MICROBIOLOGICAL TREATMENT OF DOMESTIC WASTEWATER WITH *Moringa olifera* SEEDS FROM THE CITY OF ADRAR – ALGERIA

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Abstract. Due to the lack of adequate wastewater treatment plant in Adrar, a city located in the southwestern part of Algeria, sewage waters are discharged directly into rivers. As part of the surveillance program for waterborne diseases (cholera, typhoid, hepatitis, parasitosis), and in order to assess the damage that may be caused, a study was carried out on the biological treatment with *Moringa oleifera* seeds of a few bacterial genera. Total microorganisms, total coliforms, fecal coliforms, *Staphylococcus aureus*, *Streptococcus ssp* and *Clostridium ssp*, at the collecting stations of domestic and industrial wastewater in the city of Adrar.

The results obtained confirmed that the *Moringa oleifera* seeds have a positive effect on the reduction of pathogenic microorganisms encountered in wastewater. In fact, it was found that for 5 g of *Moringa oleifera* seed powder, and for a 60-minute contact time, the concentration of total aerobic mesophilic microorganisms in wastewater passed from 14.65*108 UFC / 100mL to $190*10^6$ UFC / 100mL. For the same operating conditions (5g and 60 min), it turned out that the reduction of pathogenic bacteria in wastewater, using *Moringa oleifera* seeds, was more obvious for the genus *Clostridium*, since these microorganisms were totally eliminated. Regarding the total coliforms, it was found that the reduction percentage was 82.5%. Still under the same operating conditions (5 g and 60 min), the reduction rates found for fecal coliforms, *Staphylococcus aureus* and *Streptococus ssp* were 81.33%, 88.23% and 89.18%, respectively.

Keywords: wastewater; *Moringa oleifera*; pathogenic microorganisms; seeds; reduction rate.

INTRODUCTION

According to the World Health Organization, 80% of the diseases that affect the world's population are linked to water pollution. Indeed, most of the microorganisms which are at the origin of the great historical water-related epidemics have for normal habitat the intestines of humans and certain warm-blooded animals It is for this reason that the control and monitoring of the quality of water, especially wastewater, have become increasingly essential [5]. According to World Health Organization, Algeria is ranked among the last countries in the reuse of wastewater [2]; also, this liquid waste is one of the main environmental problems facing the region of Fez in Morocco, given the degree of pollution generated by various types of this kind of wastes (industrial and household) and its impact on surface and underground water resources [12]. Wastewater is often a reliable source of water throughout the year and contains the nutrients necessary for plant growth [22]. Nevertheless, the environmental pollution control has become a necessity in many countries in recent years, therefore, wastewater treatment is very important [10].

Wastewater is water altered by human activities as a result of domestic (household water from kitchen, bathroom, laundry detergent, as well as toilet wastewater), industrial, artisanal, agricultural use and others. These waters must be treated before being mixed with other sources of water to prevent them from being a potential source of pollution from other environments [9].

Depending on the nature and degree of pollution, different processes can be implemented for the treatment of wastewater; note that the depuration process depends on the characteristics of the dirty water and the desired degree of purification. It is widely acknowledged that biological processes make it possible to achieve fairly high yields and are very advantageous from cost effectiveness point of view, as long as they use only the purifying force of microorganisms present in water.

Despite the existence of several methods of water treatment, most still require high investment [28] and fellow researchers [1, 17, 23, 26, 27] proved that wastewater can be effectively repaired by biological treatment process and possibly reuse agricultural irrigation, it was recognized that further studies are needed to explore the benefits and limitations of reusing treated wastewater to irrigate crops.

The present study is based on the wastewater treatment technique that uses the plant *M. oleifera*. This plant is probably the most popular in the tropical species seed bank [15]. *M. oleifera* is a tree native to India. There are approximately 13 *Moringa* species which all belong to the *Moringaceae* family. Among these species, *M. oleifera* is the best known [7]. In addition, *Moringa* extracts and seeds are very effective in clarifying and purifying polluted water from the physicochemical and microbiological point of view. *M. oleifera* seeds are rich in proteins that function as a cationic polyelectrolyte on raw water and wastewater and can precipitate organic and mineral particles due to the destabilization of the colloidal system working together for sedimentation or removal by filtration [12, 23] and pathogenic bacteria use [11, 32].

The results of using moringa seeds are effective against microbes according to Odilon et *al.* [20], which is aimed at all rural residents in particular [4, 24]. *Moringa oleifera* pods are an inexpensive, indigenous and readily available raw material in large quantities that can be used for various industrial applications to reduce the cost of water treatment [5, 18, 29, 31, 32]. Promotion and development of *M. oleifera* as a natural coagulant offers many diverse advantages to many countries of the developing world. It could be viewed as sustainable, appropriate, effective and robust water treatment means. Improvements in some wastewater treatment processes may reduce the import and distribution of treatment chemicals, create new business life for farmers and job opportunities for rural people in particular.

Domestic and industrial wastewater from the city of Adrar is discharged without prior treatment into the Koussane river or in the rainwater drainage canals which are both open-air and passing through the urban and peri-urban areas of the city.

MATERIALS AND METHODS

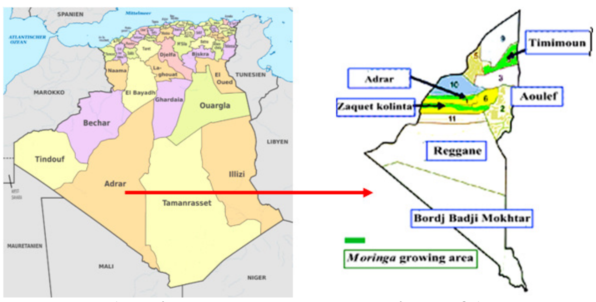
A. Sampling

The specimens were kept in sterile glass vials; they were next stored in an icebox which was kept at low temperature (4 °C) and then transported to the laboratory in the same day for microbiological analyses (ISO 5667-13:2011). The specimens were collected at the entrance of the basins of Adrar wastewater treatment plant. Note that at that level, wastewater has not undergone any treatment.

The most frequently used sampling method consists of completely filling the bottles without shaking them, while avoiding contact with air as much as possible.

B. Preparation of Moringa oleifera seeds

The collection of ripe *Moringa oleifera* seeds was carried out at the Renewable Energy Research Unit in Saharan Environment in the city of Adrar in Algeria (Figure 1). These seeds were ground according to the technique described by Folkard and Sutherland (2002). Four different masses (2.5, 5, 7.5 and 10 g) of *Moringa oleifera* seed powder were then chosen for the treatment of the previously collected wastewater samples.



Algeria **Wilaya of Adrar**

Figure 1. Growing region of *Moringa oleifera* in Adrar state (Algeria) [7]

C. Treatment process

We harvested the fruits during the month of March; the drying of these fruits is done in the open air followed by crushing. The treatment was carried out in Erlenmeyer flasks by putting the different masses of *Moringa oleifera* seed powder (2.5, 5, 7.5 and 10 g), Each weight is dissolved in 1000 mL of wastewater to be treated, each 1 liter of preparation is divided into three 3 small sterilized vials of 250mL, for a period of 90 minutes. Sampling is carried out every 30 minutes until the end of the treatment and microbiological analysis were carried out. For each sample.

All analysis were performed in triplicate.

D. Research methods and enumeration

1. Search and enumeration of total mesophilic aerobic microorganisms (ISO 4883 :2013)
2. Research and enumeration of total coliforms and fecal coliforms (ISO 4832 :2006)
3. Search and enumeration of fecal streptococci (ISO 7899-1:1984 ISO 7899-1:1984)
4. Search and enumeration of *Staphylococcus aureus* [7]
5. Search and enumeration of sulfite-reducing clostridia (ISO 15213:2003)

RESULTS

1. Total microorganisms

These are aerobic microorganisms that can multiply under ambient conditions at 30 °C and do not constitute a particular bacterial family. Their presence in wastewater within defined limits is quite normal because these microorganisms are ubiquitous and are responsible for the biological activity.

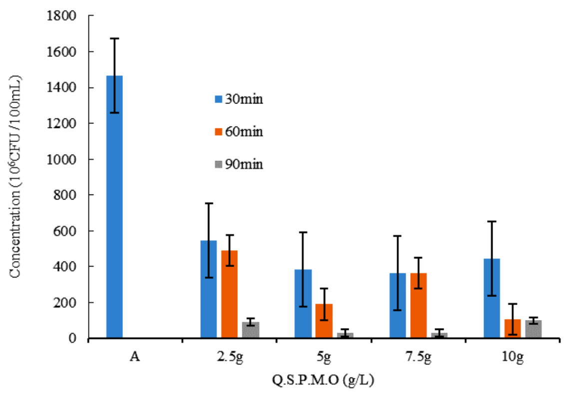


Figure 2. Evolution of total microorganisms as a function of time, for different quantities of *Moringa* seed powder QSPMO is the Quantity of *Moringa oleifera* Seed Powder

In this study of the evaluation the microbiological quality of wastewater after treatment with *M. oleifera* seeds harvested from the region of Adrar, South of Algeria, the total concentration of microorganisms in wastewater, before treatment, was very high, in the order of $14.65*10^8$ CFU / 100 mL. While, after a 30-minute treatment period, this concentration dropped, depending on the amount of *Moinga* seed powder used (Figure 2). The concentration of total mesophilic aerobic microorganisms also decreased after 60 minutes. It then continued to decline over time, up to 90 minutes of contact time, where it reached its lowest level with the value 30*10^6 CFU / 100 mL. These data shows that supplemented wastewater with *Moringa* seed powder helps to reduce or eliminate the microbial load. Moreover, the degree of depollution depends on the contact time as well as on the quantity of *Moringa* seed powder used. The 5g preparation gave the best results.

2. Total coliforms

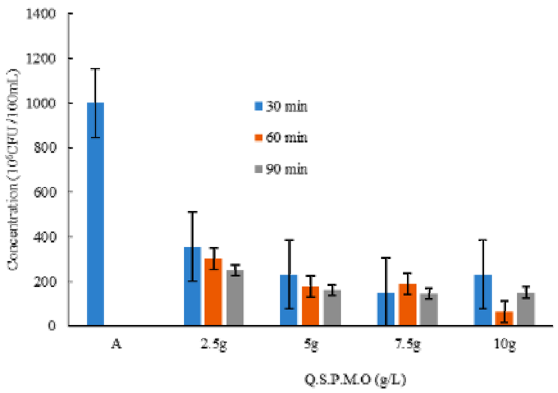


Figure 3. Evolution of total coliforms as a function of time, for different quantities of *Moringa* seed powder

Form our research and enumeration of total coliforms (ISO 4832 :2006), the obtained result as illustrate on Figure 3, reveal that the concentration of total coliforms in wastewater before treatment was 10^9 CFU / 100 mL. This concentration decreased after direct contact with *Morinaga* seed powder, for different quantities (2.5g, 5g, 7.5g and 10g), and various contact time periods (30, 60 and 90 minutes). It is important to highlight that total coliforms bacterial charge reduction is important after a treatment time period of 90 minutes for the quantity of 10g of *Morinaga* seed powder.

3. Fecal coliforms

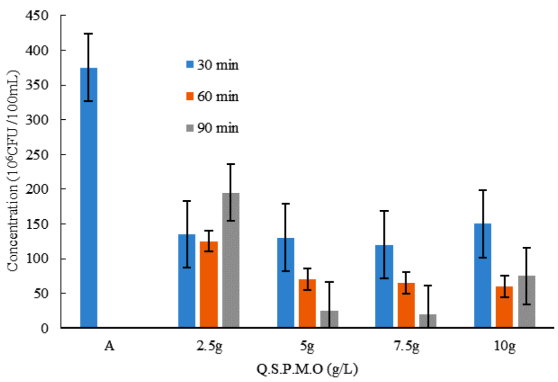


Figure 4. Evolution of fecal coliforms as a function of time, for different quantities of *Moringa* seed powder

From our investigation on the faecal coliforms according to (ISO 4832: 2006), the obtained results indicates that the concentration of faecal coliforms decreased over time during treatment Figure 4. This reduction was proportional to the duration of treatment and to the amount of *Moringa* seeds, particularly for the quantities 2.5 and 7.5g. The concentration of fecal coliforms went down from 358*10^6 CFU / 100mL before treatment to 120*10^6 CFU / 100mL after 30 minutes of contact time between wastewater and 7.5g of *Moringa* seed powder. After a 90 minutes period of treatment, and for the same amount of *Moringa*, the concentration of fecal coliforms dropped to 20*10^6 CFU / 100mL.

4. Sulfite-reducing Clostridia

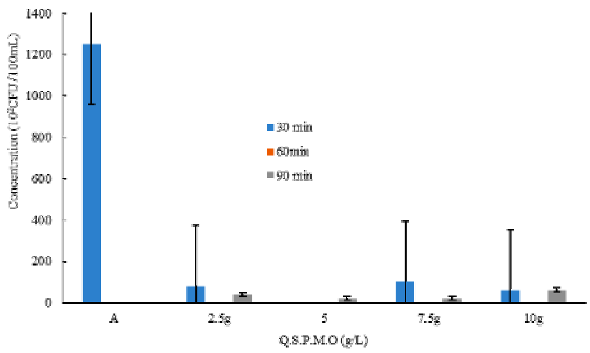


Figure 5. Evolution of clostridia as a function of time, for different quantities of *Moringa* seed powder

From the essays carried out on the research and enumeration of sulfite-reducing clostridium (ISO 15213:2003) the obtained results indicates that the concentration of clostridium in the control sample is of the order of 500*10^2 CFU / 100mL (Figure 5). After 60 minutes of treatment, by *Moringa* seed powder (5, 7.5 and 10g) , Clostridium bacteria charge decreased in wastewater treated. However, after 90 minutes, a slight increase in the concentration of bacteria was observed for the same amounts of *Moringa* seed powder.

5. Staphylococcus aureus

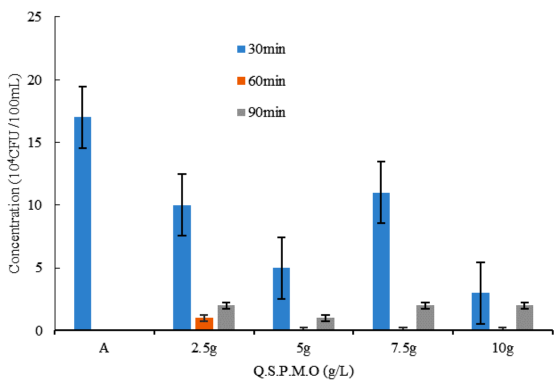


Figure 6. Evolution of *Staphylococcus* as a function of time, for different amounts of *Moringa* seed powder

The results obtained from Staphylococcus essays indicates that the concentration of *Staphylococcus aureus* decreased during the treatment period (Figure 6). This bacterial charge decrease is inversely proportional with over time. This decrease was almost in harmony with the increase in the quantity of

Moringa seeds powder quantities. It was noted that for the quantity of 10g, the number of bacteria was 11 × 10^4 CFU / 100mL and 2 × 10^4 CFU / 100mL for the treatment durations of 60 and 90 minutes, respectively.

6. Fecal Streptococci

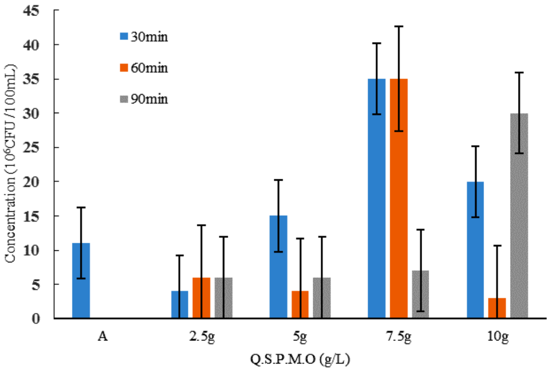


Figure 7. Evolution of fecal Streptococcus as a function of time, for different amounts of *Moringa* seed powder

Figure 7 indicates that the concentration of fecal streptococci in wastewater before treatment was 37 × 10^4 CFU / 100mL. After 30 minutes of contact time, this concentration decreased for the different quantities of *Moringa* seed powder. Indeed, it was found equal to 4, 15, 35 and 20*10^4 CFU / 100mL for the quantities of 2.5g, 5g, 7.5g and 10g, respectively. However, for a treatment time of 90 minutes, and for the same quantities of seed powder, the concentration of bacteria in wastewater was lower; they were equal to 6, 6, 7 and 11*10^4 CFU / 100mL.

DISCUSSION

In light of these results, it is clear that *Moringa* seed powder can affect several groups of bacteria (total mesophilic aerobic microorganisms, total coliforms, fecal coliforms, *Staphylococcus, Streptococcus* and sulfite-reducer clostridia).

Today, it is widely admitted that *Moringa* seed powder has a significant positive impact on wastewater since it reduces its microbial load; it has an anti-bacterial effect which is certainly attributed to the presence of three antibiotic substances, namely *pterygospermine*, *athomine* and *spirochine* whose antibacterial spectra are very broad. It is also worth noting that the antibiotic effect of Moringa seed powder depends on the physiology of microorganisms , the concentration of powder and contact time [19]. This effect can also be explained by the presence of the protein/cationic polyelectrolyte that has a coagulation effect of nutrients that are essential for bacterial growth. It is also worth noting that the antibiotic effect of *Moringa* seed powder depends on the physiology of microorganisms, the concentration of powder and contact time.

Moreover, it is important to note that the contact time is proportional to the antibiotic effect of powdered *Moringa* seeds. This means that good antibacterial efficacy requires adequate contact time. The contact time is variable; it is dependent on the germ to be treated. Indeed, ensuring adequate contact time is critical to successful wastewater treatment. The present study indicates that the optimal duration for appropriate wastewater treatment is about 90 minutes, or even more, with regard to total mesophilic aerobic microorganisms, total coliforms, fecal coliforms and clostridia. In the case of staphylococci and streptococci, it was noted that the duration of 60 minutes was more effective than the 90 minute time; this can be explained by the adaptability and resistance of these microorganisms to multiple antibiotics.

With regard to the antibacterial effect of powdered *Moringa* seeds, it was observed that in general, in the case of the 60 minutes time period treatment, the higher the amount of *Moringa* powder, the more significant the antibiotic efficacy. However, this rule is reversed for the 90 minutes time period treatment when the quantities of *Moringa* powder are very high (≥ 10g / L). This can be explained by the fact that bacteria are quite resistant to antibiotics, which constitutes a major health problem.

The antibiotic effects of *Moringa* seed powder vary from one germ to another because each bacterium has its own characteristics. Consequently, the results obtained show that the *Moringa* seed powder acts on gram-negative bacteria (total and fecal coliforms) for high concentrations (7.5 g / L, 10 g / L) and for longer treatment times (≥ 90min). On the other hand, the *Moringa* plant acts on gram positive bacteria for medium quantities of seed powder (5 g / L) and for a shorter time (60 minutes). Based on the mode of action of the antibiotics present in *Moringa* seeds, one can say that these antibiotics attack the bacterial wall (Peptidoglycan). It is important to know that *Moringa* powder inhibits the germination of clostridial spores and develops a form of resistance to various physicochemical factors; however, it remains powerless to remove them entirely.

These results are very close to those reported by Ameziane and Benaabidate [3] who revealed the presence of larger quantities of total mesophilic aerobic microorganisms, total coliforms, fecal coliforms, Staphylococcus, Streptococcus, and sulfite-reducer clostridia, with concentrations estimated at 50*10^7 UFC / 100mL, 20*10^7 FC / 100mL, 13*10^7 UFC / 100mL, 10*10^7 UFC / 100mL, 10^7 UFC / 100mL, and 10^3 UFC / 100mL, respectively.

On the other hand, the results of this study, concerning the optimal amounts of *Moringa* seed powder and contact times, are consistent with those reported by Kabore et *al.* [16] who made an attempt to optimize the effectiveness for using *Moringa oleifera* seeds for the treatment of drinking water in sub-Saharan Africa. Indeed, this author revealed that the best dosage and time for wastewater treatment are around 8g /L for 30 minutes.

It is worth indicating that our results remain below the standards recommended by the World Health Organization [22] Therefore, one may say that the water treated has an acceptable sanitary quality and can therefore be reused.

The results obtained in this study allow asserting that it is indeed possible to purify wastewater by means of *Moringa oleifera* seeds.

In light of these results, it is clear that *Moringa* seed powder has an influence on several groups of bacteria such as the total mesophilic aerobic microorganisms, total coliforms, fecal coliforms, staphylococci, streptococci, and sulfite-reducing clostridia. This powder has a significant impact on wastewater by appreciably reducing its microbial load. Its anti-bacterial effect is mainly attributed to the three antibiotic substances, pterygospermine, athomine and spirochine, which have broad antibacterial spectra. This effect can also be explained by the presence of the cationic polyelectrolyte, along with the coagulation effect of the nutrient that is required for bacterial growth. It should also be noted that the antibiotic effect of *Moringa* seed powder depends on the physiology of microorganisms, concentration of powder, and contact time. The results obtained show that the treated water having the microorganisms load shown in this study was of acceptable sanitary quality and can therefore be reused.

Further work still remains to be done in order to determine the effectiveness of this plant in the purification process of wastewater, through additional analyses, for the purpose of improving the *Moringa oleifera* seed powder-based treatment technique. Microbial analyzes of wastewater treated with different concentration of Moringa grain powder showed that the Coliform rate is less than 1000 CFU / 100mL for the concentration of 5g / L, which allows the use of this treated water for the agriculture according to Blumenthal et *al.*, 2000 [6] and Omotayo et *al.*, 2017 [21].

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