Tanzania Journal of Science 47(1): 19-33, 2021
ISSN 0856-1761, e-ISSN 2507-7961

Wastewater Clarification and Microbial Load Reduction Using Agro-Forestry and Agricultural Wastes

Joel B. Njewa, Ephraim Vunain* and Timothy Biswick
Department of Chemistry, Chancellor College, University of Malawi, P. O Box 280, Zomba, Malawi
**Corresponding author, e-mail: evunain@cc.ac.mw*

Received 19 Oct 2020, Revised 21 Dec 2020, Accepted 29 Dec 2020, Published Feb 2021

Abstract

Five agro-wastes, namely: cassava peels, Irish potato peels, *Jatropha curcas* seeds, *Moringa oleifera* seeds and rice husks were investigated as plant-based coagulants for turbidity and microbial load removal from sewage wastewater. *Moringa oleifera* and *Jatropha curcas* seeds before usage were defatted with 95% ethanol and the active coagulating component was extracted with 1.0 M NaCl. For the other three agro-wastes, their ashes were evaluated for turbidity and microbial load removal. The ash was prepared through pyrolysis of the raw biomass at a temperature of 800 °C for 3 h. The effect of pH, coagulant dose, settling time on turbidity reduction was investigated using sewage wastewater with an initial turbidity of 464.11 ± 0.13 NTU. The microbial load removal of the coagulants was investigated using three media onto which 1 mL of treated wastewater was poured and streaks were made using sterile swabs and then incubated at 37 °C for 24 h. *Jatropha curcas* and rice husks ashes exhibited the maximum turbidity reduction of 97.0% and 95.0%, respectively at pH 2, whereas for *Moringa oleifera*, the highest turbidity removal (96.0%) was achieved between pH 6 and 10. Additionally, it was observed that the electrical conductivity and total dissolved solids of the clarified wastewater increased as the doses were increased for all coagulants due to inorganic salts contained in seeds. The microbial load understudy was successfully reduced in wastewater clarified with *Jatropha curcas* and rice husks ashes, contrary to *Moringa oleifera* that increased microbial load content. The raw biomass and ashes for cassava and Irish potato peels could not clarify sewage wastewater. The results from this study have demonstrated that plant-based materials used performed effectively in turbidity and bacteria removal from sewage wastewater.

Keywords: Domestic Wastewater, Clarification, Agro-Wastes, Turbidity, Microbial Load.

Introduction

Access to safe drinking water is important in safeguarding the well-being of mankind. However, potable water availability has decreased universally due to climate change, and depletion and contamination of water resources as a result of high population growth (Okello et al. 2015). Freshwater quality is endangered because of pollution by domestic, industrial and agricultural wastes. The quantity of domestic and industrial wastewater discharged into the world's rivers is increasing at a disturbing rate rendering many surface water bodies unsuitable for domestic uses (Adeniran et al. 2017). Therefore, domestic and industrial wastewaters require appropriate treatments to remove undesirable substances before discharging to receiving water bodies.

Conventional wastewater treatment facilities generally adopt collective treatment processes (Ali et al. 2009). The two major processes in conventional water and wastewater treatments are clarification and disinfection processes (Ugwu et al. 2017). The clarification process comprises coagulation, flocculation and sedimentation sequence. The natural organic matter, turbidity, algae, colour, and microbial load are removed successfully in the clarification stage (Ghernaout and Ghernaout 2012). Coagulation is a neutralisation process that involves destabilisation of negatively charged suspended particles in the water by adding positively charged coagulant. Flocculation process occurs as a result of destabilisation process in which the destabilised particles form larger flocs that eventually settle out by gravity a process termed as sedimentation and finally removed as sludge before disinfection stage follows (Arunkumar et al.2019). The coagulation-flocculation system uses inorganic coagulants such as aluminium sulphate (alum) and polyaluminium chloride (PAC). Although these chemicals are recognised as effective, their usage is associated with some setbacks such as relatively high costs thereby limiting their usage in developing countries, less effective in low temperatures and recent scientific evidence has linked the existence of Alzheimer's disease in a human to the usage of aluminium in water and wastewater treatments (Tunggolou and Payus 2017).

In view of the foregoing, plant-based materials are gaining more attention as substitutes for synthetic chemicals for water and wastewater purification (Akhilesh and Nisa 2018). The plant-based biomaterials are presumed safe for human health, cheap, locally available and environmentally friendly (Yin 2010). It has been reported that rural communities in some African countries utilize crude seed extracts to clear turbid river water (Yin 2010). Jahn (1984) reported *Moringa oleifera* as a coagulant after studies in the Sudan showed that Sudanese village women used *Moringa oleifera* at home to clear the turbid Nile water. Active coagulants from the seeds of *Vigna unguiculata* and *Parkinsonia aculeate* have been used in Tanzania to purify drinking water in the rural communities (Marobhe et al. 2007).

The utilization of agricultural waste peels, biomass and industrial by-products as adsorbents and coagulants in water and wastewater have been reported by some researchers (Singh et al. 2018). These materials have been used as such and sometimes after some minor treatments, and are widely known as low-cost adsorbents. In Malawi, every year large quantities of agricultural and agroforestry wastes are produced through farming activities. Most of the generated wastes have no further uses and are consequently disposed of through different methods which are threats to public health and the environment. In our previous work, we demonstrated that low cost activated carbon could be produced from baobab fruit shells and that the activated carbon was very effective in the removal of methylene blue from wastewater (Vunain and Biswick 2019). In this study, *Moringa oleifera* seeds, *Jatropha curcas* seeds, cassava peels, potato peels and rice husks as agro-wastes were selected as coagulants in water clarification of wastewater collected from Chikanda Wastewater Treatment Plant (WWTP), Zomba City, Malawi. This work is, therefore, a continuation of the previous work which is aimed at adding value to the various agricultural and agroforestry wastes that are produced in Malawi.

Materials and Methods

Wastewater sample collection

Wastewater samples were collected at an influent point of the Chikanda Wastewater Treatment Plant (WWTP) before the wastewater goes through a sequence of the treatment processes (Figure 1). Some physico-chemical parameters of the wastewater such as total dissolved solids, conductivity, pH and turbidity were measured on-site using portable meters. The wastewater samples were collected in 2.5 L sterilised polyethene bottles and transported to the laboratory using iced cooler box. The samples were transferred to 20 L clean plastic bucket for homogenisation. The analysis was done within 24 h after sampling.

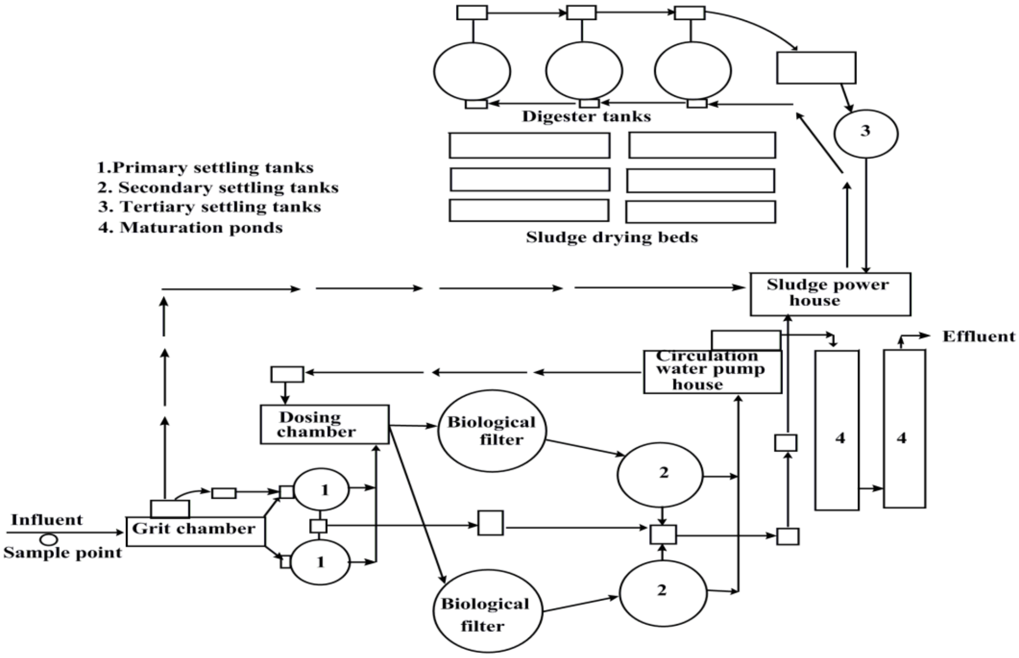

Figure 1: The schematic diagram for Chikanda wastewater treatment plant.

Preparation of raw materials powder

The cassava and Irish potato peels, and rice husks used in the study were sourced within Zomba. *Moringa oleifera* and *Jatropha curcas* seeds were collected in Machinga and Ntcheu Districts, respectively. Good matured seeds of dry pods for *Moringa* and *Jatropha* were selected and used. The seeds were removed from the pods and crushed using mortar and pestle before the extraction process. The other raw materials were sequentially washed with tap water followed by distilled water to get rid of surface impurities. They were then sun-dried for a day, and thereafter, oven-dried at a temperature of 80 °C for 12 h. Finally, they were crushed with mortar and pestle, sieved with mesh size 250 µm and stored in labelled zip-lock bags pending usage.

Extraction of *Moringa oleifera* and *Jatropha curcas* seed coagulant

To prepare the coagulant, 5 g of the *Moringa oleifera* and *Jatropha curcas* powder were blended with 100 mL of distilled water at room temperature for several minutes in order to extract the coagulating proteins of the *Moringa* and *Jatropha* powder. The resulting suspension was filtered through 250 µm sieve.

Preparation of ashes from cassava peels, Irish potato peels and rice husks

The ashes for the cassava peels, Irish potato peels and rice husks were prepared through pyrolysis using a muffle furnace. 100 g raw biomass was weighed and introduced in a preheated crucible. The crucibles and their contents were heated at a temperature of 800 °C for 3 h in a limited supply of oxygen. The furnace was allowed to cool to room temperature and crucibles were retrieved, and placed in a desiccator. The resulting residues were crushed with mortar and pestle and then sieved with 250 µm mesh size to get fine particles. The ashes were kept in airtight plastic bags until used.

Surface chemistry

The surface chemistry of the prepared materials was studied using infrared spectroscopy (Shimadzu Model 8400) using the KBr method. The spectra were recorded in the range of 400 to 4000 cm^{-1}.

Batch experimental procedure

Batch studies on the potential of cassava peels, Irish potato peels, *Moringa oleifera* seeds, *Jatropha curcas* seeds, and rice husks ashes as plant-based coagulants in turbidity and microbial loads removal from domestic wastewater were conducted at room temperature (25 °C). The raw domestic wastewater samples were first mixed thoroughly in cleaned and sterilised 20 L plastic storage buckets and homogenised. A set of six labelled beakers (500 mL) in duplicates were used. 400 mL was sampled from 20 L homogenised wastewater and introduced to each of the labelled beakers. The beakers were then dosed with different quantities of coagulants ranging from 5 mL to 25 mL for *Moringa* and *Jatropha* extracts and 20 mL to 100 mL for the biomass as well as the ashes. The beakers containing the raw wastewater were agitated at an initial speed of 150 revolutions per minute (rpm) for 10 min and finally continued at a reduced speed of 50 rpm for another 15 min. The agitation process is important as it provides thorough mixing of coagulants and sewage wastewater leading into the formation of flocs. The treated wastewaters were allowed to settle for 1-6 h, to allow coagulation, flocculation and sedimentation to complete. The supernatants were then drawn from treated wastewater for turbidity and microbiology analysis. Aluminium sulphate (1%) was used as a positive control in wastewater clarification.

Effect of solution pH and settling time on turbidity reduction

The effect of settling time on turbidity removal was studied for all the three plant-based materials under the study. 400 mL wastewater was used in the study while all other parameters were kept constant. The optimum dosage for each coagulant previously determined was adopted. The samples were first agitated with an orbital shaker with an initial agitation speed of 150 rpm for 15 min and reduced to 50 rpm for 10 min. The wastewater samples were withdrawn and allowed to sit for 5 h. Then 10 mL of treated wastewater were drawn from each beaker at a one-hour interval to determine the level of turbidity. The pH adjustments of wastewater were carried out by dropwise addition of either 0.1 M HCl or 0.1 M NaOH until the desired pH was attained. The study focused on the pH range of 2–12 with 400 mL of wastewater and the optimum dose established earlier for each coagulant was used. The beakers were mounted on the shaker and agitated at an initial speed of 150 rpm for 10 min and reduced to 50 rpm for another 15 min. After the designated time elapsed, they were withdrawn and allowed to settle for 6 h.

Microbial assay

The antibacterial assay was carried out to determine the microbiological quality in domestic wastewater. The pre- and post-treatments with various doses of plant-based coagulants were performed to observe if there was a positive or negative difference. The pour plate method was used to determine if there was a reduction in the number of colonies in the treated wastewater samples. A sequence of dilutions from 1st, 3rd and up to 5th of wastewater sample was prepared before and after treatment using sectored petri dish media. Nutrient Agar (NA), Red Violet Broth Agar (RVBA) and MacConkey Broth (MCB) were used (Almatar et al.2014). About 1 mL was sampled out and poured onto all the surfaces of the media and streaks were made using sterile swabs. The plated petri dish plates were incubated at 37 °C for 24 h. The number of colonies grown on the petri dish plates was then counted. A comparison of the number of colonies grown before and after treatment was noted. Wastewater treated with UV was used as the positive control for the microbial load content study (Macharia et al. 2016).

Results and Discussion

Surface chemistry

The FTIR spectra for the raw materials are presented in Figure 2, showing the presence of several functional groups. The peaks at 3444, 3429 and 3302 cm^{-1} are due to the stretching of OH (Magoling and Macalalad 2017). The bands at 2924–2854 cm^{-1} are associated with the asymmetric and symmetric stretching vibrations of CH (Labied et al. 2018). The bands observed at 2360-2341 cm^{-1} are associated with stretching vibrations of amines C≡N. The peaks observed at 1747–1651 cm^{-1} and 1654 cm^{-1} may be assigned to the stretching of C=O and C=C, respectively. The peaks appearing at 1593–1500 cm^{-1} and 1380–1384 cm^{-1} are attributed to stretching vibrations of COO$^-$, CH$_3$ and C=O groups, respectively (Labied et al. 2018, Rai et al. 2018). The band observed at 1458 cm^{-1} and 1238 cm^{-1} are associated with the stretching vibrations of aromatic and alkyl halides C-H. The peaks appearing at 1161–1114 cm^{-1} are linked with the vibrations of C-O (Belhamdi et al. 2016). The results have indicated the presence of the active groups such as COO$^-$, OH$^-$, CH$_3$, C=O and C–H that are responsible for flocculating activities in natural coagulants causing turbidity reduction during water and wastewater purification (Ang et al. 2020, Ramavandi 2014).

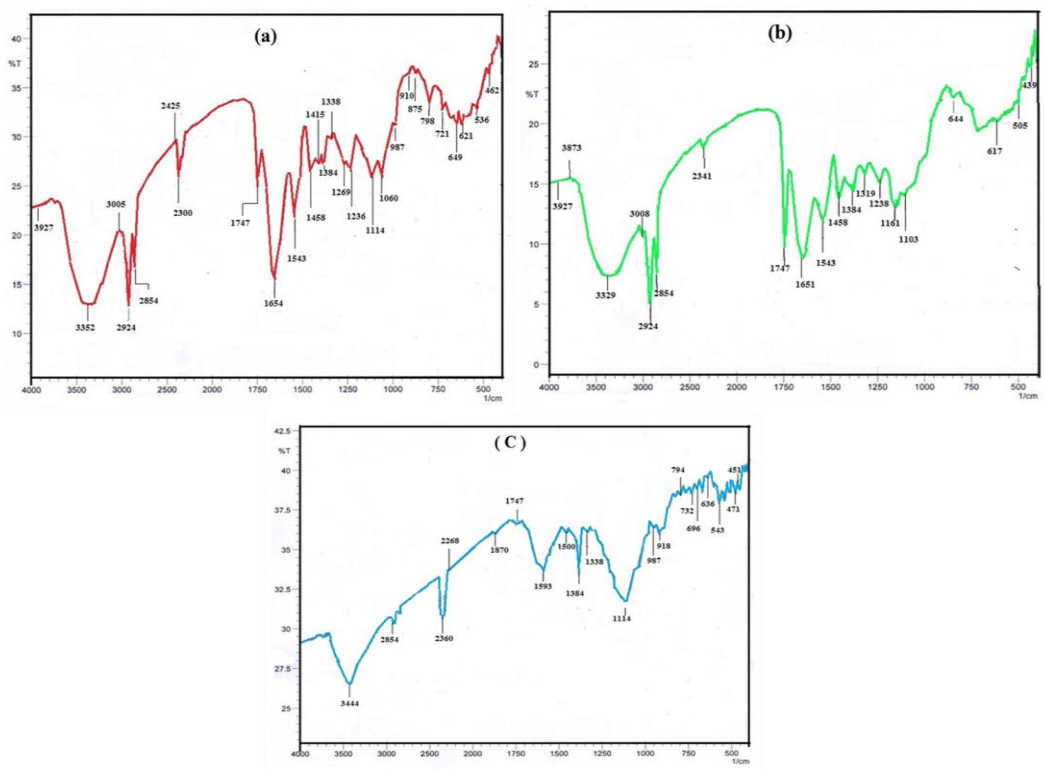

Figure 2: FTIR spectra (a) *Moringa oleifera* (b) *Jatropha curcas* and (c) Rice husks ash.

Clarification of wastewater

The turbidity removal from both water and wastewater is influenced by a number of operational parameters including dosage. Generally, insufficient dose or overdoses would affect the performance of flocculation. Solution pH is also another working parameter that requires consideration in the clarification of both water and wastewater. The pH affects the surface charge of the particles and the solubility of matter. Studying the range of pH values provides insights for optimum value in the reduction of turbidity and bacteria removal in the treatments of water and wastewater (Kian-Hen and Peck-Loo 2017). Settling time also plays a major role in the clarification stage especially in turbidity and microbial load removal. However, the raw biomass for cassava and Irish potato peels, as well as the ash, did not coagulate domestic wastewater and the results are not presented and discussed. Figure 3 displays clarified wastewater with different doses of *Moringa oleifera, Jatropha curcas*, rice husks ash and aluminium sulphate.

Figure 3: The display of clarified wastewater with different doses of (a) *Jatropha curcas* (b) *Moringa oleifera* (c) Rice husks ash and (d) Aluminium sulphate.

Effects of coagulant dosage *Moringa* extracts, *Jatropha curcas* seed extract and rice husks ash on turbidity reduction

The results for the effect of coagulant dosage on turbidity reduction are presented in Figure 6(a). *Moringa oleifera* and *Jatropha curcas* seed extracts exhibited the highest turbidity removal at a dosage of 15 mL per 400 mL. Moringa extracts reduced turbidity from 464.11 ± 0.13 to 9.01 ± 0.45 NTU (98.05%), whereas *Jatropha curcas* seed extracts decreased turbidity from 464.11 ± 0.13 to 22.68 ± 0.15 NTU (95.11%). Rice husks ash on the other hand exhibited maximum turbidity removal at a dosage of 100 mg per 400 mL, reducing the turbidity from 464.11 ± 0.13 to 18.19 ± 0.31 NTU (96.08%). Finally, the positive control aluminium sulphate with a 25 mL dose caused a reduction from 464.11 ± 0.13 to 9.83 ± 0.54 NTU (97.88%). All treated wastewaters were within the recommended value set by the Malawi Bureau of Standards for drinking water (< 25 NTU). It was observed that there was a direct relationship between turbidity removal and coagulant doses. However, higher doses for *Moringa* and *Jatropha* extracts were not effective in the clarification of sewage wastewater as they increased turbidity. This could be attributed to the fact that the high doses resulted in the saturation of the polymer bridge sites and initiated restabilisation of the destabilised particles to form inter-particle bridges (Tunggolou and Payus 2017). This suggests that the leading destabilisation mechanisms involved in *Moringa* and *Jatropha* could be adsorption and charge neutralisation (Muruganandam et al. 2017). These results are similar to those reported by Dehghani and

Alizadeh (2016), in which wastewater originating from an oil refinery was treated with 70 mg/L of *Moringa oleifera* extract and removed 63.70% turbidity. In a separate study conducted by Abidin et al. (2011), *Jatropha curcas* seed extracts with a dose of 120 mg/L, effectively removed 98% turbidity from kaolin synthetic wastewater. The capacity of *Moringa oleifera* to remove turbidity in water was also reported by Asrafuzzaman et al. (2011) in which a dose of 100 mg/L reduced turbidity from 100 to 5.8 NTU.

Effect of dosage on total dissolved solids (TDS)

The domestic wastewater was clarified with different doses of the plant-based coagulants and the TDS of the coagulated samples were measured. The results (Figure 4b) show that for the sewage wastewater clarified with *Moringa oleifera*, TDS increased from 870.37 ± 0.05 mg/L to 1183.76 ± 0.55 mg/L, whilst for *Jatropha curcas*, it increased from 870.37 ± 0.05 mg/L to 1161.83 ± 0.29 mg/L and rice husks ash increased from 870.37 ± 0.05 mg/L to 996.60 ± 0.53 mg/L and finally, water treated with aluminium sulphate, TDS increased from 870.37 ± 0.05 mg/L to 1416.52 ± 49 mg/L. The results showed that an increase in coagulant doses triggered an increase in TDS. Abidin et al. (2011) stated that *Moringa* and *Jatropha* seeds have similar chemical compositions like amino acids as well as inorganic salts such as magnesium, sodium, bicarbonate calcium and potassium. This suggests that during wastewater clarification, anions and cations are introduced especially with the increase in coagulant doses resulting in the high concentrations of the cations and anions. The increase could also be associated with the effects of the salt solution (1.0 M NaCl) used in the extraction process of coagulating proteins from the *Moringa* and *Jatropha* seeds thereby increasing TDS as the dose was increased. Aluminium sulphate produced a higher value of TDS as compared to the plant-based coagulants. This could be linked to lower sludge formation in plant-based materials than in aluminium that arises from the basis of the coagulant and coagulation processes (Beyene et al. 2016). The wastewater treated with different plant-based coagulants except for rice husks ash, gave higher values that reached the set standards of the World Health Organization (2011) for drinking water (< 1000 mg/L). These results agree well with those reported by Shan et al. (2017), in which river water was treated with *Moringa oleifera* seed cake and resulted in increased TDS from 353 ppm to 410 ppm after treatment. However, the TDS of the treated water could meet permissible standards if the seeds are purified before usage to remove salts that increase TDS during clarification process.

Effect of dosage on electrical conductivity

At the sample collection point, electrical conductivity **(**EC) was recorded as 1241.31 ± 0.31 µs/cm and brought to the laboratory for the study. After domestic wastewater was clarified with different doses of plant-based coagulants, the electrical conductivity of the treated samples were measured. It was noted that increasing the coagulant doses from 5 mL/400 mL to 100 mL/400 mL increased the electrical conductivity of the wastewater from 1241.31 ± 0.13 µs/cm to 1256.12 ± 0.06 µs/cm, 1258.49 ± 0.12 µs/cm, 1277.94 ± 0.06 µs/cm and 1245.96 ± 0.95 µs/cm for *Moringa oleifera* exctracts, *Jatropha curcas* extracts, aluminium sulphate and rice husks ash, respectively (Figure 4c). The increase in the electrical conductivity in the wastewater clarified with *Moringa oleifera* and *Jatropha curcas* may be associated with the presence of the polyelectrolytes in the seeds, as well as a contribution from the sodium chloride that was used in the extraction process (Fahey2005). The lower increase observed for wastewater treated with rice husks ash could be attributed to lower concentration of ions in the ash compared to the other. In a study conducted by Shan et al. (2017), river water treated with *Moringa oleifera* seed cake increased in electrical conductivity from 347 µs/cm to 390 µs/cm. However, in the present study, the wastewater clarified with aluminium sulphate produced the highest value increase in electrical conductivity. This could be attributed to the presence of aluminium and sulfate ions that might have increased electrical conductivity (Beyene et al. 2016). The treated wastewater samples with plant-based coagulants were within permissible limits for the Malawi Bureau of Standards (2005) in drinking water (< 3500 µs/cm).

Effect of dosage on pH

The pH affects the performance of coagulation and flocculation processes. An initial value of pH 7.3 was recorded at the sampling point. The post-treatment pH results are presented in Figure 4d, which shows that as coagulant doses increased, the pH of wastewater clarified with *Moringa*, *Jatropha* and rice husks ash increased to 7.89 ± 0.02, 7.82 ± 0.02 and 7.51 ± 0.01, respectively. However, treatment of the wastewater with alum led to a significant decrease in pH from 7.30 ± 0.01 to 6.28 ± 0.06. The pH increase in wastewater clarified with *Moringa oleifera* and *Jatropha curcas* extracts might be attributed to the presence of amino acids which were ionised and produced carboxylate and hydrogen ions (Muruganandam et al. 2017). The production of carboxylate ions after ionisation turned the water alkaline, as the amino acids accepted protons from the water thereby increasing the pH of the coagulated wastewater. The treated wastewater with aluminium registered a pH decrease because, during wastewater treatment, sulphuric acid was produced and aluminium has trivalent cations which behave as Lewis acid (Beyene et al. 2016). However, all treated wastewater samples were within the guidelines for the Malawi Bureau of Standards (2005) for drinking water (5 < pH < 9.5).

Effect of pH on turbidity reduction

The effectiveness of plant-based coagulants in turbidity removal from sewage wastewater under different pH solutions was studied (Figure 5a). The results show that *Jatropha curcas* exhibited a better performance in an acidic environment than basic. At pH 2, the maximum turbidity removal of 450.18 ± 0.72 NTU (96.99%) was achieved. Whereas *Moringa oleifera* attained the highest turbidity removal of 445.03 ± 0.51 and 445.84 ± 0.53 NTU (95.99 %) in a basic medium pH between 8 and 10. Rice husks ash, on the other hand, realized the highest turbidity removal of 441.81 ± 0.43 NTU (94.99 %) at pH 2, and finally aluminium sulphate demonstrated the best performance in turbidity removal of 461.79 ± 0.63 NTU (99.37%) at pH 6. *Moringa's* effectiveness in turbidity removal in basic solution could be connected to the arginine proteins that constitute a large percentage of total protein available in *Moringa* seed. All amino acids except arginine have been reported to exhibit negative charges in alkaline solution and that might be responsible for effective coagulation process above neutral point (Abidin et al. 2011). The rice husks ash performed differently at low and high pH. At low pH, the surface of the ash might have been protonated due to hydrogen ions from the acid promoting coagulation and flocculation processes. This resulted in destabilisation of negatively charged suspended particles resulting in low turbidity removal. However, as the pH was increased, the surfaces were deprotonated due to the hydroxyl ions resulting in a reduced rate of destabilisation of suspended particles thereby increasing turbidity removal in acidic conditions. This indicates that *Jatropha curcas* and rice husks ash perform better in acidic conditions, while *Moringa oleifera* depends on alkaline conditions.

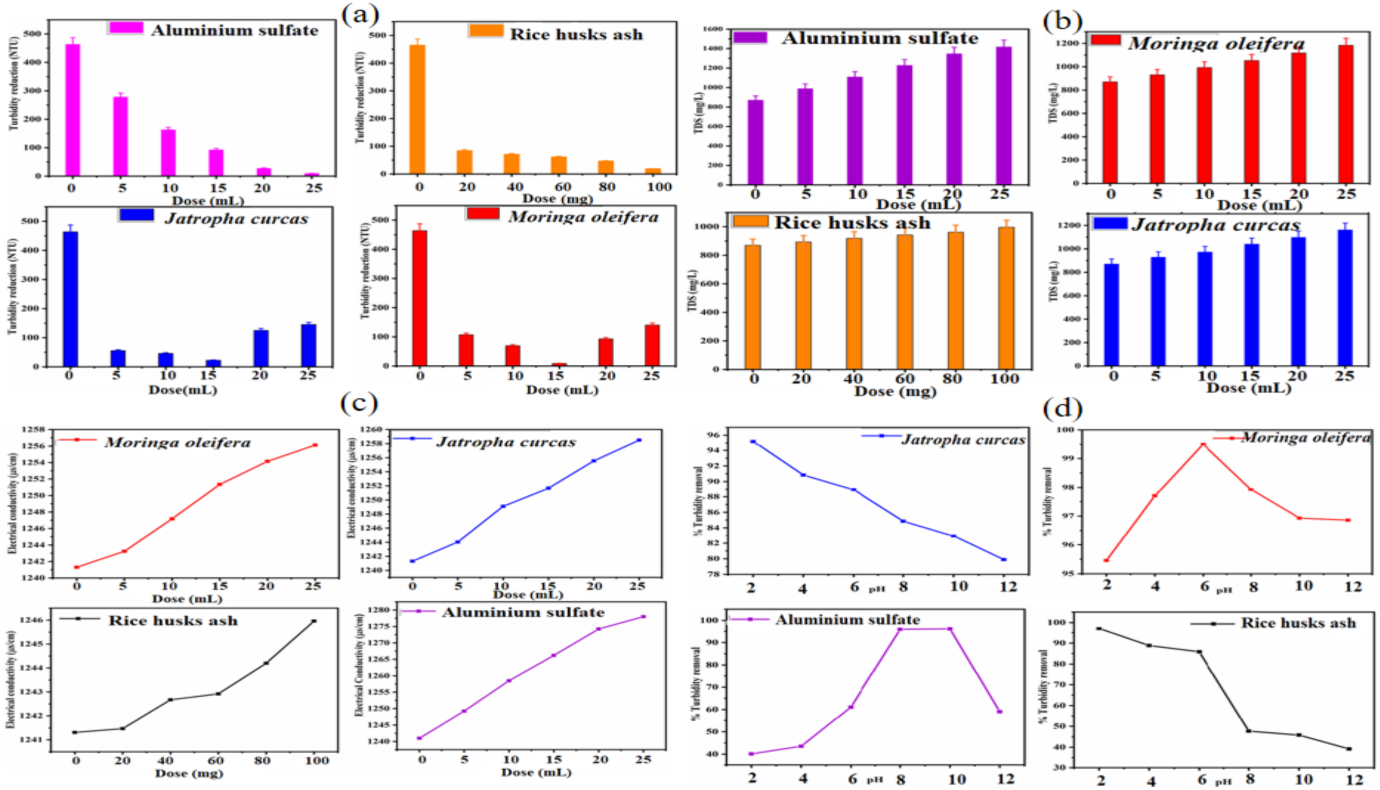

Figure 4: The effect of coagulant dosage on (a) turbidity, (b) TDS (c) Electrical conductivity and (d) pH of wastewater.

Effect of settling time on turbidity reduction

The results on effect of settling time are shown in Figure 5b. It was observed that residual turbidity removal increased as the settling time was prolonged for all the coagulants. The highest rate of turbidity removal was observed after 4 h for all the coagulants except rice husks ash which appeared after 5 h. After 4 h of settling time, the rate of turbidity removal was decreased. The positive control effectively removed 97.34% (451.77 ± 0.21 NTU), while *Moringa oleifera* successfully attained a 94.56% (438.85 ± 0.14 NTU) turbidity removal, followed by *Jatropha curcas* that achieved 92.47% (429.16 ± 0.40 NTU) and finally rice husks ash which attained 92.46% (429.13 ± 0.24 NTU). The increase in residual turbidity removal as settling time is prolonged could be associated with the settling behaviour of suspended particles plus other floc particles which are collected during their movements in the bulk of the solution forming settled particles (Mohammed and Shakir2018). These results are in line with those reported in the study conducted by Odiyo et al. (2017) in which raw river water was clarified with *Dicerocaryum eriocarpum* mucilage. The results demonstrated that an increase in settling time yielded higher turbidity removal.

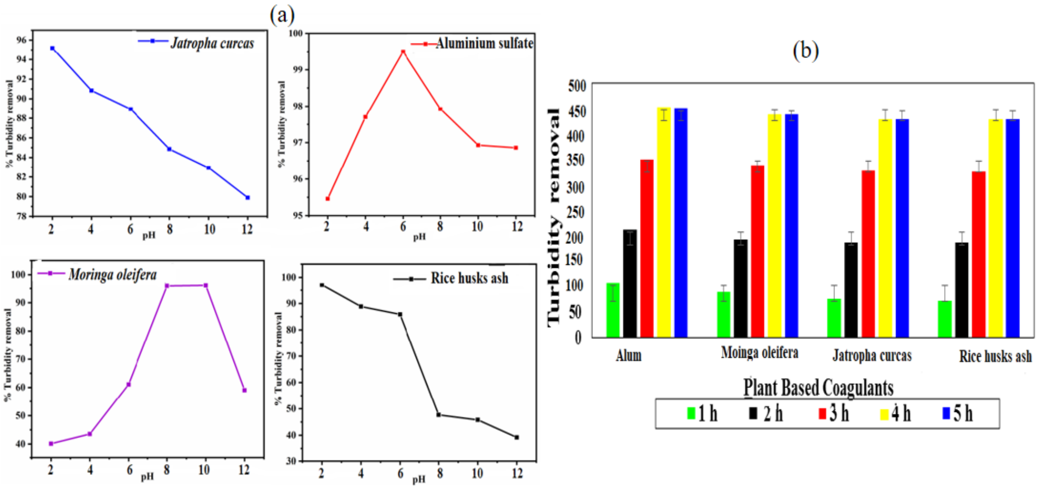

Figure 5: (a) Effect of solution pH on the efficiency of turbidity removal, (b) Effect of settling time on turbidity reduction.

Microbial load removal studies

Effect of dose on colony forming unit (CFU)

Microbial load water quality assessment is of great importance to prevent waterborne diseases. A group of bacteria usually termed as faecal coliforms are used as water quality indicators for faecal pollution of water. Their detection in low quantities indicates that the water is safe from disease-causing microorganisms. However, if faecal coliforms are detected in large quantities, it would indicate that the water contains disease-causing organisms rendering it unsafe for human consumption (Fattouh and Al-Kahtani 2002). In this study, the effectiveness of the coagulants to remove the aforementioned microbes from wastewater was investigated. The petri dishes containing the specimen were incubated for 24 h following which the colonies formed were counted using microbiological standard method (Macharia et al. 2016). The data obtained was exposed to Pearson correlation using Microsoft Excel Office Professional Plus. The significance level was set at α = 0.05 as most significant and above that value was considered as not significant.

Faecal coliforms

The faecal coliforms are a subcategory of total coliforms bacteria found in intestines and faeces of people and animals. The effects of plant-based coagulants doses on the reduction of colony forming unit (CFU) for faecal coliforms were determined and the results are presented in Figure 6a. The sewage wastewater treated with rice husks ash and *Jatropha curcas* has shown statistically higher differences of $P < 0.0004$ and $P < 0.0003$, respectively and *Moringa oleifera* was not statistically significant ($P > 0.8939$) among the doses used. The results indicated that an increase in coagulant doses of rice husks ash from 20 mg/400 mL to 100 mg/400 mL and for *Jatropha curcas* from 5 mL/400 mL to 25 mL/400 mL resulted in a reduction of CFU for faecal coliforms from 114 CFU/mL to 23 CFU/mL and 114 CFU/mL to 0 CFU/mL, respectively. However, in the wastewater clarified with *Moringa oleifera* extract, the number of colonies formed increased as the doses increased. Increasing the doses of *Moringa oleifera* seed extracts from 10 mL/400 mL to 25 mL/400 mL resulted in the growth of colonies from 139 CFU/mL to 401 CFU/mL. The increase in growth of faecal CFU witnessed is connected with the high levels of organic substrates contained in *Moringa oleifera* seeds that may be acting as the sources of food promoting the bacteria growth (Rockwood et al. 2013). The clarified domestic water samples exceeded the accepted standards for drinking water set by WHO (2008) for total coliforms (<0 CFU/100 mL).

Escherichia coli

This is another strain of bacteria which belongs to faecal coliforms associated with water pollution due to faecal contamination from infected intestines of humans, cattle and other animals (Fattouh and Al-Kahtani 2002). In this study, the capacity of plant-based materials in the elimination of *Escherichia coli* from sewage wastewater was considered. According to the results (Figure 6b), there were highly significant differences except for *Moringa*. For instance, rice husks ash and *Jatropha curcas* showed $P < 0.005$ and $P < 0.000$ correspondingly, while *Moringa oleifera* displayed $P > 0.283$. It was noted that as coagulant doses of rice husks ash and *Jatropha curcas* seed extract were increased from 20 mg/400 mL to 100 mg/400 mL and 5 mL/400 mL to 25 mL/400 mL there was a positive reduction in colony forming unit from 141 CFU/mL to 4 CFU/mL and 141 CFU/mL to 4 CFU/mL, respectively. However, sewage wastewater clarified with *Moringa oleifera* seed extracts resulted in the development of colonies despite raising the doses as was the case with the other coagulants. The colonies developed increased from 260 CFU/mL to 983.5 CFU/mL with doses of 10 mL/400 mL to 25 mL/400 mL. The increment could be linked to the fact that sewage wastewater contains food for microbes onto which they may feed on resulting in their increase. These results are similar with those reported in a study done by Maduabuchi (2018) in which the paint wastewater was treated with *Moringa oleifera* seed powder, that increased coliforms bacteria as doses and contact time rises. The treated samples did not meet the required standards for drinking water set by WHO (2008) and MBS (2005) (< 0 CFU/100 mL).

Total coliforms

Total coliforms bacteria are common in sewage wastewater environment and are regarded as harmless. They find their ways in water through faecal contamination. After exposing sewage wastewater to treatment with plant-based coagulants understudy, their effectiveness in removing total coliforms concerning dosage adjustments was carried out (Figure 6c). Results indicate a significant difference for rice husks ash ($P < 0.006$) and *Jatropha curcas* seed extract ($P < 0.003$). Whereas, *Moringa oleifera* seed extract displayed non-significant difference ($P > 0.679$). The increment in coagulant doses for rice husks ash and *Jatropha curcas* seed extracts from 20 mg/400 mL to 100 mg/400 mL and 5 mL/400 mL to 25 mL/400 mL successfully resulted into the positive reduction of colony-forming units from 375 CFU/mL to 18 CFU/mL and 375 CFU/mL to 32 CFU/mL, respectively. This was contrary to *Moringa oleifera* seed extracts that could not reduce the colony-forming units of total coliforms despite the increase in the coagulant dosage. The sewage wastewater treated with *Moringa oleifera* seed extract increased the colony growth from 375 CFU/mL to 3450 CFU/mL with dosage adjustment from 5 mL/400 mL to 25 mL/400 mL. The increment could be elucidated that there is a specific period for the reduction in microbial loads in clarified wastewater beyond that resistant strains become dormant or regrowth (Bina et al. 2010). This suggests that *Moringa oleifera* does not guarantee a hundred percent effectiveness in microbial loads removal but rather just lowers them and it is recommended to be combined with other water and wastewater treatment processes (Schwarz 2000). The treated samples exceeded permissible standards for drinking water set by WHO (2008) and MBS (2005) for total coliforms (< 0 CFU/100 mL). Figure 6d shows the pictorial media used in the study of rice husks ash, *Jatropha curcas* and *Moringa oleifera* coagulants.

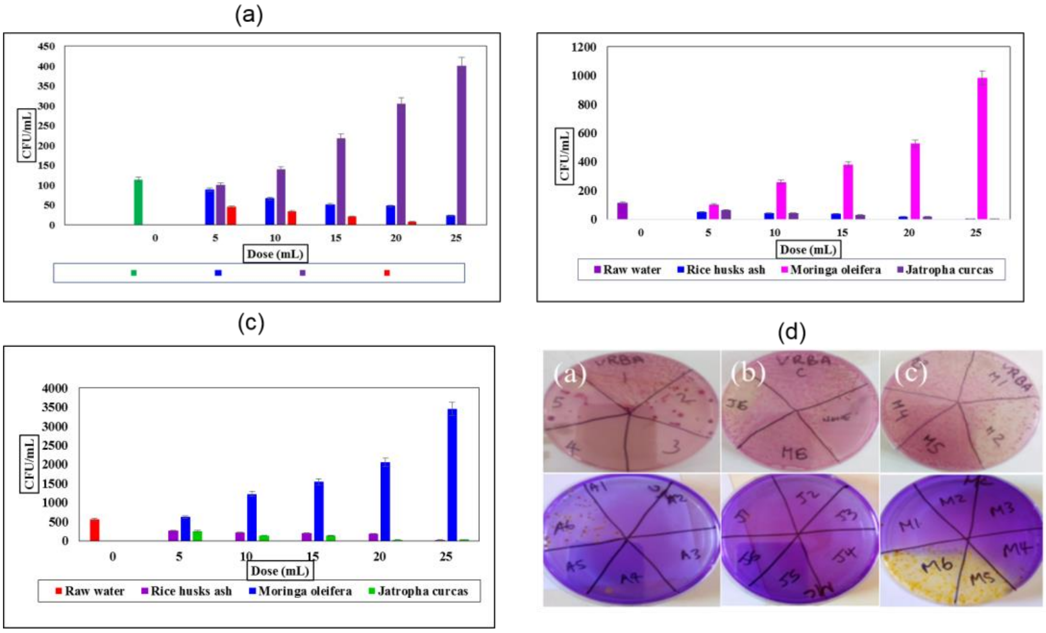

Figure 6: (a) Effect of dosage on faecal coliforms of the clarified wastewater, (b) Effect of dosage on *Escherichia coli* of the clarified wastewater, (c) Effect of dosage on total coliforms of clarified wastewater. (d) Pictorial display of media used in the study (a) rice husks ash (b) *Jatropha curcas* and (c) *Moringa oleifera*.

Conclusion

The application of plant-based flocculants is extremely significant for domestic and wastewater purification especially in developing countries which have no access to safe clean water. This goes a long way to reduce waterborne infections and avoid surface water pollution with hazardous contaminants. In this study, rice husks ash, *Jatropha curcas* and *Moringa oleifera* were used and successfully demonstrated their coagulation and flocculation capability in turbidity and microbial loads removal from domestic wastewater. The raw biomass and ashes for cassava and Irish potato peels could not coagulate wastewater. The agroforestry and agricultural residues used could be possible substitutes to replace inorganic chemical flocculants which are associated with some setbacks. The availability of these residues offers greater opportunity for their utilisation in water and wastewater purification due to the almost zero cost, environmental waste management and provision of a solution to the current situation at Chikanda wastewater treatment facility.

Conflict of interest

The authors confirm that this work has no conflicts of interest.

Acknowledgement

This research was supported by the World Academic of Science (TWAS) Research Grant Agreement (RGA): No. 17-490 RG/CHE/AF/AC_G–FR3240297725. The authors acknowledge the Department of Chemistry and Department of Biology, Chancellor College, University of Malawi for the use of their laboratories. Thanks to Jaramogi Oginga Odinga University of Science and Technology (JOOUST), Kenya, for making use of their FTIR instrument.

References

Abidin ZZ, Ismail N, Yunus R, Ahamad IS and Idris A 2011 A preliminary study on *Jatropha curcas* as coagulant in wastewater treatment. *Environ. Technol*. 32: 971-977.

Adeniran KA, Akpenpuun TD, Akinyemi BA, and Wasiu RA 2017 Effectiveness of *Moringa oleifera* seed as a coagulant in domestic wastewater treatment. *Afr. J. Sci. Technol. Innovat. Develop.* 9: 323-328.

Akhilesh M and Nisa F 2018 Clarification of water using natural coagulants of plant origin. MSc thesis. *Thapar Instititute Engineering and Technology,* India.

Ali EN, Muyibi SA, Salleh HM, Salleh MRM and Alam MZ 2009 *Moringa oleifera* seeds as natural coagulant for water treatment. *Thirteen. Int. Water Technol. Conf. Hurghada, Egypt* 13: 163-168.

Almatar M, Ali E, Chushan T and Makky E 2014 The use of *Moringa oleifera* seed as a natural coagulant for wastewater treatment and heavy metals removal. *Jokull J.* 64: 188-198.

Ang TH, Kiatkittipong K, Kiatkittipong W, Chua SC, Lim JW, Show PL, Bashir MJK and Ho YC 2020 Insight on extraction and characterisation of biopolymers as the green coagulants for microalgae harvesting. *Water* 12(5): 1388.

Arunkumar P, Kumar VS, Saran S, Bindun H and Devipriya S 2019 Isolation of active coagulant protein from the seeds of *Strychnos potatorum*–a potential water treatment agent. *Environ. Technol.* 40(12): 1624-1632.

Asrafuzzaman M, Fakhruddin ANM and Hossain A 2011 Reduction of turbidity of water using locally available natural coagulants. *Int. Schol. Res. Net. Microbiol.* 2011: 1–7.

Belhamdi B, Merzougui Z, Trari M and Addoun A 2016 A kinetic, equilibrium and thermodynamic study of l-phenylalanine adsorption using activated carbon based on agricultural waste (date stones). *J. Appl. Res. Technol.* 14: 354-366.

Beyene HD, Hailegebrial TD and Dirersa WB 2016 Investigation of coagulation activity of cactus powder in water treatment. *J. Appl. Chem.* 2016: 1-9.

Bina B, Mehdinejad MH, Dalhammer G, Rajarao G, Nikaeen GR and Attar HM 2010 Effectiveness of *Moringa oleifera* coagulant protein as natural coagulant aid in removal of turbidity and bacteria from turbid waters. *Int. J. Environ. Ecol. Engin*. 4: 261-263.

Dehghani M and Alizadeh MH 2016 The effects of the natural coagulant *Moringa oleifera* and alum in wastewater treatment at the Bandar Abbas oil refinery. *Environ.*

Health Engin. Man. 3(4): 225-230.

Fahey J 2005 Moringa oleifera : a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. *Trees for Life J.* 1(5): 1-15.

Fattouh FA and Al-Kahtani T 2002 The efficiency of removal of total coliforms, faecal coliforms and coliphages in wastewater treatment plant in Riyadh Pakistan. *J. Biol. Sci.* 5(4): 466-470.

Ghernaout D and Ghernaout B 2012 Sweep flocculation as a second form of charge neutralisation: a review. *Desalin. Water Treat.* 44: 15-28.

Jahn SAA 1984 Effectiveness of traditional flocculants as primary coagulants and coagulant aids for the treatment of tropical waters with more han a thousand fold flocculation in turbidity. *Water Supply* 2: 8-10.

Kian-Hen C and Peck-Loo K 2017 Potential of banana peels as bio-flocculant for water clarification. *Progress in Energy and Environ.* 1: 47-56.

Labied R, Benturki O, Hamitouche A and Donnot A 2018 Adsorption of hexavalent chromium by activated carbon obtained from a waste lignocellulosic material (*Ziziphus jujuba cores*): Kinetic, equilibrium, and thermodynamic study. *Adsorp. Sci. Technol.* 36: 1066-1099.

Macharia JW, Wandiga SO, Njenga LW and Madadi VO 2016 *Moringa oleifera* and ceramic filters for *Escherichia coli* and turbidity removal from drinking water . *IOSR Journal of Applied Chemistry* 9(5): 46–55.

Maduabuchi MN 2018 Bacteriological Treatment of waste water from paint industry using *Moringa oleifera* seed. *Int. J. Waste Resour.* 8(337): 2.

Magoling BJA and Macalalad AA 2017 Optimization and response surface modelling of activated carbon production from mahogany fruit husk for removal of chromium(VI) from aqueous solution. *BioResources* 12: 3001-3016.

MBS (Malawi Bureau of Standards) 2005 Guidelines for Borehole and Shallow Well Water Quality Specifications, MS 733:2005, Malawi Bureau of Standards, Blantyre.

Marobhe NJ, Dalhammar G and Gunaratna KR 2007 Simple and rapid methods for purification and characterization of active coagulants from the seeds of *Vigna unguiculata* and *Parkinsonia aculeata*. *Environ. Technol.* 28: 671-681.

Mohammed TJ and Shakir E 2018 Effect of settling time, velocity gradient, and camp number on turbidity removal for oilfield produced water. *Egyptian J. Petroleum* 27: 31-36.

Muruganandam L, Kumar MPS, Jena A, Gulla S and Godhwani B 2017 Treatment of waste water by coagulation and flocculation using biomaterials. *IOP Conf. Series: Mater. Sci. Engin.* 263(3): 1-11.

Odiyo JO, Bassey OJ, Ochieng A and Chimuka L 2017 Coagulation efficiency of *Dicerocaryum eriocarpum* (DE) plant. *Water SA.* 43: 1-6.

Okello C, Tomasello B, Greggio N, Wambiji N and Antonellini M 2015 Impact of population growth and climate change on the freshwater resources of Lamu Island, Kenya. *Water* 7: 1264-1290.

Rai BN, Giri BS, Nath Y, Bajaj H, Soni S, Singh RP, Singh RS and Rai BN 2018 Adsorption of hexavalent chromium from aqueous solution by activated carbon prepared from almond shell: kinetics, equilibrium and thermodynamics study. *J. Water Supply: Res. Technol.-Aqua* 67:724-737.

Ramavandi B 2014 Treatment of water turbidity and bacteria by using a coagulant extracted from *Plantago ovata*. *Water Resources and Industry* 6: 36-50.

Rockwood JL, Anderson BG and Casamatta DA 2013 Potential uses of *Moringa oleifera* and an examination of antibiotic efficacy conferred by *Moringa oleifera* seed and leaf extracts using crude extraction techniques available to underserved indigenous populations. *J. Int. Phytothearpy* 3: 61–71.

Shan TC, Al Matar M, Makky EA and Ali EN 2017 The use of *Moringa oleifera* seed as a natural coagulant for wastewater treatment and heavy metals removal. *Appl. Water Sci.* 7(3): 1369-1376.

Schwarz D 2000 Water clarification using *Moringa oleifera* Technical Information W1e. *Gate-Techn. Info. W1e Dep.* 1-7.

Singh NB, Nagpal G, Agrawal S, and Rachna 2018 Water purification by using adsorbents: a review. *Environ. Technol. Innovation* 11: 187–240.

Tunggolou J and Payus C 2017 Application of *Moringa oleifera* plant as water purifier for drinking water purposes. *J. Environ. Sci. Technol.* 10: 268-275.

Ugwu SN, Umuokoro AF, Echiegu EA, Ugwuishiwu BO and Enweremadu CC 2017 Comparative study of the use of natural and artificial coagulants for the treatment of sullage (domestic wastewater). *Cogent Engin.* 4(1): 1365676.

Vunain E and Biswick T 2019 Adsorptive removal of methylene blue from aqueous solution on activated carbon prepared from Malawian baobab fruit shell wastes : Equilibrium, kinetics and thermodynamic studies and thermodynamic studies. *Sep. Sci. Technol.* 54(1): 27-41.

WHO (World Health Organization) 2008 Guidelines for Drinking-Water Quality (Third edition incorporating the first and second addenda). Volume 1, Recommendations. World Health Organization, Geneva. ISBN 9789241547611.

World Health Organization 2011 Guidelines for drinking water quality, Malta: Gutenberg.

Yin CY 2010 Emerging usage of plant-based coagulants for water and wastewater treatment. *Process Biochem*. 45: 1437-1444.