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Study of *Moringa oleifera* (Drumstick) seed as natural Absorbent and Antimicrobial agent for River water treatment

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ABSTRACT

The high cost of treated water makes most people in the rural communities to resort to readily available water sources which are normally of low quality exposing them to waterborne diseases. The present study was carried out to confirm the effectiveness of seed powder extracted from mature-dried Moringa oleifera seeds which are commonly available in most rural communities. The main objective of this work is to evaluate the antimicrobial activity and efficiency of a natural absorbent from Moringa oleifera seeds in treating river water. During this study, surface water samples were collected for treatment by Moringa seeds in powdered form, resulting in an effective natural clarification agent for highly turbid and untreated pathogenic water. Various doses of Moringa seed powder viz. 50, 100 and 150 mg/l were taken and checked for the efficiency dose on raw water. After treatment of seed powder with water samples were analyzed for different parameter like pH, Turbidity, TDS, TS, Hardness, Chlorides, Alkalinity, Acidity, MPN and SPC. All parameters were reduced with increasing dose of 50, 100 and 150 mg/l seed powder respectively (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.

Key words: *Moringa oleifera*, River Water Treatment, Natural Absorbent, Antimicrobial Activity.

INTRODUCTION

Chemical coagulants like Aluminium sulphate (alum), $FeCl_2$ are used in Municipal drinking water treatment plant for purification process. This excess use of amount of chemical coagulants can affect human health e.g. Aluminum has also been indicated to be a causative agent in neurological diseases such as pre-senile dementia [1]. In rural and undeveloped countries people

living in extreme poverty are presently drinking highly turbid and microbiologically contaminated water as they lack of knowledge of proper drinking water treatment and also not afford to use highl cost of chemical coagulants. Some drinking water treatment plant in developing countries face a myriad of problems which are: large seasonal variation in raw water quality e.g. turbidity, high cost of water treatment chemicals, under dosing of chemicals leading supply of poor drinking water. 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 North-west 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 [2], and recommended it to use as a coagulant in developing countries [3]. 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 Muyibi and Evison, 1994,[1] hardness removal efficiency of *Moringa oleifera* was found to increase with increasing dosage. *M. oleifera* seeds act as a natural absorbent and antimicrobial agent as their seeds contain 1% active polyelectrolyte's that neutralize the negatively charged colloid in the dirty water. This protein can be therefore nontoxic natural polypeptide for sedimentation of mineral particles and organics in the purification of drinking water. These *seeds* are also act as antimicrobial agent against variety range of bacteria and fungi [4]. The seed contain number of benzyl isothiocyanate and benzyl glucosinolate which act as antibiotic [5]. 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 mechanism [1]. *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. According to Amagloh and Benang 2009**,** [6] 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. The use of natural materials of plant origin to clarify turbid water is not a new idea [7][8][9][10 and 11] cited by Ndabigengesere *et al*., (1995) [12] , [4] 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 (conventional chemical coagulant) [4][13]. 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 turbid. The seeds and powder can be stored but the paste needs to be fresh for purifying the water.

MATERIALS AND METHODS

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 were collected from Panchganga river water from Kolhapur city for the study purpose. 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 *M. oleifera* Seeds Powder (mg/l)
1	<50	50
2	50-150	100
3	>150	200

Plate No.1 *Moringa oleifera* tree

Plate No.2 Panchganga River, Kolhapur.

Plate No.3 Left side *Moringa oleifera* seeds with seed coating and right side seeds without seed coating

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 and 15] before and after the treatment. The efficiency dose of *Moringa oleifera* seed powder was determined.

RESULTS AND DISCUSSION

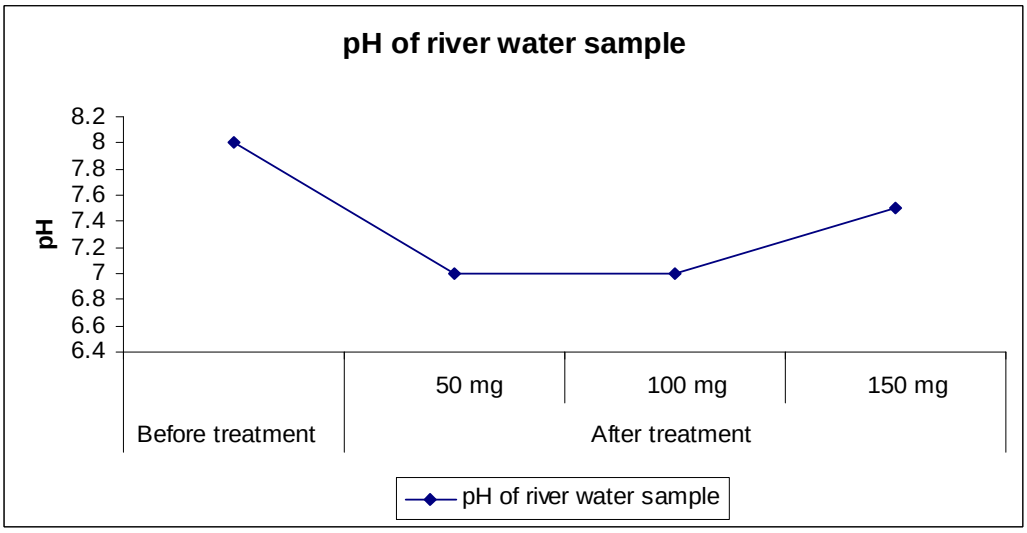
For the water samples were collected from Panchganga River, following drinking water quality parameters were analyzed before and after the treatment of various doses of *Moringa oleifera* seed powder.

Table No. 2: - Physico-chemical characters of river water before and after treatment with various doses of *M. oleifera* seed powder

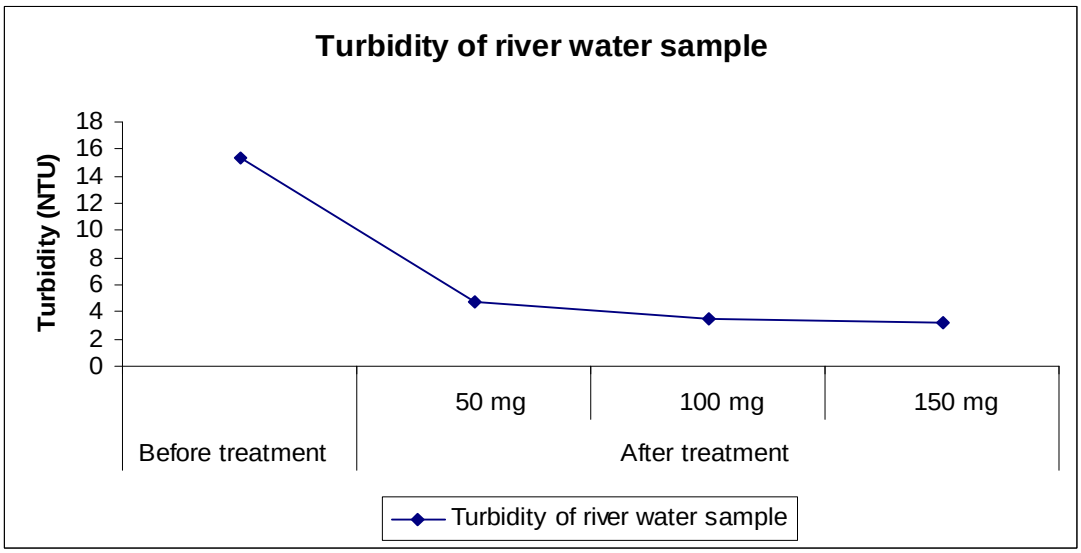
Sr. No.	Parameters	Before treatment	After treatment			WHO/USPH Standards
			50 mg/l	100 mg/l	150 mg/l	
1	Color	faint brown	Colorless	Colorless	Colorless	Colorless
2	pH	8 ± 0.11	7 ±0.04	7±0.05	7.5±0.05	6.5-8.5
3	Turbidity (NTU)	15.4±0.05	4.7±0.05	3.5±0.17	3.2±0.05	5
4	TS (mg/l)	792±0.57	500±0.56	400±0.57	400±1.15	-
5	TDS (mg/l)	652±0.57	312±0.57	230±0.28	235±0.57	500
6	Acidity (mg/l)	35±0.57	5±0.50	5±0.5	5±0.1	-
7	Alkalinity (mg/l)	104±1	92±1.15	96±0.5	96±0.28	200
8	Chloride (mg/l)	17±0.28	8.5±0.05	5.7±0.02	5.7±0.05	250
9	Hardness (mg/l)	188±0.57	168±0.57	160±0.57	156±0.28	500
10	SPC/100ml	6.2×10^{7}±0.57	1.2×10^{5}±0.57	1×10^{5}±0.57	1×10^{4}±0.57	1×10^{6}
11	MPN/100ml	2400±0.57	170±0.57	95±1	25±1.15	Nil

Values are expressed as Mean ± S. D.

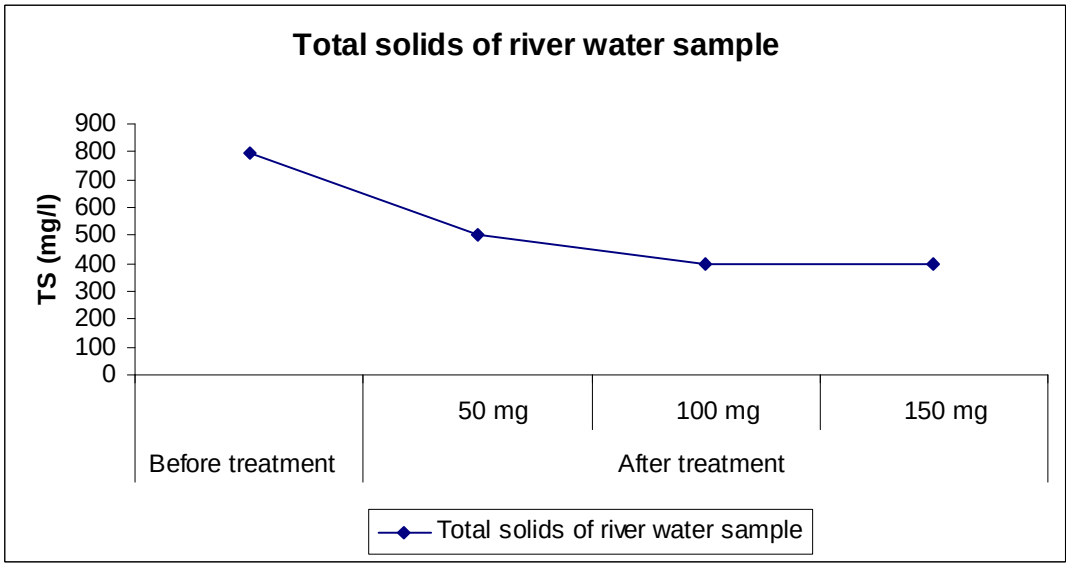
Graph No. 1 pH of river water before and after treatment of *M. oleifera* seed powder



Graph No. 2 Turbidity of river water before and after treatment of *M. oleifera* seed powder



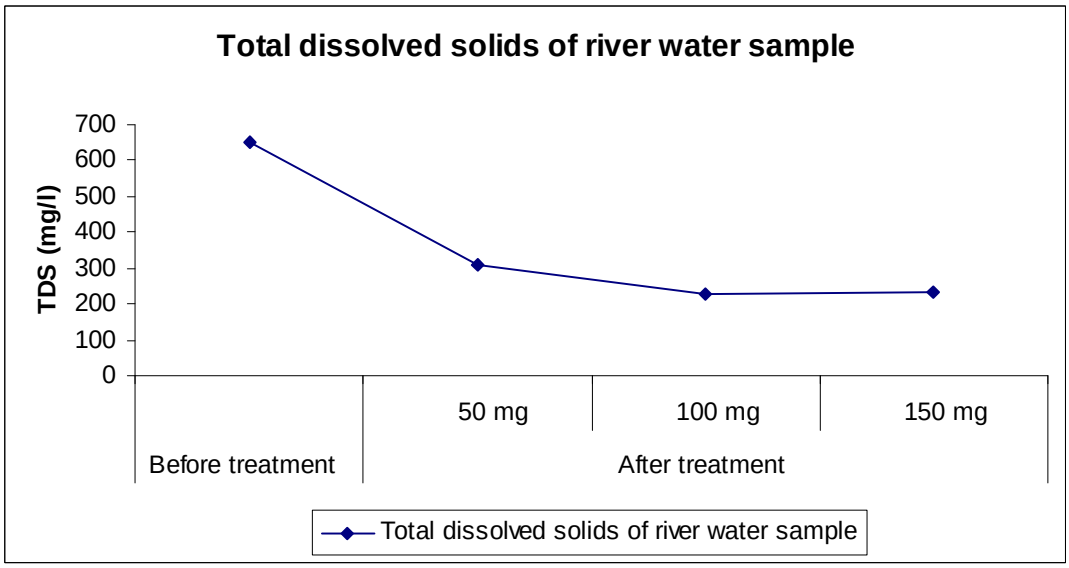
Graph No. 3 Total solids of river water before and after treatment of *M. oleifera* seed powder



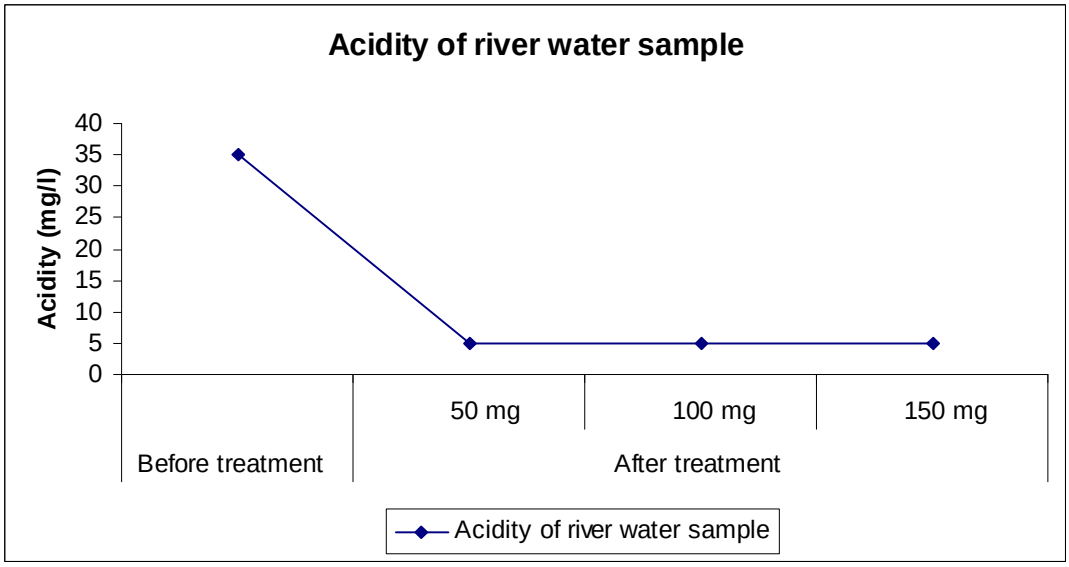
Colour: - The initial brown colour of the river water sample was completely removed after the treatment of *M. oleifera* seed powder. This suggests that the *M. oleifera* seeds show absorbent properties. Good clarification is obtained if a small cloth bag filled with the powdered seeds of the moringa is swirled round in the turbid water [9].

pH: - During the present study, treatment of *Moringa oleifera* seed powder was given to river water in different doses. During the analysis, it was observed that after treatment with *Moringa* seed powder; pH was decreased at 50 and 100 mg/l dose, but at 150 mg/l dose, it was partially increased (Graph No. 1). After treatment the range of pH was 7 - 7.5 and it is within the limit.

Graph No. 4 Total dissolved solids of river water before and after treatment of *M. oleifera* seed powder

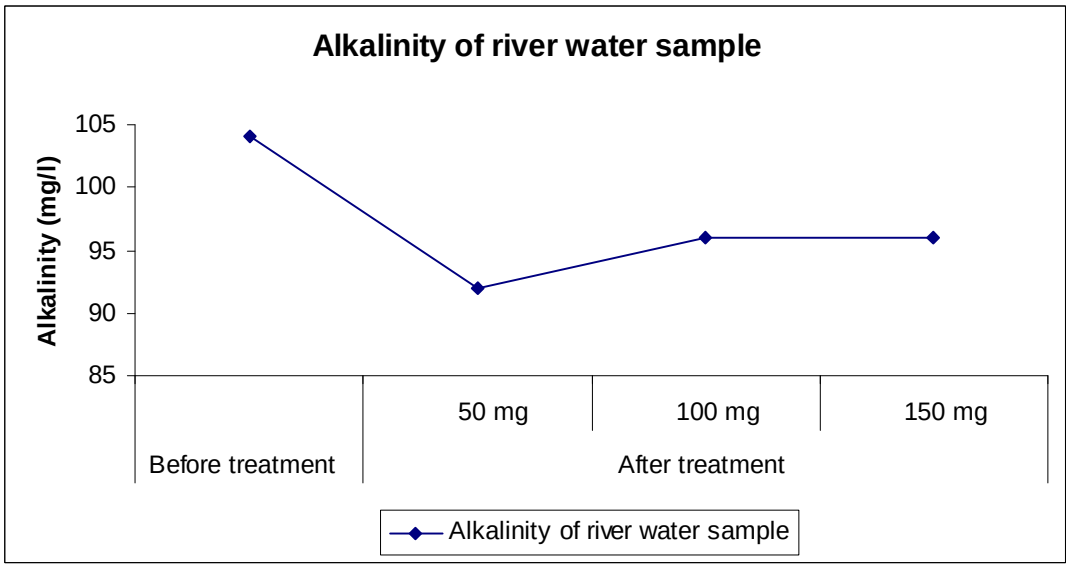


Graph No. 5 Acidity of river water before and after treatment of *M. oleifera* seed powder

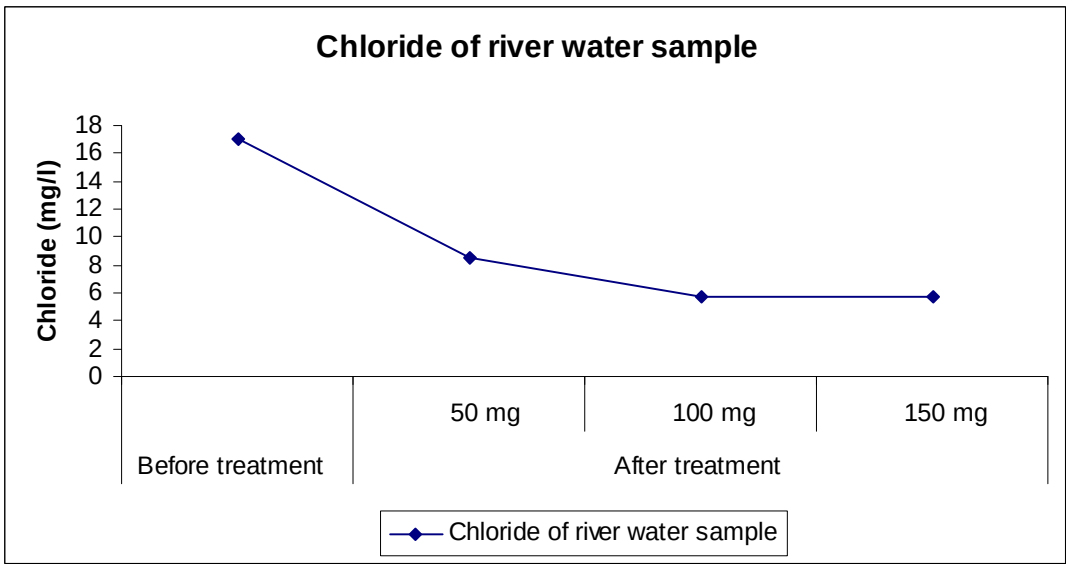


The recommended acceptable range of pH for drinking water specified by WHO (2006) is between 6.0 and 8.0. The treatments gave a pH range of 7 to 7.5 which falls within the reducing trends as the concentrations of the dosing solutions were increased. The pH increases with increasing concentrations of the *Moringa* seed powder as a *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* seed powder would accept a proton from water resulting in the release of a hydroxyl group making the solution basic [6].

Graph No. 6 Alkalinity of river water before and after treatment of *M. oleifera* seed powder

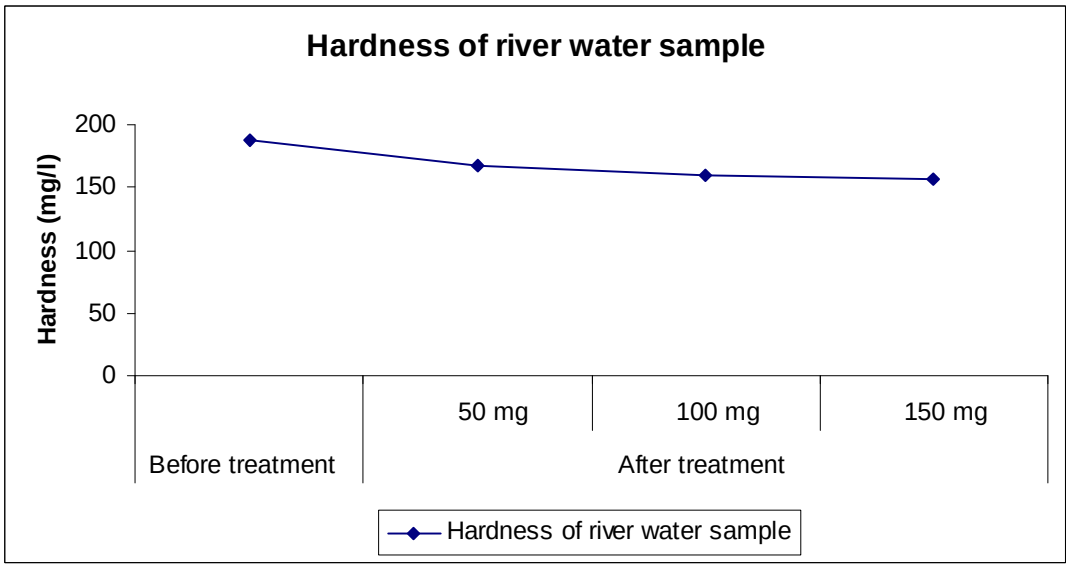


Graph No. 7 Chloride of river water before and after treatment of *M. oleifera* seed powder

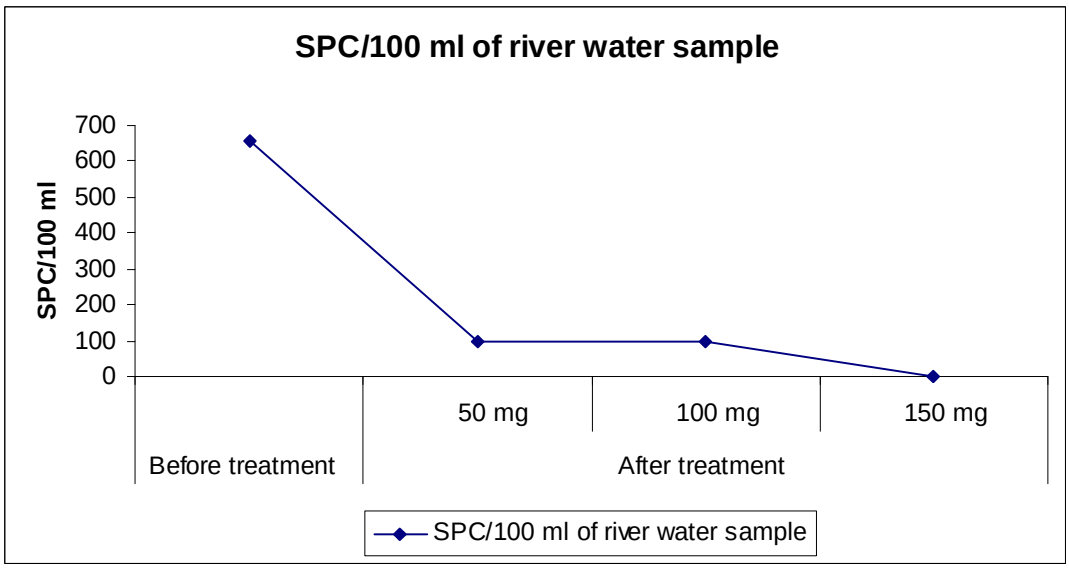


Turbidity: - It was observed that the initial turbidity was 15.4 NTU in the river water sample which was beyond the limit as per WHO standards in surface water (Graph No. 2). In the present study it was observed that the use of *Moringa oleifera* seed powder showed decreased turbidity with increased dose from 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 was settled rapidly. The overdosing resulted in the saturation of the polymer bridge sites and caused destabilization 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. *M. oleifera* seed powder removed 90-99% of turbidity in the treated water [4].

Graph No. 8 Hardness of river water before and after treatment of *M. oleifera* seed powder



Graph No. 9 SPC/100ml of river water before and after treatment of *M. oleifera* seed powder



Total solids and Total dissolved solids: - In the present study, it was observed that, the initial TS were in the range of 700-800 mg/l for the river water sample which was beyond the standard limits of WHO (Graph No. 3). In case of TDS, initial range was above 600-700 mg/l and is within permissible limits of WHO (Graph No. 4). After the treatment of *M. oleifera* seed powder, the total solids and total dissolved solids were reduced from the river water. The range of total solids was found between 350-500 mg/l and for total dissolved solids range was 200 - 350 mg/l, and these were present within the limits. *Moringa oleifera* is known to be a natural cationic polyelectrolyte and flocculent 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 [9].

Graph No. 10 MPN/100ml of river water before and after treatment of *M. oleifera* seed powder

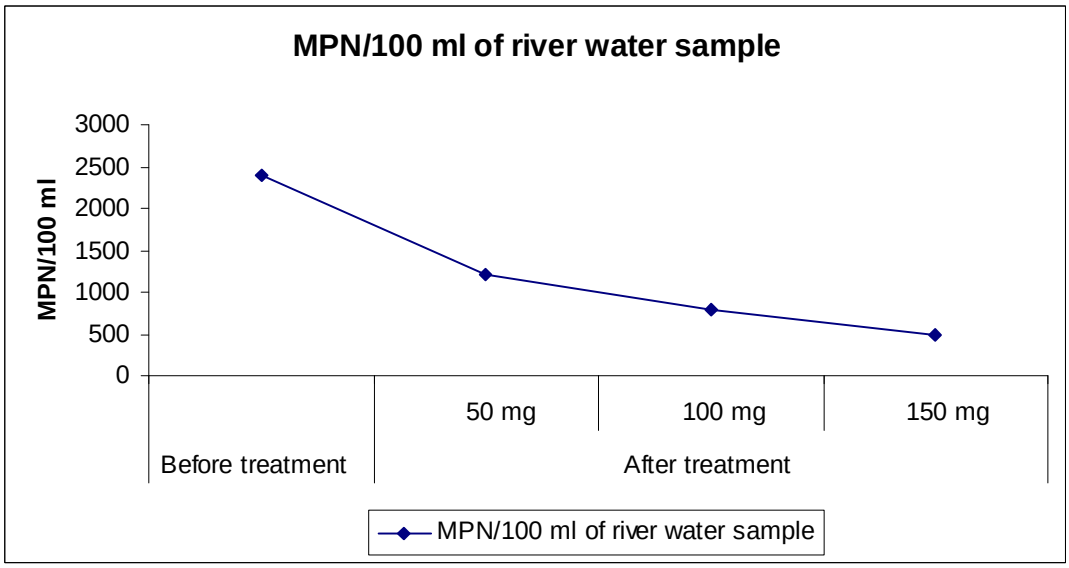


Plate No.4 Raw water sample treated with *M. oleifera* seed powder and kept on the shaker for 30 min at 110-120 rpm.

Acidity: - Acidity during the present study was observed to be 35 mg/l for the river water sample. At various doses of *M. oleifera, it* was observed that the acidity decreased and was present in the range of 5-20 mg/l within the limits of WHO standards (Graph No. 5). As the seeds of *M. oleifera* contain lower molecular weight water-soluble proteins which carry a positive charge. When the seeds were crushed and added to water, the protein produces positive charges acting like magnets and attracting predominately negatively charged particles leading to maintain acidity within range.

Alkalinity: - Alkalinity during the present study was observed to be 104 mg/l. 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 doses i.e. 100 and 150 mg/l, the alkalinity was increased (Graph No. 6). The alkalinity observed was in the range of 95-100 mg/l which was within the limit. 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, but it was found that alkalinity reduction in the coagulation of water sources using *Moringa oleifera* seeds [6].

Chloride: - Chlorides initially were 17 mg/l in the river water sample, but often treatment with *Moringa* seed reduced it three fold. It is because cations from *Moringa* seed attract the negatively charged chloride ions present in water and neutralize the chlorides. Chloride range was 5-9 mg/l in water samples and is within limits of drinking water standards (Graph No. 7).

Hardness: - Hardness ranges from 100-170 mg/l after treatment which is within the limits of WHO standards (Graph No. 8). As a polyelectrolyte it may therefore be postulated that *Moringa oleifera* removes hardness in water through adsorption and inter-particle bridging [16]. According to Muyibi and Evison, 1994 [1], as a polyelectrolyte *Moringa* seed powder *removes* hardness in water through adsorption and inter-particle bridging. Secondly, with the observation that light and slow-settling solids/flocks were formed, precipitation reaction leads 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 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 quantitatively. Due to high microbial load drinking water samples are unsafe for drinking purpose. Initial Standard Plate Count was beyond the limit of USPH standards in river water sample. 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 i.e. total bacterial count range was found in between 10^4 to 10^5 and is within permissible limit in river water (Graph No. 9). At 100 and 150 mg/l dose of *Moringa* seed powder, the plate shows very few colonies. It was also observed that the *Moringa oleifera* seed powder act as an antimicrobial agent against microorganisms [17], [5] identified the presence of an active antimicrobial agent in the *M. oleifera* seed. The active antimicrobial agent isolated was found to be 4 alpha rhamnosyloxy-benzyl isothiocyanate, and presently known as glucosidal mustard oil. It coagulates the solid matter in water so that it can be easily removed and will also remove a good portion of the suspended bacteria [3].

MPN (Most Probable Number): - MPN means total coli forms which are calculated quantitatively. The presence of coli forms indicates that the water is feacally contaminated and not safe for drinking purpose. Due to coli forms various waterborne diseases occur and therefore, MPN should be nil for drinking water. In the present study, it was observed that the initial MPN was present beyond the limits of WHO standards. After the treatment, MPN / 100 ml coli form was decreased from low dose to high level dose of *M. oleifera* seed powder**.** The MPN was present in the range 20 - 180 coli forms/ml in all samples after the treatment which indicates that it is above the limits of WHO standards (Graph No. 10). The presence of MPN gives direct proof of dangerous impurities of water, and therefore treated samples are not safe for drinking purpose.

CONCLUSION

Moringa oleifera seeds acts as a natural coagulant, flocculent, absorbent for the treatment of drinking water. It reduces the total hardness, turbidity, acidity, alkalinity, chloride after the treatment. It also acts as a natural antimicrobial active against the micro-organisms which is present in the drinking water and decrease the number of bacteria. The MPN test had shown positive which indicates the water samples are feacally contaminated and not safe for drinking. MPN test reading was reduced after treatment of higher dose at 150 mg/l of *Moringa* seed powder. If we can use combined *Moringa oleifera* seed powder and chlorine it can give best results and the water can be suitable for drinking.

Moringa oleifera seed is not giving any toxic effect. It is eco-friendly and cheaper method of purification of water and therefore can be used in the rural areas where no facilities are available for the treatment of drinking water. After the treatment of *Moringa oleifera* seed, sludge gets settled at the bottom of tank. Large scale treatment at village level produces large quantity of sludge which can be used as bio-fertilizers and it becomes an added advantage of this treatment.

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