Iranian Journal of Chemical Engineering, 17 (4), 33-48, (2020) pISSN: 1735-5397
DOI: 10.22034/ijche.2020.130360 eISSN: 2008-2355

Full Paper

Hazards Identification and the Units Assessment of a the Water Treatment Plant Against Pathogenic and Biotoxin Threats Affecting by Physicochemical Parameters

A. R. Bahramian [*]

Chemical Engineering Department, Hamedan University of Technology, P. O. Box: 65155, Hamedan, Iran

ARTICLE INFO

Article history:
Received: 2020-12-27
Accepted: 2021-04-25
Available online: 2021-05-25

Keywords:
Water Treatment Plant,
Pathogenic and Biotoxin Agents,
Hazard Identification,
Unit Assessment,
Physicochemical Parameters

ABSTRACT

In this study, the inactivation performance of units against pathogenic and biotoxin threats in a water treatment plant is studied. The assessment of the units and hazards of the water treatment plant against each of threats is studied by the RAMCAP risk analysis. The experiments showed that the Aflatoxin was eliminated only by disinfection units. The reverse osmosis unit had high efficiency in removing Ricin, while the sand filtration had the lowest efficiency in removing biotoxins. The microbial analysis showed the total coliform bacteria, thermotolerant coliform and HPC index were increased slightly by increasing the incoming water's pH and turbidity, while their count were significantly reduced by increasing the free residual chlorine. Changes in the water temperature also had minor effects on microbial indexes. The RAMCAP analysis is used to reduce the vulnerability of units to conventional threats by determining the risk values of the units and finally, to present practical solutions.

1. Introduction

Large-scale water treatment plants use a variety of methods to achieve the high-quality drinking water. The most important of these methods are chlorination, ozonation, filtration, coagulation, reverse osmosis, and the use of absorbents [1-2]. On a small scale, the UV irradiation technique is also used to purify the water. Depending on the method used to purify water for drinking purposes, some microbial and chemical threats can be expected to be removed. Indeed, the use of combined methods in water treatment units makes it possible for the purified drinking water to have the necessary standards [3]. One of the most critical concerns in a drinking water treatment plant is safeguarding the system against the threats. Among the hazards that can threaten the drinking water are biological and chemical hazards [4-7]. The use of bacteria and viruses during wars in the drinking water reservoirs to weaken the country was commonplace, and the drinking water systems have always been the target of bioterrorist attacks [5]. In this regard, the system vulnerability assessment plays an

[*]Corresponding author: bahramian@hut.ac.ir (A. R. Bahramian)

essential role in the risk analysis and management of the drinking water plants. Vulnerability refers to the extent or degree of the damage caused by the risk to the system or the community [8].

Biological hazards threatening the water quality can be caused by pathogens and biotoxin agents. In general, the ability of pathogens is greater than that of biotoxins [4]. Biological factors have characteristics that have attracted the attention of some hostile countries and terrorist groups. One of the first problems of biological hazards is that it is difficult to detect the existence and type of hazards, while they easily get transmitted from person to person. The microbial dose necessary for causing infection, which is known as the minimum infectious dose, may vary from a few to thousands of microbial cells [9]. For example, the estimated infectious dose for *Bacillus anthracis* that is used as biologial threat in the drinking water is 6000 spores via the inhalation route, while the corresponding value for *Francisella tularensis* agent is 10-50 cells by the same rout [10]. The most pathogenic agents that have been used to infect the drinking water supplies for bioterrorism purposes are bacterial species (*Bacillus anthracis, Bressinia postis*, *Vibrio cholera*, *Escherichia coli anthrohemorrhagic*), bacterial toxins (anthracinol) and noted fungal and plant toxins (*trichothecens* and *ricin*) [9-14].

Biotoxins are colorless and odorless substances, which are both toxic and biological origin coming from plants, animals or specific chemical agents. The production of biological agents may be less complex and less costly than that of the chemical poisons. The major biotoxins that have the potential for weaponization and threats to the drinking water supplies are Ricin, Saxitoxin (STX), Aflatoxin (AFT), and Anatoxin-a (ATX-a) due to their high stability in water [8, 15]. The Ricin is produced by the castor plant *R. communis*, which has a lethal dose for 50 % of the test population LD_{50}, equal to 20 mg/kg of the body weight [10]. The LD_{50} of STX, AFX and ATX-a are 3-10, 100-800, and 200 µg/kg of the body weight respectively [10]. AFT has low solubility in water and is probably heat sensitive and resistant to the chlorination [16]. Four main AFTs include B1, B2, G1, and G2, which are of significance as the direct contaminants of foods and of which molecular structures have been elucidated [16]. AFTB2 and AFTG2 were established as the dihydroxy derivatives of B1 and G1 respectively. The order of chronic toxicity is B1>G1>B2>G2 [15]. ATX-a converts to a non-toxic form in water within a few days. This toxin is relatively resistant to the chlorination and filtration methods, as mentioned methods have little effect on removing this toxin [17]. For the Botulism toxin to effectively contaminate the drinking water tank, it must enter the water after it leaves the treatment plant and must also be able to survive in the presence of free residual chlorine (FRC). So, very high amounts of the toxin are required for water tanks and therefore, it is not suitable for poisoning large water tanks. However, sunlight turns it off in 1-3 h. Botulinum toxin is also inactivated in the air within 12 h and is very sensitive to temperature and boiling conditions [15].

STX is water-soluble and resistant to acidic conditions and is stable under natural environmental conditions. STX is sensitive to an alkaline environment. This toxin is inactivated by more than 99 % after 30 minutes of exposure to a concentration of 10 mg/L of the free chlorine. Iodine at a concentration of 16 mg/L does not affect

saxitoxin. The reverse osmosis system removes saxitoxin from water up to 98.8 %, but the coagulation system does not impact on it. The efficiency of eliminating toxins from the drinking water by charcoal is low under normal conditions [15]. Ricin loses its toxicity within 10 minutes at 80 °C and is inactivated within 50 h at 50 °C but is stable at ambient temperature [8]. After 20 minutes in the presence of excess chlorine at a concentration of 100 mg/L, Ricin inactivates it up to more than 99.4 %, but remains healthy at a concentration of 10 mg/L. Iodine of up to 16 mg/L does not affect this toxin. The reverse osmosis system can effectively remove 99% of Ricin from water, but the coagulation process has no impact on its inactivation. The charcoal-containing system can effectively remove this toxin from the drinking water [18].

Hoffmann showed that the efficiency of the chlorination process to remove Microcystins toxins in the water treatment depends mainly on the chloride compounds and their concentration [19]. Aqueous chlorine and calcium hypochlorite at ≥1 mg/L remove more than 95 % of microcystins, while sodium hypochlorite at the same dose achieves 40-80 % of removal. Rositano and Nicholson [20] found that the coagulation/flocculation process is an efficient route for eliminating cyanobacterial toxins such as microcystins, STX, and ATX-a from the drinking water, while soluble cyanotoxins are not very efficiently removed from the mentioned route. Rositano et al. [21] found that ozone was the most powerful oxidizing agent that led to the destruction of cyanobacterial toxins. They showed that 1 µg/L ozone dose can eliminate 200 µg/L of Microcystin in 5 min [21]. The WHO has recently set a new provisional guideline value for Microcystin of 1.0 µg/L in the drinking water [22]. Schneider and Bláha [23] found that the cyanobacterial toxins can effectively be removed by conventional water treatment plants. They proposed the advanced oxidation processes to remove cyanobacterial toxins from the drinking water.

In this study, the performance of each unit of an urban water treatment plant against the inactivation of pathogenic and biotoxin threats is investigated experimentally. The most important biotoxin agents were Ricin, AFT, ANT-a, and STX, while the amount of HPC bacteria count, total *coliform* and thermotolerant *coliform* were determined to achieve the index of pathogenic agents. At first, the effect of physicochemical parameters including pH, temperature, turbidity and FRC of water on the inactivation rate of hazards was studied. In the second part, the assessment of water treatment units and hazards of different units of water treatment plants against each of pathogenic and biotoxin hazards was studied by the RAMCAP risk analysis.

2. Experiments

2.1. Materials and reagents

Materials used in biotoxin analysis experiments contain Ricin, ATX-a, AF-species (G1, B1, G2 and B2), and STX. Trypsin was purchased from Rocho Co. (Germany). Pure ATX-a fumarate salt was taken from Abcam@ (Cambridge, UK). Dithiothreitol (DDT) and iodoacetamide were purchased from Merck, Darmstadt, Germany. The Phosphate-buffered saline solution [137 mM KH_2PO_4, 3.2 mM KCl and 7.3 mM NaOH] was purchased from Panreac (Barcelona, Spain). The pure Aflatoxin R0 was purchased from FERMENTEK Ltd. STX dihydrochloride (6.6×10^{-2} mM in 3 mM HCl)

was purchased from National Research Council Canada. The materials used in the liquid chromatography (LC) analysis were acetonitrile (HPLC grade), methanol 96 %, trifluoroacetic acid and Chromatolith®, which were purchased from Merck, Darmstadt, Germany.

2.2. Processing of biotoxin samples

The individual extraction protocol was applied for each of the biotoxins. Samples were handled in a class 2 biosafety cabinet equipped with high energy particulate air filters because of safety requirements. The concentration of Ricin, ATX-a, AF-species (G1, B1, G2, and B2), and STX were 10, 1, 10, and 5 mg/L respectively. Samples containing Ricin were digested by trypsin following protocols similar to those described earlier [18]. Briefly, the Ricin samples were incubated with 100 mM of DDT at 60 ºC for one hour. After cooling to ambient temperature, the samples were mixed with 55 mM of iodoacetamide for one hour. The final mixture was subjected to digestion using trypsin at 38 ºC in an incubator for 10 h. The prepared samples were stored in completely closed containers for analysis.

2.3. Sample characterization

Samplings were done before and after points of the water treatment plants of Hamedan city (Ekbatan and Shahid Beheshti water treatment plants, including chlorination, ozonation, reverse osmosis, coagulation/flocculation and sand filtration units). All samples were stored near ice and transferred to a laboratory for testing in less than 2 h. Microbial tests were performed by 3759 standard of Iran. The physicochemical properties such as the pH, turbidity, and FRC of water samples were measured through the titration analysis according to 1011 standard.

2.3.1. Pathogens analysis

Samples were taken randomly and following standard conditions to prevent secondary contamination using sterile glass bottles containing thiosulfate. The HPC index, *coliforms* and physicochemical parameters including FRC, turbidity, temperature, and pH were examined. The MPN [1] method was used to count the total *coliform* [24]. The culture mediums including Lactose Broth (LB) and Brilliant Green Lactose Bile (BGB) Broth were used to study *coliform* bacteria, while R2A was used to study heterotrophic bacteria [24]. In summary, the LB culture medium was used as a possible step in the multi-tube fermentation experiment. For the drinking water, ten tubes containing 10 ml of the sample were prepared. After 24 h of incubation at 35 °C, the tubes were examined for the microbial growth and gas production. If all the samples showed negative results after 24 h, to ensure the negative results' correctness, the samples were placed in the incubator for another 24 h. No acidic reaction or gas production after 48 h indicated a negative effect, while acidic reaction or gas production after 48 h indicated a positive test. Positive tubes were transferred to the confirmation phase in a possible reaction [6, 24].

The drinking water samples that showed microbial growth without acidic reaction or gas production were also transferred to the confirmation phase. In the confirmation phase, BGB Broth fermentation tubes were used. The suspicious tubes, in which the gas or acid production were seen, were gently shaken to allow the organisms to float. Using

[1] Manufacturer Part Number

a sterile loop with 3 mm in diameter, one or more complete loops were transferred from the first stage fermentation tube to the fermentation tube containing BGB Broth in the confirmation step. The tubes in the confirmation stage were heated to 35 °C. The formation of any amount of gas in the Durham tubes at any time up to the end of 48 h of incubation indicates the response at this stage [11]. The HPC test was performed by the spread plate (Spread Plate Count) method to determine the heterotrophic bacteria's growth. The incubation temperature of culture media was adjusted at 35 °C for 48 h [11].

2.3.2. Biotoxins analysis

The LC-MS analysis was used to determine the concentration of biotoxins in the water samples obtained from the different points of water treatment plants. Briefly, sample aliquots with volumes of 50-200 µl, depending on the quantification results obtained by ELISA, were purified and concentrated by the galactose affinity chromatography [25]. In this method, the affinity separation using a galactose column (1.6×5.2 cm) of galactose substituted epoxy-activated "Sepharose" was performed and repeated three times to isolate Ricin and *R. communis agglutinin* (RCA120) from other constituents in the precipitate. Finally, the size-exclusion chromatography was used to separate Ricin from RCA120 [25]. The digests were analyzed by a high-resolution MS analyzer (nanoLC-MS, Milford, MA, USA). The retention time and the mass of the biotoxins were compared with the reference samples. The mass spectrometer was operated in a positive ion mode at 100 °C, a voltage of 40 V, the gas flow rate of 30 L/h, and gas pressure of 0.59 bar. The reverse-phase separation was carried out on a nanoACQUITY Symmetry C18 column (180 µm×20 mm; 5 µm) and a nanoACQUITY BEH130 C18 column (75 µm×250 mm). The injection volume was 5 µl, while the flow rate was set to 0.2 µl/min.

3. RAMCAP analysis

Different definitions of risk analysis have been presented in various scientific areas as the main part of risk management. The most important of these definitions consider risk as a combination of the probability of the risk severity, the severity of the risk effects, and the vulnerability of the components or units. New guidelines have been developed to assess and manage risk in water and wastewater plants. Figure 1 shows the different steps to evaluate the RAMCAP risk in water treatment plants [26]. RAMCAP was proposed the risk methodology of the choice advocated in the first official release of the National Infrastructure Protection Plan (NIPP). Based on the NIPP, RAMCAP satisfied the baseline criteria values for the risk assessment. RAMCAP is also applied based on the J100-10 American Water Works Association (AWWA) [27]. In this work, the first two stages of the assessment of units and the assessment of the pathogenic and biotoxin threats affecting the urban water treatment plant are investigated to be a model for assessing the vulnerability and risk of the water treatment plant to the threats.

Table 1 shows the criteria for the economic, functional and uniqueness of assets and facilities in water treatment plants. The economic value is the real value of an asset. The functional value means that if an asset is affected by the threat of damage, what impact it will have on the performance of the water treatment plants. As it can be seen, the

chlorination has the highest rating in the functional and uniqueness values in the indexes of the criteria of functional and uniqueness values of assets, indicating its importance in the water treatment plants. The unique value of equipment and assets is examined. In other words, if a unit is damaged by a threat, what are the problems, of which the value is numbered from one to 10 [28, 29]. The levels of criteria values for each plant, described in Table 1, were found from the AWWA J100-10 source [27], which were obtained from the completion of related questionnaires by experts in the water treatment industry.

Finally, the three described criteria are used to prioritize the assets and threats, and the vulnerability assessment of water treatment plants. The final score is obtained from the sum of the three criteria, which are presented in Table 2.

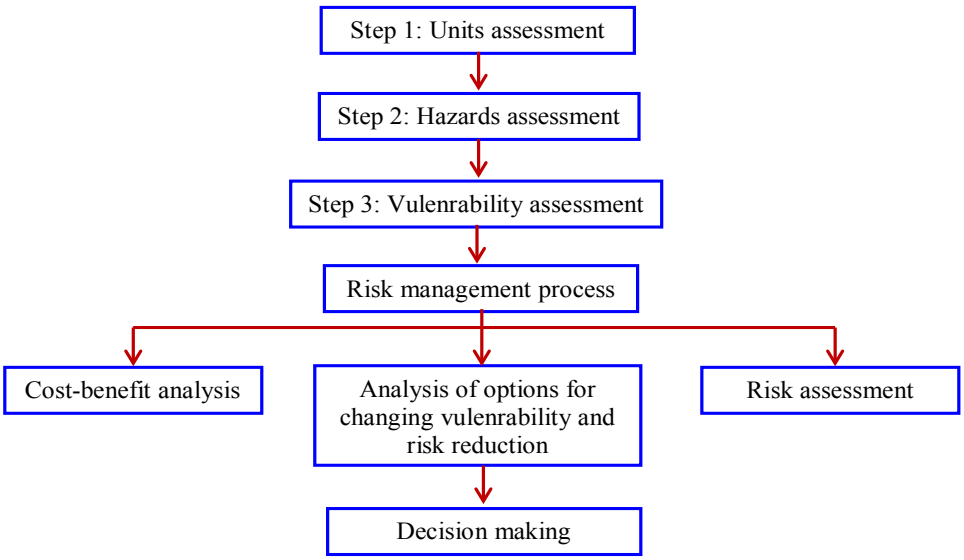

Figure 1. The Modeling of the Risk Assessment Process by the RAMCAP method [26].

Table 1
The criteria for the economic, functional and uniqueness of water treatment plant.

Row	Plants	Criteria values		
		Economic	Functional	Assets uniqueness
1	Chlorination	8	8	7
2	Ozonation	9	5	3
3	Sand filtration	2	6	2
4	Reverse osmosis	6	5	3
5	Coagulation & flocculation	4	4	6

Table 2
The scoring and quantification.

Mark	Value	Score range	
		Assets and threats	Vulnerability
A	1	1-5	1-6
B	3	6-10	7-13
C	6	11-15	14-20
D	8	16-20	21-27
E	9	21-25	28-34
F	10	26-30	35-40

4. Results and discussion

4.1. Physicochemical and microbial analysis results

Table 3 shows the chemical and microbial analysis results of the water in different points of water treatment plants. As shown, the pH of the water entering the disinfection unit is in the range of 7.1 to 7.9, which varies depending on the different measurement seasons. Usually, in spring and autumn, when the amount of the seasonal rainfall is high and the flow is more flooded, the water's pH increases. Also, the temperature of the water entering the treatment plant is different in different seasons. Clearly, in cold seasons, the water temperature is low and in hot seasons, the water temperature is affected by it. The turbidity of the water entering the treatment plant increases in spring and autumn, when seasonal floods are more likely. The increased turbidity is an essential factor in the growth and proliferation of pathogens and reducing the effectiveness of chlorine. According to the US-EPA standard, the maximum permissible water's turbidity is 5 NTU, while the optimum value is 1 NTU [30].

Table 3
The physicochemical and microbial analysis of water in different points of water treatment plants.

Physicochemical tests	Variation range	Microbial test	Variation range
FRC (mg/L)	0-1.21	Total *coliform* bacteria (MPN/100 mL)	0-1600
Temperature (^{o}C)	13.4-26.8	Fecal *coliform* bacteria (MPN/100 mL)	0-1600
pH	7.01-7.97	*Thermotolerant coliforms* (MPN/100 mL)	0-1580
Turbidity (NTU)	4.5-112.3	HPC (MPN/100 mL)	0-6501

4.2. Characterization of biotoxins

Figure 2 shows the LC-high-resolution MS analysis of the Ricin, which was was prepared by Trypsin with the initial concentration of 10 mg/L. As shown, there are several distinct peaks at the retention times of 12.9 min, 13.1 min, 13.8 min, 15.3 min and 20.8 min, which indicate the Ricin peptides obtained from chains A, and B of Ricin D, and Ricin E. The *m*/*z* range was obtained from 416 to 1139. The peaks at the retention times of 5.8 min, 9.7 min and 12.1 min represent peptides with low molecular weights and the *m*/*z* of 416, 422, and 448.7 mg/L respectively. The chemical structure of Ricin is shown in the image.

Figure 3 shows the LC-high-resolution MS analysis of the AF-types including AFB1 (1), AFG1 (2), AFB2 (3), and AFG2 (4) with the initial concentration of 10 mg/L. As it can be seen, four peaks at the retention times of 2.8 min, 3.9 min, 6.7 min, 15.3 min and 9.8 min represent the AFB1, AFG1, AFB2, and AFG2 respectively. The chemical structure of AFB1 is shown in the image.

Figure 4 shows the LC-high-resolution MS analysis of the STX with the initial concentration of 5 mg/L. The chemical structures of STX and NeoSTX are shown in the image. As it can be seen, two distinct peaks at the retention time of 3.4 min, and 9.6 min represent the STX (m/z 300.1) and NeoSTX (m/z 316.1) respectively.

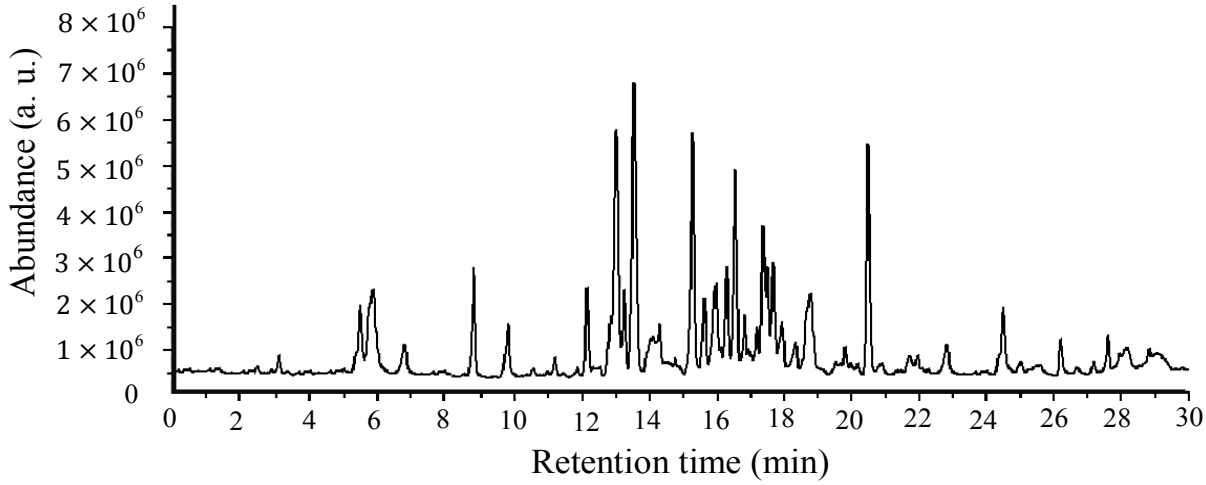

Figure 2. The LC high-resolution MA analysis of the Ricin [The chemical structure of Ricin is shown in the image].

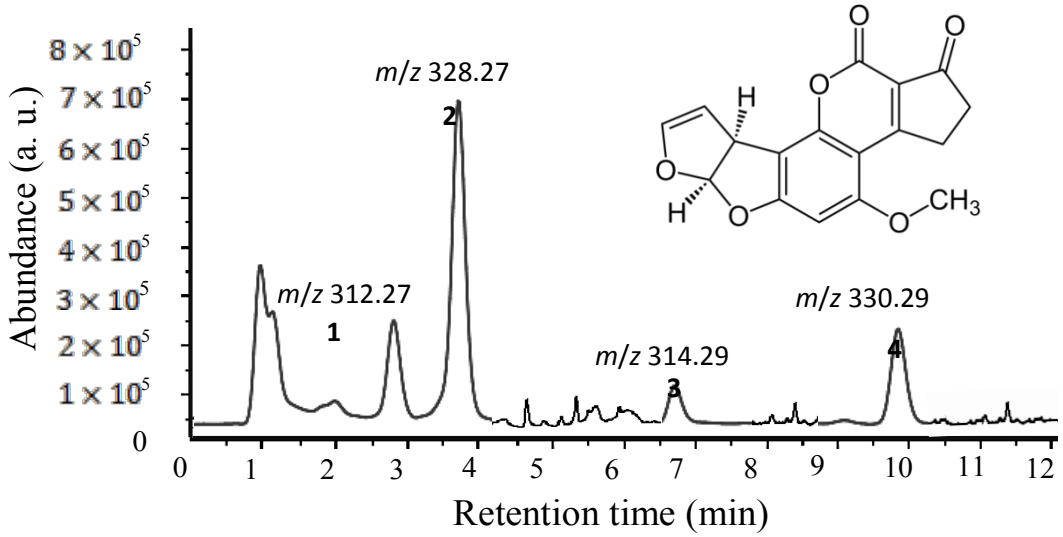

Figure 3. The LC high-resolution MA analysis of the AF-types including: (1) AFB1, (2) AFG1, (3) AFB2, and (4) AFG2 [The chemical structure of AFB1 is shown in the image].

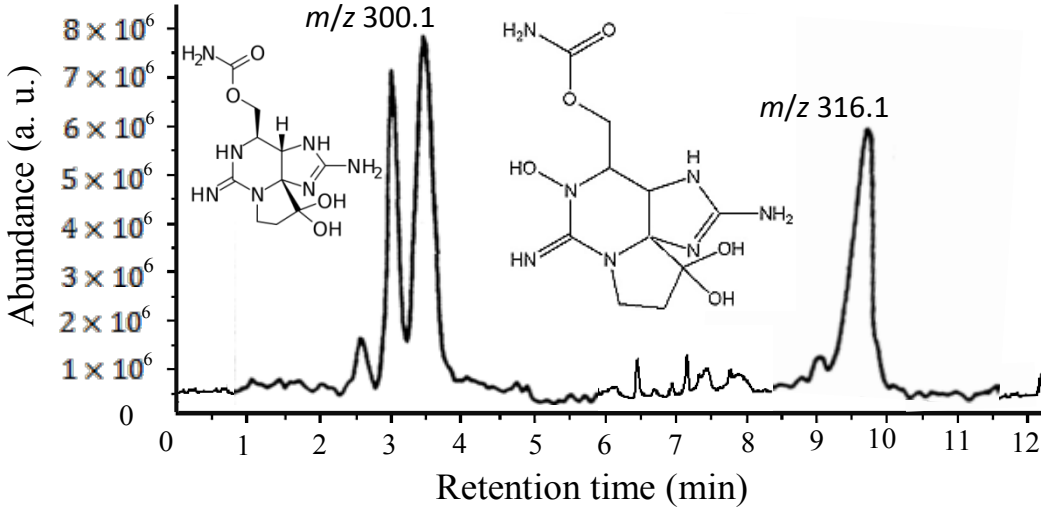

Figure 4. The LC high-resolution MA analysis of the STX, [The chemical structure of STX is shown in the image].

Figure 5 shows the LC high-resolution MA analysis of the ATX-a with the initial concentration of 1 mg/L. Chemically, ATX (inset, Fig. 5) has a semi-rigid bicyclic secondary amine structure, 2-acetyl-9-azabicyclo [4:2:1] non-2-ene (C10H15NO). A distinct peak at the retention time of 3.2 min represents the ATX-a (m/z 165.23).

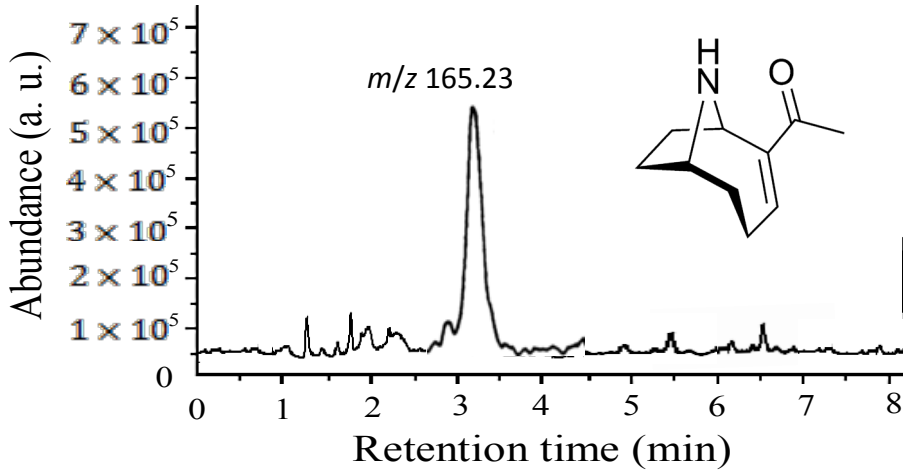

Figure 5. The LC high-resolution MA analysis of the ATX-a [The chemical structure of ATX-a is shown in the image].

4.3. Effect of physicochemical properties on pathogens analysis results

Figure 6 shows the effect of the pH (a), temperature (b), turbidity (c), and RE (d) values of water samples on the pathogens analysis results taken from before (a-c) and after (d) the chlorination plant. The microbial analysis of water samples showed that with increasing the pH of the water incoming to the disinfection unit, the number of coliform bacteria and HPC also increases nonlinearly (Fig. 6a). Figure 6b shows the effect of the inlet water temperature of the disinfection unit on removing the microbial agents. The European Union has set a guide number of at least 12 and at most 25 ^{o}C for the water. The results showed that the influence of temperature on the microbial agent and bacteriology of the water sample before entering the chlorination unit was minor. However, the results showed that increasing the temperature in the range of 22-26 °C can slightly increase microbial and bacteriological agents. This can be in the better provision of biological conditions of microorganisms in the temperature range of 28-22 ^{o}C [3, 4, 7, 29, 30]. Figure 6c shows the effect of turbidity of the water entering the disinfection unit on removing microbial and bacteriological agents. The turbidity of the water entering the chlorination unit is studied at different seasons. Figure 6c shows that the turbidity of the water entering the disinfection unit is directly related to the amount of the number of HPC bacteria, the total coliform and thermotolerant coliform, according to the Haas et al. findings [31]. The results have shown a direct relationship between the microbial parameters of water and its physicochemical properties [7]. Figure 6d shows the effect of the FRC of the effluent from the disinfection unit on removing microbial and bacteriological agents. According to the obtained results, the amount of HPC bacteria, total coliform and heatstroke can be significantly reduced by increasing the FRC. Those results showed that the effect of FRC on microbial and bacteriological factors

was more significant than thoes of other physicochemical parameters. Kelly and Sanderson [32] showed that applying an inappropriate strategy to disinfect the drinking water can lead to the regrowth of microbial and bacteriological agents in the drinking water.

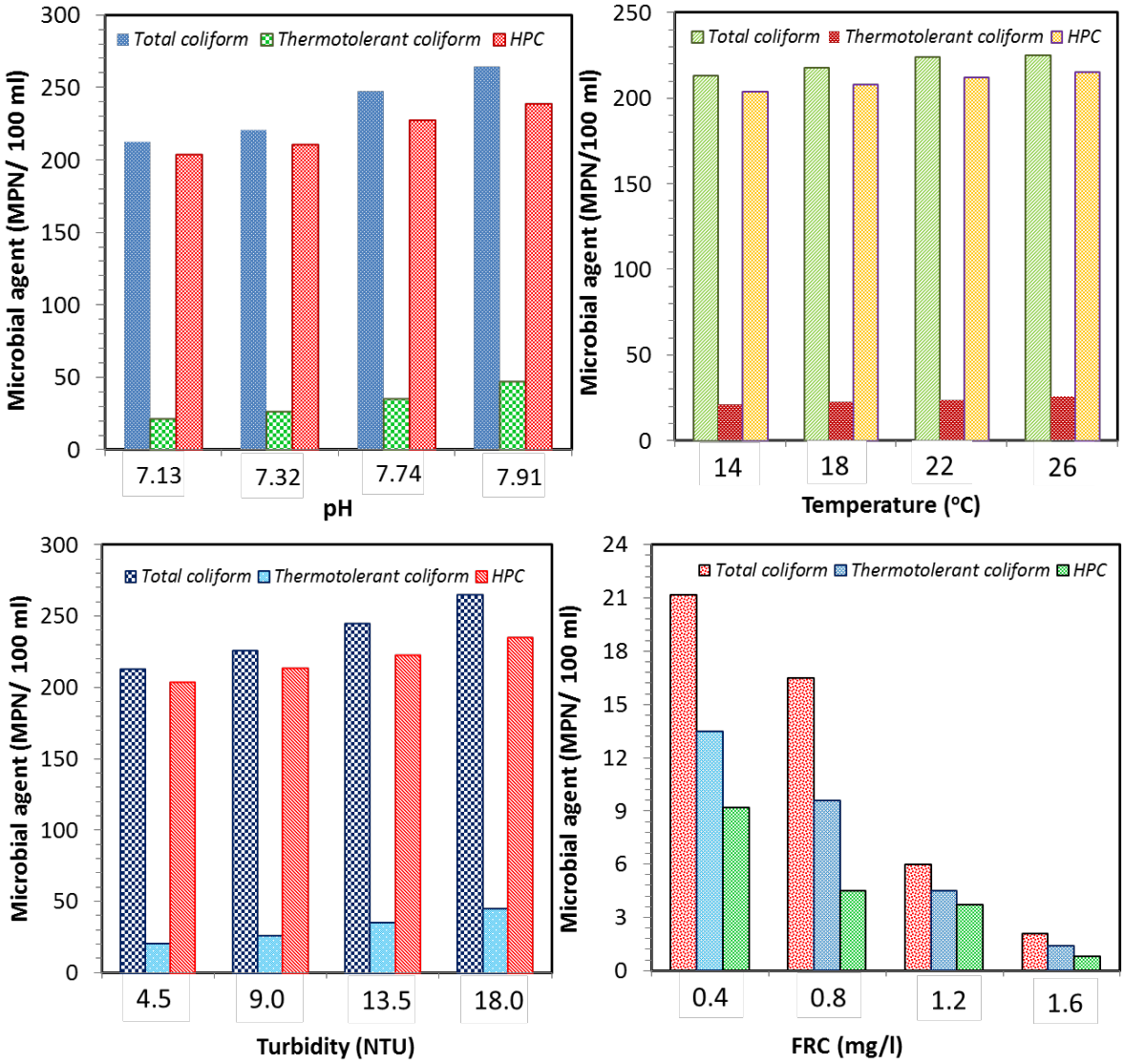

Figure 6. The effect of the (a) pH, (b) temperature, (c) turbidity, and (d) FRC values of the samples on the pathogens analysis results taken from before (a-c) and after (d) the chlorination plant.

Figure 7 shows the performance of water treatment plants to inactivate the main biotoxins. The ability to remove the biotoxin from water can be examined from two aspects: (1) the solubility of the toxin in water and (2) the inactivation power of the treatment unit. ATX-a has a high solubility in water (7.2×104 mg/L at 25 °C). The solubility of STX in water is 7.43×104 mg/L at 25 °C, which indicates the high solubility in water. The solubility of AFT in water is about 10-20 mg/L. Ricin is also a water-soluble protein. The high solubility of the mentioned biotoxins leads to a decrease in the performance of each of the water treatment plants in removing them. As shown in Fig. 7, the sand filtration unit has the lowest efficiency in removing biotoxins, while the reverse osmosis unit has a

high efficiency in removing Ricin. The results also showed that the AFT was completely eliminated by the disinfection units including chlorination and ozonation. It should be noted that the design of the disinfection units is critical in preventing the formation of other toxic and by-product compounds. Rositano et al. [20] showed that the ATX-a or STX could be destroyed neither with the chlorine doses exceeding 30-min chlorine demand nor by changes in the pH of water. Their results showed that the mentioned biotoxins (toxin concentration 20-24 µg/L) were effectively oxidized by 4 mg/L of chlorine at the pH of 7.2-7.4 [20]. The ozone is often injected into the water in the pre-coagulation stage to minimize the toxic halogen compounds formed by combining the oxidizing agent with the FRC in the coagulation stage. Hart and Stott [33] indicated that the 2 mg/L of ozone added to the raw water led to a 60 % removal of Microcystin, while the same dose added to the treated water removed toxins by 98 %. Hitzfeld et al. [8] reported that the cyanobacterial toxins containing 50-100 µg/L of Microcystin needed to be oxidized with at least 1.0 mg/L of ozone to effectively destroy the toxin, whereas ozone residuals were undetectable after 10 min.

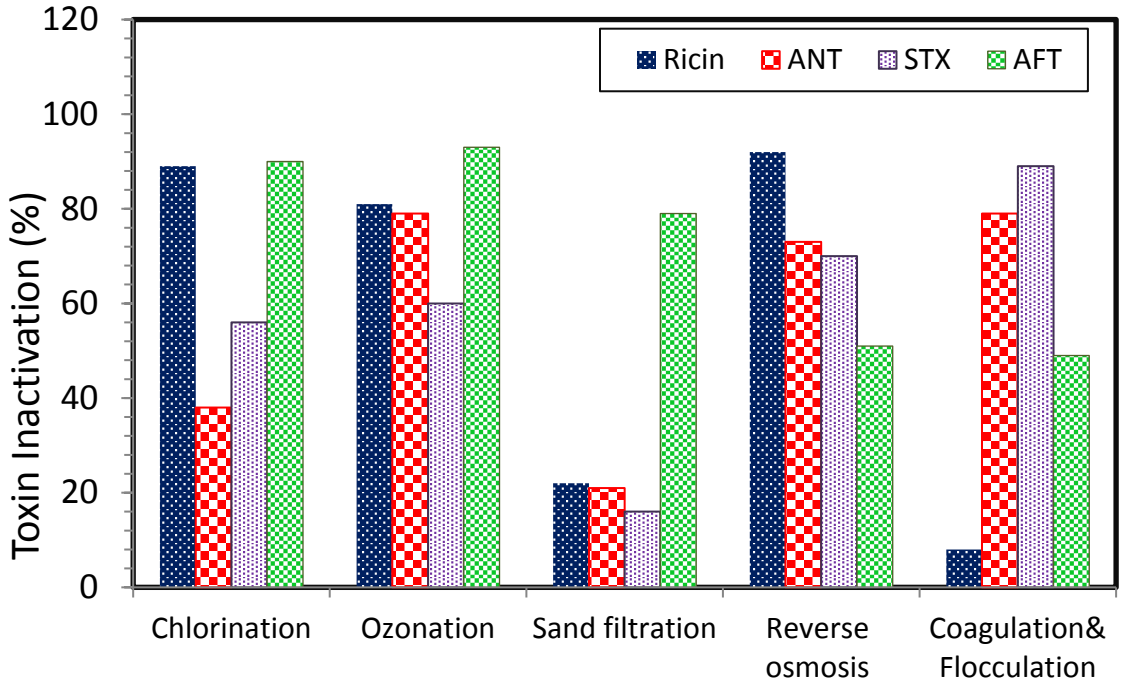

Figure 7. The performance of the water treatment plant in the inactivation of main biotoxins.

Figure 8 shows the performance of the water treatment plant to inactivate the main pathogens. As it can be seen, the disinfection processes, such as chlorination and especially ozonation, were considerably effective in reducing pathogens. Also, the use of reverse osmosis and coagulation/flocculation processes are somewhat effective in the reduction of the residual pathogens in the water. This finding is in good agreement with previous results. Kruithof [34] showed that combining physical and chemical treatment processes such as coagulation/sedimentation and oxidation/disinfection (ozone, chlorine) was useful to create multiple barriers against pathogens and microorganisms. Rose et al. [35] calculated that the amount of the concentration-time of the FRC for the

bacterial threatening agents was 271 mg.min/L for the 3-log inactivation at 5 °C. The presence of FRC in the drinking water transmission network (0.25-0.70 mg/L) can reduce the possibility of the drinking water contamination during the transmission in the network. Maul et al. [36] showed that the highest bacterial number was attributed to the lower FRC levels and the prolonged retention time of the water in the transmission network.

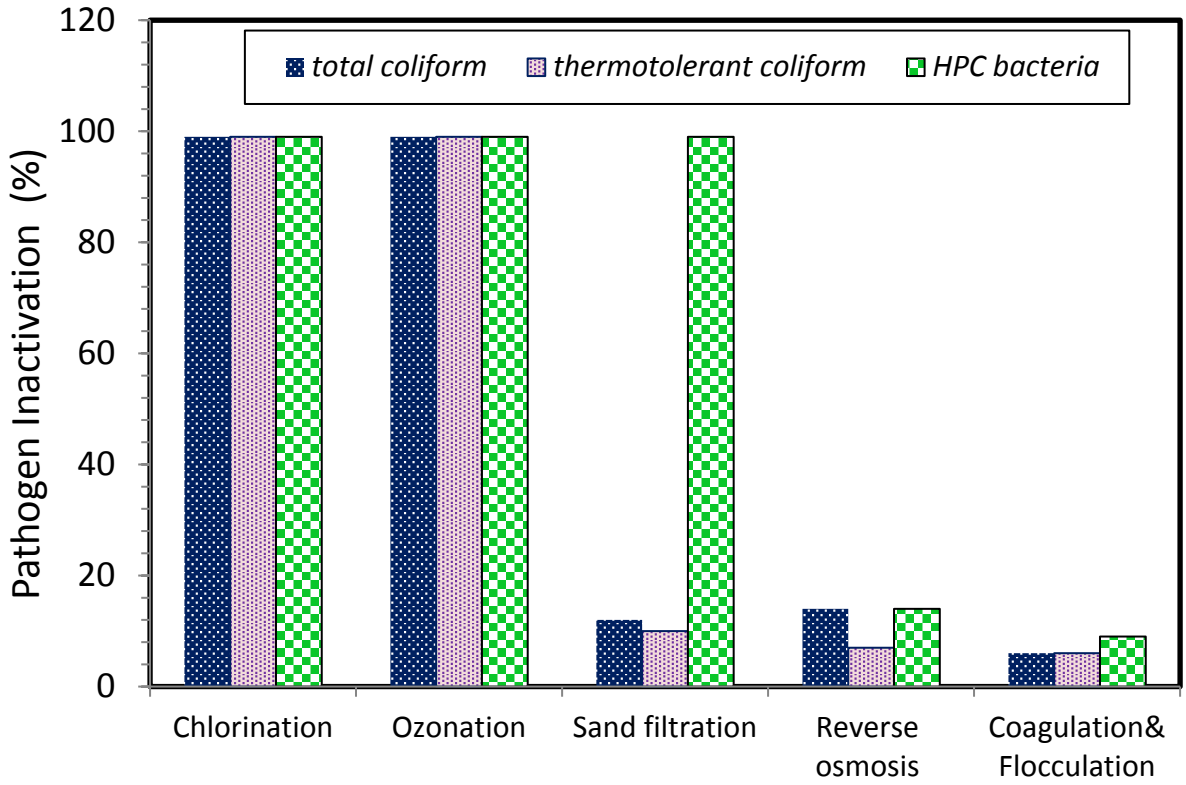

Figure 8. The performance of the water treatment plant against the inactivation of main pathogens.

4.4. RAMCAP analysis results

4.4.1. Assets assessment

Table 4 shows the results of the valuation and prioritization of the water treatment plant and the final asset rating from the sum of the three criteria selected. As it can be seen, the chlorination and ozonation units are the first priority in removing pathogens and biotoxins, while the sand filtration unit is the final stage in the water treatment plant in removing the mentioned agents.

Table 4
The results of the valuation and prioritization of the water treatment plants.

Plant	Total score	Variation range	Final score
Chlorination	23	E	9
Ozonation	18	D	8
Sand filtration	10	B	3
Reverse osmosis	14	C	6
Coagulation & flocculation	14	C	6

4.4.2. Assessment of hazards

Table 5 shows the results of the assessment of the pathogenic and biotoxin hazards in the water treatment plant. The scoring of biotoxin

and pathogenic agents were determined based on the factors including (1) the severity of the damage, (2) the history of the threat, and (3) the ability of the threat agents to enter each of the water treatment plants, which were obtained from the literatures [28, 29, 37].

Table 5
The assessment of the pathogenic and biotoxin hazards in the water treatment plant.

Hazards	Criteria values		
	Damage intensity	Threat history	Threat ability
Biotoxins	9	6	8
Pathogens	3	6	7

Table 6 shows the results of the valuation and prioritization of the pathogenic and biotoxin hazards in a water treatment plant and the final asset rating from the sum of the three criteria selected. As it can be found out, the biotoxin agents entering the water can be considered as more serious threats than pathogens for the water treatment plant. As the results of the water analysis showed disinfectant agents (chlorination and ozonation) could significantly eliminate the pathogens. Therefore, the biotoxins are considered as a more severe threat than pathogens for the water treatment plant.

Table 6
The assessment of pathogenic and biotoxin hazards in the water treatment plant.

Hazards	Total score	Variation range	Final score
Biotoxins	23	E	9
Pathogens	16	D	8

By comparing the results of Tables 4 and 6, it can be seen that chlorination and ozonation units have the highest performance in reducing pathogens. In contrast, the sand filtration unit has a functional weakness against the pathogens and biotoxins entering the water. These results were well confirmed by the experimental data obtained from the analysis of the inlet and outlet water to and from each of the mentioned units. Also, the results of the RAMCAP analysis showed that the performance of reverse osmosis and coagulation/flocculation units against pathogenic and biotoxins threats was the same.

5. Conclusions

Identifying the presence and assessing of pathogenic and biotoxin agents in an urban water treatment plant are of great importance to achieve the correct operation of the units in the face of potential threats. The LC-MS analysis results indicated that the inactivation rate of biotoxins was a function of their solubility in water and the performance of treatment units. Aflatoxin was eliminated by disinfection units, while the reverse osmosis had high efficiency in removing Ricin and the sand filtration had the lowest efficiency in removing biotoxins. The microbial tests showed that the total coliform, the thermotolerant coliform and the HPC agent were completely eliminated by the disinfection units. The use of reverse osmosis and coagulation/flocculation units is somewhat effective in the pathogens reduction. The results indicated a minor impact of the changes in the water temperature on the microbial agents. However, the pH and turbidity of the incoming water are directly related to the amount of pathogenic agents.

Also, the amount of the total coliform and HPC agent was significantly reduced by increasing the free residual chlorine. The RAMCAP risk analysis proposed that the disinfection unit was the first priority in removing pathogens and biotoxins, while the sand filtration unit was the final stage. The valuation and prioritization results showed that the biotoxins were considered a more severe threat than pathogens. The findings of this study can be applied for the practical applications to reduce the vulnerability of the treatment units to conventional threats.

Acknowledgement

The authors thank the Hamedan University of Technology for their supports under Grant No. 99.2227.

References

[1] Bose, P. and Reckhow, D. A., "The effect of ozonation on natural organic matter removal by alum coagulation", *Water Res*., **41** (7), 1516 (2007).

[2] Karnika, B. S., Daviesa, S. H. and Baumannc, M. J., "The effects of combined ozonation and filtration on disinfection by-product formation", *Water Res*., **39** (1), 2839 (2005).

[3] WHO, "Guidelines for drinking-water quality, Vol. 1, Recommendations", World Health Organization, Geneva, (2004).

[4] Figueras, M. and Borrego, J. J., "New perspectives in monitoring drinking water microbial quality", *Int. J. Environ. Res. Public Health*, **7** (1), 4179 (2010).

[5] Rahnemon, M. R., Sobhaniefar, M. J. and Haghshenas, M. R., "Smallpox and bioterrorism", *Mazand. Univ. Med Sci*., **23** (1), 122 (2013).

[6] Shukla, D., Vaghela, K. B. and Jain, N. K., "Assessment of physicochemical and bacteriological water quality parameters: A review", *Int. J. Res. Dev. Pharm. Life Sci.*, **5** (2), 1 (2017).

[7] Beauchamp, N., Lence, B. and Bouchard, C., "Technical hazard identification in water treatment using fault tree analysis", *Can. J. Civil Eng.*, **37** (6), 897 (2010).

[8] Hitzfeld, B. C., Hoger, S. J. and Dietrich D. R., "Cyanobacterial toxins: Removal during drinking water treatment, and human risk assessment", *Environ. Hlth. Persp*., **108** (1), 113 (2000).

[9] Al-Gheethi, A. A., Efaq, A. N., Bala, J. D., Norli, I., Abdel-Monem, M. O. and Ab. Kadir, M. O., "Removal of pathogenic bacteria from sewage-treated effluent and biosolids for agricultural purposes", *Appl. Water Sci.*, **8** (74), 1 (2018).

[10] Burrows, W. and Renner, S. E., "Biological warfare agents as threats to potable water", *Env. Hlth. Persp*., **107** (12), 975 (1999).

[11] Decamp, O. and Warren, A., "Investigation of *Escherichia coli* removal in various designs of subsurface flow wetlands used for wastewater treatment", *Ecol. Eng*., **14** (1), 293 (2000).

[12] Prat, R., Nofre, C. and Cier, A., "Effects of sodium hyperchlorite, ozone, and ionizing radiation on the pyrimidine constituents of *Escherichia coli*", (In French), *Annales de L'Institut Pasteur*, **114** (1), 595 (1968).

[13] U.S. EPA, Guidelines for water reuse, US Environmental Protection Agency, Office of Wastewater Management, Office of Water, Washington DC, EPA/600/R-12/618, (2012).

[14] Bastaminejad, S., Fallah, M. and

Maghsoud, A., "Detection of giardia cysts and cryptosporidium oocysts in drinking water surface suppliers, before and after treatment in hamadan", *J. Ilam Univ. Med. Sci.*, **21** (1), 29 (2013).

[15] Gopalakrishnakone, P., Balali-Mood, M., Llewellyn, L. and Singh, B. R. (eds.), Biological toxins and bioterrorism, Springer, New York, (2015).

[16] Reddy, S. V. and Waliyar, F., "Properties of aflatoxin and its producing fungi", *ICRISAT*., **10** (1), 95 (2000).

[17] De Wolf, F. A., Natural toxins, In: Moffat, A. C., Osselton, M. D. and Widdop, B. Editors, Clarke's analysis of drug and poisons, 3rd Editioin, The Pharmaceutical Press, London, p. 89 (2004).

[18] Worbs, S., Skiba, M., Söderström, M., Rapinoja, M. -L., Zeleny, R., Russmann, H., Schimmel, H., Vanninen, P., Fredriksson, S. -Å. and Dorner, B. G., "Characterization of ricin and R. *communis* agglutinin reference materials", *Toxins*, **7** (1), 4906 (2015).

[19] Hoffmann, J., "Removal of microcystin toxins in water purification processes", *Water SA*, **2** (1), 58 (1976).

[20] Rositano, J. and Nicholson, B., "Water treatment techniques for the removal of cyanobacterial toxins from water", Report 2/94, Salisbury, S. A., Australian Centre for Water Quality Research, Australia, (1994).

[21] Rositano J., Nicholson B. and Pieronne P. "Destruction of cyanobacterial toxins by ozone", *Ozone Sci. Eng*., **20** (1) 223 (1998).

[22] WHO, "Guidelines for chemical hazards in drinking-water: Microcystin-LR", WHO/SDE/WSH/03.04/57, World Health Organization, Geneva, (2003).

[23] Schneider M. and Bláha, L., "Advanced oxidation processes for the removal of cyanobacterial toxins from drinking water", *Environ Sci. Eur*., **32** (94), 2 (2020).

[24] Abo-Amer, A. E., Soltan, El. -S. M. and Abu-Gharib, M. A., "Molecular approach and bacterial quality of drinking water of urban and rural communities in Egypt", *Acta Microbiol Immunol. Hung*., **55** (1), 311 (2008).

[25] Lin, T. T. S. and Li, S. S. L., "Purification and physicochemical properties of ricins and agglutinins from *Ricinus communis*", *Eur. J. Biochem*., **105** (1), 453 (1980).

[26] Brashear, J., Olstein, M., Binning, D. and Stenzler, J., Risk analysis and management for critical asset protection for the water and wastewater sector, 2nd *Ed*., WEF., USA. (2007).

[27] Risk analysis and management for critical asset protection (RAMCAP®), Standard for risk and resilience management of water and wastewater systems, ANSI/ASME-ITI/AWWA J100-10, First Edition, (2010).

[28] Assistance infromation, Thypes of threats and how to evaluate them, 1st Ed., Passive Defense Organization, Iran, (2012).

[29] Daneshian, M., Botana, L. M., Dechraoui Bottein, M. -Y., Buckland, G., Campàs, M., Dennison, N., Dickey, R. W., Diogène, J., Fessard, V., Hartung, Th., Humpage, Leist, M. A., Molgó, J., Quilliam, M. A., Rovida, C., Suarez-Isla B. A., Tubaro, A., Wagner, K., Zoller, O. and Dietrich, D., "A roadmap for hazard monitoring and risk assessment of marine biotoxins on the basis of chemical and biological test systems", *ALTEX*, **30** (4), 487 (2013).

[30] U.S. EPA, Guidance manual for compliance with the surface water treatment rules", Turbidity Provisions, EPA/815-R-20-004, (2020).

[31] Hass, C. N., Meyer, M. A. and Paller, M. S., "Microbial alternations in water distribution systems and their relationship to physical-chemical characteristics", *J. AWWA*, **75** (1), 475 (1983).

[32] Kelly, S. M. and Sanderson, W. W., "The effect of chlorine in water on enteric viruses. II. The effect of combined chlorine on poliomyelitis and coxsackie viruses", *Am. J. Public Health*, **50** (1), 14 (1960).

[33] Hart, J. and Stott, P., Microcystin-LR removal from water, FR 0367, Marlow, Foundation for Water Research, Buckinghamshire, UK, (1993).

[34] Kruithof, J. C., Disinfection by-product formation: Dutch approach to control strategies, Microbial pathogens and disinfection by-products in drinking water, Health Effects and Management of Risks" p. 455-464, ILSI Press, Washington, (2001)

[35] Rose L. J., Rice, E. W., Hodges, L. and Shams, A. M., "Monochloramine inactivation of bacterial select agents", *Environ. Microbiol*., **73** (10), 3437 (2007).

[36] Maul, A., El-Shaarawi, A. H. and Block, J. C. "Heterotrophic bacteria in water distribution systems, I. Spatial and temporal variation", *Sci. Total Environ*., **44** (1), 201 (1985).

[37] Abbasi, S., "An experimental investigation on the effect of acid treatment of MWCNTs on the viscosity of water based nanofluids and statistical analysis of viscosity in prepared nanofluids", *Iranian Journal of Chemical Engineering (IJChE)*, **15** (3), 72 (2018).