bukar *et al.*, 2010). Reduction in BOD is closely linked to reduction in heterotrophic and coliform bacterial abundance. Ugwu *et al*. (2017) reported a 97.5% reduction in coliform counts in the treatment of sullage using MO seed powder. Likewise, reductions of 97.88 – 99.96% and 84.0% and 90.0 – 99.0% were documented for coliforms by Osei (2009) and Pritchard et al. (2010).

Better reduction results have been achieved in other studies when the MO seed-based coagulant was modified or used in combination with other organic agents that could serve as immobilisation or binding agents (Suhartini *et al*., 2013; Rosmawanie *et al*., 2018). Investigations by Okoya et al. (2020) underscored that activation of the MO seed powder produced better results in both physicochemical and biological parameters of the textile plant wastewater samples. Certain researchers, likewise, recommend the combination of the MO-based coagulant with other coagulant agents. Dehghali and Alizadeh (2016) achieved a 38.6% reduction in COD in oil refinery effluent using MO seed-based coagulant. When the MO coagulant was combined with alum, an increased reduction of about 50.4% was reached.

CONCLUSION

Based on the biodegradability indices, domestic wastewater and tainted river water were found to be susceptible to biological treatment methods. A 2% concentration of extracts from *Moringa oleifera* seed produced improvement in both the physicochemical and biological properties of sullage and tainted river water. At the end of the 7-day study, for sullage and river water samples respectively, turbidity reduced by 60% and 63.6%; total organic carbon by 62% and 68.1%; chemical oxygen demand by 68.5% and 63.7% and biochemical oxygen demand by 82.2% and 85.7%. The total heterotrophic and coliform bacteria were completely removed by the coagulant. The findings established the efficiency of *M. oleifera* seed powder as a coagulant in wastewater treatment and an agent for the improvement of surface water quality.

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CONFLICT OF INTEREST

There is no conflict of interest.

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