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

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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 Puneand needed 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 biologicql parametersin continuation with our earlier studies. For the disinfection treatmentthe commonly found plantsin the area viz. Neem, Tulsi, and Amlawere 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 resultsshowedrandom presence of total coliform and E.coli in bore well water samples indicating contaminationand the need fordisinfection.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 24.07mgfor 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 bypollutants 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 investigatedthe 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-Muthariverisneeded to be treated before using for drinking and domestic purpose[5].

Phytoremediation is one of 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. Theyare having medicinal value in traditional system of medicine [6].Antimicrobial property is reported for Tulsi, Neem and Amla in*in vitro* studies against various microorganisms [7-9]. Aqueous and alcoholic extractsof 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 anti-microbialpotentialusing on-site river samplesand 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	Manjari bk. Bridge	R
B	Manjarai-nagarbk (Right bank)	B
C	Manjarikhurd (Left bank)	B
D	KolawadiSasthe (Left bank)	B
E	Theur (near ChintamaniVidyamandir)	R
F	Theur (near ChintamaniVidyamandir) (Right bank)	B
G	Bhapkar-mala, Manjari Bk. (Right bank)	B
H	MAEER's MIT Institute of Design, Loni (Right bank)	B
I	Ashtapur (Left bank)	B
J	River Water sample, Ashtapur	R

R = River, B = Bore well

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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.

III. METHODOLOGY

A. Physicochemical analysis

Physicochemical parameters of water samples were studied as per procedures mentioned in parts of IS 3025 section ofEnvironmental 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 (PO_4) were determined as per procedure mentioned in American Public Health Association 40CFR [14].

B. Extraction

The leaves of plants of *Azadirachtaindica* (Neem), *Ocimum sanctum*(Tulsi) and *Phyllanthusemblica*(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 60^0C to dryness. After evaporation extracts are collected and stored at 4^oC [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 petrifilm. Incubated for 48 hours at 37^oC. The pink colonies that grow indicate total coliforms and blue colonies indicate *E. coli*. The colonies were counted and calculations 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 4.16 mg in fifth tube per 10 ml of river water. Similarly extract solution of 100mg/ml for Neem and 30mg/ml of 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,additionalconcentration 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 respectivelywas 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).

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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 $CaCO_3$) mg/L	340	350	470	280	400	460	250
TDS (mg/L)	630	650	1012	980	940	1230	590
Conductivity at 25 ^{0} C(μS/cm)	1125	1160	1807	1750	1678	2196	1053
DO (mg/L)	10.2	9.3	10.5	8.5	11.1	10.4	10.2
Total SO_4 (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 PO_4 (mg/L)	0.055	0.058	0.071	0.088	0.053	0.075	0.049
Temperature ^{0}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

Table 2: Preliminary phytochemical analysis

Phytochemical constituent	Amla leaves		Tulsi leaves		Neem Leaves	
	Alcext	Aqext	Alcext	Aqext	Alcext	Aqext
Steroids	-	-	+	+	-	-
Glycosides	+	+	-	-	+	+
Alkaloids	-	-	+	+	+	+
Tannins	+	+	+	+	+	+
Flavonoids	+	+	-	-	+	+
Reducing Sugars	+	+	-	-	-	-
Saponins	+	+	-	-	+	+
Anthroquinone	+	+	-	-	-	-

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.

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
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

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).
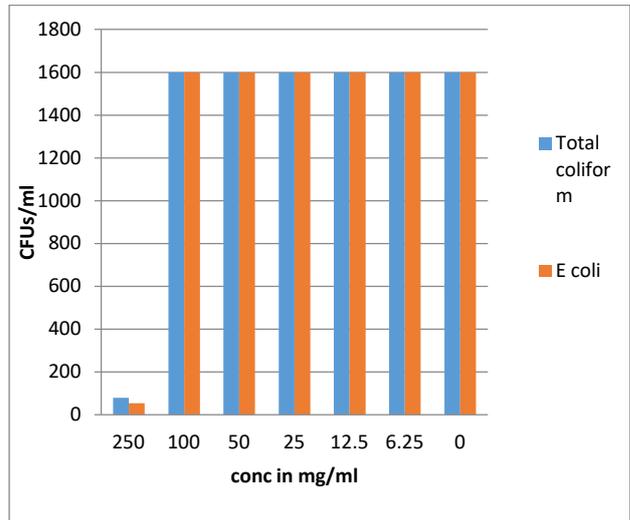

Fig 1: Dose response for aqueous extract of Tulsi
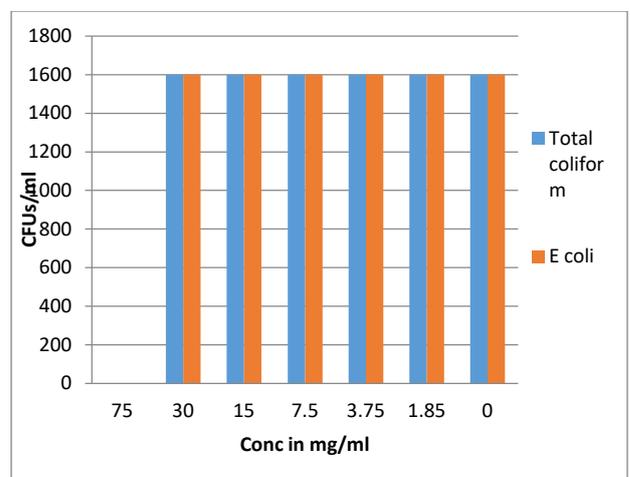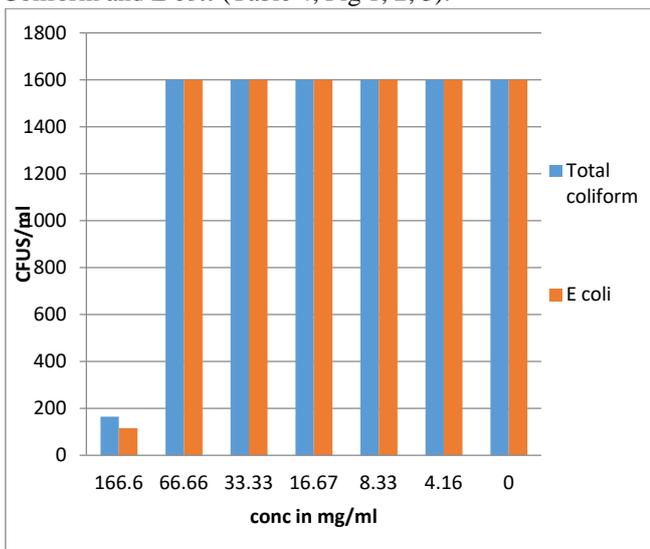

Fig 2: Dose response for aqueous extract of Neem

Fig 3: Dose response for aqueous extract of Amla
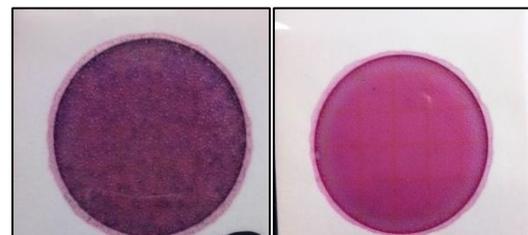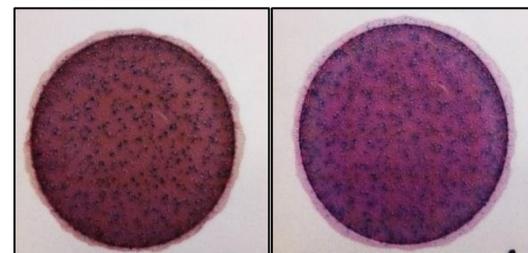
A Control 1	B Amla
C Neem	D Tulsi

Image 1: Photos of Petri film indicating presence of total coliform and *E coli* in study 2

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
	3	TNTC	TNTC

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

Fig 4: Dose response for alcoholic extract of Tulsi

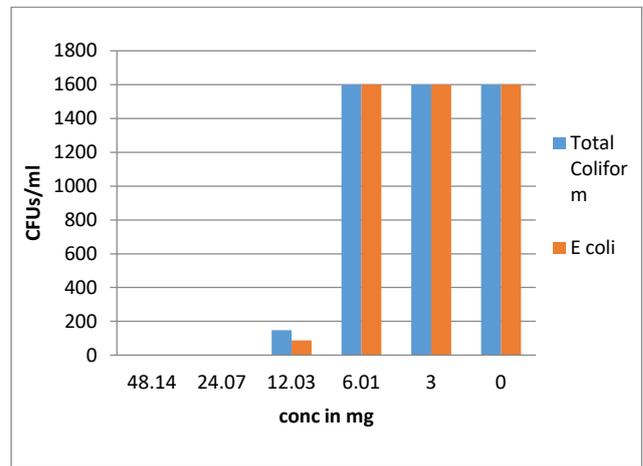**Fig 5: Dose response for alcoholic extract of Neem**

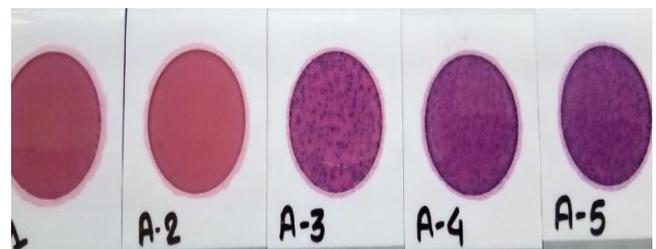**Fig 6: Dose response for alcoholic extract of Amla**

Image 2: Amla (River water treated with alcoholic extract of Amla leaves)
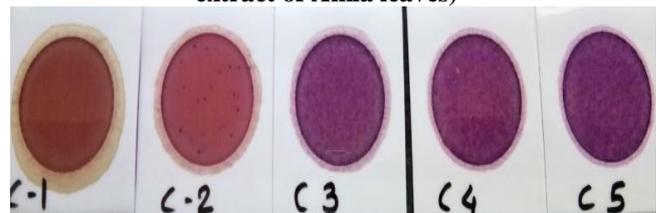

Image 3: Neem(River water treated with alcoholic extract of Neem leaves)

Image 4: Tulsi (River water treated with alcoholic extract of Tulsi leaves)

Image 5: Control (River water with 40% alcoholic water)

The alcoholic extracts showed potential result. The Tulsi at doses of 10.56mg showed moderate activity and complete inhibition of colony formation of Total Coliform and *E coli* at 21.12mg. It did not show any inhibitory activity at dose of 5.28mg, 2.64mg and 1.32mg (Table 5, Fig 4, and Image 4).
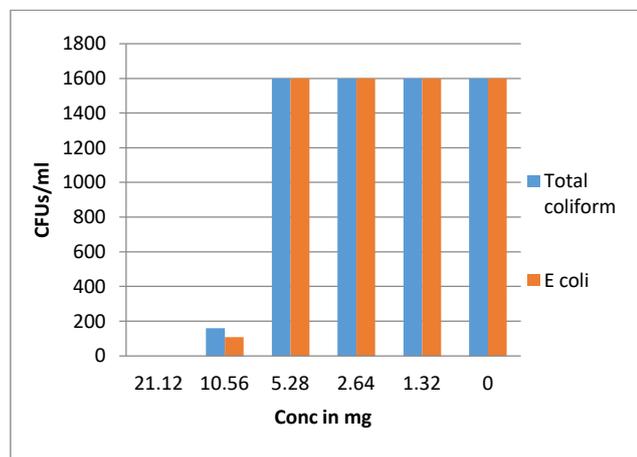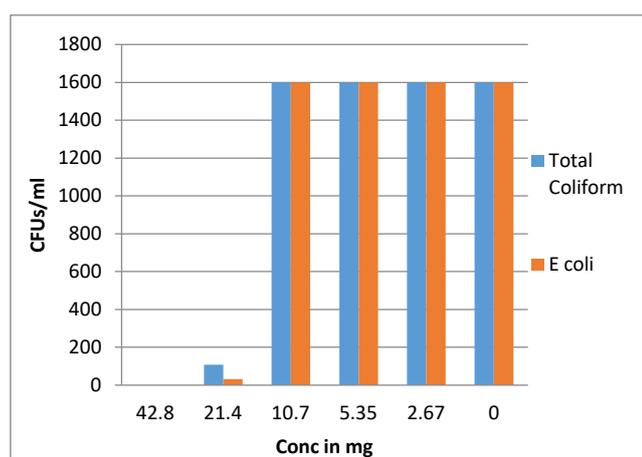
The Neem at doses of 21.4mg and 42.8mg showed moderate to complete inhibition of colony formation respectively. It did not show any inhibitory activity at dose of 10.7mg, 5.35mg and 2.67mg(Table 5, Fig 5, and Image 3).

Amla at doses of 48.14mg and 24.07mg, showed complete inhibition of colony formation, while significant inhibition at 12.03mg. It did not show any inhibitory activity at dose of 6.01mg and 3mg(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 21.12mg and Neem at 42.8mg and Amla at 24.07mg 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.

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 ofdisinfection 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 24.07mg	
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	TNTC	TNTC	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 24.07mg	
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	NIL	NIL	NIL	NIL	NIL	NIL	NIL
G	04	02	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 presenceof 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 sourcesthat can be exploited to manage the contamination of bore well water. The antimicrobial activity studied in extreme contaminated water showed good responses bythe 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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REFERENCES

1. I. A. Shiklomanov, and A. A. Sokolov, "Methodological basis of world water balance investigation and computation,"*IAHS Publication*, vol. 148, pp.77-921983.
2. H. M. Raghunath,*Groundwater*,2nded. New Delhi: Wiley Eastern Ltd, 1987.
3. M. J. Pawari, and S. M. Gavande,"Assessment of underground water quality around Hadapsar region in Pune, Maharashtra,"*IRJET*, vol. 2, no.4, pp. 943-950,2015
4. P Sahu, S. Karad, S. Chavan, and S. Khandelwal, "Physico chemical analysis of MulaMutha River,"*CiVEJ*, vol. 2, no.2, pp. 37-46,2015.
5. S. S. Deo and M. R. Gidde. "Impact of pollution on potable ground water in rural area of eastern Pune metropolitan region,"*JETIR*, vol. 6, no. 5, pp. 204-208, 2019.
6. V. M. Gogate,*Dravyagunvigyan*. 1st ed. Pune: Vaidya MitraPrakashan, 2008,
7. S Maragathavalli, S. Brindha, N. S. Kaviyarasi, B. AnnaduraiandS. K. Gangwar, "Anti bacterial activity in leaf extract of Neem (Azadirachtaindica Linn.)," *International journal of science and nature*, vol. 3, no. 1, pp. 110-113, 2012.
8. V. Kumar, A. Chakraborty, M. Kaur, S. Pandey, M. K. Jena**,**"Comparative study on antimicrobial activity of tulsi (*Ocimum sanctum*) and neem (*Azadirachtaindica*) methanol extract," *Asian Journal of pharmaceutical clinical research*, vol. 11, no. 12, pp. 514-517, 2018.
9. D.Darshan, P. Patel,N. Moitra. "Antibacterial activity of *Emblica officinalis* (Gaertn.) fruitsand *Vitexnegundo*(L.) leaves,"*Current Trends in Biotechnology and Pharmacy*,vol. 9, no. 4, pp. 357-368, 2015.
10. R. Sadul Rama, M. R. Gidde, N. K. Bipinraj, "Comparative Study of antimicrobial activity and phtochemical screening of aqueous and alcoholic leaf extract of *Occimum sanctum* on *E coli* (Fecal indicator of water pollution),"*Journal of Environmental Research and Development*, vol. 7, no.1A,pp. 312-320, 2012.
11. T. Bhattacharjee, M. R. Gidde, N. K. Bipinraj, "Disinfection of drinlking water in rural area using natural herbs," *International journal of Engineering research and development*,vol. 5, no. 10, pp. 7-10, 2013.
12. *Indian Standard drinking water specification*, 2nd Revision. New Delhi: Bureau of Indian Standards, 2012.
13. *Guidelines for drinking water quality*, 2nd ed. Geneva: World Health Organization (WHO), 1997.
14. *Standard methods for the examination of water and waste water*, 21st Ed. Washington DC: American Public Health Association, 2005.
15. K. R. Khandelwal,*Practical Pharmacognosy*. 20th ed. Pune: NiraliPrakashan, 2011.
16. *The Ayurvedic Pharmacopeia of India*, 1sted. New Delhi: Govt of India Ministry of Health and Family Welfare Dept of AYUSH, Part 1, Appendix 2 and 3, 2007.
17. L. A. Nero, V. Beloti, M, D. F. Barros, M. B. T. Ortolani, R. Tamanini and B. D. G. M Franco, "Comparison of Petrifilm Aerobic Count Plates and De Man-Rogosa-Sharpe Agar for Enumeration of Lactic Acid Bacteria,"*Journal of Rapid Methods & Automation in Microbiology*, vol. 14, pp. 249–257, 1997.
18. L. A. Watterworth, H. Schraft, "Enumeration of heterotroughs, fecal coliforms, and *Escherichia coli* in water: comparison of 3M Petrifilm plates with standard plating procedures, "*Journal of Microbiological Methods*, vol. 60, pp. 335-342, 2005.

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