

Use of *Moringa Oleifera* (Drumstick) seed as Natural Absorbent and an Antimicrobial agent for Ground water Treatment

Mangale Sapana M., Chonde Sonal G. and Raut P. D.

Department of Environmental Science, Shivaji University, Kolhapur, INDIA

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Abstract

Ground water is fresh water located in the core space of soil and rocks. Wells and springs constitute groundwater supplies. Present study deals with the suitability of the coagulation-flocculation process using *Moringa oleifera* seeds as natural absorbent and environmentally friendly antimicrobial agent for purification of ground water for drinking purpose. In present study various doses of *Moringa oleifera* seed powder like 50, 100 and 150 mg/l were taken and checked for the efficiency dose on raw groundwater. After treatment of water samples with *Moringa oleifera* seed powder were analyzed for different parameter like pH, turbidity, TDS, TS, hardness, chlorides, alkalinity, acidity, MPN and SPC. All parameters were reduced with increased dose of *Moringa oleifera* seed powder except alkalinity and pH. Application of this low cost *Moringa oleifera* seeds is recommended for eco-friendly, nontoxic, simplified water treatment where rural and peri-urban people living in extreme poverty are presently drinking highly turbid and microbiologically contaminated water.

Keywords: *Moringa oleifera*, ground water treatment, natural absorbent, antimicrobial activity.

Introduction

Presently there are no appropriate low-cost technologies available for removal of several commonly present groundwater contaminants. Fluoride is one such compound that is widely present in groundwater worldwide. Exposure to fluoride in drinking water has a number of adverse effects on human health including crippling skeletal fluorosis that is a significant cause of morbidity in a number of regions of the world. Ground water is polluted due to industrial effluents and municipal waste in water bodies. In rural and undeveloped countries people living in extreme poverty are presently drinking highly turbid and microbiologically contaminated water. Because they lack knowledge of proper drinking water treatment and they cannot afford costly chemical coagulants. To overcome chemical coagulant problems it is necessary to increase the use of natural coagulants for drinking water treatment. Naturally occurring coagulants are usually presumed safe for human health. Some studies on natural coagulants have been carried out and various natural coagulants were produced or extracted from microorganisms, animals or plants.

One of these alternatives is *Moringa oleifera* seeds. It is a native tree of the sub-Himalayan parts of Northwest India, Pakistan and Afghanistan. *Moringa oleifera* is a perfect example of a so-called "multipurpose tree". Earlier studies have found *Moringa* to be non-toxic and recommended it for use as a coagulant in developing countries. The use of *Moringa* has an added advantage over the chemical treatment of water because it is biological and has been reported as edible. According to Suleyman *et.al*¹, hardness removal efficiency of *Moringa oleifera* was found to increase with increasing dosage. *M.*

oleifera seeds act as a natural absorbents and antimicrobial agent. Its seed contain 1% active polyelectrolyte's that neutralize the negative charged colloid in the dirty water. This protein can therefore be a nontoxic natural polypeptide for sedimentation of mineral particles and organics in the purification of drinking water. *M. oleifera* seeds are also acting as antimicrobial agent against variety range of bacteria and fungi². The seed contain number of benzyl isothiocyanate and benzyl glucosinolate which act as antibiotic³. It is believed that the seed is an organic natural polymer. The active ingredients are dimeric proteins. The protein powder is stable and totally soluble in water. The coagulation mechanism of the *M. oleifera* coagulant protein has been explained in different ways. It has been described as adsorption and charge neutralization and interparticle bridging. Flocculation by inter-particle bridging is mainly characteristic of high molecular weight polyelectrolytes. Due to the small size of the *M. oleifera* coagulant protein, a bridging effect may not be considered as the likely coagulation mechanism¹. *Moringa* seeds possess antimicrobial properties reported that a recombinant protein in the seed is able to flocculate gram-positive and gram-negative bacterial cells. In this case, microorganisms can be removed by settling in the same manner as the removal of colloids in properly coagulated and flocculated water. On the other hand, the seeds may also act directly upon microorganisms and result in growth inhibition. Antimicrobial peptides are thought to act by disrupting the cell membrane or by inhibiting essential enzymes reported that *Moringa* seeds could inhibit the replication of bacteriophages. The use of natural materials of plant origin to clarify turbid water is not a new idea^{4,5} and cited by Madsenet *etal*² in 1987. According to Amagloh and Amos Benang⁷, at 95.0% confidence level, there was significant difference among all the treatments

at the varying loading dose concentrations on the pH. The treatments gave a range of 7.2 to 7.9 which falls within the reduced as the concentrations of the dosing solutions were increased. The reverse was observed with the *Moringa* treatment. Among all the plant materials that have been tested over the years, powder processed from the seeds from *Moringa oleifera* has been shown to be one of the most effective as a primary coagulant for water treatment and can be compared to that of alum a conventional chemical coagulant⁶. It was inferred from their reports that the powder has antimicrobial properties. A general rule of thumb is that powder from one *Moringa* kernel to two liters of water is a good amount when water is slightly turbid, and to one liter when water is very turbid⁸. The seeds and powder can be stored but the paste needs to be fresh for purifying the water.

Material and Methods

Materials: Study area: - Kolhapur city. Sampling sites: Ground water in open well near Sambhaji Nagar, Kolhapur.

Methodology: *Moringa oleifera* (good quality dried drumstick were selected and) wings and coat from seeds were removed. Fine powder was prepared by using mortar and pestle and this powder was directly used as coagulant. Water samples for study purpose were collected from ground water from Kolhapur city, Maharashtra. Treatment to water was given by directly using seed powder. The water quality parameters were checked before and after treatment. Doses of seed powder i.e. 50, 100 and 150 mg/l were selected for treatment by supporting table of Micheal Lea Clearing house, Low cost water treatment technologies for developing countries, Ottawa, Canada (table 1).

Table-1

Dose range of *M. oleifera* seed powder given by Micheal Lea clearing house, Low cost water treatment technologies for developing countries, Ottawa, Canada

Sr. No.	Raw Water Turbidity (NTU)	Dose Range of <i>M. oleifera</i> Seeds Powder (mg/l)
1	<50	50
2	50-150	100
3	>150	200

The coagulant was mixed with drinking water sample and kept on the shaker for 45 min at 110 -120 rpm. The settling time was 1-2 hours (depending on the water turbidity). After sedimentation, supernatant of treated water was used for test. The water quality parameters were checked for physicochemical and bacteriological parameters as per standard methods^{14,15} before and after the treatment. The efficiency dose of *Moringa oleifera* seed powder was determined.



Plate No.1
Moringa oleifera tree



Plate No.2
Moringa oleifera seeds with seed coating

Samples were collected from ground water and analyzed for physicochemical parameters and bacteriological parameters as per standard methods⁹.

Table-2

Methods used for Physico-chemical and biological study of water

Sr. No.	Parameters	Method
Physical Parameters		
1	pH	pH meter
2	Turbidity	Nephelometer
3	TS	Evaporation
4	TDS	Evaporation
5	Colour	-
Chemical Parameters		
6	Acidity	Titration
7	Alkalinity	Titration
8	Chloride	Titration
9	Hardness	Titration
Bacteriological Parameters		
10	SPC/100 ml	Quantitative
11	MPN/100 ml	Quantitative

Results and Discussion

For ground water in open well, following drinking water quality parameters were analyzed after the treatment of various doses of *Moringa oleifera* seed powder.

Table-3
Parameters studied before and after treatment of Ground water with various doses of *M. oleifera* seed powder

Sr. No.	Parameters	Before treatment ± SD	After treatment of water sample at various doses of <i>Moringa</i> seed powder ± SD				WHO/USPH Standards
		0 mg/l	50 mg/l	100 mg/l	150 mg/l		
1	pH	8 ±0.05	7 ±0.11	7 ±0.05	7.2 ±0.05	6.5-8.5	
2	Turbidity (NTU)	12.4 ±0.02	3.5 ±0.57	3.2 ±0.57	3.1 ±0.57	5	
3	TS (mg/l)	812 ±0.57	512 ±0.28	396 ±0.28	394 ±0.57	-	
4	TDS (mg/l)	712 ±0.57	350 ±0.57	216 ±0.28	212 ±0.5	500	
5	Colour	faint brown	Colourless	Colourless	Colourless	Colourless	
6	Acidity (mg/l)	50 ±0.57	50 ±0.76	40 ±0.28	30 ±0.57	-	
7	Alkalinity (mg/l)	130 ±0.1	90 ±0.57	100 ±0.57	100 ±0.28	200	
8	Chloride (mg/l)	12 ±0.57	7 ±0.57	6.5 ±0.57	5 ±0.57	250	
9	Hardness (mg/l)	190 ±0.57	110 ±0.57	105 ±0.28	100 ±0.57	500	
10	SPC/100ml	4×10 ⁵ ±0.57	1×10 ⁵ ±0.57	1×10 ³ ±0.57	1×10 ² ±0.57	1×10 ⁶	
11	MPN/100ml	1600 ±0.57	115 ±1.15	20 ±0.57	5 ±0.57	Nil	

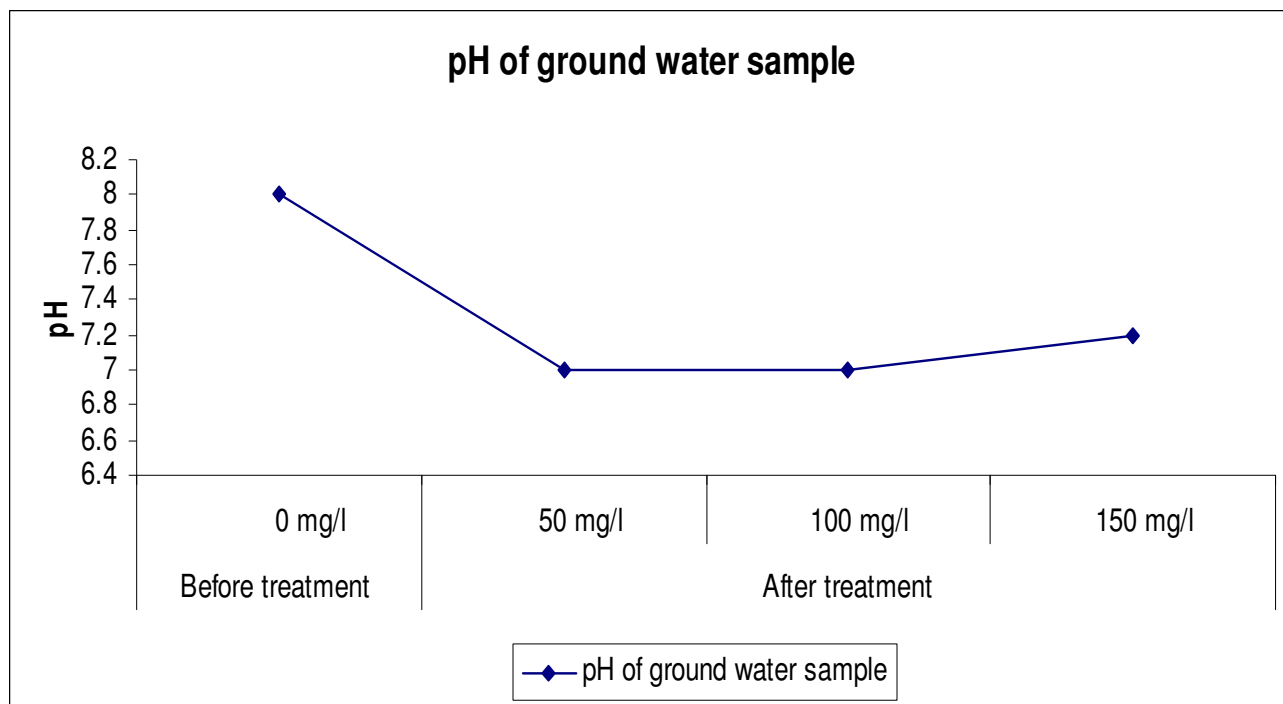


Figure 1
pH of ground water before and after treatment of *M. oleifera* seed powder

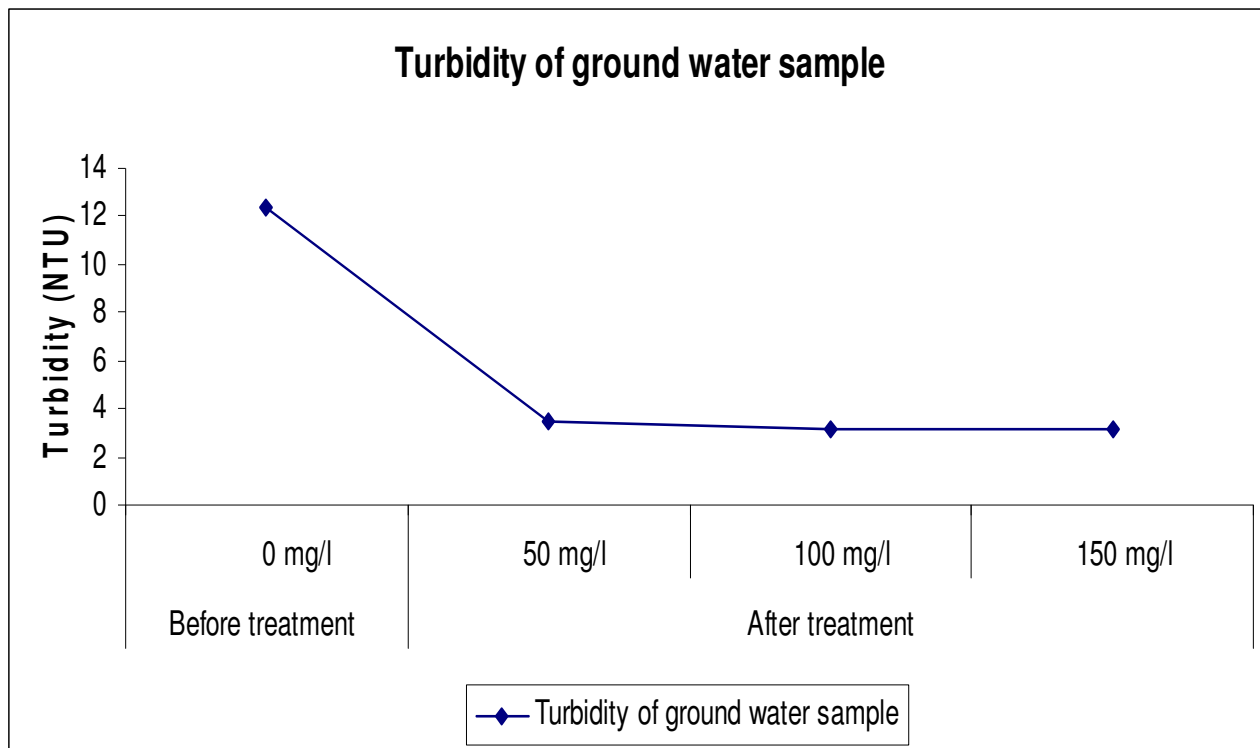


Figure-2
Turbidity of ground water before and after treatment of *M. oleifera* seed powder

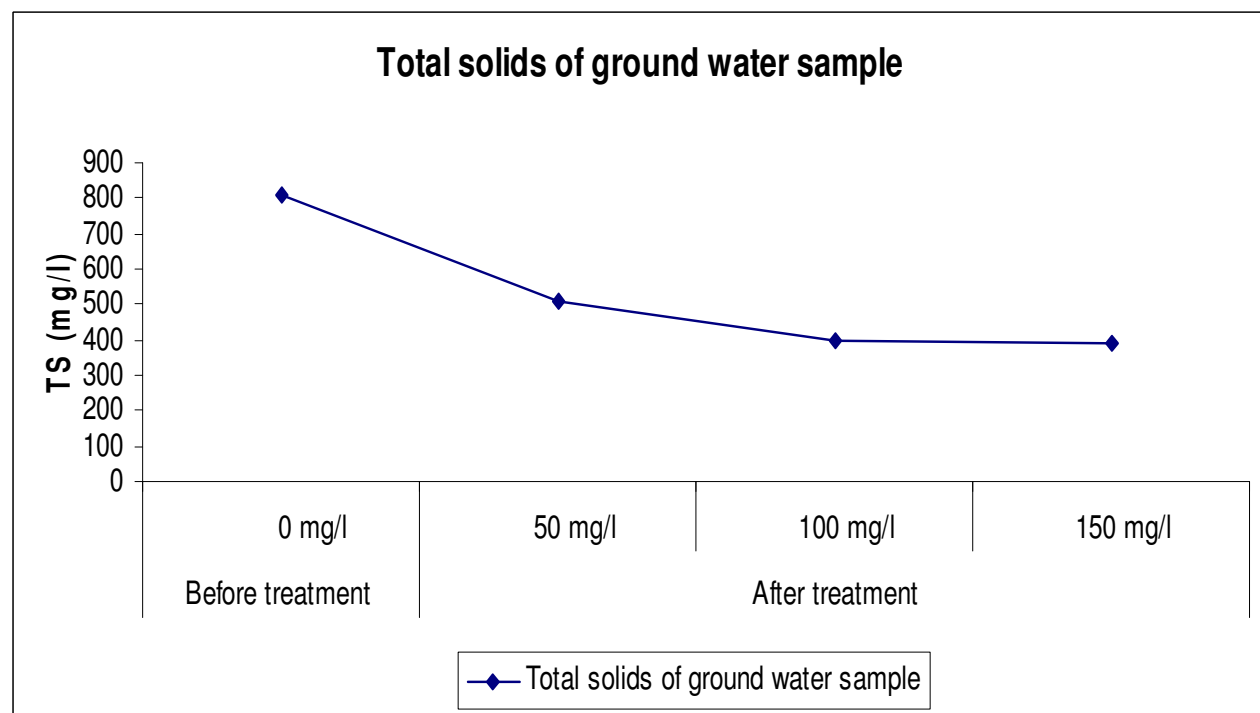


Figure-3
Total solids of ground water before and after treatment of *M. oleifera* seed powder

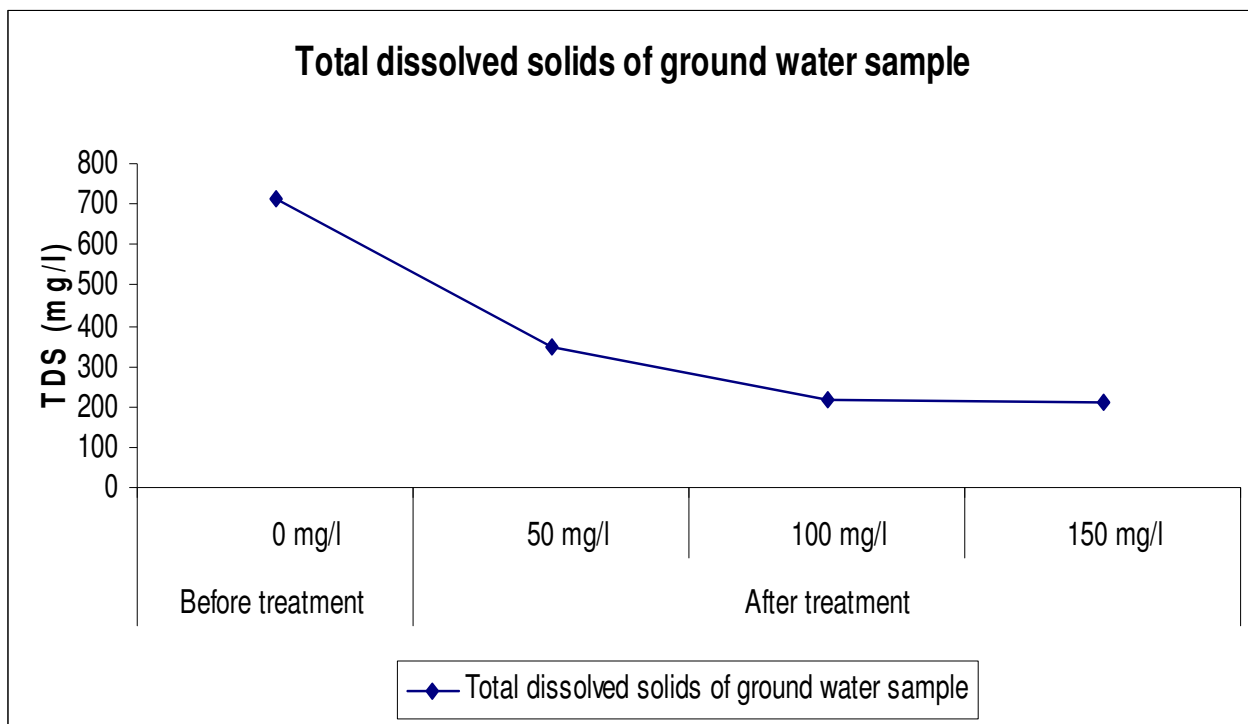


Figure-4
Total dissolved solids of ground water before and after treatment of *M. oleifera* seed powder

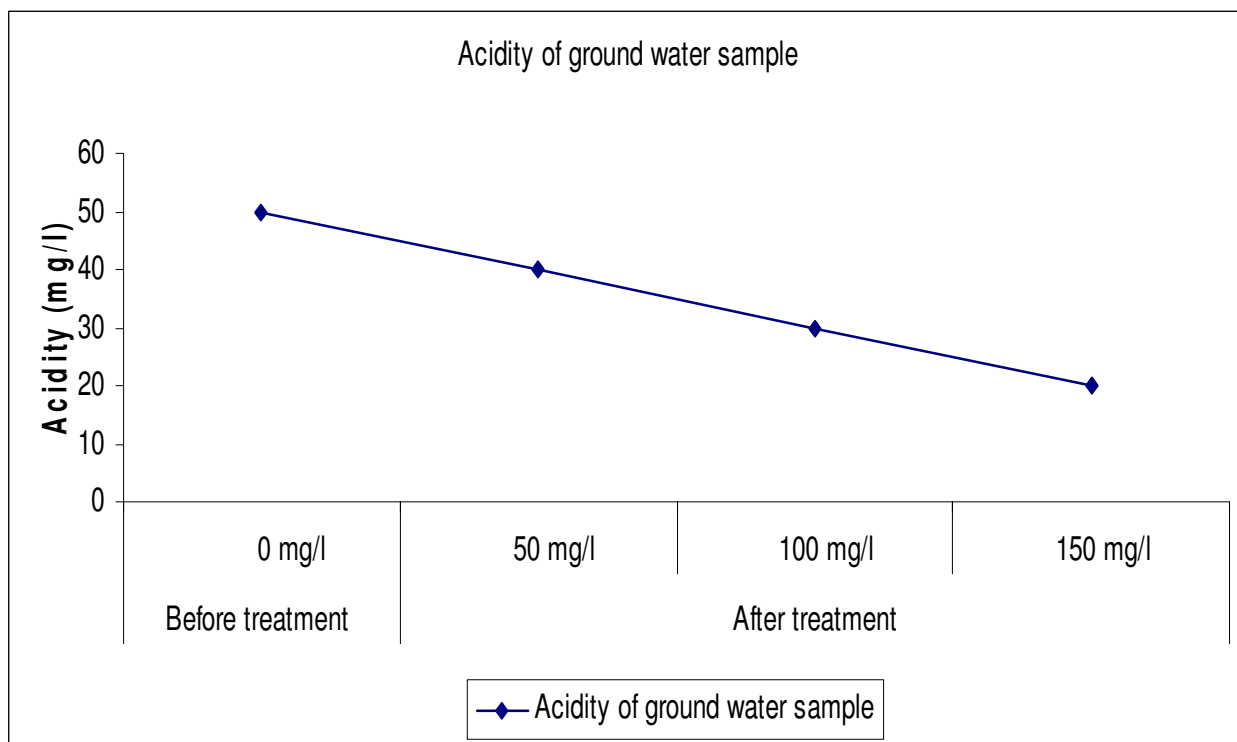


Figure-5
Acidity of ground water before and after treatment of *M. oleifera* seed powder

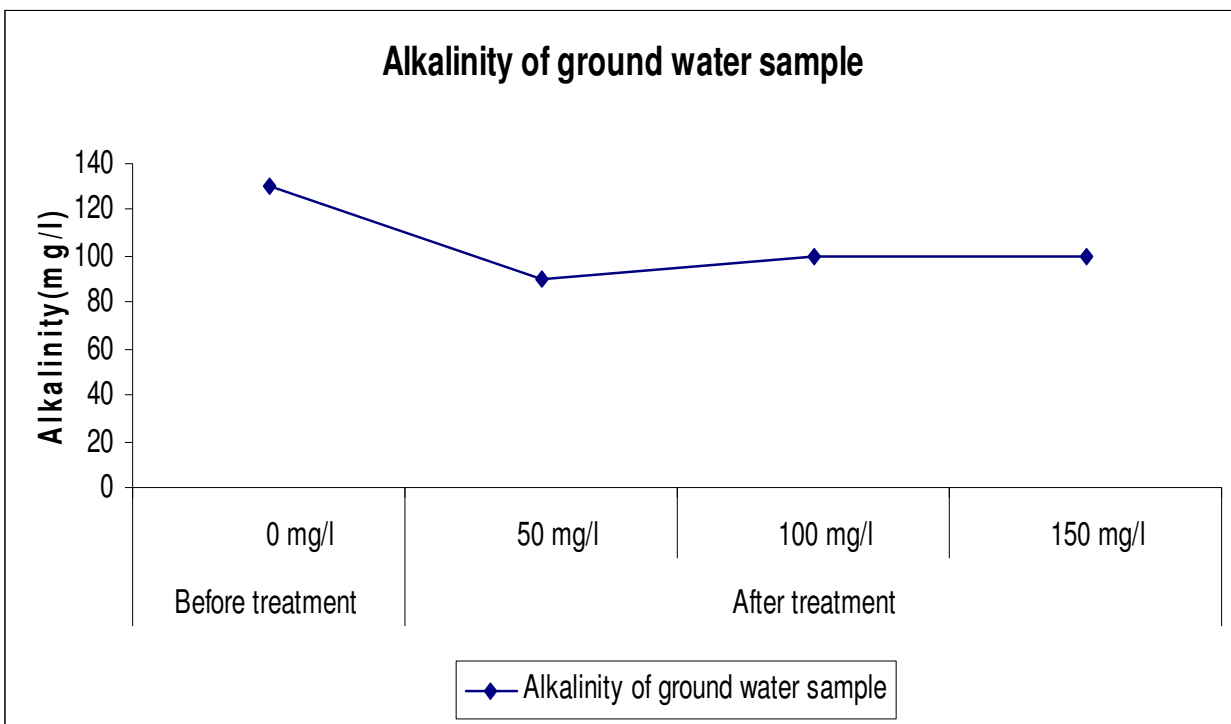


Figure-6
Alkalinity of ground water before and after treatment of *M. oleifera* seed powder

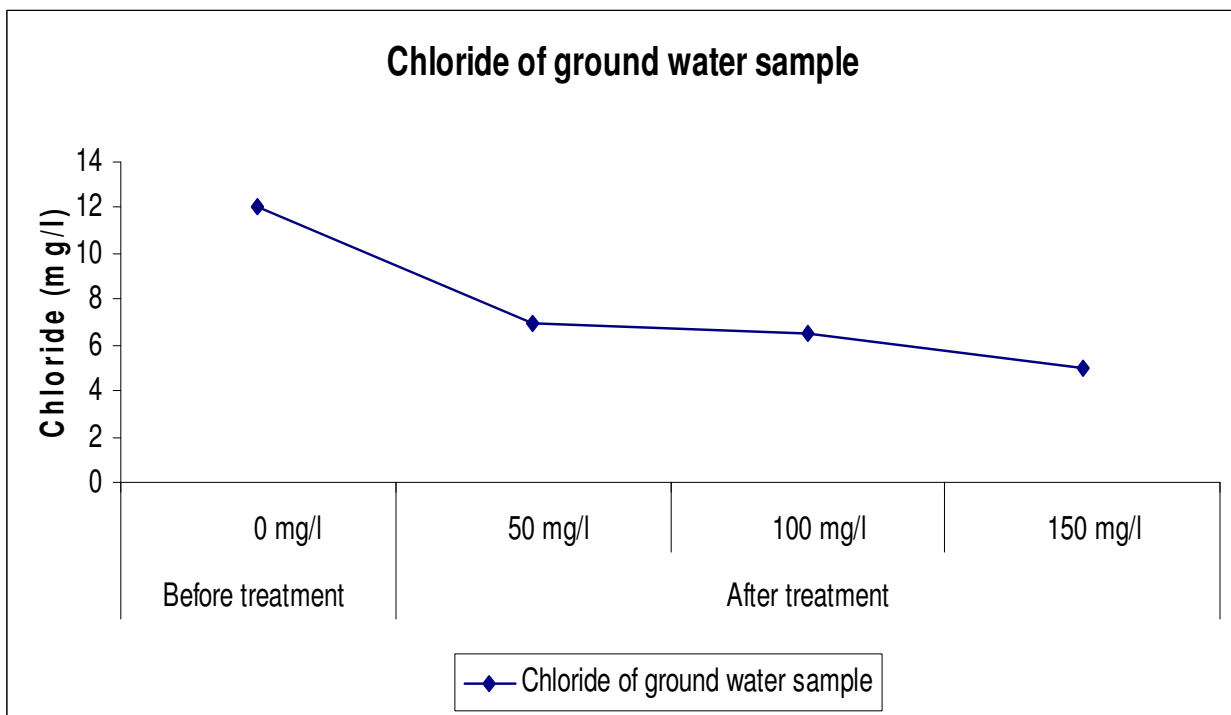


Figure-7
Chloride of ground water before and after treatment of *M. oleifera* seed powder

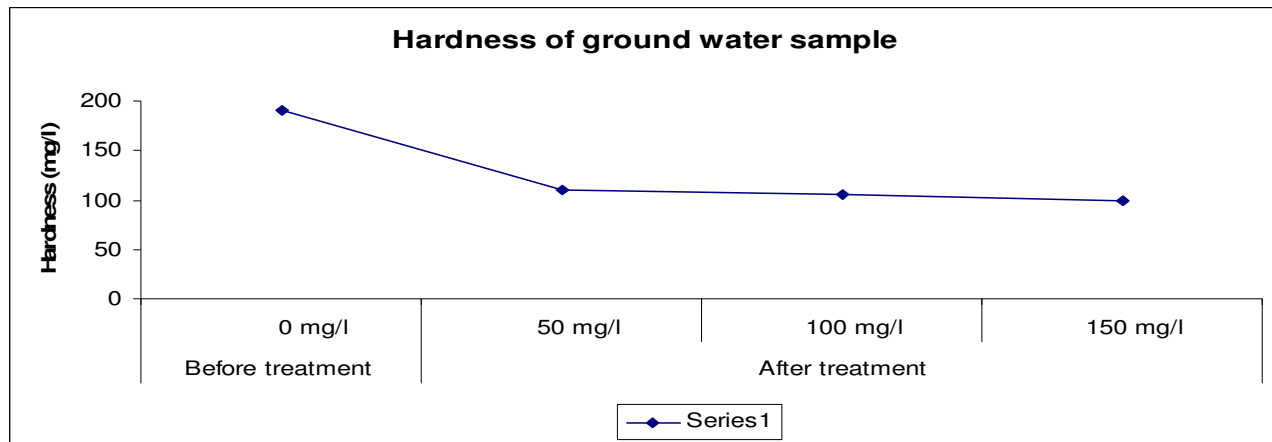


Figure-8
Hardness of ground water before and after treatment of *M. oleifera* seed powder

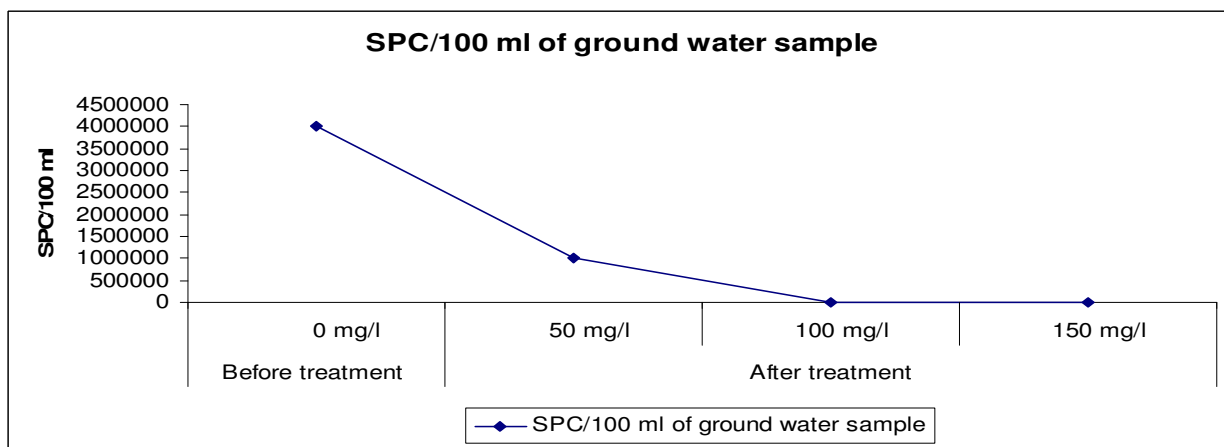


Figure-9
SPC/100ml of ground water before and after treatment of *M. oleifera* seed powder

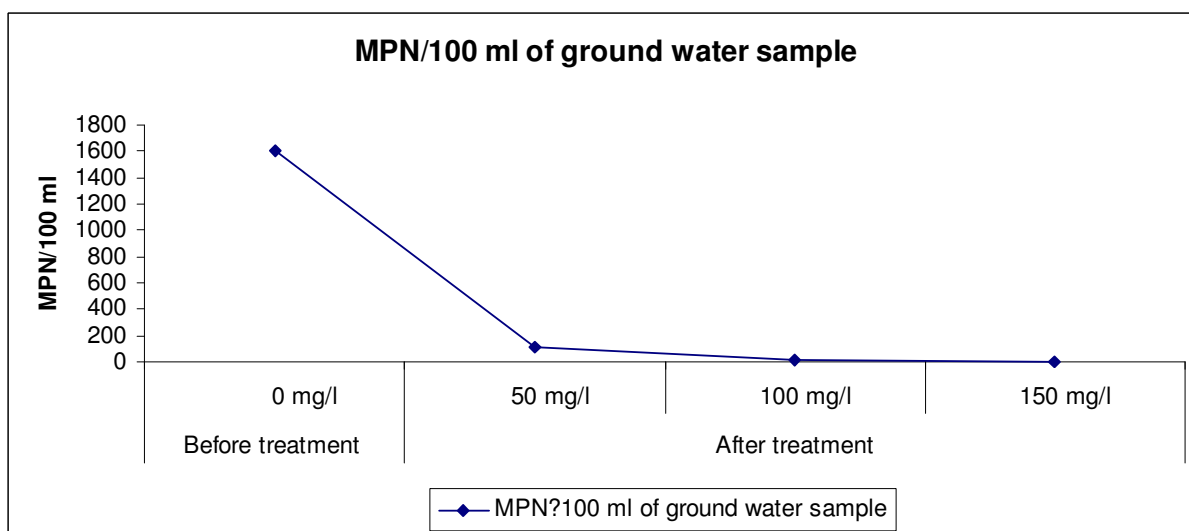


Figure-10
MPN/100ml of ground water before and after treatment of *M. oleifera* seed powder



Plate No. 3

Raw water sample treated with *M. oleifera* seed powder and kept on the shaker for 30 min at 110-120 rpm.

pH: Present study, treatment of *Moringa oleifera* seed powder was given to ground water samples in different doses. During the analysis, it was observed that after treatment with *Moringa* seed powder; pH was decreased at 50 and 100 dose, but it was partially increased at 150 mg/l dose, pH was. After treatment the range of pH was 7 - 7.5 and within the limit. The recommended acceptable range of pH for drinking water specified by WHO is between 6.0 and 8.0. The treatments gave a pH range of 7 to 7.5 which falls within the reducing trends as the concentrations of the dosing solutions were increased. The reverse was observed with the *Moringa* treatment. The pH increases with increasing concentrations of the *Moringa* as coagulant. It was reported that the action of *M. oleifera* as a coagulant lies in the presence of water soluble cationic proteins in the seeds. This suggests that in water, the basic amino acids present in the protein of *Moringa* would accept a proton from water resulting in the release of a hydroxyl group making the solution basic¹¹.

Turbidity: The initial Turbidity observed was 12.4 NTU in ground water which was beyond the limits of WHO standards. It was observed that the use of *Moringa oleifera* seed powder showed decrease in turbidity of ground water with increased dose at 50, 100 and 150 mg/l respectively. Residual turbidity reduces below 5 NTU. Due to this there was an improvement in the flock size and flock settled rapidly. The overdosing resulted in the saturation of the polymer bridge sites and caused restabilization of the destabilized particles due to insufficient number of particles to form more inter-particle bridges. The high positive charge and small size suggest that the main destabilization mechanism may could be adsorption and charge neutralization. This was also reported by Madsen *et.al*⁶, 1987 and found that 90-99% of turbidity was removed by using *Moringa* seed Powder.

Total solids and Total dissolved solids: The initial TS was in range of 700-800 mg/l for ground water which was beyond the limits of WHO. In case of TDS, initial range was 600-700 mg/l above permissible limit. After the treatment *M. oleifera* seed powder, the total solids and total dissolved solids were reduced

from ground water. The range of total solids was found in between 350-500 mg/l and for total dissolved solids range was 200-350 mg/l. These were present within the limit according to WHO standards. *Moringa oleifera* is known to be a natural cationic polyelectrolyte and flocculant with a chemical composition of basic polypeptides with molecular weights ranging from 6000 to 16,000 daltons, containing up to six amino acids of mainly glutamic acid, methionine and arginine .

Colour : The initial brown colour of ground water was completely removed after treatment of *M. oleifera* seed powder. The *M. oleifera* seeds show absorbent properties. Good clarification is obtained if a small cloth bag filled with the powdered seeds of the Maringa is swirled round in the turbid water.

Acidity: The Acidity observed was 50 mg/l for ground water. At various doses of *M. oleifera*, it was observed that the acidity decreased and found in the range of 5 - 20 mg/l which is within the limit according WHO standards. Because the seeds of *M. oleifera* contain lower molecular weight water-soluble proteins which carry a positive charge. When the seeds we crashed and added to water, the protein produces positive charge acting like magnets and attracting predominately negatively charged particles.

Alkalinity: Alkalinity during the present research work was observed to be 130mg/l for ground water. At various doses of *Moringa oleifera* seed powder, it was observed that the alkalinity reduced after the treatment at 50 mg/l dose. But at higher dose of 100 and 150 mg/l of *Moringa* seed, the alkalinity was slowly increased. The alkalinity was present in the range of 95 - 100 mg/l which was within limits of WHO standards. The slight decrease in alkalinity and pH of all water samples may be due to precipitation of insoluble products of the reaction between the *Moringa oleifera* and the hardness-causing ions similar to precipitation softening using lime/soda ash. The *Moringa oleifera* seed extract appears to have natural buffering capacity. The precipitates (solids / flocks) were light and did not settle easily. The chemical constituent of the precipitate is however not known. It was also confirmed that alkalinity reduction in the coagulation of water sources (groundwater) using *Moringa oleifera* seeds⁷.

Chloride: The Chlorides were present 12 mg/l in the ground water samples. It was observed that *Moringa* seed treatment with chloride ions reduces the chloride level, because cations from the seed attract negatively charged chloride ions present in ground water and neutralize the chlorides and therefore Chloride ions range between 5-9 mg/l in ground water samples which is within standard limit.

Hardness: Hardness was 190 mg/l for ground water sample. It was observed that hardness of water is decreased with increased dose of *Moringa* seed powder at 50, 100 and 150 mg/l of ground water. Hardness range was 100 - 170 mg/l and within the limit

of WHO standards. As a polyelectrolyte, it may therefore be postulated that *Moringa oleifera* removes hardness in water through adsorption and inter-particle bridging. According to Suleyman¹ as a polyelectrolyte it may therefore be postulated that *Moringa oleifera* removes hardness in water through adsorption and inter-particle bridging. Secondly, with the observation that light, slow-settling solids/flocks were formed and precipitation reaction lead to the conversion of soluble hardness-causing ions to insoluble compounds would also be a good prediction of the reaction mechanism. The higher value for the surface water and groundwater samples is due to the fact that they contain hardness due to calcium, magnesium and other hardness-causing substances. This implies that as the number of hardness increases, the required dosage of *Moringa oleifera* seed powder increases.

SPC (Standard Plate count): SPC means total bacterial count which is calculated by quantitatively. Due to high microbial load drinking water samples are unsafe for drinking purpose. Standard Plate Count was observed beyond the limit of USPH standards in ground water. The *Moringa oleifera* seed powder treatment had an added advantage of reducing microbial load. After the treatment, the numbers of bacterial colonies were reduced with increased dose of *Moringa* seed powder. After treatment, SPC range was found in between 10^2 - 10^5 within permissible limit in ground water. At 100 mg/l and 150 mg/l dose of *Moringa* seed, the plate shows fewer colonies. It was seen that the *Moringa oleifera* seed act as antimicrobial agent against microorganisms. Eilert et al¹², identified the presence of an active antimicrobial agent in the *M. oleifera* seed. The active isolated was found to be 4 alpha rhamnosyloxy-benzyl isothiocyanate, at present the only known glucosidic mustard oil. Coagulating the solid matter in water will also remove a good portion of the suspended bacteria.

MPN (Most Probable Number): MPN means total coliforms which are calculated quantitatively. The presence of coliforms indicates water is feacally contaminated and not safe for drinking purposes. Due to coliforms various waterborne diseases occur and therefore MPN should be nil for drinking water. MPN during the present study was observed beyond the limit of WHO standards for ground water. After treatment, MPN / 100 ml coliforms was decreased as increased dose of *M. oleifera* seed powder. MPN was found in the range 500 – 1200 coliforms/ml to all samples after the treatment which indicates above the limits of WHO standards. The presence of MPN gives direct proof of dangerous impurities in water¹³ and therefore treated Samples were indicated to be bacteriologically not safe for drinking purpose. Use chlorine with seed powder which can give MPN test negative.

Thus, effective dose of *Moringa oleifera* seed powder was 50 and 100 mg/l for reducing pH, TS, TDS, hardness, chloride, turbidity, acidity, alkalinity and for SPC and MPN the effective dose was 150 mg/l.

Conclusion

Moringa oleifera seeds acts as a natural coagulant, flocculant, absorbent for the treatment of ground water. It reduces the total hardness, Turbidity, acidity, alkalinity, chloride. It also acts as a naturally occurring antimicrobial active agent against the microorganisms which are present in the drinking water and decrease the number of bacteria. The MPN test had shown positive which indicates the fecal contamination and the water is not safe for drinking purpose. MPN was reduced after treatment of higher dose of 150 mg/l of *Moringa oleifera*. If a combined dose of *Moringa oleifera* seed powder and chlorine can give best results and ground water can be used for drinking purpose.

Moringa oleifera seed is not giving toxic effect. It is eco-friendly and cheaper method of water treatment. *Moringa* seeds can be used in the rural areas where no facilities are available for the drinking water treatment. After the treatment the sludge settled at the bottom of tank, can be used as bio-fertilizers is a added advantage of this method in rural areas.

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