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Moringa oleifera seeds extract improves the quality of treated wastewater of Byad-Elarab plant, Beni-Suef, Egypt

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Abstract

Waste generation as a result of human activities is unavoidable. A large portion of these wastes ends up as wastewater. The goal of this research is to investigate the performance of *Moringa oleifera* seeds extract (MSE) as an ecofriendly bio-coagulant in the purification of wastewater (effluent) from the Byad-Elarab treatment plant in Beni-Suef, Egypt. The physicochemical and biological properties of effluent and treated water were investigated. Results showed that MSE increased pH, electrical conductivity (EC), total dissolved solids (TDS), chemical oxygen demand (COD), and biological oxygen demand (BOD) of treated water with a concentration-based response. Contrarily, turbidity, total suspended solids (TSS), chlorides, sulphate, and total hardness were significantly decreased. Moreover, MSE inhibited total coliform, algae, and protozoa growth, particularly at high MSE dosage. According to these findings, MSE is suggested as a potential tool for improving water quality and a robust disinfectant agent for raw effluent treatment. Further research is required to investigate the viability of using MSE in fisheries effluents.

***Keywords*: **Water treatment; Wastewater; Coagulants; Biocoagulants; *Moringa oleifera*.

1. Introduction

Wastewater is typically a turbid liquid with an unpleasant odor. It usually contains large floating or suspended solids besides variable chemical and microbial pollutants [1]. Domestic sewage is a major source of pathogens (disease-causing microorganisms) which normally secreted in faeces and putrescible organic compounds due to its high concentration of dissolved and suspended pollutants. One of the most significant challenges confronting developing countries is the inadequate management of massive amounts of waste generated by a variety of human activities [2]. The process of removing contaminants from wastewater and household sewage, as well as runoffs (effluents) from domestic, commercial, and institutional sources, is known as sewage treatment. This process involves in using physical, chemical, and biological methods for wastewater purification [3, 4]. Eventually, an environmentally safe fluid waste stream (also known as treated effluent) and a solid waste (also known as treated sludge)are produced that can be disposed of or reused in further applications. Wastewater methods are critical technologies not only for our health but also for maintaining a clean and healthy environment [5, 6].

Using biological processes to eliminate high metal concentration and emerging contaminants from wastewater overcomes some of the limitations of physical and chemical treatments while also providing cost-effective metal removal [7]. Recently, there has been an increasing numbers of scientific studies focusing on the interactions of microorganisms and metals in aqueous media [8 -10].

Coagulation-flocculation is a common method has been used for removing turbidity and natural organic matter from water. This procedure involves in injecting and dispersing chemical coagulants in two steps to accelerate colloidal sedimentation [11].

Coagulants bind to the colloidal particles and dissolved organic matter, making them easy to remove *via* sedimentation, flotation, or filtration. To induce coagulation, metal salts are commonly used. Colloidal destabilisation occurs as a result of addition of these salts due to electronegative charge neutralisation of colloids, resulting in the formation of micro-flocs [12]. Flocculation is the slow mixing of micro-flocs with synthetic polymers such as polyacrylamide that bonds

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them together. A simple separating procedure is then used to remove the flocs [13].

Coagulants include natural coagulants, synthetic organic polymers, and inorganic coagulants. The two most widely used primary coagulants are aluminium and iron (III) salts [14,15]. A coagulant is a chemical compound that generates diametrically opposed ionic charges to those of colloidal turbid particles in water. The repelling charges on colloidal particles are neutralised by coagulants, resulting in a jelly-like spongy mass known as a floc. Flocculation causes coagulated particles to grow in size and density,causing particle settling in a solution or wastewater to occur at a faster rate [16].

Greener wastewater treatment processes have grown in popularity because they are more environmentally friendly and provide a variety of other benefits, such as cost savings, reduced by-product generation, and increased biodegradability [17]. Extracts of *Moringa oleifera* seeds, *Cactuslatifaria*, *Prosopisjuliflora* [18], Water hyacinth (*Eichhornia crassipes*), duckweed, seaweed, and alligator weeds have been frequently used in water purification for man consumption [19,20]. Based on existing reports, *Moringa* seed powder is a non- toxic compound, has antimicrobial properties [21] and is advised for usage as a coagulant in economically developing countries. Several inspections have shown that *Moringa* seeds can be used as a biocoagulant [17] to enhance the physicochemical properties of contaminated water. *M. oleifera* coagulats *via* adsorption and neutralization mechanisms [22, 23].

M. oleifera dust has been previously shown to reduce high and low turbidity levels in surface water [24] with a bacterial removal range of 90-99% [15]. *M. oleifera* is a vegetable in the Brassica order and a member of the *Moringaceae* family, which consists of a single genus and 13 known species [25] .As a result, the current study was initiated to assess *M. oleifera*'s efficacy as a coagulant and disinfectant for wastewater treatment.

2. Materials and methods:

2.1. *M. oleifera* coagulant stock solution preparation:

M .oleifera seeds were brought from the tree forest of Bayad-Elarab plant, Beni-Suef, Egypt. The dried seed kernels were finely ground to create an aqueous extract. Tap water was mixed with the powdered seeds (2 g seeds powder in 100 ml water) to make a 2% suspension. The suspension was vigorously shaken for 30 minutes with a magnetic stirrer to promote water extraction of the coagulant proteins, and then passed through filter paper (Whatman No.1). To avoid any ageing effects (such as a change in pH, viscosity, and coagulation activity), the solutions were vigorously shaken before using and refrigerated. The stock solution was used to make concentrations of 100 mg/l, 200 mg/l, and 400 mg/l [26].

2.2. Experimental design:

Wastewater samples were obtained from the effluent of the Beni-Suef wastewater treatment plant (Bayad-Elarab).A coagulation experiment was carried out with a variety of coagulant dosages from the respective stock solution.

Group 1: Effluent wastewater (control group**)** consisting of 3 replicates

Group 2: The raw water sample (100 ml of effluent water) was given a dose of100 ml of *M. oleifera* seed MSE at concentration of 100 mg/l.

Group 3: 100 ml of effluent water was given a dose of 100 ml MSE at concentration of 200 mg/l.

Group 4: 100 ml of effluent water was given a dose of 100 ml MSE at concentration of 400 mg/l. Each mixture was mixed thoroughly in 500 ml beakers for 60 seconds and was quickly stirred. To promote flocculation of suspended and colloidal particles, mixture was slowly and gently mixed at 15 to 20 rotations per minute for 30 seconds. The beakers were carefully removed from the jar test setup and the contents were allowed to settle [26]. The resulting supernatant was removed and subjected to physicochemical and biological bioassays.

2.3. Analytical methods:

2.3.1. Determination of physical, chemical, and biological parameters:

The pH was determined using an electrometric pH meter (ORION 2 STAR). Electrical conductivity (EC) and total dissolved solids (TDS) were determined using conductivity meter (Jenway 4320). Residual turbidities were measured in Nephelometric turbidity units with a turbiditymeter (NTU). The dichromate closed reflux method was used to conduct chemical oxygen demand (COD) according to ASTM [27]. Total suspended solids (TSS) were determined using standard methods [28], procedure number 2450D. The Biological oxygen demand (BOD) was measured according to the standard methods, procedure number 5210B [28]. Total hardness and chloride were determined using potentiometric titration according to standard methods [28]. Sulphate was measured using a Spectrophotometer (HACH DR 3900) according to method described by standard procedures, procedure number 8051 [28]. Total coliform bacteria were counted using the membrane filter procedure number 9222B. [28]. Microscopic examinations of algae and protozoa slides revealed cultural and morphological characteristics were done using optical microscopy (Olympus BH2).

2.4. Statistical analysis

Analysis of variance (ANOVA) and regression analyses was performed by WASP-Web Agri Stat Package statistical analysis software. Treatment means were separated using Duncan's multiple range test [29]. All analyses were conducted at the significance value of $p \leq 0.05$.

3. Results:

1. MSE improved water quality parameters of water effluent

The water quality is determined through a set of chemical and biological parameters such as TSS, TDS,BOD, and COD. Results in Fig 1 revealed that MSE significantly decreased ($p \leq 0.05$) total suspended solids (TSS) of water effluent with a concentration-based response. Where the initial TSS of raw water effluent (290 mg/l)was significantly decreased to 116 mg/l at concentration of (100 mg/l MSE), and further decreased to 90 mg/l at 200 mg/l MSE. Whilst, the highest reduction of TSS (40 mg/l) was observed at 400 mg/l MSE (Fig.1a).

Application of MSE at dosages of 100, 200, and 400 mg/l slightly increased the total dissolved solids (TDS) levels from 1064 mg/l of raw effluent water to 1080 mg/l, 1083 mg/l, and 1084 mg/l, respectively. Obviously, increasing MSE concentration showed a comparable effect on TDS level at $P \leq 0.05$(Fig.1b).

Similarly, treatment of effluent with MSE significantly increased the COD values to180, 220 and 460 mg/l at applied dosages of 100, 200, and 400 mg/l (Fig 1c).Also, BOD levels were significantly induced to 180 mg/l by 200 mg/l MSE and to 250 mg/l by 400 mg/l MSE when compared to raw effluent water at $P < 0.05$. Obviously low MSE concentration had no effect on BOD of water effluent (see Fig 1d).

pH value is a critical factor which implies water quality, results in Fig. 1.e revealed that MSE increased water alkalinity with increasing MSE concentration. Where pH values of raw effluent increased from 8 to 11.12 by 100 to 400 mg/l MSE.

Likewise, MSE positively affected water electrical conductivity (EC). Notably, increasing applied MSE dosage had comparable effects on EC values of water effluent (Fig.1 f). Results of EC are in consistent with obtained results of TDS (Fig.1b)

2. MSE improved physicochemical properties of water effluent

In fact, the physical and chemical properties of treated water are decisive factors that imply feasibility of its usage in multiple applications (e.g fisheries fields, forest irrigation and microalgae mass production). Our results indicated that MSE positively affect physical properties of water effluent with a concentration-dependent response. For example, 400 mg/l of MSE significantly decreased turbidity and water hardness to 60 NTU and 100 mg/l compared to initial values of water effluent (320 NTU and 510 mg/l) (Fig.2 a,b). Additionally, both sulphate and chloride contents were progressively decreased with increasing MSE level. Where, the highest reduction in sulphate and chloride contents (90 mg/l, 208 mg/l) were obtained by 400 mg/l MSE when compared to (458 mg/l and 911 mg/l) of raw water effluent (Fig.2 c,d)

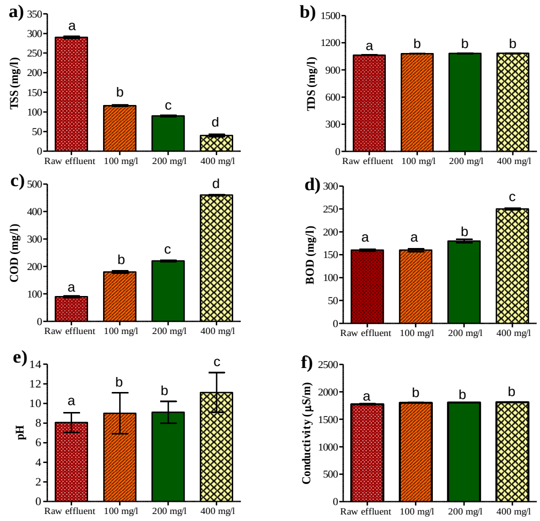

Fig.1 Influence of different *M.oleifera* seeds extract (MSE) concentrations on water quality parameters of Byad-Elarab plant; (a) Total suspended solids (TSS), (b)Total dissolved solids (TDS), (c) Chemical oxygen demand (COD), (d) Biological oxygen demand (BOD), (e) pH and (f) Electrical conductivity (EC) of effluent water. Results are expressed as mean of three independent replicates± standard error (SE). Different letters indicate statistical significance among treatments at $P < 0.05$.

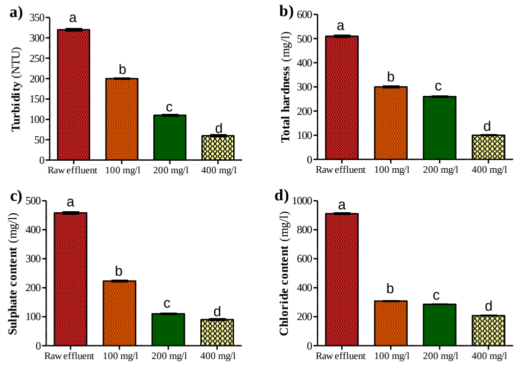

Fig.2. Effect of different *M. oleifera* seeds extract (MSE) concentrations on physicochemical properties of treated effluent of Byad-Elarab plant; (a) Turbidity (NTU), (b) Total hardness (mg/l), (c) Sulphate content (mg/l), and (d) Chloride content (mg/l). Results are expressed as mean of 3 independent replicates ± standard error (SE). Different letters indicates statistical significance among treatments at $P < 0.05$.

3. MSE inhibited growth of coliform, algae and protozoa

Microbiological analysis of water is principally based on examination of fecal indicator bacteria and emerging pathogens. Our microbiological investigation srevealed that MSE tremendously inhibited growth of different groups of emerging pathogens that originally found in water effluent. For instance, 100 and 200 mg/l MSE significantly decreased total coliform bacteria (6-4 CFU/100 ml $\times 10^3$), total algae (10-8 organism/ml) and total protozoa counts (6-4 organism/ml)compared to 37×10^3 CFU/100 ml, 40 and 25 organism/ml of initial water effluent. Interestingly, 400 mg/l MSE completely inhibited growth of all emerging pathogen groups (Fig.3).

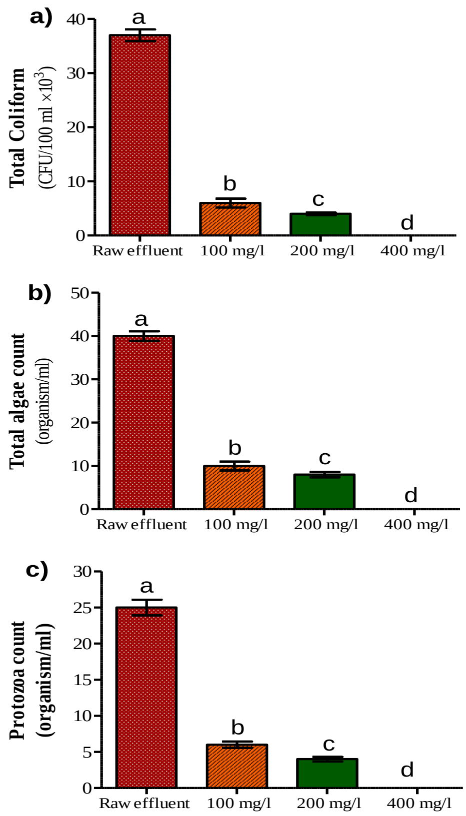

Fig.3. Effect of different *M. oleifera* seeds extract (MSE) concentrations on microbial analysis of water effluent of Byad-Elarab plant; (a) Total coliform (CFU/100 ml) $\times 10^3$, (b) Total microalgae count (organism/ml), and (c) Total Protozoa count (organism/ml).Results are expressed as mean of 3 independent replicates ± standard error (SE). Different letters indicate statistical significance among treatments at *P< 0.05*.

4. Discussion

Due to shrinkage of fresh and clean water supply, purification of water effluent is seen as sensible and realistic solution. Among myriads options, bioremediation of wastewater effluents has been recently reviewed by [3,4,7].

In the present study, MSE biocoagulant was found to improve water quality by decreasing TSS by 86.2%. It has been previously reported that the isolated *Moringa* flocculants, mainly comprised of basic polypeptides with molecular weights ranging from 6000 to 16000 Daltons, are the specific causes of water clarifiers [30]. Our results indicated that there were an increases in the electrical conductivity (EC) and TDS levels after treatment with three MSE concentrations, which are similar to the findings of Arnoldsson**,** *et al* [31] and Shan, *et a*l [32].

In the current study, treating effluent water with different doses of MSE increased the level of COD. Our finding is in accordance with results of Shan, *et a*l [32] and Effendi, *et al* [33]. Water-treated moringa seeds induced the COD values due to decomposition of residual organic content [31]. Herein, MSE dosages progressively increased the BOD value with a concentration-based response, particularly when 400 mg/l MSE was used. This finding is consistent with those of Shan,*et al* [32].The presence of natural organic chemical compounds in MSE could increase BOD value of treated effluent. Increasing coagulant concentration due to MSE, induced water alkalinity where water pH significantly increased. According to previous studies, the seeds' water-soluble cationic proteins are responsible for *M. oleifera's* coagulant action. This implies that in water, the basic amino acids found in *Moringa* protein would accept a proton from the water, resulting in the release of a hydroxyl group, rendering the solution to alkaline. [26]. Based on the obtained results, we assume that MSE-treated effluent isn't suitable for drinking however, it may serve as a suitable medium for propagation of alkalinity-tolerant micarolage. Previous studies indicated that *Spirulinaplatensis*, a blue green microalga is tolerant to high pH and their growth, protein and carbohydrate contents were significantly improved at pH ≥ 10 [34, 35]. Thereby, its biomass can be used for production of a wide range of natural bioproducts.

Our study indicated that increasing the applied concentration of MSE significantly reduced water turbidity. This effect could be attributed to the seeds' chemical structure, which contains water soluble proteins with a low molecular weight [36]. These proteins have a positive overall charge in water which electrostatically attract the negatively charged water pollutants such as sand, silt, clay, and bacteria [30]. MSE has the ability to reduce both low and high turbidity levels in surface water [37]. The MSE-based protein has been elucidated to act similarly to a synthetic positively charged polymer coagulant. When it is added to raw water, it binds to the predominantly negatively charged particulates, which contribute in raw water turbidity [38]. As a result, *Moringa* solution demonstrated effective turbidity removal, which is consistent with previous reports [39 – 43]. Compared to low turbidity levels, *M. oleifera* seed extract was more effective in removing high turbidities [44].

Sharmila, *et al* [45] discovered that *M. oleifera* extract could reduce total solids in domestic wastewater by

36%, from 80 mg/l to 51.2 mg/l. A flocculating protein found in *M. oleifera* seeds acts as a turbid water clarifier [44, 45].

Yarahmadi, *et al* [46] compared the efficiency of MSE to that of polyaluminium chloride, and found that the latter was more efficient in minor turbidity water level. In addition, application of various MSE doses resulted in decreasing sulphate content and water hardness of raw effluent .Our results are consistent with those obtained by Muyibi, and Evison, [24] and Chaudhuri, and Khairuldin [48].

According to our findings, MSE treatment lowered chloride levels. This can be attributed to negatively charged chloride ions in water being drawn to the cations in *Moringa* seed, where the chlorides are neutralized [49].

According to WHO, the highest microbial risks are associated with wastewater discharges in fresh water webs and costal seawaters. These effluents are usually contaminated with human or animal feces and emerging pathogens. Thus, development of ecofriendly and efficient methods to eliminate effluent pathogens is a serious demand.

Herein, MSE treatment inhibited the coliform growthin water effluent, with a concentration-dependent response. The microbial inhibition potential of MSE could be attributed to total phenolic compounds found in MSE. Many authors proposed that phenolic compounds inhibit microorganisms growth due to iron deficiency or hydrogen bonding with vital proteins such as microbial enzymes[50].In general, phenolic compounds have shown good antimicrobial potency against Gram-positive bacteria, with their effect proportional to concentration; at low concentrations, they can stimulate protein denaturation, leading to irreversible cell modification and death [51]. Antimicrobial activity may also be due to the presence of a short peptide which may work directly on microorganisms and inhibit growth by disturbing cell membrane synthesis or synthesis of essential enzymes [52]. The antimicrobial and preservative properties of *M. olifera* extract are thought to be related to phytochemical components of its seeds such as phenolics and tannins [53].

Noor, *et al* [54] achieved 96% turbidity scrapping in wastewater using *moringa* seeds containing an antibacterial factor called glucomoringin (GMG), which is reported to discard three logs (99.99%) of coliforms from water [55]. Other investigations have found that the antimicrobial properties of *moringa* seeds protein are related to membrane fusion [56]. *M. oleifera* seed antimicrobial factor (rhamnosyloxy benzyl-isothiocyanate) could remove coliform bacteria from water [33].

Similarly, in this study, MSE treatment also decreased algae count, and completely inhibited by 400 mg/l MSE. These findings are consistent with Shehata, *et al* [56] and Ibrahim, *et al* [58]. As demonstrated in our present study, the MSE is effective biocoagulant in protozoa removal, which is primarily concerned with removing helminth eggs from turbid wastewater, and these findings are consistent with previous research [59 –61].

5. Conclusion

The present study suggests that MSE is effective biocoagulant to treat wastewater at a concentration of 400 mg/l due to its antimicrobial activity against pathogenic microorganisms. As it significantly reduced the total coliform, protozoa, and total algae counts. It also reduced turbidity, TSS, chlorides, sulphate, and total hardness. It should be noted that it has a significant positive impact on the pH, electrical conductivity, TDS, COD, and BOD levels. Thus, MSE could be a beneficial biocoagulant and disinfectant for the clarification of wastewater.

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