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**MORINGA SEED POWDER - A POWERFUL BIOFLOCCULENT FOR DISINFECT
PAMPA RIVER DRINKING WATER SYSTEMS AS AN ALTERNATE TREATMENT
STRATEGY**

SAJUDEEN PA^{1*}, MOHAMMED ASHIQ², JAYACHANDRAN K³ AND BIJU A¹

1: Post graduate and Research Department of Zoology, St. Stephens College, Pathanapuram

2: DDRS SRL, Department of Molecular Biology and Genetics, Trivandrum

3: School of Biosciences, Mahatma Gandhi University, Kottayam

***Corresponding Author: Dr. Sajudeen PA; E Mail: sajudeenpa@gmail.com; Ph- +919447594052**

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ABSTRACT

Water is one of the most important commodities which Man has exploited than any other resource for sustenance of his life. River Pamba is the lifeline of Central Travancore. The variations in the ecological balance of river Pamba adversely affect the living organisms as well as the human beings who depend upon this river. Chlorine has been most widely used disinfectant for the purification of water systems throughout the world. Pamba river water with high amount of faecal coliform and suspended solids was subjected to chlorination dosage of 10mg/l for 30 minutes contact time followed by chlorination with sodium thiosulphate, Superchlorination resulted in the doubling of COD and BOD indicating the formation of more recalcitrant highly toxic chlorination derived by-products. As chlorination produces byproducts like Trihalomethanes and Halo acetic acids, we have developed alternate treatment strategies. It has been proved that chemicals are not a safe option for water disinfection. The newly formed compounds were extracted and were subjected to structural analysis. The data obtained indicated the presence of Trihalomethane, Haloacetic acid as the toxic chlorination derived by-products. Organic substances like

biofloculants, are better and dependable choice for aggregation of large flocs. Moringa seed powder mediated flocculation was used for the aggregation of large flocs due to slow process of sedimentation. Chlorination cannot be totally avoided. Chlorination only at low dose is recommended. Treatment with a natural bio flocculants like Moringa seed powder followed with mild chlorination was proved to be a safe practice of disinfection. This is the focus of our present investigation.

Keywords: Trihalomethane, coagulation, flocculation, Chemical Oxygen Demand, Biological Oxygen Demand

INTRODUCTION

Coagulation and flocculation plays a dominant role in water and wastewater treatment schemes, including those incorporating membrane treatments. Flocculation process was used commonly for water treatment to remove turbidity and natural organic matter. The process occurs in two stages to accelerate the colloids sedimentation by the injection and the scattering of chemical coagulants. These coagulants aggregate the colloidal particles and dissolved organic matter and easily eliminate them by sedimentation, flotation or filtration. Coagulation is generally induced by metals salts. The most widely and commonly used are aluminum and iron salts. The commonly used metal coagulants fall into two general categories: those based on aluminum and those based on iron. The aluminum coagulants include aluminum sulfate, aluminum chloride and sodium aluminate. The iron coagulants include ferric

sulfate, ferrous sulfate, ferric chloride and ferric chloride sulfate. Other chemicals used as coagulants include hydrated lime and magnesium carbonate. The effectiveness of aluminum and iron coagulants arises principally from their ability to form multi-charged polynuclear complexes with enhanced adsorption characteristics. The nature of the complexes formed may be controlled by the pH of the system. The coagulants are classified into inorganic, synthetic organic polymer and natural coagulants. The two most commonly used as primary coagulants are aluminum and iron (III) salts. However, the use of such chemicals, particularly aluminum, may have several environmental consequences [10]. It has been proved that synthetic chemicals are not a safe option for water disinfection whereas organic substances bio flocculants are better suited for water treatment [7].

Moringa Seed Powder as a Bioflocculant

There are various new water purification techniques which have come up to purify water for example by using rechargeable polymer beads, using the seeds of *Moringa oleifera* tree, purifying water by using aerobic granular sludge technology etc. All these techniques are being developed to ensure that in near future everyone will have access to clean and pure water and that too at an affordable cost.

The latest research has established that crushed moringa seeds are capable of attracting and sticking fast to bacteria and viruses that are found in contaminated and turbid water. The seeds produce positive charges like magnets attracting negative elements of bacteria and other toxic particles. This inspired the development of a revolutionary new natural sanitation water treatment that uses moringa seeds to purify water. Moringa seed oil also has potential for use as a biofuel Moringa seed. The seed of the Moringa tree (*Moringa oleifera*) is a natural flocculent/coagulant. The dried Moringa seeds or seed powder is added to water to treat turbid or cloudy water by pulling together floating particles-including dirt, other solids, and some germs and worms-and when the water settles, the particles sink to the bottom of the water

container. This method reduces dirt and germs but the water is not completely free of germs and therefore Moringa seeds should not be used as the only method of treating water. *Moringa oleifera* seeds acts as natural coagulant, flocculent, absorbent for the treatment of drinking water. It reduces the total hardness, turbidity, acidity, alkalinity, chloride after the treatment. Besides it is natural antimicrobial agent against the microorganisms which is present in the drinking water. In the present research work the pampa river water which is highly contaminated during the pilgrimage season was taken for analysis and effect of moringa seed powder was compared with usual chlorination procedure and attempt was done to reduce the dosage of chlorination.

MATERIALS AND METHODS

PREPARATION OF MORINGA OLEIFERA SEED POWDER

Ripped fruits (pods) of *M. oleifera* were collected from near Mahatma Gandhi University and near Pandalam area, Pathanamthitta, Kerala during the early rainy season and cracked to obtain the seeds. The seed were fine powdered for analysis.

Sample Treatment

The seeds were peeled to obtain the nuts and dried in an oven for 1hr. Thereafter, the dried seeds were ground and preserved in

air tight container for further use. Five different concentrations of the solutions for the loading dose were prepared by weighing 5.0, 10.0, 15.0, 20.0, 25.0 mg/l of Moringa powder separately into a beaker containing 1000 ml of distilled water. The Pamba river water sample with respective concentrations of Moringa seed powder were mixed in beakers and stirred using a magnetic stirrer to obtain a clear solution and allowed to stand for half an hour for settling down the contents. A 1000 ml of Pamba river water with no Moringa powder was kept as the control treatment.

Estimation of COD

Chemical Oxygen Demand was determined following the official method mentioned in APHA 2005 [2].

Detection of MPN (Total coliform using most probable number (MPN) procedure)

In determining the most probable number of coliforms that were present in each of the treated water samples, the multiple tube fermentation method was adopted. Lactose broth was used as the medium for the bacteria growth. Two types of the Mac Konky broth were prepared. These were the single strength broth (SSMB) and the double strength lactose broths (DSMB) of 3 tube method were followed.

Solvent extraction

Samples after chlorination was subjected to solvent extraction and the extracted samples were spectroscopically analysed. The samples were collected in the sterilized cans of 2.5 liters. The different parameters were analyzed according to the standard methods adopted by APHA 1995 [1].

FT/IR analysis

The extracted samples were subjected to spectroscopic analysis FT/IR using standard procedures at STIC, CUSAT, Cochin.

Chlorination

Chlorination experiment consisted of dosing different portion of the sample with varied chlorine amounts and mixing on a multi position magnetic stirrer for 30 min. A control sample to which no chlorine and moringa seed powder was added, was also processed similarly. At the end of contact time residual chlorine was measured and a calculated amount of 0.025 N sodium thiosulphate solution was added for complete chlorination. The amount of thiosulphate added was carefully measured to give an equivalent amount just enough to remove the residual chlorine and avoid any excess thiosulphate. Then a sampling programme was conducted to investigate the formation of

disinfection byproducts (DBPs) and dissolved organic carbon. Changes in the concentration of dissolved organic carbon, the Trihalomethanes potential and the haloacetic acid potential in the finished drinking water were evaluated.

RESULTS

Complete elimination of high amount of coliform by super chlorination is not a safe option for water disinfection. Hence attempts were made to bring alternate strategies for initial elimination of coliforms contributing to only mild chlorination towards the end.

Natural Organic substances are the best and dependable choice for flocculants. Bioflocculants are the best option for water treatment as they do not cause any harmful byproducts nor they will be affecting the polluting factors of the contaminated water.

In the present study Moringa seed powder was used as the bioflocculant.

Moringa seed powder was added at different dosage and its effect on MPN and COD was evaluated (**Table 1**).

The initial value of MPN was the highest and the COD was 17 mg/l. Upon treatment with Moringa seed powder the MPN got considerably reduced to 190 at 20 mg/l of Moringa seed powder. However the COD remained almost constant at 18 mg/l. 30 mg/l Moringa seed powder brought down the MPN to 36 where the COD remained unaffected at 18 mg/l.

In the **Figure 4**, band at 2880 cm^{-1} indicated Aliphatic CH_2 , band at 1258 cm^{-1} indicated CH_2 , band at 1100 cm^{-1} indicated C Cl bond and band at 700 cm^{-1} indicated the presence of residual chlorine Cl^- .

In the FT/IR analysis of the Moringa seed powder treated sample **Figure 5** shows that there is no bands at 2880 cm^{-1} , 1258 cm^{-1} , 1100 cm^{-1} , 700 cm^{-1} , indicate Aliphatic CH_2 , CH_2 , C Cl bond Cl^- .

Table 1: Effect of Moringa seed powder on TC and COD

| Trial | Moringa Seed Powder Dosage (mg/l) | MPN | COD mg/l |
|-------|-----------------------------------|------|----------|
| 1 | 0.0 | 1300 | 17±0.12 |
| 2 | 5.0 | 1220 | 17±0.35 |
| 3 | 10.0 | 760 | 18±0.19 |
| 4 | 15.0 | 370 | 18±0.198 |
| 5 | 20.0 | 190 | 18±0.198 |
| 6 | 25.0 | 36 | 18±0.198 |

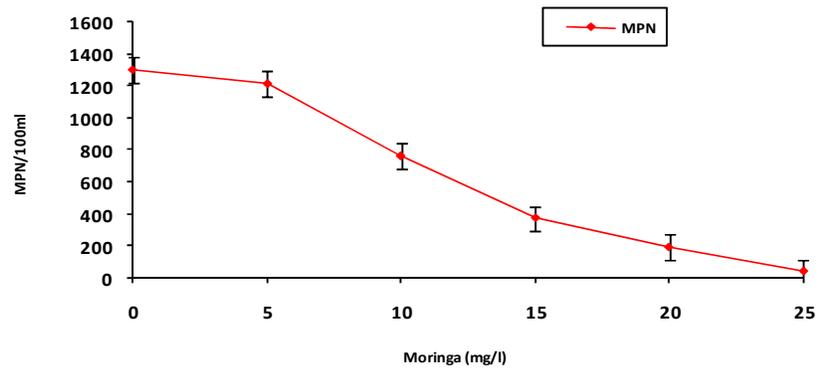


Figure 1: Effect of Moringa seed powder on MPN of Pampa river water sample

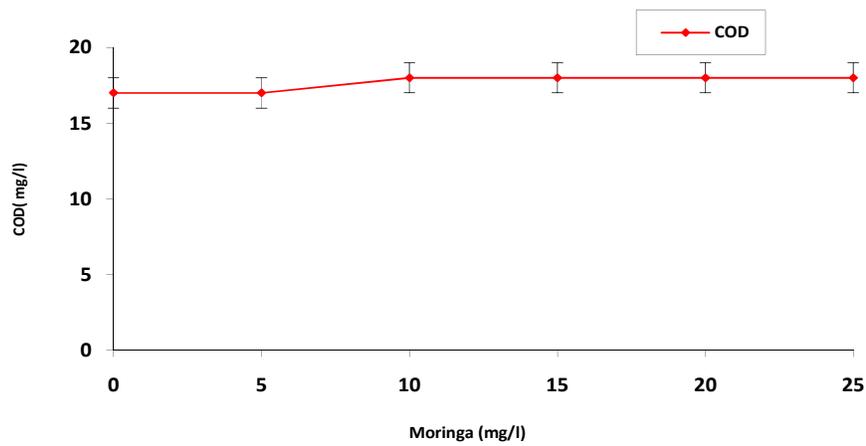


Figure 2: Effect of Moringa seed powder on COD

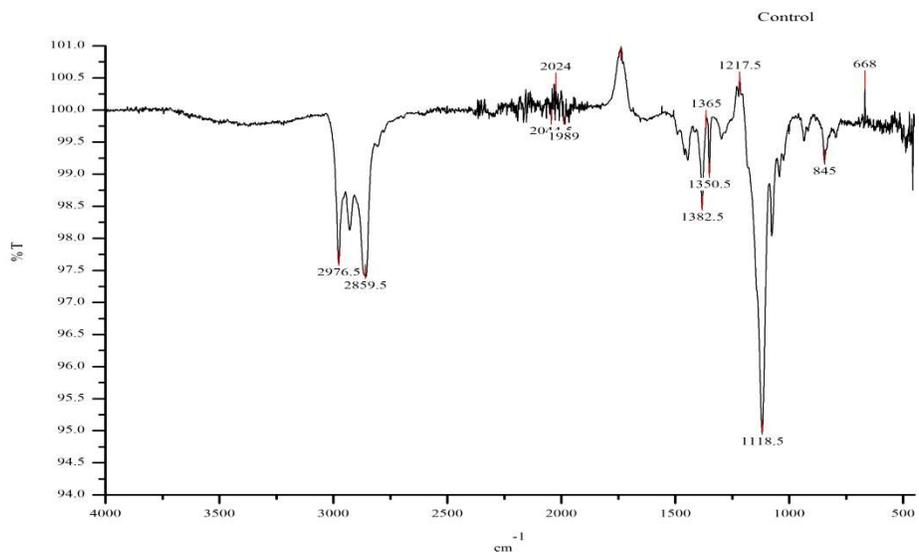


Figure 3: FT/IR spectrum of pamba river water sample (untreated)

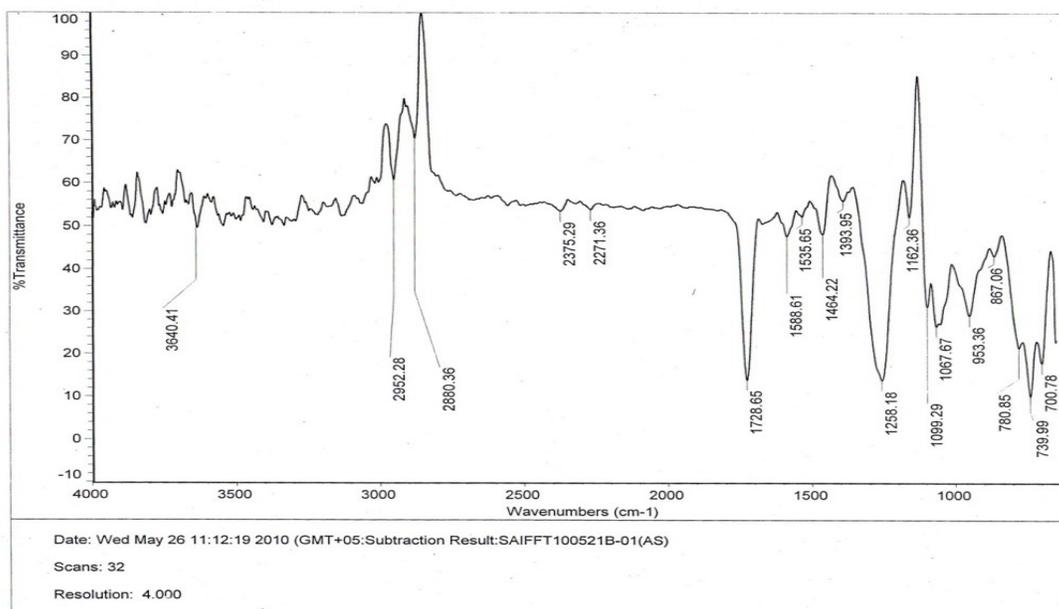


Figure 4: FT/IR of the ether extract of Pamba river water sample after chlorination

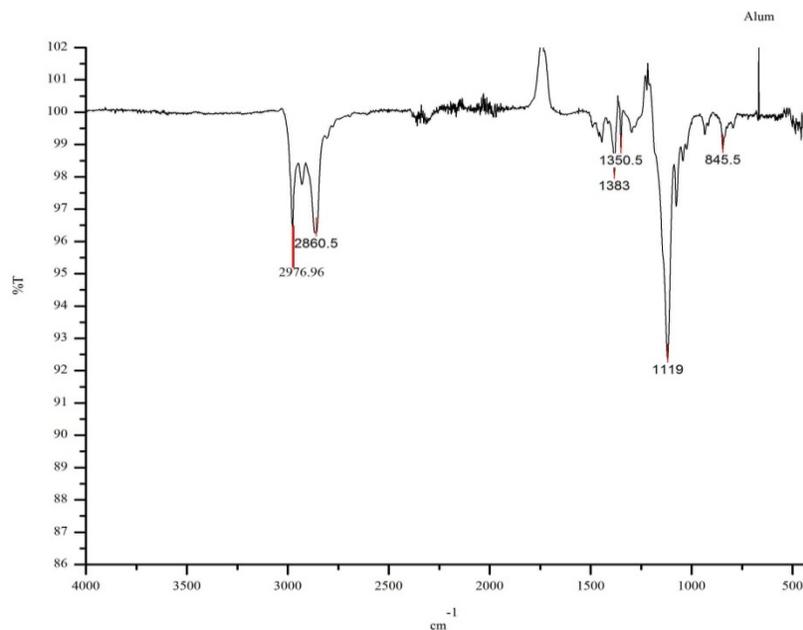


Figure 5: FT/IR spectrum of Pamba river water sample treated with Moringa seed powder

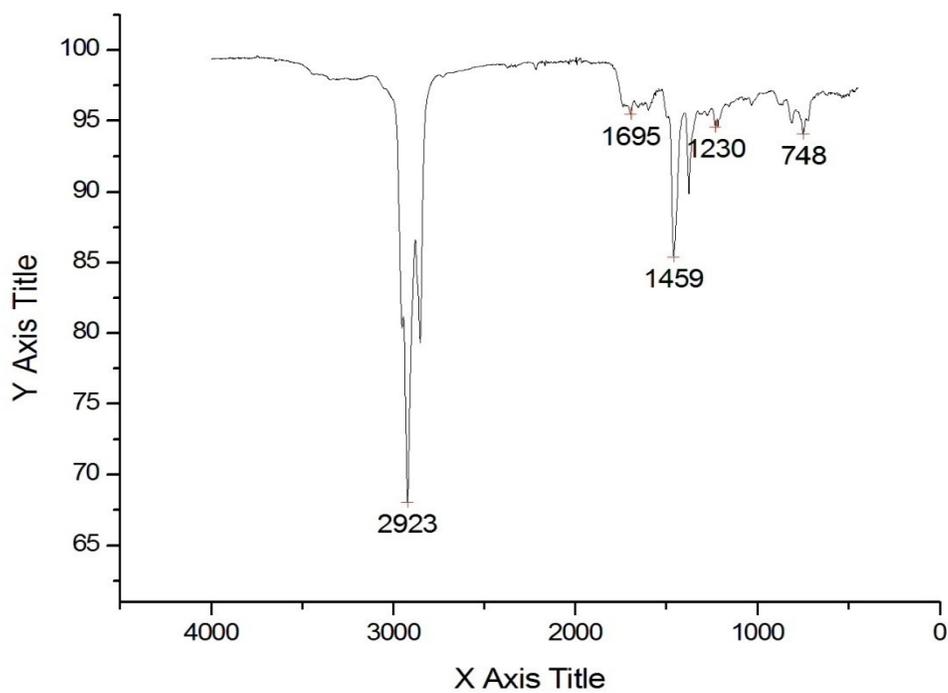


Figure 6: FT/IR analysis of the polluted water sample after integrated treatment of the water sample with the combinations of Moringa seed powder followed by low dose chlorination

DISCUSSION

The Pamba river water is heavily polluted with respect to high coliform and suspended solids. The MPN test had shown consistently positive result which indicated the water samples as feacally contaminated and not safe for drinking. The Most Probable Number (MPN) representing Total Coliforms were extremely high and represented heavy fecal contamination.

Equally, the water sample carried high value for the suspended solids which on the other side enhanced the polluting load of the sample. The removal of the suspended solids is generally regarded as the primary requisite for effectively treating any contaminated water systems. The most convenient and effective method of suspended solids removal is by sedimentation. It is based on the difference in the density between the bulk of the liquid and the sold particles. The settling may be discrete settling or zone settling. It may be by induced coagulation or by flocculation with flocculent aids. The coagulation or flocculation mediated sedimentation facilitates better sedimentation of the suspended matter resulting the co precipitation of coliform.

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 [4]. As a polyelectrolyte it may therefore be postulated that *Moringa oleifera* removes hardness in water through adsorption and inter-particle bridging [5]. It was also observed that the *Moringa oleifera* seed powder act as an antimicrobial agent against selected microorganisms. 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.

The adsorption of metals using *Moringa* is limited to the adsorption surface. This is because *Moringa* is a cationic polyelectrolyte of short chain and low molecular weight [6]. Heavy metals and solids have high charges and *Moringa* colloidal surface will remove high percentage of metals compared to other seeds. The mechanism that brings about the adsorption of heavy metals is through the positive metal ion that forms a bridge among the anionic polyelectrolyte and negatively charged

protein functional groups on the colloidal particle surface. There is formation of complexes for heavy metals with the organic matter of *Moringa* and protein content of seed. Due to its hydrophilic character, several hydrogen bonds are formed among polyelectrolyte and water molecules [8]. Polyelectrolyte coagulant aids have structures consisting of repeating units of small molecular weight forming molecules of colloidal size that carry electrical charges or ionisable groups that provide bonding surfaces for the flocs. Adsorption describes attachment of ions and molecules from seed protein by means of specific mechanisms [3]. The flocculation activities of *Moringa* seeds are based on the electrostatic patch charge mechanism. The Studies have shown that the seeds have the capability to adsorb metal cations and attract highly toxic compounds [6]. The *Moringa* had the potential to adsorb the heavy metals from the leachate and from industrial wastewater. This research shows that the method can be used for heavy metal removal from drinking water and wastewater. In this study the local *Moringa* seeds did not significantly have toxic effects but aided in improving the water quality for drinking purposes. The mechanism of coagulation with the seeds of *Moringa oleifera* consists of adsorption and neutralization of the

colloidal positive charges that attract the negatively charged impurities and metals in water. The results obtained in this study were comparable with the performance achieved in heavy metal removal by previous research work using *Moringa oleifera* extracts.

Treatment of contaminated water with *Moringa* seed powder considerably reduced the coliform count to 35 where it was 450 in the case of Alum treatment. More than that alum treatment slightly enhanced the COD where *Moringa* seed powder treatment was not found to influence the COD.

The FT/IR analysis of the chlorinated Pamba river water (**Figure 4**) showed all evidences for the formation of chlorination derived byproducts but there were indication of modified organic compounds in the case of FT/IR spectrum of *Moringa* seed powder treated (**Figure 5**) water sample.

The FT/IR analysis of the polluted water sample after integrated treatment of the water sample with the combinations of *Moringa* seed powder followed by low dose chlorination (**Figure 6**) shows that no chlorination derived byproduct formation and the treatment was a promising one.

Moringa seed powder mediated flocculation results in the aggregation of large flocs due to slow process of

sedimentation. The suspended high amount of coliforms might have got co-sedimented along with the large flocs formed during the treatment. The sedimented moringa seed powder along with the sedimented bacteria could be separated, dewatered, and filter pressed to get ideal soil conditioner and fertilizer [9].

Moringa plant are best cultivated in Kerala and the thus the moringa seed can be extensively collected as a small scale industry, The collected seeds can be crushed and pretreated to generate moringa seed powder on a large scale basis. This kind of a new strategy holds grater promise as a small scale industry offering job opportunities for large number of village women encouraging women empowerment. The strategy can be adopted in all villages as a special programme on sustainable basis for the primary treatment of the contaminated water systems. These studies have confirmed that the seeds are highly effective in removing suspended particles from water with medium to high levels of turbidity Moringa seeds are less effective at treating water with low levels of turbidity). The MPN test had shown positive results which indicated the water samples are faecal contaminated and not safe for drinking. MPN test reading was reduced after treatment of higher dose at 25mg/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. Chlorination cannot be totally avoided and can be recommended only at low dose. Super chlorination is to be avoided and alternate strategies like coagulation combined with low level chlorination is to be adapted as large scale water treatment methodologies.

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