International Journal of Microbiological Research 5 (3): 171-178, 2014
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2014.5.3.8621

# Bacteriological Assessment of Drinking Water in Flood Affected Areas of District Charsadda, KPK Pakistan

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**Abstract:** The present study was conducted to analyze the bacterial contamination and its count in three water sources i.e. wells, tube wells and hand pumps in three selected areas of district Charsadda viz Charsadda town, Gulabad and Sukkar. Charsadda town and Gulabad were selected as areas representing the flood affected areas, while Sukkar was selected as representative of the area not affected by flood. A total of 64 water samples were analyzed for Total Plate Count (TPC), Coliform, Fecal coliform and *E. coli*. Four, eight and 33 (total 45) water samples were analyzed from the wells of Charsadda town, Gulabad and Sukkar, respectively. Only three and four water samples were analyzed for bacteriological count from tube wells water of Charsadda town and Sukkar respectively. From hand pump water four, three and five (total 12) samples were examined from Charsadda town, Gulabad and Sukkar, respectively. Gulabad Bala 2 had TPC value of.$6X10^4$/mL which was the highest among all the well water samples from Charsadda town, Gulabad and Sukkar. Among all the three water sources from the selected areas of district Charsadda the well water of Sukkar Payan 3 had the lowest TPC value of $1.92X10^2$/mL. In tube well water high TPC value was that of Qazi Khel Charsadda which was $6.6X10^3$/mL. The TPC value of hand pump water samples of Charsadda town 2 was $7.7X10^4$/mL which was the highest among all the three water sources. *E. coli* was present in all the three water sources (wells, tube wells and hand pumps) from Charsadda town, Gulabad and Sukkar areas. It was concluded from the present study that water in district Charsadda was contaminated and do not fit for drinking purposes and water contamination was not limited to areas affected by 2010 floods.

**Key words:** Drinking Water · Coliforms Bacteria · *E. coli* · Flood · Health Issues

## INTRODUCTION

Water is one of the essential natural resources given by the creator. In Holy Quran it's stated that "We have kept alive everything from water". So it proves that without water life can't go on. We can live without food for many days or weeks, but without water we can die less than a week [1]. Water is a basic requirement for all living creatures; among them the most important use is drinking [2]. It has been examined that surface water is not suitable for drinking purposes due to organic, inorganic and biological components. Other purposes include cooking, bathing, washing and use in industries and agriculture [3]. The nature of drinking water is very essential to human being, because it is connected to human life [4].

Clean and safe drinking water is one of the most important requirements for healthy human life [5]. Water quality get weaken due to increase in population, industrial development and unhygienic conditions in all parts of the world [6]. In Pakistan fresh water sources are glaciers, rivers and lakes but due to low rainfall and snowfall, Pakistan is suffering from water deficit. So to solve this problem, people rely on ground water. It is an important natural resource for various human activities [7]. In developing world water pollution is one of the alarming issues, especially caused by animal waste, industrial, household effluent and heavy metals. High concentration of the heavy metals like arsenic and lead has been found in water both in developed and developing countries [8].

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Contaminated water may cause direct threat to human health. So the purity and contamination of water is one of the major problems of the world. Rivers, streams and other different reservoir may look clean and have no undesirable odor or taste. Pathogens found in water are not harmful but also invisible to naked eye which may be odorless and tasteless. These bacteria can cause more serious illnesses, such as severe diarrhea, hepatitis or typhoid fever. Water taken from different reservoirs should always be free of contamination or disinfected before being used for drinking or cooking [9].

Analysis of water quality can be done by counting the visible colonies of indicator bacteria, e.g. *total coliform, fecal coliform* and *E. coli* [10]. *Salmonella typhi* causes typhoid fever and *Vibrio cholera* causes cholera. Both are the important pathogenic bacteria transmitted by water route [11]. *Pseudomonas aeruginosa* found very commonly in nature and can be isolated from a large number of natural sources. An attention has been given to this bacterium because of its increasing significances as a human pathogen and its high resistance to most antibiotics [12].

Therefore the present study were to analyze the bacterial contamination and its count in three water sources i.e. wells, tube wells and hand pumps in three selected areas of district Charsadda viz. Charsadda town, Gulabad and Sukkar. Charsadda town and Gulabad were selected as representative of the flood affected areas, while Sukkar was selected as representative of the area not affected by flood. Comparison of the levels of contamination in drinking water in the two sets of study areas was aimed at understanding the effects of the flood in the quality of drinking water in the study area.

## MATERIALS AND METHODS

There are three drinking water sources available in district Charsadda i.e. wells, tube wells and hand pumps. Almost 80% residents in Charsadda use drinking water from the wells, which is perhaps the oldest source of water in this area.

In July 2010 many areas of district Charsadda were hit by a flood, which caused huge contamination in the water of district Charsadda. Present study was conducted to analyze the bacterial contamination and its count in three water resources i.e. wells, tube wells and hand pumps in three selected areas of district Charsadda viz. Charsadda town, Gulabad and Sukkar. Charsadda town and Gulabad were selected as areas representing the flood affected areas, while Sukkar was selected as representative of the area not affected by flood. A total of 64 water samples were analyzed for Total Plate Count (TPC), Coliform, Fecal coliform and *E. coli*. Four, eight and 33 water samples were analyzed from the wells of Charsadda town, Gulabad and Sukkar, respectively. From hand pumps four, three and five water samples were examined from Charsadda town, Gulabad and Sukkar, respectively. No tube well was found in Gulabad area, whereas four and three water samples were analyzed for bacteriological count from tube wells of Charsadda town and Sukkar, respectively.

Microbiological analyses of water samples were performed as described in Standard Methods for the Examination of Water and Wastewater; total coliform and thermo-tolerant bacteria were determined by means of Standard Total Coliform Fermentation Technique, including presumptive, confirmed and completed phases [13].

**Sample Collection:** Samples of water (100 mL) from wells, tube wells and hand pumps were collected from Charsadda town, Gulabad and Sukkar areas. All these water samples were analyzed bacteriologically for TPC, Coliform, Fecal coliform and *E. coli*. A total of 64 water samples were collected in sterilized bottles from wells (45), tube wells (7) and hand pumps (12).

### Bacteriological Analysis of Water Sample

***Method for Total Plate Count:*** The TPC is designed to provide the estimate of the total number of aerobic organisms in a particular sample. Two drops (125µL) of water sample, with the help of a dropper, were added to the sterilized Petri dish containing Plate Count Agar (PCA). The Petri dishes containing water samples were spread with spreader onto the agar plates. These Petri dishes were incubated at 35°C for 48 hours. After 48 hours these Petri dishes were examined for the colonies of bacteria on the surface of agar. After incubation, colonies were counted by colony counter.

***Coliform and Fecal Coliform:*** Incubated tubes containing water samples with Lactose broth at 35±2°C for 48 hours. Examined tubes after 48 hours for gas production. Re-incubated negative tubes for additional 24 h. Examined second time for gas. Performed a confirmation test on all presumptive positive tubes.

**Procedure for Fecal Coliform:** Tubes having 10mL Escherichia coli (E.C) broth with inverted Durham tubes were inoculated from the presumptive fermentation tubes showing gas and were incubated at 44.5°C for 24 hours

and were e xamined for gas production. Fecal coliforms were calculated from MPN tables, out of ten tubes [14].

***Procedure for E. Coli:*** Ethyline Methyline Blue (EMB) agar was used for the presence or absence of *E. coli*. All the tubes of E.C broth showing gas were sub-cultured by streaking on EMB agar plates and were incubated at 35°C for 18 to 24 h. After 24 hours incubation the typical colonies will be confirmed by biochemical tests [15].

**Multiple Tube Techniques:** In this experiment (Multiple Tube Fermentation Techniques), a set of ten tubes (for every water sample) were inoculated with one mL water sample and nine mL medium. After appropriate inoculation, the tubes were examined for the diagnostic reaction, gas production for Coliform. The multiple tube techniques yield the statistically derived Most Probable Numbers (MPN) of organisms per aliquot (usually one mL) of water. Each set (ten tubes for every water sample) were scored for the number of positive tubes and the score of set was then used for estimating the MPN. The advantage of this technique is that it will detect very small organisms, much less than one per mL which would otherwise require inoculation of large volumes.

**Confirmation Test by Kits:** *E. coli* O157 (Pathogenic strain) LATEX TEST REAGENT KIT is an agglutination test kit for the presumptive identification of *E.coli* serogroup O157 antigen on laboratory culture media.

**Test Procedure:** A total of 64 confirmation tests by kits were performed for the presence or absence of *E. coli* O157 in the water samples of selected areas. All reagents were allowed to come to room temperature before use. The *E.coli* O157 Latex Reagent and Negative Control Latex Reagent were tested with the positive control antigen prior to running test specimens. The *E.coli* O157 Latex Reagent showed positive agglutination and the Negative Control Latex Reagent showed no agglutination within two minutes. It indicated that the reagents retain their activity. Test material was obtained by culturing water specimens. Selected suitable colonies from the agar medium surface.

Placed one drop of *E. coli* O157 Latex Reagent on a test circle on one of the test cards provided. Using a sterile pipette added one drop of the test specimen to the test circle then mixed with the Latex Reagent using one of the mixing sticks provided.

## RESULTS AND DISCUSSION

Present study was conducted to analyze the bacterial contamination and its count in three water resources i.e. wells, tube wells and hand pumps in three selected areas of district Charsadda viz. Charsadda town, Gulabad and Sukkar. Charsadda town and Gulabad were selected as areas representing the flood affected areas, while Sukkar was selected as representative of the area not affected by flood. A total of 64 water samples were analyzed for TPC, Coliform, Fecal coliform and *E. coli*. Four, eight and 33 (45) water samples were analyzed from the wells of Charsadda town, Gulabad and Sukkar, respectively. From hand pumps four, five and three (12) water samples were examined from Charsadda town, Gulabad and Sukkar, respectively. No tube well was found in Gulabad area, whereas from tube wells of Charsadda town and Sukkar four and three water samples were analysed respectively for bacteriological investigation.

A total of 45 water samples were analyzed from the wells of Charsadda town, Gulabad and Sukkar areas for bacteriological count. Four samples were analyzed from the wells of Charsadda town eight samples were analyzed from the wells of Gulabad area (one sample from each well). Sukkar is a rural area, 33 water samples were analyzed for bacterial count (one sample from each well).

Charsadda town water was less contaminated as compared to Gulabad and Sukkar as Umerabad 1 Charsadda had TPC value of $6.7X10^3$/mL which was very less than the TPC value of Gulabad Payyan 1.

The TPC value of well water of Gulabad area showed that its water was much contaminated. The TPC value of Guabad Payyan 1 was 64640/mL while Gulabad Payyan 2 well water was fit for drinking as the TPC value was only 640/mL

The well water from the Sukkar area was also unsafe for drinking purposes. In this area Sukkar Koroona 2 had TPC value of 2048/mL while the lowest one was that of Sukkar Garhi 2 (240/mL), which was fit for drinking.

The statistical analysis of the data for the well water showed significant differences in various areas of district Charsadda ie. Charssada town,Gulabad and Sukkar. (Table 1).

The water of Charsadda town tube well was not fit for drinking according to WHO standard as Kazi Khel Charsadda water had TPC value of 6560/mL. The tube well water from Sukkar area was also unsafe as it had high contamination. The TPC value of Bero Sukkar Bala 1 was 3280/mL.

Among all the three sources of water from Charsadda district highest contamination was found in the drinking water of hand pump from Charsadda town 2. It had TPC value of 77440/mL. The hand pumps water of Gulabad was also contaminated as Gulabad chowk 1 had TPC value of 45600/mL.

Charsadda is an urban area, three samples one from each tube well was analyzed for bacterial count. The tube well water of this area was unfit for drinking as Mean, Std. Deviation and TPC per mL showed. E. coli were found in the water samples of this area. Sukkar is a rural area, four samples one from each tube well were analyzed. Although the values of Mean and Std. Deviation were not much high but the values of TPC / mL indicated that the water was not safe for drinking purposes (Figures 4 and 5).

From the hand pump of Charsadda town four samples (one from each hand pump) of water were analyzed for bacterial count. It was found that the water of hand pump in Charsadda town was high contaminated among all the three water sources (well, tube well and hand pump), as values of Mean, Std. Deviation and TPC/mL showed. E. coli were also found in the water of this area. Three water samples (one from each hand pump) were analyzed from Gulabad area, this area was much affected by the flood of 2010. The effect of flood still found in the water of this area as abundant microbes were found in the water. Sukkar was a flood free zone, five samples from the hand pump of this area were analyzed for bacterial count (one sample from each hand pump), the water of this area was germs free and fit for drinking purposes because Mean and Std. Deviation were low, no E. coli were found in the water. Although the values of TPC/mL were high (Table 6 Figures 6, 7 and 8).

The statistical analysis of the data for the hand pump water showed no significant differences in various areas of district Charsadda ie. Charssada TOWN, Sukkar and Gulabad (Table 7).

Table 1: Mean of TPC/mL of well water of District Charsadda

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | Minimum | Maximum |
|---|---|---|---|---|---|---|---|
| Gul Abad | 8 | 3256.50 | 3810.421 | 1347.187 | 70.91 | 6442.09 | 84 |
| Charsadda | 4 | 302.00 | 369.814 | 184.907 | -286.46 | 890.46 | 40 |
| Sukkar | 33 | 448.85 | 551.191 | 95.950 | 253.40 | 644.29 | 24 |
| Total | 45 | 934.93 | 1932.288 | 288.048 | 354.41 | 1515.46 | 24 |

Table 2: Mean and Standard Deviation of the Bacterial count of hand pump water samples collected from different localities of district Charsadda

| Location | No. of Samples | Total | Coliform/100mL | F.coliform/100mL | E. coli |
|---|---|---|---|---|---|
| Charsadda town | 4 | Mean | 13.05 | 8.3 | Present |
| | | Std. Deviation | 7.9 | 5.3 | |
| Gulabad | 3 | Mean | 12.03 | 4.2 | Present |
| | | Std. Deviation | 8.9 | 3.6 | |
| Sukkar | 5 | Mean | 2.8 | 1.54 | Nil |
| | | Std. Deviation | 1.015 | 0.48 | |
| Total | 12 | Mean | 25.3 | 1.17 | Present |
| | | Std. Deviation | 18.6 | 3.5 | |

Table 3: Mean of TPC/mL of hand pump water of district Charsadda

| | | | | | 95% Confidence Interval for Mean | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | ---------- | | | |
| Area | N | Mean | Std. Deviation | Std. Error | Lower Bound | Upper Bound | Minimum | Maximum |
| Gulabad | 3 | 2147.33 | 2354.052 | 1359.113 | -3700.46 | 7995.12 | 62 | 4700 |
| Charsadda | 4 | 3158.00 | 4432.267 | 2216.133 | -3894.73 | 10210.73 | 41 | 9680 |
| Sukkar | 5 | 264.40 | 140.379 | 62.779 | 90.10 | 438.70 | 132 | 502 |
| Total | 12 | 1699.67 | 2852.511 | 823.449 | -112.73 | 3512.07 | 41 | 9680 |

Table 4: Most Probable Number of drinking water of district Charsadda

| | | | Test for Coliform /100 mL | | | Test for Fecal coliform (/100mL) | |
|---|---|---|---|---|---|---|---|
| | | | No of tubes giving +ive reactions out of 10 | | | No of tubes giving +ive reactions out of 10 | |
| Sample ID No | Name of Area | Source | LB | BGB | MPN/100mL | EC | MPN/100 mL |
| 1 | Gulabad Mera | well | 3 | 3 | 3.6 | 2 | 2.2 |
| 2 | Gulabad Mera | well | 3 | 3 | 3.6 | 3 | 3.6 |
| 3 | Gulabad | well | 2 | 2 | 2.2 | 2 | 2.2 |
| 4 | Gulabad Chowk 1 | well | 1 | 1 | 1.1 | 0 | <1.1 |
| 5 | Gulabad Bala 1 | well | 8 | 8 | 16 | 6 | 9.2 |
| 6 | Gulabad Bala 2 | well | 10 | 10 | >23 | 8 | 16 |
| 7 | Gulabad Payan 1 | well | 10 | 10 | >23 | 8 | 16 |
| 8 | Gulabad Payan 2 | well | 1 | 1 | 1.1 | 8 | 16 |
| 9 | Tabligh MKZ Charsadda | well | 2 | 1 | 2.2 | 0 | <1.1 |
| 10 | Umar abad 1 Chd | well | 1 | 1 | 1.1 | 1 | 1.1 |
| 11 | Umar abad 2 Chd | well | 0 | 0 | <1.1 | 0 | <1.1 |
| 12 | Umar abad 3 Chd | well | 4 | 3 | 5.1 | 3 | 3.6 |
| 13 | Sukkar Bala 1 | well | 5 | 5 | 6.9 | 4 | 5.1 |
| 14 | Sukkar Bala 2 | well | 6 | 6 | 9.2 | 4 | 5.1 |
| 15 | Sukkar Nationalabad 1 | well | 4 | 4 | 5.1 | 3 | 3.6 |
| 16 | Sukkar Nationalabad 2 | well | 6 | 6 | 9.2 | 5 | 6.9 |
| 17 | Sukkar Mayan 1 | well | 3 | 3 | 3.6 | 2 | 2.2 |
| 18 | Sukkar Mayan 2 | well | 1 | 1 | 1.1 | 0 | <1.1 |
| 19 | Sukkar Payan 1 | well | 3 | 3 | 3.6 | 2 | 2.2 |
| 20 | Sukkar Payan 2 | well | 6 | 6 | 9.2 | 5 | 6.9 |
| 21 | Sukkar Payan 3 | well | 0 | 0 | <1.1 | 0 | <1.1 |
| 22 | Sukkar Payan 4 | well | 4 | 4 | 5.1 | 3 | 3.6 |
| 23 | Sukkar Koroona 1 | well | 2 | 2 | 2.2 | 3 | 3.6 |
| 24 | Sukkar Koroona 2 | well | 8 | 8 | 16 | 7 | 12 |
| 25 | Sukkar Koroona 3 | well | 3 | 3 | 3.6 | 2 | 2.2 |
| 26 | Sukkar Koroona 4 | well | 2 | 2 | 2.2 | 2 | 2.2 |
| 27 | Berosukkar 1 | well | 3 | 3 | 3.6 | 2 | 2.2 |
| 28 | Berosukkar 2 | well | 4 | 4 | 5.1 | 3 | 3.6 |
| 29 | Berosukkar 3 | well | 3 | 3 | 3.6 | 2 | 2.2 |
| 30 | Berosukkar 4 | well | 3 | 3 | 3.6 | 2 | 2.2 |
| 31 | Sukkar Garhi 1 | well | 3 | 3 | 3.6 | 2 | 2.2 |
| 32 | Sukkar Garhi 2 | well | 1 | 1 | 1.1 | 0 | <1.1 |
| 33 | Sukkar Garhi 3 | well | 3 | 3 | 3.6 | 0 | <1.1 |
| 34 | Sukkar Garhi Payan 1 | well | 6 | 6 | 9.2 | 5 | 6.9 |
| 35 | Sukkar Garhi Payan 2 | well | 1 | 1 | 1.1 | 0 | <1.1 |
| 36 | Sukkar Garhi Payan 3 | well | 3 | 3 | 3.6 | 2 | 2.2 |
| 37 | Sukkar Garhi Chowk | well | 1 | 1 | 1.1 | 0 | <1.1 |
| 38 | Berosukkar Ali koroona 1 | well | 1 | 1 | 1.1 | 0 | <1.1 |
| 39 | Berosukkar Ali koroona 2 | well | 1 | 1 | 1.1 | 0 | <1.1 |
| 40 | Berosukkar Ali koroona 3 | well | 0 | 0 | <1.1 | 0 | <1.1 |
| 41 | Berosukkar Ali koroona 4 | well | 2 | 2 | 2.2 | 1 | <1. |
| 42 | Berosukkar Bala 1 | well | 2 | 2 | 2.2 | 1 | 1.1 |
| 43 | Berosukkar Bala 2 | well | 3 | 3 | 3.6 | 1 | 1.1 |
| 44 | Berosukkar Mayan | well | 3 | 3 | 3.6 | 1 | 1.1 |
| 45 | Berosukkar Payan | well | 0 | 0 | <1.1 | 0 | <1.1 |
| 46 | Sukkar Bala 1 | Hand pump | 2 | 2 | 2.2 | 1 | 1.1 |
| 47 | Sukkar Bala 2 | Hand pump | 3 | 3 | 3.6 | 1 | 1.1 |
| 48 | Sukkar Mayan | Hand pump | 1 | 1 | 1.1 | 1 | 1.1 |
| 49 | Sukkar Payan 1 | Hand 1pump | 3 | 3 | 3.6 | 2 | 2.2 |
| 50 | Sukkar Payan 2 | Hand pump | 3 | 3 | 3.6 | 1 | 1.1 |
| 51 | Charsadda Town | Hand pump | 0 | 0 | <1.1 | 0 | <1.1 |

Table 4: Continue

| Sample ID No | Name of Area | Source | Test for Coliform /100 mL<br>No of tubes giving +ive reactions out of 10<br>LB | BGB | MPN/100mL | Test for Fecal coliform (/100mL)<br>No of tubes giving +ive reactions out of 10<br>EC | MPN/100 mL |
|---|---|---|---|---|---|---|---|
| 52 | Charsadda Town 1 | Hand pump | 7 | 7 | 12 | 5 | 6.9 |
| 53 | Charsadda Town 2 | Hand pump | 10 | 10 | 23 | 8 | 16 |
| 54 | Charsadda Town 3 | Hand pump | 8 | 8 | 16 | 6 | 9.2 |
| 55 | Gulabad Chowk 1 | Hand pump | 10 | 10 | 23 | 9 | 16 |
| 56 | Gulabad Village Awami | Hand pump | 7 | 7 | 12 | 6 | 9.2 |
| 57 | Gulabad Chowk 2 | Hand pump | 0 | 0 | <1.1 | 0 | <1.1 |
| 58 | Sukkar Bala 1 | T. Well | 2 | 2 | 2.2 | 1 | 1.1 |
| 59 | Sukkar Bala 2 | T. Well | 3 | 3 | 3.6 | 2 | 2.2 |
| 60 | Berosukkar Bala 1 | T. Well | 2 | 2 | 2.2 | 1 | 1.1 |
| 61 | Qazi Khel Charsadda | T. Well | 4 | 4 | 5.1 | 2 | 2.2 |
| 62 | Charsadda Town 1 | T. Well | 3 | 3 | 3.6 | 2 | 2.2 |
| 63 | Umerabad 1 Charsadda | T. Well | 2 | 2 | 2.2 | 1 | 1.1 |
| 64 | Charsadda Town 2 | T. Well | 0 | 0 | <1.1 | 0 | <1.1 |

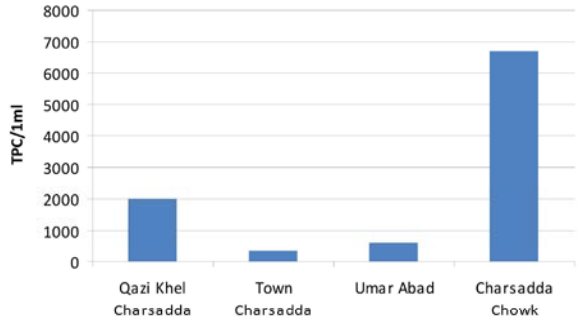


Fig. 1: TPC of well water from Charsadda

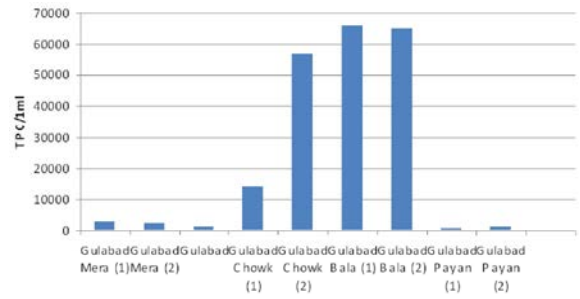


Fig. 2: TPC of well water from Gulabad

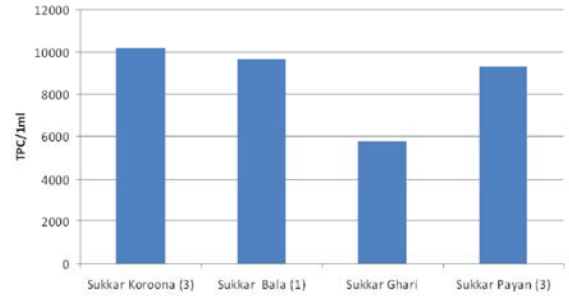


Fig. 3: TPC of well water from Sukkar

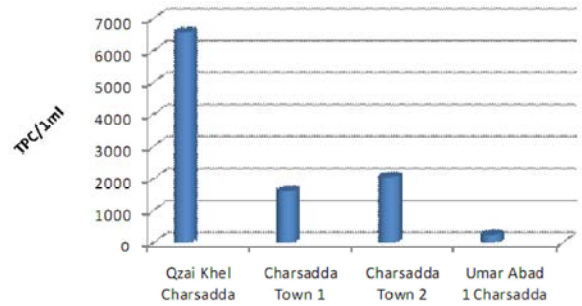


Fig. 4: TPC of Tube well water from Charsadda

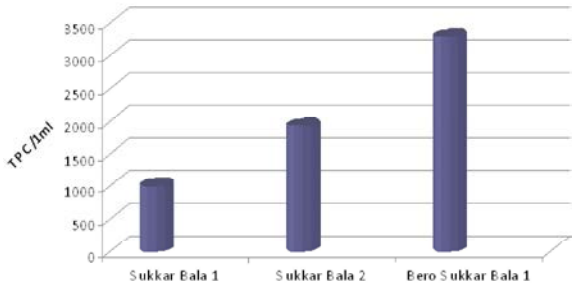


Fig. 5: TPC of Tube well water from Sukkar

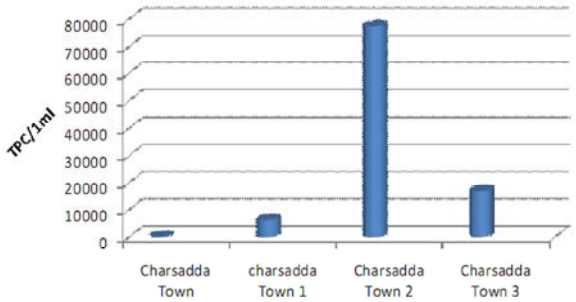


Fig. 6: TPC of Hand pump water from Charsadda Town

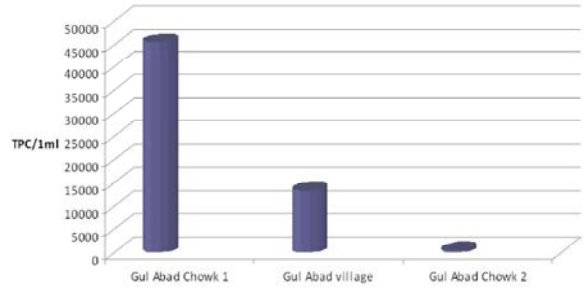


Fig. 7: TPC of Hand pump water from Gulabad

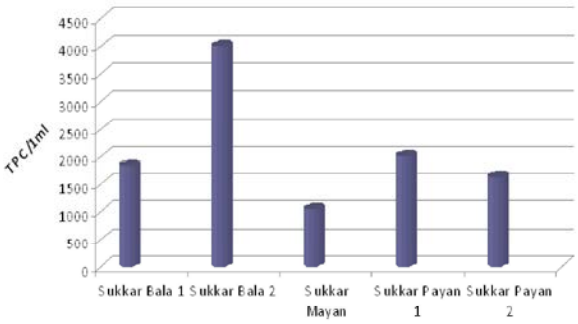


Fig. 8: TPC of Hand pump water from Sukkar

- Geometric mean of well water of District Charsadda = 28083
- Geometric mean of hand pump water of District Charsadda= 502.198
- Geometric mean of tube well water of District Charsadda= 205.52

Here Geometric Mean for hand pump water was lager for well water was second larger and for tube well water was smaller so here the average bacteria for the water of tube well was smaller. So we suggest that the tube well water of district Charsadda was better than the well and hand pump water.

## CONCLUSION

It was concluded from the present study that the reason for increased Plate Count of water was the heap present near to the water points. All these factors contribute to increase Plate Count, Coliform and Fecal coliform which lead to hazard for public health. It is imperative for the Government to create awareness among the masses about the importance of water for life, the microorganisms present in the water sources, which may play a role for the provision of safe drinking water to the public.

## ACKNOWLEDGEMENT

We are thankful to PCSIR Laboratories Complex Peshawar, for laboratories facilitation.

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