Applied Ecology and Environmental Sciences, 2020, Vol. 8, No. 6, 478-484
Available online at http://pubs.sciepub.com/aees/8/6/21
Published by Science and Education Publishing
DOI:10.12691/aees-8-6-21

Phytoremediation in Disinfection of Water from Underground Sources: Perception Study

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Received September 11, 2020; Revised October 12, 2020; Accepted October 21, 2020

Abstract Application of phytoremediation in disinfection was studied using extracts of Tulsi, Neem and Amla in underground water in rural area of eastern Pune metropolitan region. The perception study involved understanding the potential of hydroalcoholic extracts of leaves of Tulsi, Neem and Amla in disinfection of underground water. Secondly optimizing the dose and time required for complete disinfection by the extracts. Thirdly a liquid herbal preparation for disinfection using extracts was formulated and studied, and also further used for achieving complete disinfection of onsite households' water. The disinfection effect of crude powder of leaves of plants was also noted. Study area being agrarian area, a survey was taken to create awareness and highlight the outcome of the studies done so far. The 3M petri film method was used for disinfection study. The extracts of Tulsi, Neem and Amla completely disinfected 10ml of water at a dose of 10.56mg, 21.4mg and 12.03mg respectively. Herbal preparation containing 90mg extracts completely disinfected 500ml of underground water of study sites and households. The responses of crude powder were very less potent as compared to extracts. Finally, the results conclude that phytoremediation by commonly and abundantly available plants can be used for disinfection.

Keywords: *disinfection, ground water, contamination, phytoremediation, perception*

Cite This Article: Sunil S Deo, and Milind R Gidde, "Phytoremediation in Disinfection of Water from Underground Sources: Perception Study." *Applied Ecology and Environmental Sciences*, vol. 8, no. 6 (2020): 478-484. doi: 10.12691/aees-8-6-21.

1. Introduction

In our earlier study, we have monitored the quality of ground water by physicochemical and biological parameters at different seasonal time points in the rural area of eastern metropolitan region of Pune (Study area). 7 sites (B, C, D, F, G, H, I) of ground water near the bank of Mula-Mutha River were selected for sampling. Along with issue of hardness the main concern highlighted by the study was frequent faecal contamination at sites C, F, and G (Figure 1, Figure 2, Figure 3) [1,2]. In the antimicrobial study done earlier, Amla, Neem and Tulsi showed potential to disinfect the onsite underground water samples. The alcoholic extracts of leaves were observed to be more potent than the aqueous extracts [2,3].

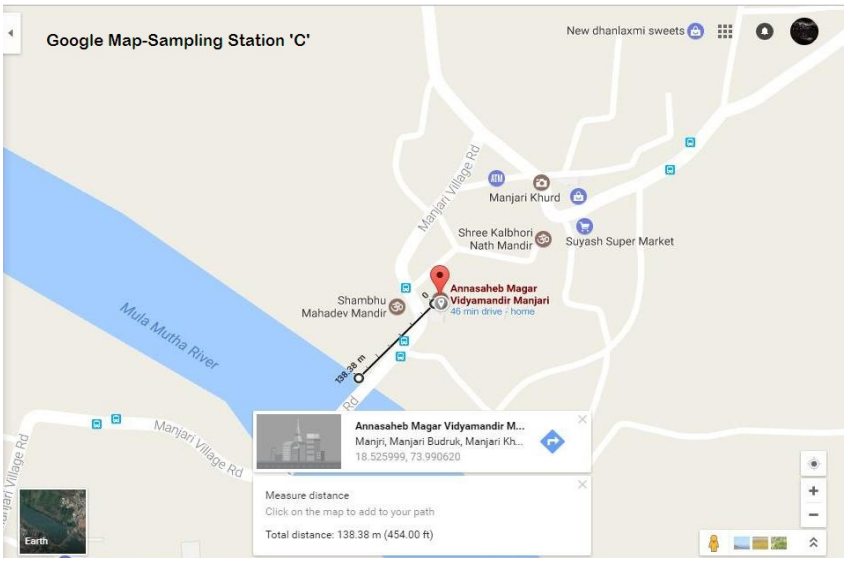

Figure 1. Sampling Station C (Manjari (Khurd) 18°31'33.6"N 73°59'26.2"E) (Annasaheb Magar school 138.3m from bank of Mula Mutha river)

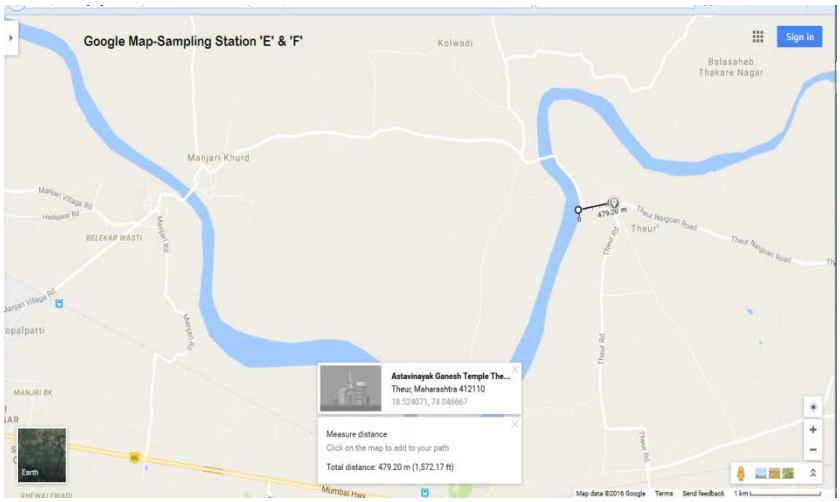

Figure 2. Sampling station F (Theur, 18°31'26.7"N 74°02'48.0"E) (Chintamani Vidyamandir 479.2m from bank of Mula Mutha river)

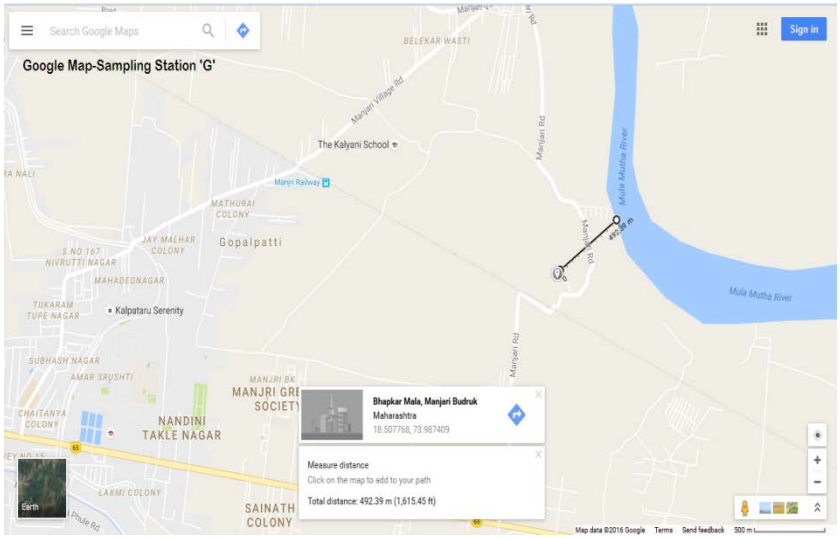

Figure 3. Sampling station G (Manjari bk 18°30'28.0"N 73°59'14.7"E) (Bhapkar Mala 492.39m from bank of Mula Mutha river)

Amla, Tulsi and Neem are the commonly available plants in the study area. On the basis of our earlier study results, we conducted the perception study in the locality. The study mainly focused on validating the disinfection process by plant leaves hydroalcoholic extracts. Secondly formulating a liquid herbal preparation and testing and using it to disinfect underground and household water in the study area. The households selected were those that were using underground water from constantly contaminated sites. Thirdly a survey was conducted to understand people's view about underground water quality highlighted by our earlier studies in their locality [1] and the use of medicinal plants for disinfection.

1.1. Study Area

Out of underground bore well sites (B, C, D, F, G, H, I) studied earlier, sites C, F and G showed consistent contamination [1]. Hence additional underground bore well sampling sites along the sites C, F and G were selected for further exploration (Table 1). Water samples were collected in sterilized glass bottles. Sampling locations of bore wells were around approximately 500m from the banks of Mula-Mutha River.

Table 1. Study area

Sites	Name of sampling stations
C, C1	Annasahebmagar school, Manjari khurd (Left bank)
F, F1	Chintamani Vidyamandir, Theur (Right bank)
G, G1, G2	Bhapkar-mala, Manjari Bk. (Right bank)

2. Material and Methods

2.1. Extraction

The leaves of plants of *Phyllanthus emblica* (Amla), *Azadirachta indica* (Neem) and *Ocimum sanctum* (Tulsi) were obtained from local area of eastern part of Pune metropolitan city and were properly identified. The leaves were washed, shed dried and powdered mechanically with standard method using mortar and pestle. The leaves powder was then extracted with 40% alcohol using Soxhlet apparatus. The dissolved extracts were filtered through Whatman filter paper (No.1). Supernatants of each extracts were evaporated at 60°C to dryness. After evaporation extracts were collected and stored at 4°C [4].

2.2. 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 [5,6].

2.3. Estimation of Total Phenolic Constituents

Total phenolic constituents were determined by Yang *et.al.* One gram of extract was taken, 50 ml of 95% ethanol was added to it. This was stored for 48 hrs at 0°C. It was filtered. 1ml of filtrate was taken in test tube. To it, 1 ml of 95% ethanol and 5ml of distilled water were added. Folin-Ciocalteau reagent (50%, 0.5 ml; Sigma) were added. After 5 min, 1ml of 5%Na_2CO_3 was added and mixed homogenously. This mixture was kept in dark for one hour. The absorbance was measured at 725nm. Standard curve was prepared with gallic acid in 95% ethanol. Total phenolics content was expressed as milligrams per grams of extract [7,8].

2.4. Estimation of Total Alkaloid by Gravimetry

5g of the powdered drug was extracted repeatedly using (3 X 50 ml) 0.1N H_2SO_4 in an ultrasonic bath. The solution was filtered. The mixed acid solution was washed with 4 successive quantities of 25 ml of chloroform (washing each chloroform solution with 20ml of acid). The chloroform washing was rejected, acid solution basified with dilute ammonia solution and extracted with (5 X 20 ml) diethyl ether. The combined diethyl ether extracts were washed with 5ml distilled water and ether evaporated to dryness in a weighed beaker on a water bath. Residue dried to constant weight at 105°C [5].

2.5. Estimation of Total Tannins

2 g of sample was defatted with 25 ml petroleum ether for 12 h. The marc was boiled for 2 h with 300 ml of double distilled water. Cooled, diluted up to 500 ml and filtered. 25 ml of this infusion was taken into 2-litre porcelain dish and added to it 20 ml Indigo solution and 750 ml double distilled water. Titrated it with 0.1N potassium permanganate solution, 1 ml at a time, until blue solution changed to green. Thereafter it was added drops wise until solution became golden yellow in colour. Similarly, 20-ml Indigo solution and 750 ml of double distilled water mixture was titrated. The difference between two titrations in ml was noted. Each ml of 0.1N potassium permanganate solution is equivalent to 0.004157 g of total tannins [7,8].

2.6. Petri Film Method

Biological analysis of water was done by using Petri film method. In this method 1 ml of water sample was added to 1 petri film. Incubated for 48 hours at 37°C. The pink colonies that grow indicated total coliforms and blue colonies indicated *E. coli*. The colonies were counted and calculations done to give colony forming units (CFUs)/ml of water [9,10].

2.7. Dose Response Study Using Bore Well Water

Dose response study was carried out to study disinfection potential of extracts in samples of bore well water of site C. Alcoholic extracts in concentration of 21.12 mg/ml, 42.8 mg/ml, and 24.07 mg/ml of Tulsi, Neem and Amla respectively was prepared in 40% alcohol. Serial dilution method was used. In separate sets for each extract 1 ml of extract was added to test tube containing 9 ml of water from site C. Shaken to mix properly. 5 ml of this treated mixture was added to second tube containing 5 ml of water from site C. Shaken and mixed well. 5ml of this second tube was added to third tube containing 5 ml water from site C. Shaken to mix well. Same steps were repeated for fourth and fifth tube. This resulted in addition of extracts 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; 24.07mg, 12.03mg, 6.01mg, 3.00mg and 1.5mg of Amla in 10 ml of 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 water from site C and 1ml 40% alcohol.

2.8. Time Response Study Using Bore Well Water

Determination of optimum contact time required for alcoholic extracts of Amla, Neem and Tulsi to cause complete disinfection was done using petri film method. For study purpose, water sample of bore well site G was used. For disinfection study three containers containing each 1 lit of bore well water from selected site G was taken. Extract in concentration of 12.03 mg/ml, 21.4 mg/ml and 10.56 mg/ml of Amla, Neem and Tulsi respectively in 40% alcohol was prepared. 10 ml of this prepared extract was added respectively in three containers containing bore well water. Stirred to mix properly. In fourth container 10ml of 40% alcohol was added as control. 1 ml test sample was collected from these three containers at contact times of 5, 10, 20, 30, 40 and 60 minutes after extract addition and analysed by petri film method in duplicate. A further study was carried out at contact times of 0.5, 1, 2, 3, 4 and 5 minutes.

2.9. Herbal Preparation and Its Disinfection Potential

An liquid herbal preparation was formulated by taking Amla, Neem, and Tulsi alcoholic extracts in weight ratio of 1:1:1 and dissolving in 40% hydro alcoholic medium. For the determination of disinfection potential, water from sampling site G1 was used for study. In 10 beakers each 500 ml of water from site G1 was taken and to it herbal preparation containing 18mg, 36mg, 54mg, 72mg, 90mg, 180mg, 270mg, 360mg and 450mg of mixture of extracts containing herbal preparation was added separately. A control containing 10ml of 40% alcohol was set. Stirred and kept for 30 minutes. Further analysed by Petri film method in duplicate.

2.10. Perception Study

Water samples from the bore well sampling sites identified for the study viz. C, C1, F, F1, G, G1 and G2 was tested for contamination. Except for site C1 sample, rest of the samples showed contamination. The contaminated sites were further selected for sampling of water from households that are using these waters. 500ml of water from each 3 households for each sites was taken and treated with 90mg mixture of extracts of herbal preparation. Kept for 30 min and water was tested by Petri film method in duplicate.

2.11. Effect of Crude Drug

Crude powder of leaves of Amla, Neem and Tulsi was studied by petri film method at dose of 3g for its potential to disinfect 50ml of water using water samples from site.

2.12. Survey

A survey was conducted to create awareness and get opinions of local people using these bore well water, about all the studies conducted so far.

3. Results and Discussion

3.1. Phytochemical Analysis

Preliminary phytochemical analysis shows presence or absence of important chemical constituents in extracts that are responsible for the various activities of the plants (Table 2). The antioxidant principles like tannins, phenolics and alkaloidal principles were determined quantitatively (Table 3).

Table 2. Preliminary phytochemical analysis of extracts

Phytoconstituents	Amla	Tulsi	Neem
Steroids	-	+	-
Glycosides	+	-	+
Alkaloids	-	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Reducing Sugars	+	-	-
Saponins	+	-	+
Anthroquinone	+	-	-

Table 3. Quantitative estimation of important phytoconstituents

Phytoconstituents	Amla	Tulsi	Neem
Total Alkaloids %w/w	--	1.1	0.9
Tannins in % w/w	1.1	0.8	1.5
Phenolics in (GAE)mg/g	27 mg	59 mg	12 mg

GAE = Gallic acid equivalent.

3.2. Dose Responses of Extracts in Bore Well Water

The water of bore well sampling site C was used for study. The Tulsi at concentration prepared at 10.56 mg/ml showed complete inhibition of total Coliform in bore well water. While the lower doses of 5.28 mg/ml, 2.64 mg/ml, 1.32 mg/ml showed significant reduction in colony count. The Neem at dose of 21.4 mg/ml showed complete inhibition of Total Coliform colony formation, while lower doses of 10.7 mg/ml, 5.35 mg/ml, 2.65 mg/ml showed significant lowering of colony count. On the other hand Amla at dose of 12.03 mg/ml showed complete inhibition of total Coliform colony formation, while lower doses of 6.01 mg/ml, 3mg/ml, 1.5mg/ml showed significant lowering of colony formation (Table 4). The result showed that there was significant reduction in colony formation at the studied doses of extracts in bore well samples compared to the earlier study done for river water samples [2].

Table 4. Total coliform and E.coli CFUs count during dose response study in bore well water

Group	Extract dose in mg	Total Coliform CFUs/ml	E.coli count CFUs/ml
Control	-----	55	Nil
Tulsi	21.12	Nil	Nil
	10.56	Nil	Nil
	5.28	1	Nil
	2.64	2	Nil
	1.32	3	Nil
Neem	42.8	Nil	Nil
	21.4	Nil	Nil
	10.7	2	Nil
	5.35	3	Nil
	2.65	3	Nil
Amla	24.07	Nil	Nil
	12.03	Nil	Nil
	6.01	2	Nil
	3	3	Nil
	1.5	4	Nil

3.3. Contact Time Responses of Extracts with Bore Well Water

The study was done using bore well water sample from site G. Extracts with concentration of 10.56 mg for Tulsi, 21.4 mg for Neem and Amla at dose of 12.03 mg was used for study. The extracts were added to water and stirred. Sampling of water to be analyzed was done at time points 5, 10, 20, 30, 40 and 60 minutes. Water samplings were analyzed by petri film method (Table 5).

It was observed that the dose of 10.56 mg for Tulsi, 21.4 mg for Neem and Amla at dose of 12.03 mg was able disinfect the water within five minutes of contact with bore well water (Table 5).

The study was repeated with contact time of 30, 60, 120, 180, 240 and 300 seconds. The result shows that Tulsi and Amla was able to disinfect the water samples with minimum contact time of 240 seconds. While Neem required contact time of 300 seconds for complete disinfection. The lower contact time of 30, 60, 120, 180 seconds did not show complete disinfection for Tulsi and Amla, while it did not show complete disinfection up to 240 second for Neem (Table 6).

Table 5. Total coliform and *E.coli* CFUs count

Group	Contact time with extracts in minutes	Total Coliform CFUs/ml	E.coli count CFUs/ml
Control	-----	28	8
Tulsi	05	Nil	Nil
	10	Nil	Nil
	20	Nil	Nil
	30	Nil	Nil
	40	Nil	Nil
	60	Nil	Nil
Neem	05	Nil	Nil
	10	Nil	Nil
	20	Nil	Nil
	30	Nil	Nil
	40	Nil	Nil
	60	Nil	Nil
Amla	05	Nil	Nil
	10	Nil	Nil
	20	Nil	Nil
	30	Nil	Nil
	40	Nil	Nil
	60	Nil	Nil

Table 6. Total coliform and *E.coli* CFUs count

Group	Contact time with extracts in seconds	Total Coliform count CFUs/ml	E.coli count CFUs/ml
Control	-----	24	6
Tulsi	30	12	2
	60	12	1
	120	4	Nil
	180	5	Nil
	240	Nil	Nil
	300	Nil	Nil
Neem	30	15	2
	60	14	Nil
	120	8	Nil
	180	6	Nil
	240	2	Nil
	300	Nil	Nil
Amla	30	13	2
	60	13	3
	120	6	Nil
	180	1	Nil
	240	Nil	Nil
	300	Nil	Nil

3.4. Formulation of Herbal Preparation

For perception study a liquid herbal preparation was formulated using the hydroalcoholic extracts of Tulsi, Neem, and Amla in 1:1:1 ratio. This herbal preparation was studied in dose response manner by petri film method to identify the dose that can be used for perception study. The results shows that the minimum extracts concentration that showed complete disinfection was 90mg (30 mg Tulsi + 30mg Neem + 30mg Amla). Extracts added less than 90mg showed partial inhibition of total coliform and *E.coli* (Table 7, Figure 4 & Figure 5).

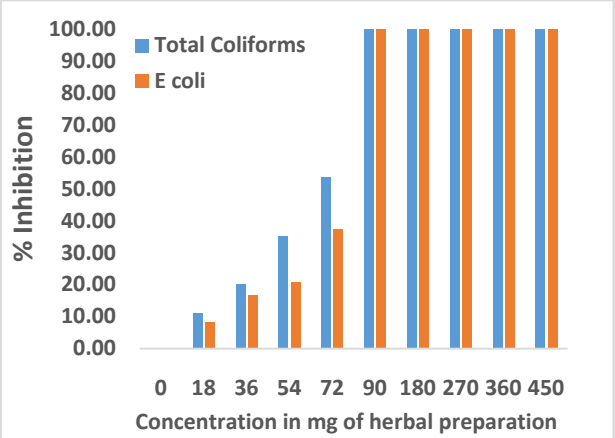

Figure 4. Inhibition of CFUs by herbal preparation

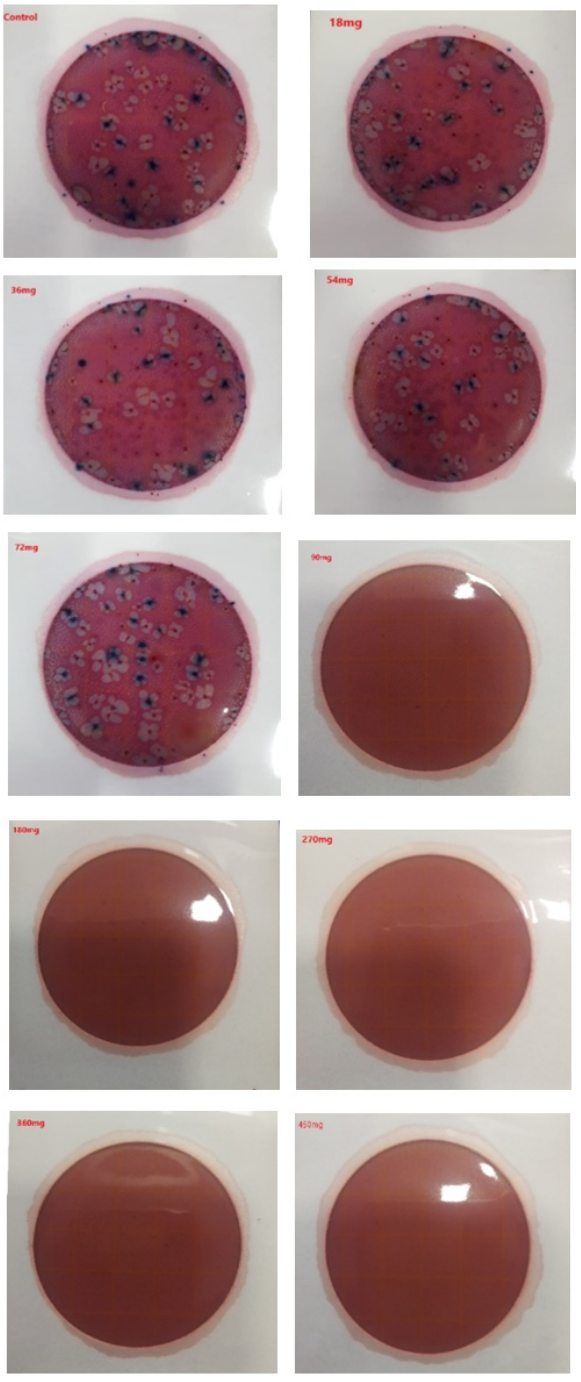

Figure 5. Photos of petri films for dose response of herbal preparation

Table 7. Dose response study of herbal preparation

Group	Dose in mg	Total Coliform CFUs/ml	% Inhibition	*E.coli* CFUs/ml	% Inhibition
Control	0	54	0	24	0
Test (Herbal Prepn)	18	48	11.1	22	8.3
	36	43	20.3	20	16.6
	54	35	35.1	19	20.8
	72	25	53.7	15	37.5
	90	Nil	100	Nil	100
	180	Nil	100	Nil	100
	270	Nil	100	Nil	100
	360	Nil	100	Nil	100
	450	Nil	100	Nil	100

3.5. Disinfection by Herbal Preparation in Household Water

Bore well samples from sites C, C1, F, F1, G, G1, G2 were collected for study (Table 8).

Out of the two sites C and C1, the site C showed contamination by both total coliform and *E coli* while site C1 showed no contamination. F and F1 sites showed contamination with total coliform. Site G showed highest contamination. The samples of sites G, G1 and G2 showed presence of both total coliform and *E coli*. (Table 8, Figure 6)

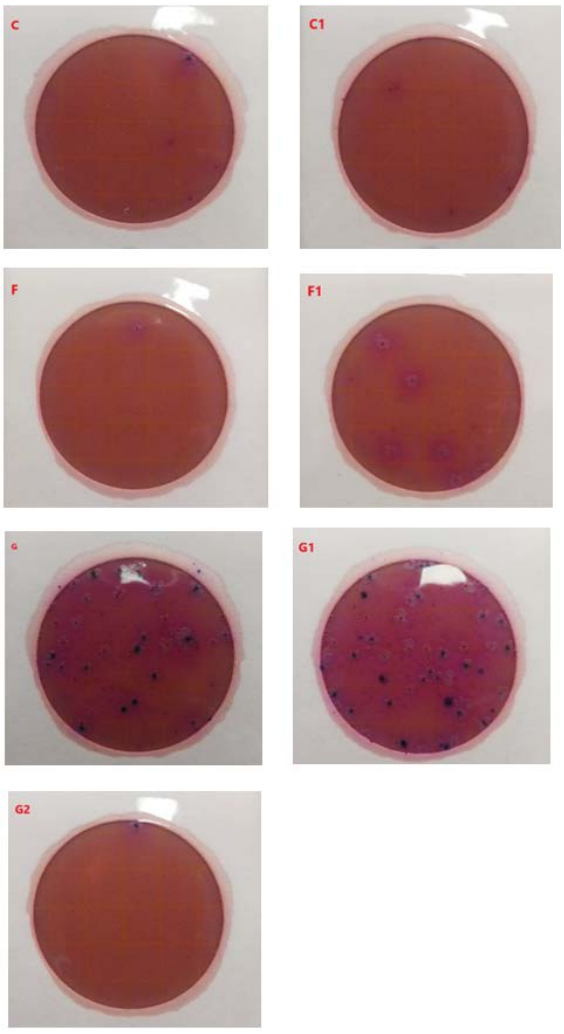

Figure 6. Photos of petri films of bore well samples without treatment

Table 8. Total Coliform and E coli CFUs count in bore well water samples

Bore well site	Total Coliform CFUs/ml	E.coli count CFUs/ml
C	3	1
C1	Nil	Nil
F	1	Nil
F1	8	Nil
G	41	16
G1	55	23
G2	1	1

Table 9. Disinfection in water samples of house holds

Bore well site	House	% disinfection
C	1	100
	2	100
	3	100
C1	1	100
	2	100
	3	100
F	1	100
	2	100
	3	100
F1	1	100
	2	100
	3	100
G	1	100
	2	100
	3	100
G1	1	100
	2	100
	3	100
G2	1	100
	2	100
	3	100

Water samples from at least three households collected from each of the identified sites C, C1, F, F1, G, G1 and G2 were disinfected by herbal preparation and analyzed by petri film method. The result showed complete disinfection of water samples collected from households of these consistently contaminated sites (Table 9).

3.6. Disinfection by Crude Powder of Leaves

Crude dried plant material of leaves of Tulsi, Neem and Amla was studied for its potential to disinfect the bore well water sample. The water sample from site G1 was used for study. The water sample was treated separately with 3g of plant material. There is complete inhibition of total coliform and *E.coli* by crude Tulsi leaves. There is nearly 85% inhibition of total coliforms and complete inhibition of *E.coli* by crude Amla leaves. There is nearly 61% inhibition of total coliform and 79% inhibition of *E.coli* by crude Neem leaves when compared to control (Table 10, Figure 7 & Figure 8).

Table 10. Total coliform and *E.coli* CFUs count during crude drug treatment

Group	Qty	Total Coliform CFUs/ml	% inhibition	E.coli count CFUs/ml	% inhibition
Control		54	0	24	0
Tulsi	3g	Nil	100	Nil	100
Amla	3g	8	85.19	Nil	100
Neem	3g	21	61.11	5	79.16

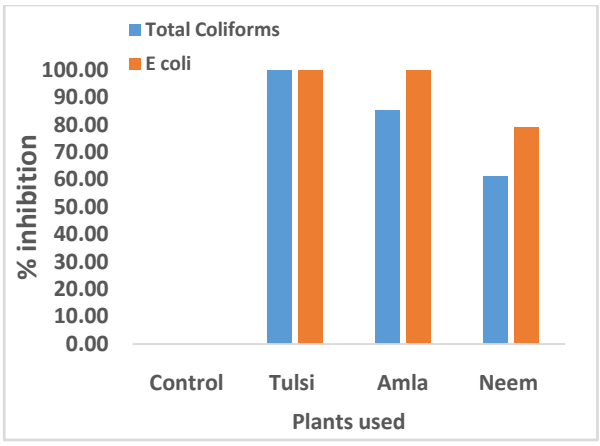

Figure 7. Inhibitory responses by crude drugs

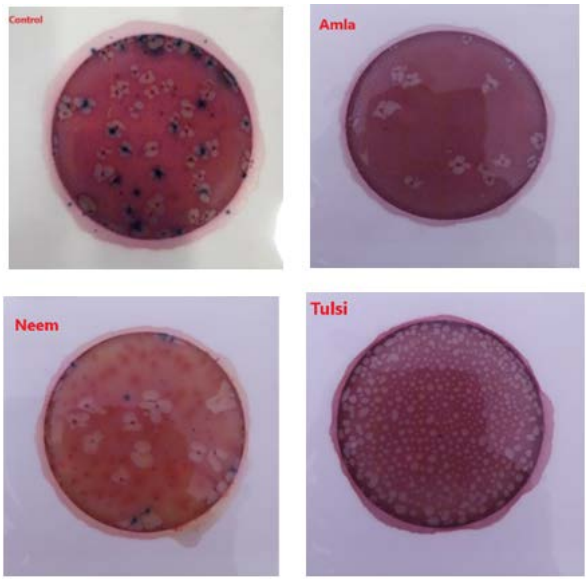

Figure 8. Photos of petri films for inhibitory responses by crude drugs

Survey responses shows that 90% people were unaware about the status of contamination and quality of underground water in their area. 20 % people were using only surface water while 80% people were using both underground and surface water. People found the phytoremediation and perception study done in their area useful to them.

The consistant contamination of sites C, G and F is due to industrial effluents constantly getting dumped in the river. Moreover, the area around bore wells being unhygenic due to effluents of households and cattle sheds getting accumulated around the bore well sites. Very high colony count of total Coliforms and *E. coli* indicate the faecal contamination of water. The presence of the total Coliforms and *E coli* bacteria are indicative of the risks of waterborne pathogens in water.

As the study area is agricultural area surface water is insuffcient for both agriculture and domestic use.

The river being highly contaminated is unsuitable for agrarian use. Tulsi, Neem and Amla being abundantly available in the area and are generally known for their traditional medicinal use. The anti microbial property of Tulsi, Neem and Amla is exploited successfully as remediation for disinfection of underground water.

4. Conclusion

With respect to our earlier studies, the study concludes that the water samples from site C, G and F are consistantly contaminated. Herbal preparation made of leaves extracts of plants like Tulsi, Neem and Amla are effective to disinfect the contaminated water as observed in the perception study.

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