IMPACT OF DEGRADED WATER ON IMMUNE RESPONSE: SURVEY OF LAHORE, PUNJAB, PAKISTAN IN 2017.

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خلاصه

Abstract

An immune response can be affected by inhaling toxins. We investigated whether an intake of degraded water can affect immunity. The home tap-water was collected from different regions of Lahore. Different water parameters were measured in laboratory. Information on sources of water intake, diseases acquired and the type of environmental pollution exposure was gathered. The complete blood count test was also conducted with few residents. The Mann-Whitney U and t-tests were applied to compare deranged values. The 76.72% of high EC, ~43% of high Total and Fecal Coliforms, 36.20% of high TDS values were found. Overall, 54% high monocytes, 38% high RBCs, 32% high Ab. monocytes, 22% high lymphocytes and 18% low granulocytes were found. There were 33.57% use of filtered water, 28.32% of tap-water use and 26.22% of mineral water was reported. The diarrhea was 27.62%, cholera 20.98% and typhoid was 19.93%. Poor conditions of roads and street were 46.50% and air pollution was 38.46%. We reported cell-lines variations in the form of monocytosis, lymphocytosis and granulocytopenia indicating an altered immune response in a group of individuals who were exposed to polluted drinking water and the environment. Raised levels of monocytes and lymphocytes show hyper immune response in individuals. An active and hyper immune cell line indicate the presence of infectious causing microorganisms in the human population of Lahore.

Introduction

The primary source of drinking water for the majority of people in Pakistan is groundwater (Pakistan-WWF 2007). Lahore is a second largest city, where almost all populations depend upon groundwater for their drinking water needs (Ahmad *et al.*, 2012). It is of utmost necessary to monitor the quality of water more frequently. The present study was focused to determine the extent of water quality around the city Lahore (Punjab), Pakistan by measuring following some of the physical/chemical/biological parameters in laboratory of Civil Engineering Department, National University of Computer & Emerging Sciences (NUCES), Lahore. The home tap-water (n=116) sampling was done from the following eleven regions of Lahore: EME DHA, Johar Town, Faisal Town, Township, Allama Iqbal Town, Model Town, Wapda Town, Canal View Society, Gulberg

III, Mughal-Pura and Gulshan Ravi. Following chemical parameters: pH, EC (electrical conductivity) in µs/cm, DO (dissolved oxygen) in ppm, TDS (total dissolved solids) in ppm, TSS (total suspended solids) in ppm, Total Hardness (as CaCO₃) in ppm Sodium (Na+) in ppm and Potassium (K+) in ppm, one physical parameter, i.e., Turbidity in NTU (Nephelometric Turbidity Units), and following biological parameters: Total Coliform in MPN (most probable number) and Fecal Coliform in MPN from different equipments and standard methods. We tried to determine the commonest source of drinking water in order to figure out the underlying risk of waterborne viral or infectious or diseases among citizens. A comprehensive survey of 286 residents of above mentioned regions was made for the collection of information regarding environmental pollution, type of infectious/viral diseases, heart disease, hypertension or any cancer reported in each home within at least last three years. In order to assess the primary status of immunity among residents, few individuals (n=50) were randomly tested for basic complete blood count (CBC) parameters as well, so that correlative inferences could be made regarding waterborne diseases. It is known that an abnormal immune response can lead to infections and infection fighting cell lines, such can be raised during recovery phase. Intake of degraded water, can affect immune response and that was the purpose of this research. It was reported by Calderon-Garciduenenas et al., (2008), that exposure to air pollution exposure can cause, 'an altered brain innate response', 'neuroinflammation', 'Alzheimer's disease' or 'Parkinson disease' etc. Yet, immune response alteration needs to be confirmed in case of degraded drinking water intake or usage. We tried to look that if an intake of degraded water, can affect immune response from CBC tests and survey analyses from the population of Lahore.

Materials and Methods

Study Areas and Sampling

The home tap-water (n=116) sampling was done from the following eleven regions of Lahore: EME DHA, Johar Town, Faisal Town, Township, Allama Iqbal Town, Model Town, Wapda Town, Canal View Society, Gulberg III, Mughal-Pura and Gulshan Ravi. The tap-water was collected in cleaned 500 mL plastic bottles. A *Google Map* was generated to highlight the selected regions.

Tap-Water's Chemical/Physical/Biological Parameters' Testing:

The samples were analyzed for following chemical parameters: pH, EC (electrical conductivity) in μ s/cm, DO (dissolved oxygen) in ppm, TDS (total dissolved solids) in ppm, TSS (total suspended solids) in ppm, Total Hardness (as CaCO₃), in ppm Sodium (Na+) in ppm and Potassium (K+) in ppm, one physical parameter i.e., Turbidity in NTU and following biological parameters: Total Coliform in MPN and Fecal Coliform in MPN from different equipment using standard methods.

Measurement of pH and Electrical Conductivity (EC):

The pH is a measure of alkalinity or acidity of a solution. The pH of water samples was measured from standard method number 4500-H⁺ B from Digital Multi-Parameter (model: inoLab Multi 9420) of Germany. According to EPA (Environmental Protection Agency) and WHO (World Health Organization) the permissible pH of drinking water is between 6.5- 8.5 (Mohsin *et al.*, 2013; Pakistan Environmental Protection Agency, 2008).

Measurement of Electrical Conductivity (EC):

The electrical conductivity is a magnitude of water to conduct electricity and it is associated with the dissolved salt concentrations in water. The EC of water samples was measured from standard method number 4500-H⁺ B (WHO-WMO-No.1113, 2013; Clesceri *et al.*, 2005) from Digital Multi-Parameter (model: inoLab Multi 9420) of Germany. The permissible limits of EC should not exceed \leq 400 as mentioned by the WHO (Mohsin *et al.*, 2013).

Measurement of Dissolved Oxygen (DO):

The Dissolved Oxygen (DO) is related to non-compound free oxygen levels available in water (Fondriest Environmental Inc., 2017). The dissolved oxygen was measured from the standard method number 2430 B (Clesceri *et al.*, 2005) from DO meter (model DO 200A) from Eco Sense (USA). The range of DO meter should be from 0 to 20 ppm for DO. The permissible limit by ISI10500-91 for DO is 5 mg/l or 5 ppm (Raj and Thakur, 2017). We took 6 ppm as standard DO value in this study.

Measurement of Total Dissolved Solids (TDS):

The Total Dissolved Solids are those solids which dissolve into water and can pass through the filter paper. The lower the TDS, value, the better will be the water quality. The Total Dissolved Solids (TDS) was measured from standard method 2540 C (Clesceri *et al.*, 2005) from the Handheld TDS meter (model: CON 5/TDS) of Eutech Singapore. The permissible limit of TDS is 500 ppm by USEPA (US EPA, 2017). *Measurement of Turbidity:*

The Turbidity is a relative clarity of water- a measure of cloudiness of water i.e., not clear due to dissolved or suspended sediments and visible particles or sediments in the suspension in water. The turbidity of water was measured from standard method number 2130 B from (Clesceri *et al.*, 2005) Portable Turbimeter (model: Turb

430 IR/T) of WTW Germany, based on the principle of Nephelometry. For drinking water, the permissible value of turbidity should be < 5 NTU as per WHO (Sehar *et al.*, 2011).

Measurement of Total Hardness:

The Hardness of water was determined from standard method number 2340 C (Clesceri *et al.*, 2005) with following apparatus: titration flask, burette & pipette, difference buffer solutions and indicators. The allowable range of Total Hardness as CaCO₃ is 500 ppm by WHO (Mohsin *et al.*, 2013). The Total Hardness was determined by following Complexo-metric titration: A 25 ml of water sample was taken in a titration flask and added 25 ml distilled water in it to dilute the water solution (to avoid formation of CaCO₃). A distilled water does not participate in ion exchange in reaction. Added 1-2 ml of Buffer Solution (Ammonia Buffer NH₄Cl + NH₄OH) to maintain pH in solution. Added a small amount of EBT (Echochrome Black T) as an indicator. As a result of addition of EBT, the solution of color changed to wine red. Titrated it against 0.01 M EDTA solution. Added EDTA (Ethylene Diamine Tetra Acetic Acid) from a burette until color changed to blue. We tested three samples and determined the mean volume of titrant used. The Total Hardness (mg/lit) as CaCO₃ was calculated from the formula.

Total Hardness as $CaCO_3 (mg/l) = (Volume of Titrant Used \times 1000) / Volume of the Sample.$ Determination of Total Coliform

The *Escherichia coli* and *Fecal Coliform* bacteria must not be detected in any 100 ml sample of drinking water, otherwise there will be a substantial risk of waterborne diseases. According to the WHO, the amount of *Total* and *Fecal Coliforms* in drinking water should be zero (Fondriest Environmental Inc., 2017; Pakistan Environmental Protection Agency, 2008). All gram negative, aerobic, facultative anaerobic, rod shaped and non spore forming bacteria are included in the *Coliform* group. The growth of *Fecal Coliform* bacteria in ambient water develops from the spillage from nonpoint sources of human and animal wastes or domestic sewage (Toronto and Region Conservation Authority, 2010). The test of detecting *Total Coliform* was carried out from standard method number 9221 C from 'Multiple Tube Fermentation Technique' (Clesceri *et al.*, 2005; Bhandari *et al.*, 2015). Many pathogens emanate from polluted water with human excrements (Chapman, 1996). The 'Multiple Tube Fermentation Technique' (Clesceri *et al.*, 2005; Bhandari et al., 2015). Many pathogens emanate from polluted water with human excrements (Chapman, 1996). The 'Multiple Tube Fermentation Technique' (Clesceri *et al.*, 2005; Bhandari et al., 2015). Many pathogens emanate from polluted water with human excrements (Chapman, 1996). The 'Multiple Tube Fermentation Technique' (Nest probable number) in order to estimate *Coliforms* ('American Public Health Association', 1989; 'American Water Association', 'Water Environment Federation', 1994).

We used strong, thick walled glass or plastic bottles free of contamination to collect samples for microbiological analysis. The samples bottles were kept at about 4°C immediately after collection during transportation. Those samples which arrived after 24 hours were discarded. Following apparatus was used: autoclave, incubator, sample bottles, fermentation tubes with inverted vials, dilution bottles, pipettes and pipette stand. Lactose broth and distilled water were used as reagents. The procedure was as follows: for potable water, arranged ten fermentation tubes in a rack with inverted vials. Before sterilization, dispensed an ample medium to cover inverted vials at least partially after sterilization. Sterilized fermentation tubes containing medium along with other necessary glass apparatus in an autoclave for 15 minutes at 121° C. Removed fermentation tubes from autoclave as soon as the chamber pressure was reached zero. We never re-autoclaved the medium. Dispensed 10 ml of sample in each tube and incubated the inoculated tubes at 35 ± 0.5 °C. After 24 ± 2 hours, shook each tube gently and examined it for any gas or acidic growth ('American Public Health Association', 1989; 'American Water Association', 'Water Environment Federation', 1994). As absence of gas or acidic growth was found, the samples were re-incubated and re-examined at the end of 48 ± 2 hours. Recorded the presence or absence of gas or acid produced in fermentation tubes. Any absence of acidic growth or gas formation at the end of 48 ± 2 hours of incubation constituted a negative test. Any formation of acidic growth in the tubes within 48 ± 2 hours constituted a "positive presumptive reaction". Submitted these tubes with 'positive presumptive reaction' to the 'confirmed phase'. Shook samples and diluted them vigorously for about 25 times and repeated the same procedure mentioned.

For the confirmed phase, following reagents were used: 'Brilliant Green Lactose Bile Broth' (BGLB) and distilled water. Following apparatus was used: fermentation tubes with caps, inverted vials, sterile metal loop of 3 mm diameter and a sprit lamp. The procedure was as follows: before sterilization, dispensed sufficient medium, to cover inverted vials at least partially after sterilization. Submitted all primary tubes showing any amount of gas or acidic growth within 24 ± 2 or 48 ± 2 hours of incubation to the confirmed phase ('American Public Health Association', 1989; 'American Water Association', 'Water Environment Federation', 1994; 'Corpus Christi Bay National Estuary Program', 1998). When active fermentation or acidic growth appeared in the primary tubes earlier than 24 hours, it then transferred to the confirmatory medium, preferably without waiting for full 24 ± 2 hours period to elapse. When additional primary tubes showed an acidic growth at the end of a 48 ± 2 hours incubation period, submitted these to the confirmed phase (US EPA, 2017). Gently shook or rotated primary tubes showing gas or acidic growth to re-suspend the organisms. Took a metal inoculating loop of 3 mm diameter and heated it on the spirit lamp till it had become a red-hot. Cooled the loop to room temperature and with its help, transferred a loop full of culture to fermentation tube containing brilliant green lactose bile broth. Incubated the inoculated 'Brilliant Green Lactose Bile Broth' tube for 48 ± 2 hours at

 $35 \pm 0.5^{\circ}$ C. The formation of gas was observed in inverted vial of the 'Brilliant Green Lactose Bile Broth' broth fermentation tube at any time within 48 ± 2 hours, constituted a 'positive confirmed phase'. Calculated the MPN value from the number of 'positive, brilliant green lactose bile' tubes ('American Public Health Association', 1989; 'American Water Association', 'Water Environment Federation', 1994) from the following formula:

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\frac{MPN}{100_{mL}} = \frac{No.of \text{ positive tube} \times 100}{\sqrt{m_L \text{ of sample in negative tubes}} \times (m_L \text{ of sample in all tubes})}
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Determination of Fecal Coliform:

This group comprises those Coliform Bacteria whose origin is feces i.e., the intestines of warm-blooded animals, which can induce waterborne diseases. This test differentiates between Coliforms of fecal origin and Coliforms of non-fecal origin. The test Total Coliform was carried out from standard method number 9221 C from 'Multiple Tube Fermentation Technique' (Clesceri et al., 2005). The 'Multiple Tube Fermentation Technique' may be used to obtain statistically valid MPN (most probable number) estimate of the Coliform. We used strong, thick walled glass or plastic bottles free of contamination to collect samples for microbiological analysis. Following reagents were used: EC (Escherichia Coli) medium and distilled water. Following apparatus was used: fermentation tubes with caps, inverted vials, sterile metal loop of 3 mm diameter and a sprit lamp. The procedure was as follows: we filled fermentation tubes with desired broth and inserted inverted vial including a sufficient medium which covers the inverted vial at least partially after sterilization. Sterilized the tubes containing medium and other necessary glassware at 121°C for 15 minutes in an autoclave. Submitted all presumptive fermentation tubes showing any amount of gas or heavy growth within 48 hours of incubation of the confirmed test ('American Public Health Association', 1989: 'American Water Association', 'Water Environment Federation', 1994). Gently shook or rotated the presumptive fermentation tubes showing any gas or heavy growth. Took a 3 mm diameter metal loop and heated until it became a red-hot on a sprit lamp. Cooled the loop to room temperature and with the help of this, the loop transferred growth from each presumptive fermentation tube to EC (*Escherichia coli*) broth. Incubated inoculated EC broth tubes at $44.5 \pm 0.2^{\circ}$ C for 24 ± 2 hours (Al-Lahham et al., 2003). Any gas formation in an EC broth culture within 24 hours was considered a positive reaction indicating *fecal Coliform* presence. Calculated the MPN value from the number of positive 'Brilliant Green Lactose Bile' tubes (US EPA, 2015; 'American Public Health Association', 'American Water Works Association', 'Water Environment Federation', 20th Edition, 1994) from following from same formula.

Determination of Sodium and Potassium Levels:

The allowable range of Sodium is 200 ppm and for Potassium is 12 ppm by WHO (Mohsin *et al.*, 2013). The Sodium and Potassium in water samples were measured using Flame Photometer (model: M360) from Sherwood Flame Photometer. The standard method number was 3500-Na D for Sodium and 3500-K B for Potassium (Clesceri *et al.*, 2005). Following reagents were used: 1000 ppm Na stock solution (prepared from NaCl) and 1000 ppm K stock solution (prepared from KCl). Following procedure was adopted: turned on the air supply as well as the gas supply from a cylinder. Placed a beaker of distilled water on right side of a flame photometer and inserted the nebulizer in the beaker. The meter, then displayed a certain reading. With the help of the blank, the control was set to the value of zero. Now replaced the distilled water with the standard stock solution. Again placed the distilled water. When meter showed a zero reading, it meant that the calibration is fine, otherwise repeated formation of standard stock solution. After the calibration, placed the desired sample and recorded the readings in ppm.

Survey: Environmental Factors, Water Intake Source and Clinical History

The information on the local environmental factors such as: air pollution, industrial area, roads & street conditions, agricultural exposure, municipal fall out and the presence of animal manure was gathered from the residents (n = 286) of the above mentioned eleven regions of Lahore. The information on the following types of drinking water intake was also gathered: mineral water, tap water, filtered water, bore water, tube-well, hand pumps, ground water, etc. The information was gathered from each family regarding several infectious and viral diseases reported in the last three years: Diarrhea, Cholera. Dysentery, Typhoid fever, Ebola fever, Lyme, Gastric Ulcer, Influenza, Dermatitis, Dental caries, Hepatitis, Yellow fever, Naegleria, Shigellosis, Polio, Giardia, Botulism, Cryptosporidiosis, Tuberculosis, Measles, Malaria, Pneumonia, Dengue fever and Swine Flu etc. Further, information regarding cancer, heart disease and hypertension was also gathered from each family.

Complete Blood Count (CBC) Test

The complete blood count (CBC) was conducted randomly from one non-smoker person of each family (n=50) at the time of sampling on informed consent voluntarily. The values of the following CBC test were taken: hemoglobin (HGB- g/dl), white blood cells (WBC-10⁹/l), platelets (PLT-10⁹/l), hematocrit (HCT-%), red blood cells (RBC-10¹²/l), lymphocytes (LY %), monocytes (MO %), granulocytes (GR %), absolute

lymphocytes count (Ab. LY absolute- $10^{9}/1$), absolute monocytes count (Ab. MO absolute- $10^{9}/1$) and absolute granulocytes count (Ab. GR absolute- $10^{9}/1$). At the time of sampling, the person whose blood sample was drawn, was not having any disease.

Statistical Analysis

The frequency of each deranged (high/low) water parameter's frequency along with frequency of each normal (within range) value was calculated. Similarly, each mentioned CBC parameter's mean, standard deviations, maximum and minimum values were calculated. The frequency of each deranged (high/low) CBC parameter's frequency along with frequency of each normal (within range) value was calculated.

The Shapiro-Wilk (W) Statistics were applied to separate normalized distributed or non-normalized distributed water and CBC parameters based on the p-value. Those parameters whose p-value in Shapiro-Wilk (W) test was greater than 0.050 was normalized, and those parameters (water or CBC) whose p-value was lesser than 0.050 was considered non-normalized. The Mann-Whitney U test for non-normalized parameters (water or CBC) values was applied to compare deranged values (high or low) of different parameters of water samples with the normal values found. The significance level was 0.05 (two tailed). Whereas, the two tailed t-test (independent samples) on normalized parameters (water or CBC) values was applied to compare deranged values with the normal values found. The significance level was 0.05 (two tailed). Whereas, the two tailed t-test (independent samples) on normalized parameters of water samples with the normal values found. The significance of water samples with the normal values found. The significance of water samples with the normal values found. The significance of water samples with the normal values found. The significance of water samples with the normal values found. The significance of water samples with the normal values found. The significance of difference was considered at p values <0.050.

A Table was maintained to summarize with percentage prevalence-PP (%) results from the survey analysis related to source of water-intake, infectious diseases reported in 1-3 years, environmental pollution exposure, cancer reported and hypertension (yes/no).

Results

The **Figure 1** shows a *Google earth map* showing study sights. Eleven regions were selected for water sampling (n=116) to monitor quality.

Mean, Maximum/Minimum and Deranged Values of Physical-Chemical Parameters of Tap-Water in All Regions:

The following acceptable ranges were considered: 6.5-8.5 for pH, 400 (μ S/cm) for EC (electrical conductivity), 6 ppm for DO (Dissolved Oxygen), 500 ppm for TDS (Total Dissolved Solids), 500 ppm for Total Hardness as CaCO₃, 200 ppm for Sodium, 12 ppm for Potassium, less than 5 NTU for Turbidity, 0 MPN for both *Total Coliform* and *Fecal Coliform*. The following parameters' mean values were out of range: EC at 679.413 (μ S/cm), TDS at 508.245 (ppm), *Total Coliform* at 3.767 MPN and *Fecal Coliform* at 3.577 MPN (**Table 1**). The following parameters' mean values were not out of range: pH: 7.482, DO: 5.050 (ppm), Total Hardness: 230.663 (ppm), Sodium: 73.784 (ppm), Potassium: 11.314 (ppm) and Turbidity: 1.611 (NTU).

The **Table 2** shows different parameters' mean and maximum/minimum values of tap-water samples of each region separately. All regions' pH values were within the range. The maximum value of water pH (8.40) was reported from Wapda town and the minimum value (6.40) was reported from Gulshan Ravi.

The mean values of EC were found high compared to the accepted range in the tap-waters of all mentioned regions: EME DHA (677.33 μ S/cm), Johar Town (586.176 μ S/cm), Faisal Town (567.956 μ S/cm), Township (620.333 μ S/cm), Iqbal Town (928.100 μ S/cm), Model Town (627.875 μ S/cm), Wapda Town (745.00 μ S/cm), Canal View (728.750 μ S/cm), Gulberg III (778.000 μ S/cm), Mughalpura (697.428 μ S/cm) and Gulshan Ravi (757.000 μ S/cm). Among all regions, the mean (928.100 μ S/cm) of EC was found high in the tap water of Iqbal Town region. The maximum value of EC (1412 μ S/cm) was reported from the Model Town region among all other regions which was the only value lesser than 400 (WHO limit is \leq 400).

The mean values of DO was found high compared to the accepted range (6 ppm) in the tap-waters of Mughalpura (6.271 ppm). The maximum value of DO (9 ppm) was reported in the tap-waters of EME DHA and Johar Town regions. The minimum value of DO (0.50 ppm) was reported in the tap-waters of Gulberg-III regions. The low values of DO was considered in the range 0.5-3.9 ppm.

The mean values of TDS were found high compared to the accepted range (500 ppm) in the tap-waters of the following regions: Johar Town (533.058 ppm), Faisal Town (541.695 ppm), Township (531.444 ppm), Iqbal Town (593.444 ppm), Wapda Town (524.400 ppm), Canal View (514.125 ppm) and Mughalpura (570.571 ppm). Among all regions, the mean (593.444 ppm) of TDS was found high in the tap water of Iqbal Town region. The maximum value of TDS (1146.00 ppm) was reported from Mughalpura region among all other regions.



Fig.1. Google Earth Map Showing Study Sights. Eleven Regions Were Selected for Water Sampling (n=116) to Monitor Quality.

Parameters	Acceptable Values	Mean	Max.	Min.	Std. Deviation
pН	6.5-8.5	7.482	8.90	6.40	0.43803
EC (µS/cm)	≤400	679.413	1412.00	326.00	237.255
DO (ppm)	5	5.0509	9.00	0.50	1.768
TDS (ppm)	Up to 500	508.245	1146.00	212.00	160.300
Total Hardness (ppm)	Up to 500	230.663	476.00	48.00	105.320
Sodium (ppm)	Up to 200	73.784	211.00	14.00	50.018
Potassium (ppm)	<i>Up to 12</i>	11.314	40.00	0.50	8.561
Turbidity (NTU)	< 5	1.611	4.30	0.01	1.305
Total Coliform (MPN)	0	3.767	30.00	0.00	6.046
Fecal Coliform (MPN)	0	3.577	25.00	0.00	5.439

 Table 1. Tap-Waters' Parameters' Mean, Maximum and Minimum Values of All (n=116) Tap-Water

 Samples from All Regions (EME-DHA, Johar Town, Faisal Town, Township, Iqbal Town, Model Town,

 Wapda Town, Canal View, Gulberg III, Mughalpura and Gulshan Ravi).

Key- EC: Electrical Conductivity, DO: Dissolved Oxygen, TDS: Total Dissolved Solids; Max: Maximum, Min: Minimum

The mean values of Potassium were found high compared to the accepted range (12 ppm) in the tap-waters of the following regions: Iqbal Town (13.80 ppm) and Mughalpura (18 ppm). The maximum value of Potassium (40.00 ppm) was reported from Iqbal Town and Mughalpura regions. The minimum value of Potassium (0.50 ppm) was reported from tap-waters of the Canal View region.

All mean values of Total Hardness were lesser than 500 ppm in tap-waters of all regions. The maximum value of Total Hardness (476 ppm) was reported from Gulshan Ravi region among all other regions. The minimum value of Total Hardness (48 ppm) was reported from the EME DHA region among all other regions.

All mean values of Sodium were lesser than 200 ppm in tap-waters of all regions except in Iqbal Town region (995.500 ppm). The maximum value of Sodium was reported from EME DHA (208.00 ppm) and Gulberg III (211.00 ppm) regions among all other regions

					Parar	neters				
	pН	EC	DO	TDS	Total Hardness	Sodium	Potassium	Turbidity	Total	Fecal Coliforn
Location		(µS/cm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(NTU)	Coliform(MPN)	(MPN)
	6.5-8.5	≤400	6	Up to 500	Up to 500	Up to 200	Up to 12	<5	0	0
EME	Mean:7.611	Mean:677.33	Mean:5.555	Mean:497.555	Mean:177.11	Mean:79.556	Mean:9.222	Mean:1.881	Mean: 0.556	Mean: 0.444
DHA	Max:8.90	Max:1012.00	Max:9.00	Max:895.00	Max:325.00	Max:208.00	Max:21.00	Max:3.11	Max:3.00	Max:2.00
n=9	Min:6.70	Min:432.00	Min:3.10	Min:212.00	Min:48.00	Min:21.00	Min:2.00	Min:0.05	Min:0.00	Min:0.00
Johar	Mean:7.530	Mean:586.176	Mean:4.447	Mean:533.058	Mean:211.352	Mean:74.823	Mean:10.00	Mean:1.771	Mean: 2.294	Mean:2.352
Town	Max:8.20	Max:811.00	Max:9.00	Max:722.00	Max:431.00	Max:168.00	Max:21.00	Max:4.30	Max:14.00	Max:12.00
n=17	Min:6.80	Min:423.00	Min:1.90	Min:346.00	Min:49.00	Min:55.00	Min:3.00	Min: 0.01	Min:0.00	Min:0.00
Faisal	Mean:7.405	Mean:567.956	Mean:5.026	Mean:541.695	Mean:262.869	Mean:39.347	Mean:9.869	Mean:1.478	Mean: 6.826	Mean:3.521
Town	Max:8.20	Max:1051.00	Max:8.20	Max:788.00	Max:456.00	Max:166.00	Max:23.00	<i>Max</i> :3.11	Max:30.00	Max:16.00
n=23	Min:6.90	Min:380.00	Min:3.10	Min:346.00	Min:154.00	Min:29.00	Min:1.00	Min: 0.01	Min:0.00	Min:0.00
Town	Mean:7.322	Mean:620.333	Mean:5.055	Mean:531.444	Mean:228.666	Mean:64.333	Mean:10.555	Mean:1.682	Mean: 3.888	Mean: 2.888
Ship	Max:8.00	Max:1077.00	Max:8.60	Max:792.00	Max:418.00	Max:135.00	Max:24.00	Max:3.11	Max:10.00	Max: 6.00
n=9	Min:6.60	Min: 326.00	Min:1.80	Min:287.00	Min:98.00	Min:15.00	Min:3.00	<i>Min</i> : 0.01	Min:0.00	Min:0.00
Iqbal	Mean:7.380	Mean:928.100	Mean:5.220	Mean:593.600	Mean:208.100	Mean:99.500	Mean:13.80	Mean:2.218	Mean:2.000	Mean:2.800
Town	Max:8.10	Max:1134.00	Max:7.90	Max:981.00	Max:331.00	Max:190.00	Max:40.00	<i>Max</i> :3.11	Max:8.00	Max:12.00
n=10	Min:6.80	Min:692.00	Min:2.80	Min:291.00	Min:108.00	Min:39.00	Min:1.00	<i>Min</i> : 0.01	Min:0.00	Min:0.00
Model	Mean: 7.537	Mean:627.875	Mean:5.237	Mean:375.250	Mean:250.625	Mean:50.125	Mean:9.625	Mean:1.498	Mean:4.750	Mean: 4.875
Town	Max: 8.10	Max:1412.00	Max:8.60	Max:526.00	Max:356.00	Max:81.00	Max:31.00	Max:4.12	Max:10.00	Max:10.00
n=8	Min: 7.20	Min:381.00	Min:2.90	Min:256.00	Min:101.00	Min:29.00	Min:1.00	<i>Min</i> : 0.01	Min:0.00	Min:0.00
Wapda	Mean:7.383	Mean:745.00	Mean:5.933	Mean:524.000	Mean:254.666	Mean:112.166	Mean:9.00	Mean:0.943	Mean:2.166	Mean:2.333
Town	Max:8.40	Max:1054.00	Max:7.00	Max:786.00	Max:438.00	Max:176.00	Max:21.00	Max:3.11	Max:8.00	Max:8.00
n=6	Min:6.90	Min:586.00	Min:4.30	Min:252.00	Min:110.00	Min:55.00	Min:2.00	<i>Min</i> : 0.01	Min:0.00	Min:0.00
Canal	Mean:7.387	Mean:728.750	Mean:5.475	Mean:514.125	Mean:229.750	Mean:67.000	Mean:10.937	Mean:1.943	Mean:2.250	Mean:2.000
View	Max:8.10	Max:1341.00	Max:7.60	Max:699.00	Max:351.00	Max:126.00	Max:28.00	Max:2.90	Max:7.00	Max:6.00
n=8	Min:6.90	Min:390.00	Min:2.90	Min:370.00	Min:106.00	Min:16.00	Min: 0.50	<i>Min</i> : 0.01	Min:0.00	Min:0.00
Gulberg	Mean:7.527	Mean:778.000	Mean:4.018	Mean:438.272	Mean:210.0909	Mean:97.909	Mean:11.363	Mean:0.945	Mean:3.272	Mean:5.454
III	Max:8.10	Max:1149.00	Max:8.10	Max:599.00	Max:408.00	Max:211.00	Max:24.00	<i>Max</i> :3.11	Max:7.00	Max:25.00
n=11	Min:6.80	Min:345.00	Min: 0.50	Min:283.00	Min:67.00	Min:14.00	Min:3.00	<i>Min</i> : 0.01	Min:0.00	Min:0.00
Mughal	Mean:7.871	Mean:697.428	Mean:6.271	Mean:570.571	Mean:226.571	Mean:104.042	Mean:18.00	Mean:1.547	Mean: 3.714	Mean:5.000
pura	Max:8.20	Max:1241.00	Max:8.20	Max:1146.00	Max:446.00	Max:198.00	Max:40.00	Max:2.90	Max:10.00	Max:22.00
n=7	Min:7.60	Min:402.00	Min:3.90	Min:384.00	Min:95.00	Min:34.00	<i>Min</i> :1.00	<i>Min</i> : 0.01	Min:0.00	Min:0.00
Gulshan	Mean:7.387	Mean:757.000	Mean:4.700	Mean:396.125	Mean:264.625	Mean:84.250	Mean:16.250	Mean:1.766	Mean:6.250	Mean: 9.000
Ravi	Max:7.90	Max:1113.00	Max:7.00	Max:512.00	Max:476.00	Max:169.00	Max:32.00	Max:4.19	Max:22.00	Max:25.00
n=8	Min:6.40	Min:547.00	Min:2.60	Min:290.00	Min:89.00	Min:20.00	Min:2.00	Min:0.05	Min:0.00	Min:0.00

Table.2. Different Parameters' Mean, Maximum and Minimum Values of Tap-Water Samples of Each Region .

Key- EC: Electrical Conductivity, DO: Dissolved Oxygen, TDS: Total Dissolved Solids, Max: Maximum, Min: Minimum

(6.250 MPN). The highest mean value (6.826 MPN) was reported from Faisal Town region's tap-waters. The maximum value (22 MPN) was reported from Gulshan Ravi region's tap-water.

All mean values of Turbidity were lesser than 5 NTU in tap-waters of all regions. The maximum value (4.30 NTU) of Turbidity was reported from the tap-waters of Johar Town region. The minimum value (0.01 NTU) was reported from the following regions: Johar Town, Faisal Town, Township, Iqbal Town, Model Town, Wapda Town, Canal View, Gulberg III and Mughalpura.

The mean values of Total Coliform were found high compared to the accepted value (0 MPN) in the tapwaters of all mentioned regions: EME DHA (0.556 MPN), Johar Town (2.294 MPN), Faisal Town (6.826 MPN), Township (3.888 MPN), Iqbal Town (2.000 MPN), Model Town (4.750 MPN), Wapda Town (2.166 MPN), Canal View (2.250 MPN), Gulberg III (3.714 MPN), Mughalpura (3.714 MPN) and Gulshan Ravi

CBC	HGB	WBC	PLT	НСТ	RBC	LY	MO	GR	Ab. LY	Ab. MO	Ab. GR
Parameters	(g/dl)	$(10^{9}/l)$	$(10^{9}/l)$	%	$(10^{12}/l)$	%	%	%	(10%/l)	$(10^{9}/l)$	$(10^{9}/l)$
Normal Range	12.0-	4.0-	150-	36.0-	3.8-	20.0-	3.0-	50.0-	1.30-	0.15-	2.50-
	20.0	11.0	400	50.0	5.5	40.0	7.0	75.0	4.00	0.70	7.50
Mean Values	14.882	8.545	264.68	43.57	5.436	32.53	8.062	58.85	2.767	0.665	5.111
			0	2		2		2			
Max. Values	17.43	13.04	385.00	54.42	6.95	47.50	16.62	77.00	4.57	1.48	8.28
Min. Values	9.30	4.12	165.00	27.61	3.61	15.50	1.50	31.20	1.28	0.19	1.91
Std. Dev.	1.4193	1.845	64.959	5.487	0.6922	7.458	3.477	8.992	0.8603	0.2882	1.4101
		5	4	9		0	0	8			
1	14.20	12.12	278	42.42	5.29	28.10	06.20	65.80	3.40	0.75	7.97
2	13.40	8.43	165	40.01	6.71	47.40	08.00	44.50	4.00	0.68	3.76
3	14.30	7.87	176	44.12	5.69	28.00	06.60	65.40	2.20	0.52	5.15
4	16.60	10.21	320	49.28	5.95	36.90	08.00	55.20	3.76	0.81	5.63
5	15.20	10.72	249	45.09	5.41	36.00	05.90	58.00	3.86	0.63	6.22
6	16.50	07.28	361	49.85	6.01	30.20	04.20	65.60	2.20	0.30	4.78
7	12.40	05.15	222	37.73	4.44	24.90	06.30	68.80	1.28	0.33	3.54
8	15.40	11.31	355	45.43	5.23	40.20	06.80	53.00	4.55	0.77	5.99
9	09.30	07.98	385	30.61	4.92	32.00	07.30	60.70	2.55	0.58	4.84
10	15.50	09.9	245	46.42	5.50	33.30	05.50	31.20	3.29	0.55	6.06
11	15.10	08.22	220	45.01	5.70	44.30	07.10	48.70	3.64	0.58	4.00
12	15.33	08.78	200	44.50	5.40	20.90	10.60	68.50	1.83	0.93	6.02
13	15.30	13.04	208	43.84	5.01	35.00	01.50	63.50	4.57	0.19	8.28
14	16.10	10.74	303	47.99	5.65	27.50	01.80	70.70	2.96	0.19	7.59
15	12.90	08.66	357	37.79	4.55	26.40	06.60	67.00	2.29	0.57	5.80
16	14.40	10.13	289	42.80	5.40	25.80	10.70	63.40	2.62	1.09	6.42
17	15.70	04.12	315	47.19	5.46	41.90	11.90	46.30	1.73	0.49	1.91
18	14.50	05.25	172	42.73	4.52	39.00	13.20	47.70	2.05	0.70	2.51
19	15.00	06.14	165	45.17	5.80	37.20	10.40	52.50	2.28	0.64	3.22
20	14.60	07.76	281	43.92	5.29	41.00	07.40	51.50	3.18	0.58	4.00
21	13.50	08.17	368	39.17	4.31	28.20	07.60	64.10	2.31	0.62	5.24
22	14.20	07.46	225	42.08	5.05	28.40	10.00	61.60	2.12	0.74	4.59
23	16.20	08.57	283	48.32	5.61	29.80	06.90	63.40	2.55	0.59	5.43
24	15.30	08.12	249	42.26	5.07	32.80	03.70	63.50	2.66	0.30	5.16
25	17.40	09.84	318	52.42	6.27	15.50	07.50	77.00	1.53	0.74	7.57
26	15.80	08.56	236	47.88	5.81	44.50	05.80	49.70	3.81	0.50	4.25
27	15.20	07.80	196	45.50	5.45	30.00	16.10	54.00	2.34	1.25	4.21
28	15.60	08.91	226	46.32	5.94	26.40	16.60	57.10	2.35	1.48	5.09
29	13.80	07.39	293	39.90	3.61	41.80	13.70	44.40	3.09	1.01	3.29
30	14.21	12.14	277	42.42	5.29	28.10	06.20	65.80	3.40	0.75	7.97
31	13.42	8.42	166	40.01	6.76	47.50	08.00	44.50	4.00	0.68	3.76
32	14.31	7.81	177	44.12	5.69	28.00	06.61	65.40	2.20	0.52	5.15
33	16.61	10.22	321	49.28	6.95	36.90	08.03	55.20	3.76	0.81	5.63

 Table 3. Complete Blood Count (CBC) Parameters of All Individuals (n=50).

34	15.22	10.72	248	29.61	5.41	36.00	05.91	58.00	3.86	0.63	6.22
35	16.52	07.29	365	49.85	6.20	30.20	04.21	65.60	2.20	0.30	4.78
36	12.43	05.14	224	37.73	4.44	24.90	06.32	68.80	1.28	0.33	3.54
37	15.43	11.33	358	45.43	5.23	40.20	06.83	53.00	4.55	0.77	5.99
38	15.01	06.18	166	45.17	5.86	37.20	10.40	52.50	2.28	0.64	3.22
39	14.62	07.76	288	27.61	5.29	42.00	07.41	51.50	3.18	0.58	4.00
40	13.52	08.17	366	39.17	4.31	28.20	07.60	64.10	2.31	0.62	5.24
41	14.29	07.44	228	42.08	5.05	28.40	10.01	61.60	2.12	0.74	4.59
42	16.28	08.55	287	48.39	5.69	29.80	06.90	63.40	2.55	0.59	5.43
43	15.35	08.13	243	41.26	5.07	32.80	03.71	63.50	2.66	0.30	5.16
44	17.43	09.88	318	54.42	6.22	15.60	07.50	77.00	1.53	0.74	7.57
45	15.88	08.54	236	47.88	6.81	44.60	05.82	49.70	3.81	0.50	4.25
46	15.22	07.84	197	45.50	5.46	30.00	16.10	54.00	2.34	1.25	4.21
47	15.69	08.93	229	49.32	5.95	26.40	16.62	57.10	2.35	1.48	5.09
48	13.54	08.14	367	33.17	4.31	28.22	07.60	64.10	2.31	0.62	5.24
49	14.24	07.45	224	42.08	5.08	28.41	10.51	61.60	2.12	0.74	4.59
50	16.26	08.58	289	48.39	5.69	29.80	06.91	63.40	2.55	0.59	5.43

Key- HGB: Hemoglobin, WBC: White Blood Cells, PLT: Platelets, HCT: Hematocrit, RBC: Red Blood Cells, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, LY: Lymphocytes, MO: Monocytes, GR: Granulocytes

Mean, Maximum/Minimum and Deranged Values of CBC Parameters:

The normal range of each CBC parameter is mentioned in **Table 3.** Only one CBC parameter Monocytes' (MO) mean value (8.062 %) was found deranged from the normal range. Mean value of hemoglobin (HGB) was 14.882 g/dl for 49 normal range values; mean value of white blood cells (WBC) was $8.545 \ 10^{9}/1$ for 48 normal range values, mean value of platelets (PLT) was 264.680 $10^{9}/1$ for 50 normal range values, mean value of hematocrit (HCT) was 43.572 % for 45 normal range values, mean value of RBC was $5.436 \ 10^{12}/1$ for 31 normal range values, mean value of lymphocytes (LY) was 32.532 % for 37 normal range values, mean value of granulocytes (GR) was 58.852 % for 39 normal range values, mean value of absolute lymphocytes count (Ab. LY) was $2.767 \ 10^{9}/1$ for 45 normal range values, mean value of absolute monocytes count (Ab. MO) was $0.665 \ 10^{9}/1$ for 34 normal range values and mean value of absolute granulocytes count (Ab. GR) was $5.111 \ 10^{9}/1$ for 43 normal range values (**Table 3**).

Maximum value of hemoglobin (HGB) was 17.43 g/dl; maximum value of white blood cells (WBC) was 13.04 10^{9} /l, maximum value of platelets (PLT) was 385.00 10^{9} /l, maximum value of hematocrit (HCT) was 54.42 %, maximum value of RBC was 6.95 10^{12} /l, maximum value of lymphocytes (LY) was 47.50 %, maximum value of monocytes (MO) was 16.62 %, maximum value of granulocytes (GR) was 77.00 %, maximum value of absolute lymphocytes count (Ab. LY) was 4.57 10^{9} /l, maximum value of absolute monocytes count (Ab. LY) was 4.57 10^{9} /l, maximum value of absolute monocytes (CR) was 8.28 10^{9} /l (Table 3).

Minimum value of hemoglobin (HGB) was 9.30 g/dl; minimum value of white blood cells (WBC) was 4.12 10^{9} /l, minimum value of platelets (PLT) was 165.00 10^{9} /l, minimum value of hematocrit (HCT) was 27.91 %, minimum value of RBC was 3.61 10^{12} /l, minimum value 15.50 %, minimum value of monocytes (MO) was 1.50 %, minimum value of granulocytes (GR) was 31.20 %, minimum value of absolute lymphocytes count (Ab. LY) was 1.28 10^{9} /l, minimum value of absolute monocytes count (Ab. MO) was 0.19 10^{9} /l and minimum value of absolute granulocytes count (Ab. GR) was 1.91 10^{9} /l (Table 3).

Results of Survey for: Environmental Factors, Water Intake Source and Clinical History:

The most popular home water filter in Pakistan is a 'Triple Water Filters" which is based on three staged system (**Figure 2**). First stage compartment of water filter uses Polypropylene yarn cartridge which removes suspended particles up to 5 microns (e.g., sand, rust, silt etc.). The second stage compartment uses coconut base activated carbon filter which removes organic chemicals, pesticides, herbicides and chlorine to improve the color and taste of water. The third and last stage includes an ultraviolet disinfection to kill microorganisms with germicidal lamp which emits '>30,000 micro-watt seconds per square centimeter of UV energy'. **Table 4** summarizes the results of the survey conducted from 286 houses in Lahore of eleven mentioned regions. Most of the residents (33.57%) were taking filtered water as drinking water. The second most (28.32 %) drinking water source was the tap-water from city water supplies. The third most (26.22%) source of drinking water was from the mineral water (i.e., bottled brands) available from local markets in sealed bottles. The other sources were as follows: bore-water systems in houses (8.39%) and tube-well/hand pumps (3.5%). We found that the trend of water boiling water is declining, as now people have option to buy bottled mineral water.

According to survey of waterborne infectious and viral diseases reported in past 3 years in 286 families: the prevalence of diarrhea was highest (27.62%), second highest (20.98%) disease reported was cholera, third highest (19.93%) disease reported was typhoid, fourth highest (15.73%) disease reported was dysentery and fifth highest disease was reported influenza (12.58%). Other prevalence of reported diseases were as follows: Malaria (11.54%), Hepatitis (10.49%), Dental Caries (10.13%), Gastric Ulcer (5.24%), Pneumonia (2.79%), Yellow Fever (2.79%), Measles (2.45%), Dengue Fever (1.4%), Yellow Fever (1.4%), Tuberculosis (1.4%) and Dermatitis (1%). The cancer was reported only 6.3% among 286 families. Heart disease was reported only 15.38% and hypertension was reported 45.1%.



Fig.2: Home Installed Triple Water Filter .

(Aqua Water Technologies. 2012; http://startraders.biz/triple-water-filter)

Associated with environmental pollution exposure, in most of the residential locations, the poor conditions of roads and street were 46.50%. The air pollution was the second major (38.46%) cause of poor environment and the municipal fall-out was the third most (8.4%) cause of contaminated environment. Other exposures were from industrial areas (5.24%), agricultural sites (4.9%) and from animal manure (2.79%).

Results of Shapiro-Wilk (W) Statistics for Physical-Chemical Parameters of Tap-Water:

Table 5 shows the results of Shapiro-Wilk statistics. The Kurtosis values of pH, EC, DO, TDS, Total Hardness, Sodium, Potassium, Turbidity, Total Coliform and Fecal Coliform are as follows: -0.136, 0.238, -0.348, 2.433, -0.698, 0.209, 0.916, -1.366, 5.702 and 5.002 respectively. The Shapiro-Wilk (W) values of pH, EC, DO, TDS, Total Hardness, Sodium, Potassium, Turbidity, Total Coliform and Fecal Coliform are as follows: 0.983697, 0.909154, 0.982247, 0.901482, 0.962630, 0.868872, 0.905804, 0.863155, 0.664799, 0.685899 respectively. Only two parameters pH and DO were found with normalized distribution and rest of the parameters were found with non-normalized distribution, decided on the p-values.

Results of Mann Whitney U Test to Compare Deranged (high) Water Parameters:

For pH, Turbidity and Total Hardness, there were all normal values with following mean values 7.48, 1.611 NTU, 230.66 ppm respectively (**Table 6**). The parameter TDS has 42 high values (mean 645.333), parameter EC has 89 high values (mean 76.72), parameter DO has 30 high values (mean 7.39), parameter Sodium has 2 high values (mean 209.5), parameter Potassium has 41 high values (mean 20.731), parameter Total Coliform has 50 high values (mean 8.58) and parameter Fecal Coliform has 54 high values (mean 8.13) (**Table 6**). The parameter DO has 32 low (27.58%) values (mean 2.953) (**Table 6**).

Overall, the highest number (76.72%) of high EC values was found among all water parameters. Second highest number (~43%) was with high values of both Total Coliform and Fecal Coliform, third highest (36.20%) high values was found with TDS, fourth highest (35.34%) with high values of Potassium and fifth highest (27.58%) was with low values of the DO and sixth highest (25.86%) was with high values of DO (**Table 6**). All deranged differences were found statistically significant at either p<0.0001 or p<0.00001.

The Mann Whitney U test was applied to those water parameters which were found with non-normalized distribution with deranged high values only. The U value for TDS was 40, U value for Potassium was 3, U value for *Total Coliform* was 32.5 and U value for *Fecal Coliform* was 0 (**Table 6**). The test was not applicable on parameter Sodium due to small sample size of deranged high values. All these parameters' (TDS, Potassium, Total Coliform and Fecal Coliform) deranged high values were at significant difference (p value<0.00001) when compared to normal values.

Parameters	Categories	Number	PP %
	Filtered Water	96	33.57
	Tap Water	81	28.32
Water-Intake	Mineral Water	75	26.22
Source	Bore Water	24	8.39
	Tube-Well/ Hand-Pump	10	3.5
	Diarrhea	79	27.62
	Cholera	60	20.98
	Typhoid	57	19.93
	Dysentery	45	15.73
	Influenza	36	12.58
	Malaria	33	11.54
	Hepatitis	30	10.49
	Dental Caries	29	10.13
Infectious/Viral Disease	Gastric Ulcer	15	5.24
	Pneumonia	8	2.79
	Yellow fever	8	2.79
	Measles	7	2.45
	Dengue Fever	4	1.4
	Swine Flu	4	1.4
	Tuberculosis	4	1.4
	Dermatitis	3	1.05
	Roads/Street Conditions	133	46.50
	Air Pollution Exposure	110	38.46
Environmental Pollution	Municipal Fall Out	24	8.4
	Industrial/Factory Area	15	5.24
	Agricultural Exposure	14	4.9
	Animal Manure	8	2.79
Cancer	Yes	18	6.3
	No	268	93.71
Heart Disease	Yes	44	15.38
	No	242	84.62
Hypertension	Yes	129	45.1
	No	157	54.9

Table 4 Survey	Analysis-Percentage	Prevalence of Each	Parameter's	Category (n-286)
Table 4. Sulvey	Analysis-1 el centage	I revalence of Each	I al ameter 5	Category $(n-200)$.

Sr. No.	Water Parameter	Kurtosis	Shapiro-Wilk Statistics (W)	Shapiro-Wilk Statistics p-value	Normalized/Non Normalized
1	pН	-0.136	0.983697	0.172>0.050	Normalized
2	EC (µS/cm)	0.238	0.909154	0.000001<0.050	Non Normalized
3	DO (ppm)	-0.348	0.982247	0.128>0.050	Normalized
4	TDS (ppm)	2.433	0.901482	0.000000<0.050	Non Normalized
5	Total Hardness (ppm)	-0.698	0.962630	0.0025<0.050	Non Normalized
6	Sodium (ppm)	0.209	0.868872	0.000000<0.050	Non Normalized
7	Potassium (ppm)	0.916	0.905804	0.000001<0.050	Non Normalized
8	Turbidity (NTU)	-1.366	0.863155	0.000000<0.050	Non Normalized
9	Total Coliform (MPN)	5.702	0.664799	0.000028<0.050	Non Normalized
10	Fecal Coliform (MPN)	5.002	0.685899	0.000002<0.050	Non Normalized

Table 5. Shapiro-Wilk Statistics (W) Test Results (*n=116*) for Water Parameters.

Water Parameter	Total Normal Values with Mean	Total High Deranged Values with Mean	Total Low Deranged Values	U-Value	p-value
TDS (ppm)	74 (mean: 420.445); 63.79%	42 (mean: 645.333); 36.20%	-	40	<0.00001†
Total Hardness (ppm)	116 (mean: 230.66); 100%	-	-	Test not applicable	-
Sodium (ppm)	114 (mean: 71.403); 98.27%	2 (mean: 209.5); 1.72%	-	Test not applicable *	-
Potassium (ppm)	75 (mean:6.1); 64.65	41 (mean: 20.731); 35.34%	-	3	<0.00001†
Turbidity (NTU)	116 (mean: 1.611); 100%	-	-	Test not applicable	-
Total Coliform (MPN)	66 (mean: 0); 56.89%	50 (mean:8.58); 43.10 %	-	32.5	<0.00001†
Fecal Coliform (MPN)	65 (mean: 0); 56.03%	51 (mean: 8.13); 43.96%	-	0	<0.00001†

*due to small sample size

† Highly significant

.

Results of t-Test (Independent Samples) to Compare Deranged (low/high) Water Parameters:

The t-test was applied to those water parameters which were found with the normalized distribution with deranged low/high values only (**Table 7**). The parameter EC's high values were found at significant difference with p value<0.00001 (t:7.2353;df:114), when compared to normal values. The parameter DO's high values were found at significant difference with p value<0.00001 (t:15.8333;df:82), when compared to normal values. The parameter DO's low values were found at significant difference with p value<0.00001 (t:15.2823;df:84), when compared to normal values (**Table 7**).

Water Parameter	Total Normal Values with Mean	Total High Deranged Values with Mean	Total Low Deranged Values with Mean	t	df	95% CI	p-value (two- tailed)
рН	116 (mean: 7.48); 100%	-	-		Test no	t applicable	
EC (µS/cm)	27 (mean: 438.814); 23.27%	89 (mean: 752.404); 76.72%	-	7.2353	114	227.73 to 399.45	<0.0001†
					For high va	lue comparison	
DO (ppm)	54 (mean:	30 (mean:	32 (mean:	15.8333	82	-2.708 to - 2.104	<0.0001 †
	4.99);	7.39);	2.953);	For low value comparison			
	46.55%	25.86%	27.58%	15.2823	84	1.772 to 2.303	<0.0001†

 Table 7. t -Test (Independent Samples) Results For Normalized Distributed Water Parameters.

Results of Shapiro-Wilk (W) Statistics for CBC Parameters:

Table 8 shows the results of the Shapiro-Wilk statistics. The Kurtosis values of HGB, WBC, PLT, HCT, RBC, LY, MO, GR, Ab. LY, Ab. MO and Ab. GR are as follows: 2.938, 0.108, -1.132, 0.902, 0.097, 4.066, 6.601, -0.407, 0.548, 0.298, -0.75, 1.309 and -0.128 respectively. The Shapiro-Wilk (W) values of HGB, WBC, PLT, HCT, RBC, LY, MO, GR, Ab. LY, Ab. MO and Ab. GR are as follows: 0.926071, 0.964522, 0.946681, 0.938611, 0.975955, 0.817845, 0.840763, 0.955806, 0.902216, 0.960478, 0.937833, 0.901586 and 0.971904. Following parameters were found with normalized distributions: HGB, PLT, HCT, MO, Ab. LY and Ab. MO. Following parameters were found with non-normalized distributions: WBC, RBC, LY, GR and Ab. GR.

Table 8. Shapiro-Wilk Statistics (W) Test Results (n=50) For CBC Parameters.

Sr. No.	CBC	Kurtosis	Shapiro-Wilk	Shapiro-Wilk	Normalized/Non
	Parameter		Statistics (W)	Statistics p-value	Normalized
1	HGB	2.938	0.926071	0.0039<0.050	Non Normalized
2	WBC	0.108	0.964522	0.137>0.050	Normalized
3	PLT	-1.132	0.946681	0.024<0.050	Non Normalized
4	HCT	0.902	0.938611	0.011<0.050	Non Normalized
5	RBC	0.097	0.975955	0.396>0.050	Normalized
6	LY	-0.407	0.955806	0.059>0.050	Normalized
7	MO	0.548	0.902216	0.0005<0.050	Non Normalized
8	GR	0.298	0.960478	0.093>0.050	Normalized
9	Ab. LY	-0.75	0.937833	0.011<0.050	Non Normalized
10	Ab. MO	1.309	0.901586	0.005<0.050	Non Normalized
11	Ab. GR	-0.128	0.971904	0.97>0.050	Normalized

Results of Mann Whitney U Test to Compare Deranged (low/high) CBC Parameters:

There were no deranged high values of HGB and PLT (**Table 9**). The parameter HCT had 2 values high (mean 53.42), MO has 27 (mean 10.217) values high, Ab. LY has 3 values high (mean 4.556 10⁹/l), Ab. MO has 16 values high (mean 0.948 10⁹/l), WBC has 5 values high (mean 11.988 10⁹/l), RBC has 19 values high (mean 6.08310¹¹/l), LY has 11 values high (mean 43.218%), GR has 2 values high (mean 4.556) and Ab. GR has 6 values high (mean 4.825 10⁹/l) (**Table 9**). Highest number (54%) of MO (monocytes) values were found high among all CBC parameters. (**Table 9**). Second highest (38%) high values were found with RBC and the third highest (32%) high values were found with Ab. MO.

There were no deranged low values of PLT, WBC, RBC and Ab. MO (**Table 9**). The parameter HGB has only 1 value low (09.30), parameter HCT has 3 values low (mean 29.276 %), parameter MO has 4 values low (mean 10.70 %), Ab. LY has 2 values low (mean 1.28 10⁹/l), parameter LY has 2 values low (mean 15.55 %), parameter GR has 9 values low (mean 45.188) and parameter Ab. GR has only 1 value low (1.91) (**Table 9**).

The highest number (54%) of high MO values were found low among all CBC parameters. Second highest (18%) low values were found with GR and third highest (6%) high values were found with HCT (**Table 9**).

Overall, the highest number of high MO (54%) values were found high among all CBC parameters. Second highest high values (38%) was found with RBC, third highest high values (32%) were found with Ab. MO, fourth highest high values (22%) were found with LY and fifth highest low values (18%) were found with GR (**Table 9**). All deranged differences were found statistically significant at either p<0.0001 or p<0.00001.

The Mann Whitney U test was applied to those CBC parameters which were found with non-normalized distribution with deranged (low/high) values only. The U value for MO was 0 for high values and U value for Ab. MO values were 12 for high values. The test was not applicable to the following parameters: HGB and low values of MO, due to small deranged sample size (**Table 9**). All these parameters' (high values of MO and Ab. MO) values were at significant difference (p value<0.00001) when compared to normal values (**Table 9**).

CBC Parameter	Total Normal Values with Mean	Total High Deranged Values with Mean	Total Low Deranged Values with Mean	U-Value	p-value
HGB (g/dl)	49 (mean: 14.875)	-	1 (15.33); 2%	Test not appli	cable*
PLT (10 ⁹ /l)	50 (mean: 264.68)	-	-	Test not appl	icable
HCT (%)	45 (mean: 44.088)	2 (mean: 53.42); 4%	3 (mean: 29.276); 6%	Test not applicable* and low va	0
MO (%)	39 (mean: 6.132)	27 (mean: 10.217); 54%	4 (mean: 10.71); 8%	0 Test not applicabl values	<0.00001† e* for low
Ab. LY (10 ⁹ /l)	45 (mean: 2.714)	3 (mean: 4.556); 6%	2 (mean: 1.28); 4%	Test not applicable* for both high and low values	
Ab. MO (10 ⁹ /l)	34 (mean: 0.532)	16 (mean: 0.948); 32%	-	12	<0.00001†

Table 9. Mann Whitney U Test Results for Non-Normalized Distributed CBC Parameters .

*due to small sample size

† Highly significant

Results of t-Test (Independent Samples) to Compare Deranged (low/high) CBC Parameters:

The t-test was applied to those CBC parameters which were found with the normalized distribution with deranged low/high values only. The parameter WBC's high values were found at significant difference with p value<0.00001 (t:5.59;df:48), when compared to normal values. The parameter PLT's high values were found at significant difference with p value<0.00001 (t:-4.60;df:48), when compared to normal values. The parameters LY's high values were found at significant difference with p value<0.00001 (t:-9.64;df:46), when compared to normal values. The parameters LY's high values were found at significant difference with p value<0.00001 (t:-70;df:46), when compared to normal values. The parameter GR's low values were found at significant difference with p value<0.00001 (t:-7.70;df:46), when compared to normal values. The parameter Ab. GR's high values were found at significant difference with p value<0.00001 (t:-7.60;df:46), when compared to normal values. The test was not applicable to low values of LY, high values of GR and low values of Ab. GR (**Table 10**).

Discussion

The current study was aimed to evaluate the extent of tap-water quality from city line (tap-water) and assessment of different waterborne diseases, drinking water source, type of pollution and incidence of cancer from a comprehensive survey analysis from residents of EME DHA, Johar Town, Faisal Town, Township, Allama Iqbal Town, Model Town, Wapda Town, Canal View Society, Gulberg III, Mughal-Pura and Gulshan Ravi was conducted. The home tap-water sampling was done from the following eleven regions of Lahore: EME DHA, Johar Town, Faisal Town, Township, Allama Iqbal Town, Model Town, Faisal Town, Township, Allama Iqbal Town, Model Town, Faisal Town, Canal View Society, Gulberg III, Mughal-Pura and Gulshan Ravi. Overall, the highest number (76.72%) of high EC values was found among all water parameters. Second highest number (~43%) was with high values of both Total Coliform and Fecal Coliform, third highest (36.20%) high values was found with TDS, fourth highest (35.34%) with high values of Potassium and fifth highest (27.58%) was with low values of the DO and sixth highest

(25.86%) was with high values of DO (**Table 6**). All these deranged differences were found statistically significant at either p<0.0001 or p<0.00001 as compared to normal values. In all water samples, the three parameters pH, Total Hardness and Turbidity were found within normal range in all mentioned regions' tap-waters. Sodium levels were only 1.72% deranged. Overall, the mean values were deranged in the following regions: Iqbal Town (EC & Sodium), Mughalpura (DO, TDS & Potassium), Faisal Town (Total Coliform) and Gulshan Ravi (Fecal Coliform). Most significant deranged mean values were found in EC values, TDS values, Total Coliform values and Fecal Coliform values in tap-water samples of the following regions: Iqbal Town, Mughalpura, Faisal Town and Gulshan Ravi respectively. Most significant deranged maximum value was found in EC, TDS, Potassium, Total Coliform values and Fecal Coliform in following regions: Model Town, Iqbal Town, Mughalpura and Gulshan Ravi respectively.

CBC Parameter	Total Normal Values with Mean	Total High Deranged Values with Mean	Total Low Deranged Values with Mean	t	df	95% CI	p-value (two- tailed)
WBC (10 ⁹ /l)	48 (mean: 8.412)	5; mean (11.988); 10%	-	5.59	48	4.728 to 8.598; 10.68 to 13.29	<0.0001†
RBC (10 ¹² /l)	31 (mean: 5.0396)	19 (mean: 6.083); 38%	-	-7.60	48	4.870 to 5.210; 5.866 to 6.301	<0.0001†
LY (%)	37 (mean: 30.273)	11 (mean: 43.218); 22%	2 (mean: 15.55); 4%	-9.64	46	28.98 to 31.57; 40.84 to 45.59	<0.0001†
GR	39 (mean:	2 (mean:	9 (mean:	Test not applicable* for low values Test not applicable* for high values			
(%)	61.074)	4.556); 4%	45.188); 18%	7.70	46	59.28 to 62.87; 41.45 to 48.93	<0.0001†
Ab. GR (10 ⁹ /l)	43 (mean: 4.807)	6 (mean: 7.825); 12%	1 (1.91); 2%	-7.60	47	4.528 to 5.087; 7.007 to 8.573	<0.0001†
				Te	est not a	pplicable* for low va	alues

Table 10. t - Test (Independent Samples) Results For Normalized Distributed CBC Parameters.

*due to small sample size

† Highly significant

In our research, measurement of Turbidity showed that there were all normal (<5 NTU) within range values of it in all tap-water samples of all regions. However, maximum (4.30 NTU) but within ranged value was reported from the tap-water samples of Johar Town. Sometimes, there may some geographical reasons are behind for such deterioration of water (Malana and Khosa, 2011). After snow melt and precipitation, the resulting floods in hilly areas can sometimes change turbidity of water (Kistemann et al., 2002). Another groundwater quality monitoring was done by Hagras in 2013 across Punjab. Hagras (2013) reported an overall good physical quality of groundwater, but in few samples, there were presence of colors because of high levels of dissolved substances and high levels of turbidity. The higher levels of turbidity (>5 NTU) were found among the waters of Guirat, Rawalpindi and Bahawalpur. A water quality monitoring was done in different cities of Pakistan by Pakistan Council of Research in Water Resource. Water samples of all provinces were found contaminated from microbes and bacteria, including high levels of turbidity, nitrates and arsenic (Soomro et al., 2011). In current research, DO's (dissolved oxygen) mean value was deranged slightly high (6.271 ppm) from normal value (6 ppm) in tap-water samples of Mughalpura region. Whereas, the maximum value (9 ppm) of DO was reported from tap-water samples of EME DHA and Johar Town, whereas, minimum value (0.50 ppm) was reported from tap-water samples of Gulberg III. In current research, a 1% dermatitis were reported from a survey of 286 houses of Lahore. In current research, the measurement of Total Hardness showed that there were all normal (500 ppm) within range values of it in all tap-water samples of all regions. However, maximum but within the ranged value of Total Hardness (476 ppm) was reported from tap-water samples of Gulshan Ravi and minimum value (48 ppm) was reported in tap-water samples of EME DHA. In current research, the mean value of TDS among all regions, the mean (593.444 ppm) of TDS was found high in the tap water of Iqbal Town region. The maximum value of TDS (1146.00 ppm) was reported from Mughalpura region among all other regions. Water with the lowest levels of TDS concentration may also not accepted because it can cause it flat with insipid taste. The elements or compounds as TDS in water, can affect encrustation or corrosion in water distribution networks and systems (Sawyer and McCarty, 1967; Edwards, 2004). It is known that high TDS levels of greater than 500 ppm can cause significant scaling of boilers, pipes, geysers, steam irons and kettles (Tihansky, 1974). Further, scaling effect can decrease the service life of common household appliances

(McQuillan and Spenst 1976). Russian epidemiological studies had reported that varied levels of TDS in drinking water can be found with mineral deficiencies and as a result, there may be risks of goiter, gastric and duodenal ulcers, chronic gastritis, hypertension, coronary heart disease and several diseases in infants such as anemia, jaundice and even growth disorders (Mudryi 1999; WHO, 2005). In current research the hypertension was reported 45.1% and gastric ulcer was reported 5.4%. Lutai in 1992 conducted a large cohort epidemiological research in Russian adults, children and pregnant women, as they were taking water with low mineral contents. Later on, the higher incidences of hypertension, ischemic heart disease, chronic gastritis, gastric and duodenal ulcers, goiter and nephritis were reported. Moreover, children were found with inactive physical development with growth abnormalities. The pregnant women were suffering from anemia and edema and their newborns showed increased morbidity. The least morbidity was linked with water having, magnesium levels of 17-35 mg/L, calcium levels of 30-90 mg/L and TDS of about 400 mg/L (for bicarbonate waters). In current research according to a survey of 286 families, the cancer was reported 6.3%. Craun and Mccabe (1975) reported that elevated levels of TDS in drinking water can cause coronary heart disease, cardiovascular disease, arteriosclerotic heart disease and cancer. In current research, among all water samples (of all mentioned regions), the most deranged parameter was EC (electrical conductivity) as its mean value 679.413 µS/cm was found high, with a maximum value (1412.00 µS/cm) was reported from Model Town. Whereas, it should be equal to lesser than 400 µS/cm. According to the survey of Punjab groundwater quality in 2013, however, the EC value in eleven cities of Pakistan (Islamabad, Rawalpindi, Gujrat, Lahore, Sialkot, Shiekhupura, Gujranwala, Faisalabad, Kasur, Bahawalpur and Multan) ranged from 170-7930 µS/cm (Hagras 2013). According to another survey of Bahawalpur, Pakistan, the values of TDS, EC, Hardness and pH were found high as compared to WHO allowed limit. This region considerably been affected from waterborne diseases such as diarrhea, cholera (Mohsin et al., 2013). The levels of Total Dissolved Solids and Electrical Conductivity in drinking water were extremely elevated as researched by Malana and Khosa (2011). A study was conducted to monitor the physico-chemical parameters in drinking water samples collected from different urban areas of Faisalabad. The results showed that pH, P-alkalinity, calcium and sulfates were within allowable limits, whereas conductivity, total dissolved solids, m-alkalinity, total hardness, sodium, potassium and chloride concentrations of most of samples were above the permissible and safe limits (Saeed et al., 2012). There were no deranged mean value of Sodium in any of tap-water samples among all regions. However, the maximum value (211.00 ppm) was reported from the tap-water samples of Gulberg III and minimum value (15 ppm) from the tap-water samples of Township region. The existence of saline groundwater (Ahmad, 1993; Ahmad, 1974) in the south of Lahore is a substantial threat to the aquifer waters. There is a danger of deterioration of the aquifer water quality if the saline water finds a way to enter into the city area. The washing of such saline water, if occurs, then it could be troublesome mitigating it. In current research, the mean value of Potassium was deranged (18 ppm) from the normal value (12 ppm) was found in the tap-water samples of Mughalpura with a maximum value of 40 ppm. Whereas, the minimum value (0.50 ppm) was reported in the tap-water samples of Canal View. Increased potassium levels in drinking water can pose significant health impacts in patients with heart and kidney diseases, coronary artery disease, diabetes, hypertension, adrenal insufficiencies etc. (WHO, 2009). An excess of Potassium in drinking water can lead to dehydration (Radojevic and Bashkin, 1999).

In current research, the high mean (6.826 MPN) value of Total Coliform measurement was reported from the tap-water samples of Faisal Town. Whereas, the maximum value (22 MPN) of Total Coliform was reported in tap-water samples of Gulshan Ravi. Very fewer tap-water samples were having 0 MPN values of Total Coliform. The high mean (9 MPN) value of Fecal Coliform measurement was reported from the tap-water samples of Gulshan Ravi, with a maximum value (25 MPN). The maximum value of Fecal Coliform was also found in the tap-water samples of Gulberg III. Very fewer tap-water samples were having 0 MPN values of Fecal Coliform. According to the WHO amount of total and fecal Coliforms in drinking water should be zero (Pakistan Environmental Protection Agency, 2008). A groundwater quality monitoring was done by Hagras in 2013 across Punjab found that all water samples were contaminated with bacteria. A water quality monitoring was done in Lahore near River Ravi to study the prevalence of microbial contamination in drinking water associated with the waterborne diseases. Several significant diseases were found including malaria, jaundice, diarrhea, eye and skin problems, especially in monsoon and summer seasons, because 92% of the water was not safe to drink. According to the survey in the region near Ravi, 82% of people were not treating their home drinking water by either boiling or through filtration (Qureshi et al., 2011). Jabeen et al., (2011) have also reported health impacts of unsafe polluted water in rural and urban areas, they observed following ailments, gastric problems (53%) allergies (33%), diarrhea (27%), skin infection (23%), typhoid (20%) and hepatitis (13%). According to our survey of 286 residents in current research, the most common source form of drinking water was from home-installed triple water filter. We found that the trend of water boiling water is found to decline as now people have opted to buy bottled mineral water. The next common source was the intake of direct tap-water. Use of mineral water (sealed bottles) was also found common, but lesser than filtered water or direct tap-water. Regarding survey of infectious waterborne and viral diseases reported in past 3 years; the prevalence of diarrhea was found highest among all diseases. Next following were also found most commonly

among citizens, i.e., cholera, typhoid, dysentery and influenza. Some prevalence of following reported diseases were as follows: Malaria, Hepatitis, Dental Caries, Gastric Ulcer, Pneumonia, Yellow Fever, Measles, Dengue Fever, Yellow Fever, Tuberculosis and Dermatitis. Cancer was reported only 6.3% among 286 families. Heart disease was reported only 15.38% and hypertension was reported 45.1%. Poor conditions of roads and street were found associated with environmental pollution exposure in most of the residential locations. Air pollution was the second major cause of poor environmental conditions, the municipal fall-out was the third most causes of contaminated environmental condition. Other exposures were from industrial areas, agricultural sites and from animal manure. Ahmad *et al.* (2012) conducted a study to affirm the intrusion of sewage water into the ground water of Lahore city. Water samples of Lahore Canal were tested and it was found that "all the injector pumps of shallow depth are polluted due to sewage water intrusion". However, deep tube wells were somehow found free from contaminants (Ahmad *et al.*, 2012).

A water quality survey was conducted by Yasar *et al.* (2011) in Gangapur, Lahore and samples were collected from tube wells, hand pumps and motor pumps. In this region, waterborne diseases were common as the drinking water was being polluted with *Fecal Coliform*. Further, improper water supply system, poor sanitation and drainage were found (Yasar *et al.*, 2011). Another survey was held by 'Pakistan Council of Research in Water Resources' (PCRWR), and it found that more than eighty one thousand cases of waterborne diseases were reported in BHU (Basic Health Units) in Rawalpindi, Pakistan (Tahir and Bhatti, 1994). In recent, contamination of nitrate in drinking water sources has also been reported in Pakistan, which has been documented as one of the main quality issues in Pakistan. The highest concentration of contaminants up to 23% was reported in water samples collected from Baluchistan and Punjab (Hussain *et al.*, 2002). A drinking water quality monitoring of water purification systems was conducted in Karachi, Pakistan. According to the survey, 66% of people were using different home water purification methods and 58% were using boiled water. Only 16% water samples were found free from *Coliforms* (Luby *et al.*, 2000).

There is another concern that many water purification and filtration systems may also remove essential minerals and ions which are important to human health. Many epidemiological studies around the globe has found that the soft water intake with low minerals like calcium and magnesium contents is linked with increased mortality and morbidity in patients with cardiovascular disease (Donato et al., 2003; Nardi et al., 2003) including risks of bone fractures (Verd Vallespir et al., 1992), pre-term birth and low birth weight (Yang et al., 2002), certain neurodegenerative diseases (Jacqmin et al., 1994), higher risks of motor neuronal disease (Iwami et al., 1994), pregnancy disorders (preeclampsia) (Melles and Kiss 1992) and some types of cancer as well (Yang et al., 1997; Yang et al., 1998; Yang et al., et al., 2000). According to our in the current research survey of 286 residents, the most source form of drinking water was from home-installed triple water filter. The filtration system has been described in 'Results' section. This system, though, claim a good water filtration, but since there is no confident surety for a safer drinking means. Although, the use of UV (ultraviolet) wavelengths is an effective mean of killing harmful bacteria in the water, but there are some disadvantages associated with it. The water which is subject to pass over the UV wavelengths, must not be having with high values of turbidity as the cloudiness present in the water can produce hindrance of UV light absorption and thereby reducing its ability to destroy microorganisms. Another significant disadvantage is that a home base UV water purification system does not incorporate residual treatment, thereby again, there will be a greater chance of bacterial growth. An incidence of bladder cancer in Washington County (Freedman et al., 1997) was found in association with duration of residence with chlorinated surface water. Several other studies have reported colon (Young et al., 1984), kidney, pancreas, brain (Cantor et al., 1996) and liver (Wilkins et al., 1981) cancers as a result of exposure to chlorinated by-products. An incidence of childhood leukemia was suspected from contaminated drinking water in Woburn, Massachusetts (US) (Byers et al., 1988).

The complete blood count (CBC) test was conducted in order to assess the primary status of immune response among some of the residents which were randomly selected from each mentioned regions. An altered immune response and intake of contaminated and poor quality can induce infectious or viral diseases. In CBC (complete blood count) test, the mean values of all these parameters (Hemoglobin, WBC, Platelets, Hematocrit, RBC, Lymphocytes, Granulocytes, Ab. Lymphocytes, Ab. Monocytes and Ab. Granulocytes) were found normal in all 50 blood samples except with the Monocytes (mean: 8.062 %) with a maximum value reported as 16.62 %. Overall, the highest number (54%) of high monocytes levels were found high among all CBC parameters. Secondly, (38%) high levels were found in RBCs, third highest (32%) high levels were found with absolute monocytes count, fourth highest (22%) high values were found with lymphocytes and fifth highest (18%) low values were found with granulocytes (Table 9). All these deranged differences were found statistically significant at either p<0.0001 or p<0.0001 as compared to normal values. It is known that the hematopoietic system with major system includes "bone-marrow", with its functional cells, carry oxygen in the blood, mitigate with infections with immune response, ensures coagulation of blood and keeps intact the blood vessels (Smirnova, 2010; Shin et al., 2010; Fliedner et al., 2012). Myelopoietic marrow cell renewal system generates mature granulocytes i.e., neutrophils, eosinophils, and basophiles, in a stream of circulating blood (NATO handbook, 1996). The immune system fights in case of infectious or cancerous diseases. Any harmful or

toxic exposure can increase or decrease this system (Godekmerdan et al., 2004). The agranulocytes comprised of monocytes and lymphocytes. A lymphocytes are a kind of WBC, resides in lymphatic tissues which participates in immunity (Al-Zubaydi, 2017). Largest of white blood cells are monocytes that are formed in bone marrow to flow into the bloodstream. The macrophages are the major scavenger cells of immune system (Al-Zubaydi, 2017). An individual may have some symptoms associated with altered monocytes levels. We looked hematological impacts of the residents of Lahore who are being exposed to an unsafe, polluted and bacterial contaminated water. Rapidly dividing bone marrow cells are more vulnerable to any harmful exposure. The bone marrow's precursor cells are organized to differentiate into certain different cell lines. These cells perform their functions upon their maturity states in the blood stream. An immune response is dependent on the functions performed by granulocytes and lymphocytes emerging from bone marrow (Smirnova, 2010; Shahid et al., 2014). Granulocyte cells are greatly involved in immune response which comprises macrophages and neutrophils. In case of infection, these cells cause inflammation, as granulocytes gather at the site of infection to release substances in order to destroy hostile agents. Cytokines are released by macrophages to deal with infectious fevers. The antigen-specific cells activated by the receptors of lymphocytes acted on each disposed cell (Formentis, 2010; Gridley et al., 2009; Curtin et al., 2005). In our research, a significant number of individuals (54%) were found higher levels of Monocytes along with absolute counts. Raised levels of monocytes (monocytosis) can be resulted in cases of blood disorders, in some carcinomas or chronic infections etc. (Territo, 2017). Few studies are available on altered levels of monocytes in fish in polluted aquatic environments (Adham et al., 2011; Zhelev et al., 2016), immune responses of aquatic animals (Khan and Thulin, 1991; Ali et al., 2008; de Swart et al., 1994). But still there is no affirmed study available to know the incidence of poor immune response including hyper monocytes from polluted water. Higher levels of lymphocytes were also found in individuals (22%) which indicate the lymphocytosis. Moreover, 18% of people were having low levels of granulocytes (granulocytopenia). With modulated immune response, it is known that the granulocytes may show an initial increase before they finally depress (Dainiak, 2002). The granulocytopenia is defined as reduced number of blood granulocytes (type of WBC), namely neutrophils, eosinophils and basophils. The cell-lines variations in the form of monocytosis, lymphocytosis and granulocytopenia reported in current, indicating an altered immune response in group of individuals who were exposed to polluted drinking water and environment. Raised levels of monocytes and lymphocytes show a hyper immune response in individuals. An active immune cell lines indicates the presence of infectious causing microorganisms in population of Lahore.

The citizens of Lahore, not only experiencing unsafe with altered DO (dissolved oxygen) levels of drinking water, but also inhaling polluted air (38.46%) from poor roads an street conditions (46.5%). Elevated levels of RBCs (38%) were found in residents of Lahore. It has been long observed the linkage between depressed atmospheric oxygen pressure and higher RBCs in humans (Haase, 2010). It was mentioned by Luttmann-Gibson et al., (2014) that growing exposure of air pollution, including the non-traffic pollutant (SO₄²⁻) from industrial sources, can change oxygen saturation that may show "particle induced pulmonary inflammatory" or "vascular responses". It was reported that subject who was exposed to air pollution, were having lower oxygen saturation levels, which induces a risk of cardiovascular ailments (Luttmann-Gibson et al., 2014). When the condition of hypoxia prevails, the bone marrow further activates to produce more red blood cells (RBCs). With the stimulus of hypoxia, there would be more release of erythropoietin from kidney leading to more production of RBCs with the raised retic count, as a result of the compensatory mechanism initiated by the bone marrow (Dainiak, 2002). A study provided some evidence related to the exposure of pollution and induction of hypoxia (Pope et al., 1999). They observed association between elevated pulse rate and previous exposure to particular air pollution (Pope et al., 1999). A pathophysiological pathway found associated with particulate air pollution and cardiopulmonary death is thought to be existed. Approximately, 28% of drinking water samples were found in lower levels of dissolved oxygen in the current research. A hypoxia (low oxygen) of water can pose significant impacts on aquatic life. Such water hypoxia can be resulted from many factors such as excess nutrients and nitrogen and phosphorus contents (US EPA, 2017). Adequate oxygen levels allow our body to successfully fight all microorganisms that are harmful to our body. It also allows us to detoxify chemical pollutants. With hypoxial conditions, a body could sufficiently suffer to deal with the infections caused by microorganisms. Record of air bubbles in fossil amber has shown that in the early history of life, 35% of oxygen was present in the air. But now the levels have been dropped to 20% approximately. In polluted regions, the oxygen content is only between 12-15%, whereas it is known that below 7% of oxygen content, it is impossible to sustain a human life (TNHP, 2017). Why individuals (38%) of current research were suffering from raised red blood cells (hypoxia)this may refer to the inhaling of polluted air and/or intake of lower dissolved oxygen content. Further investigation is required to affirm this suspicion.

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