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Determination of Phytochemical Properties of Moringa Oleifera Seed Powder as Bio-Coagulant in Wastewater and Groundwater Treatment

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Abstract: *This study was design to assess Moringa oleifera, seed powder as bio-coagulant in wastewater and groundwater treatment. Fresh, healthy and matured seeds of Moringa oleifera, were bought from Ekeonunwa market Owerri and processed into fine powders. Qualitative phytochemicals screening of aqueous extracts of the seed powders were carried out. Physicochemical parameters such as colour, odour, appearance was checked using ten (10) different observers while pH, turbidity, electrical conductivity (EC), total dissolved solids (TDS), biological oxygen demand (BOD), dissolved oxygen (DO) and chloride (Cl-) were assessed according to standard technique. The result revealed the presence of phytochemicals; alkaloids, flavonoid, phenols, tannins, steroids, saponins, and anthraquinonesin M. oleifera, the treatment of groundwater indicates a change in colour, odour, appearance, and pH, which compare favorably with the control while temperature, EC, TDS, DO, BOD and Cl- were statistically the same before and after treatment,while the turbidity increases across the different treatment.*
Keywords: *Moringa oleifera*, powder, bio-coagulant, wastewater, groundwater, treatment

INTRODUCTION

Coagulation and flocculation are physicochemical processes that are often used at the beginning or end of wastewater treatment processes. In conventional treatment processes, many different types of coagulants are commonly used depending on the chemical features of the contaminants present in the water (Vieira et al., 2010). In general, coagulants are classified as inorganic, as well as synthetic organic, or natural organic polymers (Vieira et al., 2010).

An ideal water has some characteristics like clear, colorless, tasteless, odorless, pathogen-free, harmful chemical-free and non-corrosive. Water is also expected not to leave sediment in all distribution organs as this will help to prevent the occurrence and the spread of water borne diseases. To achieve this standard, there is one common technique applied in water treatment process, which is coagulation-flocculation. Coagulation is the process of coagulating colloidal particles due to the addition of synthetic materials to neutralize charged particles thus forming a precipitate due to the force of gravity and it is achieved by the use of coagulant obtain from synthetic materials such as ferrous sulfate (Fe(SO4), aluminium sulfate or alum (Al2(SO4)3), and Poly Aluminium Chloride (PAC) (Al2(OH)3Cl3) (Vieira et al., 2010).

Coagulationisoneofthemostcommonwaystoreducethepollutantcontentsinthe water body that are present as turbidity, color and organic matters. Separation of these colloids can be done by the addition of synthetic coagulant or bio-coagulant followed by slow agitation (flocculation) that causes coagulation of colloidal particles so they can be separated by sedimentation.

Moringa oleifera is a fast-growing, drought-resistant tree that can reach a height of 10-12m. It is them ostwidely cultivated specie so monogeneric family, the *Moringaceae, which* is indigenous to South Asia, where it grows in the Himalayan foothills from Northeastern.

MATERIALSANDMETHODS

The study will be carried out in Imo state Nigeria, lying within latitude 40451N and 70151 N and longitude 60501 E and 70251 E (Wikipedia, 2020). It has a total area of 5,100 square kilometres and a population of 4.8 million persons. The capital city is Owerri. Imo has three geopolitical zones namely; Owerri, Orlu, and Okigwe and twenty-nine local government areas (Wikipedia, 2020). There are two distinct seasons within this area, namely; rainy seasons, which begins in the month of April and lasts until October, with annual rainfall varying from 1,500-2,200mm (60-80 inches), while the dry seasons is ushered in by harmattan period and are characterized by hot weather and low humidity (Wikipedia, 2021). The rainy season is associated with very high humidity of about 80-85% with very heavy rainfall. Temperature varies according to season between 250c to 320c in sunny days. The forest/vegetation in Owerri is a rain forest with lots of plants diversity, growing under the described climatic conditions. The population is predominantly Igbos and Christians (Wikipedia, 2020).

Samples collection and authentication

Plant Seed Samples

The three different leguminous plant seeds were collected from Owerri metropolis. Matured and naturally dry seedpods of *Moringa oleifera* (brown in colour) was harvested from a healthy Moringa tree in a botanical garden of Federal University of Technology, Owerri. Waste water and Ground water sample Collection

Wastewater used in this study was collected randomly at different point (upper stream, mid- stream and downstream) from Somachi slaughterhouse in Owerri municipal into 10 litres gallon while groundwater was collected from different borehole in Eziobodo village into 10litres gallon. The water samples were transported immediately to the laboratory for analysis.

Preparation of Samples

Preparation of Seeds of *Moringa oleifera*

The methodology of Dehghani & Alizadeh, (2016) with slight modification was used. The seeds of *Moringa oleifera* were dehusked manually using kitchen knife and then, dried in an oven at 350C for 5 hours to make sure they are properly dried before triturating into fine powder with home blender and then, stored in an airtight plastic container for further use.

Stock solution preparation

The methodology of Hoa & Hue, (2018) was adopted with slight modification, different grams of the seeds powder (15g,30g and45g) for each sample wasdissolvedin300mLofdistilledwatertoprepare a fresh stock solution that was used in the treatment process. The mixture was blended with magnetic stirrer for 30 minutes at high speed to extract the active proteins from the powdered seed samples. The suspension was filtered through a Whatman No 1. filter paper into a beaker to obtain a homogenous stock solution which is free from suspended materials.This solution was to be prepared fresh each time it was to be used in water treatment and kept in a cool place with a minimum temperature of 200C to prevent changes in pH and viscosity (Katayon et al., 2006).

Physicochemical Parameters

Physicochemical parameters such as colour, odour, taste, appearance, turbidity, pH, electrical conductivity, total dissolved solid, biological oxygen demand, dissolved oxygen, and chloride ion were determined following the standard protocols and methods of American Public Health Organization (APHA) (1995) and American Society for Testing and Materials (ASTM) using different calibrated standard instruments. The temperature, pH, and conductivity were analyzed on-site immediately after samples collection.

Determination of Temperature

Temperature of the samples were determined by on-site and laboratory. The temperature was measured using mercury-in-glass thermometer. The value of each sample was taken after submerging the temperature probe in the water sample and holding for a couple of minutes to achieve a stabilized reading. After the measurement of each sample, the probe was rinsed with deionized water to avoid cross contamination among different samples.

Determination of pH

The pH of the water samples was measured by using a pH meter (model HI 98130 HANNA). The pH meter was calibrated, with three standard solutions (pH 4.0, 7.0, and 10.0), before taking the measurements. The value of each sample was taken after submerging the pH probe in the water sample and holding for a couple of minutes to achieve a stabilized reading. After the measurement of each sample, the probe was rinsed with deionized water to avoid cross contamination among different samples.

Determination of Electrical Conduction

The conductivity of the samples was measured using a conductivity meter (model HI 98130 HANNA). The probe was calibrated using a standard solution with a known conductivity. The probe was submerged in the water sample and the reading was recorded after the disappearance of stability indicator. After the measurement of each sample, the probe was rinsed with deionized water to avoid cross contamination among different samples.

Determination of Turbidity

The turbidity of the watersamples was measured using spectrophotometer (Unico S-2150 model). Each sample was poured in a cuvette and then placed inside the cuvette holder inside the spectrophotometer and the absorbance of is read against visible light of 450nm, the value of the absorbance was recorded. The cuvette was rinsed with deionized water to avoid cross contamination among different samples.

Determination of Total Dissolved Solid(TDS)

The TDS of the water samples were determined by the gravimetric method as described bySawyer et al. (1994). The weight of empty filter paper was weighted and recorded as W1for each sample, then a known volume of the sample was measured and filtered. After filtration, the filter paper was air-dried and the weight was read and recorded as W2. The value of TDS was calculated by using the formula below;

$$\text{TDS} = \frac{W2 - W1}{Amount\ of\ sample} \times 1000$$

Determination of Biological Oxygen Demand

Biochemical oxygen demand was determined using azide modification of Winkler's method.BODbottlewas prepared and incubatedat 20°C for5 days in thedarkforeach sample. Afterfive days, incubated BOD bottle was poured with mixing 2 mL of orthophosphoric acid, which was shaken gently and titrated with sodium thiosulphate to the end point where there was change in colour. The titre value represents dissolve oxygen on day five. BOD was then calculated as the difference between dissolve oxygen on day one and that on day five.

Determination of Disolved Oxygen

Dissolved Oxygen (DO) was determined using azide modification of Winkler's method as decribed by Biwas, (2015). 200 mL of the water sample was carefully transferred into a 300 ml BOD bottle. 1 mL of manganese sulphate solution was added followed by 1 mL of the alkaline alkali-iodide-azide reagent. The resulting mixture was titrated against 0.025 N sodium thiosulphate to the end point where there was colour change, the titre value was recorded as DO.

Phytochemical Analysis

The preliminary qualitative phytochemical screening was carried out according to the method described by Harbone (1998), Parekh & Chanda (2007). The extractsfrom the different samples were assessed for the presence/absence of

the following phytochemicals parameter: saponins, flavonoids, alkaloids, tannins, phenols, anthraquinones and steroids.

TestforAlkaloids

Methodology is as reported by Ejikeme et al., (2014). Two (2) ml of extract will beaddedto2ml of concentrated hydrochloric acid. Then few drops of Mayer's reagent will be added. Presence of green color or white precipitate indicates the presence ofalkaloids.

TestforTannins

About 1g of the plantextract will be dissolved in 5ml of distilled water, filtered and ferric chloride reagentadded to the filtrate.Ablue-black,green, orblue-green precipitate indicate the presence of tannins

Testforsaponins

About 1 g ofthepowdered sample will be dissolved in a 20 ml distilled waterand thenboiled in a water bath, before filtering. 10ml of the filtrate will be mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth, which shows the presence of saponins.

Testforflavonoids

Five (5) ml of dilute ammonia solution will be added to a portion of aqueousfiltrateoftheextract followedbyadditionofconcentratedH2SO4.Ayellowcolouration observed indicates the presence of flavonoids. The yellow colouration disappeared on standing. Few drops of 1% aluminum solution will be added to a portion of each filtrate. A yellow colouration will be observed, thus, indicating the presence of flavonoids.

TestforPhenols

Two (2) ml of distilled water followed by few drops of 10% ferric chloride will be added to 1mlof the extract. Formation of blue or green color indicates presence of phenols.

TestforAnthraquinones

To 1 ml of the extract add few drops of 10% ammonia solution and the appearance of pink color precipitate indicates the presence of anthraquinones.

TestforSteroids

To 1 ml of the extract, equal volume of chloroform will be added and a few drops of concentrated sulphuric acid. The appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

Statistical Data Analysis

The data were analyzed using Analysis of Variance (ANOVA). All analysis was determined at significant level of $P = 0.05$.

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RESULTS

Phytochemical Analysis of the Moringa oleifera Seed Sample used as Bio-coagulant

The phytochemical parameter of the plant seed samples used as bio-coagulant is shown in table 1. The result reveals the presence of the following phytochemicals; alkaloids, flavonoids, phenols, tannins, steroids, saponins and anthraquinones in *M. oleifera* seed.

Table 1: Phytochemical Analysis of Moringa oliefera Seed Sample used as Bio-coagulants

S/N	PARAMETERS	*Moringaoleifera*
1	**Alkaloids**	**+**
2	**Flavonoids**	**+**
3	**Phenols**	**+**
4	**Tannins**	**+**
5	**Steroids**	**+**
6	**Saponins**	**+**
7	**Anthraquinones**	**+**

Values

Legend:

+ = Present

-=Absent

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Physicochemical parameters of slaughtered house water and groundwater before and after treatment

The result of physicochemical analysis before treatment and after treatment with alum shows significant difference in the colour, odour, appearance, turbidity, temperature, dissolved oxygen, biological oxygen demand and chloride ion after treatment with alum for slaughterhouse waste water, as there was an increase in the mean value of colour from (33-100), odour (92-100), appearance (31- 100), dissolved oxygen (4.20-5.78) and chloride ion (1.42-2.14) and decreased in turbidity (0.86-0.13), Temperature (31-29), and biological oxygen demand (7.59-2.05). Groundwater before treatment and slaughterhouse wastewater after treatment were significantly higher than slaughterhouse wastewater before treatment in colour, odour and appearance

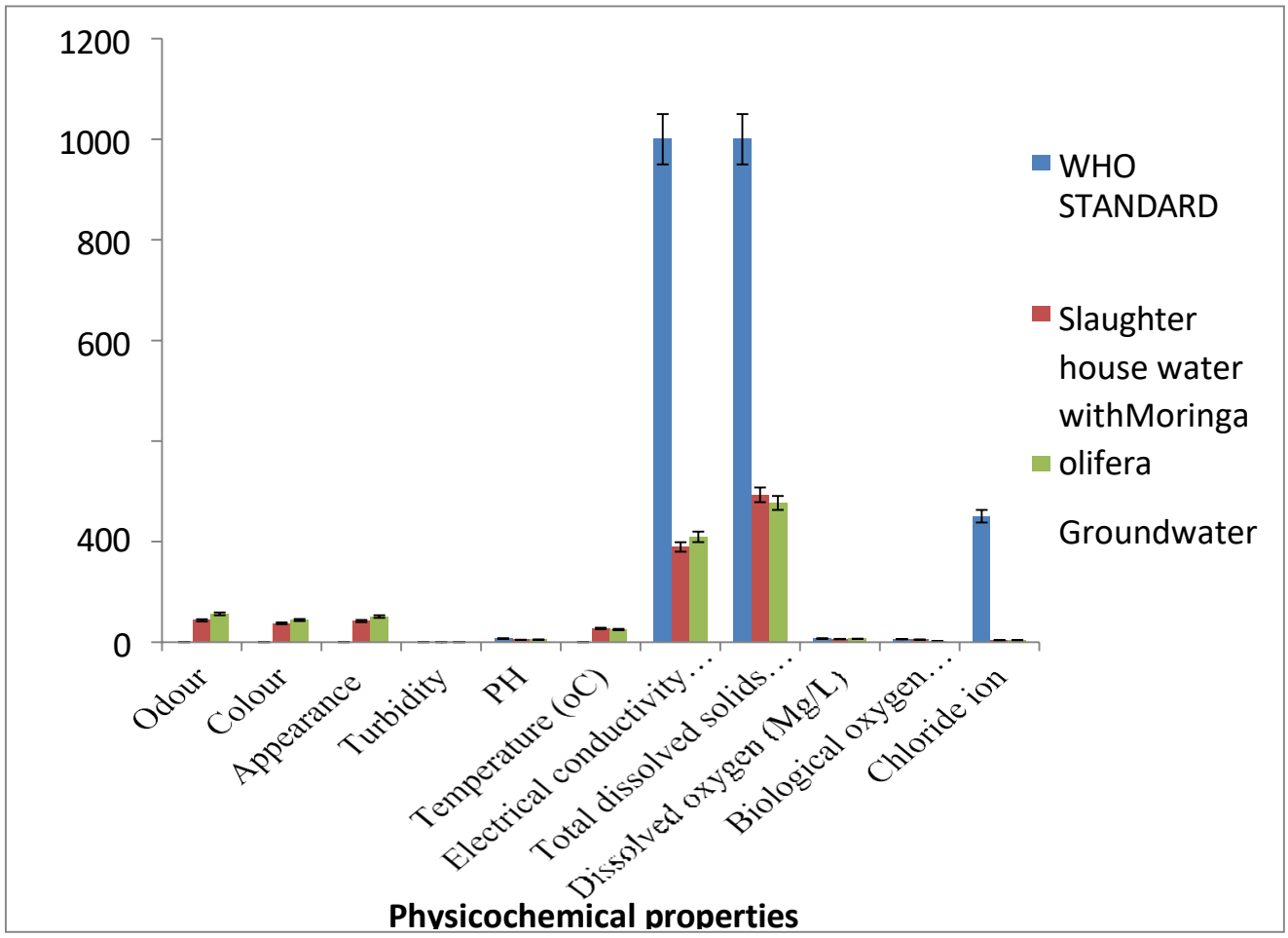

Figure2: Overall physicochemical properties after treat

The result of phytochemical analysis shows that the aqueous extract of the three different indigenous leguminous plant seed powders (*Moringa. oleifera*, *Afzelia. africana,* and *Muccuna. flagellipes*) have the presence of alkaloids, flavonoids, phenols, tannins, steroids, and anthraquinones. While steroids were only present in *M. oleifera*. This implies that *M. oleifera* has all the phytochemical parameters assessed while *A. Africana* and *M. flagllipes* lack steroids but have the rest phytoconstituents.This result is in line with works of Okwu & Okoro, (2007); Nweze & Nwaform (2014); Ojiako, (2014); Elzein *et al*. (2018) and *Olorunmaiye et al*. (2019).

Physicochemical properties of the water samples before and after treatment

Slaughterhouse Wastewater(SHW)

The physicochemical properties of the slaughterhouse wastewater (SHW) before treatment shows that the water was brown in colour and poor in appearance but almost odourless. The turbidity as measured with spectrophotometer is 0.86, pH (6.62) was within WHO standard, while electrical conductivity (195), total dissolved solids (327), dissolved oxygen (4.20) and chloride ion (1.42) were all below WHO standard. Biological oxygen demand (7.59) was above WHO standard. After treatment with alum, which serves as the control, the wastewater becomes colourless, odourless with a better appearance and a significant decreased in turbidity (0.86-0.13), temperature (31-29) and biological oxygen demand (7.59-2.05). The decrease in electrical conductivity (195-187) and total dissolved oxygen (327-305) were not significant while the increase in pH (6.62-7.12) and chloride ion (1.42- 2.14) were statistically significant different at p=0.05.

The result of the physicochemical properties of the slaughter house waste water treated with 15g/300mL stock solution (SS) from the different three plant samples used in this present study at a concentration of10mLand20Mlss revealed a significant difference in the colour, odour, appearance, turbidity, pH, Temperature, dissolved oxygen, biological oxygen demand and chloride after treatment.

CONCLUSSION

This present study revealed that leguminous plant seed powders from *Moringa oleifera* used as bio-coagulants for treating wastewater showed possible potential in treating wastewater but not groundwater. *Moringa oleifera* showed the highest potential with evidence of a sharp reduction inturbidity, colour, odour, TDS, BOD,EC and increased in DO, Cl^- after treatment as compared to the untreated wastewater

samples. Again *M. oleifera* was also seen as the best in the reduction of total coliforms and some pathogenic bacteria resulting from the wastewater samples.

RECOMMENDATION

- The use of *M.oleifer a* shouldbead opted in waste water treatment both locally and commercially.
- Further research should be carried out on the plant materials.

The use of synthetic coagulants should be discouraged

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