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Research article

Mono and polycyclic aromatic hydrocarbons in waterpipe wastewater: Level and ecotoxicological risk assessment

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ABSTRACT

Increasing of tobacco consumption around the world has led to the production of a large volume of waterpipe wastewater that enter the environment (e.g., coastal areas)and threaten aquatic creatures. However, until now, no research has been carried out on the amounts of monocyclic and polycyclic aromatic hydrocarbons (PAHs) in hookah wastewater. In the current study, the levels of PAHs and BTEX compounds in waterpipe wastewater resulting from the use of different tobacco brands were determined and their eco-toxicological effects were also evaluated. The mean levels of ΣPAHs in waterpipe wastewater of Al Tawareg, Al-Fakher, Nakhla, Tangiers and traditional tobacco brands samples were 3.48 ± 1.65, 3.33 ± 1.52, 3.08 ± 1.25, 2.41 ± 0.87 and 0.70 ± 0.13 μg/L, respectively. The mean levels of ΣBTEX in waterpipe wastewater of Al Tawareg, Al-Fakher, Nakhla, Tangiers and traditional tobacco brands samples were also 2.53 ± 0.61, 2.65 ± 0.78, 2.51 ± 0.72, 2.35 ± 0.56, and 0.78 ± 0.12 μg/L, respectively. The maximum level of PAHs and BTEX compounds in all brands/flavors samples were for naphthalene (Naph) and toluene, respectively. The concentrations of some PAHs (fluoranthene (Flrt), anthracene (Ant), benzo(b)fluoranthene (BbF), benzo(b)fluoranthene (BkF), benzo (g,h,i)perylene (BghiP) and dibenzo (a, h) anthracene (DahA)) and BTEX compounds (benzene) in the waterpipe wastewater samples were more than recommended guidelines and standards by the international reputable organizations such as World Health Organization (WHO) for water quality. Waterpipe wastewater can be introduced as an important origin for the release of these dangerous contaminants into the environmental matrixes. Therefore, more stringent regulations should be considered for the safe disposal of such hazardous wastes including waterpipe wastewater.

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1. Introduction

In the last decade, the use of waterpipe has increased significantly all over the world, including in the Middle East countries (i.e. Iran), Arabian countries, North Africa, Eastern Europe, and Western countries, including the United States [1–3]. In each of these regions, waterpipe is known by different names such as hookah, argileh, narghil, shisha, and hubble-buble [4,5]. Various tobacco products such as traditional tobacco, fruit-flavored tobacco (known as Moassel), tobacco-free herbal Moassel, opium and cannabis are smoked by the waterpipe device [6]. A waterpipe consists of different components: a water bowl (containing about 70% water), a vertical tube inside, a head containing tobacco and a flexible hose connected to the mouthpiece. Smoke is gradually produced by burning the tobacco product that is inhaled by the waterpipe user through the hose [7,8]. Schematic of a routine hookah machine is depicted in Fig. S1. The smoke produced in waterpipe contains high levels of various chemicals such as aromatic hydrocarbons, toxic elements, tar, carbon monoxide, aromatic amines, nitrosamines, nicotine and even radionuclides [8,9]. Therefore, some of the pollutants in the waterpipe smoke accumulate in the water jar as it passes through the bowl of the hookah and become a potential source of many toxic pollutants [10,11].

There are many toxic chemicals in the smoke and remaining burning of tobacco including heavy metals [12–14], aromatic amines [15,16], BTEX compounds [17], and PAHs [18,19] and a lot of other toxics [20]. Aromatic hydrocarbons, a group of dangerous pollutants, which include a range of chemical compounds with one or more benzene rings are considered as priority pollutants and were detected in waterpipe smoke [21]. BTEX compounds are mono-aromatic hydrocarbons that have been also detected in smoke and tobacco remaining [17,22,23]. Previous studies have shown that these compounds can have many acute and chronic side effects including weakness, fatigue, confusion, nausea, loss of appetite, neurological disorders and even cancer [24,25]. In addition to the health effects on humans, these compounds can cause adverse effects on living organisms if they reach the environmental matrices [26]. PAHs compounds have numerous health and eco-toxicological effects and have shown sufficient evidence of mutagenicity/genotoxicity in human cells and other living organisms [27,28]. Benzene also has detrimental impacts on humans and aquatic creatures, such as affecting the bone marrow, reducing red blood cells/leading to anemia, affecting the immune system, and increasing the likelihood of infection [29].

It has been reported in several studies that fresh tobacco, waterpipe smoke, burnt tobacco waste, and cigarette butts contain high amounts of PAHs and BTEX [17–19,30–32]. Therefore, when using a waterpipe, the water in the bowl of the waterpipe is in the path of the sucked smoke, it can be imagined that this water may contain large amounts of pollutants. Therefore, the discharge of produced wastewater after using waterpipe into the environment may have the potential to make several environmental hazards [26,33], including adverse effects on aquatic ecosystems [17,34]. Therefore, to determine the level of waterpipe wastewater hazards it is highly necessary to investigate the amounts of toxic contents including mono- and poly-cyclic hydrocarbons. However, as far as we know, there is no research on the levels of BTEX and PAHs compounds in waterpipe wastewater. So in this study, for the first time, the contents of BTEX and PAHs compounds in the traditional tobacco waterpipe wastewater (TWPW) and different fruit-flavored tobacco waterpipe wastewater (FWPW) samples have been investigated. Also, based on the measured concentrations levels of BTEX and PAHS compounds, the eco-toxicological risks of these pollutants for aquatic ecosystems were evaluated.

2. Materials and methods

2.1. Reagents and solutions

The mixed PAHs standard solution, standard of benzo [a]pyrene-d$_{12}$ (BaP-d$_{12}$) as well as chrysene-d12 (CHR-d$_{12}$) were provided from Sigma-Aldrich (Sigma-Aldrich (Supelco, Inc, Ireland Ltd, MORE)). The mixed BTEX standard (2000 μg/mL in methanol, 1 mL) was also procured from Supelco™ and utilized to prepare the standard solutions. The anhydrous sodium sulfate (Na_2SO_4), potassium hydroxide (KOH), dichloromethane (DCM), *n*-hexane, ethanol and methanol were also obtained from Merck (Merck Millipore (Darmstadt, Germany)). *N*-hexane and GFL analytical water treatment system (GFL, Germany) as lab water source were applied to provide the working samples and standard solutions. A stock BTEX solution (10 mg/L) was prepared by *n*-hexane, and then an appropriate levels of standard were prepared by further dilution of the stock solution in ultra-pure water. Moreover, ethylbenzene-d10 (2000 μg/mL) as internal standard procured from Supelco™. Stock solutions of CHR-d12 and BaP-d12 (as internal standards), and standard of PAHs/BTEX were also provided at levels of 0.5 and 10 mg/L, respectively, and stored at −4 °C until further analysis.

2.2. Collection of waterpipe wastewater samples

After a field survey of available tobacco in the Iranian market, the most popular brands among fruit-flavored tobaccos were Al-Fakher, Nakhla, Al-Tawareg and Tangiers brands and the most popular flavors were mint-peach, watermelon ice, blueberry cream and orange flavors. After refer to waterpipe cafés in Bushehr city, from each brand and each mentioned flavors, 5 samples of freshly smoked waterpipe watewater were collected. In addition to flavored tobaccos, 5 samples of freshly smoked waterpipe wastewater from traditional tobacco were also collected. The categories and the number of samples taken from each category are shown in Fig. S2. All samples were put in proper foil-wrapped glass containers, and transferred to the lab and stored at −4 °C until pollutants analysis.

2.3. Sample preparation and extraction

2.3.1. Extraction of hydrocarbon compounds from waterpipe wastewater

A PAL RTC auto sampler, equipped with SPME fibers and PAL SPME Arrows were used for sample extraction. The sorption phase (20-mm) was selected for PAL SPME Arrow to boost fixed and full immersion within extraction procedure. For extraction, wastewater samples were transported to a stirring station according to an IKA-Mag RCT basic (IKA-Werke GmbH & CO KG, Staufen, Germany) and stirred continuously at 1500 rpm and 35 °C for 10 min as a temperature pre-equilibration time and then during sample extraction. Parallel to the sample pre-equilibration, the preconditioning of SPME fiber or PAL SPME Arrow was done at 200 °C under a stream of nitrogen. Then, the septum of the sample flacons was pierced by the fiber and the absorption phase was continuously stirred in the sample for 70 min. To ensure fixed and full immersion within extraction procedure, the sample flacon penetration depth was set to 55 mm. When extraction process was finished, the extraction fiber was moved to the GC injector and desorption was done at 280 °C for 5 min. Afterwards it was put in the SPME fiber conditioning station at 200 °C for 20 min for cleaning.

2.4. Gas chromatography-mass spectroscopy (GC/MS) instrumentation

All examines were performed by using an Agilent (Palo Alto, CA) 7820A gas chromatograph equipped with a 5977E mass spectrometer. The separation of target pollutants was carried out on an HP-5MS UI column (30 m, 0.25 mm i.d, 0.25 μm, J&W Scientific, Folsom, CA). In this analysis, the carrier gas was helium with a flow rate of 1.2 mL/min. Then, 1 μl of each sample was injected into the GC-MS device, and the temperature in the injection point was set at 310 °C. The temperature of the oven was programmed as follow: 2 min at 80 °C and then raising to 280 °C at a ramp of 30 °C/min and keeping for 1.83 min. The temperature of detector was set at 280 °C. The mass detection was operated at 70 eV electron impact and selective ion monitoring (SIM) mode. The temperature of both MS transfer line and ion source were set to 230 °C and ion quadrupole temperature was 150 °C. Agilent Chemstation was applied for data evaluation. Each analysis was read triplicate and the mean levels were reported.

2.5. Quality control/quality assurance (QC/QA)

The range of limit of detection (LOD), and limit of quantitation (LOQ) values of measured PAHs in waterpipe wastewater were 0.11–0.81 ng/L and 0.34–2.57 ng/L, respectively. The range of LOD, and LOQ of measured BTEX in waterpipe wastewater were also 0.23–0.33 ng/L and 0.69–0.98 ng/L, respectively. The accuracy and precision of the chromatography analysis was also done inter/intra-day and its observations are reported in Table S1. The recovery rate and the accuracy of PAHs analysis in waterpipe wastewater were 85.7–113% and 0.46–15.1%, respectively. The corresponded values for BTEX analysis in waterpipe wastewater were 87.6–112% and 0.52–14.4%, respectively. Thereupon, the observed quality control data (LOD, LOQ and recovery rates) verify the applicability of the analysis approach utilized to quantify the concentrations of PAHs/BTEX in waterpipe wastewater samples.

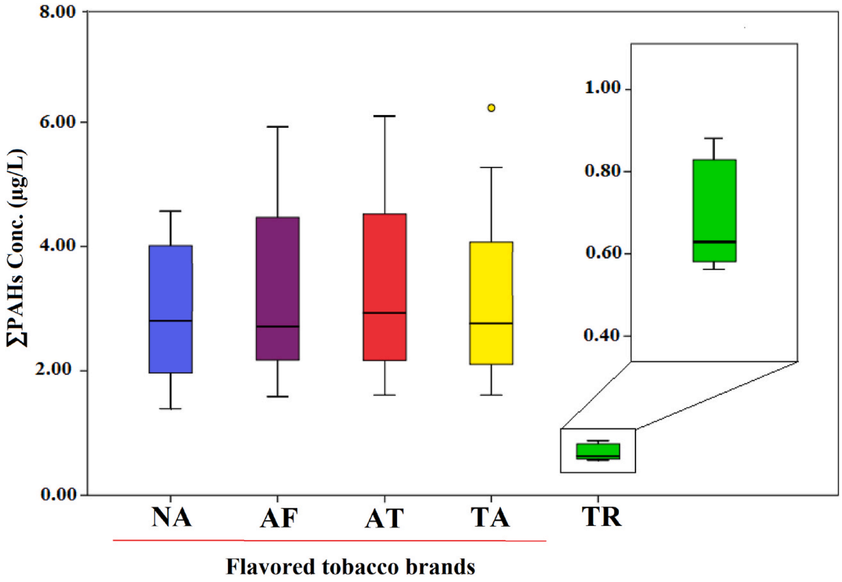

Fig. 1. The ΣPAHs concentration (μg/L) in waterpipe wastewaters from flavored tobacco brands (NA: Nakhla, AF: Al-Fakher, AT: Al-Tawareg, TA: Tangiers and TR: traditional).

2.6. Data analysis

In this study, the data analysis was done by SPSS Statistics version 22 software. Kolmogorov-Smirnov test was utilized for evaluation of the normality of data. ANOVA test was applied to assess the statistically significant difference between target pollutants contents in studied tobacco brands and flavors. The $p\text{-value}<0.05$ was considered as the statistical criterion for decision about significance of differences.

3. Results and discussion

3.1. The levels of BTEX and PAHs in waterpipe wastewater in different brands of tobacco

The concentration levels of ΣBTEX and ΣPAHs in waterpipe wastewater samples collected from different fruit-flavored tobacco brands and traditional tobacco are depicted in Figs. 1–2. As seen, BTEX and PAHs compounds were detected in all studied waterpipe wastewater samples.

The average levels of ΣPAHs in waterpipe wastewater samples of Al-Fakher, Nakhla, Al Tawareg, Tangiers and traditional tobacco brands were 3.33 ± 2.71, 2.41 ± 2.41, 3.01 ± 2.76, 3.48 ± 2.93 and 0.70 ± 0.13 μg/L, respectively while average concentration levels of ΣBTEX were 2.66 ± 2.50, 2.65 ± 2.49, 2.53 ± 2.49, 3.48 ± 2.93, and 0.78 ± 0.12 μg/L, respectively. The order of ΣPAHs concentration in waterpipe wastewater samples from different brands was as: Al Tawareg > Al-Fakher > Tangiers > Nakhla≫> traditional; while in the case of the ΣBTEX was as: Al Tawareg > Al-Fakher > Nakhla > Tangiers ≫> traditional. It can be also said that the concentration sequence of both mono/poly aromatic compounds in different tobacco brands was nearly similar. The findings showed that the concentration levels of hydrocarbons (BTEX and PAHs) in wastewater samples obtained from flavored tobacco were considerably more than traditional tobacco wastewater samples. These observations can be attributed to flavoring agents, high amounts of synthetic additives, essential oils and chemicals that are added to the flavored tobacco formulation to create an attractive taste [35,36]. Both PAHs and BTEX compounds are mainly produced during incomplete combustion [37,38]. Consequently, the organic substances combustion in tobacco has led to the production of these pollutants and their releasing into the water of the waterpipe bowl [20]. In other words, one of the main ways of producing mono/poly aromatic compounds is the incomplete burning of organic materials, and at the higher temperature of the waterpipe head, the organic materials in the structure of tobacco are pyrolyzed into smaller and more unstable components [39]. These smaller molecules combine together during a series of reactions affected by free radicals and lead to the formation of stable aromatic hydrocarbons [40,41]. Therefore, when a person smokes a waterpipe, the smoke containing mono/poly aromatic hydrocarbon compounds passes through the waterpipe bowl and creates wastewater with hydrocarbon pollutants [32,42].

There are no similar study regarding the amount of hydrocarbons (BTEX and PAHs) in waterpipe wastewater for comparison with our findings. In our previous study, which investigated the release of PAHs compounds from burnt waterpipe wastes into water environments, it was found that the concentration of PAHs compounds in leachates ranged from of 0.11 to –3.64 μg/L [32]. In another study, the levels of ΣPAHs in different tobacco flavors were observed in the range of 47.48–29.75 μg/g [43]. Dobaradaran et al. [18], also found the levels of ΣPAHs in freshly cigarette butt samples within the range of 14.4–35.9 μg/g. Dobaradaran et al. [19], also observed that the leachate level of ΣPAHs from freshly cigarette butt into water environments was in the range of 3.3–5.7 μg/L. In another study, Dobaradaran et al. reported that the leaching concentrations of BTEX compounds from freshly cigarette butt into different water environments within the range of 0.09–0.9 μg/L [17].

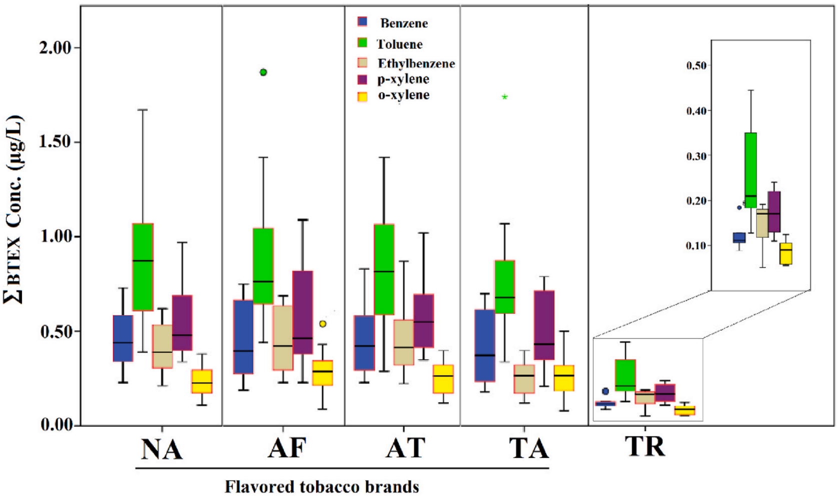

Fig. 2. The BTEX concentration (μg/L) in waterpipe wastewaters from flavored tobacco brands (NA: Nakhla, AF: Al-Fakher, AT: Al-Tawareg, TA: Tangiers and TR: traditional).

The levels of all 16 PAHs and BTEX compounds in waterpipe wastewater samples collected from Al-Fakher, Nakhla, Al Tawareg, Tangiers and traditional tobacco brands are given in Table 1. As seen; naphthalene (Naph) was the dominant PAHs compound in the waterpipe wastewater samples collected from all studied brands.

The mean level of this pollutants in waterpipe wastewater samples obtained from the Al-Fakher, Nakhla, Al Tawareg, Tangiers brand and traditional tobaccos were 0.63 ± 0.52, 0.41 ± 0.33, 0.79 ± 0.63, 0.75 ± 0.68, and 0.08 ± 0.07 μg/L respectively. Naphthalene is a chemical with known toxic effects, which can enter the aquatic environment by discharging waterpipe wastewater into the environment and cause many adverse effects on aquatic life (fish, algae, etc.) [44]. In our previous study, it was also reported that naphthalene had the maximum concentration levels in the burnt tobacco wastes [8]. Our findings are in agreement with the study reports performed on tobacco waste [43] and the study done on the fresh/old cigarette butts [19]. In both studies the predominant detected PAHs was naphthalene. As seen in Table 1, benz(a)anthracene (BaA) and Indeo [1,2,3-cd] pyrene (IndP) were also detected in high levels in all target tobacco brands which are in the list of probable carcinogens based on the classification of the Environmental Protection Agency (EPA) [45].

Among BTEX compounds, toluene had the maximum concentration levels. The amount of this compound in waterpipe wastewater samples from Al-Fakher, Nakhla, Al-Tawareg, Tangiers brands and traditional tobacco samples were 0.89 ± 0.33, 0.86 ± 0.31, 0.83 ± 0.31, 0.75 ± 0.28 and 0.26 ± 0.11 μg/L, respectively. In a research by Dobaradaran et al. [17] on the release of BTEX from cigarette butts into aquatic environments, the maximum levels was related to toluene and the mean leached concentration was reported as 0.39 ± 0.90 μg/L. In research by Ghobadi et al. [22], the average level of benzene, toluene, ethylbenzene, o-xylene, and p-xylene in urine of waterpipe smokers were about 470, 670, 130, 90, and 45 ng/g, respectively, which toluene and benzene had the maximum level [22]. These findings indicate that waterpipe wastewater is a toxic wastewater that can be a carrier of many dangerous pollutants entering the environment and cause eco-toxicological effects in aquatic environments [34].

3.2. *MAHs/PAHs contents of waterpipe wastewater in different flavor*

The average concentrations of ΣPAHs and BTEX in waterpipe wastewater samples from different flavors and traditional tobacco are shown in Figs. 3–4, respectively. As seen, the averages concentrations of ΣPAHs in waterpipe wastewater samples from four mint-peach, watermelon-ice, blueberry-cream, orange flavors and traditional tobacco were 2.69 ± 0.62, 2.38 ± 0.58, 4.90 ± 1.11, 2.33 ± 1.23 and 0.70 ± 0.63 μg/L respectively. Also, the average levels of ΣBTEX in waterpipe wastewater samples from four mentioned flavors and traditional tobacco were 2.15 ± 0.46, 2.36 ± 0.46, 2.98 ± 0.80, 2.56 ± 0.66 and 0.70 ± 0.13 μg/L respectively. Both ΣPAHs and BTEX concentrations in different tobacco flavors followed the sequence of blueberry -cream ≫ mint-peach ≫ watermelon-ice > orange ≫> traditional.

According to this trend, waterpipe wastewater from the smoke of the blueberry-cream flavored tobacco had the highest concentration levels of ΣPAHs and BTEX. These observations can be related to the higher fat content in blueberry-cream flavored samples. Incomplete combustion of this tobacco with high fat content will result to formation of a high concentration levels of PAHs and BTEX [46–48]. It has been reported that high fat content is an important agent in the production of PAHs and BTEX compounds during materials combustion [37,48,49].

The individual concentration levels of all 16 PAHs and BTEX compounds in waterpipe wastewater samples of four studied flavors and traditional tobacco are provided in Table 2. The maximum levels of PAHs and BTEX compounds were related to naphthalene and

Table 1

Mono- and poly-aromatic hydrocarbons concentration levels (mean ± SD, μg/L) in waterpipe wastewater samples collected from different brands.

Hydrocarbons	Compounds	Flavored tobacco brands				Traditional
		Nakhla	Al- fakher	Al Tawareg	Tangiers	
PAHs	Napthalene (Naph)	0.41 ± 0.32	0.63 ± 0.40	0.85 ± 0.48	0.78 ± 0.42	0.08 ± 0.04
	Acenaphthylene (Acen)	0.21 ± 0.12	0.24 ± 0.15	0.27 ± 0.22	0.23 ± 0.14	0.07 ± 0.01
	Acenaphthene (Ace)	0.27 ± 0.18	0.21 ± 0.18	0.26 ± 0.22	0.20 ± 0.10	0.10 ± 0.04
	Fluorene (Flu)	0.25 ± 0.22	0.28 ± 0.16	0.29 ± 0.27	0.24 ± 0.19	0.12 ± 0.09
	Anthracene (Ant)	0.15 ± 0.12	0.31 ± 0.27	0.35 ± 0.28	0.26 ± 0.24	0.07 ± 0.04
	Phenanthrene (Phen)	0.20 ± 0.17	0.32 ± 0.26	0.22 ± 0.20	0.17 ± 0.11	0.05 ± 0.02
	Fluoranthene (Flrt)	0.12 ± 0.11	0.25 ± 0.24	0.41 ± 0.34	0.23 ± 0.14	0.04 ± 0.01
	Pyrene (Pyr)	0.11 ± 0.11	0.15 ± 0.14	0.15 ± 0.12	0.25 ± 0.16	0.07 ± 0.01
	Benz(a)anthracene (BaA)	0.09 ± 0.07	0.17 ± 0.14	0.19 ± 0.16	0.20 ± 0.19	0.02 ± 0.01
	Chrysene (Chr)	0.11 ± 0.08	0.14 ± 0.08	0.08 ± 0.07	0.20 ± 0.19	0.03 ± 0.02
	Benzo(b)fluoranthene (BbF)	0.07 ± 0.06	0.13 ± 0.13	0.09 ± 0.06	0.08 ± 0.06	0.02 ± 0.01
	Benzo(k)fluoranthene (BkF)	0.09 ± 0.06	0.11 ± 0.09	0.06 ± 0.04	0.08 ± 0.06	0.02 ± 0.01
	Benzo(a)pyrene (BaP)	0.07 ± 0.06	0.10 ± 0.08	0.06 ± 0.03	0.07 ± 0.06	0.03 ± 0.05
	Indeo [1,2,3-cd] pyrene (IndP)	0.08 ± 0.04	0.11 ± 0.09	0.07 ± 0.05	0.07 ± 0.04	0.10 ± 0.07
	Benzo (g,h,i)perylene (BghiP)	0.09 ± 0.06	0.12 ± 0.07	0.09 ± 0.05	0.08 ± 0.04	0.09 ± 0.06
	dibenzo (a, h) anthracene (DahA)	0.10 ± 0.06	0.10 ± 0.06	0.07 ± 0.07	0.05 ± 0.03	0.03 ± 0.001
BTEX	Benzene	0.46 ± 0.14	0.45 ± 0.19	0.44 ± 0.17	0.41 ± 0.18	0.03 ± 0.05
	Toluene	0.87 ± 0.31	0.90 ± 0.34	0.84 ± 0.31	0.76 ± 0.29	0.03 ± 0.02
	Ethylbenzene	0.42 ± 0.12	0.45 ± 0.16	0.45 ± 0.15	0.40 ± 0.15	0.02 ± 0.01
	o-xylene	0.52 ± 0.18	0.60 ± 0.25	0.56 ± 0.18	0.50 ± 0.19	0.02 ± 0.01
	p-xylene	0.23 ± 0.08	0.29 ± 0.10	0.25 ± 0.09	0.26 ± 0.09	0.03 ± 0.05

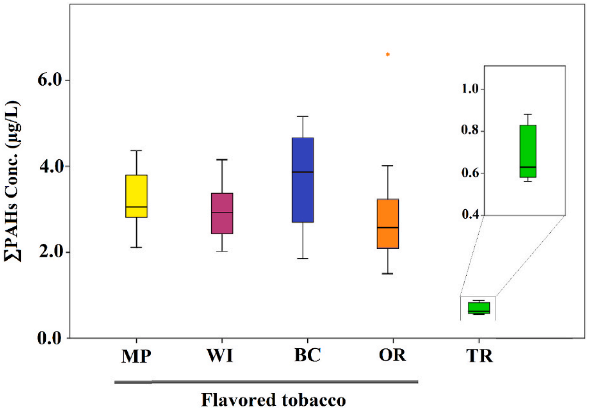

Fig. 3. The ΣPAHs concentration (μg/L) in waterpipe wastewaters from flavored tobacco flavors (MP: Mint peach, WI: Watermelon ice, BC: blueberry cream, OR: Orange and TR: traditional). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

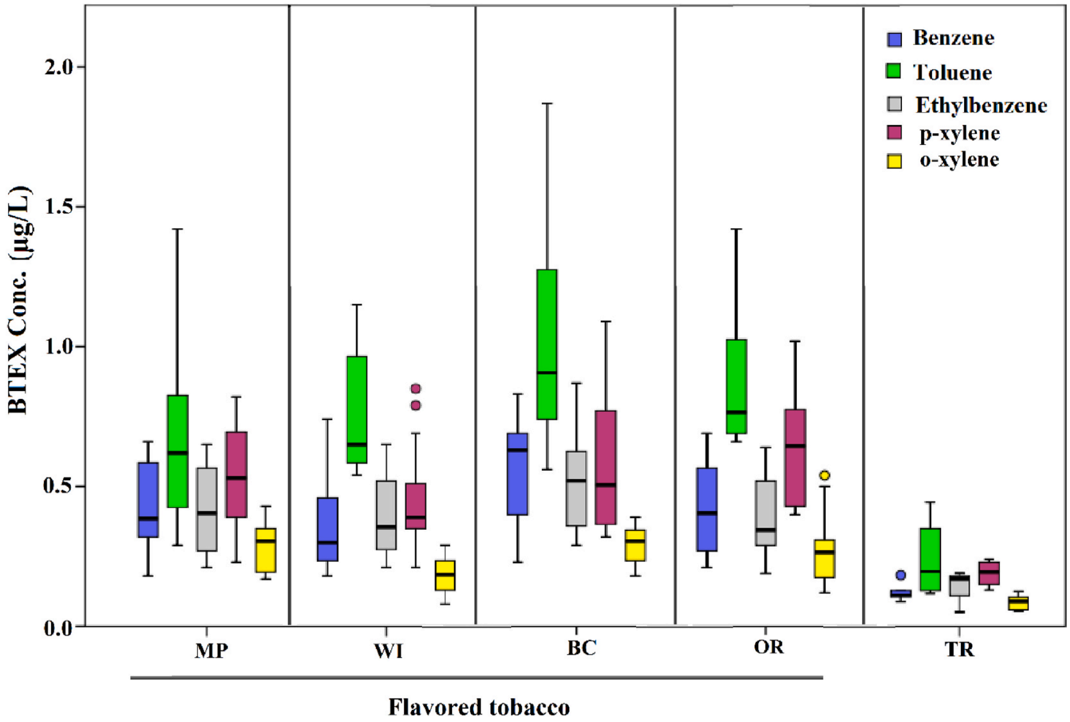

Fig. 4. The BTEX concentration (μg/L) in waterpipe wastewaters from flavored tobacco flavors (MP: Mint peach, WI: Watermelon ice, BC: blueberry cream, OR: Orange and TR: traditional). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

toluene, respectively. The average levels of naphthalene in wastewater samples of watermelon-ice, mint-peach, blueberry-cream and orange flavors and traditional tobacco were 0.45 ± 0.18, 0.58 ± 0.32, 1.15 ± 0.47, 0.50 ± 0.34, and 0.08 ± 0.04 μg/L, respectively. In our previous study, it was also observed that naphthalene had the maximum concentration in the in the PWTWs of fruit-flavored tobacco samples [8]. The average concentrations of toluene in wastewater samples of watermelon-ice, mint-peach, blueberry-cream, and orange flavors as well as traditional tobacco were 0.76 ± 0.21, 0.69 ± 0.30, 0.78 ± 0.24, 1.03 ± 0.38 and 0.14 ± 0.05 μg/L, respectively. Similar findings were reported in Dobaradaran et al. study and toluene had the highest concentration value among BTEX in cigarette butts leachates [17]. In another research conducted by Polzin et al. [50], it was reported that toluene (4.5–82.4 μg/cig) had the highest and o-xylene (0.2–3 μg/cig) had the lowest levels among BTEX compounds in various cigarette brands sold in the United States [50].

The comparison of BTEX and PAHs concentration levels in waterpipe wastewaters from different brands and traditional tobacco are

Table 2

Mono- and poly-aromatic hydrocarbons concentration levels (mean ± SD, μg/L) in waterpipe wastewater samples from different flavors.

Hydrocarbons	Compounds	Tobacco Flavors				Traditional
		Mint-peach	Watermelon-ice	Blueberry -cream	Orange	
PAHs	Naph	0.58 ± 0.32	0.45 ± 0.18	1.15 ± 0.47	0.50 ± 0.34	0.08 ± 0.04
	Acen	0.21 ± 0.10	0.17 ± 0.10	0.39 ± 0.21	0.18 ± 0.13	0.07 ± 0.01
	Ace	0.21 ± 0.13	0.21 ± 0.14	0.29 ± 0.25	0.19 ± 0.18	0.10 ± 0.04
	Flu	0.26 ± 0.13	0.14 ± 0.07	0.47 ± 0.28	0.20 ± 0.15	0.12 ± 0.09
	Ant	0.27 ± 0.23	0.18 ± 0.15	0.48 ± 0.31	0.14 ± 0.10	0.07 ± 0.04
	Phen	0.16 ± 0.13	0.24 ± 0.23	0.34 ± 0.24	0.18 ± 0.14	0.05 ± 0.02
	Flrt	0.21 ± 0.20	0.21 ± 0.15	0.45 ± 0.33	0.15 ± 0.13	0.04 ± 0.01
	Pyr	0.12 ± 0.11	0.11 ± 0.10	0.26 ± 0.21	0.12 ± 0.10	0.07 ± 0.01
	BaA	0.15 ± 0.13	0.14 ± 0.09	0.27 ± 0.18	0.14 ± 0.13	0.02 ± 0.01
	Chr	0.09 ± 0.07	0.10 ± 0.08	0.18 ± 0.17	0.10 ± 0.08	0.03 ± 0.02
	BbF	0.07 ± 0.05	0.07 ± 0.05	0.14 ± 0.13	0.07 ± 0.05	0.02 ± 0.01
	BkF	0.06 ± 0.05	0.08 ± 0.05	0.13 ± 0.10	0.05 ± 0.04	0.02 ± 0.01
	BaP	0.07 ± 0.06	0.05 ± 0.03	0.12 ± 0.09	0.07 ± 0.04	0.03 ± 0.05
	Indp	0.07 ± 0.06	0.08 ± 0.04	0.11 ± 0.08	0.08 ± 0.05	0.10 ± 0.07
	BghiP	0.09 ± 0.06	0.10 ± 0.05	0.11 ± 0.07	0.08 ± 0.04	0.09 ± 0.06
	DahA	0.07 ± 0.05	0.09 ± 0.05	0.10 ± 0.06	0.07 ± 0.06	0.03 ± 0.001
BTEX	Benzene	0.43 ± 0.15	0.36 ± 0.16	0.56 ± 0.17	0.42 ± 0.15	0.03 ± 0.05
	toluene	0.69 ± 0.30	0.76 ± 0.21	1.03 ± 0.38	0.88 ± 0.24	0.03 ± 0.02
	Ethylbenzene	0.43 ± 0.14	0.38 ± 0.14	0.51 ± 0.15	0.39 ± 0.13	0.02 ± 0.01
	O-xylene	0.53 ± 0.17	0.45 ± 0.17	0.58 ± 0.24	0.62 ± 0.19	0.02 ± 0.01
	P-xylene	0.29 ± 0.17	0.18 ± 0.06	0.29 ± 0.07	0.27 ± 0.11	0.03 ± 0.05

summarized in Table S2. The average levels of PAHs (except acenaphthylene (Acen), acenaphthene (Ace), Fluorene (Flu), pyrene (Pyr), benzo(b)fluoranthene (BkF), and benzo(a)pyrene (BaP)) as well as all BTEX compounds in different brands of fruit-flavored tobaccos and traditional tobacco were statistically considerably different ($p < 0.05$). The comparison of mono- and poly-aromatic hydrocarbons concentration levels in waterpipe wastewater samples from different flavors and traditional tobacco are summarized in Table S3. The average levels of all PAHs (except Ace, Pyr, Chrysene (Chr) and dibenzo (a, h) anthracene (DahA)) as well as BTEX compounds in different flavors and traditional tobacco were statistically considerably different ($p < 0.05$).

3.3. Eco-toxicological risk assessment of PAHs and BTEX in waterpipe wastewater

Based on our searches, it was observed that there is no established quality standard in the field of PAHs and BTEX compounds in waterpipe wastewater and discharge of these wastewaters into aquatic environments. Therefore, in the present study, the average detected levels for some compounds of PAHs in waterpipe wastewater were compared with the European environmental quality standards (EQSs) (Table 3 and Fig. 5) [51]. These guidelines/values are recommended standards to safeguard the aquatic organism and human health against dangerous pollutants. As seen, the detected levels of Flrt, Ant, BbF, BkF, benzo (g,h,i)perylene (BghiP) and DahA compounds in the waterpipe wastewater exceeded the recommended guidelines. The levels of BaP exceeded all recommended values

Table 3

Comparing the levels of PAHs (μg/L) waterpipe wastewaters samples with the Water Framework Directive (WFD) standards.

Component		Naph	Flrt	Ant	BaP	BbF	BkF	BghiP	Indp
Concentration level (μg/L)	**Flavored tobacco**	0.57	0.25	0.27	0.07	0.09	0.08	0.08	0.1
	Traditional tobacco	0.08	0.04	0.07	0.03	0.01	0.02	0.01	0.001
AA-EQS[a] Inland surface Waters[b]		2	0.0063	0.1	0.00017	Footnote[d]	footnote	footnote	footnote
AA-EQS[a] Other surface Waters		2	0.0063	0.1	0.00017	footnote	footnote	footnote	footnote
MAC-EQS[c] Inland surface waters[b]		130	0.12	0.1	0.27	0.017	0.017	0.0082	Not applicable
MAC-EQS[c] Other surface waters		130	0.12	0.1	0.027	0.017	0.017	0.00082	Not applicable

- AA-EQS: Annual average- European environmental quality standards.
- MAC-EQS: Maximum allowable concentration- European environmental quality standards.
- EQS: European environmental quality standards.
- WFD: Water Framework Directive.

[a] This parameter is the EQS expressed as an annual average value (AA-EQS). Unless otherwise specified, it applies to the total concentration of all isomers.

[b] Inland surface waters encompass rivers and lakes and related artificial or heavily modified water bodies.

[c] This parameter is the EQS expressed as a maximum allowable concentration (MAC-EQS). Where the MAC-EQS are marked as not applicable, the AA-EQS values are considered protective against short-term pollution peaks in continuous discharges since they are significantly lower than the values derived on the basis of acute toxicity.

[d] For the group of priority substances of polyromantic hydrocarbons (PAH), the biota EQS and corresponding AA-EQS in water refer to the concentration of benzo(a)pyrene on the toxicity of which they are based. Benzo(a)pyrene can be considered as a marker for the other PAHs, hence only benzo(a)pyrene needs to be monitored for comparison with the biota EQS or the corresponding AA- EQS in water.

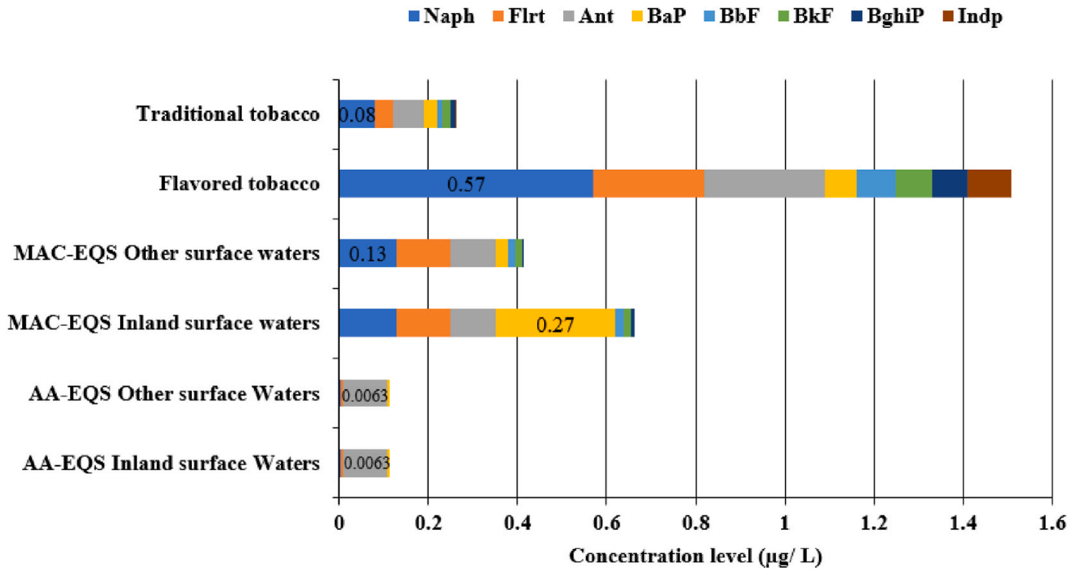

Fig. 5. The levels of PAHs (μg/L) in waterpipe wastewater samples compared with different standards.

(MAC-EQS Inland surface waters standard is exception), but the level of Naph was below these limits.

Due to the acute and chronic toxicity of PAHs, these chemicals can have harmful ecotoxicological effects on the entire aquatic system by bioaccumulation in aquatic organisms and severely threaten the aquatic environment [52–55]. Finally, they also affect the human health through the entry into the food chain [51]. As well as, in the case of BTEX compounds in waterpipe wastewater, there is no specific instruction to safeguard aquatic organisms from these dangerous pollutants. Therefore, the average levels of BTEX detected in different kinds of tobacco were compared with European environmental quality standards (EQSs) as Water Framework Directive (WFD) standards. It is worth mentioning that this standard is the only approved guideline for benzene, whose average annual concentration for inland surface waters and other surface waters is 10 and 8 μg/L, respectively. For other compounds including toluene, ethylbenzene and xylene, there are no guidelines or limits set. Our findings showed that benzene level in waterpipe wastewater is below the suggested WFD standards by EQSs levels for inland and other surface waters. Dobaradaran et al. [17], also found similar observations for benzene in leachates of cigarette butt.

Because of the increase in tobacco consumption around the world, a huge volume of waterpipe wastewater is produced annually, which can enter the environment and water bodies and threaten the life of aquatic organisms. In addition, a large volume of waterpipe wastewater is released into the different environmental matrices globally and enters surface water and in some areas where there is almost no water treatment and people rely on the quality of surface water, it is possible PAHs and BTEX compounds have been found to have adverse effects on the health of consumers. In addition to PAHs and BTEX compounds, tobacco wastewater may have large quantities of refractory contaminants such as heavy metals, and other hazardous chemicals. According to these facts, regulatory monitoring are required to reduce the detrimental impacts of all tobacco-related wastes (waterpipe effluent, cigarette butts, burnt tobacco waste, and discarded e-cigarettes), which are ubiquitous and pervasive environmental issues.

4. Limitation and ideas for future researches

As this research is a primary report on aromatic hydrocarbons in hookah wastewaters, there were several limitations which could be attractive ideas for future researches. The first thing is that other kind of liquids (milk, juice, etc.) may utilized in the hookah bowl instead of water and, therefore, it would be important to quantify the pollutants level in these matrixes and compare them with water. In addition, it was observed that the flavored tobacco have a higher level of hydrocarbons. It is necessary to evaluate the flavoring agents that contain higher amount of pollutants and replaced with better substances that can be utilized instead to decrease health hazards. Moreover, although this work recommends that hookah wastewater could be an origin for hazardous contaminants, the impacts of these pollutants on living creatures have not been assessed. Therefore, more comprehensive study is needed to evaluate the toxicity of this kind of wastewaters on living organisms and also the possible wastewater treatment techniques [56,57] for this kind of wastewater.

5. Conclusion

In the present study, the levels of mono and polycyclic hydrocarbon compounds in waterpipe wastewater of different tobacco (flavored and traditional) samples were determined. Higher levels of these pollutants in waterpipe wastewaters of the fruit-flavored tobacco than traditional tobacco wastewater. Naphthalene and toluene had the maximum observed concentration levels among PAHs and BTEX, respectively. The levels of some PAHs and BTEX in the studied waterpipe wastewater exceeded the guidelines of surface water recommended by the WHO and other international agencies. Due to increasing tendency to use waterpipe and consequently the large amount of post-consumption tobacco waste, high amounts of PAHs and BTEX compounds will be released via both wastewater and tobacco waste of waterpipe into the environment and the aquatic environment. Consequently, these pollutants can cause momentous hazards to aquatic environments and also jeopardize human health through the entry into human food chain. Therefore, regulatory rules and regulations should select suitable options with careful considerations for the safe disposal of such hazardous wastes and/or even changing the content of waterpipe tobacco to avoid producing of higher levels of hazardous pollutants such as PAHs and BTEX that found in flavored products.

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

Additional data from the study are available by request to the corresponding author by email.

CRediT authorship contribution statement

Nima Rashidi: Writing – original draft, Formal analysis, Data curation. **Mohammad Reza Masjedi:** Writing – review & editing, Methodology, Funding acquisition. **Hossein Arfaeinia:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Sina Dobaradaran:** Writing – review & editing, Methodology, Conceptualization. **Seyed Enayat Hashemi:** Writing – review & editing, Validation, Methodology. **Bahman Ramavandi:** Writing – review & editing, Validation, Methodology. **Roshana Rashidi:** Investigation, Data curation. **Sara Dadipoor:** Software, Methodology. **Farshid Soleimani:** Writing – review & editing, Writing – original draft, Formal analysis.

Declaration of competing interest

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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Appendix A. Supplementary data

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