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INVESTIGATION ON WATER TREATMENT WITH OIL EXTRACTED *MORINGA OLEIFERA* SEED POWDER IN ETHIOPIA

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ABSTRACT

One of the major challenges of developing country like Ethiopia is quality of drinking water. The high cost of treated water make large population in the rural communities to resort to readily available water sources, which are normally of low quality exposing them to waterborne diseases. In the present study an attempt has been made to treat the unsafe drinking water by naturally occurring *Moringa Oleifera* (MO) seed powder. The results show at 200 mgL^{-1} and 2 hours both, concentration of MO and contact time respectively. The treated water samples falls within WHO drinking water standards in terms of physico-chemical properties like color, EC, pH, TDS, concentration of magnesium, calcium, chlorides and bacteriological contents. Locally available *Moringa Oleifera* was found to be effective, cheap and natural coagulant, flocculent, absorbent for the treatment of unsafe drinking water. *Moringa Oleifera* showed anti bacterial activity, the number of bacterial colonies were reduced with increased dose of *Moringa Oleifera* seeds.

KEYWORDS: *Moringa Oleifera*, Antimicrobial activity, physico-chemical parameters for water quality.

INTRODUCTION

Water is a resource that is essential for life and is required by almost every living organism. This resource is however becoming very limited in its pure state due to many anthropogenic means of contaminations. About 1.2 billion people are drinking unsafe water worldwide. The vast majority of these people are located in sub-Saharan Africa, South Asia and East Asia. Countless lives are lost annually due to drinking and using contaminated water (WHO, 2014).

Increasing population density, scarcity and pollution of surface water and in organic contents of ground water is a serious problem for drinking water supplies in Ethiopia. Furthermore, drinking water may not be available where the people live, and Ethiopian women often have to walk for an hours to get the daily water for a family. Although, Ethiopia being a developing country where 22.4 % of people live below the poverty line (WHO, 2014) expensive conventional methods of assuring water quality are unsustainable.

In addition to the high cost of importing water treatment, chemicals like Alum , a common coagulant. A number of researchers have also found that Alum residue in water may be carcinogenic (Varadajan *et al,* 2008; Muhammad *et al*, 2011 and Reza *et al,* 2011). *Moringa* coagulant shows a major advantage compared to aluminium sulphate; it does not affect neither pH and alkalinity nor conductivity and TDS, whereas aluminium sulphate influences all of these water quality parameters (Ghebremichael *et al*, 2005). According to Federal Ministry of Water Resources Ethiopia (FMOWR, 2004) data, approximately 37.3 % of the Ethiopian population drinking treated water and 62.7 % was relied on sources that are unimproved, such as ponds, lakes, rivers and open dug wells. In addition, the coverage by improved technology varied significantly between regions, from 8% in Afar, 24% in Somali and to 96 % in Addis Ababa ((FMOWR, 2006). Thus only 24% people of Ethiopian Somali region were drinking treated water others are drinking at unsafe water from different sources.

In Ethiopia, the diarrhea morbidity is around 17 % (Daniel, 2008). The most common source of drinking water for the rural people is ground water from boreholes, shallow wells and springs (Marques *et al,* 2012). Groundwater is usually consumed without any form of treatment. Water is a medium of thousands of micro-organisms some of which are disease-causing pathogens (e.g.bacteria, viruses, protozoa and helminths) in water.

In Somali regional state two types of *Moringa* seed are available. *Moringa Oleifera* and *Moringa stenopetala* plant. However, *Moringa Oleifera* is selected due to more coagulating activity than *Moringa stenopetala*, *Moringa Oleifera* (MO) is a tropical, multipurpose tree whose seeds contains high quality edible oil (up to 40% by weight) and water soluble proteins that act as effective coagulants for water and waste water treatment and also the antimicrobial activity, turbidity removal, hardness removal of MO seed where conducted by different researchers (Khan *et al,* 2003, Ghebremichael *et al,* 2005).

This study was designed to reduce the use of carcinogenic organic solvents and to save the economic cost invested to extract oil from MO seed powder as well as to convert unsafe drinking water in to safe drinking water by using locally available *Moringa Oleifera* seeds.

MATERIAL AND METHODS

Study Area: Siti Zone Regional State bordered by Somalia's Awdal Region to the east; Dire Dawa and Jigjiga Zones to the southeast; Oromia Region to the Southwest, and Afar Region to the west with annual rainfall is between 500-700 mm. The source of water in Siti zones are boreholes, seasonal river beds, Pool and pods and rare base flows.

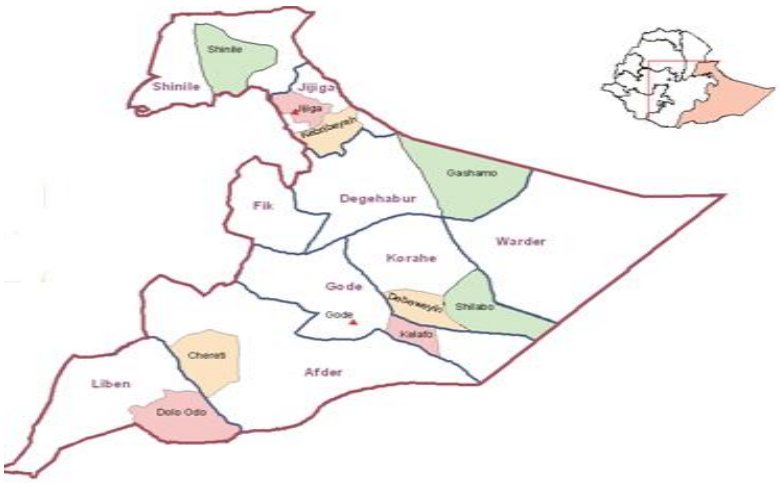

Figure. 1: Picture of Zones of Somali regional states of Ethiopia.

Sampling Methods

The matured seed of *Moringa Oleifera* was collected from Adgahala kebele of Siti Zone during dry season (January –May). The raw water was taken from four sources (two surface and two ground water) from the Siti Zones, Shinile woreda, Adgahala Kebeles of Somali regional states of Ethiopia. The collected water Samples were transported to laboratory and stored at 22-25 ^{0}C for further analysis. Conductivity, pH measurements and bacterial examination was investigated in Jig Jiga University. Whereas, concentration of Magnesium and Calcium in water samples and fat contents of MO seed was conducted in Haramaya University of Instrumental and nutritional laboratory whereas as well as Jar test was done in Addis Ababa University.

Figure. 2: Surface and ground waters sampling area in Siti Zone of Somali regional state, Shinille, Ethiopia.

Experimental Procedures

Preparation of Oil Extracted *Moringa Oleifera* Seed powder: For preparation seed powder for water treatment purpose, the seedpods were first allowed to dry naturally on the tree prior to harvesting. Dry MO seeds were collected and stored at room temperature (22-25 ^{0}C). The seed kernels of *Moringa Oleifera* were also removed by physical methods.Then Selected MO seed kernels were boiled in water at 80 ^{0}C for 30 minutes. Boiled seeds were dried in oven at 80 ^{0}C for 2hrs and grinded by using mortar and pastel. For each treatment, a paste of seed kernel powder with water was prepared and was stirred for 10-15 minutes to release the active components of seed in water with small amount of Sodium chloride (Bina, 2010). The water samples were treated with the seed extracts and were stirred rapidly for about 2-5 minutes and slowly for 10-15 minutes. Than samples were left undisturbed for 2 hours for formation of flakes and permitted the impurities to sink to the bottom of the beaker. The water samples were filtered using muscling clothes and stored for analysis.

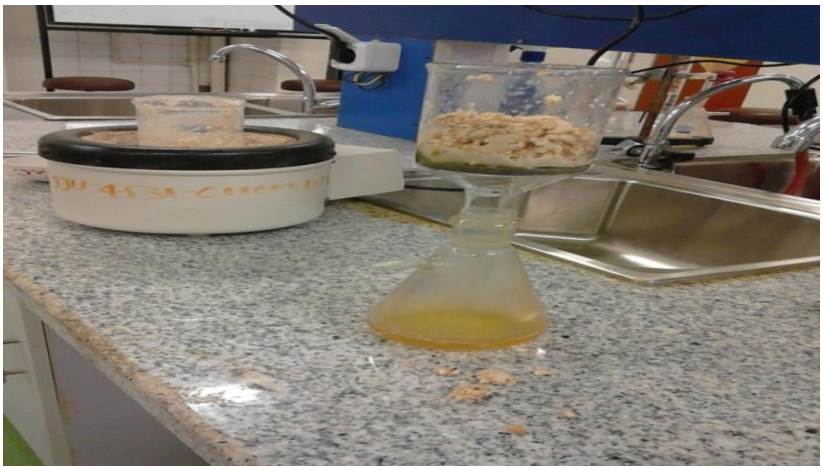

Figure. 3: Sample *Moringa Oleifera* seed powered preparations.

Fat contents of *Moringa Oleifera* seed powder

Fat content of MO seed was measured for boiled seed with distilled water and the fat content of seed was calculated by the following formula.

$$\% \text{ Oil in MO seed} = \frac{[(\text{Oil+flash weight}) - (\text{Flash weight})] \text{ X } 100\%}{2}$$

Effect of operation parameters on efficiency of oil extracted *Moringa Oleifera* seed powder on treatments of drinking water

Effect of Concentration load of *Moringa Oleifera* Seed powder

The effect of MO seed powder on efficiency of treatment of drinking water was observed by taking different amounts of *Moringa Oleifera* seed (50 mgL^{-1} to 300 mgL^{-1}) each time at constant intervals at pH 7.

Effect of Contact Time

The effect of contact time of MO seed on untreated water was studied by fixing other parameters constant, concentration of MO (200 mgL^{-1}), and pH of 7.04, with varying Contact time in the range of (1hr to 5 hrs). The water was stirred rapidly for two minutes and then slowly for 15 minutes.

Then the bucket of water was left undisturbed for 1to 5 hours and water was filtered through a clean cloth or filter paper then TDS, Ca, Mg and total hardness of drinking water parameters were studied.

Disinfection of water samples

A 2L filtrates of sample treated with MO seed powder was taken to clean transparent plastic containers (highland). The filtered water sample was stored in tightly closed, clear plastic containers and exposed to sunlight for 2hr period, after which a representative sample of each filtrate was taken for laboratory examination.

Water Quality Parameters

Drinking water, or potable water, is defined as having acceptable quality in terms of its physical, chemical, bacteriological parameters so that it can be safely used for drinking and cooking (WHO, 2014). WHO defines drinking water to be safe if no any significant health risks during its lifespan of the scheme and when it is consumed.

Water quality analysis

Effect of pH

Effect of pH on water treatment of oil extracted MO seeds was investigated over a pH range from 6.**5** to **9** keeping other parameters constant at 150 mgL^{-1} concentration of MO and 2hr contact time.

Total dissolved solids (TDS)

TDS contents after and before treatment by oven dry method. 100 mg weight of empty dish and 200 mg weight of empty dish plus and weight of water treated by MO was poured to evaporating dish and heated for 105 ^{0}C for 1 hour in oven. Then after all water samples were evaporated, the dishes were kept in desiccators for cooling for one hour. Final weight of evaporating dish was taken for TDS calculation (Eman *et al*, 2009).

Determination of Calcium and Magnesium concentration

The amount of calcium (Ca) and magnesium (Mg) in the samples before and after treatment with MO seed was determined by absorbance measurements conducted by using AAS (BUCK) instrumental Model, 210VGP) with current of 1amper , air/ acetylene flame and width slit 0.7nm (Kim and Cardone, 2005).

Determination of concentration of Chlorides

9.0 g of $AgNO_3$ was weighed out, transferred to a 500 mL volumetric flask and made up to volume with distilled water. The resulting solution was approximately 0.1M. This solution was standardized against NaCl. Reagent-grade NaCl was dried over night and cooled to room temperature. 0.2500 g Portions of NaCl were weighed into Erlenmeyer flasks and dissolved in about 100mL of distilled water. In order to adjust the pH of the solutions, small quantities of $NaHCO_3$ was added. About 2 mL of K_2CrO_3 was added and the Solution was titrated to the first permanent appearance of red $AgCrO_3$ then concentration of chloride was calculated at equivalent point. Concentration of Chloride is a calculated at equivalent point by the following formula (Samia *et al*, 1979).

$$Chloride\ conc.\ in \frac{mg}{L} = \frac{(Vs - Vb)\ x\ N\ x\ equivalent\ of\ chloride\ \ x\ 1000}{Volume\ of\ sample}$$

When Vs = volume silver nitrate for sample

Vb = Volume silver nitrate for blank and N = normality of $AgCrO_3$

Bacterial Examination

Water samples treated with MO seeds were filtered and filtrate was placed in the direct sunlight in a clear bottle for two hours then sample was taken for Antimicrobial examination by colony factor according to the method given by **(**Dhanalakshmi, *et al*, 2013).

pH and Electrical Measurements

Two samples (ground and surface water) three for each replicate was taken to 500 ml beaker. EC was measured by using calibrated conductivity (Model 2061, Germany) the two electrodes were inserted in sample water for different concentration of MO seed treatments the conductance was recorded (Abdulmoneim and Abu Zaid 2011).

RESULTS AND DISCUSSION

Fat contents of *Moringa Oleifera* seed powder

MO seed powder was investigated after grinding the matured seed contains 36.4 % of fats while after boiling in water at 80 ^{0}C for 30 minutes the fat content was reduced to 22.20 % this means 77.80 % oil of MO seed was removed.

Physical Properties analysis

Color: Color of drinking water is one of the parameter that affects quality of drinking water. Also Drinking water should be free of color. However, the initial brown color of both Surface and ground water sample at Shinile zone of Adgahala kebele as shown in figure 4 was completely removed after the treatment with of MO seed powder. This suggests that the MO seeds show absorbent properties of color forming metals and also flocculation of TDS of water.

Figure. 4: Color removal application of *Moringa Oleifera* seed powder.

Electrical conductivity (EC)

Electrical conductivity was a measures the saltiness of the water. As shown in Figure 5 and Table-1 EC of water sample before treatments is very high which is above WHO drinking water standard due to high temperature and salinity of water in Siti zone of Adgahala kebele. However, after treatment of sample water with natural coagulant MO, EC decreasing at alarming rate due to hardness and negatively charged salts adsorbed on the surface of proteins in MO seed which are accounts for EC. Upon addition of 250 mgL^{-1} of MO seed the EC of sample water reduced to 1600- 1400 µS cm^{-1} which is in the range of WHO drinking water standards.

Table. 1: Effect of *Moringa Oleifera* seed powder for removal of EC of sample waters.

Concentration of MO in mgL^{-1}	EC of sample surface water in μScm^{-1}	EC of sample ground water in μScm^{-1}
0	2700	2300
50	2637	2140
100	2567	2064
150	2300	1873
200	2138	1649
250	1875	1478
300	1579	1369

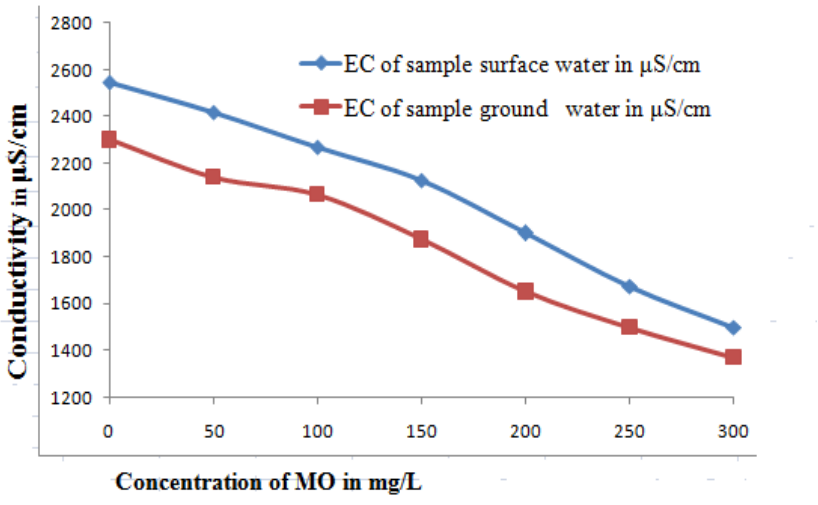

Figure. 5: Electric Conductivity versus Concentration of *Moringa Oleifera* seed graph

Chemical Analysis of Water Treated with *Moringa Oleifera* Seed Powder.

Effect of pH on treatment of *Moringa Oleifera* seed powder

As shown in Table-2, Figure 6 the sample water from surface and ground the results show weak base characters with pH of 7.9 and 8.3 respectively ground water without any

treatments. However; upon addition of MO seed powder the pH reduced from 7.04 - 7.5 which nearly neutral. Even though, the recommended acceptable range of pH for drinking water specified by WHO (2014) is between 6.5 and 8.0. The treatments gave a pH range of 7.04 to 7.5 that fit World health organization (WHO) and Ethiopian Standard Agency (ESA) for drinking water 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 MO and the hardness causing ions similar to precipitation softening using lime/soda ash. The MO seed extract appears to have natural buffering capacity. The precipitates (solids / flocks) were light and did not settle easily and separated from water by filtration using Whatman filter paper/traditionally pure clothes. However; the pH increases with increasing concentrations of the MO seed powder may be due to increasing excess positive protein may account for increasing pH. 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 (Witek-Krowiak *et al*, 2011).

Table. 2: Effect of pH on efficiency of *Moringa Oleifera* seed powder on sample water treatment.

Concentration of MO added in mgL^{-1}	pH of Surface water	pH of ground water
0	7.9	8.3
50	7.65	7.78
100	7.33	7.45
150	7.08	7.12
200	7.04	7.09
250	7.06	7.13

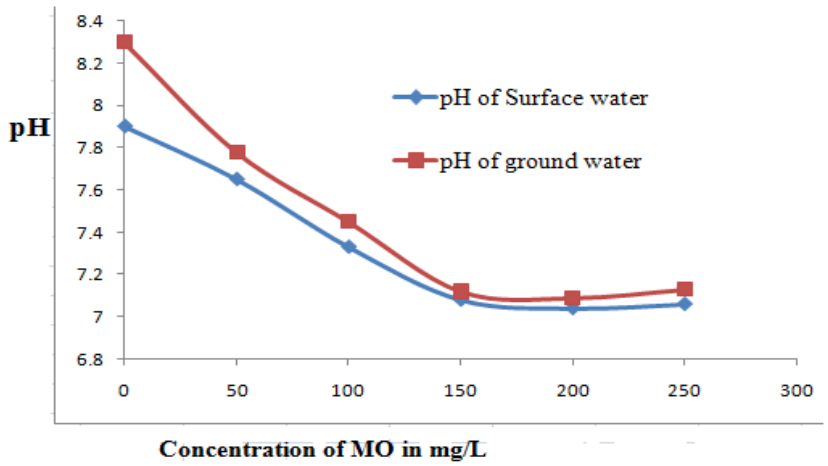

Figure. 6: Effect of pH on efficiency of *Moringa Oleifera* seed powder on unsafe water treatments.

Studies were carried on by securing a few milligrams of pure protein for the characterization of coagulant activities of MO. Also, the extraction of the proteins using 1M sodium chloride solution gave enhanced coagulation at significantly reduced dosage compared to water extracted material (Akhtar *et al*., 2006).

Total dissolved solids (TDS)

As shown in Table 3 and Figure 7 water samples analysis of both (surface and ground), results showed that without any addition of MO seeds was in the range of 1585- 1885 mgL^{-1} due to high concentration of soluble salts magnesium and calcium in the area. After addition MO seed powder total dissolved solids were reduced due to peptide protein in MO seed high settling powder to form flakes to decrease TDS of drinking water. The range of total dissolved solids after treatment with MO seed was found to be 967-1205 mgL^{-1} upon addition of MO seed which fits WHO standard limits for drinking water.

Table. 3: Doze related response of water samples treated with *Moringa Oleifera* seed powder.

Concentration of MO in mgL^{-1}	TDS in ground water in mgL^{-1}	TDS in surface water in mgL^{-1}
0	1320	1467
50	1228	1393
100	1125	1374
150	1071	1271
200	1004	1167
250	998	1085
300	967	1006

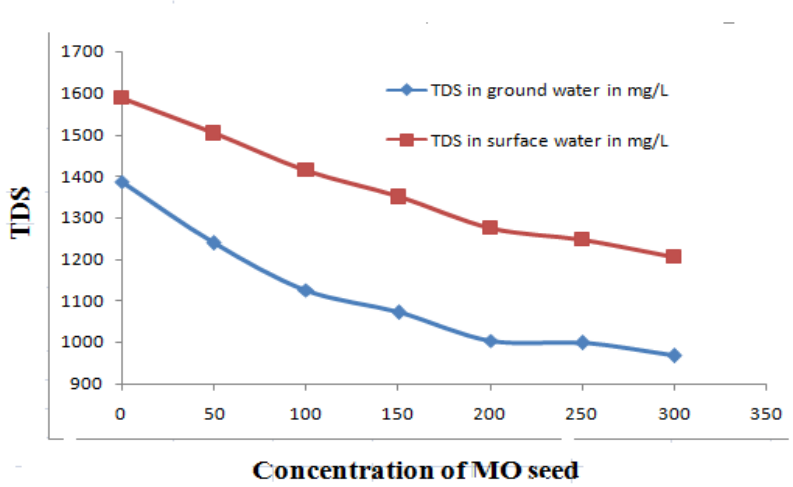

Figure. 7: Efficiency of *Moringa Oleifera* seed powder on removal of TDS of waste water.

Removal of calcium and magnesium from treated water samples

Atomic absorption spectroscopic (AAS) data shown in Table 4, the concentration of hardness causing both calcium and magnesium was determined after treatment with MO seed and without treatment. Then concentration of both Ca and Mg was calculated from standard curve at regression value of R^2 = 0998 and 0.999 respectively for calcium and magnesium as shown in figure 6. The concentrations of both magnesium and calcium without treatment was 120 mgL^{-1} and 100 mgL^{-1}respectively. However, when 200 mgL^{-1} of MO seed was added the concentration of both calcium and magnesium was decreased from 43 and 57 mgL^{-1} respectively which goes with the WHO and ESA standard limits.

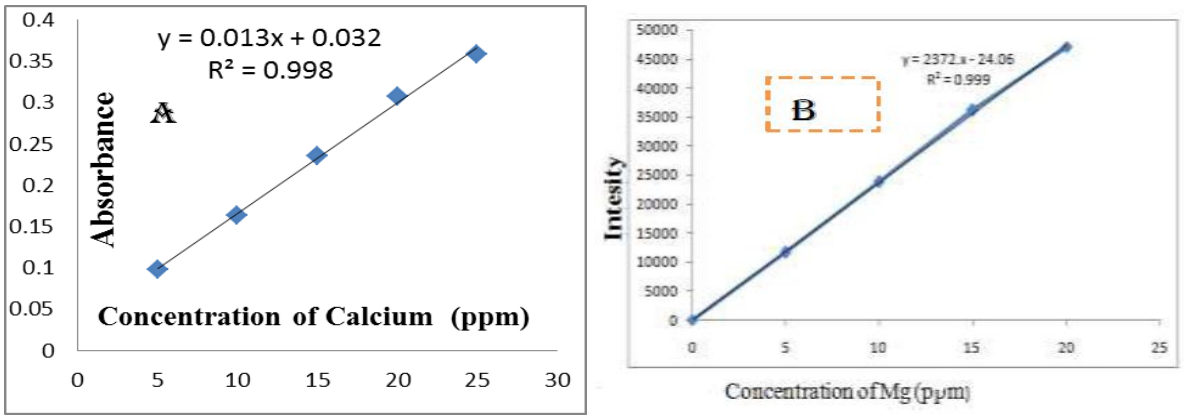

Figure. 6: AAS Calibration curve for Calcium and Magnesium.

Table. 4: Atomic Absorbance of calcium and magnesium in water samples.

No.	Sample water from Shinille Zone	Mg -AAS Result at DFx20	Ca-AAS Result at DFx10
1	Without treatment	0.391	0.236
2	With MO seed treatment	0. 251	0.123

The mechanism for softening was found to be due to adsorption with the adsorption isotherm approximating to the Langmuir type, and conversion of soluble hardness-causing ions to insoluble products by precipitation reactions. Removal efficiency was found to increase with increasing dosage of *Moringa oleifera* (Patil *et al*., 2012). *Moringa stenopetala* and *Moringa oleifera* seed powder techniques in developing countries was well known source of natural water clarifiers, is effective in heavy metal detoxification of water and also are effective sorbents for removal of heavy metal and volatile organic compounds in the aqueous system (Li *et al*, 2010).

Removal of chlorides from water by natural coagulant *Moringa Oleifera* seed powder

As shown in Table 5 and figure 7 Chlorides concentration without any treatment for surface and ground water was 323mgL^{-1} and 624 mgL^{-1} respectively which were above WHO standards (< 250 mgL^{-1}) but upon addition of extracted MO seed the chloride concentration decreased from 122 and 372 mgL^{-1} that is very much within the limits of WHO drinking water standards. Chloride removal properties MO seed is due to the positively charged soluble proteins in the MO attract the negatively charged chloride ions present in water and neutralize the chlorides.

Table. 5: Removal of chlorine by *Moringa Oleifera* seed from sample drinking water.

Concentration of added MO seed in mgL^{-1}	Concentration of Chloride from ground water in mgL^{-1}	Concentration of Chloride from surface water in mgL^{-1}
0	423	549.80
50	387	499.56
100	317	448.06
150	299	343.12
200	249	307.75
250	234	319.91
300	196	232.34

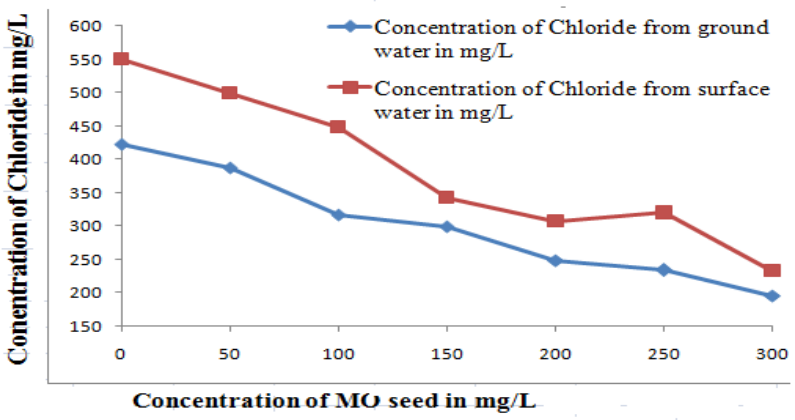

Figure. 7: Efficiency of *Moringa Oleifera* seed powder for removing Chlorine from water samples.

Chemical Oxygen Demand (COD) and Biological oxygen Demand (BOD)

Chemical oxygen demand (COD) is another measure of organic material contamination in water specified in mgL^{-1},it's defined as the amount of dissolved oxygen required to cause chemical oxidation of the organic material in water. Both BOD and COD are key indicators of the environmental health of a surface water supply. They are commonly used in waste water treatment but rarely in general water treatment (Krishnamurthy, 1990). The Chemical

Oxygen Demand (COD) of the water sample was in the range of 10 to 14.5 mgL^{-1}respectively for ground and surface water in Siti Zone Somali regional state before treatments however, after treatments with oil extracted MO seed powders it reduced to 2.7and 4.2mgL^{-1} for both ground and surface water. Accordingly, minimum amount of COD was recorded in for ground water. If COD is higher the sample waters will be considered containing greater number of microorganisms. Standard limit of COD was not given both by WHO and ESA. However, the lower concentration of COD determines the safe drinking water (Pereira and Arruda, 2003). Similarly the Biological Oxygen Demand (BOD) was in the range of 4 to 10 mg/L for sample water of ground and surface water before any treatments, while after treating with *Moringa Oleifera* seed in reduced to 1.8 and 2.3 mgL^{-1}.Biological Oxygen Demand (BOD) measures the amount of oxygen used by microorganisms in this case of bacterium, to oxidize organic matter present within the samples.

Bacterial Examination: As shown in Table 5, the colony factor unit shows the concentration of microorganism in water sample was beyond the limit of WHO and ESA standards in sample water Siti zone of Shinille woreda. However, after addition of MO seed powder and exposed to UV radiation reducing microbial load at high variation happens. After the treatment, the numbers of bacterial colonies were reduced with increased dose of MO seed powder from 77 x 10^4 to 4 x 10^4 $CFUmL^{-1}$. It was also observed that the MO seed powder also has antibacterial properties against Gram-positive bacteria as similarly reported (Abdulmoneim and Abu Zaid, 2011). The active antimicrobial agent isolated was found to be *4 α-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.

Table. 5: Physico-chemical Parameters of Drinking Water before and after Treatments by MO and Comparative analysis with WHO and ESA standard

No	Quality of drinking water Parameters	Before treatment	After treatment by MO seed	WHO drinking water standards	Ethiopian standards agency (ESA)
1.	Color	Brown	Colorless	Colorless	Colorless
2.	pH	7.9- 8.07	7.04 - 7.13	6.5 - 8.5	6.5 - 8.5
3.	TDS	1385- 1587mgL^{-1}	967 - 1205 mgL^{-1}	< 1500 mgL^{-1}	< 1000 mgL^{-1}
4.	Ca	15.06 mgL^{-1} at x 20 DF	100 mgL^{-1}	< 75 mgL^{-1}	< 75 mgL^{-1}
5.	Mg	2.58 mgL^{-1} at x 20 DF	120 mgL^{-1}	< 50 mgL^{-1}	< 50 mgL^{-1}
6.	Chloride	624-324 mgL^{-1}	122-372 mgL^{-1}	< 250 mgL^{-1}	< 250 mgL^{-1}
7.	Total viable organisms	77 x 10^4 - 43 x 10^4 CFU	19 x 10^1 - 4 x 10^4	Must not be	Must not be

	(Bacteriological), colonies per mL		CFU	detectable	detectable
8.	Conductivity	2300-2700 µS cm^{-1}	2000 - 1250 µS cm^{-1}	< 1500 µS cm^{-1}	< 1500 µScm^{-1}
9.	BOD mgL^{-1}	4-10 mgL^{-1}	1.8- 2.3 mgL^{-1}	-	-
10.	COD mgL^{-1}	11-16 mgL^{-1}	2.7-4.2 mgL^{-1}	-	-

CFU; Colony factor unit, DF: Dilution factor

CONCLUSION

The present study concludes that Moringa Oleifera seeds acts as a natural coagulant, flocculent, adsorbent for the treatment of drinking water.The challenge of 36-40 % oil content of *Moringa Oleifera* seed in water treatments accounts for development of BOD and COD which may accounts for the growth of microorganisms rather than antimicrobial activities of *Moringa Oleifera* seed coagulant activities. However, boiling of powder seed in pure water can reduce 63.37% its oily nature. *Moringa Oleifera* seed powder show best coagulation activity for Chlorides, Calcium and Magnesium and antimicrobial activity also. The treatment of both surface and ground water samples with *Moringa Oleifera* seed powder showed improved quality as well as suitable for human consumption as per WHO norms.

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