

Removal of cyanobacterial toxins from drinking water sources by aluminium sulphate treatment

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Abstract. Cyanobacterial toxins are very harmful and known as hepatic toxins and a major cause of liver damage. They can easily grow in water sources under specific conditions of temperature and small nutrition. In this study, the cyanobacterial toxins were identified from different samples of drinking water sources and blood of that water consumers like animals (cattle & buffaloes) and liver infected people. The values of cyanobacteria toxins (microcystins) were very much correlated in each type of samples in the designed study but high values of toxins were seen in canal water samples. It is dangerous for both human and animals as shown in results because of high values of microcystin were present in cattle and buffaloes blood samples. Aluminium sulphate was used to treat contaminated water which is an inorganic molecule and its reaction is very fast with dissolved particles in water samples. The effectiveness of aluminium sulphate as coagulant in the coagulation/flocculation process for removing the Cyanobacteria toxins from 1-0.1 mg/L on average from all different types of water sample sources (ground water, canal water and water storage tanks in houses). It was found as very useful to clean the drinking water by using its different concentration like 5, 10, 15, 20 and 25 mg/L. This study reveals a drastic picture of drinking water conditions in Pakistan however, the contaminated water can be treated effectively by using aluminium sulphate.

Keywords: Wastewater treatment, Cyanobacteria, Microcystins, Toxins, Aluminium sulphate.

Introduction

It is commonly observed that drinking water supply system in some parts of world, as well as in Pakistan, has exhibited an increase in the growths of cyanobacterial blooms and contaminated the water. Cyanobacterial blooms contribute to an objectionable odour and taste which is produced in water and cause difficulties in water treatment systems. However, the key anxiety to the growing

cyanobacterial blooms is the increasing their production and as to the discharge of cyanotoxins. Cyanotoxins can cause serious diseases and an infection to consumers if their concentrations are higher. Ingesting and inhalation of toxins produced by cyanobacterial species is a great cause of poisoning in people and animals. These cyanobacteria may create specific microcystins with hepatotoxic chemical reaction in both humans and animals (Antoniou et al., 2005). Now to date,

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microcystins have been identified as more than 60 variants of structure and microcystin-LR look as one of the most usually microcystins in drinking water source across the world (Henderson et al., 2010; Ho et al., 2011). Therefore, the research has motivated on this area of particular toxin. As increasing the concern with health problems, the World Health Organization (WHO) recognized the guideline of drinking water is level of 1.0 mg/L for particular microcystin-LR as (MC-LR) (Codd et al., 2005).

A recent method for removal of cyanobacterial toxins with the help of chemical coagulants such as aluminium sulphate (AS) has been used by several (de la Cruz et al., 2011). Treatment of Water with AS coagulant creates low slurry volume and biodegradable sludge produces which does not affect the pH of water. Previous studies have shown the efficiency of AS in reducing microorganisms and turbidity in raw drinking water (Dixon et al., 2011a). Additionally, AS can stop the growth of some cyanobacterial species related to such as *Microcystis aeruginosa* (Dixon et al., 2011b) and AS is also the active chemical agent in aqueous medium and more effective in stopping the growth. Moreover, the cost of AS coagulant would be less when it compared with drinking water borne diseases treatment conventionally (Falconer and Humpage, 2006).

Another effective method for removing of cyanotoxins and cyanobacteria is coagulation process with some effective chemicals like ferrous or aluminium salts for removing the dissolved organic matters like toxins. Aluminium salts have strong positive and negative charges to attract organic molecules. It is common satisfactory way to remove cyanobacterial cells and cyanotoxins because of big size and high molecular weight of these molecules (Falconer, 2005). Coagulation/flocculation procedure are separately very effective in the purpose of removing of cyanobacterial cells and cyanotoxins, so these processes for the drinking water treatment of removing the cyanobacterial blooms and cyanotoxins becomes feasible (Lehman, 2007).

Current study proves the effectiveness of the aluminium sulphate coagulation/flocculation process in the removal of cyanobacterial cells and cyanotoxins.

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 21.34 m (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 °C at the time of sample collection (Eaton et al., 1995).

Sampling from water storage tank

Random water samples were collected from different water storages tanks of houses with the depth of 0.5 m and frequency of samples (n = 116). The average height of storage tanks was 2.13 ± 0.27 m (7 ± 0.9 feet) and width 0.91 ± 0.12 m (3 ± 0.4 feet) and the temperature of the day at sampling was 27 °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 °C.

Analytical methods

Parameters like turbidity and colour were analysed on a spectrophotometer (Shumedzu 2011), according to the recommended procedure by the Standard Methods (Eaton et al., 1995). pH was determined by using a pH digital meter (Coloria 2011), operating under the manufacturer's methodology. Removal of cyanotoxins and cyanobacterial cells was examined by using the Utermöhl Method (Utermöhl, 1958), as to methodology adopted by Lund et al. (1958), which includes the sediment organisms counting on an especial chamber under an inverted microscope of good quality (Nkurunziza et al., 2009).

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, 10, 15, 20, 25 and 30 mg/L was prepared using calculated amount of AS salt dissolved in deionised water. The coagulation/flocculation process followed the condition as under rapid mixing chemicals reagents on 100 rpm for mixing time 5 min and for slow mixing use 10 rpm and time duration 10 min (Nwaiwu and Lingmu, 2011; Eaton et al., 1995).

Results

Toxins analysis

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. The concentrations of toxin level in different samples varied significantly as in canal water samples, the toxins with very high values as microcystins 22 ± 2 mg/L with range of 18-23 as shown in Table 1. The toxins with very low values were observed in ground water samples as microcystins 2.3 ± 0.2 mg/L and in range 1-2.9 detected, respectively as shown in Table 1.

Several factors contribute to the production the toxins like environmental conditions and nutritional requirement, so algae and Cyanobacteria can grow in surface water as faster and toxic microcystin are produced quickly. The microcystins toxin limit is 1 mg/L as determined by WHO in drinking water but other toxins like botulinum and shiga have no standard limit given in quality of drinking water. Water temperature varied from 11 °C to 20 °C during our study due to extreme change in weather condition about 16 °C to 20 °C, such changes temperature are characteristic for this area. Human and animals blood analysis showed that toxin values had a correlation between them and both of their food chain suffering from hazardous toxins shown in Table 1. Sampling area includes human or animals suffering from a disease or a type of illness related with gastro or liver functions (Ode et al., 2005).

It is shown very clearly from Table 2 that toxins producing rate depends on microbes cyanobacterial species because it is only organism which responsible the microcystin hapatoxins.

Table 1. Data of water & blood (human & animals) samples analysis.

Sampling type	Microcystin (toxin)	
	Mean \pm S.D	Range
Canal water samples (mg/L)	22 ± 2	18-23
Storage Tanks water samples (mg/L)	5.5 ± 0.3	4.5-6.8
Ground Water samples (mg/L)	2.3 ± 0.2	1-2.9

Table 2. Detection of microbes in different water samples analysis.

Microbes	Water storage tanks samples detection in percentage (%) per samples		Ground Water samples detection in percentage (%) per samples		Canal water samples detection in percentage (%) per samples	
	Mean \pm S.D	Range	Mean \pm S.D	Range	Mean \pm S.D	Range
Cyanobacteria	63 \pm 2.5	60-66	42 \pm 1.9	40-44	97 \pm 2.7	95-99

Table 3. Data of aluminium sulphate as coagulant doses for removing toxins from drinking storage tank water samples.

Coagulant dose (AS) (mg/L)	Microcystin (mg/L) (actual value)		Microcystin (mg/L) value after treatment		Microcystin values after treatment (%)	
	Mean	Range	Mean	Range	Mean	Range
2	5	5-6	4.2	3- 4.5	84	82-84
4	5	5-6	3	2-4	60	58-62
6	5	5-6	2	1.5-2.8	40	38-42
8	5	5-6	1	0.5-1.9	20	19-21
10	5	5-6	0.1	0.1-0.5	2	.09-3

Effect of AS on toxins removal

Microcystins was existed 84% in range of 82-84% and 91.66% of botulinum in range of 89-93% with dose of 2 mg/L (aluminium sulphate), which needed to increase the efficacy of coagulant dose. By using coagulant 10 mg/L, 0.066% with range 0.01-0.1% shiga toxin, microcystin 2% with range 2-3% and botulinum toxin 2.5% with range 0.09-3% was removed, removed (Table 2). Different doses of coagulant aluminium sulphate were used as 2, 4, 6, 8 and 10 mg/L which showed good results even no need of high dose or concentration of coagulant aluminium sulphate because water samples from water storage tanks had very low values of toxins in it (Table 2). It is mentioned in literature about calculation of coagulant dose that if pollutants load is high, then more dose of coagulant is needed for proper removal of organic molecules dissolved in it (Ozmen et al., 2006).

In addition, AS consists of aluminium as metal which is most reactive and ability to losing of electron and form chemical bond to other non-metals. In these studies, we observed that increasing amount of coagulant could increase the toxins removal rates under the controlled conditions of temperature and pressure (Figure 1).

AS is very efficient to remove toxins from canal water samples by using high dose of coagulant as canal water observed often highly contaminated with carbon based dissolved solid like microbes 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. It is clearly seen in Table 3 that toxins like as microcystins had 18.18% values with range 16-22%, respectively found at same coagulation dose 25 mg/L showing the removal efficiency of toxins by using aluminium sulphate. During our sampling, 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 stomach disorder. Actually, in 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 ineffective results on water treatment properly and appears the toxic effects on public health by drinking.

In general, 95-97% organic molecules (toxins) were removed with treatment of coagulant aluminium sulphate using the different doses that depend on

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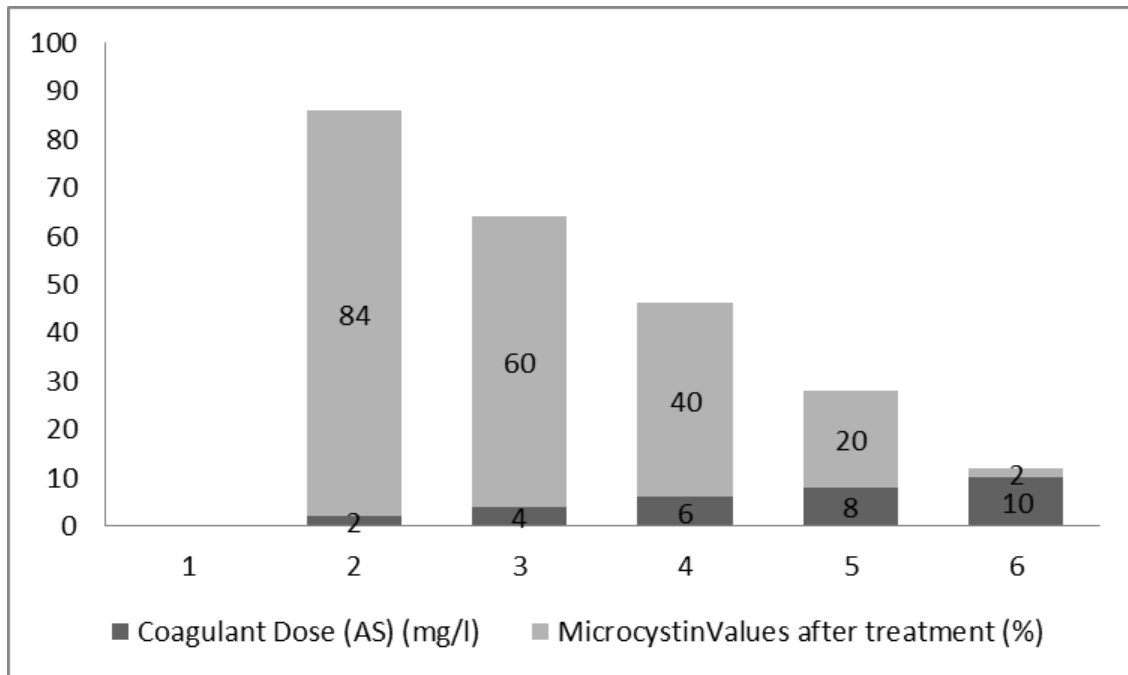


Figure 1. The graph shows the different dose of coagulant effect on removing toxins in a sample of water storage tanks.

concentration of toxins in drinking water as seen in Tables 3, 4 and 5 in different samples. The greatest toxins reduction was observed in the water storage tanks samples and ground water samples, where 95% and 97% toxins removal were found with aluminium sulphate concentration of 40 mg/L. 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 that presence of toxins and dissolved organic carbon can be removed effectively with high concentration aluminium sulphate treatment. As indicated in Table 4, removal of toxins with organic matter of low molecular weight depends on the coagulant concentration and need to use this strong coagulant for canal water treatment for drinking purposes. The maximum removal of organic compound was observed in the samples of canal water with values as 22 mg/L in Table 1 and it is also reported

that the removal efficiency of coagulant depend on its molecular weight.

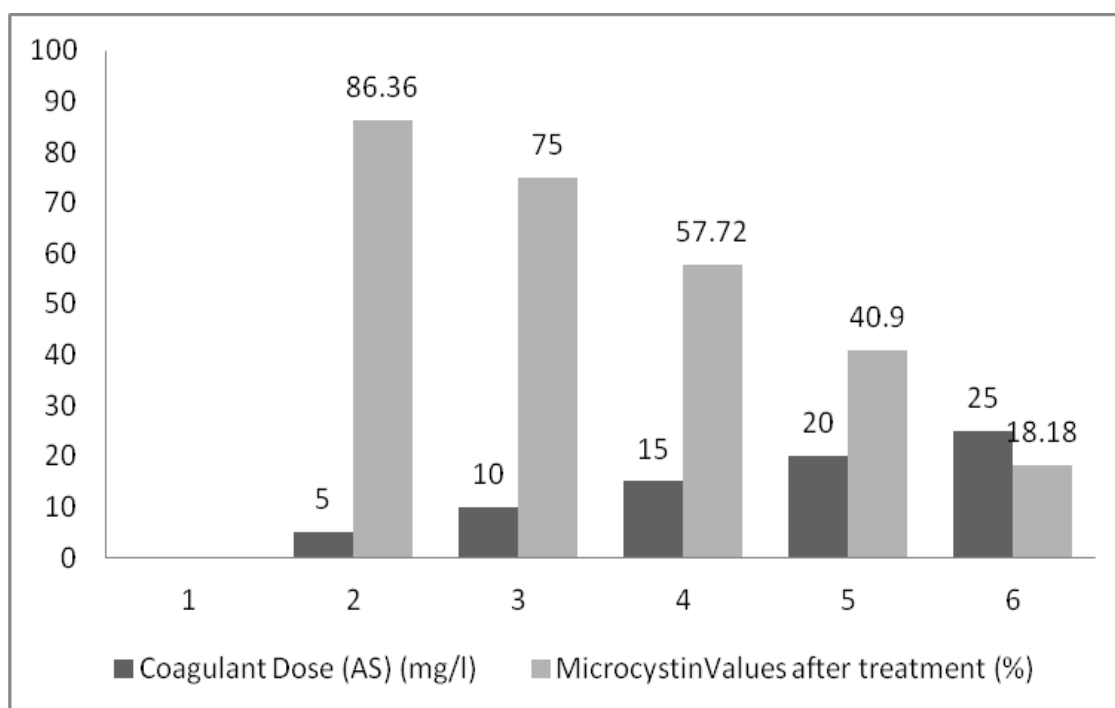
However, the efficiency of coagulation for removing of cyanobacterial cells may be pushed with some influences like as shape and size of the toxins related cyanobacteria, presence or absence of mucilage sheath and the composition of the cell wall, etc. The cyanobacterial materials can be removed by taking the coagulation/flocculation process is directed on the similar principles which applicable for removing the suspended particles in colloids (Paller, 2006), according to Benhardt and Clasen (1991). As spherical structures and smooth surface of Cyanobacteria can be destabilized with using the coagulation process mechanism and this process make to the cause of charge neutralisation on cyanotoxins as seen in current study as seen Figures 2 and 3 (Teixeira et al., 2006).

Table 4. Data of aluminium sulphate as coagulant doses for removing toxins from drinking canal water samples.

Coagulant Dose(AS) (mg/L)	Microcystins (mg/L) (actual value)		Microcystins (mg/L) (Value after treatment)		Microcystins Values after treatment (%)	
	Mean	Range	Mean	Range	Mean	Range
5	22	16-23	19.0	16.0-20.5	86.36	83-37
10	22	16-23	16.5	10.0-17.7	75.00	74-78
15	22	16-23	12.7	6.4-13.9	57.72	55-60
20	22	16-23	9.0	7.0-10.5	40.90	38-42
25	22	16-23	4.0	2.5-5.0	18.18	16-22

Table 5. Data of aluminium sulphate as coagulant doses for removing toxins from drinking ground water samples.

Coagulant dose (AS) (mg/L)	Microcystin (mg/L) (actual value)		Microcystin (mg/L) value after treatment		Microcystins values after treatment (%)	
	Mean	Range	Mean	Range	Mean	Range
1.0	1.2	0.9-2.0	1.0	0.8-1.6	83.33	80-85
1.5	1.2	0.9-2.0	0.7	0.5-1.0	58.33	55-63
2.0	1.2	0.9-2.0	0.5	0.3-0.9	41.66	39-43
2.5	1.2	0.9-2.0	0.3	0.1-0.5	25.00	23-27
3.0	1.2	0.9-2.0	0.1	0.0-0.1	8.33	7-10

**Figure 2.** The graph shows the different dose of coagulant effect on removing toxins in samples of Canal water.

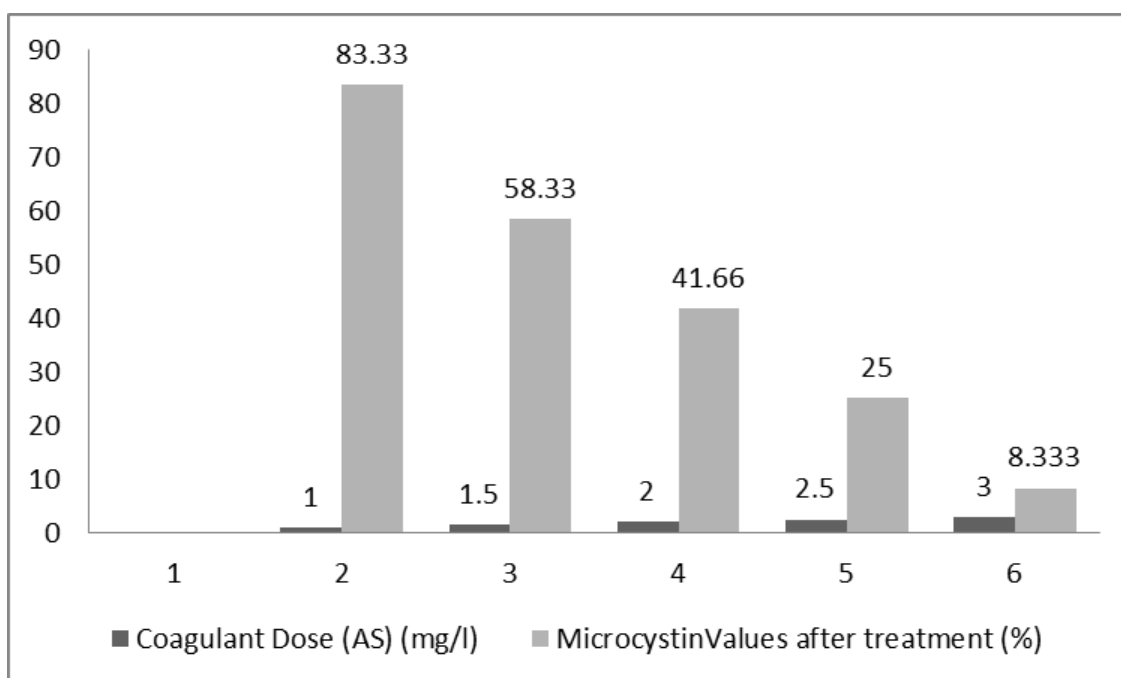


Figure 3. The graph shows the different dose of coagulant effect on removing toxins in the samples of ground water.

Discussion

The first aim of study is to detect the toxins and their producing microbes in different water samples. All the nations of world are very conscious on drinking water quality, so effective efforts in form of research and study make on this issue. *E. coli* related with coliform family which is basic indicator of faecal contamination which is also known as disease causing pathogen. Microbial quality is totally depending on presence of coliform group *E. coli*. recognised as pathogenic microbes and their metabolise chemical known as toxins (Simeonov et al., 2003). Qualitative and quantitative composition within bacterial pathogens and their metabolites toxins in samples of drinking water of canal, ground and water storage tanks were probably due to contamination of sources of drinking water by human or animal excreta. The major problem in the country in rural as well as urban areas is bacteriological contamination in drinking water due to lack of management. Pipes leakage near water supply system is also source of contamination in the drinking water. Human activities also the cause of chemical and biological contamination such as

hazard waste disposal on land or discharge into water streams. The extremely hazardous sources of contamination are floods, excessive monsoon rains, fungicides, herbicides, pesticides and untreated municipal and hospital waste (Singh et al., 2007).

Public health is only protected by launching drinking water quality criteria and standards that are safe medically and chemically balanced. The objectionable quality seen of water as bad tasting, brackish, colour, foul smelling and turbid in different cities of Pakistan in previous studies. The concern authorities are answerable for water quality checks quality with basic parameters like as bacterial contaminations and toxicity according to given standards by government (Singh et al., 2004). This study was limited to the rural areas of District Sheikhpura not urban area that the enumeration of faecal coliform (*E. coli*) bacteria and their toxin from drinking water of rural area of City Sheikhpura water in higher concentration, since the presence of toxins and other microbe's identification is actual mirror about contaminated drinking water quality.

The results in Tables 1 and 2, showed the presence of toxins and bacterial

faecal coliform group was noted higher in canal water as compare to water storage tank and ground water samples, but this important bacterial group found in large numbers as per 100 mL water samples. Microbes finding possibility is high in hot season as compare in winter months results as given in Table 2. The temperature increases in summer season as 27 °C to 35 °C, then this increased the microbial growth as colony forming units per 100 mL (cfu/100 mL). Diarrhoea incidences cases with large number were reported in hot season, data had shown from district hospitals on diarrhoea related diseases and this is also correlated in the present picture. It has shown from literature citations that waterborne pathogens problems are the seasonal and situational. Present Research in duration 2010-2014, it observed in Sheikhpura City that faecal coliform (*E. coli*) per 100 mL as per sample for potable water quality was not under WHO drinking water guidelines. It is also reported that high incubation rate of microcystins is very ideal in hot months starting from May to August. The supplies of potable water of rustic area are superficial water from the canal water sources which having tributary appearance because these are unprotected and uncovered from contamination sources (Singh et al., 2005).

However, the result of our study is far from WHO standards for cyanobacteria and coliform as microbiological quality and biological pollutants in potable water, it is the indication of toxins and bacterial species can also assessed at the consumers level. In this research use the different point for sampling sources such as canal water, ground water and household water. It is evident from the study that water quality further deteriorated at the consumer level because no action plan implemented against pollution control. The Mandialli is the one rural area of Sheikhpura City have 1,000 people houses from use the source of district information office, the population are using the potable water source of canal with slightly conventional treatment because canal water is unprotected totally from contamination sources of sewage water pollutants and industrial water pollution. Total 116 samples was collected as 100% but 98 samples means 84.48%

were showed polluted with microbial contamination and toxins, where observed high level contamination problems. Quantity of collected samples results from rural area under study matches with research results as given by many other researchers from many other countries (Westrick et al., 2010).

It is evident from the present study, results showed the similarities occurred as other under developing countries, many ways did not used in rural area of Pakistan to correct quality wise of drinking sources of water in public insert for domestic purposes. Same results derived as given by the study's results from (Singh et al., 2005), it reported that in most of the developing countries, the quality of drinking water very underprivileged as 10,000 faecal coliform bacterial species 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 (WHO, 2009). South Asia including Pakistan and India, findings related both rural and urban water reservoirs is almost same as repeatedly polluted with human coliform group. South Asia have 85% of potable water sources coming from an human faecal contaminated sources which often cause disease and illness in common people. Certainly, contamination with human faeces in water is almost usual, approximately found in South Asia and it is understand as the normal water quality (Newcombe, 2010).

The areas under study, contamination of potable drinking water from metropolitan supplies have the similar arrangement such as other cities municipalities of Pakistan Country. The drinking water pollution in level of basic water source is very common in almost large cities due to the water storage reservoirs have opened and exposed to human and animal actions. Qualitative determination of cyanobacteria in from of Algae in different water samples from rural area of City of Sheikhpura was possible because due to inadequate way to control the quality of potable water. Physical condition is very effective on producing water borne pathogens and their metabolites toxins such as temperature, light and

humidity. In this study, the pathogenic occurrence of microbe cyanobacteria in samples of potable water is relative to increase sources of contamination as toxins. Microbiological analysis of water samples taken by different sources like ground, canal and water storages tanks of area understudy, presence of these microbes is the position may show public health threat with facing bacterial pathogens from waterborne disease. Cyanobacteria and microcystins toxins were in current work as isolated quantitatively 92% of samples of water as described in Table 1. The occurrence of microbes in water samples possibly will be due to unhygienic situation of water sources with some nutrition. If microbes are the associate with the algae family, however it is possible existing with the faecal matter of human and animal.

Conversely, observations due to microcystins toxins from laboratory results presented the toxins which are created by the cyanobacteria species as found slow growing. The major aspects in measuring for the removal of cyanobacteria toxin from water treatment which includes removing the soluble and suspended substance removes. All The toxins that under studied are microcystins, shiga toxin and botulinum toxins resolvable in water. In present study, some physical and chemical factors play an important role in microbes growth increasing with their metabolise like toxins, these factors discussed one as Temperatures, it is reported in this study the samples of water is effected increasing by increasing the strength of sunlight as temperature average raises from 16 °C to 50 °C during study in sampling area. The environments for microbe's growth, incubation temperature particularly have effect on their existence with metabolites as toxins (Zamyadi et al., 2012). During summer season, microbes were isolated in high numbers at temperature 22 °C to 27 °C and very small in number identified in winter season.

High temperature is commonly documented as a vital adjusting feature of microbial progress like bacterial, algal evolution. Environments under water high temperatures can increase microbial growth and but, at the minimum temperature where microbial movement seen is observed

varies in the different co-ordination. It is reported that increases in bacterial activity occurred in the day during algal blooms in White Clay Creek. The microbes react speedily to indicate at higher temperatures with dissolved organic matter are helped to increase growth. The quality of natural organic matter as well organic pollutants in surface water is degrades by solar radiation. In summer seasons, waterborne bacterial pathogens growth was achieved but not as same in midwinter that is indicated the potential influence of temperature on waterborne microbial pathogens occurrence (Singh et al., 2007).

Second is as pH, the 6.5-8.5 pH of most drinking water ranges, if some changes occur within this value as result of carbonate, bicarbonate or carbon dioxide dissolving in system of water supplies because of increased carbon dioxide concentration in water shows lower pH and as a decrease values reason high. Basically, the pH is the corrosively of the water, so small changes in pH has no non-stop influence on consumers health. At below pH 8.0, it is ensure about water clarification and chlorine disinfection occurs (Singh et al., 2004). The normal of pH is very important because of water flow in pipes system may effect quality of pipes which used in water supplies, so it must need proper pH monitoring system for maintaining the pH of water. pH of water may change its taste, odor and appearance (Singh et al., 2005).

The aim of this research was to identify the treatment characteristics of toxins and organic matter preferentially removed by 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. 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 abstracted from full-

scale and pilot-scale plants identified toxins and organic matter preferentially removed.

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, showed as results in Tables 3, 4 and 5 of bench and pilot-scale study. Furthermore it was observed, that chemical treatment is useful for suspended material such as macroscopic algae during solid-liquid separation by settling (Westrick et al., 2010). 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 (Newcombe, 2010).

Conclusions

Cyanobacteria toxins (cyanotoxins) have become an important group of toxic compounds in the environment. Microcystins belong to the most hazardous cyanotoxins with respect to their acute and chronic toxicity and common environmental occurrence. Microcystins occur frequently in the environment, as was clearly confirmed in this study. Algal or cyanobacterial toxins limits in drinking water is 1 mg/L and *E coli* from human as well as animal sources per 100 mL of drinking water used for drinking purposes with zero value has been recommended by WHO worldwide to monitor the quality of drinking water. High value of microcystin toxins were seen in canal water samples it must needs treatment for human and animal consumption because much amount accumulation can cause high chances of liver cancer. However low values found in ground and water storage tanks samples and this situation with less toxic as compare to canal water samples but it also needs some treatment for making better quality.

Conflict of interest statement

Authors declare that they have no conflict of interests.

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