International Journal of Advanced Engineering Research and Science (IJAERS) *[Vol-3, Issue-11, Nov- 2016]*
https://dx.doi.org/10.22161/ijaers/3.11.2 *ISSN: 2349-6495(P) | 2456-1908(O)*

Microbiological Botulinum Toxins Removing From Drinking Water Sources by Treatment of Coagulation Process

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Abstract— *Water is a very important nutrient and responsible to maintain good health as well as proper performing the body functions, Water can remove the harmful toxins from the body.*

Infective disease produced by pathogenic microbes like bacteria, parasites and viruses including their metabolites as toxins are the known as most common and common health risk which connected with unsafe drinking water. It is expected; around 1.1 billion people worldwide have to drink unsafe drinking water per day. More than 95 % of these deaths are possible in low-income countries, where numerous causes like malnutrition, poor hygiene and sanitation create the immune deficiencies and specially factor such as unsafe drinking of water strongly affected on it.

In the present study, C. Botulinum as bacterial specious and its related toxin botulinum toxins are detected in samples of ground water, water storage tanks and canal water but low values of toxins present in ground water sample and high values find in canal water sample.Coagulation process is used for removing the Botulinum toxins from drinking water source and giving the amazing results as show 92-97% toxins removes from drinking water samples by using the coagulant aluminium sulphate.

Keywords— Botulinum Toxin, Coagulation process, C. Botulinum, Removing toxin, Aluminium Sulphate.

I. INTRODUCTION

C. Botulinum was first discovered by E. van Ermengem in 1897 afterward his study on foodborne microbes and disease in Ellezelles, Belgium. Botulism is Foodborne microbe and found rare, but contaminated products with this may exposed to humans and animals. C. Botulism can produce a neuroparalytic illness causing by the contamination with it. Foodborne botulism can create an emergency of a medical and a public health and to avoiding this problem need effective messaging between clinicians and public health departments [1].

The types of C. Botulinum from A to G are different by only the antigenic characteristics found in the neurotoxins which they produced. C. Boulinum with Types A, B, E, and only F in rare cases found which cause illness in humans and animals. Especially C. Bolulinum with Types C and D cause illness in mammals and birds and type G are identified in 1970 which cause of infection in humans or animals [2].

The word of Botulism read from the language Latin as botulus, and its meanings is sausage. Botulism word was first time familiar in Europe and several cases were registered which caused by house fermented sausages. Historically importance, four different forms of botulism can observe, but depending on the mode of reaction of these toxins. Wound infection botulism is produce by c. botulinum that multiplies and then produce toxin in a contaminated wound of human or animals. Botulism is due to the harmful production of toxins by spores of C. Botulinum in the intestine, and Teen-ager or adult infected mostly [3, 4].

Botulism is a thoughtful and unusual, paralytic disease which caused by neurotoxins produced by the common bacterium specious as name Clostridium Botulinum, C. Botulinum found all over the world in samples of soil and ocean sediment. Usually, the bacterium can survive in the special environment as 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 most strong toxins. Botulinum toxin is produced by Clostridium Botulinum which 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

https://dx.doi.org/10.22161/ijaers/3.11.2 *ISSN: 2349-6495(P) | 2456-1908(O)*

due to c. botulinum as wound infection can also cause of spores transformation from person to person [5, 6].

Our study goal is to identify botulinum toxins in different samples and remove the toxins of botulinum species using coagulation method with economical ways.

II. MATERIALS AND METHODS

Area of Study

Sheikhupura is a city of Punjab, Pakistan which is located about 36 Km from Lahore. Sheikhupura lies 31° 42' 51.16 "N latitude and 73° 59' 3.49" E longitude. The city is well connected with its surrounding big urban centres like Faisalabad 94 Km, Sargodha 143 Km and Gujranwala 54 Km. Sheikhupura is also a railway junction. Importance of Sheikhupura city is due to its commercial zones and large number of industrial units which can contaminate water.

Ground water sampling

Random ground water samples were collected from different houses by 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. All sample collect in sterilized PVC bottles. The bottles were filled up to 100 % of the volume capacity (1 L). The temperature was 20 ^{0}C at the time of sample collection [7].

Sampling from water storage tank

Random water samples were collected 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 was 7±0.9 feet and width 3±0.4 feet and the temperature of the day at sampling was 27 ^{0}C. All samples were collected in sterilized PVC bottles and were filled 100% of the volume capacity (1 L).

Canal water sampling

Random canal water samples were collected from different source points and the frequency of samples (n= 10). All samples were collected in sterilized PVC bottles which were filled 100 % of the volume capacity and actual capacity of the bottle was 1 L. The temperature of sampling day was 16 ^{0}C.

Analytical methods

Parameters like turbidity and colour were analysed on a spectrophotometer (Shumedzu 2011), according to the recommended procedure by the Standard Methods (APHA, 2005). pH was determined by using a pH digital meter (coloria 2011), operating under the manufacturer's methodology . Removal of toxins was examined by using the Utermöhl method (1958), which includes the sediment organisms counting on an especial chamber under an inverted microscope of good quality [1].

Isolation and Identification of C. Botulinum

Agar Base Media composition is used and media solution is prepared as the Suspension 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 microbe's contamination, aseptically add sterile 50 ml of Egg Yolk as Emulsion. Allow mixing well and transferring into sterile Petri plates for microbial growth [7].

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.

Confirmatory Biochemical Tests for C. Botulinum growth

Further confirmatory test for C. Botulinum growth in avoiding the any error in this research, conduct the biochemical and physical tests as below.

Catalase and in dole Tests

C. Boulinum confirmatory test method is involved standard test method as When bacterial growth mixed with reagent, if reaction give colour red to yellow, test will be positive and indicate presence of microbial growth [8].

Gram Staining

The microbial growth from media was spread and fixed on a clean glass slide and stained by putting the colour dye named as crystal violet on slid for 30 seconds and then washed it with distilled water. Then Gram's iodine was added on it for 10 seconds, after this, washed with tap water and uses decolourised as 95% Acetone alcohol solution and added finally safranin as secondary dye for 30 seconds. Final step was washed with tap water and dried in normal air. It is observed the slide under microscope with oil immersion objective of power 100X [9].

Aluminium Sulphate (AS)

Aluminium sulphate with a chemical formula $Al_2(SO_4)_3 \cdot 14H_2O$ was used in these experiments. Different composition of solutions (5 to 30mg/l) was prepared using AS as calculated amount of salt dissolved in deionised water. The flocculation/coagulation process under condition of rapid mixing regents and chemicals on (116 rpm) for mixing time (7 min) and for slow mixing use (27 rpm) and , time duration (11 min) [10].

Toxins Detection Methods

Only ELISA method is used for toxins analysis which still very useful and reliable in analytical testing field. The samples were analysed inside Chemical Bio-Tech laboratories, staffed with technicians and they skilled to handle the safely about botulinum toxin. Every day, fresh reagents were prepared

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from standards solutions in distilled water. Prepared the each sample was in its own container and labelled with identification number of sample that also noted in a laboratory record book with particulars of the sample preparation. Before analysis of each sample, the verification of staff recorded about the sample identification with number on a sample data sheet. After complete the analysis, sample results recorded on the sample data sheet. All test samples were three times repeated for analysis [11].

III. RESULTS

Microbe C. Botulinum detection and Toxins Analysis in drinking water samples

Toxins concentrations in the canal Water samples, water storage tanks samples and ground water samples were monitored using ELISA method which is very reliable for a detection toxin from microbes. To find the different concentrations of toxin level in different samples and there was seen that in canal water samples the toxins with very high values as Botulinum toxin (10.5±0.7) mg/l with range of 9-11.5 as shown in table-1. The toxins with very low values was observed in ground water samples as botulinum toxin (1.2±0.1) mg/l and in range 0.5-1.4, respectively as shown in tables 1&2.

Table.1: Detection of Microbes in Different Water Samples Analysis

Microbe	*Water Storage Tanks samples (%)*		*Ground Water samples (%)*		*Detection in Canal water Samples (%)*	
	Mean±S.D	*Rang*	*Mean±S.D*	*Rang*	*Mean±S.D*	*Rang*
C. Botulinum	*49±1.2*	*47-52*	*40±1.2*	*38-43*	*92±2*	*90-95*

Several factors contribute to the production the toxins like environmental condition and nutritional requirement, so C. botulinum can grow in surface water as faster and toxin botulinum produced quickly. Only microcystins toxin limit is 1 mg/l under the WHO in drinking water but other toxins like botulinum have no standard limit given in quality of drinking water. Water temperature varied from 11 to 20 ^{0}C, during our study due to extreme change in weather condition about 16 to 20 ^{0}C, such changes temperature are characteristic for this area. Table-2 was showed the concentration of Botulinum toxins found in different types of drinking water samples that depend on presence of microbe C. btulinum in quantity but these values of toxins are not in normal range.

Table.2: Water Analysis Data of different Samples

Sampling Type	Botulinum Toxin	
	Mean±S.D	Range
Canal water samples (mg/l)	10.5±0.7	9-11.5
Storage Tanks water samples (mg/l)	1.2±0.1	0.3-1.9
Ground Water samples (mg/l)	1.2±0.1	0.5-1.4

Effect of coagulant aluminium sulphate on Botulinum toxins removing

In **table-3**, could be seen that aluminium sulphate create good results to remove the toxins from canal water samples by using high dose of coagulant because canal water observed often time highly contaminated with carbon based dissolved solid like as microbes biomass and their metabolites as toxins. However, these organic materials have great capacity to be dissolved in water and make the source of contamination commonly in canal water.

Table.3: Data of Aluminium Sulphate as Coagulant Doses for Removing Toxins from Canal Water Samples

Coagulant Dose (mg/l)	Botulinum toxin(mg/l) (Actual value)		Botulinum toxin (mg/l) (after treatment)		Botulinum toxin after treatment (%)	
	Mean	Range	Mean	Range	Mean	Range
5	10.5	9-11.5	9	5-10	85.71	83-86
10	10.5	9-11.5	7	3-8.5	66.66	63-68
15	10.5	9-11.5	5	1-7	47.61	45-49
20	10.5	9-11.5	3	0.5-5	28.57	26-29
25	10.5	9-11.5	1	0.5-2	9.52	7-11

It is clearly seen in table-3 that value of Botulinum toxins had 1 % values with range 2-3 at AS dose 10 mg/l and 9.52 % values with range 7-11 respectively found at same coagulation dose 25 mg/l. it means that percentage of above given values show removal efficiency of different toxins by using the Aluminium Sulphate coagulation and further idea is created that need of high dose for proper coagulation process in canal water samples.

During sampling process, It was came into our notice that all the animals and some human use canal water as direct source of drinking and domestic purpose. The high level of confederation of toxin in canal water is the basically source of disease of liver, skin and disorder stomach function for equally both animals and humans as well.

https://dx.doi.org/10.22161/ijaers/3.11.2 *ISSN: 2349-6495(P) | 2456-1908(O)*

Gradually used the aluminium sulphate in different dose for removing of toxins because here objective was to find optimum dose value for treatment of water to drinking purpose. Increase the dose of aluminium sulphate step wise is the chance to minimise error in these batch experiments. Actually, in under developing countries facing the problems related with water treatment is badly effected due to mismanagement of handling the chemical dose in coagulation process which may show un- effected results on water treatment properly and appears the toxic effects on public health by drinking.

In general, organic molecules as toxins removed 95-97% in these results with treatment of coagulant aluminium sulphate using the different dose that depend on concentration of toxins in drinking water as seen in tables (3,4&5) in different Samples.

Table.4: Data of Aluminium Sulphate as Coagulant Doses for Removing Toxins from Storage Tank Water Samples

Coagulant Dose (AS) (mg/l)	Botulinum toxin(mg/l) (Actual value)		Botulinum toxin (mg/l) after treatment		Botulinum toxin after treatment (%)	
	Mean	Range	Mean	Range	Mean	Range
2	1.2	0.3-1.9	1.1	0.8-1.5	91.66	89-93
4	1.2	0.3-1.9	0.9	0.6-1.2	75	74-76
6	1.2	0.3-1.9	0.7	0.5-0.9	58.33	56-60
8	1.2	0.3-1.9	0.2	0.3-0.5	16.66	14-18
10	1.2	0.3-1.9	0.1	0.1-0.2	2.5	2-3

In table-5, it had significantly observed the low values of toxins seen in ground water drinking samples and these values were much correlated with table-4 because ground water stored in water storage tanks.

Table.5: Data of Aluminium Sulphate as Coagulant Doses for Removing Toxins from Ground water samples

Coagulant Dose (mg/l)	Botulinum toxin(mg/l) (Actual value)		Botulinum toxin (mg/l) after treatment		Botulinum after treatment (%)	
	Mean	Range	Mean	Range	Mean	Range
1	1	0.5-1.2	0.9	0.5-1.1	90	89-92
1.5	1	0.5-1.2	0.7	0.4-0.9	70	68-72
2	1	0.5-1.2	0.5	0.3-0.7	50	47-53
2.5	1	0.5-1.2	0.2	0.1-0.4	20	18-22
3	1	0.5-1.2	0.0	0-0.2	1	0.5-1.7

The greatest toxins reduction was observed in the water storage tanks samples and ground water samples, where 95.0 % and 97.0 % toxins removal (figure 1,2&3) were found with aluminium sulphate (4 to10 mg/l) by concentration. Even uses of Aluminium sulphate with Lower dosages effective for the destabilization of toxins and other organic materials because of amino groups in the toxins have high charge density.

Figures (1, 2&3) were gave a critical picture in which this research came on one point that hazards toxins in drinking water sources were able to remove using the different chemical treatment process but aluminium sulphate dosing could be applied in control range for getting the maximum removing efficiency. And dosing of aluminium sulphate depends on nature of toxins concentration in different types of drinking water sample.

The treated water was observed as lowest values in the canal water as (0.7-0.8 mg/l), water storage tanks as 0.5-0.21 mg/l, ground water as 0.31-0.1 mg/l previous up and low values of treated water as seen in tables (3,4 & 5). The removal of dissolved organic carbon as toxin and other organic matter was increased with the removal of turbidity and colour and it is reported the presence of toxins and dissolved organic carbon can be removed effectively with treated the aluminium sulphate as a coagulant in high concentration.

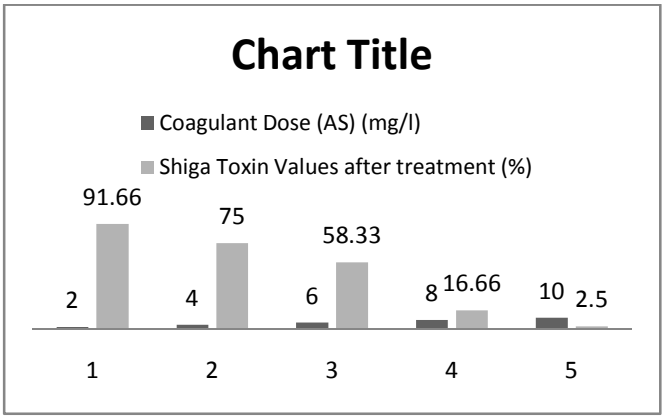

Fig.1: The graph shows the different dose effect on removing toxins in samples of drinking water storage tank

Given the results in tables (3,4 & 5) indicate that remove of toxins with organic matter of low molecular weight depends on the coagulant concentration and need to use this strong

https://dx.doi.org/10.22161/ijaers/3.11.2 *ISSN: 2349-6495(P) | 2456-1908(O)*

coagulant for canal water treatment for drinking purposes. The organic compound maximum removals observed in the samples of canal, ground water and water storage tanks samples waters with low values of Botulinum as shown in figure 1, 2 & 3 and it is also reported that the removal efficiency of coagulant depend on its molecular weight.

The results in tables (1&2) showed the presence of bacterial C. Botulinum and toxins was noted higher in canal water as compare to water storage tank and ground water samples. So, detection of bacterial group in a large number implies that the contaminated drinking water may be responsible for increasing number of water borne diseases in the country. It is evident from the study that water quality further deteriorated at the consumer level [14].

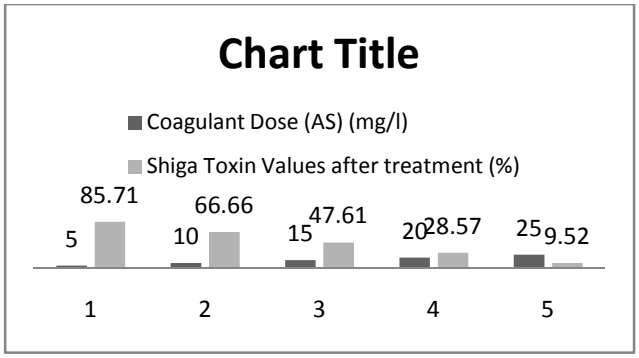

Fig.2: The graph shows the different dose effect on removing toxins in samples of Canal water

After the coagulation treatment with aluminium sulphate with high dose, the toxin decreased about 0.8 mg/l in most all samples of the treated water. Figure 2&3 showed that the drinking water is fusible is useful for drinking and other domestic uses.

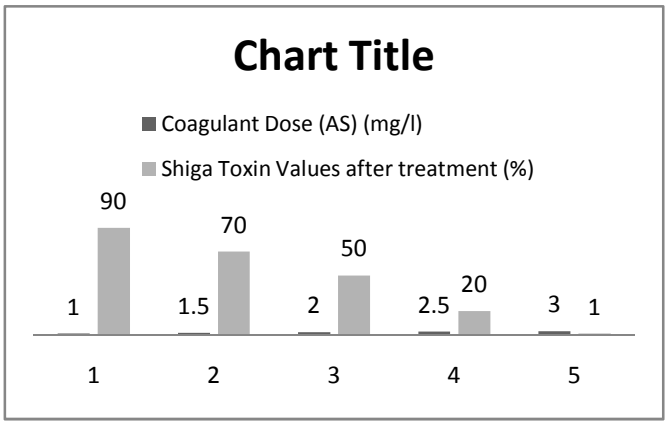

Fig.3: The graph shows the different dose effect on removing toxins in ground water sample

This is also formation in literature that by-products chlorinated water likes as trihalomethanes therefore will be less but organic matter mostly found in non-humic form with low weight.

IV. DISCUSSIONS

Identification of C. botulinum in water samples from area of city Sheikhupura was not thinkable due to non-professional way to control the quality of drinking water. Physical condition of temperature, light and humidity is very effective on producing water borne pathogens and their metabolites toxins. In the study, the pathogenic Microbe C Boulinum in samples of potable water is relative to sources of contamination [12, 13].

In the present study, these results had showed some similarities observed as in under developing countries like rural area of Pakistan has not correct quality of drinking sources of water for domestic purposes. Same results derived as given by the study's results from Brooks *et al*. (2011), it reported that in most of the developing countries, the quality of drinking water very not good as find some bacterial specious as per 100 ml of potable water used in domestic purpose. The recommend drinking water values of all limits under WHO guidelines is 100 ml per sample for detecting the total coliform group [15].

Toxins removes from drinking water samples by using the coagulant water treatment and boiling is also give more than 95% result for toxins removing. The diseases i.e. Diarrhoea, Dysentery, Gastroenteritis, Typhoid fever and Cholera and other water related nosocomial infection in health care setting may be the result of consumption of such polluted water [16].

The spores due to C. Botulinum, present in soil and then germinate for the produce of toxins and it caused by injecting the drugs that are contaminated by 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 [17].

Infected injection is one cause of accidental botulism in pharmaceutical preparations may cause of botulinum neurotoxin, Such as four cases registered in December 2004 in state of Florida, cosmetic injection with contaminated with botulinum toxin that was not allowed for human purposes. It is not cases reported in the UK, but Inhalation of botulism has confirmed as three cases reported by veterinary technicians in Germany in 1962. C. boulinum toxin is a high possible route for releasing. Botulism due to Water borne may also be produce by ingestion of toxin. Accidental inhalation botulism, three cases were registered [18].

https://dx.doi.org/10.22161/ijaers/3.11.2 *ISSN: 2349-6495(P) | 2456-1908(O)*

This group is belong to bedrail specious and produce the Botulinum toxins, Our findings reveal the presence of C. Botulinumin 67% in drinking water samples collected from water storage tanks and 45 % detect in ground water in this study as given in table-1. The C. Botulinum and its toxins represented as faecal pollution of potable water and this is also an indicator organism as pathogenic if detected in raw sea foodstuff and any other foods. C. Botulinum denotes asrisk alarm of public health and C. Botulinum knows as pathogenic bacterium [19].

In case when the long-time abdominal infection and catheterization of bladder toxicities feeling is already reported due to producing C. Botulinum and their toxin. The isolation rate of C. Botulinums was significantly greater in summer months than in winter months in drinking water all samples from khanpur rural area in city sheikhupura, respectively. Samples of the study area were contaminated with C. Botulinum during three successive study years and displayed some toxicity level in blood samples as given in table-1. The always occurrence of organism and their toxins show the unhygienic condition of quality of drinking water in specific region. Both anthropogenic and from animals are the main source of contamination of this microorganisms due to the drinking water reservoirs was remained unprotect and open and facing activities of animals and human. Moreover, the chlorine treatment of potable water for domestic purposes was insufficient before the proper chlorine treatment [20, 21].

Botulinum toxins producing rate depend on C. Botulinum bacteria, high concentration of botulinum toxins seen in canal water as see table-2. Botulinum toxins effected on paralysis disease directly both male and female, and this was seen during interviews before sampling [22].

V. CONCLUSION

Almost the world's population now presently facing the deficiencies of potable water with better quality, with using of correct and applicable technology or water purifications above methods for domestic water are a wonder full solution of these problems with very low prices.

The removal of toxins inform of organic matter was manage to minimise the toxicity in the experimental steps from drinking water samples. Coagulation experiments conducted to know actual effective and improved dose used to optimise for coagulation process for maximum removal of toxins from drinking water. Selected aluminium sulphate coagulation dosing (10mg-27mg) for treating the contaminated drinking water from different samples canal water, water storage tanks and ground water, this dose does not causes of toxicity to human and animal health as the aluminium residues present in drinking water supplies.

Hygiene information is very important for better utilization of safe water drinking. Moreover, the procedure involved for drinking water management system and how to store at the domestic level, it is need to increase knowledge of individual and community about the awareness of water hygiene and public health.

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