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Effect of boiling on removing of shiga toxins from drinking water samples

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Abstract
Shiga toxin producing *E. coli* has been considered an emerging food borne pathogen that causes haemorrhagic colitis, abdominal pain, and occasional fevers; along with haemolytic uremic syndrome that develops in 5-8% of cases. The main natural reservoir of shiga toxins is ruminants such as cattle and; however, faecal contamination during manual milking, along with poor hygiene practices during cheese preparation, allows for the presence of toxins in raw milk. These conversational water treatment methods of drinking water are needed to modifications for disinfection of microbes and removing their toxins from drinking water sources with very low cost, this is the main objective of this present study.
ELISA method is used for detection of toxin level in drinking water which is reliable, microbial contamination is confirmed by media cultural growth. Ferrous salt is used as coagulant before boiling that makes it effective treatment.
After treatment of conventional method of pasteurization with addition of ferrous salt, results revealed that 99% toxin of shiga removed with achievement of maximum success. The high values shown of toxins in canal water sample as 23.5mg/l and very low value seen in ground water samples as 0.7 mg/l. Proper pasteurization of contaminated water with coagulant agent gives the reduced value of toxins and contamination which is highly best for safe water drinking.

Keywords: Toxins, boiling water, microcystins, coagulation

1. Introduction
The environmental pollution is a common problem in developing countries, these developing countries ensure their pollution share in environment due to their increasing rate of industrialization and discharge the uncontrolled of waste into the environment. According to a survey directed by the United Nations (2006), 62% of Pakistan's urban living people and 84% of Pakistan's rural residents does not properly treat their drinking water, as a results it was recorded that more than 100 million of diarrhoea cases registered in the hospitals of Pakistan. These figures further lead to about 40% expiries in this country as an outcome of contaminated drinking of water. Shiga toxin is the prototype of family and it is a group related to exotoxins with specific structure and functions. In 1897, the Japanese microbiologist Professor Kiyochi Shiga characterized the bacteriological source of infections as being produced by *S. dysenterie* [1, 2].

It was identified a group of *E. coli* that are able to kill Vero cells in culture by secreting a factor into the tissue culture medium. These bacteria were named Vero toxin creating *E. coli* represented as (VTEC). By the early 1980s, Alison O'Brien and her research group discovered, *E. coli* formed a toxin very similar like Shiga toxin, therefore, named of this microorganism is Shiga toxin making *E. coli* (STEC). Later, it became clear; VTEC and STEC are two names describing the same organisms and toxins. Shiga toxin 1represened as (Stx1) formed by *E. coli* is nearly same as the Shiga toxin prototype, but they only differ by a one amino acid in a catalytic. STEC produces both Stx1 and Stx2 variants. Stx2-producing STEC have been linked epidemiologically to more severe disease in infected humans and neurological symptoms due to strains producing of Stx1 [3].
Shiga toxin family members consist of a single enzymatically active A-subunit (molecular mass of 32 kDa) and five identical B-subunits (pentamer with each B fragment weighing at 7.7 kDa) allowing the toxin to bind to the target cell surface receptors [4]. Toxin-induced lipid partition drives nano compartmentalization at the cytoplasmic level to induce the recruitment of an intracellular sorting machinery and toxin translocation [5].
Many other farm animals such as sheep, goats, pigs, and turkeys can also shed STEC in their faces. The most common route of transmission to humans is via ingestion of contaminated

foods and water. In the process of grinding beef, pathogens contaminating the surface of the meat transfer to the interior; therefore, to ensure complete killing of the bacteria, ground beef should be cooked all the way through. Other contaminated food source including, unpasteurized milk as fresh produce, and drinking water. Epidemiological studies suggest that these food sources were contaminated with bovine and feral swine faecal materials [6, 7].

This research paper is attentive on the different waterborne pathogen *E. coli* and their toxins that are detected and removed from supply of ground water, storage water tanks and canal water.

2. Martials and methods

2.1 Collection of Water Samples

Sheikhupura District under study and research, random samples were collected of the different types. All the samples were carried to chemical Biotech Labs. Sheikhupura, where various chemical analysis and other tests were performed. Total 116 random samples were collected from Ground water, water storage tanks and Canal water under stick quality parameters. All samples were collected in sterilized PVC bottles as the container and water sample container were filled up to 100 % of the volume as actual capacity of the container that had I litre or 1000 ml. when sample collection was started the temperature of the day was 20 ^{0}C [8].

2.2 Isolation and Identification of Escherichia coli

Media composition (MacConkey agar) was used to dissolve the dehydrated medium in the water and transfer this in a flask, and then fixed the temperature at 121ºC on autoclave for 16 min. The next step was allowed to cool the media to normal position from 44 to 47 °C before adding the supplement as potassium tellurite and cefixime in low concentration. pH adjustment was very important from 7 to 8 at 27°C after sterilisation process. It was needed to prepared final plates that may preserve at 5°C for 11 days period. According to method Petri plates were on incubation at temperature 37 ºC for 24 hours duration, process was repeated for water samples testing. If growths of microbes are seen then result of sample is considered as positive otherwise negative. Microbiological final confirmation was done possibly by biochemical reaction [9].

2.3 Laboratory Testing Method of Shiga Toxins

The Premier EHEC test kit utilizes monoclonal anti-Shiga toxin capture antibodies absorbed onto the bottom of micro wells. When testing, 100 µL samples were added to each well mixed thoroughly with the pipette and incubated at room temperature (RT) for 1 hour of the 96-well plates. Then Wells were washed according to manufacturer's instructions. Volumes of 100 µL of polyclonal anti-Shiga toxin antibodies provided by the kit were added into each well, mixed well as before, and incubated at RT for 30 min. Wells were washed again to remove unbound antibody. Aliquots of 100 µL of enzyme-conjugated anti-IgG polyclonal antibody was added into each well, mixed and incubated at RT for 30 min. After washing to remove unbound conjugate, 100 µL of substrate were added to each well and incubated for 10 min at RT of plates, add 100 µL as stop solution into wells. And then measured absorbance at 450 nm by using a Bio-Tek Kinetics Reader [10].

2.4 Boiling experiments

Ferric Chloride salt of 5 mg concentration as coagulant was used to add to one litre drinking water samples (Canal water, storage tanks water, ground water). The boiling procedure was adopted on same condition. The boiling procedure was adopted as using a 2700 W electric kettle with the capacity of 1.5 Litre. Water boiling was started at specific point where the heat source rotated on off automatically. The research experiments were performed for the three times with same device as TOSHIBA-97. Boiled water that taken by electric kettle was preserved at room temperature as 27 ºC for 10 minutes. At the time of analysis, temperature of boiled water was 27 ºC of all drinking water samples.

3. Results

3.1 Estimation of Toxins and Microbes

In the present study it was observed that the contamination rate is maximum found as 98 % due to E Coli in canal water samples and toxin level as (16.5±0.9) can be seen in table 1. Toxins production rate depends on microbial growth rate but environmental conditions are very important factors for this present study. These values had a hazard level as against that given by the WHO 0.1 ppm. The untreated or inadequately treated water to the population, results in the waterborne diseases in that area. The health effects of that unsafe water are clear from data presented in this study. All over the world toxins are known as notorious for making the complications in humans and animals.

Table 1: Detection of Microbes and toxins in Different Water Samples Analysis

Sampling Type (mg/l)	Shiga Toxin		*E. Coli* Detection in samples (%)	
	Mean±S.D	Range	Mean±S.D	Range
Canal Water	16.5±0.9	10-19	98±2.1	97-99
Storage Tanks Water	1.8±0.1	0.5-3	53±1.5	45-55
Ground Water	0.7±0.02	0.5-1.2	45±1.3	40-50

3.2 Effect of Boiling On Toxins Removal from Canal Water

Boiling effects on water quality were appeared as same on canal Water, water storage tanks and ground water samples because boiling time increasing was reduced the values of toxins respectively as given in the below table 2.

In figure 1, it was shown that toxicity reduced of drinking water samples due to removing the toxins and removal rates that depends on time durations. It is confirmed from published literature that water boiling point is 100 ^{0}C and need a constant supply of heat for this process but it depends on this how much dissolved and suspended solids are remained in water. Because if water samples have more suspended and dissolved organic solids then it needs more heat supply as compare to normal water drinking samples.

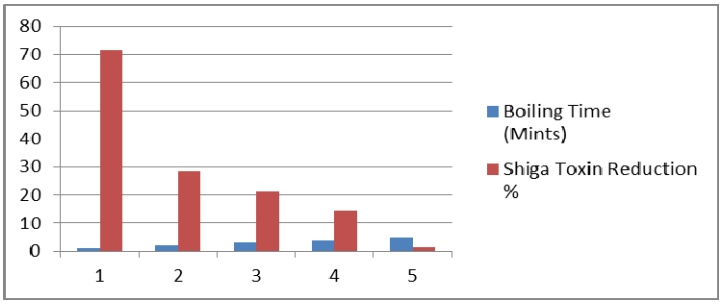

Fig 1: Graph showing the effects of boiling on removing of toxins in samples of Canal water

It was seen that dissolved toxins found with high concentration in canal water samples as shown in table 2, it needs high amount of heat and long time because toxins can volatile and removed. Boiling process has both of advantages as disinfection and toxins removing from the water supply system because at hundred degree centigrade temperature kills the almost microbes according to literature.

Table 2: Effect of boiling duration on removing the Toxins Levels in Drinking Canal Water

Boiling Time (Mints)	Shiga Toxin					
	Actual Value mg/l		Reduced value after treatment mg/l		Values after treatment (%)	
	Mean±S.D	Range	Mean±S.D	Range	Mean±S.D	Range
1	16.5±1.2	9.5-20	10.9±0.4	9.5-11	66.0±3.9	63-69
2	16.5±1.2	9.5-20	8.5±0.3	8-9.2	51.51±3	49-53
3	16.5±1.2	9.5-20	4.7±0.2	4-5.2	28.48±2.6	26-30
4	16.5±1.2	9.5-20	2.2±0.1	1.9-2.9	13.33±1.2	11-15
5	16.5±1.2	9.5-20	0.4±.01	0.16-1	0.90±0.1	0.7-1.3

It has shown from results of the present research that the drinking water quality standards of toxins and bacteriological contamination are not fulfilled the normal values limits given by World Health Organization. The disinfection process for treatment of drinking water has important role in reducing the waterborne diseases. Government departments and health authorities must take the serious notice on condition of contaminated drinking water and try to correct it for saving the public health which is suffering from drinking water contamination with pathogens and chemical toxins.

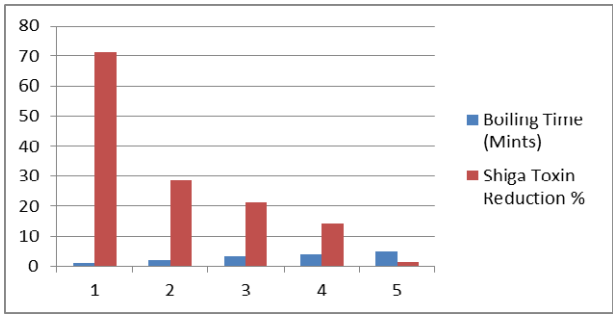

Fig 2: Graph showing the effects of boiling on removing of toxins in ground water storage tanks samples

3.3 Effect of Boiling On Toxins Removal from Water Storage Tanks

In figure 2, it was observed that toxin concentrations reduced mostly in experiments of water boiling. Toxins removal rates were same as noticed in experiments related as in boiling studies. After treatment of potable water with chlorine, very little residues of chemicals were detected because series of experiments conducted on confirmation of the actual dose.

Consequently, the organic compounds are degraded; it is possible due to hydrolysis as excess water with heating effect and then volatilizing is occurred during the pasteurization. This does not undergo base-catalysed hydrolysis and do not increase their toxicity to that of the degradation of natural compounds, so reducing concentrations of toxic organic compound may be due to volatilization, possibly.

Table 3: Effect of boiling duration on removing the Toxins Levels in samples Storage Tanks

Boiling Time (Mints)	Shiga Toxin					
	Actual Value mg/l		Reduced value after treatment mg/l		Values after treatment (%)	
	Mean± S.D	Range	Mean± S.D	Range	Mean±S.D	Range
1	1.5±0.3	0.5-2	1±0.03	0.7-1.5	66.66±4	63-68
2	1.5±0.3	0.5-2	0.7±0.02	0.5-1	46.6±3.7	44-49
3	1.5±0.3	0.5-2	0.3±0.01	0.1-0.5	20±1.7	17-22
4	1.5±0.3	0.5-2	0.21±0.01	0.0-0.3	14±0.9	11-17
5	1.5±0.3	0.5-2	0.01±0.001	0.0-0.1	0.66±0.1	0.4-0.9

3.4 Effect of Boiling On Toxins Removal from Drinking Ground Water

Based on study results, preceding research was cited on the effects of boiling and heating on removing of toxins were hooked on organics nature of compound as stability, volatility and water temperature. Boiling or heating also had deep effects on drinking water quality and residues dissolving in it. If the water treated chemicals residual is increased in considerable amount in any a drinking water supply system then there is increase in the cancer risk. In figure 3, it was established the good relationship between boiling time and reduction of toxins values.

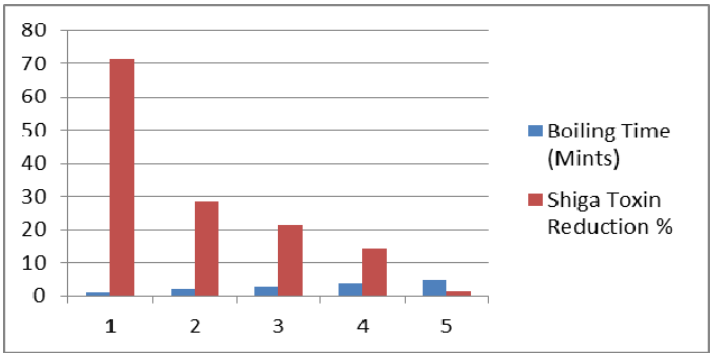

Fig 3: Graph showing the effects of boiling on removing of toxins in ground water samples

As table 4 also give same expression from figure 3, In general a higher isolation rate was obtained because the water borne pathogens are mesophilic, can best grow at the temperature ranges of the drinking water of area under study. However, other factors such as organic matter, water level and drought may influence the bacteriological quality of water. It will be interesting to evaluate and determine these parameters for proper understanding of microbial pathogens in drinking water.

Table 4: Effect of boiling duration on removing the Toxins Levels in Groundwater samples

Boiling Time (Mints)	Shiga Toxin					
	Actual Value mg/l		Reduced value after treatment mg/l		Values after treatment (%)	
	Mean±S.D	Range	Mean±S.D	Range	Mean±S.D	Range
1	0.7±0.1	0.5-1	0.5±0.04	0.4-0.9	71.42±3	69-73
2	0.7±0.1	0.5-1	0.2±0.02	0-0.1	28.57±2	26-30
3	0.7±0.1	0.5-1	0.15±0.01	0-0.1	21.42±1.9	19-22
4	0.7±0.1	0.5-1	0.1±0.01	.01-0.1	14.28±1	12-16
5	0.7±0.1	0.5-1	0.01±0.0002	0-0.1	1.4±0.02	0.9-2

4. Discussion

The presence of other organic substances consumed chlorine, requiring higher coagulant doses for degradation of cylindrospermopsin. A cylindrospermopsin concentration of 20-24 mg/l was effectively destroyed by a coagulant dose of 4 mg/l at pH 7.2-7.4. A concentration of 100 mg/l was reduced to <0.2 mgl^{-1} after 27 minutes contact and coagulant residues are with 0.53 mg/l concentration. Impact on the treatment efficiency by aluminium sulphate on other suspended material such as silt has been reported in any previous study on full-scale or pilot-scale. However it could be questioned whether a continuous solid build-up could affect the process; such as by interfering with the coagulant dosing, releasing turbidity into the treated water or potentially becoming a nutrient source for bacteria, leading to a biofilm formation on the resin beads, previously seen to affect organic matter adsorption on other exchange resins [11, 12].

Long history of disinfection with Chlorine, it is used bleach as a chemical agent in the disinfection of drinking water in 1897 in UK. Biggest successes in the health sector are modification of harmless potable water supplies. Chlorine as treatment agent for drinking water had started with Chicago and Jersey City of America in 1908 and that was helped to bane the spreading of disease like typhoid, fever cholera and hepatitis. Drinking water treatment with filtration and chlorination has assisted to reduce the illnesses in under developing states like Pakistan. Uses of chlorine was used generally in drinking water management for the decontamination process [13]. However, source of surface water used for domestic purposes and then it needs some special treatment as filtration, sedimentation and coagulation formerly decontamination. Another method for disinfection process includes ozone, chlorine and chloramines are used as the last block against microbes. The situation in the area under study was also observed same. Chlorine was not used adequately; that is why it was present in meager amount or completely absent in water samples tested in this study (Figure. 1).

Chlorine Practice in case of water management is effective as use of lethal hypochlorite for microbes disinfection as they are the most commonly used as disinfectant process in drinking waters. It is reported the *H. pylori* may enter into water supplies by only possible way from human urine contamination into the water system; *H. pylori* stay alive inside water supply arrangements due to less level of disinfectant of chlorine in water distribution system. It is evident from the results (high coliform and faecal coliform count at all sampling levels) and potable water standards are further deteriorated of the water supply scheme may be due to the leakage of pipes, where sewage water enters into the municipal water. At the level of customer the potable water is getting further contaminated because of the non-properly managed and uncovered storage tanks [14].

It has been reported that the microbiological quality of water was not be satisfactory 36% samples were found to be contaminated with waterborne pathogens during microbiological study of drinking water supplied to the schools in Karachi. Another study on ground water quality conducted by Habib *et al*. (2009), the faecal coliform was isolated in most of the sample of groundwater in kasur Pakistan [15].

But one problem is connected to this techniques, boiling can apply on domestic level it cannot be practiced on large scale such as municipal water or any network related big distribution for water supply system.

5. Conclusion

Shiga toxins are very dangerous in any drinking water supply; it needs to remove safely from drinking water sources. The results in this study lead to conclusion that the boiling process

with addition of chemicals is very good at consumer level. It is therefore, recommended that following steps may be taken by the relevant authorities to curtail the bacterial contamination of water supplies for drinking purpose: commonly, the water distribution systems of Pakistan in small cities and rural areas not proper design according to working in developing countries.

Canal water sample are not easy to treat because it has high amount of toxins as compare to other samples of ground water and water storage tanks. Toxins removing process depend on boiling durations and addition of chemicals as coagulants.

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