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# Water Treatment and Purification using *Moringa Oleifera* Seed Extract

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**ABSTRACT**

Water is the most abundant chemical and important natural resource. Various concentrations of water at given place contributes to water quality. The suitability of water and its specific use are evaluated by examining its quality parameters. The adverse health effects have been observed in developing countries due to drinking contaminated water. The natural resources have serious threat due to development and urbanization in countries. People are made to use low quality water because of high cost of treated water which results in exposing them to waterborne diseases. The seed extract of Moringa oleifera is used for purification of drinking and wastewater due to presence of soluble cationic coagulant. It has capability to reduce the turbidity from water. In the present study, the collected water samples were examined with various physical, chemical and biological parameters. Obtained values of each parameters were compared with standard values set by World Health Organization.

***KEYWORDS:*** *Moringa oleifera, Physico-chemical, Bacteriological, Parameters, Seed extract, Purification*

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## INTRODUCTION

Water is an essential natural resource, it is the source of sustenance for all living organisms, ecological system, food production and economic development (Saxena *et.al.*, 2011). Sustenance of human health and influence of life clean and safe drinking water is major requirement. The vital concern of developing countries throughout the world is provision of good quality, clean, purified and safe water (Khan *et.al.*, 2013). Most widely circulated and distributed abundant substance is water. In total there is 1400 million billion liters of water out of which only 3% is fresh water and 1% is available for portable use (Hujare *et.al.*, 2008). Water is used for various activities of human life like drinking, cooking and agricultural purposes as well as regulation of human body such as nutrient transport, thermal regulation, digestion, metabolic activities and flush of toxic waste (Kumar *et.al.*, 2013, Abera *et.al.*, 2011). As water is directly connected to human welfare its quality checking and maintaining is vital concern to mankind. Food security, health and hygiene mismanagement of water resources has strong socio-economic repercussions (Khalid *et.al.*, 2007). For use of water in different areas in have requirements, composition and purity is to be analyzed on regular basis to confirm suitability for its use ( Saxena *et.al.*, 2011). According to study of world health organization inadequate sanitation, polluted water or unavailability of water are the main reasons of about 80% of all the sickness, illness and diseases (Vagarali *et.al.*, 2011). The pathogens presenting serious threat to disease if found in water include *Salmonella species, Shigella species, Escherichia coli, Vibrio cholerae, Yersinia enterolitica, Cmpylobacter species.* There is requirement of systemic water quality monitoring for environmental and public health (Kumar *et.al.*, 2013). The water treatment procedures of many emerging countries involves coagulation, flocculation, sedimentation and disinfection which are expensive because of high cost involved for clarification and assessing the chemical coagulants including alum (Sunita *et.al.*, 2014). The high cost associated with water treatment leads to use of readily available source for people which is of low quality expose them to water borne diseases (Vrushali *et.al.*, 2018). *Moringa oleifera* is found to be a viable coagulant for chemical replacement. The seed extract of *Moringa oleifera* have water soluble cationic protein which act as primary coagulant in process of water clarification treatment having the ability to reduce the turbidity (Md Saduzaman *et.al.*,2013).

## MATERIALS AND METHODS

### Collection of water sample

The two water samples were collected in clean and surface sterilized bottles from local area of Bhiwandi, Thane, Maharashtra.

### Collection of *Moringa oleifera* seeds

*Moringa oleifera* seeds were separated from fresh drumsticks, the seed were dried in sunlight for few days and powdered using a mixer grinder for further experimental purposes.

**Estimation of Alkalinity**

The alkalinity of water sample is its quantitative capacity to neutralize a strong acid to a designated pH caused by $HCO_3$ and $OH^-$ ions in water. Before titration process standardization of HCl was carried and end point was notes from yellow to orange colour (x $cm^3$). The first alkalinity titration is phenolphthalein alkalinity which is carried using 50$cm^3$ water sample with 0.1N $Na_2S_2O_3$ solution and 1%phenolphthaleim as indicator titrated against standard HCl solution. End point is determined when pink color just disappear or fade(y $cm^3$). Alkalinity results were reported in terms of *mg* of $CaCO_3$.

**Estimation of Chemical Oxygen Demand (COD)**

Organic substances present in water sample are oxidized by $K_2Cr_2O_7$ in acidic medium. The sample was heated in presence of $Ag_2SO_4$ as catalyst, $HgSO_4$ was used for removal of chloride interference. The excess of $K_2Cr_2O_7$ was titrated back against ferrous alum solution. Amount of oxidisable organic matter measured as oxygen equivalent is proportional to $K_2Cr_2O_7$ consumed. In two round bottom flasks few porcelain pieces, 1g 20% $HgCl_2$, 5$cm^3$ $K_2Cr_2O_7$ conc.$H_2SO_4$, 50 $cm^3$ of 0.025N $K_2Cr_2O_7$ and 5 $cm^3$ of 1% $Ag_2SO_4$ solutions was added. In last add 25 $cm^3$ of distilled and sample waters in respective tubes and keep for refluxing on boiling water bath for 90 minutes. Now, titrate the sample after cooling with 0.025M ferrous alum using indicator 0.025M ferroin. End point will be from green to red colour. This method determined the COD value of 50 mg/$dm^3$ or more.

**Estimation of Biological Oxygen Demand (BOD)**

Biological oxygen demand is the amount of dissolved oxygen needed by aerobic biological organism to break down organic matter present in given water sample at certain temperature over a specific time period. Samples were labelled as sample1 (Day 1&5) and sample2 (Day 1&5). To both the samples 2.0 ml of $MnSO_4$ and 2.0ml of alkali iodide azide were added. Precipitate of $MnSO_4$ was formed which was dissolved by adding 2.0ml of $H_2SO_4$. It was titrated against sodium thiosulphate using starch as indicator and readings were recorded in terms of 0.0125N sodium thiosulphate for titration. The BOD value was expressed in milligrams of oxygen consumed per liter of sample on both day 1 and day 5 for both blank and test respectively.

Calculation:

Amount of $Na_2S_2O_3$ = Sample 1– Sample 2

BOD in mg/L = A*Normality of $Na_2S_2O_3$*8*1000*dilution factor / Amount of sample

**Isolation and Identification of Bacteria**

Selective and differential media was prepared as per standard protocol and after sterilization agar plates were prepared under aseptic conditions. Different water samples collected; were streaked on different media. The plates were incubated at 37$^0$C for 24 hours to observe bacterial growth. Biochemical tests were done to identify the microorganisms.

**Most Probable Number (MPN)**

Most probable number analysis is a statistical method based on the random dispersion of microorganism per volume in a given sample. For presumptive test 10ml water sample was inoculated in three double strength Lauryl Tryptose Broth (LTB) tubes. In these tubes, 0.1ml and 1ml of water sample was added in three single strength LTB tubes. The tubes were incubated at 37$^0$C for 48 hours for gas production observation. Tubes showing positive presumptive test (gas production) were inoculated one loopful in sterile BGLB tubes and incubate at 37$^0$C for 48 hours and check for gas production in BLGB tubes constitutes positive confirmed test.

**Determination of Initial Number of Organisms Present in Water Sample**

Firstly, 1.0ml of both water samples were poured in sterile petri plates and then at about 45$^0$C R2A agar media was poured, as agar solidified the plates were kept for incubation at 37$^0$C for 5 days. Regularly observation was taken and results were noted.

**Addition of *Moringa oleifera* seed powder in water samples**

The water samples were treated in the addition of *Moringa oleifera* seed powder at different concentrations of 0.2% to 2.4% gm of seed powder. This added water sample were kept into purification for 10 days.

**Determination of Final Number of Organisms Present in Water Sample**

The treated and control water sample were examined on the basis of appearance, taste, pH, odour and microbial evaluation, total bacterial count were taken for each treated sample and control by pour plate method. Both the samples were filtered with whatsman filter paper and 1ml of treated sample was added on R2A agar media plate and incubated for 24 hours. The evaluation of bacterial count was observe and compared to untreated water sample.

**RESULTS AND DISCUSSION**

**Collection of Water Samples**

Water samples from lake and tap were collected in sterile plastic bottles to avoid additional contamination due to non-sterile bottles. The bottles were transported immediately to laboratory with appropriate care and stored at 4$^0$C till further processing.

**Collection of Seed Powder**

*Moringa oleifera* seeds were collected from fresh drumstick plant, dried on sunlight for few days and then powdered using grinder for further test.

**Determination of pH in water samples**

Digital pH meter is used to check the hydrogen ion concentration in both water samples. The readings were taken after 1 minute constant value (Kanase *et.al.*, 2016). The results are observed as given below in table no.1

**Table no.1: Determination of pH in water samples**

| Water Samples | pH |
|---|---|
| Sample 1 | 7.2 |
| Sample 2 | 7.2 |

Drinking water with a pH between 6.5 and 8.5 is generally considered satisfactory. The hydrogen ion (pH) in both the samples is 7.2 which is within the safe limit set by WHO standard and hence will not cause any harmful effect.

**Determination of Alkalinity in water samples**

The water samples were checked for alkalinity, measured by content of hydroxide and carbonate ion present in samples.

It is titrated with standard HCl, result are displayed in table no.2

**Table no.2: Determination of alkalinity in water samples**

| Water Samples | Alkalinity (mg/L) |
|---|---|
| Sample 1 | 350 |
| Sample 2 | 330 |

Acid neutralizing capacity is alkalinity of water due to presence of carbonates and bicarbonates. The range set by WHO is 500mg/L (Kanase *et.al.*, 2016). Both the samples showed alkalinity in range of 400mg/L.

**Determination of Chemical Oxygen Demand in water samples**

For both the samples chemical oxygen demand was determined by using dichromate refluction method. The results are as displayed in table no.3

**Table no.3: Determination of chemical oxygen demand**

| Water Samples | COD (ppm) |
|---|---|
| Sample 1 | 200 |
| Sample 2 | 719 |

Oxygen is important component as it is required for degradation of organic matter hence COD is important parameter. Both the samples showed a large variation in chemical oxygen demand.

**Determination of Biological Oxygen Demand in water samples**

Biological oxygen demand of both the samples were tested, the maximum value as per WHO standard is 2.0mg/L for drinking purpose. The results are shown in table no.4

**Table no.4: Determination of biological oxygen demand**

| Water Samples | Day 1 (mg/L) | Day 5 (mg/L) | Results (mg/L) |
|---|---|---|---|
| Sample 1 | 13.5 | 2.9 | 1160 |
| Sample 2 | 15.0 | 3.2 | 1180 |

The tests showed wide range obtained under BOD test with minimum value 2.9mg/L observed. No similar results were obtained. Sample1 and sample2 showed BOD as 1160mg/L and 1180ma/L respectively.

**Determination of Most Probable Number (MPN) Method**

Determination of most probable number for both the water samples was done using multiple tube method. The results are given in table no.5

**Table no.5: Determination of most probable number**

| Water Samples | 10 ml | 1.0ml | 0.1ml | MPN Index per 100ml |
|---|---|---|---|---|
| Sample 1 | 0 | 1 | 0 | 2 |
| Sample 2 | 1 | 1 | 0 | 4 |

According to WHO standard for drinking water quality there should be < 1 MPN/100ml of total coliforms and no *Escherichia coli* in 1ml of water (Olowe *et.al.*, 2016) reported MPN index in the range of 2-60 MPN/100ml. Both the water samples used for this study showed MPN index 2 to 4 MPN/100ml.

**Determination of Initial Number of Organisms in water samples**

Pour plate method was used to determine initial count of organisms in untreated water sample, they were observed and found that lake water (sample1) contain large number of organism as compare to tap water (sample2). The results are as shown in table no.6

**Table no.6: Determination of initial number of organisms**

| Water Source | Physical appearance | Number of organisms (cfu/ml) |
|---|---|---|
| Sample 1 | High yellow | 1800 |
| Sample 2 | Yellow | 1500 |

**Isolation and Identification of Microorganisms**

Microbiological and biochemical investigations were carried out of various water sample. Olowe *et.al.*, 2016, Vagarali *et.al.*, 2011, Abera *et.al.*, 2011 reported presence of *Escherichia coli* in the range of 10% to 17.3%. The present study of both water samples confirmed presence of *Escherichia coli*.

**Determination of Final Number of Organisms in water samples**

The water sample was treated with *Moringa oleifera* seed extract thus the treated sample was checked for reduction in total bacterial count, it was observed that highest bacterial reduction count was on 2.4% of plant extract by pour plate method. The results are displayed in table no.7

**Table no.7: Determination of final number of organisms**

| Water Samples | Physical appearance | pH | 0.2% | 0.6% | 1.0% | 2.2% | 2.4% | Control |
|---|---|---|---|---|---|---|---|---|
| | | Number of organisms (cfu/ml) | | | | | | |
| Sample1 | Clear | 7.0 | 1600 | 300 | 90 | 50 | 20 | 3800 |
| Sample2 | Clear | 7.0 | 1100 | 900 | 400 | 50 | 10 | 2500 |

Reduction in total bacterial count was observed that 2.4%. Similar results with reduction in bacterial count were observed by Yongabi, *et.al.*, 2011.

**CONCLUSION**

Water plays important role for sustenance of industries, agriculture and human existence. The healthy water ecosystem is depended on physico-chemical and bacteriological characteristics. It is observed that water gets polluted significantly due to human activities, seed of *Moringa oleifera* were used for water purification. Alkalinity and pH of both the samples were as per WHO standard, the COD of sample 2 was not found to permissible limit where as BOD of both the samples had unacceptable values. MPN index indicates presence of greater number of bacteria and was positive for *Escherichia coli*. The turbidity and bacterial count was reduced by using *Moringa oleifera* seed extract,

2.4% of powder has ability to reduce the impurities from water.The study can be used to determine fungal, algal, viral count present in water samples. *Moringa oleifera* is a natural coagulant produces biodegradable environment friendly sludge as compared to chemical coagulant. Properties of *Moringa oleifera* like adsorbent, coagulant, dewatering and desalting agent can be checked and used for removal of heavy metals from water.

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