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Effect of Boiling on Removing of Microcystins Toxins from Drinking Water Samples

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Abstract: ***The Population has been living more than 37% in the under developing countries, they need to access on safe drinking water as their basic right. Every year, the 875 million cases of diarrhoea and 4.7 million deaths are facing in these countries because primarily deficiencies occur in drinking water supply. These countries have needed more than $150 billion, it is expected estimate for their requirement to establish full safe water drinking supply coverage system and this calculated total amount may not be raise by them in the near future. For above purpose, it is needed to know the usefulness of boiling, chlorination and heat pasteurization methods for disinfection of microbes as (E. Coli, C. Boulinum and Cyanobacteria) in drinking water sources. The lab testing under the reliable method was used for detecting the microbial contamination and toxins these including the proper sampling of wastewater, then detection of toxins by using the ELISA method and microbial contamination by plate growth method. Simple Water boiling method is used with adding the small amount of coagulant as Ferric Chloride. The study showed that the toxins level was very high as 22 mg/l of microcystins in canal water that depend on microbial contaminations. In this research, 90-98% toxins were removes from drinking water samples by using the boiling process with some amount chemical as ferrous sulphate for drinking water treatment. Conclusions: the conclusion isdrowning from this study as finding very low consternation of toxins are observed as 0.2 mg/l with zero microbial contamination after treatment of boiling of drinking water under effective designed method.***

Keywords: Toxins, Boiling Water, Microcystins, Coagulation

1. Introduction

A common person consuming 3000 ml of water daily, this is approximately 3 litres. This analysis do not give actual idea for essential variations in intake of drinking water for individuals that depend on factors such as body type, age groups and level of physical fitness. Moreover, approximately half of the net weight of food is water, so this is normal requirement for water need in human body. Finally, the necessary amount of water does not fix, it is varied and depends on person to person, and each person should receive a desirable amount of drinking water. Approximately, Drinking water should take 70 ounces per day and this quantity may feel like a huge amount, but this quantity is necessary for normal health of person because water makes up approximately 60% of a person's body weight, and it is also most large amount compound found in the human body because all biochemical reactions take place in water. Finally, water act as an active participant in those important chemical reactions which are important for metabolic reactions. Water can remove the harmful toxins from the body, it carries nutrients and transfer to important vital organs and it enters to the cells and around them, and act as a special solvent for minerals, vitamins, amino acids, glucose and other nutrients [1, 2].

Drinking water supplies contaminated with Cyanobacteria toxins is a main cause of a health hazard for human beings, domestic animals both large and small, and wildlife animals. Cyanobacteria can be produced both toxins microcystins and nodularin which are known as the hepatotoxinsthat are powerful tumour promoters and can bind to serine and threonine protein phosphatase enzymes with slow the protein activity by reaction mechanism. The results of this hepatotoxicity from the entering ability of microcystins and nodularin to hepatocytes process where they make strong cause of hyper phosphorylation of liver proteins and destruction of liver cells [3].

A report (2013) according to Pakistan Economic Survey, it is also estimated the financial loss, this state is bearing Rs.112 billion loss per every year as drinking water, hygiene and sanitation connected diseases, but need the budget nearly Rs. 300 million per day for safe drinking and proper sanitation. It is calculated in Pakistan that 44 percent of the population have lacked access to safe drinking water in urban area, while 90 per cent of the population with lacks access to safe drinking water in rural areas. According to a report from Pakistan Council of Research in Water Resources, One important indication about the problems, it is expected about 200,000 teen-agers die every year in Pakistan due to diarrhoeal infections [4, 5].

This research project is focused on the cyanobacterial pathogens and their metabolites as toxins that are detected in samples of ground water, storage water tanks and canal water.

2. Materials and Methods

Collection of Water Samples
In selected area under study and research, it collected random samples of the following types but all the samples were taken in sterilized container for detecting microbe's presence as Cyanobacteria and their related toxin as Microcystins toxin under strike slanders of quality. All the samples were shifted to chemical Biotech Labs. Sheikhupura, where perform chemical analysis and other tests. Environmental parameters of lab were as given in table as below [6].

Ground water sampling
Collect random ground water samples from different houses by red motor water pump with power capacity 1 horse power and the frequency of samples (n= 116). In this area ground water 70 feet below from earth surface.

Sampling from water storage tank
Collect random water sampling from different water storages tanks of houses with the depth of 0.5m and frequency of samples (n= 116). The average height of storage tanks are 7±0.9 feet and width 3±0.4 feet and the temperature of the day when collect the samples was 27 ^{0}C.

Canal water sampling
Collect random canal water samples from different source point and the frequency of samples (n= 10). All sample collect in sterilized PVC bottles as the container and water sample container were filled 100 % of the volume capacity and actual capacity of the container is I litre or 1000 ml the temperature of day when sample collection was 16 ^{0}C.

Isolation and Identification of Cyanobacteria
Medium (**EMB, OXOID England**) dissolved in 1000 ml of deionized water, and then it need autoclave for 15min at 121 °C. Incubated the Petri plates for 24 hours at 37 ºC, and then process repeated for other water samples used in this research. It was Appeared the colonies after incubation on the growth medium and number of positive (NP) samples recoded for this research. Final confirmation was made by biochemical reaction.

The medium is made with adjust to pH 7.6-7.8 with 1M NaOH or HClputin deionised water and maintain the pH at 7.5. Autoclave the microbial media at 121°C, cool and mix aseptically. Add 15g non-nutrient agar per litre of medium into deionised water and maintain the pH at 7.5. The Petri plates were incubated at 37 ºC for 24 hours and the process was repeated to other replicates of water samples. Appears the colonies after incubation on the growth medium and number of positive (NP) samples recoded for this research. Final confirmation was made by biochemical reaction [7, 8].

Confirmatory Tests for Cyanobacteria and Algae growth
Microscopic observation was performed to verify a normal and healthy appearance of the inoculums culture of both Cyanobacteria and Algae and to observe any abnormal appearance of the algae and Cyanobacteria at the end of the test for taking the best quality in research.

Microcystins Toxin Testing Method
ELISA is the most reliable method for rapid screening of samples for detection of microcystins because its sensitivity and specificity very clear with ease operation in devices. ELISA assay provide the information about the total toxin concentration in the sample. If the water samples very clear or filtered, then the testing were started with following assay protocol with better sensitivity, where the analysis of the three microcystins calibrators are 0.16, 1.5 and 2.5 ppb performed, were diluted as 1:3 by adding 100 µL of each to 200 µL of kit water and then give the concentrations of calibrators as 0.05, 0.2 and 0.83 ppb respectively. The technique is involved by the adding of 50 µL of negative control, each calibrator with each sample put in the wells and get 50 µL of microcystin with added assay diluent and was incubated for 30 minutes on a shaker [9, 10].

Boiling experiments
5 mg of Ferric Chloride salt (coagulant) was added in each one litre samples of drinking water (Canal water, ground water, storage tanks water) and boiling procedure adopted. The boiling experiments were done by using a 2700 W electric kettle with the capacity of 1.5 Litre having the 14 cm of pot diameter. When the water boiling started at specific point then heat source was automatically rotated on off. In this research, experiments were performed the three times on this device (TOSHIBA-97). Boiled and treated water from electric kettle was preserved at room temperature (27 ^{o}C) for 10 minutes for chemical and microbiological analysis under standard procedure.

At the time of analysis, temperature of boiled water was 27 ^{o}C of drinking water samples [11, 12].

3. Results

Results from chemical analysis of toxins microcystins first based on systematically microbes growth identification of microcystins toxins producing by species cyanobacteria that indicate in all the samples of ground water, storage water tanks and water have values (41±4)%, (65±4.3) % and (95±3) %, respectively (shown in table-1). This pictured is very clear about toxins that are dissolved in different samples as given in table -1,
In table-1, it is shown that values of toxins depend on presence of microbe (cyanobacteria) activity that related with the nature of weather conditions as humidity and temperature. Sampling collection temperature was 39 ^{o}C during the summer season which was ideal for microbes growth.

Table 1: Values (Mean±S.D) of cyanbectria microbe and microcystins toxins

Toxins indicator	Canal water samples		Gounod water samples		Storage water tanks samples	
	Mean±S.D	Range	Mean±S.D	Range	Mean±S.D	Range
Cyanobacteria (%)	95±3	92-97	41±4	38-44	65±4.3	63-67
Microcystins (mg/l)	21±2.5	19-23	2.4±0.7	2-3	5.7±0.9	4-7

Effect of Boiling On Toxins Removal From Canal Water

In this study some new bacterial pathogens was isolated and identified from water samples tested for example cyanobacteria. These bacteria are not included in the list of bacterial pathogens of water previously. These findings could be valuable or a breakthrough in the inclusion of these species as waterborne pathogens.

In figure 1, it was observed the boiling time very effective with regard the removing of toxins from samples of canal drinking water. From time 1 to 5 minuts, it has seen the boiling time can maximize the removal value of toxins because organic compounds are volatile by nature and they cane evaporate on heating or boiling.

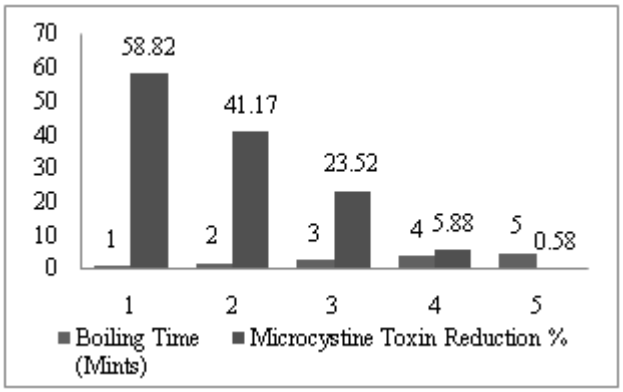

Figure 1: Graph showing the effects of boiling on removing of toxins in samples of Canal water

In table 2, it was shown that toxicity reduced of drinking water samples due to removing the toxins and removal rates that dependent on time durations. The aim of this research was to identify the treatment characteristics of toxins and organic matter preferentially removed by boiling and coagulant method to help assessing its effectiveness to treatment of waters from dissimilar source. Impact on process of this method on downstream coagulation and chlorination was investigated as well to assess any potential impact on water quality.

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

Boiling Time (Mints)	Microcystins					
	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	22±2.4	16-23	16±2	15.2-17	72.72±5	70-74
2	22±2.4	16-23	10.6±1.5	9-11	48.18±4.3	45-51
3	22±2.4	16-23	7.9±0.9	7-8.5	35.90±3.6	33-38
4	22±2.4	16-23	3±0.5	2.7-3.9	13.63±2	11-15
5	22±2.4	16-23	0.17±0.3	0.5-1	0.77±0.1	0.5-0.9

Effect of Boiling On Toxins Removal From Water Storage Tanks

In water samples of storage water tanks, it was observed that low toxins detected but toxins values indicates that no proper arrangement of cleaning system there and this was reasons to grows and isolate the cynobectrias. The work undertaken during this research allowed to achieve all these objectives and to obtain a cheaper method for removing toxins and organic matter into the mechanisms involved from polluted water. Continuous bench-scale tests on sources of water as well as the investigation of regenerate solutions are abstracted from full-scale and pilot-scale plants identified toxins and organic matter preferentially removed.

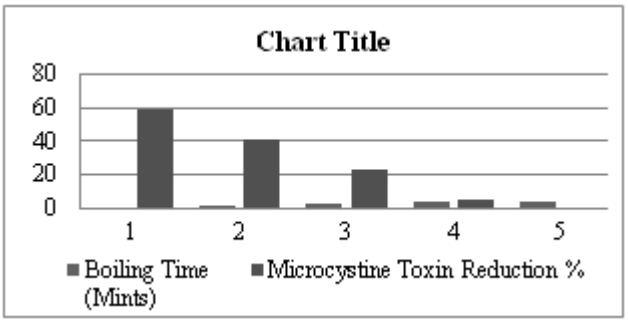

Figure 2: Graph showing the effects of boiling on removing of toxins in samples of water storage tanks

In table – 3, water samples of tanks water have toxins average values (5±1.2) mg/l but adding some salt and boiling can reduce the values of toxins gradually, at 5 mints 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. 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.

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

Boiling Time (Mints)	Microcystins					
	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	5±1.2	5-6	3.5±0.7	2.7-4.5	70±6.8	67-73
2	5±1.2	5-6	2.7±0.4	1.9-3.5	54±4	52-56
3	5±1.2	5-6	1.5±0.2	0.5-2	30±2.7	27-33
4	5±1.2	5-6	0.5±0.1	0.2-0.9	10±1.2	7-13
5	5±1.2	5-6	0.01±0.01	0-0.3	0.2±0.001	0.1-0.4

Effect of Boiling On Toxins Removal from Drinking Ground Water

It was observed high removal of toxins achieved by aluminium sulphate coagulant as compare to boiling method. Increasing the time from 1 to 5 minutes, which was within the parameters indicated removing of toxins, is part of this study to enhance organics removal in figure 3 and table 4. Hundred percent (100%) microbes were killed with 2-min boil and ninety seven percent (97 %) of toxins was removed on 5 min. boiling due to large volatizing process of toxins during water boiling as shown in table 4 as water samples from canal source. Basically canal water samples was most polluted water samples and even no capable and fit for animals drinking as compare to water storage tanks and ground water samples.

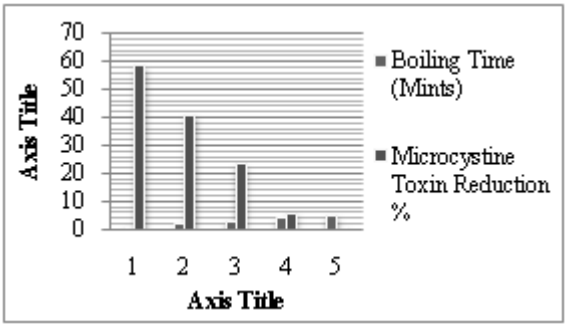

Figure 3: Graph showing the effects of boiling on removing of toxins in samples of drinking ground water

In figure 3, it was showed that increasing the boiling time from 1 mins to 5mins was reduced the toxins values that dissolved in the ground water samples. The ground water samples are already low in toxins values but high in the Microcystins toxins values due to maximum expose time with high contamination source as microbial growth of cynobectira and Microcystins that are more stable toxin as compare to high values as understand.

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

Boiling Time (Mints)	Microcystins					
	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	1.7±0.3	0.9-2	1±0.2	0.5-1.5	58.82±3.1	56-60
2	1.7±0.3	0.9-2	0.7±0.1	0.4-1	41.17±2.9	38-43
3	1.7±0.3	0.9-2	0.4±.01	0.1-0.7	23.52±1.7	21-25
4	1.7±0.3	0.9-2	0.1±0	0-.01	5.88±1.2	3-7
5	1.7±0.3	0.9-2	0.01±0.1	0-0.1	0.58±0.1	0.3-1.7

4. Discussion

It was also observed in this study that when values of toxin with low concentration then colour also seen little removed with using of coagulant aluminium sulphate. Specific colour and toxins removes showed the same trend in same way as if a toxin concentration is low then colour of treated water was also clear. Nitrogen based biological compound inside samples of canal water can be detached by aluminium sulphate setting if the matter is based on organic compound a minor in quantity, Pietsch et al. (2001) initiate that the removal of nitrogenous matter is problematic to attain with simple coagulation in some cases and the nitrogen based compound are detached by microbial degradation and zonation processes [13,14].

Moreover, Vilge-Ritter et al. (1999) reported that bio-organic based compounds resemble to if a minor in percentage in the any kind of samples of water; their elimination is so much poor because Aluminium and ferric salts not able to coagulate them. Removal of cyanobacteria and algae in coagulation and clarification process is dependent on optimization of chemical doses of coagulation with Aluminium coagulants [15].

Specific dose of Coagulant is essential to removal cyanobacteria and algal cell which is relative to the cell number of logarithm (Perelle et al., 2005). Minimizing turbidity in jar test is not sufficient to remove algae and cyanobacteria toxin. Cyanobacteria will not be removed on insufficient coagulant dose of aluminium sulphate.Aluminium sulphate dosed at 20 mg/l (in table 3) without polymer addition removed about 80 percent of the toxicity from neurotoxic bloom of microcystins, Coagulation had an ability to eliminate the toxins in water samples in several studies. These studies tested the coagulant as Aluminium sulphate in different concentrations. Coagulation and clarification studies have had mixed results on cell lysis and the subsequent release of cyanobacterial algal toxins [16, 17].

Results of this study shows that microcystin toxins are produced by cyanobacteria that is commonly called blue-green algae although it is really an algae and algal toxins are released from cyanobacteria as a bloom nears the end of its lifecycle, or the cells are lysed (split apart) and the toxins are released. A third study showed chlorination attained in significant decrease of microcystins from 0.4-0.6mg/l of levels in canned water samples. Two studies indicated the no visible elimination of anatoxin-a from chlorination process. Rositano and Nicholson (1994) also showed with a dose of 15 mg/L at pH 7 with chlorination of anatoxin-a ineffective for contact time 30 minutes, 16 per cent removal providing only was occurred. However, chlorination was very effective to destroying microcystins and shiga toxin after 30 minutes interaction time at 8≥pH [18, 19, 20].

A combination of boiling and coagulant dose may effects the good impact on removal efficiently by 85 to 90 % of toxins is cited in literature but depend on water treated coagulation dose as 20 mg/l to 30 mg/l, it is showed as results in table 4 for bench and pilot-scale study. Furthermore it was observed in this thesis that chemical treatment is useful for suspended material such as macroscopic algae during solid-liquid separation by settling. It is proved from this research the boiling process can breaking down organic based nature of toxins convert into light gases that evaporate into atmospheric medium. It is very helpful study and discussion about boiling facts of drinking water in absence of chlorination compounds in drinking water [21, 22].

5. Conclusion

One of the most important techniques in drinking water is boiling that are used in the world famously. In the present study, we use three different contaminated drinking water samples from canal, ground and water storage tanks. So, different drinking source water has different abnormal values of microcystins toxins as (22, 10. 5) mg/l.

Microcystins toxins in fresh drinking water source are usauall across the whole world. Microcystins toxins are known as great hapatoxins and can create a sevier problems in liver function performances. Microcystins toxins source is only green algae which are known as cyanobacterial species. Each type of samples give special boiling treatment, as results find the concentration of microcystins toxins going toward decrease with increasing the boiling times. These experiments repeat many times accordingly but find same results.

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