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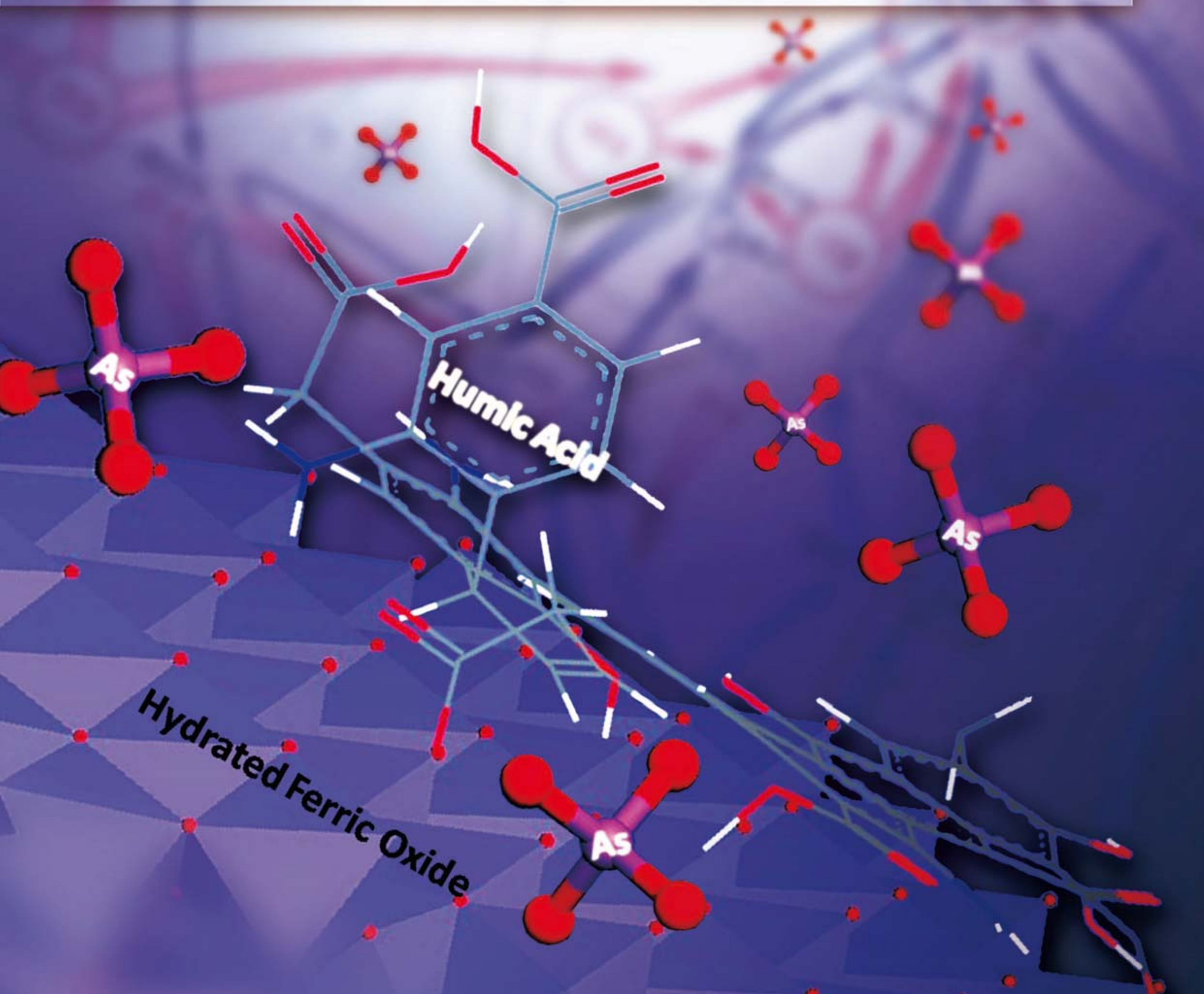

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Efficacy of two chemical coagulants and three different filtration media on removal of *Aspergillus flavus* from surface water

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ABSTRACT

Aquatic fungi are common in various aqueous environments and play potentially crucial roles in nutrient and carbon cycling as well as interacting with other organisms. Species of *Aspergillus* are the most common fungi that occur in water. The present study was undertaken to elucidate the efficacy of two coagulants, aluminum sulfate and ferric chloride, used at different concentrations to treat drinking water, in removing *Aspergillus flavus*, as well as testing three different filtration media: sand, activated carbon, and ceramic granules, for their removal of fungi from water. The results revealed that both coagulants were effective in removing fungi and decreasing the turbidity of drinking water, and turbidity decreased with increasing coagulant concentration. Also, at the highest concentration of the coagulants, *A. flavus* was decreased by 99.6% in the treated water. Among ceramic granules, activated carbon, and sand used as media for water filtration, the sand and activated carbon filters were more effective in removing *A. flavus* than ceramic granules while simultaneously decreasing the turbidity levels in the test water samples. Post-treatment total organic carbon (TOC) and total nitrogen (TN) concentrations in the experimental water did not decrease; on the contrary, TN concentrations increased with the increasing dosage of coagulants. The filtration process had no effect in reducing TOC and TN in tested water.

Introduction

The occurrence of fungi in drinking water is considered water contamination; Sammon et al. (2010) investigated the qualitative and quantitative composition of fungi in water collected from different parts of a municipal water treatment plant. Previously, several studies, for example, Arvanitidou et al. (2000), Hageskal et al. (2006), and Grabinska-Loniewska et al. (2007), had reported the occurrence of fungi (*Aspergillus* spp.) in drinking water. These fungi, *A. flavus*, *A. parasiticus*, and *A. nomius*, are known to produce aflatoxins (Manonmani et al., 2005). Also, aflatoxins G2 and B2, produced by *A. flavus*, have been detected in stored water (Paterson et al., 1997). In addition, *Aspergillus* spp. cause many diseases, and also are among a growing list of allergens that can aggravate asthmatic responses (Green et al., 2003; Basilico et al., 2007), as well as asthmatic lung disease characterized by increased Th2 cytokine generation, IgE, IgG, Eosinophilia, airway hyper-responsiveness, and airway remodeling (Hogaboam et al., 2005).

In the past, some studies have been made on the occurrence of fungi in water collected at the outlet from water treatment plants to remove fungi for water safety. Sammon et al. (2010) showed that coagulation/flocculation, sand filtration, and chlorination were highly effective in removing micro-fungal contaminants from raw water. Similarly, Saprykina et al. (2009) investigated the effects of coagula-

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tion/flocculation in removing yeast fungi, *Candida* sp., and their results revealed that this process was effective for the removal of these fungi from water. Zemmouri et al. (2012) used this process for water clarification and reported a decrease in turbidity; this process also was investigated in wastewater for removal of algal-bacterial biomass (Godos et al., 2011), as well as for the removal of melanoidins from biologically-treated molasses (Liang et al., 2009).

In the past, the research focus has been on effect of the process of water treatment on removal/decrease of some microorganisms such as bacteria, yeast fungi, algae, etc., but there is no previous investigation on the effects of this process on removal of fungi.

Therefore the present study was undertaken to investigate the level of decrease/removal of *A. flavus* from water through coagulation followed by filtration. The study also sought to evaluate suitable filtration media, such as sand, activated carbon, and ceramic granules, for use in removal of fungi from water samples. To the authors' knowledge, this is the first report on the type(s) of study described herein.

1 Materials and methods

1.1 Chemicals and equipment

A Jar Test Apparatus Coagulation Machine (Wuhan Hengling Technology Co., Wuhan, China) was used in the experiments. To measure total organic carbon (TOC) and total nitrogen (TN), an auto-analyzer (TOC-V CPH, Shimadzu, Japan) was used. The coagulants, aluminum sulfate ($Al_2(SO_4)_3{\cdot}18H_2O$) and ferric chloride ($FeCl_3{\cdot}6H_2O$), were obtained from Sinopharm Chemical Reagents Co., Shanghai, China; while Malt Extract Agar (MEA) media culture was obtained from Base Bio-Technology Company, Hangzhou, China. *Aspergillus flavus* was isolated in the laboratory from field-collected samples of untreated water.

1.2 Water preparation

1.2.1 Artificial contaminated water

In the laboratory, an 80 × 80 × 100 cm plastic tank was used to store the contaminated water (tap water with *A. flavus* spore suspension) in the dark. The fungal spores used for the contamination purpose were collected from media culture patato dextrose agar at 7 days, and were suspended in water and incubated at room temperature for three months during the summer season to allow fungal *A. flavus* re-growth in contaminated water without the addition of any chemicals.

The qualitative and quantitative (number of fungal colonies per mL) composition after pre-, and post-treatments (coagulation as well as filtration) was determined using MEA media culture.

1.2.2 Surface water

Surface water samples for the study were periodically collected, as needed, from Xing-Ling-Wan Lake, located at 24°28′47.41′′N and 118°05′21.91′′E, Fujian Province, Jimie, Xiamen, China. The water quality of these samples was determined. This lake receives water from the Jiu Long River; the resident lake water is known to be highly contaminated by different microorganisms; also, turbidity levels of this water are usually much higher than other prevailing surface waters in the city (Al-Gabr et al., 2014). **Table 1** shows the characteristics of waters.

1.3 Water characteristics

Water pH (laboratory-contaminated as well as field-collected surface water samples) and turbidity (only field-collected samples) were determined. The water temperature was adjusted using the coagulation machine before initiating any water treatment. The TOC and TN were measured using the TOC auto-analyzer.

1.4 Comparison between chemical coagulants

One liter of water from the laboratory and/or field source was used for each coagulation and filtration evaluation. Aluminum sulfate and ferric chloride, each at 10, 20, 30, 40, and 50 mg/L concentration, were used as coagulants. The coagulation was conducted in glass jars of the coagulation machine. Three replicates of each concentration of a coagulant were used. Six jars with provision of automatic addition of the coagulant chemicals were employed. The water temperature in the jars was adjusted before initiating the experiment. The experiment was initiated by rapid mixing (300 r/min for one min) of contents in the jars (coagulation machine), followed by slow agitation at 60 r/min for 15 min. The flocs that formed in the jars, including fungal mass and spores, were allowed to settle in the jar for 45 min. Thereafter, a 50 mL sample of water from near the middle of the jar (from a 50 mL sterilized test tube that was located near the jar middle) was collected and plated in MEA culture media for 7 days; the culture Petri dishes were checked daily to determine qualitative and quantitative (number of colonies) of fungi. After collecting a 50 mL water sample from the jar, the remaining water

Table 1 Characteristics of field-collected water and tap water

Parameters	Surface water	Tap water
Total organic carbon (TOC) (mg/L)	8.40 ± 1.46	2.20 ± 146
Total nitrogen (TN) (mg/L)	8.43 ± 2.22	3.65 ± 2.22
DO (mg/L)	6.12 ± 1.40	7.41 ± 1.23
Cl_2 (mg/L)	0.10 ± 0.13	0.43 ± 0.53
Total Cl_2 (mg/L)	0.18 ± 0.21	0.57 ± 0.62
pH	7.15 ± 0.46	6.89 ± 0.43
Temperature (°C)	25.3 ± 4.86	23.13 ± 4.62
Turbidity (NTU)	41.15 ± 17.56	0.36 ± 0.89

in the jar (except for nearly 50 mL water at the bottom of the jar, which was discarded) was carefully filtered through sand, activated carbon, or ceramic granules.

1.5 Comparison between three filtration columns

Columns of the three different filtration media, sand, activated carbon, and ceramic granules, were used to determine the most effective medium for the removal of *A. flavus* from drinking water. The dimensions of each column were 50 cm height and 2.1 cm diameter. The bottommost layer (10 cm thick) in each column was gravel with an overlying layer of filtering media. After coagulation at different concentrations of each coagulant, water from each sample was passed through the column and the filtrate was collected to determine qualitative and quantitative *A. flavus* content. Each evaluation was repeated three times, and in total, nine replicates of a coagulant were used at the various employed concentrations.

1.6 Isolation procedure

Pre-treatment and post-treatment water samples were collected in 50 mL sterilized plastic test tubes, then using the direct plate spread method, 0.1 mL water was spread on the plate using an L-shaped glass rod. Three replicates for each sample were tested and thus there were nine replicates in each treatment. The plates were incubated at 28 ± 0.5 °C, and were checked every day for two weeks to count the number of fungal colonies (CFU/mL).

1.7 Statistical analysis

Analysis of variance was conducted to analyze data collected in all experiments using the SPSS software program, version 11.5. Mean values (different coagulant concentrations and filtration media) were compared by using one-way ANOVA and student's *t*-test ($P \leqslant 0.05$).

2 Results and discussion

2.1 Water quality

The turbidity data are presented in (**Fig. 1**), while pH data are not included because no difference of pH values was noted between the treatments. Water turbidity was observed to have an inverse relationship with the concentration of used coagulants. The results showed the highest turbidity levels at low concentration (10 mg/L) of the coagulants, and the lowest turbidity at 50 mg/L. Aluminum sulfate appeared to decrease turbidity levels more than ferric chloride; the level of turbidity was reduced up to 92% by aluminum sulfate. This finding is in agreement with Konieczny et al. (2006) who reported a 100% reduction of turbidity level with aluminum sulfate, although they had employed ultra-filtration. Ferric chloride reduced turbidity by 79.8% in the present investigation, and this result is compatible with the findings of Qin et al. (2006) and Zemmouri et al. (2012), who reported up to 97% turbidity removal from surface water. Konieczny et al. (2006) also reported up to 89% turbidity removal from surface water using ferric chloride, while Lee and Westerhoff (2006) showed 70% turbidity removal using aluminum sulfate. Among the different filtration media used in the present study, sand and activated carbon filtration were more effective in reducing turbidity than ceramic granules. However, turbidity levels in all water samples declined after processing. Coagulation and filtration was most effective, at < 5 NTU, and in surface water, aluminum sulfate coagulant after filtering through sand decreased turbidity by 90%, and activated carbon by 91.5%; the same values for the ferric-chloride-treated water were 89.8% (sand) and 89.1% (activated carbon).

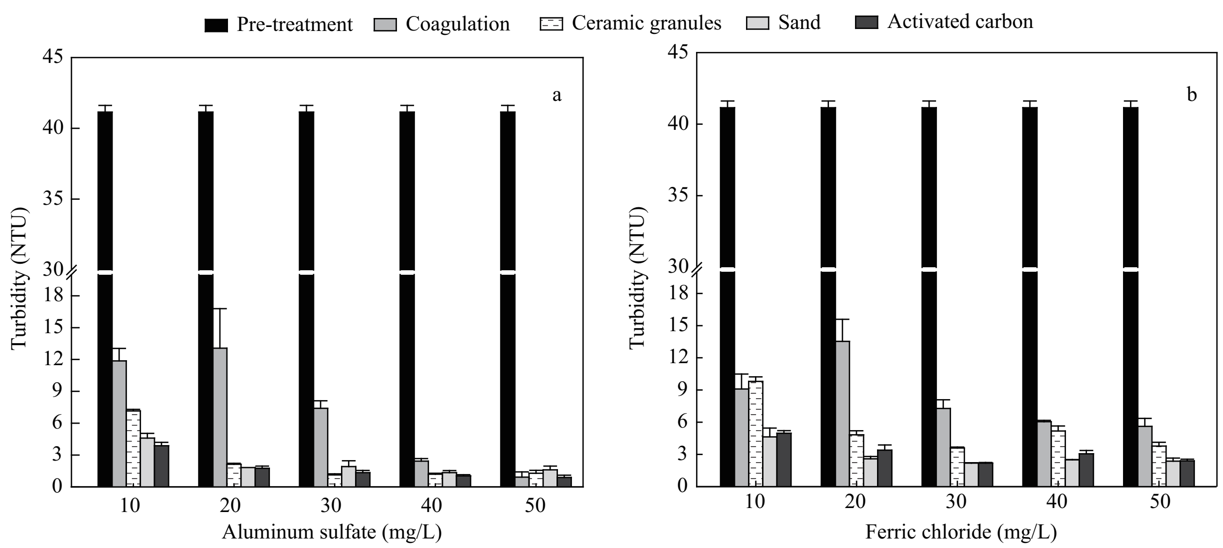

Fig. 1 Effects of water coagulation using aluminum sulfate (a) and ferric chloride (b); and filtration through sand, activated carbon, or ceramic granules, on turbidity of water. Data are expressed as mean ± SD.

The results related to TOC and TN revealed that the used coagulants at different concentrations as well as the three different filtration media did not decrease the concentration levels of these chemical parameters, as no statistically significant ($P > 0.05$) difference between these parameters was noted between pre- and post-treatment data. On the contrary, TN was observed to have a positive correlation with aluminum sulfate in the coagulation process (**Table 2**), and filtration had no effect in decreasing the TN concentration. This result is in disagreement with the study of Konieczny et al. (2006), who had used coagulation and microfiltration, and had reported a 100% reduction of TOC. On the other hand, Zularisam et al. (2009) noted that dosage of aluminum in coagulation treatment was the most significant factor that influences the natural organic matter removal.

2.2 Composition of fungi

2.2.1 Effects of coagulants

Figure 2a shows that reduction of *A. flavus* obtained by using aluminum sulfate was greater than that resulting from ferric chloride at all five employed concentrations of the two coagulants. The lower rate of 10 mg/L of each coagulant concentration reduced the fungi less than the highest rate of 50 mg/L level; the numbers of colonies of *A. flavus* at 10 mg/L level were 402 CFU/mL and 495 CFU/mL using aluminum sulfate and ferric chloride, respectively, whereas at 50 mg/L, these colonies amounted to 35.5 CFU/mL (aluminum sulfate) and 62.2 CFU/mL (ferric chloride). At range of 20–50 mg/L, aluminum sulfate reduced *A. flavus* by 91.3% and ferric chloride by 87.5%, but these concentration levels of the two coagulants did not provide a complete reduction of the fungus.

The results related to surface water (**Fig. 2b**) showed that the percent removal of fungi was directly related to the increase in concentration of the coagulants, and aluminum sulfate was more effective than ferric chloride. Statistical analyses of data revealed that the reduction of *A. flavus* was significantly ($P < 0.01$) related to the concentration of each coagulant. Also, there was a significant ($P < 0.01$) difference between the post-treatment reduction of fungi resulting from aluminum sulfate and ferric chloride treatments. Based on the present result of post-treatment *A. flavus* reductions of 90.6% (aluminum sulfate) and 85.9% (ferric chloride), it can be suggested that these coagulants may be used in water treatment plants for removal of *A. flavus* from drinking water. Previously, Saprykina et al. (2009) had reported a reduction of 99.7% of the fungus *Candida albicans* from water by coagulation using aluminum dihydrosulfate, and Godos et al. (2011) reported the removal of microorganisms such as alga-bacterial biomass from wastewater at a pig farm by 66%–98% using ferric chloride and ferric sulfate, while Shen et al. (2011) reported removal of algae by 96%–98% after processing waste water through coagulation and chlorination. Although previous studies have shown higher levels of removal of microorganisms (algal-bacteria and algae) from wastewater than in the present study, it is likely that the coagulation behavior of fungi may be different from some other microorganisms mentioned above, resulting in relatively lower levels of their reduction.

2.2.2 Effects of different filtering media

Figure 3 shows that sand filtration and active carbon filtration were more effective in removing *A. flavus* than filtration with ceramic granules. Sand filtration removed 91.6% fungal spores after the coagulation process with aluminum sulfate, whereas active carbon after ferric chloride coagulation reduced fungi by 95.6%. At aluminum sulfate dosages of 20, 30, 40, and 50 mg/L, the fungal percentage reductions were 99.5%, 99.8%, 99.9%, and 100%, respectively. After coagulation with ferric chloride,

Table 2 Effects of water treatment with the coagulants, aluminum sulfate or ferric chloride, on concentrations of TOC and TN in surface water filtered through the columns of sand, activated carbon, or ceramic granules

	Coagulant (mg/L)	Pre-treatment (mg/L)	Aluminum sulfate				Ferric chloride			
			Post-coagulation (mg/L)	Post-filtration (mg/L)			Post-coagulation (mg/L)	Post-filtration (mg/L)		
				Sand	Activated carbon	Ceramic granules		Sand	Activated carbon	Ceramic granules
TOC	10	8.40	8.37	5.77	8.88	7.71	8.37	7.83	5.42	8.79
	20		7.57	6.86	4.24	6.96	7.55	8.36	4.81	6.97
	30		5.02	6.58	7.47	11.3	7.97	7.10	4.47	6.80
	40		5.53	6.24	3.58	7.23	6.67	6.91	5.12	6.19
	50		5.42	7.88	4.44	5.39	6.42	6.44	5.25	6.4
TN	10	8.43	5.83	5.22	5.92	6.03	8.43	8.25	5.46	8.01
	20		5.89	5.89	5.01	5.88	8.26	8.21	6.57	8.04
	30		5.74	5.69	5.44	6.02	8.18	8.15	7.01	8.07
	40		29.4	28.7	32.1	28.6	8.17	8.03	7.3	8.06
	50		29.1	27.9	30.9	27.4	7.98	8.09	7.29	7.96

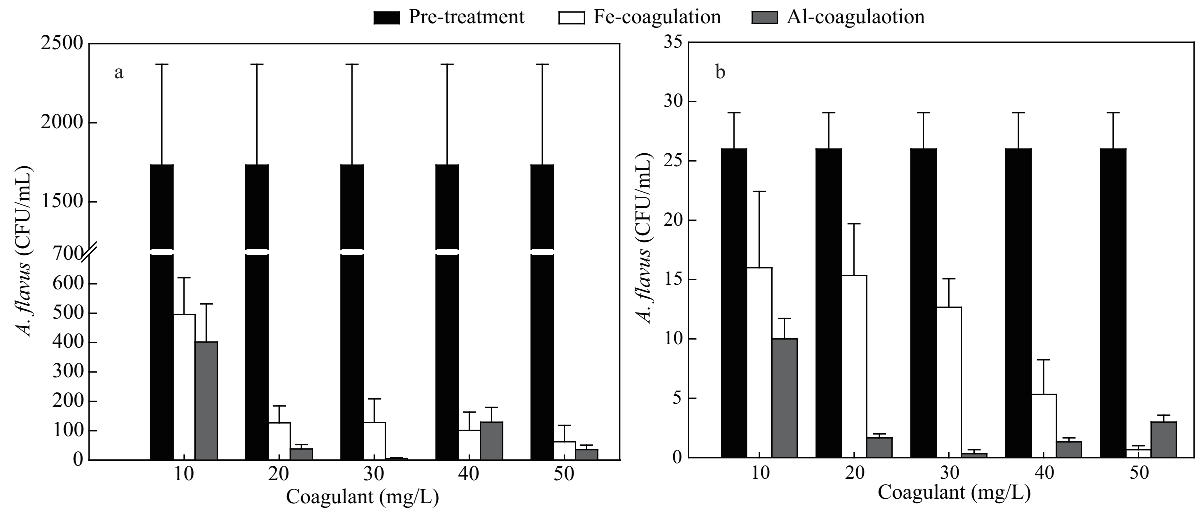

Fig. 2 Effects of aluminum sulfate and ferric chloride, used as coagulants at five different concentrations for removal of *Aspergillus flavus* from artificial contaminated water (a) and surface water (b). Data are expressed as mean ± SD.

and the treated water passed through the sand column, the results showed that dosages of 20, 30, 40, and 50 mg/L, respectively reduced 99.8%, 100%, 100%, and 100% of the fungi.

Activated carbon removed *A. flavus* by 94.9% and 97.4% after treatment with aluminum sulfate and ferric chloride, respectively, whereas aluminum sulfate at concentrations of 20, 30, 40, and 50 mg/L, respectively reduced *A. flavus* by 99.3%, 99.6%, 99.9%, and 98.7%. Ceramic granule filtration reduced *A. flavus* by 32.0% and 42.5% after coagulation with aluminum sulfate and ferric chloride, respectively. The ceramic granule filtration after coagulation with aluminum sulfate at 20, 30, 40, and 50 mg/L reduced fungi by 91.3%, 96.5%, 98.9%, and 83.9%, respectively. At the same dosages of ferric chloride, the fungi were reduced by 98.7%, 98.1%, 98.6%, and 87.1%, respectively.

The present study revealed that the coagulants prior to filtration effectively removed *A. flavus*, and ferric chloride was more effective than aluminum sulfate in reducing fungi in laboratory-maintained water. These results are in agreement with the findings of Godos et al. (2011), who reported 66%–98% of algal-bacterial biomass removal from a pig farm wastewater in the presence of ferric salts at concentrations of 150–250 mg/L. However, a comparison between results from tap water and field-collected highly contaminated water from Xing-Ling-Wan Lake, Jimei, Xiamen, showed aluminum sulfate was more effective in filtration than ferric chloride (**Fig. 4**). The various filtration media. Sand, activated carbon, and ceramic granules after treatment with aluminum sulfate removed the selected fungi by 98.7%, 97.4%, and 95.0%, respectively, while ferric chloride removed the fungi by 87.2%, 95.0%, and 93.6%, respectively. Sand and activated carbon were more

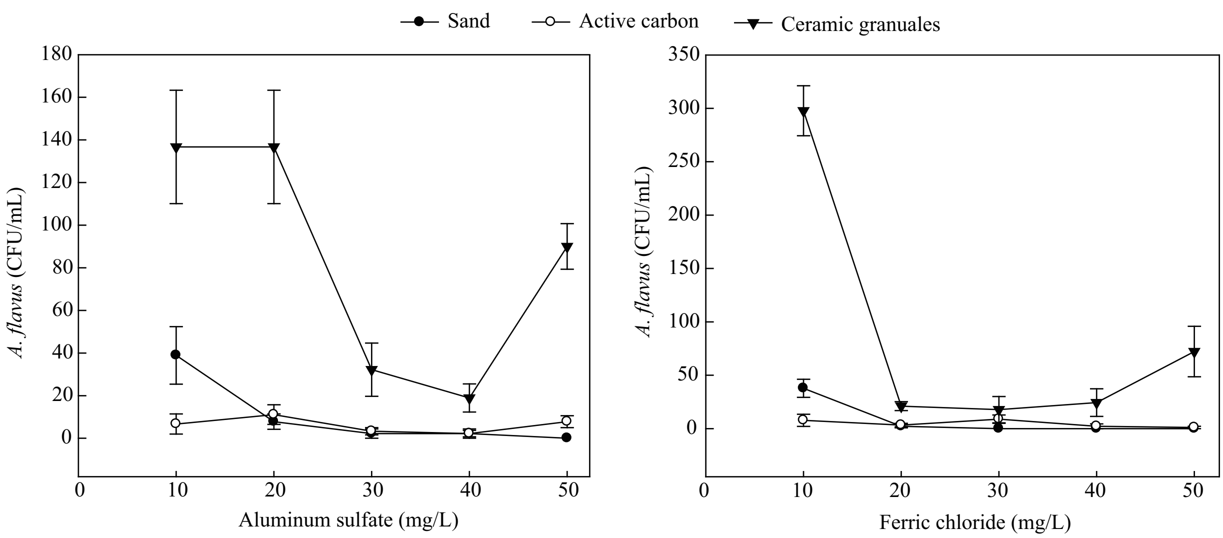

Fig. 3 Effects of different filtration media on removal of *A. flavus* from aluminum sulfate-treated water and ferric chloride-treated water. Data are expressed as mean ± SD.

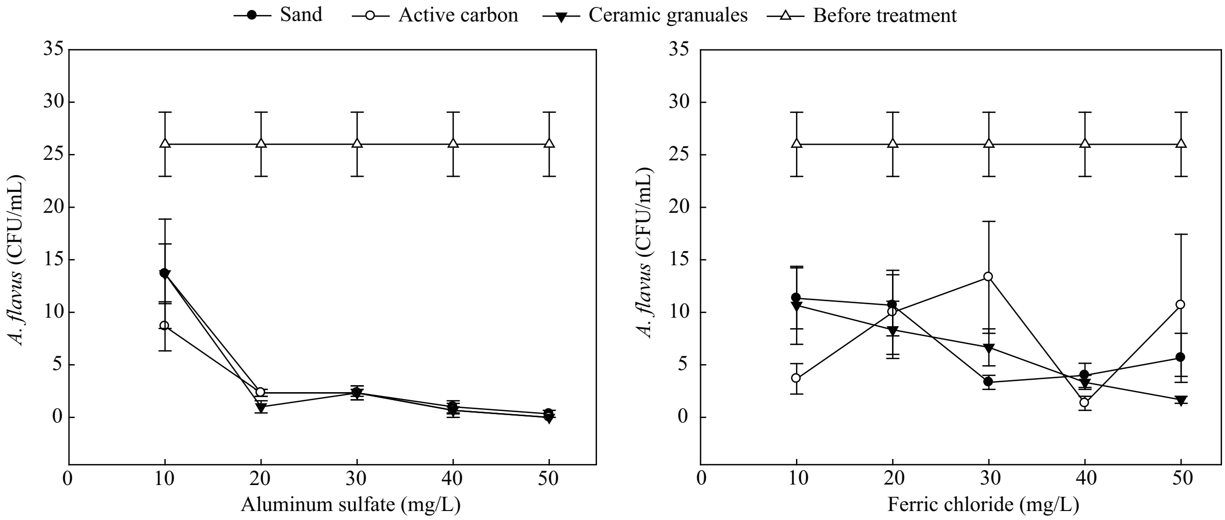

Fig. 4 Effects of different filtration media on removal of *A. flavus* from surface water treated with aluminum sulfate and ferric chloride. Data are expressed as mean ± SD.

effective in removing *A. flavus* than ceramic granules. It is evident from **Table 3** that many fungal species, including *A. flavus*, remained in field-water samples after processing (coagulation and filtration), although in very low quantities compared to their prevailing levels of pre-treatment. Sammon et al. (2010) had shown that coagulation and filtration, followed by chlorination, were very effective in removal of micro-fungal contaminants from the raw water. Grabinska-Loniewska et al. (2007) found that the fungi were abundant in water systems.

This study showed that these processes effectively removed genus *A. flavus*, but still there was some occurrence of the fungus after filtration, at low percentages but enough to re-grow in suitable media or as a bio-film in water systems. Doggett (2000) investigated the formation of bio-films in municipal water distribution systems, where the presence of *Aspergillus* spp., *Penicillium* spp., *Cladosporium* sp., *Phoma* sp., and some other species was reported. Likewise some genera such as *Penicillium*, *Fusarium*, and *Trichoderma* were also found. In addition, *Aspergillus* spp. cause many diseases, and are among a growing list of allergens that can aggravate asthmatic responses and threaten immune-compromised patients (Spreadbury et al., 1993; Green et al., 2003; Hogaboam et al., 2005), and exposure to filamentous fungi may result in a wide variety of health problems in humans (Hageskal et al., 2006;

Table 3 Dominant fungi found that remained in field-collected (Xing-Ling-Wan Lake, Jimei, Xiamen, China 2012) water samples (filtrate) after treating the samples with the coagulants (aluminum sulfate or ferric chloride), and filtering through various media (sand, active carbon, or ceramic granules)

Coagulants		Filtration medium		
		Sand	Active carbon	Ceramic granules
Aluminum sulfate	40 mg/L	*Fusarium oxysporium*	*A. flavus* *Trichoderma* sp.	*Fusarium* sp. *Trichoderma* sp. *Penicillium* sp.
	50 mg/L	*Fusarium oxysporium*	*A. flavus* *Trichoderma* sp.	*Fusarium* sp. *Trichoderma* sp.
Ferric chloride	40 mg/L	*Fusarium* sp. *Trichoderma* sp. *Exophiala* sp. *Penicillium* sp.	*A. flavus* *Exophiala sp.* Unknown *Trichoderma* sp.	*A. flavus* *Exophiala* sp. *Aspergillus* sp. *Penicillium* sp. *Trichoderma* sp.
	50 mg/L	*Fusarium* sp. *Penicillium* sp. *Exophiala* sp.	*A. flavus* *Trichoderma* sp. *Penicillium* sp.	*A. flavus* *Exophiala* sp. *Penicillium* sp. *Aspergillus* sp. Unknown

Basilico et al., 2007).

3 Conclusions

Previously, numerous studies on the occurrence (qualitative and quantitative) of fungi in water from a variety of sources have been reported. However, the present study to the authors' knowledge is the first one which presents information on the removal of *A. flavus* from drinking water. The study is of significant importance because of the known production of aflatoxins (causing health hazards for humans) by *Aspergillus* spp.

Aluminum sulfate and ferric chloride, used as coagulant chemicals, were effective in removing *A. flavus* from laboratory-maintained contaminated water, particularly at concentrations of 30–50 mg/L. Also, the sand and activated carbon filtration media proved to be more effective in the removal of *Aspergillus* spp. from water than ceramic granules. However, the level(s) of overall removal of fungi in the present study were probably not sufficient, because the very low levels of fungi remaining in the processed water could re-grow in large numbers as bio-films in water systems. Therefore, further research work is needed on disinfection processes in removal of this fungus.

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