Managing Pollution Impacted Potable Ground Water in Rural Area of Eastern Pune Metropolitan Region: Disinfection by Common Plants

Sunil S. Deo, Milind R. Gidde

Abstract: Potable ground water quality is at risk due to contamination by pollution, sewage, industrial effluents along the nearby areas of Mula-Mutha river banks in eastern metropolitan region of Pune and need treatment especially disinfection for making it domestically usable. The phytoremediation is one of the important useful method when considered for treatment of water in rural area. The study involved monitoring the quality of ground water in the selected areas along the banks of the river using physicochemical and biological parameters in continuation with our earlier studies. For the disinfection treatment the commonly found plant species viz. Neem, Tulsi, and Amla were selected and for the water samples, onsite river water samples and bore well water samples were used. The plants leaves were extracted by water and alcohol and the extracts in dose response manner were used to treat the water. The treated water was monitored for presence of Total coliform and E.coli, the indicators of contamination, using petri film method. The results showed the presence of total coliform and E.coli in bore well water samples indicating contamination and need for disinfection. The disinfection study showed that alcoholic leaves extracts were more potent in disinfecting the water samples than aqueous extracts. The complete disinfection by alcoholic extracts was shown at concentration of 21.12mg, 42.8mg and 24.07mg for Tulsi, Neem and Amla respectively.

Keywords: Disinfection, ground water, contamination, Neem, Tulsi, Amla

I. INTRODUCTION

One of the purest and reliable source for drinking purpose is the groundwater constituting about 30% of the world’s total fresh water and 99% of its total stock of liquid fresh water [1, 2]. Mula-Mutha river and bore wells located on banks of river area is a major source of water for drinking, domestic and agriculture in the eastern Pune metropolitan area. But due to urbanization and industrialization the water quality is deteriorated by pollutants and nutrients through the sewage, industrial effluents, agricultural runoff etc. into the water of Mula-Muthariver, which brings about a series of changes in the physicochemical and biological characteristics of water [3, 4].

In our earlier studies we have investigated the water quality of samples collected from Mula-Mutha river stream and bore wells located on banks of river in eastern part of Pune metropolitan area to understand pollution status in these water bodies.

We monitored the bore well water during seasonal time points to see if there is any variation in water quality. Results showed that the physicochemical parameters like hardness, total dissolved solids (TDS) and conductivity for bore well samples were high. Total suspended solids (TSS) levels were high for river water samples. There was definite presence of total coliform and E.coli in river water and random presence of total coliform and E.coli in bore well samples. Therefore our study indicated that water from bore wells adjacent to Mula-Muthariver is needed to be treated before using for drinking and domestic purpose [5].

Phytoremediation is one of the important tool that offers solutions in various diseases. The plants like Neem, Tulsi and Amla found abundantly in nearby areas of selected sampling stations were selected for study. They are having medicinal value in traditional system of medicine [6]. Antimicrobial property is reported for Tulsi, Neem and Amla in vitro studies against various microorganisms [7-9]. Aqueous and alcoholic extract of Tulsi were found to be effective against E.coli [10]. In a comparative study of Neem and Tulsi, the alcoholic extracts were able to decrease most probable (MPN) count in well, lake and river water [11].

In present study the water from the selected sampling stations were monitored for physicochemical and biological parameters in continuation with earlier studies. The selected plants were studied for determining the antimicrobial potential using on-site river samples and disinfection effect was tested in bore well water samples collected from several sites along banks of river.

II. MATERIALS

### Water sampling stations during study [5]

| Name of sampling station | A Manjarai bk. Bridge | B Manjarai-nagarbk (Right bank) | C Manjarihirur (Left bank) | D KolawadiSante (Left bank) | E Theur (near ChintamaniVidyamandir) | F Theur (near ChintamaniVidyamandir) (Right bank) | G Bhapkars-mala, Manjari Bk. (Right bank) | H MAEER’s MIT Institute of Design, Loni (Right bank) | I Ashitapur (Left bank) | J River Water sample, Ashitapur |
|------------------------|----------------------|-------------------------------|---------------------------|-----------------------------|---------------------------------|---------------------------------------------|------------------------------------------|---------------------------------------------|-----------------------|-----------------------------|
| R = River, B = Bore well |

Water samples were collected in sterilized glass bottles. Sampling locations of bore wells were around approximately 1 to 1.5 km from the banks of Mula-Muthariver.
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III. METHODOLOGY

A. Physicochemical analysis

Physicochemical parameters of water samples were studied as per procedures mentioned in parts of IS 3025 section of Environmental Protection and Waste Management (CHD 32) of Bureau of Indian Standards (BIS). pH, total suspended solids (TSS), total hardness (TH), total dissolved solids (TDS), and conductivity, total sulphates, total chlorides, temperature were determined [12, 13].

Chemical Oxygen Demand (COD), Dissolved oxygen (DO), total phosphates (PO₄) were determined as per procedure mentioned in American Public Health Association 40CFR [14].

B. Extraction

The leaves of plants of Azadirachta indica (Neem), Ocimum sanctum (Tulsi) and Phyllanthus emblica (Amla) are obtained from local area of eastern part of Pune metropolitan city and are properly identified. The leaves are washed, shed dried and powdered mechanically with standard method using mortar and pestle. The leaves powder is then extracted with distilled water and alcohol using Soxhlet apparatus. The dissolved extracts are filtered through Whatman filter paper (No.1). Supernatants of each extracts are evaporated at 60°C to dryness. After evaporation extracts are collected and stored at 4°C [15].

C. Preliminary Phytochemical evaluation

The extracts were evaluated for the presence of phytoconstituents viz. alkaloids, glycosides, tannins, saponins, steroids, reducing sugars, anthroquinone, flavonoids by chemical tests as per the procedures given in Ayurvedic Pharmacopoeia [16].

D. Petri film method

Biological analysis of water was done by using Petri film method. In this method 1 ml of water sample is added to 1 petri film. Incubated for 48 hours at 37°C. The pink colonies that grow indicate total coliforms and blue colonies indicate E. coli. The colonies were counted and calculation done to give colony forming units (CFUs)/ml of water [17, 18].

E. Determination of antimicrobial potential of aqueous extracts

The biological analysis of river consistently showed high contamination and hence it was used as water sample for antimicrobial studies and method used was petri film.

In study 1, extract solution of Tulsi at concentration 66.66mg/ml was prepared in water. 1 ml of this extract was added to test tube containing 9 ml of river water. Shaken to mix properly. 5 ml of this treated mixture was added to second tube containing 5 ml of river water. Shaken and mixed well. 5ml of this second tube was added to third tube containing 5 ml river water. Shaken to mix well. Same steps were repeated for fourth and fifth tube. This resulted in addition of extract in the range of 66.66mg in first tube, 33.33mg in second tube, 16.67mg in third tube, 8.33mg in fourth tube and 4.17 mg in fifth tube per 10 ml of river water. Similarly extract solution of 100mg/ml for Neem and Amla was prepared and serially diluted in river water to give addition of 100mg, 50mg, 25mg, 12.5mg and 6.25mg extract in 10ml river water for Neem and for Amla resulted in addition of 30mg, 15mg, 7.5mg, 3.75mg, and 1.85mg extract in 10ml river water. After 30 minutes of contact time the microbial analysis was done in duplicate by petri film method.

In study 2, additional concentration dose of 166.65mg/ml, 250mg/ml, and 75mg/ml of Tulsi, Neem and Amla respectively was prepared and 1ml extract added in 9 ml river water separately and further studied by similar procedure.

F. Determination of anti-microbial potential of alcoholic extracts

Alcoholic extract in concentration of 21.12 mg/ml, 42.8 mg/ml, and 48.14 mg/ml of Tulsi, Neem and Amla respectively was prepared in 40% alcohol. Serial dilution and treatment of river water was followed as per the earlier procedure of aqueous extract. This resulted in addition of extract in concentration range of 21.12mg, 10.56mg, 5.28mg, 2.64mg and 1.32mg serially of Tulsi; 42.8mg, 21.4mg, 10.7mg, 5.35mg and 2.67mg of Neem; 48.14mg, 24.07mg, 12.03mg, 6.01mg and 3.00mg of Amla in 10 ml river water in each tube. After keeping for 30 minutes microbiological study was done by petri film method in duplicate. A control in duplicate was set with 9ml river water and 1ml 40% alcohol.

G. Disinfection of water samples from bore well by potential extracts of plants

Water from sampling stations (A to J) was collected. For each sample of water 4 test tubes were taken and 9 ml of sample water was added to each tube. In first tube acting as control 1 ml of 40% alcohol was added. In 2nd, 3rd and 4th tube 1 ml of extract containing 21.12mg/ml of Tulsi, 42.8mg/ml of Neem and 24.07mg/ml of Amla respectively was added. Extract was prepared in 40% alcohol. Shaken to mix properly and kept for 30 minutes. Further analyzed in duplicate by petri film method. Similar procedure was repeated for rest of the water samples. The water samples were collected in monsoon 2018 and during winter 2018.

IV. RESULTS AND DISCUSSION

A. Physicochemical and biological analysis

In continuation of earlier studies [5] to monitor the water samples of bore wells along the nearby regions of banks of Mula-Muthariver, the samples were monitored in summer 2019 for physicochemical and biological properties. The results confirm the earlier observations and were nearer to normal range for physicochemical parameters, except for TDS and conductivity, which are slightly high especially in sample H. The results are consistent with earlier observations regarding random presence of total Coliforms and E. coli (Table 1).

B. Extraction and phytochemical analysis

Percentage extractability in water and ethanol for Neem leaves was observed to be 10.5% and 9.2% respectively; for Tulsi it was noted to be 12.5% and 9.6% respectively, while for Amla it was noted 6.25% and 13.52% respectively.
Observations of preliminary phytochemical investigation are denoted as presence or absence of important phytochemicals like steroids, glycosides, alkaloids, tannins, flavonoids, reducing sugars, saponins and anthroquinones in alcoholic and aqueous extracts (Table 2). The phytochemicals are important constituents responsible for various biological activities shown by these plants.

Table 1: Physicochemical and biological properties of water samples of bore well sites (Summer 2019 samples)

| Parameters         | B   | C   | D   | F   | G   | H   | I   |
|--------------------|-----|-----|-----|-----|-----|-----|-----|
| pH                 | 7.72| 7.92| 7.22| 7.57| 7.12| 7.53| 8.1 |
| COD (mg/L)         | 9.6 | 19.2| 28.8| 38.4| 24  | 38.4| 28.8|
| TSS (mg/L)         | 4   | 7   | 9   | 8   | 11  | 15  | 7   |
| Turbidity (NTU)    | 1   | 1   | 2   | 1   | 2   | 2   | 1   |
| TH (as CaCO₃) mg/L | 340 | 350 | 470 | 280 | 400 | 460 | 250 |
| TDS (mg/L)         | 630 | 650 | 1012| 980 | 940 | 1230| 590 |
| Conductivity at 25°C (μS/cm) | 1125| 1160| 1807| 1750| 1678| 2196| 1053|
| DO (mg/L)          | 10.2| 9.3 | 10.5| 8   | 11.1| 10.4| 10.2|
| Total SO₄ (mg/L)   | 72.44| 79.02| 115.24| 95.49| 81.49| 161.34| 80.67|
| Total Cl (mg/L)    | 74.97| 84.97| 134.95| 119.96| 114.96| 129.95| 74.97|
| Total PO₄ (mg/L)   | 0.055| 0.058| 0.071| 0.088| 0.053| 0.075| 0.049|
| Temperature °C     | 31  | 30  | 32  | 31  | 31  | 30  | 30  |
| Coliform CFU/ml    | Nil | 8   | 1   | 12  | 64  | Nil | 13  |
| E.Coli CFU/ml      | Nil | Nil | Nil | Nil | 17  | Nil | Nil | Nil |

Table 2: Preliminary phytochemical analysis

| Phytochemical constituent | Amla leaves | Tulsi leaves | Neem Leaves |
|--------------------------|-------------|-------------|-------------|
|                          | Alcex t     | Aqex t      | Amla leaves | Tulsi leaves | Neem Leaves |
| Steroids                 | -           | +           | +           | -           | +           |
| Glycosides               | +           | -           | -           | +           | +           |
| Alkaloids                | -           | +           | +           | +           | +           |
| Tannins                  | +           | +           | +           | +           | +           |
| Flavonoids               | +           | +           | -           | +           | +           |
| Reducing Sugars          | +           | +           | -           | -           | -           |
| Saponins                 | +           | +           | -           | +           | -           |
| Anthroquinone            | +           | +           | -           | +           | -           |

Table 3: Anti-microbial study of aqueous extracts in river water (Study 1)

| Group | Conc of extract in mg/10ml R.W | Total Coliform CFUs/ml | E.coli count CFUs/ml |
|-------|--------------------------------|------------------------|----------------------|
| Control | TNTC                          | TNTC                   | TNTC                 |
| Tulsi | 66.66                          | TNTC                   | TNTC                 |
|      | 33.33                          | TNTC                   | TNTC                 |
|      | 16.67                          | TNTC                   | TNTC                 |
|      | 8.33                           | TNTC                   | TNTC                 |
|      | 4.16                           | TNTC                   | TNTC                 |
| Neem | 100                            | TNTC                   | TNTC                 |
|      | 50                             | TNTC                   | TNTC                 |
|      | 25                             | TNTC                   | TNTC                 |
|      | 12.5                           | TNTC                   | TNTC                 |
|      | 6.25                           | TNTC                   | TNTC                 |
| Amla | 30                             | TNTC                   | TNTC                 |
|      | 15                             | TNTC                   | TNTC                 |
|      | 7.5                            | TNTC                   | TNTC                 |
|      | 3.75                           | TNTC                   | TNTC                 |
|      | 1.85                           | TNTC                   | TNTC                 |

**TNTC** = Too numerous to count i.e 1600+ colonies /ml

C. Antimicrobial study

Petrifilm plates are widely used because of the cost-effectiveness, simplicity, convenience, and ease of use. Effect against total Coliform and E. coli are good indicators for disinfection hence, was selected for determination of anti-microbial potential of plant extracts.
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Table 4: Anti-microbial study of aqueous extracts in river water (Study 2)

| Group | Conc of extract in mg/10ml R.W | Total Coliform CFUs/ml | E. coli count CFUs/ml |
|-------|-------------------------------|------------------------|-----------------------|
| Control | ----                        | TNTC                   | TNTC                  |
| Tulsi  | 166.65                       | 164                    | 116                   |
| Neem   | 250                          | 79                     | 54                    |
| Amla   | 75                           | NIL                    | NIL                   |

TNTC = Too numerous to count i.e 1600+ colonies /ml

The result did not show any antimicrobial activity for aqueous extracts of Tulsi at concentration range of 66.66 mg, 33.33mg, 16.67mg, 8.33mg and 4.16mg (Fig 1); Neem at concentration range of 100mg, 50mg, 25mg, 12.5mg and 6.25mg in 10 ml river water (Fig 2); and Amla at concentration range of 30mg, 15mg, 7.5mg, 3.75mg and 1.85mg in 10 ml river water (Fig 3). As there was no activity observed at studied concentrations, the higher dose of aqueous extract 166.65mg, 250mg, 75mg of Tulsi, Neem and Amla respectively was studied. The results showed significant reduction in CFUs/ml of total Coliform and E. coli. For Tulsi (Image 1D) and Neem (Image 1C) while Amla (Image 1B) showed complete inhibition of total Coliform and E. coli (Table 4, Fig 1, 2, 3).

Table 5: Anti-microbial study of alcoholic extracts in river water

| Group | Conc of extract in mg/10ml R.W | Total Coliform CFUs/ml | E. coli count CFUs/ml |
|-------|-------------------------------|------------------------|-----------------------|
| Control | ----                        | TNTC                   | TNTC                  |
| Tulsi  | 21.12                         | NIL                    | NIL                   |
|        | 10.56                         | 160                    | 108                   |
|        | 5.28                          | TNTC                   | TNTC                  |
|        | 2.64                          | TNTC                   | TNTC                  |
|        | 1.32                          | TNTC                   | TNTC                  |
| Neem   | 42.8                          | NIL                    | NIL                   |
|        | 21.4                          | 108                    | 31                    |
|        | 10.7                          | TNTC                   | TNTC                  |
|        | 5.35                          | TNTC                   | TNTC                  |
|        | 2.67                          | TNTC                   | TNTC                  |
| Amla   | 48.14                         | NIL                    | NIL                   |
|        | 24.07                         | NIL                    | NIL                   |
|        | 12.03                         | 148                    | 88                    |
|        | 6.01                          | TNTC                   | TNTC                  |

TNTC = Too numerous to count i.e 1600+ colonies /ml
The alcoholic extracts showed potential results. The Tulsi at doses of 10.56 mg showed moderate activity and complete inhibition of colony formation of Total Coliform and E. coli at 21.12 mg. It did not show any inhibitory activity at dose of 5.28 mg, 2.64 mg and 1.32 mg (Table 5, Fig 4, and Image 4). The Neem at doses of 21.4 mg and 42.8 mg showed moderate to complete inhibition of colony formation respectively. It did not show any inhibitory activity at dose of 10.7 mg, 5.35 mg and 2.67 mg (Table 5, Fig 5, and Image 3). Amla at doses of 48.14 mg and 24.07 mg showed complete inhibition of colony formation, while significant inhibition at 12.03 mg. It did not show any inhibitory activity at dose of 6.01 mg and 3 mg (Table 5, Fig 6, and Image 2).

D. Disinfection study

For the disinfection study the water samples were collected from sampling stations (A to J) and treated with optimized alcoholic extracts of plants and evaluated by petri film method. Results of disinfection effect in the water samples collected during monsoon 2018 is given in (Table 6). River samples A, E, J and bore well sample C, G, H showed significant presence of total Coliforms. River samples A and E and bore well sample G showed presence of E. coli. Alcoholic extracts of Tulsi at 21.12 mg and Neem at 42.8 mg and Amla at 24.07 mg showed complete inhibition of total Coliform bacteria and E. coli CFUs indicating total disinfection of water samples of river as well as bore well (Table 6).

The disinfection study was repeated in bore well water samples collected in winter season.
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Bore well sample B, C, D, F, G, and I showed significant presence of Coliform bacteria and absent in H. While bore well sample C and G showed presence of E. coli (Table 7). Alcoholic extracts of Tulsi at 21.12mg and Neem at 42.8mg and Amla at 24.07mg showed complete inhibition of total Coliform bacteria and E. coli CFUs confirming the earlier response of disinfection of water samples of bore well (Table 7).

Table 6: Disinfection study of bore well water samples and river water samples by potential extracts (Monsoon season 2018)

| Water Sample | Control | Tulsi Extract 21.12 mg | Neem Extract 42.8 mg | Amla Extract 24.07 mg |
|--------------|---------|------------------------|----------------------|-----------------------|
| Stations     | TC CFU/ml | E. coli CFU/ml | TC CFU/ml | E. coli CFU/ml | TC CFU/ml | E. coli CFU/ml | TC CFU/ml | E. coli CFU/ml |
| A            | NIL     | NIL                 | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |
| B            | NIL     | NIL                 | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |
| C            | 06      | NIL                 | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |
| D            | NIL     | NIL                 | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |
| E            | 260     | 64                  | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |
| F            | 03      | NIL                 | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |
| G            | 166     | 94                  | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |
| H            | 09      | NIL                 | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |
| I            | NIL     | NIL                 | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |
| J            | 156     | NIL                 | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |

TC = Total coliform; TNTC = Too numerous to count i.e 1600< colonies /ml

River Sample: A, E, J  
Bore well: B, C, D, F, G, H, I

Table 7: Disinfection study of bore well water samples by potential extracts (Winter season 2018)

| Water Sample | Control | Tulsi Extract 21.12 mg | Neem Extract 42.8 mg | Amla Extract 24.07 mg |
|--------------|---------|------------------------|----------------------|-----------------------|
| Stations     | TC CFU/ml | E. coli CFU/ml | TC CFU/ml | E. coli CFU/ml | TC CFU/ml | E. coli CFU/ml | TC CFU/ml | E. coli CFU/ml |
| B            | 01      | NIL                 | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |
| C            | 03      | 02                  | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |
| D            | 03      | NIL                 | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |
| F            | 06      | 02                  | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |
| G            | 04      | NIL                 | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |
| H            | NIL     | NIL                 | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |
| I            | 01      | NIL                 | NIL     | NIL         | NIL     | NIL         | NIL     | NIL         |

TC = Total coliform; TNTC = Too numerous to count i.e 1600< colonies /ml

River Sample: A, E, J  
Bore well: B, C, D, F, G, H, I

V. CONCLUSION

Physicochemical and biological study of water samples showed constant presence of total coliform and E coli at all seasonal times of year. This indicates disinfection of water in these areas is necessary before its use for drinking purpose. In the eastern metropolitan region of Pune, the plants are one of important source that can be exploited to manage the contamination of bore well water. The antemicrobial activity studied in extreme contaminated water showed good responses by the studied plants. The optimized doses showed complete disinfection of all river and bore well samples. The result shows that the alcoholic leaves extracts have more potential than aqueous leaves extracts indicating presence of phytoconstituents in the plants that are having antimicrobial properties to potentially disinfect.

This study gives an opportunity to further explore the use of these plant materials for onsite disinfection of water sources by perception study and design an application tool that can be easily applicable especially in rural area.

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