

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

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Abstract: *C. Botulinum* growing the spores is found all over the world in the samples of soil and marine sediments and a normal healthy person can consume the spores. But when food is contaminated and the spores grow in anaerobic conditions then toxin is created with highly poisonous and toxic when ingested it. In food-borne botulism cases are linked with home conserved meats as fish and vegetables. The conventional methods of chemical treatment of drinking water are included as disinfections, chemical coagulation treatment, heat pasteurization, and filtration. These were very useful drinking water treatment methods but this time needed how to apply this conventional method on present complex chemical contamination situation of drinking water sources. In the present study, the enumeration of *C. Botulinum* as bacterial species detected on different media but their related metabolites toxins like botulinum was tested by ELISA method. In this study a new bacterial pathogen was isolated and identified from water samples tested for example *C. Botulinum*. Toxins of botulinum is seen with very high concentration as 10.5 mg/l in the canal and water storage tanks due to high microbial growth detected by simple conventional method of media culture. Boiling water with chemical treatment give maximum reduction as 99% of toxins level of different sample which very useful for domestic level. Increasing the toxins level in any living organism can create the chronic and acute diseases which is both harmful for society and economics.

Keywords: Toxins, Boiling Water, Microcystins, Coagulation

1. Introduction

Clostridium Botulinum is an anaerobic bacterial species with spore-forming and it produces a chemical toxins compound family of botulinum neurotoxin that are consisting of seven serotypes, A to G. These toxins are mostly known as strong natural food producing poisoning agents. It is a toxin related family where neurotoxins are offered resistance against the acidic environment and protease-rich environment of the gastric tract and their reaction mechanism make the reasons paralysis of the host in humans. Other botulinum toxins Serotypes A, B, and E are normally related with disease in humans, whereas toxin serotype F is associated less and serotypes C and D are not often connected with human botulism and infection. Botulinum toxin Serotype G has not been associated to human botulism for disease infection. *C. botulinum* is basically pathogen related with low-acid canned foods (LACF) and this type of bacteria may be originated in LACF medium [1, 2].

Botulinum toxin has 7 neurotoxins types as A, B, C, D, E, F, and G which are very different by antigenically and serologically but have similar structure. Disease in Human due to botulism is caused mostly by types A, B, E, and F (rarely). C and D Types can cause toxicity and disease only in animals. Several botulinum toxins have individual strengths and extra care is required to assure right use and need avoid from medication errors [3].

This infection is exceptional in the UK but collective in Eastern and Southern Europe due to more home preservation practice. The disease caused by B toxin which produced by bacteria and growing in canned food that had ineffectively heat treated, used to flavour to yoghurt products [4].

The spores due to *C. Botulinum* are found in soil and then germinate for the produce of toxins. It is caused by injecting the drugs that are contaminated by these spores. Wound botulism is very common form in the UK and there have 100 clinical cases diagnosed of wound botulism from 2002 to 2007, but all related cases are illegal injecting drugs [5].

In adults, small number of cases has reported worldwide, but disease in person results from absorption of *C. botulinum* spores by germination of the stomach and then production of toxin *C. botulinum*. In the UK and USA, botulism has related with the ingestion of corn syrup, honey and environmental sources as dust or soil of spores.

This study has based on the detection and removed of waterborne pathogen botulinum and their toxins from ground water, storage water tanks and canal water [6, 7].

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 *C. Botulinum* and related toxins such Botulinum under strike

slanders of quality. Samples were shifted to Lab where perform chemical analysis and other tests. 116 random samples are collected from each type of sampling from Ground water, water storage tank, Canal water. All sample collect in sterilized PVC bottles as the container.

Isolation and Identification of C. Botulinum

Media composition (**Agar Base**) used as Suspension is prepared by dissolving 37 grams media components in 500 ml ionized water and then heat it to its boiling state for complete mixed medium. Sterilize at 121°C by autoclaving at pressure 16 lbs at time 15 minutes and then keep the temperature from 50-55°C to avoid microbes contamination, aseptically add sterile 50 ml of Egg Yolk as Emulsion. Allow to mix well and transfer into sterile Petri plates for microbial growth. Incubated the Petri plates at 37 °C for the period of 24 hours. This process was repeated to other water samples for this test. Appears the colonies after incubation on the nutrient agar and number of positive (NP) samples recoded for this research. Final confirmation was made by biochemical reaction [8, 9].

Confirmatory Biochemical Tests For C. Botulinum growth

Further confirm C. Botulinum growth in avoiding the any error in this research, conduct the biochemical and physical tests (**Gram Staining**).

Preparations

Suspend 37 grams in 450 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50-55°C and aseptically add sterile 50 ml Egg Yolk Emulsion (FD045) and reconstituted contents of 1 vial of CBI Supplement (FD049). Mix well and pour into sterile Petri plates [10].

Laboratory Testing Method of Botulinum Toxin

The procedure of testing is involved the following steps for investigating liquid samples for the identification of botulinum toxin (Brooks et al., 2011), dilution buffer of 50: 1L was added to all wells that hold a sample, test sample of 50 mL was added to the dilution buffer and incubated the plates for one hour at 25 °C temperature.

Washed three times Plates with 200: 1L of ELISA washes buffer per Well. Detect antibody, was added of 100: L to each sample well and incubated the plates for one hour at 25 0C temperature and Washed Plates three times with 200: 1L of ELISA washes buffer per well. Added 100: 1L of substrate solution to each sample well, the plates incubated the plates for one hour at 25 °C temperature. Read the results visually. Colour changed of Different Wells that one differences in the negative and positive control as having given in results of liquid samples. The procedure took approximately 5 hours [11,12].

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 °C) for 10 minutes for chemical and microbiological analysis under standard procedure.

At the time of analysis, temperature of boiled water was 27 °C of drinking water samples [13, 14].

3. Results

Analysis of C. Botulinum and Botulinum Toxin

In table 1, it shown that chemical analysis of water samples for determination of toxins values as how much hazard for microbes but some Chemical pollution of drinking water with advanced treatment can be removed by simple boiling process. As drinking water have high amounts of toxins that is not fit for boiling process. Chemical analysis is done for treatment purposes and for removing contamination by adopting suitable process. For example, if calcium is high in waters then White precipitates are deposited in bottom of container during boiling process. In these cases, the boiling container must be washed accurately after use but tastes of Boiled water may not good. Then, temperature of boiled water can be reduced as cooled down at room *temperature for getting good taste.*

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

Sampling Type (mg/l)	C. Botulinum detection (%)		Botulinum Toxin	
	Mean±S.D	Range	Mean±S.D	Range
Canal Water	92±2	90-95	10.5±0.7	9-11.5
Storage Water Tanks	49±1.2	47-52	1.2±0.1	0.3-1.9
Ground Water	40±1.2	38-43	1.2±0.1	0.5-1.4

Effect of Boiling On Toxins Removal from Canal Water

Canal water has highly polluted with toxins as result shown in table 1 but boiling activity prove the lower of toxins due to breakdown of toxins compounds (figure 1 & table 2). Boiling process of drinking water is depend on fuel sources, if fuel sources available local then boiling process cost is affordable. Otherwise it not feasible and economical source for boiling process. Boiled process of drinking water can be caused of burn injuries due to handled non-systematic. Due to long time exposure with the fire or smoke, water boiling persons may be associated with respiratory illnesses. Due to this reasons indoor boiling space should be well ventilated.

Protozoa are the most common infection and disease causes agents in humans and animals. So drinking of raw water without treatment can causes of diseases that have significant public health impacts with socioeconomic. Water has an important role to plays the transmission of pathogens.

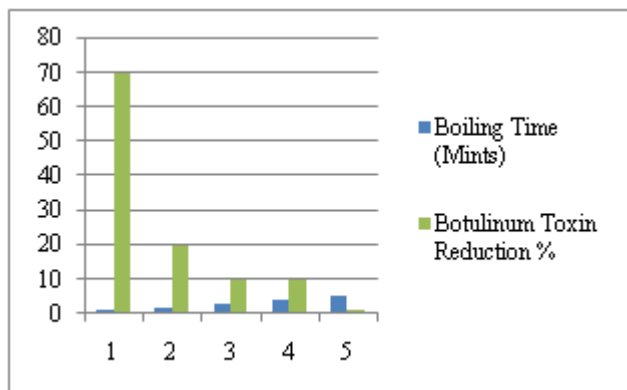


Figure 1: Graph showing the effects of boiling on removing of toxins in samples of Canal water

The waterborne transmission has real challenges for its control because large number of the pathogens produce or cysts or eggs and cysts which have strongly resistant to the processes used for the drinking water disinfection. But this is also possible in some cases as difficult to removing by processes of filtration.

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

Boiling Time (Mints)	Botulinum 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	10.5±0.9	9-11.5	7.9±0.5	7-8.4	75.23±4.9	72-78
2	10.5±0.9	9-11.5	5.5±0.4	4.9-6.7	52.38±3.8	49-55
3	10.5±0.9	9-11.5	3.2±0.3	2.9-4	30.47±2.9	27-33
4	10.5±0.9	9-11.5	1.1±0.1	0.7-1.5	10.47±1.9	8-12
5	10.5±0.9	9-11.5	0.3±0.09	0.5-1	0.85±0.07	0.6-1

Hot water can help to keep regular of your bowel movements with healthy and pain free and Dehydration can create the result in chronic problems as constipation disease. When the stool accumulates inside the intestine then the movement of the bowel relatively becomes slow. It is always suggested that you may consume a glass of hot or warm water on every morning when stomach is empty. Warm water decomposes the any foodstuffs inside stomach or intestine that can make the movement of the particles so smooth and less painful through the intestine

Effect of Boiling On Toxins Removal from Water Storage Tanks

In figure 2 and table 3, treatment effects of boiling are very useful for removing the toxins and only remaining as (0.83±0.1) in storage tanks. Boiled water has a great capacity to maintaining the body metabolism that is to normalize blood chemicals. The uncontaminated boiled water is very best to lose extra weight according to conducted by different studies. This is also an additional bonus, boiled water have ability to help for break down the deposited fat in body tissues that can causes of obesity in males and females.

Boiled water drinking habits is a good practice for prevention of common colds, coughs and a sore throat due to allergic and microbial agents. It dissolves phlegm and also helps to

remove it from your respiratory tract. As such, it can provide relief from a sore throat. It also helps in clearing nasal congestion. Some medical studies shows that boiled hot water can help to maintain the normal menstrual cycle in females. The heat of the boiled water appears the soothing effect on the abdominal muscles and no fat deposition possibility here.

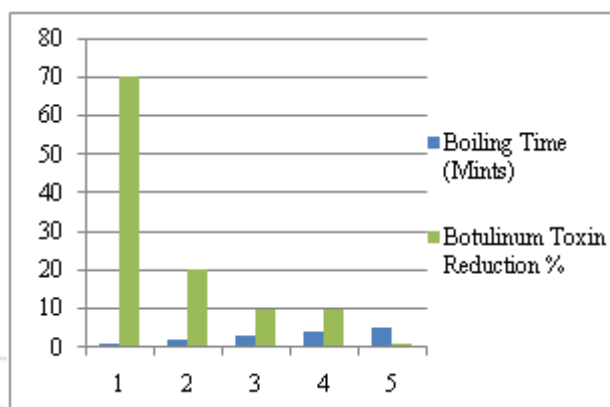


Figure 2: Graph showing the effects of boiling on removing of toxins in storage tanks samples

According to Winder’s study hot water is a very beneficial in food digestion because removal of unwanted materials. Some Studies have been shown that drinking of cold water before or after lunchtime can make the deposited the oil or fat present in the consumed diets. This situation can lead that fat be deposited on the wall of intestine towards inside that can cause intestinal cancer as eventual result. Conversely, if we replace a glass of cold water with hot then we can free from this bad situation and finally can say hot water is very helpful for the digestion.

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

Boiling Time (Mints)	Botulinum 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	1.2±0.2	0.3-1.9	0.9±0.1	0.7-2	75±5	72-77
2	1.2±0.2	0.3-1.9	0.5±0.1	0.4-0.7	41.66±3.7	38-45
3	1.2±0.2	0.3-1.9	0.3±0.1	0.2-0.5	25±2.9	23-27
4	1.2±0.2	0.3-1.9	0.1±0.01	0.0-0.1	8.33±1.8	5-10
5	1.2±0.2	0.3-1.9	0.01±0.02	0.0-0.1	0.83±0.1	0.6-1

Effect of Boiling on Toxins Removal from Drinking Ground Water

In table 3, it has shown clearly that toxins are reduced by (0.83±0.1) % after 5 mints boiling which are good results. As boiled hot water has the strange quality as helping to body for detoxification. Because if take boiled hot water to drink then feel to rise the body temperature and finally start the sweating. This process is very useful for removing the fat molecules and other toxins which are staying in body. Experimentally, if want this to happen because it helps to release toxins from your body chemically and cleanse it properly. If feel order problems against optimal results, add a little squeeze of lemon before drinking of water.

A little hot drinking water has good result to enhance the blood circulation that is an important for proper growth of muscles and activities of nerve. An addition benefits as to keeps your nervous systems are healthy due to breaking down of deposited fat.

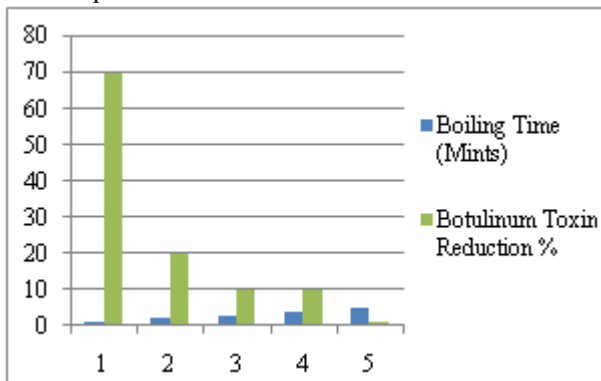


Figure 3: Graph showing the effects of boiling on removing of toxins in ground water samples

In figure 3, it can be seen clearly that toxins removal mechanism depend on boiling time duration and it is also good for health naturally as According to some research studies, it has been show that boiled drinking water supports to help to repair the skin cells that is causing to rise the elasticity of upper surface of skin that are affected by hurtful free chemical radicals. Purified drinking water can improve the damaged skin into smoother. Drinking hot water has **Benefits** on skin as for obtaining the shiny and soft hair. Hot drinking water supports the energetic activities to hair roots for strong growth. This is important for your hair growth as the natural vitality.

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

Boiling Time (Mints)	Botulinum 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±0.2	0.5-1.2	0.7±0.2	0.5-1	70±4	67-72
2	1±0.2	0.5-1.2	0.2±0.01	0.1-0.4	20±3	18-22
3	1±0.2	0.5-1.2	0.1±0.01	0-0.1	10±1.8	8-12
4	1±0.2	0.5-1.2	0.1±0.001	0-0.1	10±1.5	8-11
5	1±0.2	0.5-1.2	0.01±0	0-0	1±0.1	0.7-1.4

4. Discussion

Botulism is a thoughtful and unusual, paralytic disease that is caused by neurotoxins and it is produced by the common bacterium species as name Clostridium Botulinum. C. Botulinum is found all over the world in samples of soil and ocean sediment. Usually, the bacterium can survive in the special environment of a resting spore. On the other hand, in low oxygen environments (anaerobic) such as in case of canned foods, intestinal tract, deep wounds and spores germination convert into active bacteria, then it multiply with passage of time and produce neurotoxin. C. Botulinum creates 8 types of different toxins (from A to H) which are known as the strongest toxins. Botulinum toxin is produced by Clostridium Botulinum that is a gram-positive anaerobic bacterium group. Clinical disease related to

botulism can take place with ingestion of contaminated food and then settlement of bacterial growth inside the gastrointestinal tract. The infection due to c. botulinum as wound infection can also cause of spore's transformation from person to person. Food net active surveillance program is indicated that there are 459 and 264 cases of STEC per million people in 2009, respectively [15, 16, 17].

As earlier studies on treatment of potable water using with chlorine have shown the results which showed that different optimum dose of chlorine can disinfect the microbes in different types of samples effectively. Some studies showed a 5 mg/l was ineffective for destroying toxin extracts but it is demonstrated that joint flocculation management procedures which involved chlorination 0.7 mg/l dose also successful for killing of microbes (Rangel et al). Dissolved microcystins, Botulinum toxin and shiga toxin in the range 5-10 mg/l, showed at pH 5, removal was more than 93 % within 30 minutes but at elimination touched only 85 percent 22 hours after. Cylindrospermopsin can be effectively degraded by chlorination. With cylindrospermopsin concentrations of 17-185 mg/l, the concentration of residues of chlorine was 0.5 mg/l as sufficient to disinfect more than 99.5 % of microbes. At pH range 6 to 9, a great cause of microbe's deaths occurred rapidly within very short of time [18, 19].

Boiling with addition of chemicals alum and ferrous also used as disinfectant but new thing in this study is specific dose is determined for three samples of drinking water with no residue of chemical left in treated water because it can cause cancer in humans. Another comparative studies show that Contaminated canal water for drinking purpose needs dose 3mg/l of chlorine for complete disinfection without residues and 1.5 mg/l dose of chlorine is required for treating the drinking water of storage tanks without any toxicity causing by chlorine residues [21]. Chemical coagulation as treatment of drinking water with Aluminium Sulphate was very effective for removing toxins (shiga toxin, Botulinum toxin and Microcystins) from drinking water as well use FeCl₃ salt as coagulation process and results of two coagulant were confirmed that removing of toxins from FeCl₃ as compare to Aluminium salt. In the process of filtration as final treatment was used the Granular Activated Carbon material which proved very useful for maintains the taste and natural smell of drinking water in the experimental research study [20, 21].

Some pervious research has proposed the most economical method in treating the drinking water with coagulation treatment process for humans, the cost is Rs.1.25 per litre calculated but in case of open surface water is only Rs.0.15 cost. After treatment, it is sure to safe water for drinking and domestic purpose [22, 23].

5. Conclusion

Due to incomplete of an infrastructure for detection and recording infection disease due to contaminated potable water and its source, a little information is available on water borne diseases in Pakistan. Excess amount of Toxins in human body can initiate different diseases and this need remove from water supply properly. Remarkable results given in treatment with boiling and mixing chemical like

coagulant aluminium sulphate and ferric chloride for removing of toxins in drinking water samples of canal, storage and ground along with coagulation and boiling methods.

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