
Physicochemical and Bacteriological Study of Sewage Before and After Treatment with *Moringa Oleifera* Seeds at Brazzaville (Congo)

Moyen Rachel^{1, *}, Serge Mdjouetede¹, Nguimbi Etienne¹, Baloki Ngoulou Tarcisse¹,
Matini Laurent²

¹Laboratory of Cellular and Molecular Biology, Sciences and Techniques Faculty, Marien Ngouabi University, Brazzaville, Congo

²Laboratory of Applied Mineral Chemistry, Sciences and Techniques Faculty, Marien Ngouabi University, Brazzaville, Congo

Email address:

rmoyen@yahoo.fr (M. Rachel)

*Corresponding author

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Abstract: Within the framework of the mobilization of water resources and the supply of drinking water to the populations as well as the valuation of local materials, four (4) samples of piped water from the districts of Brazzaville were analyzed for their physical quality. chemical and microbiological before and after treatment with *Moringa oleifera* seed powder. The physicochemical parameters were determined using standard devices. As for the microbiological parameters, they were analyzed by conventional microbiological methods which consisted of isolation on selective media, identification using cultural, morphological and biochemical criteria and then counting. The results obtained concerning the physicochemical parameters showed that the raw water exhibited pH values ranging from 6.4 to 7.02; temperatures ranging from 25 to 25.8 with high values of turbidity and dry matter. However, low levels of magnesium and calcium ions have been noted. Regarding the microbiological parameters, this water exhibited bacteria of the genus *Staphylococcus* as well as fecal and total coliforms, a large number of which were unidentified. The genera *Salmonella* and *Shigella* have been identified as well as the species *E. coli*, an indicator of contamination. After clarification of the *Moringa* beans with powder, the pH values varied from 5.40 to 4.02; there is a decrease in turbidity as well as the dry matter. Microbiologically, the clarified water contained unidentified coliforms with persistence of *Salmonella* and *Staphylococci* in low proportions. As for *E. coli* and *Shigella*, they have been totally eliminated. After clarification and decantation, the results show that all the microorganisms are found concentrated in the sludge. Before treatment, the cloudy pipe water exhibited high concentrations of organic and mineral chemicals. These analyzed waters contained several types of germs in very high numbers. The muddy water is decanted, clarified and purified by the powder of *Moringa oleifera* seeds in less than 2 hours. However, there is a decrease in the number of germs in the clarified water with the formation of an activated sludge. *Moringa oléifera* is therefore a local plant material that can be used in the treatment of wastewater to prevent possible contamination.

Keywords: Muddy Water, Pipeline, Quality, Treatment, *Moringa Oléifera*

1. Introduction

Water is a commodity that conditions people's lives at all times and in all places. The survival of mankind therefore depends on the ability to share and manage this vital resource. In arid and semi-arid zones, this vital and symbolic resource profoundly conditions societies. This is why, in these areas,

there is often recourse to groundwater resources, which are true secret reserves, long unknown [1]. According to UNESCO, the world's population has grown from 3 billion to 6.8 billion people, resulting in a growing need for drinking water. However, an estimated 1.3 billion people do not have access to safe drinking water, the majority of whom are in Africa [2]. Every year, approximately 1.5 million children die

from water-borne diseases. The degradation of water quality in rivers, lakes and groundwater has a direct impact on ecosystems and human health. Such a situation represents a terrible human tragedy and a major obstacle to development [2]. *Moringa oleifera* is a plant that grows in all regions of the world. It abounds in several virtues: medicinal, physiological, biochemical etc... All these virtues are related to the chemical composition of the plant in general and in particular to that of its seeds. Indeed, *Moringa oleifera* seeds contain lipids, carbohydrates and mucilaginous proteins [3]. Thus, we want to take advantage of the virtues of *Moringa oleifera*, an available natural resource, in order to reduce the dependence on expensive chemical products and to bring a solution in the treatment of sewage water for a possible redistribution.

2. Materials and Methods

2.1. Material

The study material has been constituted:

Samples of sewage water from four (4) districts of Brazzaville: La Glacière, (Bacongo), Diata (Makelekele), Plateau des 15 ans and Mougali center (Mougali).

The powder of oven-dried *Moringa oleifera* seeds. The seeds are crushed in a mortar and then finely ground using a Moulinex grinder. The following culture media were used for the isolation of microorganisms before and after clarification of the different water samples: EMB Agar, Mac conkey Agar, Chapman Agar, SS Agar.

2.2. Methods

2.2.1. Physicochemical Study

The different parameters were analyzed before water treatment and after water treatment with *Moringa* powder.

(i). pH, Temperature and Electrical Conductivity

These parameters were measured using the electrochemical method with a pre-calibrated multifunction pH meter. The two electrodes of the instrument are directly immersed in the sample to be analyzed. The pH of the solution, the temperature (in °C) and the electrical conductivity (in $\mu\text{S}/\text{cm}$) are automatically displayed on the instrument's screen.

(ii). Turbidity

It was determined by measuring the absorbance with a spectrophotometer at a wavelength of 520nm using an apparatus (REf apparatus). It is expressed in Formazine Unit (FTU). These units are practically equivalent to Jackson units (JTU) and nephelometric units (NTU). The turbidity value expressed in FTU is read directly from the display [4].

(iii). Calcium

The test is used to determine the hardness of the water and is called the Calcium Hardness Test or Palin Test. It is based on the Calcicol Indicator Reagent method. Calcium ions react especially with the Calcicol indicator in an alkaline solution to give an orange coloration. The reagent itself gives a purple color in the presence of an alkaline solution. After sample

preparation and staining, the reading is taken with the spectrophotometer at 570 nm [4].

(iv). Magnesium

Magnesium gives an orange coloration in the presence of a naturally yellow reagent in solution. The color developed is proportional to the magnesium concentration of the sample to be analyzed. The magnesium is measured at 520 nm on the photometer using the Wavelength bar. To determine the magnesium hardness in mg/L CaCO_3 multiply by 4.2 [4].

(v). Suspended Matter

TSS are defined as the residues retained on a filter membrane of 0.45 μm nominal porosity after drying at 105°C. The presence of TSS causes turbidity in the water and can affect the growth of various living organisms. A 100 mL water sample is filtered through a filter membrane with a nominal porosity of 0.45 μm , previously rinsed and dried at 105°C. The material retained on the filter is dried at 105°C and weighed with the filter. The mass of TSS is obtained by differentiating the mass of the filter including the dried residue from the initial mass of the filter, taking into account the filtered volume [4].

2.2.2. Microbiological Analysis

The study was done with untreated water samples and samples treated with 100g of *Moringa oleifera* powder. The inoculum consisted of 10 mL of the different raw water samples, 10 mL of the supernatant collected after treatment for each sample and then the settling sludge.

(i). Isolation

2 μl of these samples were inoculated on Petri dishes containing selective and non-selective culture media, and incubated in an oven at 37°C for 18 to 24. The following media were used:

Mac Conkey Agar medium, an isolation medium for Enterobacteriaceae;

Chapman Agar medium, a selective medium for bacteria of the genus *Staphylococcus*

EMB Agar medium is used to isolate and identify *Escherichia coli* and *Enterobacter* as well as gram-negative intestinal bacteria;

SS Agar medium is a selective and differential medium for the isolation of pathogenic enteric bacilli belonging to the genera *Salmonella* and *Shigella*.

(ii). Identification

It was made from the cultural, morphological and biochemical characteristics. The morphological characteristics were determined by microscopic examination of the colonies observed in the fresh state and after staining, and the morphological characteristics were determined by microscopic examination of the colonies observed in the fresh state and after staining. Biochemical characteristics were determined from the metabolic reactions of the different colonies. The production of certain enzymes such as catalase, coagulase, sugar fermentation was studied.

(iii). Enumeration of Bacteria in Liquid Medium

It was carried out by the method of dilution in liquid medium then seeding on solid medium. The bacteria present in the inoculum are enumerated on the surface of an agar medium. Each isolated bacterium gives rise to a colony or CFU for "Colony Format Unit". Three boxes are inoculated by dilution. Petri dishes are incubated at 37°- 45°C for 24-48 hours. The NPP method, derived from Mac Crady's studies, consists of interpreting the results by comparing the three test tubes and their results [5]. After incubation, record for each test tube whether the result is positive or negative. For each test tube, assign a number equal to the sum of the positive tubes Table 1.

3. Results**3.1. Raw Water****3.1.1. Physico-chemical Parameters**

The values of the different parameters measured are presented in the table below. According to the results of the physico-chemical parameters of the raw water in Table 2, the and 25.8°C, the E. C. between 100.8 and 1562 µS/cm. The

Ca²⁺ and Mg²⁺ concentrations vary between 7 and 12 mg/L, 1 and 10 mg/L respectively. Turbidity is less than 5 FTU and TSS are between 4.03 and 4.99 mg/L.

3.1.2. Bacteriological ANALYSIS

The culture media used allowed us to highlight all families of Enterobacteriaceae, specifically *E.coli*, *Salmonella* spp, *Shigella* spp and *Staphylococcus*. Table 3 gives us the quantities of germs contained in the muddy water in the pipes.

3.2 Water Clarified with *Moringa Oleifera* Seed Powder**3.2.1. Physico-chemical Parameters**

The values of the different physicochemical parameters measured are recorded in Table 4.

3.2.2. Bacteriological Analysis

The results of the bacteriological analysis are shown in Table 5.

3.3. Activated Mud

Microbiological analyses performed on the activated sludge yielded the following results Table 6.

Table 1. Example: With three dilution tubes.

Dilution	10 ⁰			10 ⁻¹			10 ⁻²			10 ⁻³		
Results	+	+	+	+	+	-	+	-	-	-	-	-
Figures attributed to each positive dilution	3			2			1			0		

Table 2. Values of measured physico-chemical parameters.

Samples	pH	T (°C)	C. E	TURB	Ca ²⁺	Mg ²⁺	MES
			µS/cm	FTU	mg/L	mg/L	mg/L
1	6,4	25,7	1562	< 5	7	10	4,14
2	6,65	25,8	100,8	< 5	12	6	4,99
3	6,36	25,8	910	< 5	8	1	4,62
4	7,02	25,8	1017	< 5	10	4	4,03
WHO standards	6,5-9,00	12-30°C		5	70	50	< 1

pH: Hydrogen potential, T (°C): Temperature in degrees Celsius, C. E: Electrical conductivity; TURB: Turbidity; Ca²⁺: Calcium ion, Mg²⁺: Magnesium ion, MES: Suspended matter.

Table 3. Quantity of bacteria.

Samples	Other unidentified coliforms	E.coli	Salmonella spp	Shigella spp	Staphylococcus
1	3.875.10 ⁵	-	15.10 ⁴	125.10 ³	205.10 ⁶
2	62.375.10 ⁴	21.10 ⁴	-	14.10 ⁴	28.125.10 ⁴
3	5.125.10 ⁵	135.10 ³	12.10 ⁴	13.10 ⁴	1.875.10 ⁵
4	6.125.10 ⁵	-	-	11.10 ⁴	28.875.10 ⁴

pH: Hydrogen potential, T (°C): Temperature in degrees Celsius, C. E: Electrical conductivity; TURB: Turbidity; Ca²⁺: Calcium ion, Mg²⁺: Magnesium ion, TSS: Suspended solids.

Table 4. Values of the physico-chemical parameters.

Samples	pH	T°C	C. E µS/cm	TURB FTU	Ca ²⁺ mg/L	Mg ²⁺ mg/L	MES mg/L
1	5,40	25,7	119,9	< 5	6	4	1,06
2	4,72	25,8	2,30	< 5	11	7	1,08
3	5,40	25,8	121,2	< 5	3	1	1,04
4	4,05	25,8	156,7	< 5	9	3	1,07

pH: Hydrogen potential, T (°C): Temperature in degrees Celsius, C. E: Electrical conductivity; TURB: Turbidity; Ca²⁺: Calcium ion, Mg²⁺: Magnesium ion, TSS: Suspended solids.

Table 5. Values of bacteriological parameters.

Samples	Other unidentified coliforms	E.coli	Salmonella spp	Shigella spp	Staphylococcus
1	205.10 ⁶	-	11.10 ⁴	-	19.10 ⁷
2	24.375.10 ⁴	-	-	-	-
3	2.625.10 ⁵	-	-	-	-
4	3.125.10 ⁵	-	-	-	-

Table 6. Quantity of germs isolated.

Samples	Other unidentified coliforms	E.coli	Salmonella spp	Shigella spp	Staphylococcus
1	325.10 ⁶	-	115.10 ³	-	175.10 ⁶
2	30.625.10 ⁴	15.10 ⁴	-	11.10 ⁴	1.625.10 ⁶
3	2.875.10 ⁵	105.10 ³	-	115.10 ³	16.10 ⁷
4	35.10 ⁵	-	-	105.10 ³	185.10 ⁶

4. Discussion

4.1. Physico-chemical Parameters

The main parameters of interest in the proper functioning of a biological treatment plant, carried out in our study are: pH, temperature, turbidity, electrical conductivity, TSS, calcium and magnesium. Temperature and pH influence the multiplication of bacteria. An excess of TSS can cause clogging of the pre-treatment system. Also, waste water with too much organic matter will be incompletely treated, as it requires more intense microbial activity.

From our results, it appears that the values of pH, temperature, electrical conductivity, turbidity, calcium, magnesium and suspended solids are respectively between 4.05 and 7.02; 25.7° and 25.8°C; 2.30 and 156.7 µS/cm; less than 5; 3 and 12 mg/L; 1 and 10 mg/L and 1.04 and 4.99 mg/L. The pH values of the raw water do not fully meet the WHO standards for wastewater discharge, which recommend a pH between 6.5 - 9.00, however the pH of the water treated with *Moringa oleifera* seed is below the standards.

Our raw water pH results (6.36 to 7.02) differ from those found by Makhoukh M and al. [6] in Eastern Morocco pH=7.04 to 7.89, by Abouelouafa and al. [7], in Morocco pH=5.4 to 8.05, by Guamri and al. [8], pH=7.2 in Morocco, by Belghyti and al [9] in Rabat pH=7.5 by Nola et al.[10]; pH=7.24, by Balok et al.[11] 8.47 to 9.49 and by Pkia Eddabra [12] pH=7.71.

Our temperature values range from 25.7°C to 25.8°C. These results disagree with those found by Makhoukh M and al [6] in eastern Morocco in the study of surface water in the dry season, by Guamri and al. also by Belghyti and al [8, 9] in Rabat, by Abouelouafa and al, Pkia Eddabra, Parihar [7, 12, 13] and Baloki and al. [11] on household wastewater, whose respective averages are: 29°C, 20.8°C, 21, 5°C; 21.11 to 22.95°C; 17 to 29°C; 28 to 31°C and 22.03 to 23.55°C.

From our study, it appears that the average electrical conductivity of the raw water ranges from 910 to 1562 µS/cm. Our results differ from those found by Amghar et al. [16] in Ghana E. C.=4520 µS/cm, [10], E. C.=6320 µS/cm. For some sites, they are close to those found by Pkia Eddabra [13] in India, where the respective averages range from 1510 to 3660 µS/cm and Baloki [11] in Congo Brazzaville 518.37 to

2660.11 µS/cm and quite close to those found by Mukhopadhyay et al., and by Ouali et al [14, 15] in Morocco, whose averages are: 1230 to 1466.68 µS/cm; 1360.5 µS/cm. In comparison with the USAID standard, these waters should be used with caution since the electrical conductivity is less than 3000 mg/L. The average values recorded do not exceed the Moroccan standard for surface water (2700µS/cm). Our results could be explained on the one hand by the presence of several dissolved solids (strong mineralization) and on the other hand by a continuous supply of household waste.

The average turbidity is less than 5 NTU. Our results are similar to those found by Mukhopadhyay et al. and by Srivastava et al. [14, 17] whose averages range from 1.5 to 3.5 NTU, from 0.3 to 1 NTU. This differs from those found by Baloki et al. and by Attab [18, 11] whose means range from 161 to 453 NTU and 100 to 482 NTU. The lowest turbidity value is. The turbidity values can be explained by the high content of colloidal material of mineral or organic origin (sands, microorganisms.). The values for temperature, turbidity, calcium and magnesium are in accordance with the WHO standards for wastewater discharges which recommend respectively between 12-30°C; 5; 70 mg/L and 50 mg/L.

As for the suspended matter, the results of our work give values higher than those recommended by the WHO standards [21].

The Suspension matter of the measured raw waters vary from 4.03 to 4.99. Our results are different from those reported by Niang Sophie [19]. for the waste water of Dakar at Senegal. And those reported by Makhoukh and al. and also Belghyti and al. in Rabat [6, 9]. whose values vary between 33 and 651 mg/L; 123 mg/L (S1) and 2730 mg/L (S2); 513 mg/L and 482 mg/L and 122 mg/L to 1067.25 mg/L [11]. These results can be explained by the presence of poorly biodegradable materials and/or by a continuous supply of household waste [20].

Studies in Thyolo, southern Malawi, have shown that the turbidity of impure water (turbidity of 1000 FTU) could be reduced to less than 10 FTU by coagulation/sedimentation. The same experiment carried out with sand filters gave a final turbidity of 1 FTU. A filtration using the sand gravity technique finally gave a water turbidity generally below 5 MPO [21]. The temperature, suspended solids and pH values obtained in our work are lower than those reported by Yao Akpo [22] which are 29.2°C for temperature, 699 mg/L for

suspended solids and 7.7 for pH. The average TSS values recorded in the water clarified with Moringa oleifera make it possible to assess the purifying performance of this plant.

4.2. Bacteriological Parameters

The raw muddy waters analyzed are highly loaded with Enterobacteriaceae and Staphylococci with the presence of *E. coli*, *Salmonella* spp and *Shigella* spp depending on the samples. This bacterial load is much higher than that reported by Aboukacem and al. [23] which is 107 to 109 CFU/100mL. However, a decrease or total disappearance of the bacterial load in the clarified water can be observed depending on the samples and the amount of Moringa oleifera incubated. This bacterial load, which settles, becomes more abundant in the sludge. These results clearly show the purifying performances of Moringa oleifera. The purifying performances of the Moringa oleifera seed powder according to its concentration are demonstrated in the sample where unidentified coliforms, *Salmonella* spp, *Shigella* spp and Staphylococci are observed at high levels. Clarification with 25g of powder led to a decrease of the entire load. However, with 50 g of powder a total disappearance of *Shigella* spp. is observed, so this concentration is bactericidal for *Shigella*.

In the raw water of sample number 2 we note the presence of unidentified Enterobacteriaceae, *E. coli*, *Shigella* spp and Staphylococcus, after treatment with Moringa oleifera seed powder dosed at 50 g we notice the decrease of Enterobacteriaceae numbers and the elimination of *E. coli*, *Shigella* spp and *Staphylococcus*. The same results were observed for samples number 3 and 4 treated with the seed powder at doses of 75 g and 100 g respectively with elimination of *Salmonella* spp. These results show that the coliform load of the treated water is higher than the WHO standards for wastewater discharge into the natural environment, which is 1000 CFU/100mL. According to the WHO, the presence of *E. coli* in these waters is an indicator of fecal pollution. Studies conducted in Thyolo, southern Malawi [24], at a river water treatment plant that is pumped 24 m³/day to an upper tank/mixer where shelled Moringa oleifera seeds are dosed at 200 mg per liter, showed that coliforms were reduced to 96%. These results are related to the properties of the Moringa oleifera seed powder.

5. Conclusion

The results of our study show that sewage water contains both chemical and microbiological pollutants at high concentrations. The use of Moringa oleifera seed powder at different concentrations not only clarifies the muddy water in less than 2 hours, but also shows the decantation of biological pollutants; this explains the purifying power of Moringa oleifera seed powder by taking advantage of its bacteriostatic and bactericidal activities. These properties of Moringa oleifera seeds can be exploited in arid countries on a large scale as part of the mobilization of drinking water resources in place of chemical coagulants and flocculants.

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