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Research Paper / Araştırma Makalesi

Effects of Humic Acid and Bromide on Trihalomethane Formation during Water Disinfection with Chlorine

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ABSTRACT

Chlorination is one of the most important methods used in water disinfection. Chlorine reacts with natural organic substances in water and causes the formation of disinfection byproducts that might cause health problems. The predominant by-product of chlorination is trihalomethanes. Humic substances, which make up the majority of natural organic substances, are the primary precursors of trihalomethanes. In this study, the effect of different doses of chlorine on the formation of chloroform, bromodichloromethane, dibromochloromethane and bromoform in the presence of natural organic matter and bromide in drinking water was evaluated. Artificial raw water samples prepared with the addition of 2, 3 and 5 mg/L humic acid representing natural organic matter were subjected to chlorination at doses of 1, 2 and 3 mg/L and analysed on the 0th, 3rd and 7th day. The only trihalomethane formed was chloroform with a concentration of 20.52-131.13 µg/L. Increased humic acid and chlorine levels resulted in increased chloroform content. Free chlorine in the water caused chloroform formation to continue even on the 7th day. Accordingly, the amount of chloroform formed increased with the contact time. While the chlorine dose was constant, increased humic acid resulted in decreased free chlorine. To evaluate the effect of bromide on trihalomethane formation, 200 µg/L bromide was added to 2 mg/L humic acid containing water, and 1 mg/L and 2 mg/L chlorination was applied. At the end of the chlorination process in bromide-free waters, only 23.46-41.90 µg/L of chloroform was formed. In the presence of bromide, chloroform, bromodichloromethane, dibromochloromethane and bromoform were formed and the total trihalomethane level increased to 50.03-85.59 µg/L. While the ratio of brominated trihalomethane increased, the amount of chlorinated species decreased.

Keywords: Water, Disinfection, Chlorine, Bromide, Trihalomethanes, Humic acid

Suların Klorla Dezenfeksiyonunda Trihalometan Oluşumuna Hümik Asit ve Bromürün Etkisi

ÖZET

Suların dezenfeksiyonunda kullanılan en önemli yöntemlerden biri klorlamadır. Klor, sudaki doğal organik maddelerle reaksiyona girerek sağlık sorunlarına neden olabilecek dezenfeksiyon yan ürünlerinin oluşmasına neden olur. Klorlamanın baskın yan ürünü trihalometanlardır. Doğal organik maddelerin çoğunluğunu oluşturan hümik maddeler, trihalometanların birincil öncülleridir. Bu çalışmada farklı düzeylerde uygulanan klorun doğal organik madde ve hümik asit varlığında kloroform, bromodiklorometan, dibromoklorometan ve bromoform oluşumuna etkisi incelenmiştir. Denemede kullanılan su örnekleri, doğal organik maddeyi temsilen deiyonize suya 2, 3 ve 5 mg/L hümik asit eklenmesiyle hazırlanmış ve 1, 2 ve 3 mg/L düzeyinde klorlanarak 0., 3. ve 7. günlerde analiz edilmiştir. Oluşan tek trihalometan 20.52-131.13 µg/L düzeyindeki kloroform olmuştur. Artan hümik asit ve klor düzeyleri kloroform artışına yol açmıştır. Suda bulunan serbest formdaki klor 7. günde bile kloroform oluşturmuştur, dolayısıyla temas süresi de artışa etki etmiştir. Sabit klor dozunda artan hümik asit miktarı serbest kloru azaltmıştır. Trihalometan oluşumuna bromür iyonunun etkisini anlamak üzere 2 mg/L hümik asit içeren suya 200 µg/L bromür eklendikten sonra 1 mg/L ve 2 mg/L düzeyinde klor uygulanmıştır. Bromür içermeyen sularda klorlama işlemi sonunda sadece 23.46-41.90 µg/L düzeyinde kloroform oluşmuştur. Bromür varlığında ise kloroform, bromodiklorometan, dibromoklorometan ve bromoform oluşarak toplam trihalometan düzeyi 50.03-85.59 µg/L'ye yükselmiştir. Bromlu trihalometan oranı yükselirken klorlu türlerin miktarı azalmıştır.

Anahtar Kelimeler: Su, Dezenfeksiyon, Klor, Bromür, Trihalometanlar, Hümik asit

INTRODUCTION

Disinfection which is the process of removing pathogenic organisms, is a necessary water treatment step for food industry, and avoid infectious diseases transmitted with potable water [1]. The disinfectants used in the disinfection of water, while inactivating the microorganisms that cause diseases, react with the organic and inorganic substances in the water and cause the formation of disinfection byproducts (DBPs) with toxic effects [2-4]. Different DBPs can be formed depending on the type and amount of the disinfectant used and the organic and inorganic substances in the water. Chlorine, chloramine, chlorine dioxide, ozone and UV are widely used in the disinfection of water [5-6]. Chlorine, which is widely used worldwide due to its microbial efficiency, low cost, ease of supply and application, and protection against microbial contamination that may occur in pipelines, causes the formation of trihalomethanes (THMs). Gaseous chlorine (Cl_2), sodium hypochlorite (NaClO) or calcium hypochlorite ($Ca(ClO)_2$) is widely used in the disinfection of water with chlorine [4]. The greatest advantage of chlorination is that chlorine remains in water as residual chlorine after dosing, and disinfection activity continues in distribution and storage systems. Adding chlorine as a disinfectant to water causes the formation of hypochlorous acid (HOCl) and hypochlorite ions (OCl^-), expressed as free chlorine, have disinfectant properties and the ability to react with organic substances [5]. The chlorine balance of water depends on pH, and the pH value is a critical parameter that can affect both disinfection efficiency and the formation of DBPs in different manners [7].

After the discovery of halogenated DBPs in disinfected waters, researchers have conducted many studies focusing on the toxicity, formation mechanisms, precursors, speciation, control and removal technologies of DBPs [8-10]. DBPs are secondary pollutants formed as a result of the oxidation of natural organic matter (NOM) and inorganic compounds with disinfectants. NOMs, the precursors of DBPs, are primarily responsible for imparting taste, color and odor to water. NOMs, which also affect water quality, are a heterogeneous mixture of carbohydrates, proteins, lignin, and organic acids of various origins and types [6,11].

DBPs consist of a complex mixture having more than 700 defined and many more undefined types [4]. The key factors controlling the formation of DBPs are the character of the raw water source and characteristics of the disinfectant. The spectrum of NOM and halogenated molecules in water, pH, temperature, contact time, distribution system, disinfectant and concentration are variables that affect the formation of DBPs [12-13]. Many countries and international organizations have introduced legal regulations that determine the maximum levels of the most common DBPs due to the potential health risks that may arise from DBPs [14]. DBPs that occur because of chlorination vary according to raw water and disinfection conditions, but the dominant fraction is THMs. Haloacetic acids (HAAs), halonitromethanes, haloacetonitriles (HANs), chloramines, chlorophenols, halofuranones and chloral hydrate can be formed with THMs [12]. It has been shown that humic species (humic acid and fulvic acid) constitute a large part of natural organic substances, which are the precursors of DBPs; these structures are effective in the formation of THM and humic acid (HA) exhibits higher chlorine reactivity than fulvic acid [6]. Humic materials in the surface water originated from the degradation of plants, animal remains and/or soil runoff [11].

THMs are compounds formed by the replacement of active groups in organic compounds in water with halogens such as chlorine, bromine, and iodine and formulated as CHX3 [15]. These include chloroform (TCM), bromodichloromethane (BDCM), dibromochloromethane (DBCM), bromoform (TBM), dichloroiodomethane, bromodichloroiodomethane, chlorodiiodomethane, dibromoiodomethane, bromodiiodomethane and iodoform compounds [16]. The main THM compound formed is chloroform, and the presence of bromine in water leads to the formation of bromine and chlorine-based THM compounds such as BDCM and DBCM together with TBM [17]. Since there is little iodine in surface waters, the TCM, BDCM, DBCM and TBM compounds are expressed as total THM (TTHM or THM4), and limitations have been made by conducting many studies on them [16]. Many countries and international organizations have set a maximum limit for THMs, which is 100 µg/L in Europe, Canada and Turkey and 80 µg/L in the USA [12]. Since THMs are a hazard to human health, understanding the effects of humic acid on water disinfection with chlorine in order to control their formation is important for safe drinking water supply [11]. Chloroform and bromodichloromethane are included in the 2B group in the cancer classification made by the International Agency for Research on Cancer (IARC) and are listed as substances that possibly carcinogenic to humans. Dibromochloromethane and bromoform are among the compounds (Group 3) that not classifiable as to its carcinogenicity to humans [18].

Inorganic components in water affect the formation of byproducts during disinfection, and one of the most important inorganic components studied extensively is bromide, the precursor ion of TBM formation [3]. Bromide ions are found in aquatic environments worldwide and are generally at low levels in freshwater. However, depending on the water pollution, salt water intake and geology, higher levels can be found in waters that are close to salt deposits, estuarine waters and waters where urban-industrial wastewater discharges are made [19-20].

Bromide concentrations in natural waters are higher in oceans but generally vary between 4 and 1000 µg/L [21]. On the other hand, its concentration in natural spring waters in Europe varies between 30-200 µg/L, sometimes up to 500 µg/L [22]. The critical value of 50 µg/L bromide in waters is environmentally important. For this reason, the bromide concentration should be checked before disinfection in water [21]. During the disinfection of water containing bromide with chlorine, the bromide ion reacts with $HOCl/OCl^-$ and oxidizes to hypobromous acid (HOBr), which is much more reactive than HOCl. HOBr then reacts with THM precursors to form brominated THMs [23]. Brominated DBPs are known to be more toxic than chlorinated analogues [15, 24]. It has been reported in the literature that bromide ions are approximately 20 times more active against organic substances than chlorine. Therefore, besides the total amount of by-products, species distribution is also a major concern [7].

The most basic method for the control of THMs is the optimization of the chlorine dose. However, reducing the chlorine dose should not result in inadequate disinfection. The use of alternative disinfectants is also one of the methods. The formation of other by-products should be taken into account when choosing an alternative disinfectant. Other approaches to prevent the presence of THM in drinking water: (i) Source control; that is, reducing the precursors, especially humic substances, in the water before disinfection (ii) Removing the formed THMs from the water with appropriate technologies. The coagulation/flocculation process applied during treatment eliminates some natural organic matter. In addition, the use of additional techniques such as adsorption, membrane filtration or ion exchange resin before chlorination increases NOM removal. Air stripping process, adsorption by granular activated carbon, membrane process or UV-based processes are applied to remove the formed THMs [17].

Since chlorine is widely used in the disinfection of water, many studies have focused on the THM precursor HA. In this study, by applying different levels of chlorine to water, the formation of THM compounds, e.g., TCM, BDCM, DBCM and TBM, was investigated. In this context, humic acid was added to deionized water disinfected with UV to represent the real raw water to ensure that the experiments were carried out under controlled conditions, and the effect of bromide ions (Br^-) on THM formation was also examined.

MATERIALS and METHODS

Materials

Artificial raw waters were prepared using deionized water (18.2 MΩ·cm ultrapure water produced by a Sartorius-Arium 611VF model pure water device) disinfected with UV. The stock (5000 mg/L) humic acid solution was prepared using humic acid sodium salt (Sigma-Aldrich H16752) and kept in the dark at 4°C for a maximum of 20 days.

Chlorine solution was freshly prepared at a concentration of 1% (= 10000 mg/L) using 7% stock sodium hypochlorite (Merck 1056142500) solution.

A dosing solution at a concentration of 10 mg/L was prepared from a stock solution containing 1000 µg/mL Br^- (Bromide, High-Purity Standards) in water.

Certified standard solutions: 1000 µg/mL Br^- in water (Bromide, High-Purity Standards).

Certified reference material: SM 137. 1 mg/L bromide, 10 mg/L chloride, 1 mg/L fluoride, 1 mg/L nitrate, 5 mg/L phosphate and 10 mg/L sulphate in water (SM-137-801, High-Purity Standards).

Certified standard solution: 200 µg/mL TCM, 200 µg/mL BDCM, 200 µg/mL DBCM and 200 µg/mL TBM in methanol (THM mix, High-Purity Standards).

Internal Standard (IS): 2000 µg/mL fluorobenzene in methanol (AccuStandard).

Experimental Design

Water samples containing 2 mg/L, 3 mg/L and 5 mg/L humic acid were prepared using stock humic acid solution in deionized water. The water samples at different concentrations were subjected to chlorination at doses of 1 mg/L, 2 mg/L and 3 mg/L. The disinfected water samples were kept in sealed amber-coloured glass bottles under room conditions to prevent the loss of volatile byproducts, and samples were analysed on the 0th, 3rd and 7th day. All experiments, including blind ones, were performed in triplicate at 18.5±3°C to minimize the effect of temperature variation.

In order to investigate the effect of bromide ion, 200 µg/L Br^- was added to water containing 2 mg/L humic acid, and 1 mg/L and 2 mg/L chlorination was performed.

Disinfection by Chlorination

For 1 and 3 liter volumes, necessary amounts of chlorine solution (for 1, 2 and 3 mg/L) were added to amber-coloured bottles. For sufficient effectiveness of chlorine, a 30-min contact time was given, and afterwards, the analyses were performed. While the stock chlorine solution was kept in the dark at 4±2°C, the dosing solution was freshly prepared before disinfection.

Analysis of pH, Conductivity, Temperature and Colour

Determinations of pH and conductivity were made according to ISO 10523 [25] and EN 27888 [26], respectively, with a Thermo Orion Star 215 Model pH and conductivity measuring device. Temperature was determined with the thermometer included in the same device and colour was measured at 465 nm as a PtCo unit by using a Hach Lange DR 2800 spectrophotometer, respectively [27-28].

Chlorine Analysis

The amounts of free chlorine and bound chlorine in the water samples were determined titrimetrically according to the ISO 7393-1 method [29]. This method is suitable for the determination of total chlorine in the range of 0.03 - 5 mg/L. In the assay pH 6.5 buffer solution, N,N-diethyl-1,4-phenylenediamine sulphate (DFD) solution, ammonium iron (II) sulphate titration solution and potassium iodide (KI) were used. All solutions were kept in the dark at +4°C and used considering the times specified in the method. Bound chlorine was calculated by subtracting the free chlorine content from the total chlorine content.

Bromide Analysis

Bromide analyses were performed by liquid chromatography according to the EN ISO 10304-1 method [30]. The working conditions were as follows:

Device: Thermo Dionex ICS-5000+ Ion Chromatography; pump unit: isocratic pump (DP); eluent generator: EGC III KOH (produces KOH for anion analysis); detector: conductivity (DC); column: Dionex AS19 Analytical column - 4x250 mm; front column: Dionex AG19 Guard column - 4x50 mm; suppressor: anion suppressor, CSRS 4 mm; autosampler: AS-DV; injection volume: 25 µL; firmware: Chromelon; mobile phase: produced automatically from deionized water with an eluent generator. The retention time was 14.45 min, and the limit of quantification (LOQ) was 20 µg/L.

Calibration: Calibration standards were prepared in deionized water at concentrations of 0.020-0.050-0.125-0.25-0.5-1 mg/L. Standard solutions were prepared in PE bottles and kept at 4 °C in the dark. The calibration graph ($y = 1.0072x-0.0363$, $R^2 = 0.9999$) was drawn with 6 points.

Trihalomethane Analysis

Total THM analyses were performed on a purge and trap (P&T) gas chromatography-mass spectrometry (GC-MS) system according to the EPA method [31]. The purge and trap extraction method does not require any preparation. Water samples were filled to the brim in 40 mL glass vials so that no air gap was left and then injected directly into the device. The working conditions were as follows:

P&T terms: purge and trap unit: Teledyne Tekmar/Atomx (Serial No: US13302007); injection volume: 5 mL, transfer line temperature: 140 °C, valve oven temperature: 140 °C, and purge flow rate: 40 mL/min.

Trap10 (tenax): purge time: 10 min, trap temperature: 180°C, dry purge time: 1 min, desorp preheating temperature: 245°C, desorp temperature: 250°C, desorp time: 2 min, bake temperature: 260°C, and bake time: 2 min.

GC-MS conditions: device: Agilent Technologies 7890A GC System (Serial Number: CN12481071).

Inlet temperature: 180ºC, split ratio: 5:1, flow rate: 1.2 mL/min, column: Agilent DB-624, 30 m x 0.25 mm x 1.40 µm, detector: Agilent Technologies 5975C inert XL MSD with Triple-Axis Detector, acquisition mode: SIM, and runtime: 19.79 min.

Oven: The oven temperature program is single ramp. After being held constant at 40°C for 4 minutes, it increases by 10°C per minute until reaching 220°C, where it is held steady for 2 minutes at this temperature.

In all analyses, an internal standard solution (fluorobenzene) prepared at a concentration of 125 mg/L and loaded in the device was used. Samples were placed in 40 mL screw septum-capped glass vials.

Calibration: TCM, BDCM, DBCM, and TBM working standards were prepared as mixtures from the intermediate stock solution diluted at a concentration of 2 mg/L in methanol (Sigma-Aldrich 24229) in the range of 0.2 - 100 (0.2-0.5-1-5-10-25-50-100) µg/L using deionized water. The calibration curve was plotted using 25 µg/L fluorobenzene as an internal standard. To determine the high concentrations of TCM, a curve in the range of 5 - 200 (5-10-25-50-100-200) µg/L was also drawn. Data (curve equation, linearity, LOQ, retention time and ions) for the tested THMs are given in Table 1.

Statistical Analysis

The effect of different chlorine doses applied to water samples with different concentrations of humic acid on trihalomethane formation was evaluated using variance analysis in three replicate factorial patterns. According to the results of variance analysis, the differences between groups were evaluated using the One-way-ANOVA test. The possible difference caused by chlorine doses in the effect of bromide ions on by-product formation was evaluated by one-way analysis of variance (ANOVA). Open source R statistics software [32] and SPSS package program were used in the statistical calculations.

Table 1. Limit of quantification (LOQ), retention time, fragmentation ions and linearity data for trihalomethanes

Trihalomethane	Equation (y=ax+b)	Linearity	LOQ (µg/L)	Retention time (min)	Ions (m/z)
TCM	y=0.9760x -0.0034	R^2=0.9993	0.2	10.26	83, 85, 47
BDCM	y=0.9619x+0.0031	R^2=0.9992	0.2	11.56	83, 85, 129
DBCM	y=0.9369x+0.0221	R^2=0.9988	0.2	12.67	129, 127, 131, 79
TBM	y=0.9141x+0.0385	R^2=0.9986	0.2	13.73	173, 171, 175, 105
Fluorobenzene (IS)	-	-	-	10.92	96, 70, 95, 97

RESULTS and DISCUSSION

Effects of Different Chlorine and Humic Acid Doses on THM Formation

According to the results, the chlorination process led to the formation of only TCM in water samples containing different amounts of HA. Other THM compounds were not formed. The amount of TCM formed depending on the chlorine and HA doses and analysis day is shown in Table 2. When the chlorination dose is constant, the amount of TCM formed is directly related to the amount of HA in water. As the amount of HA increased, the amount of TCM formed increased. At the same time, when the amount of HA was constant, the formed TCM amount increased as the applied chlorine dose increased.

Table 2. Effects of various chlorine and humic acid doses on chloroform formation [a, b, c]

Chlorine (mg/L)	Humic acid (mg/L)	Chloroform (µg/L)		
		Day 0	Day 3	Day 7
Control (0)	2	0	0	0
	3	0	0	0
	5	0	0	0
1	2	20.52±1.25^{aA}	21.02±1.56^{aA}	20.37±1.42^{aA}
	3	29.50±3.95^{aA}	32.35±3.59^{aA}	34.50±3.97^{aB}
	5	43.73±5.68^{aB}	46.64±5.46^{aB}	45.84±3.57^{aC}
2	2	28.98±0.95^{aA}	33.70±2.04^{abA}	39.30±4.23^{bA}
	3	57.22±7.53^{aB}	60.59±8.25^{aA}	60.73±6.71^{aA}
	5	108.59±11.81^{aC}	108.22±11.44^{aB}	106.92±10.92^{aB}
3	2	36.18±1.15^{aA}	38.85±1.91^{abA}	45.19±3.27^{bA}
	3	71.47±8.69^{aB}	76.05±9.48^{aB}	83.15±8.99^{aB}
	5	120.42±7.31^{aC}	124.79±7.08^{aC}	131.13±6.70^{aC}

[a]: Values shown in the table are average values of three replicates (±standard deviation). [b]: For the same humic acid amount, different letters (a-b) in the same line indicate statistically significant differences ($p<0.05$). [c]: For the same chlorine amount, different letters (A-C) in the same column indicate statistically significant differences ($p<0.05$).

Contact time, which is also responsible for the formation of THMs, is a critical factor for inactivating microorganisms. Many studies have shown that THM levels increase with increasing contact time. It has been reported that THM formation is faster in the beginning and slows down over time [17].

THM formation, which starts with disinfection in the water treatment process, can continue in the distribution system due to the release of residual free chlorine in the water. In a study, it was observed that by increasing the contact time from 30 min to 24 hours, there was a two- to three-fold increase in the total THM and haloacetic acid concentration. DBPs can continue to form as long as residual disinfectant and precursor are present, and the rate of formation varies greatly according to the properties of organic matter [13]. In another study showing the effect of contact time, the concentrations of total THMs and individual compounds increased with a longer contact time, but the concentration of haloacetic acid showed little change with the contact time. The greatest change in concentration with contact time was obtained for TCM. The amount of TCM, which was 0.2 - 2 µg/L in one hour of contact time, increased to 2.5 - 4 µg/L after 24 hours [33]. In another study, the concentration of THMs in the distribution system increased by an average of 36% [34]. The available information shows that THMs occur with a rapid onset formation during chlorination, followed by a formation that continues with a slower increase rate over time. During chlorination, both the reactive group and free chlorine are reduced, which reduces the reaction rate and inhibits the formation of THMs [7].

In our study, when the chlorine dose was constant, the amount of TCM formed increased linearly with the contact time. The presence of free chlorine in the environment caused TCM formation to continue. Since the samples were kept closed, increased levels of TCM were detected even at the end of the 7th day. The EU limit is 100 µg/L for THMs in drinking waters. The TCM content was determined to be 20.52 - 131.13 µg/L in this study. As a result of chlorination of 5 mg/L HA with 2 mg/L and 3 mg/L Cl_2, it was observed that the amount of TCM formed at the end of day 0, day 3 and day 7 varied between 106.92 and 131.13 µg/L, which exceeded the legislative limit.

Effect of Bromide Ion on THM Formation

After 1 and 2 mg/L chlorine treatment, only TCM was formed in no bromide-free samples, while BDCM, DBCM, TBM and TCM were formed in waters containing bromide. It has been determined that Br^- ions are more reactive to NOMs than chlorine and that the type of THM changes due to the presence of bromide. When the formation of brominated THM increases, the amount of chlorinated THM species decreases. The applied chlorine dose was effective on the amount of TCM formed. The amounts of TTHMs formed at the end of the 1 mg/L and 2 mg/L chlorination processes were 50.03 µg/L and up to 85.60 µg/L, respectively (Fig. 1).

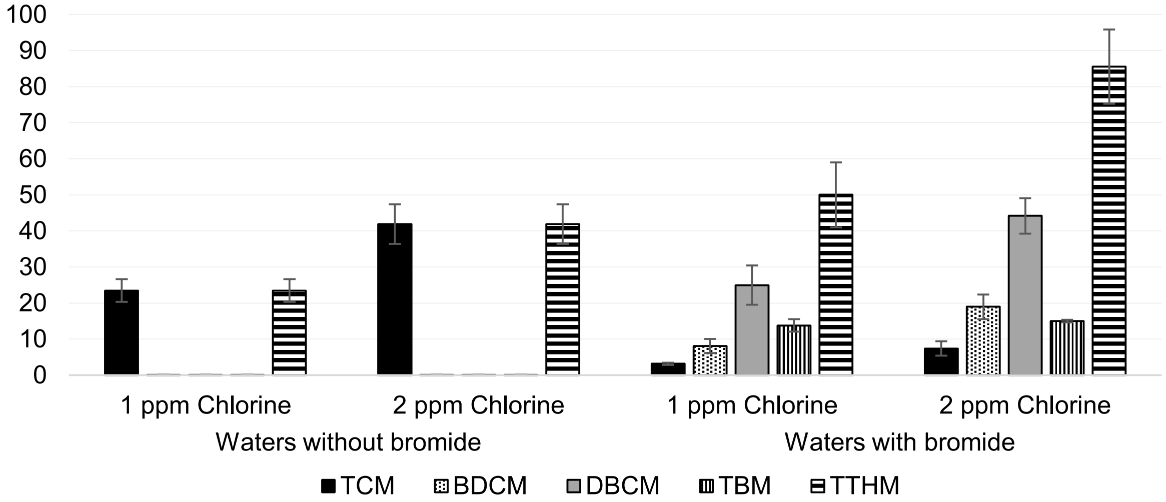

Figure 1. Effects of bromide on THM formation (µg/L) with different chlorine doses. [a]TCM: Trichloromethane (chloroform); BDCM: Bromodichloromethane; DBCM: Dibromochloromethane; TMB: Tribromomethane (bromoform); TTHM: Total THMs. [b]THMs were not formed in control (0 mg/L chlorine) samples

In a study conducted by Sakai et al [8], TBM could not be determined in the chlorination of waters containing different levels of total organic carbon (1.45 - 6.84 mg/L) and Br^- (32-222 µg/L), while the highest TCM was formed among the four tested THM types, followed by BDCM. While the DBCM content showed a high correlation with bromide, no relationship was observed between organic matter and DBCM formation.

In our study, when waters containing 200 µg/L Br^- were subjected to a 1 mg/L chlorination process, the order of formed compounds was found to be DBCM> TBM> BDCM> TCM THM. When the amount of chlorine is increased to 2 mg/L, there is a shift from brominated DBPs to chlorinated byproducts, as chlorine can compete with the more reactive Br^-. By observing an increase in the amount of BDCM, the order changed to DBCM> BDCM> TBM> TCM (Fig. 1). This finding shows the importance of bromide and chloride levels in determining the distribution of THMs formed. In a study investigating the effect of bromide ion amount on THM formation during chlorination [35], the order of DBCM> TBM> BDCM> TCM in the presence of 200 µg/L Br^-, 9.61 µg/L DBCM, 4.49 µg/L TBM, 4.47 µg/L BDCM and 3.65 µg/L TCM were observed.

In a study on chlorination of different concentrations of Br^-containing waters, the order of the amount of THMs formed at low bromide ion contents was TCM> DBCM> BDCM> TBM. With increasing Br^-, the content of TCM decreased sharply, and BDCM and DBCM levels decreased after rising in the first stage, while the TBM concentration increased continuously. At high bromide ion concentrations (>500 µg/L), TBM becomes the main component [24]. In another study where similar results were obtained, TCM was the main component, with a proportion of 40 - 98% in spring water forming 14.2 - 143 µg/L TTHM, followed by BDCM [34].

Padhi et al [13] found 38.3 µg/L THMs in sea water, 18.8 µg/L in river water and 21.5 µg/L in tank water at a 1 mg/L Cl_2 dose and 30 min contact time. TBM has been observed to be the only dominant species constituting approximately 90% of the total THMs in seawater containing high amounts of bromide. It has been reported that the distribution of THM species in river water is in the order of TCM ~ DCBM> CDBM> TBM, and the distribution between chloro and bromo analogues of THMs primarily depends on the bromide concentration in raw water.

The bromine atom has a higher molecular weight than chlorine. Therefore, the mass concentration of by-products increases upward with increasing Br^- level [17]. In our study, it was found that the presence of 200 µg/L bromide caused an average 108.76% increase in the amount of TTHM in chlorination processes. The amount of TTHM, which was 23.46 µg/L in the 1 mg/L chlorination process, increased to 50.03 µg/L in the presence of bromide. Likewise, for 2 mg/L chlorination, these values increased from 41.90 µg/L to 85.59 µg/L. Chlorination of water typically yields chloroform from total THM compounds. Chlorination of bromine-containing waters resulted in the formation of Br-THM, and the presence of Br and chlorinated by-product species shifted towards mixed brominated-chlorinated THMs.

Effect of Treatments on Free, Total and Bound Chlorine Levels

The effects of HA content and chlorine dose on the amount of free chlorine, total chlorine and bound chlorine are given in Table 3.

Table 3. The effects of humic acid content and chlorine dose on the amount of free chlorine, total chlorine and bound chlorine [a, b, c]

Residue chlorine	Humic acid (mg/L)	Chlorine (mg/L)			
		0	1	2	3
Free chlorine	2	0^{a}	0.73±0.03^{bA}	1.50±0.04^{cA}	2.31±0.01^{dA}
	3	0^{a}	0.62±0.03^{bA}	1.47±0.03^{cA}	2.29±0.06^{dA}
	5	0^{a}	0.54±0.09^{bA}	1.290±0.16^{cA}	1.64±0.18^{cB}
Total chlorine	2	0^{a}	0.73±0.03^{bA}	1.51±0.05^{cA}	2.38±0.02^{dA}
	3	0^{a}	0.64±0.02^{bA}	1.49±0.04^{cA}	2.36±0.08^{dA}
	5	0^{a}	0.55±0.09^{bA}	1.31±0.17^{cA}	1.65±0.18^{cB}
Bound chlorine	2	0^{a}	0.006±0.006^{aA}	0.006±0.003^{aA}	0.07±0.02^{bA}
	3	0^{a}	0.02±0.01^{abA}	0.02±0.01^{abA}	0.07±0.03^{bA}
	5	0^{a}	0.01±0.01^{aA}	0.01±0.01^{aA}	0.006±0.006^{aA}

[a]: Values shown in the table are average values of three replicates (±standard deviation). [b]: For the same humic acid amount, different letters (a-c) in the same line indicate statistically significant differences ($p<0.05$). [c]: For the same chlorine amount, different letters (A-B) in the same column indicate statistically significant differences ($p<0.05$).

According to the results, while the chlorine dose was constant, the increase in the amount of HA caused a decrease in the amount of free chlorine. When the amount of HA was constant, the amount of free chlorine increased as the chlorine dose increased. The term "residual disinfectant" is used for the concentration of chlorine detected in the water after the disinfectant has been applied in the disinfection process. The effectiveness of chlorine depends on the amount applied and the residual disinfectant concentration. The stability of the disinfectant under the application conditions and the presence of chlorine-requiring substances in the disinfection environment greatly affect the residual disinfectant level. Natural organic matter, PAHs, pesticides, and metals in water react with chlorine and therefore increase the demand for disinfectants in practice. Residual disinfectants, i.e., free chlorine, can significantly affect disinfection and by-product formation [36-37]. Accordingly, the results show that increasing the amount of HA increases the need for disinfectants.

Effects of Treatments on pH, Electrical Conductivity and Colour

The effects of the different chlorine and HA doses on pH, electrical conductivity and colour are given in Table 4. The increase in the chlorine dose caused an insignificant increase in the pH. It has been reported that pH is effective for DBP formation and THM formation increases with increasing pH [17]. In a study, it was observed that increasing the pH from 5.0 to 9.0 increases the formation of THM during chlorination [2]. However, the effect of pH on THM formation was not investigated in our study. It was observed that the increased active chlorine and HA content resulted in increased conductivity. While increasing the HA dose increased the colour, some colour loss occurred with oxidation caused by chlorine.

Table 4. The effects of humic acid content and chlorine dose on several analytical parameters [a, b, c]

Analyte	Humic acid (mg/L)	Chlorine (mg/L)			
		0	1	2	3
pH	2	5.67±0.16^{aA}	6.31±0.18^{bA}	6.87±0.01^{cA}	7.18±0.09^{cA}
	3	6.21±0.27^{aA}	6.34±0.09^{aA}	6.94±0.08^{bA}	7.32±0.12^{bA}
	5	6.50±0.32^{aA}	6.95±0.41^{aA}	7.21±0.28^{aA}	7.34±0.30^{aA}
Conductivity (μS/cm)	2	1.69±0.07^{aA}	8.99±0.1^{bA}	16.73±0.11^{cA}	24.40±0.22^{dA}
	3	2.19±0.29^{aA}	9.11±0.54^{bA}	16.39±13.46^{cA}	23.75±0.93^{dA}
	5	3.47±0.55^{aB}	16.09±2.91^{bB}	23.74±2.92^{cB}	26.40±0.99^{cA}
Colour (PtCo)	2	33.00±2.08^{aA}	24.33±0.67^{bA}	23.33±1.20^{bA}	20.67±0.33^{bA}
	3	47.33±3.71^{aA}	34.33±4.70^{abA}	31.00±4.00^{bA}	29.67±3.93^{bA}
	5	94.67±6.64^{aB}	75.67±4.63^{bB}	68.67±2.40^{bB}	64.67±3.33^{bB}

[a]: Values shown in the table are average values of three replicates (±standard deviation). [b]: For the same humic acid amount, different letters (a-d) in the same line indicate statistically significant differences ($p<0.05$). [c]: For the same chlorine amount, different letters (A-B) in the same column indicate statistically significant differences ($p<0.05$).

Effects of Bromine on Free, Total and Bound Chlorine and Several Water Quality Parameters

The effects of chlorine dose and bromine addition at a constant HA dose of 2 mg/L on the amount of free chlorine, total chlorine and bound chlorine and several other water quality parameters are given in Table 5. The bromide ion did not have a significant effect on pH, electrical conductivity, free chlorine or colour. pH along with bromide ions is an important factor in the formation of THM. Therefore, by-product formation should be considered together with factors that can act simultaneously, such as bromide, pH, chlorine dose, amount of HA, and contact time. It was shown that an alkaline pH resulted in increased THM formation [7]; however, in our study, the effect of bromide ions on pH was not evaluated.

Table 5. The effect of bromide presence on some water quality parameters and residual disinfectant levels with various chlorine (chl.) treatments [a, b]

Analyte	2 mg/L humic acid			2 mg/L humic acid and 200 µg/L Br^-		
	Chlorine (mg/L)					
	0	1	2	0	1	2
pH	5.90 ± 0.26^{A}	6.62 ± 0.21^{B}	7.03 ± 0.17^{C}	6.35 ± 0.16^{B}	6.59 ± 0.15^{B}	6.99 ± 0.13^{C}
Conductivity (µS/cm)	1.64 ± 0.04^{A}	8.97 ± 0.05^{B}	16.66 ± 0.03^{C}	1.73 ± 0.13^{A}	8.37 ± 0.53^{B}	15.46 ± 0.72^{C}
Temperature (°C)	18.80 ± 1.6^{B}	18.40 ± 1.1^{B}	18.40 ± 1.2^{B}	16.40 ± 0.1^{A}	17.30 ± 0.2^{AB}	17.70 ± 0.7^{AB}
Colour (PtCo)	30.00 ± 1.0^{C}	23.00 ± 1.0^{B}	22.00 ± 2.0^{B}	28.00 ± 2.0^{C}	19.00 ± 0.0^{A}	17.00 ± 1.0^{A}
Bromide (µg/L)	-	-	-	150.00 ± 17.32^{B}	28.00 ± 1.16^{A}	26.00 ± 2.00^{A}
Free chl. (mg/L)	-	0.63 ± 0.03^{A}	1.52 ± 0.06^{B}	-	0.58 ± 0.01^{A}	1.38 ± 0.04^{B}
Total chl. (mg/L)	-	0.65 ± 0.02^{B}	1.55 ± 0.05^{C}	-	0.58 ± 0.01^{A}	1.39 ± 0.04^{C}
Bound chl. (mg/L)	-	0.02 ± 0.01^{A}	0.03 ± 0.02^{A}	-	0.00 ± 0.00^{A}	0.01 ± 0.00^{A}

[a]: Values shown in the table are average values of three replicates (±standard deviation). [b]: For each of the analytes, different letters (A-C) indicate statistically significant differences ($p<0.05$), and at least one common letter indicates that the difference is nonsignificant ($p>0.05$).

CONCLUSION

Increasing the amount of HA and chlorine increased the formation of THM, which has negative effects on health. In the European Union, the limit value for the total amount of THM in drinking water is 100 µg/L. Chlorination of 5 mg/L HA-containing waters with 2 mg/L and 3 mg/L Cl_2 resulted in TCM contents between 106.92 µg/L and 131.13 µg/L at the end of days 0, 3 and 7. All of these values exceeds the legislative limit. This means that chlorination of high HA-containing waters with 2 or 3 mg/L chlorine is not suitable for such waters. In this type of water, the amount of HA should be reduced by treatment techniques such as coagulation and activated carbon adsorption, or alternative disinfection methods such as ozonation should be evaluated.

When the chlorine dose was constant, it was found that increased contact time (0, 3 and 7 days) increased TCM formation. The presence of free chlorine in water samples caused continuous TCM formation. This shows that THM formation, which starts with disinfection, can continue in the distribution network when residual free chlorine is found in the water.

As a result of chlorination, only TCM was formed in water samples containing different amounts of HA representing natural organic substances. In the presence of bromide in water, bromine-containing halomethanes are formed as a result of chlorine treatment. DBCM occurred as the dominant species.

The presence of bromide ions in water increased THM formation from 23.46 - 41.90 µg/L to 50.03 - 85.59 µg/L and changed the THM diversity. Therefore, the presence and amount of bromide in the water is a critical factor to consider when choosing the disinfection method.

When the effects of chlorine and HA applications on free, total and bound chlorine levels and pH, electrical conductivity and colour are examined, an increase in the chlorine dose generally caused an increase in the amount of free, total and bound chlorine, pH and electrical conductivity, and a decrease in colour. As the content of HA increased, the electrical conductivity and colour increased, while the amount of free, total and bound chlorine decreased.

Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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