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Bioaccumulation of polycyclic aromatic hydrocarbons from leachates of waterpipe tobacco wastes on *Peronia peronii* species from the Persian Gulf region

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This study examines the bioaccumulation factor (BAF) of polycyclic aromatic hydrocarbons (PAHs) in *Peronia peronii* (as the bio-indicator organism) exposed to the leachate resulting from fruit-flavored and traditional burnt tobacco wastes. The ΣPAHs concentrations in the muscle samples of *P. Peronii* of exposed group I (organisms exposed to the leachate resulting from fruit-flavored waterpipe tobacco wastes) and exposed group II (organisms exposed to the leachate resulting from traditional waterpipe tobacco wastes) and control group (exposed to natural seawater) were 37.49 ± 12.9, 9.32 ± 3.8, and 3.89 ± 1.9 ng/g, respectively. Furthermore, there was a significant difference between the mean values of all ΣPAHs compounds in the three groups (group I, group II, and control). The mean Log BAFs in *P. Peronii* exposed to fruit-flavored and traditional tobacco waste leachates were ranged from 1.70 to 3.61 and 2.49 to 3.07, respectively. The mean Log BAFs of none of the PAHs compounds did not exceed 3.7 (the limit set as "bio-accumulative") in none of the leachates (fruit-flavored or traditional). In the organisms exposed to the leachates resulting from fruit-flavored tobacco waste, Log BAFs of Benzo[b] fluoranthene (BbF), benzo(a)anthracene (BaA), and chrysene (Chr) lay within the range of 3.3–3.7 ("potentially bio-accumulative"). However, in the organisms exposed to the leachates resulting from traditional tobacco waste, the mean Log BAFs of all compounds did not reach even 3.3. The findings of our study indicate that leachates from tobacco waste, pose significant environmental and health hazards. Considering the prevalence of tobacco smoking, suitable strategies should be employed for management of these hazardous wastes to protect the environmental health.

Keywords Waterpipe Tobacco wastes, PAHs, *Peronia peronii*, Bioaccumulation

Tobacco smoking is a significant global health issue, contributing to over 8 million deaths annually, and millions of diseases and disabilities are attributed to this risk factor[1,2]. These mortalities and diseases burden the healthcare system of countries[3]. Waterpipe tobacco smoking (WTS) (known as Hookah, Ghalyan, Ghalun, Shisha, Argileh, and Nargile) is one of the old methods of tobacco consumption, further adding to this burden[4]. Waterpipe tobacco smoking first emerged some hundred years ago in the Middle East and India. However, it has gradually become an inappropriate entertainment among the youths and an important public health concern in worldwide[1,5]. In this method of smoking, the mixture of tobacco and the flavorings are put in the waterpipe head and then covered using a holed aluminum foil[6,7]. Next, ignitive charcoal is placed on a reticular foil, whereby heat is applied and tobacco is burnt[8]. The resulting fume then goes through a water bowl, and is finally smoked by the smoker through the opening of a flexible hose connected to the waterpipe[4,8]. Waterpipe tobacco comes in various types, primarily categorized into traditional and fruit-flavored varieties. One of the most commonly

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used types is fruit-flavored tobaccos, known as Maassel, Mu'assel, and/or Moassel[8,9]. One third of this product is tobacco and the remaining two thirds contain various types of flavoring, humectants, and sweeteners[10].

While academic research and regulatory attempts have focused on mitigating the health effects of waterpipe[11–13], similar attention should be paid to the environmental side effects resulting from "releasing the wastes post waterpipe tobacco consumption". Recent studies indicate that approximately 71% of fresh tobacco used in waterpipe sessions becomes burnt tobacco waste, which is often discarded improperly, leading to potential pollution[14]. In addition, waterpipe tobacco smoking generates various types of waste such as charcoal, ash, and wastewater that can negatively impact the environment[7,15]. It has been reported that fresh (unsmoked) waterpipe tobacco or tobacco fume contains large amounts of toxic pollutants such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), nitrosamines, BTEX (benzene, toluene, ethylbenzene and, xylene) and aldehydes[8,16–18]. Thus, tobacco wastes may also contain large amounts of these hazardous chemicals, causing serious risks for the receiving environments[19]. The release of tobacco waste into the environment poses significant risks, particularly through the leaching of harmful pollutants into aqueous media such as surface and groundwater[20,21]. Upon entrance of these toxic wastes into water bodies, they can be ingested by animals thereby, seriously harming them. In addition, the toxic leachate of these wastes may jeopardize the aquatic life[20,21].

The increasing prevalence of waterpipe consumption, particularly in urban areas, is raising environmental concerns, especially regarding waste management. Mobile waterpipe supply stations have been deployed in parks and coastal areas, leading to significant waste release into coastal environments. Upon entrance of tobacco wastes, the biodiversity is threatened, and both animal and plant species in the aquatic ecosystem may be harmed[22]. Exposure to the toxins resulting from tobacco wastes (i.e. cigarette butts) induces a wide range of effects including toxicity, embryotoxicity, genotoxicity, neurotoxicity, psychotropic effects and behavioral changes, mortality, biochemical harms, changes in the body weight and composition in different creatures[22–27]. In addition to different toxic effects, the pollutants present in tobacco wastes may have different fates and/or potentials for bioaccumulation[25]. Nevertheless, The environmental impact of tobacco waste, particularly cigarette butts, has been increasingly recognized[28,29]. In contrast burnt tobacco as one of the primary wastes of tobacco products has remained neglected. Our previous studies indicated that waterpipe tobacco wastes can be a source of pollutant emissions (i.e. PAHs, BTEX, and heavy metals) into aquatic media[6,8,30]. PAHs are a class of organic compounds formed primarily during the incomplete combustion of organic materials, such as tobacco, coal, oil, and wood[31,32]. The conditions that facilitate PAH formation include high temperatures and low oxygen levels, which lead to the pyrolysis of complex organic compounds[30,33]. PAHs are characterized by their low water solubility, low volatility, and high octanol/water partition coefficients (Kow), which significantly influence their behavior in the environment and their accumulation in living organisms[34,35]. In addition, these compounds have carcinogenic, mutagenic, and toxic properties with a wide range of adverse effects on creatures in different processes such as reproduction, development, metabolism, osmotic regulation, behavior, and survival[34,36]. As a result, monitoring PAHs is crucial due to their significant health negative impacts on humans and ecosystems. *Peronia peronii* is a gastropod mollusk species belonging to the *Onchidiidae* family, inhabiting in the Persian Gulf and Oman Sea. In this study, this organism has been used as a bio-indicator for evaluating bioaccumulation of PAHs (e.g., naphthalene (Naph), acenaphthylene (Acen), acenaphthene (Ace), fluorine (Flu), anthracene (Ant), phenanthrene (Phen), fluoranthene (Flrt), pyrene (Pyr), benz_a_anthracene (BaA), chrysene (Chr), benzo_b_fluoranthene (BbF), benzo_k_fluoranthene (BkF), benzo_a_pyrene (BaP), indeno_123cd_pyrene (IP), benzo_ghi_perylene (BghiP), and dibenz_a_h_anthracene (DahA) in the leachates of the waterpipe tobacco wastes. *P.peronii*is an effective bioindicator because of its sensitivity to environmental fluctuations in intertidal zones, which provides insights into water quality and sediment conditions for pollution studies[37,38]. Furthermore, as a native species of the Persian Gulf, it plays a significant role in local ecosystem health, which enhances its importance for conservation initiatives and environmental monitoring[39,40]. In this study, the leachate of two types of tobacco wastes (traditional tobacco wastes (TTW) and flavored tobacco wastes (FTW)) were used for evaluating and comparing the bioaccumulation of PAHs on *P. peronii* body. The specific objectives of this study were to: 1) identify and determine the amount of leachable PAHs in the leachate resulting from two types of tobacco wastes (TTW and FTW), and 2) evaluate whether these organic compounds have bioaccumulation potential in *P. peronii*.

Materials and methods

Collecting the tobacco waste samples

In this study, tobacco waste samples were collected from one of the highly consumed brands of flavored tobacco as well as one type of highly used traditional tobacco in the waterpipe cafés across Bushehr city. 10 tobacco waste samples were gathered from each tobacco type (flavored and traditional). According to our previous study[30], Al-mahmoud brand with the orange-creame taste was one of the highly consumed flavored tobaccos, whose wastes had the most considerable amounts of PAHs. Thus, in this study, the wastes of this tobacco (orange-creame flavor- Al-mahmoud) were procured. Note that all of the tobaccos were smoked under equal conditions (1 one hour of smoking per each session), after which the remaining wastes in the waterpipe head were collected. The collected tobacco waste samples were placed inside a closed-lid opaque glass container, then foil wrapped, and transferred to laboratory in the cold box, and kept at −4°C until analysis.

Preparing the tobacco waste leachate and calculating the number of required organisms

In order to prepare the leachate required for performing the bioaccumulation experiments, 30 g of the tobacco wastes of each type of tobacco (flavored and traditional tobacco) was weighed and added to one liter of natural seawater (prepared from the Persian Gulf), and was kept at room temperature in the laboratory in a still state for 72 h. Thereafter, the supernatant was filtered using the Whatman filter grade 42, whereby lower concentrations

of the leachate (1.875, 3.75, 7.5, and 15 g/L) were prepared with × 0.5 dilution sequence. Next, the pH of the samples was adjusted to 7.5. Subsequently, to determine the acute toxicity (96 h LC_{50}), for the leachate of each of the flavored and traditional tobacco wastes, five concentrations (1.875, 3.75, 7.5, 15, and 30 g/L) as well as time of 96 h were considered; for each run, five organisms were exposed. Each run was performed with four replications. Specifically, 200 *P. peronii* organisms (two types of leachate * 5 concentrations * 5 organisms * 4 replications) were considered for examining the 96h lethal concentration 50 toxicity ($96LC_{50}$). One hundred organisms were also considered in the natural seawater as the control group. After this stage, one-third of the 96-h LC_{50} was considered for chronic exposure (90 days). Finally, for each leachates of tobacco wastes, 30 organisms were considered. In total, 60 *P. peronii* were used for evaluating the bioaccumulation of PAHs compounds resulting from the exposure to leachates of two types of tobacco wastes. In addition, 30 organisms in the aquarium containing natural seawater were also considered as the control group.

Preparing the bioindicator organisms and conducting bioaccumulation experiments

The bioindicator organisms required for the present study (*P. peronii*) were collected from the TIDAL coastal region and Hara forests along the Persian Gulf, Bushehr Port, Iran, which were transferred to the laboratory in suitable plastic tanks. In the laboratory, the organisms were kept inside glass pre-designed and prefabricated aquaria (50 cm * 35 cm * 35 cm) containing a water depth of 10 cm. Natural seawater from the Persian Gulf was collected and processed through a series of steps at the Research Institute of the Persian Gulf University. The seawater was pumped directly from the Gulf and filtered, aerated and, stored in tanks for aquarium use. The pH values were stabilized at 7.5, Dissolved Oxygen (DO) levels were 5–6 mg/L, temperature levels ranged from 24°C to 32°C, and salinity was 35%. All of the aquaria as well as the quality of the water present in them were checked regularly in order to ensure the health of the creatures. Aerators devised in the aquaria performed aeration for the oxygenation organisms along the experiments at a constant rate. The water of the aquaria were replaced every other days, and natural lifecycle governed in the aquaria. The organisms were acclimatized to the laboratory environment, after which the experiments were performed. The organisms were exposed to different leachate concentrations (1.875, 3.75, 7.5, 15, and 30 g/L) for 96 h, and at the end of the experimental period at hour 96, a survival count was done. The mean 96-h lethal effect concentration ($96h\text{-}LC_{50}$) was determined and obtained as 7.46 and 9.38 g/L for traditional and flavored tobacco waste leachates, respectively. Next, one-third of $96h\text{-}LC_{50}$ values (2.49 and 3.13 g/L for traditional and flavored tobacco wastes respectively) was considered for bioaccumulation experiments. In these experiments, the organisms were exposed to the tobacco waste leachates for 90 days (chronic exposure), and then the accumulation rate of PAHs compounds in their muscle tissues were investigated.

Extracting and analyzing PAHs in the leachates and tissues samples

Extracting PAHs in the leachate and tissues samples

Extraction of the PAHs from the leachate was performed according to our previous studies[30,41]. The details of the extraction process are also provided in the supplementary file. For PAHs extraction from seawater, the approach used by Nan Xiang et al.[42], was applied. Firstly, 10 mL of HPLC-grade dichloromethane, methanol, and ultrapure water (in sequence) were applied to activate the SPE Solution Classic C18 cartridge. Then, a volume of 5 mL of HPLC-grade methanol was mixed with 0.5 L of the water samples. The resulting mixture was thoroughly blended and subsequently subjected to pre-concentration using the solid-phase extraction (SPE) technique at a flow rate of 5 mL/min. The cartridge underwent vacuum drying for 15 min after percolation. After that, 12 mL of HPLC-grade dichloromethane (DCM) was used to elute the PAHs on three separate occasions. Residual water was then eliminated using anhydrous sodium sulfate (which had been activated at 450 °C for 6 h) in the packing column. The obtained eluents were concentrated by applying a mild flow of dry nitrogen (≥ 99.999%) in a water bath set at 45 °C. The residue was finally dissolved in 1 mL of HPLC-grade methanol for quantification by GC–MS analysis. For extracting PAHs from the muscle tissues, the method utilized by Tatiana Recabarren-Villal´on[34] was employed. For this purpose, two g of the homogenized dry muscle tissue of the organism was weighed, spiked with of internal/external standards and digested under reflux with methanol. Thereafter, potassium hydroxide (0.7 molar) and triple distilled water were added to it, which was left untouched for 2 h for reflux. To extract the non-saponification part, hexane was used. Dehydrated sodium sulfate was employed for drying the organic phase, and the final sample was concentrated in rotary evaporator with thermostatic bath at a low temperature up to 5 mL. Thereafter, concentration continued with slow nitrogen flow with high purity, until the sample size reached 1.5 mL. For greater clearance of the sample, extraction was placed inside an alumina-silica gel column (2:1). Next, PAH were washed with a suitable volume of hexane- dichloromethane mixture. After that, the final volume of the solutions was reduced to 5 mL with a rotary evaporator and to 1.5 mL under nitrogen flow. Ultimately, after passing through a head syringe 0.45 µm Filter, the solutions were placed inside a 1.5 mL vial for measurement with GC–MS. Note that the all samples were as analyzed three times.

Analysis of PAHs

PAHs were quantified using an Agilent (Palo Alto, CA) 7820A gas chromatograph coupled to a 5977E mass spectrometer. Analyte separation was performed on an HP-5MS UI, 30 m, 0.25 mm i.d, 0.25 µm film thickness column from J&W Scientific (J&W Scientific, Folsom, CA). Specifically, 1µL of the sample was injected into GC–MS device in split-less state, with the injection point temperature being 310°C. Helium gas (1.2 mL/min) functioned as the carrier gas in the analyses. The thermal program of the oven was as follows: remaining at 80°C for 2 min, followed by temperature elevation to 280°C at 30°C/min rate, and staying at this temperature for 1.83 min. The detector temperature was set at 280°C. Mass spectrometer was adjusted at 70 eV electron impact, and set in the selective ion monitoring (SIM) mode. The MS transfer line and ion source were both set at 230°C, while

that the ion quadruple was set at 150°C. For instrument control, collection, management, and evaluation of data, Agilent Chemstation was used.

Quality control and quality assurance

For quality control and quality assurance of analysis of PAHs, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) were determined. The unexposed organism samples were considered as the blank matrix in the analysis of organism samples. The water samples without tobacco wastes were also regarded as the blank matrix in the analysis of the tobacco waste leachate samples. The 16-compound PAHs mixture was used as the external standard, while CHR-d_{12} and BaP-$d1_2$were employed as the internal standards. For each PAH compound, a calibration curve was plotted to guarantee precise and accurate quantification. This was accomplished by spiking eight different levels of the blank sample, which acted as the baseline for comparison. Alongside the blank, internal standards were spiked at a concentration of 10 µg/kg to provide a reliable reference point for the measurements. An external standard with a concentration of 50 µg/kg was also incorporated in the process. Together, these components allowed for the development of a robust calibration curve, which aids in accurately determination of PAH levels in subsequent analyses. The accuracy and precision of the method were determined by spiking three concentrations (5, 10, and 20 µg/kg in the muscle tissue samples and 5, 10, and 20 µg/kg in the leachate samples) of the internal standard into the blank matrix with three replications as well as analysis as intra-and inter-day. LOD and LOQ in the samples were determined based on the method proposed by Ripp et al[43]..

Statistical analyses

Statistical analyses of the data were done using SPSS statistics 22.0-IBM. The Kolmogorov–Smirnov test was used to determine the normality of the obtained. Then, the statistically significant difference between the analyte concentration in the samples of the groups (leachate resulting from flavored and traditional tobacco wastes) was examined using ANOVA and Tukey Post Hoc tests. $P < 0.05$ was considered as the statistical significance.

Results and discussion

Validation parameters of PAHs analysis

The validation parameters of PAHs analysis in the leachate samples resulting from the tobacco wastes and the muscle tissue of the organisms including LOD, LOQ, accuracy, and precision for quality assurance are provided in Table S1. According to this table, the LOD values of PAHs measurement lied within the range of 0.12–0.91 ng/L in the tobacco waste leachate samples, and within 0.19–0.67 µg/kg in the muscle tissues. The respective values of LOQ were 0.38–2.51 and 0.57–1.89 µg /kg. The LOD and LOQ values proposed by the European commission regulations[44] for PAHs are < 0.3 µg /kg and < 0.9 µg/kg respectively.

Furthermore, the samples were analyzed as inter-and intra-day as well, with the accuracy and precision of the obtained analysis presented Table S1. According to these findings, the recovery rate of measurement of PAHs in the muscle tissue samples lied within the range of 76.90–118%, and the accuracy of analysis within 1.29–16.90%, while in the leachate samples resulting from the waterpipe tobacco wastes, the values were 86.40–113% and 0.72–13.70% respectively. As observed in Table S1, some of the recovery rates have been higher than 100%. Mean recovery rates above 110% can be considered as poor separations. In the present study, the mean values of recovery in most of the samples for majority of the PAHs were close to 100%. Nevertheless, in several samples, for some of the compounds, recovery rate above 100% was also observed which can be attributed to the spiking at a very low level (5 µg/kg). Indeed, those values observed for the precision test in this study fulfill the maximum requirements determined for low spiking levels up to 120%[44]. Thus, using the obtained validation data, the practicality of the analytical method used for determining the amount of PAHs in the muscle tissue samples and waterpipe tobacco waste leachates in this study can be confirmed.

PAHs in the tobacco wastes leachates

The concentration values of PAHs compounds in the leachate samples resulting from flavored and traditional tobacco wastes are presented in Table 1 and Fig. 1. As observed, in the leachate of wastes of both traditional and flavored tobacco types, all PAHs have been detected, possibly suggesting that the waterpipe burnt tobacco is an important source of these organic and hazardous pollutants[30]. Figure 1indicates that the ΣPAHs concentration in the leachate samples resulting from flavored and traditional tobacco wastes as well as seawater has been 81.23 ± 17.8, 15.23 ± 3.2 and 4.25 ± 1.3 ng/L respectively. A cursory look indicates that the leachate resulting from flavored tobacco wastes has significantly contained higher values of ΣPAHs concentration than traditional tobaccos ($P < 0.05$). Moreover, the ΣPAHs levels in the leachate from both types of tobacco were significantly greater than those detected in seawater samples. These observations can be attributed to additives, flavorings, sweeteners, essences, and other organic chemicals which are added to this type of tobacco along the processing[8,10]. Additives in tobacco products, particularly organic substances, can significantly alter the chemical composition of smoke when tobacco is burned. These additives often lead to the formation of hazardous compounds, including PAHs, which are known carcinogens[45,46]. Indeed, the incomplete combustion of organic materials is one of the main mechanisms of production of PAHs; at high temperatures and along the pyrolysis process, the macromolecules of organic compounds are broken down into finer and unstable molecules, and then energy is released[47]. These smaller components, in response to blending with radicals, create larger products, which are indeed stable and polycyclic aromatic hydrocarbons[45–48]. In addition, flavored tobaccos have a fatter nature, and previous studies have reported that the fat content can lead to the production and formation of higher amounts of PAHs during the burning of materials[49,50]. Moreover, the high yields of PAHs in mainstream hookah smoke are primarily attributed to the charcoal used for heating the tobacco[51]. The relationship between PAHs in hookah smoke and unburned charcoal extracts, as well as their emissions, indicates a significant correlation

PAHs	Waterpipe tobacco waste leachate types									Seawater		
	Fruit-flavored tobacco					Traditional tobacco						
	Mean ± S.D	Min	Max	Comparison*	Comparison**	Mean ± S.D	Min	Max	Comparison**	Mean ± S.D	Min	Max
Naph	6.73 ± 4.9	2.40	19.16	< 0.001	< 0.001	1.61 ± 1.5	0.43	5.21	< 0.001	0.34 ± 0.2	0.12	0.73
Acen	15.41 ± 12.4	6.19	48.04	< 0.001	< 0.001	2.97 ± 2.3	1.12	8.69	< 0.001	0.50 ± 0.2	0.24	0.87
Ace	10.36 ± 5.6	1.44	18.76	< 0.001	< 0.001	1.10 ± 0.5	0.57	2.20	< 0.001	0.20 ± 0.1	0.12	0.25
Flu	9.37 ± 2.9	4.96	12.89	< 0.001	< 0.001	0.89 ± 0.4	0.35	1.29	< 0.001	0.21 ± 0.1	0.15	0.27
Ant	8.29 ± 3.9	3.17	14.63	< 0.001	< 0.001	1.15 ± 0.5	0.53	2.04	< 0.001	0.40 ± 0.2	0.17	0.83
Phen	3.26 ± 1.9	1.34	7.55	< 0.001	< 0.001	1.89 ± 1.8	0.28	5.08	< 0.001	0.72 ± 0.6	0.13	1.87
Flrt	4.24 ± 4.2	0.47	12.43	< 0.001	< 0.001	1.49 ± 0.8	0.32	2.25	< 0.001	0.54 ± 0.3	0.28	1.12
Pyr	3.49 ± 2.4	0.82	8.41	< 0.001	< 0.001	0.72 ± 0.4	0.24	1.52	< 0.001	0.19 ± 0.1	0.14	0.23
BaA	1.72 ± 1.7	0.42	6.53	< 0.001	< 0.001	0.97 ± 0.6	0.18	1.90	< 0.001	0.24 ± 0.1	0.04	0.40
Chr	2.58 ± 2.3	0.78	8.41	< 0.001	< 0.001	0.91 ± 0.4	0.38	1.52	< 0.001	0.47 ± 0.5	0.17	1.67
BbF	1.54 ± 1.2	0.23	4.21	< 0.001	< 0.001	1.12 ± 0.5	0.57	1.98	< 0.001	0.23 ± 0.1	0.12	0.42
BkF	3.43 ± 2.01	1.07	6.49	< 0.001	< 0.001	1.00 ± 0.5	0.39	1.93	< 0.001	0.65 ± 0.6	0.13	2.23
BaP	4.23 ± 4.2	1.07	13.75	< 0.001	< 0.001	1.05 ± 0.9	0.27	2.49	< 0.001	0.45 ± 0.2	0.22	0.67
IP	4.93 ± 2.8	1.00	7.97	< 0.001	< 0.001	1.22 ± 0.3	0.62	1.52	< 0.001	0.26 ± 0.1	0.13	0.32
DahA	2.90 ± 1.7	1.19	4.90	< 0.001	< 0.001	1.29 ± 0.7	0.19	2.41	< 0.001	0.50 ± 0.3	0.22	1.22
BghiP	3.60 ± 2.4	1.13	8.04	< 0.001	< 0.001	0.85 ± 0.3	0.51	1.45	< 0.001	0.29 ± 0.1	0.16	0.56

Table 1. Statistical analysis of PAHs concentration levels (ng/L) in traditional and fruit-flavored waterpipe tobacco waste leachates (n = 10). * Comparison with traditional tobacco waste leachate. ** Comparison with seawater.

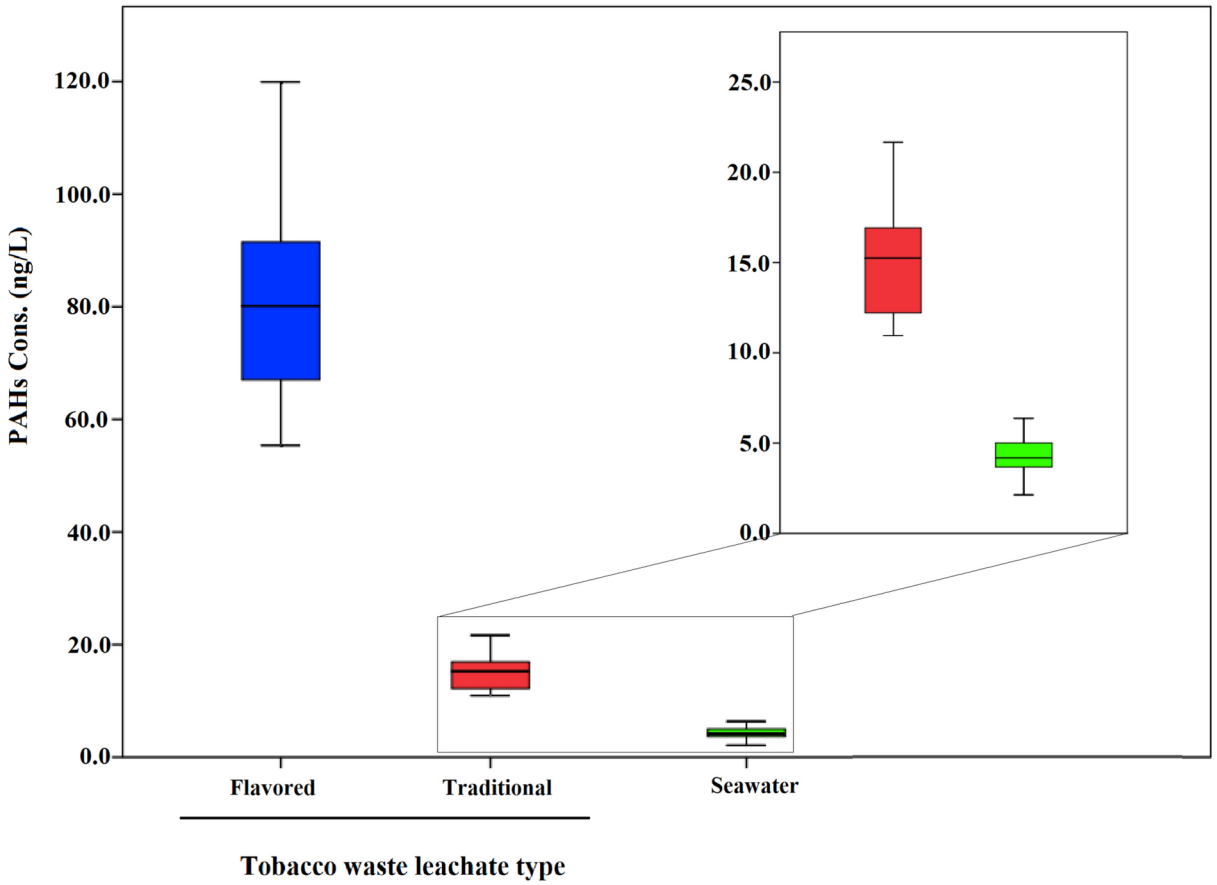

Fig. 1. The mean concentration levels of PAHs (ng/L) in traditional and fruit-flavored burnt tobacco leachates (n = 10) as well as seawater (n = 10). The deference among the concentrations in three leachate types was significant (p-value>0.05).

that supports the hypothesis of charcoal-derived PAHs in hookah smoke[51]. Therefore, the use of charcoal in hookah smoking poses significant health risks to hookah users, primarily due to the production of toxins and their release in smoke and tobacco waste.

The individual concentrations of each of the 16 PAHs in the leachate resulting from flavored and traditional tobacco wastes are also presented in Table 1. The highest concentrations in the leachate resulting from both types of tobacco wastes (flavored and traditional) have been related to low molecular weight PAHs. In the leachate resulting from flavored tobacco wastes, the highest concentration has been associated with acenaphthylene (15.41 ± 12.4 ng/L), acenaphthene (10.36 ± 5.6 ng/L) and fluorene (9.37 ± 2.9 ng/L). In the leachate resulting from the traditional tobacco wastes, the highest concentrations have also been related to acenaphthene (2.97 ± 2.3 ng/L), phenanthrene (1.89 ± 1.8 ng/L) and naphthalene (1.61 ± 1.5 ng/L). These observations can be attributed to lower $K_{ow}$and in turn the high tendency and solubility of these compounds in aqueous media[52]. The findings of the present study have been in accordance with other studies examining the extent of emission of PAHs compounds from tobacco wastes (cigarette stub and waterpipe tobacco) into aqueous media[21,30]. Also, in another study, it was reported that the extent of emission of PAHs with 3- and 4- benzene rings from bitumen and asphalt into aqueous solutions were studied, being 0.1–180 and 0.1–5 ng/L, respectively[53].

PAHs in the muscle samples of *P. peronii* exposed to tobacco leachates

The concentration values of PAHs in the muscle samples of *P. peronii* exposed to the leachate resulting from flavored and traditional waterpipe tobacco wastes are presented in Table 2 and Fig. 2. As seen in Fig. 2, the ΣPAHs concentration in the muscle samples of exposure group I (organisms exposed to the leachate resulting from flavored tobacco) and exposed group II (organisms exposed to leachate resulting from traditional tobacco) and control group (exposed to natural seawater) were 37.49 ± 12.9, 9.32 ± 3.8, and 3.89 ± 1.8 ng/g respectively. As seen, the extent of accumulation of these pollutants has been significantly higher in the exposure groups compared to the control ($P < 0.05$). This suggests that releasing tobacco wastes into the environment and their entrance to the coasts and aqueous environments can lead to their accumulation in aquatic creatures thereby threatening water ecosystems[6,8]. In other words, thus tobacco wastes can function as a carrier for hazardous pollutants (e.g., PAHs), transfer these pollutants into different environments including coasts and seas, thereby causing toxicities for the aquaculture[6,8].

The individual concentrations of PAHs compounds in the muscle samples of *P. peronii* exposed to waterpipe tobacco wastes leachates are outlined in Table 2. As observed, the highest concentrations are related to high molecular weight compounds such as chrysene, benzo(b)fluoranthene, and benzo(a)anthracene with levels of 8.29 ± 5.6, 6.26 ± 4.8, and 6.08 ± 5.1 ng/g, respectively. It is observed that the composition pattern of PAHs in the leachate of tobacco wastes of the muscle tissue of the exposed organism has not been similar. These observations can be attributed to the different potential of bioaccumulation of PAHs[34]. The metabolism of many pollutants including PAHs occurs through cytochrome P450 enzyme system[54]. Low molecular weight PAHs are more volatile and can evaporate quickly from surface waters, which might reduce their availability for bioaccumulation in aquatic organisms[55]. However, they can still be present in interstitial water, making them accessible to infaunal organisms like amphipods and polychaetes[55]. These compounds tend to be more bioavailable in water[56], leading to uptake primarily through ventilation of interstitial water rather than ingestion of sediment. High molecular weight PAHs are hydrophobic and tend to bind strongly to organic matter in sediments, reducing

	Groups											
	EG1: exposed to traditional waterpipe tobacco waste leachates					EG2: exposed to fruit-flavored waterpipe tobacco waste leachates				CG: Control Group		
PAHs	Mean ± S.D	Min	Max	Comparison with EG2	Comparison with CG	Mean ± S.D	Min	Max	Comparison with CG	Mean ± S.D	Min	Max
Naph	2.41 ± 0.5	1.54	3.10	< 0.001	< 0.001	1.23 ± 0.7	0.33	2.19	< 0.001	0.23 ± 0.1	0.11	0.52
Acen	2.01 ± 1.6	0.81	6.26	< 0.001	< 0.001	0.93 ± 0.7	0.35	2.75	< 0.001	0.42 ± 0.3	0.17	1.30
Ace	0.88 ± 0.5	0.35	1.97	< 0.001	< 0.001	0.60 ± 0.4	0.18	1.27	< 0.001	0.26 ± 0.1	0.16	0.36
Flu	0.72 ± 0.3	0.21	1.23	< 0.001	< 0.001	0.43 ± 0.2	0.17	0.84	< 0.001	0.21 ± 0.1	0.13	0.30
Ant	2.31 ± 0.9	1.08	3.58	< 0.001	< 0.001	0.73 ± 0.6	0.18	1.89	< 0.001	0.22 ± 0.1	0.14	0.33
Phen	2.64 ± 1.7	0.30	5.48	< 0.001	< 0.001	0.83 ± 0.5	0.19	1.61	< 0.001	0.40 ± 0.2	0.18	0.76
Flrt	2.30 ± 1.7	0.43	5.32	< 0.001	< 0.001	0.58 ± 0.1	0.43	0.71	< 0.001	0.30 ± 0.1	0.17	0.56
Pyr	1.78 ± 1.3	0.32	4.38	< 0.001	< 0.001	0.71 ± 0.6	0.22	2.19	< 0.001	0.18 ± 0.1	0.10	0.23
BaA	6.08 ± 5.0	0.29	13.93	< 0.001	< 0.001	1.16 ± 1.4	0.12	3.67	< 0.001	0.28 ± 0.1	0.15	0.44
Chr	8.29 ± 5.6	1.18	18.39	< 0.001	< 0.001	0.76 ± 0.6	0.25	1.67	< 0.001	0.27 ± 0.2	0.13	0.56
BbF	6.26 ± 4.8	1.02	17.03	< 0.001	< 0.001	0.72 ± 0.6	0.18	2.05	< 0.001	0.12 ± 0.1	0.11	0.44
BkF	2.05 ± 1.4	0.42	5.28	< 0.001	< 0.001	0.73 ± 0.7	0.16	2.18	< 0.001	0.20 ± 0.1	0.13	0.29
BaP	1.73 ± 1.4	0.38	4.33	< 0.001	< 0.001	1.17 ± 0.7	0.34	2.18	< 0.001	0.26 ± 0.1	0.16	0.41
IP	1.89 ± 0.8	0.55	2.81	< 0.001	< 0.001	0.58 ± 0.4	0.33	1.62	< 0.001	0.19 ± 0.1	0.16	0.22
DahA	1.55 ± 1.3	0.19	4.38	< 0.001	< 0.001	0.59 ± 0.2	0.35	0.94	< 0.001	0.16 ± 0.0	0.11	0.34
BghiP	1.30 ± 0.8	0.35	2.83	< 0.001	< 0.001	0.35 ± 0.2	0.16	0.78	< 0.001	0.20 ± 0.2	0.13	0.44

Table 2. Statistical analysis of PAHs concentration levels (ng/g) in tissue samples of *P. peronii* exposed to traditional and fruit-flavored waterpipe tobacco waste leachates (n = 10).

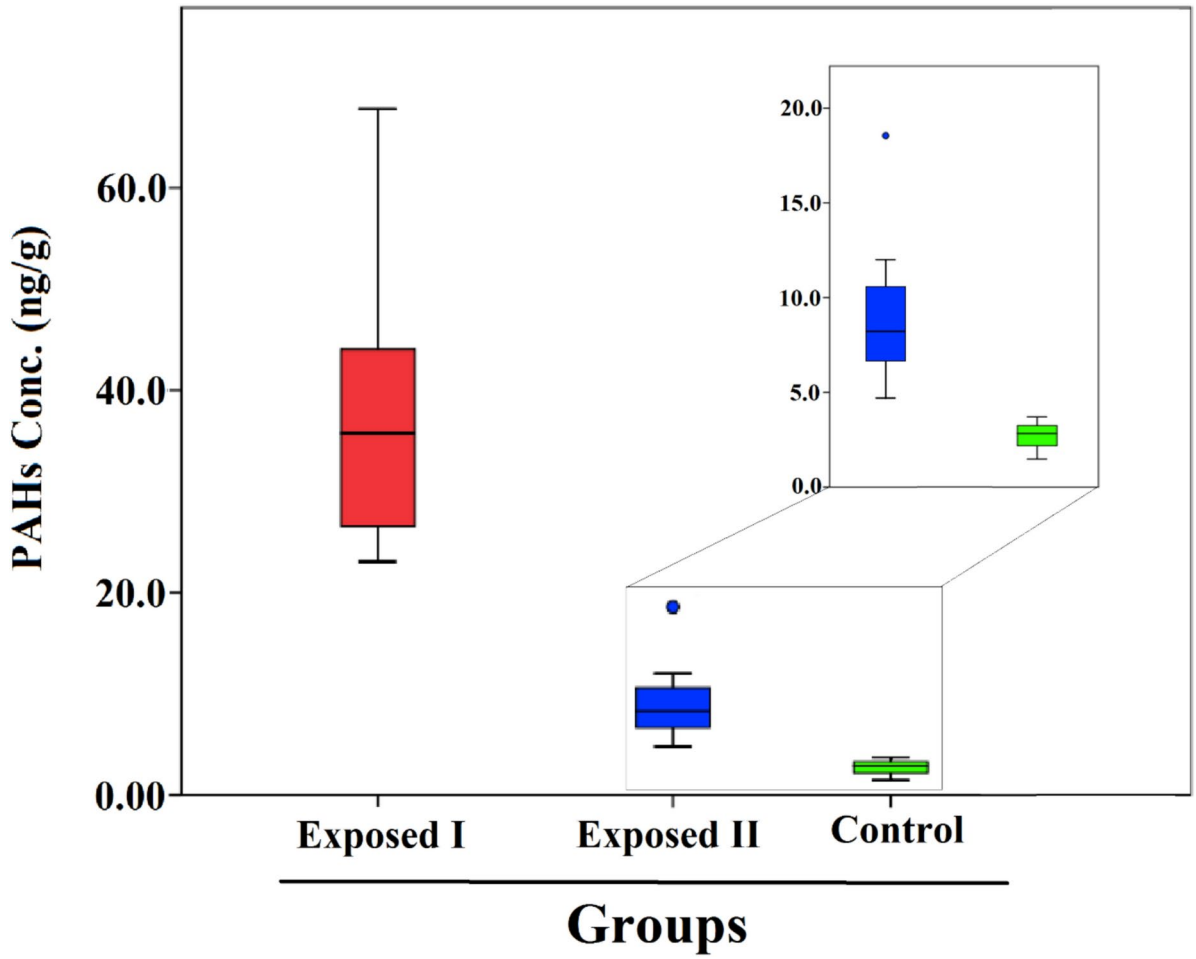

Fig. 2. The mean concentration levels of PAHs (µg/g) in tissue samples of *P. peronii* exposed to traditional and fruit-flavored burnt tobacco leachates (n = 10). Group I (organisms exposed to the leachate resulting from flavored tobacco); group II (organisms exposed to leachate resulting from traditional tobacco) and control group (exposed to natural seawater). The deference among the concentrations in three exposed groups was significant (p-value>0.05).

their bioavailability for many organisms[57,58]. This binding can limit their uptake by species that primarily feed on sediment, such as deposit-feeding polychaetes, although these organisms can still accumulate significant amounts through ingestion[55]. High molecular weight PAHs are more likely to be accumulated through ingestion of contaminated sediment rather than through water[55]. Their strong affinity for organic matter means they are less available in dissolved form but can be consumed by organisms that ingest sediment[55]. The feeding mode of an organism significantly affects PAH bioaccumulation. Non-deposit feeders like amphipods may accumulate PAHs mainly from water, while deposit feeders like polychaetes accumulate them from ingested sediment[55].

Bioaccumulation factor (BAF)

Study of bioaccumulation of pollutants is useful for better understanding the migration and transformation in natural systems (aquatic and dry) as well as determining their possible eco-toxicological effects[59,60]. These studies establish a scientific approach not only for evaluating the risk of pollutants for the ecosystem but also for assessing the health effects for humans[34,61]. Bioaccumulation indicates the ratio of chemical pollutants among living organisms and the matrices of their surrounding environment[59,62]. Indeed, this method presents valuable information of pollutants including the chemical stability properties as well as their manner of accumulation in living organisms. This information can be utilized for environmental and human health risks assessment[63,64]. Considering the carcinogenicity of some PAHs compounds, their increasing emission in the environment should be considered as a warning[60,62]. Since in the present study the pattern and concentration levels of PAHs compounds were different in the tobacco wastes leachates and the muscle tissue samples of *P. peronii*, bioaccumulation factor (BAF) in *P. peronii* was considered as an indicator. In the present study, the bioaccumulation factor (L/Kg) was calculated according to Eq. 1, and as the ratio of PAHs concentration in the muscle tissues of *P. peronii* to its concentration in the leachate.

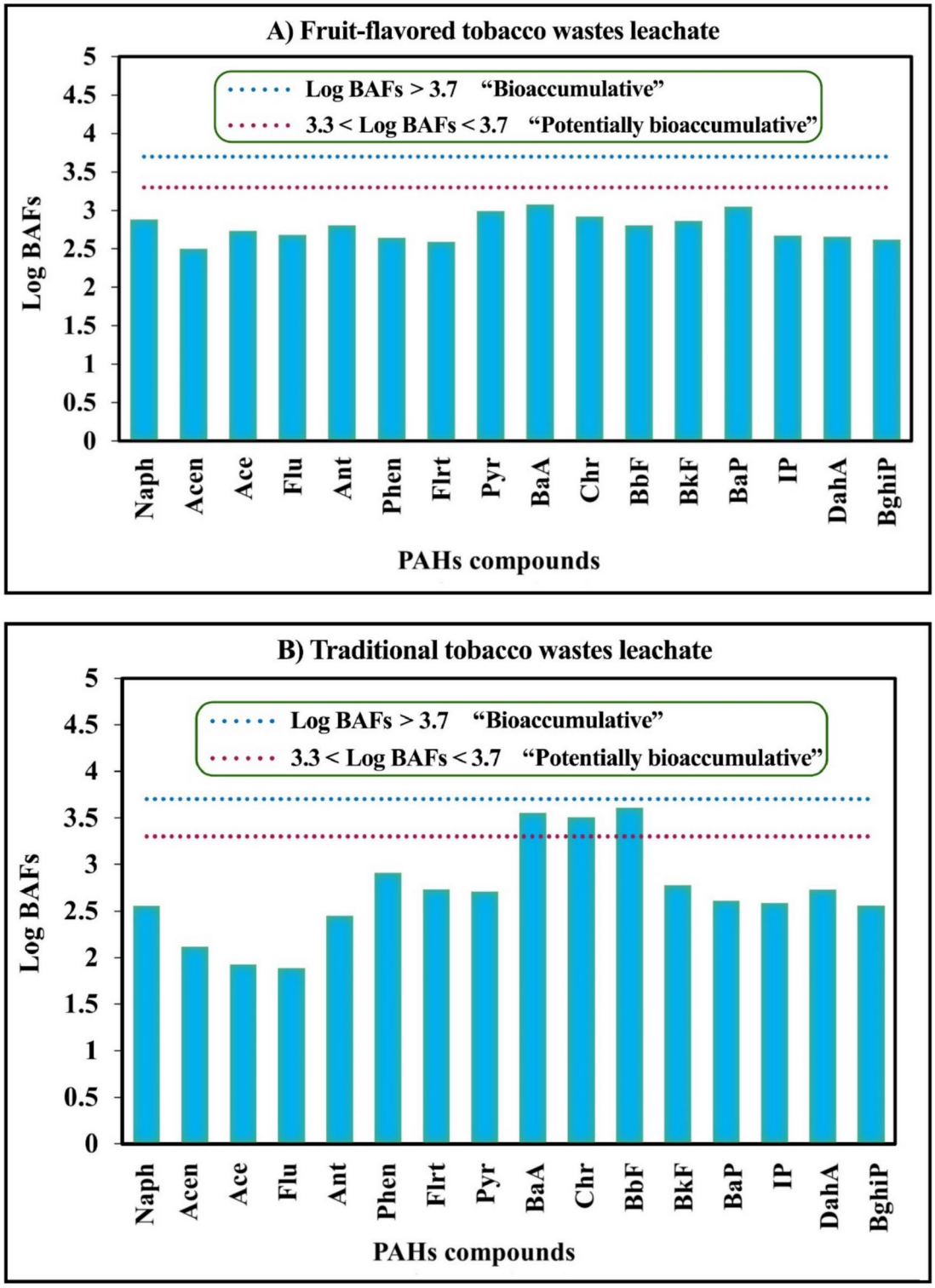

Fig3. The mean Log BAFs in *P. Peronii* exposed to the flavored and traditional tobacco waste leachates.

$$BAFs = \frac{C_O}{C_L} \quad (1)$$

In this equation, C_0 represents the concentration of PAHs compounds in the body of the bioindicator organism (ng/kg) and C_L denotes the concentration of these compounds in the leachate (ng/L). One BAF was calculated for each of the PAH compounds (individual BAF) and one total BAF was calculated from the total concentration of PAHs in the muscle tissue of *P. peronii* and tobacco wastes leachates, with its results presented in Fig. 3.

As shown, the mean Log BAFs in *P. peronii*exposed to the flavored tobacco waste leachates lied within the range of 1.70–3.61 and the traditional counterpart 2.49–3.07. According to the classification of the European Chemicals Agency (ECHA), chemicals with BAF larger than 5000 L/kg (Log BAFs > 3.7) fall in the category of "bioaccumulative" compounds while the chemicals with BAF between 2000 and 5000 L/kg (3.3 < Log BAFs < 3.7) fall in the category of "potentially bioaccumulative"[65,66]. According to this classification, the Log BAFs did not exceed 3.7 for any of the PAHs compounds in any of the leachates (flavored and traditional). In the organisms exposed to the leachates resulting from flavored tobacco wastes, Log BAFs of BbF, BaA, and Chr compounds lied within the range of 3.3–3.7 ("potentially bioaccumulative"). However, in the organisms exposed to the leachates resulting from traditional tobacco wastes, the mean Log BAFs of all compounds did not reach even 3.3. The difference between the mean Log BAFs of PAHs compounds in the organisms exposed to the leachates resulting from flavored and traditional tobacco wastes was not statistically significant ($P > 0.05$). Minwei Han et al. reported that eight compounds of PAHs including Acen, Ant, Pyr, Flu, Chr, BaA, and BbF were found as bioaccumulative in some of the corals in the coasts of the southern China Sea[66]. In another study, the BAFs values of PAHs was 0.3–8 for different species of fish hunted from the much polluted Bahia Blanca estuary (BBE) in Argentina (34). High molecular PAHs may bioaccumulate more because of their environmental persistence[67], resulting in long-term exposure to these pollutants despite lower bioavailability. Moreover, some aquatic organisms might possess specific physiological mechanisms that enable them to efficiently absorb HMPAHs, even when these substances have low solubility. Future comprehensive research needs to focus on more rigorously examining our interpretations better to understand these recruitment mechanisms and their ecological consequences.

It should be noted that due to the numerous influential variables such as i) the characteristics of the aquatic creatures: species, age, gender, diet, physiology, behavior, metabolic features, the mucus of aquatic creatures, nutrition and trophic strategies, ii) compounds hydrophobicity, and iii) metabolization of PAHs into their metabolites in the body of living creatures, etc., precise judgment about the bioaccumulation pattern of PAHs is challenging[34,36,68]. Typically, there is an inverse relationship between octanol–water partition coefficients (K_{OW}) and the extent of bioaccumulation of compounds. Accordingly, the bioaccumulation of PAHs compounds in aquatic creatures is usually higher for low molecular weight PAHs (lower K_{ow}) compared to high molecular weight PAHs (larger K_{ow})[69]. This is due to the solubility of PAHs compounds diminishes with elevation of molecular weight. Thus, high molecular weight PAHs tend to absorb particles and sediments, and are less bioavailable to aquatic creatures[34]. PAHs also after entrance into the human body and other living creatures change into daughter compounds (metabolites and intermediates). Thus, the measurements are consistently underestimated[70]. The mucus of aquatic creatures may also play a key role in the bioaccumulation of PAHs in their body. PAHs accumulate in the mucus of aquatic creatures, and thus accumulation of PAHs in the muscle tissue diminishes. In other words, the mucus of these creatures aggregates the hazardous pollutants in the water, and functions as a protective layer, protecting them against the threats posed by these pollutants[71].

Conclusion

The findings of the present study indicated that the bioaccumulation of PAHs in two groups of *P. peronii* exposed to the leachates of flavored and traditional waterpipe tobacco wastes has increased compared to the control group. Indiscriminate discharge of tobacco wastes into coastal environments can adversely affect the quality of seawater and threaten the life of aquaculture. Since PAHs are among pollutants with bioaccumulation properties, the long-term exposure of aquatic organisms to these pollutants can lead to numerous adverse effects in them. Thus, to effectively address the environmental challenges posed by burnt tobacco waste, it's essential to adopt a comprehensive approach that includes management measures and regulatory frameworks. Furthermore, since these wastes can be categorized as hazardous wastes, it is suggested to isolate and manage them separately and discharge them in specific landfill sites with constant monitoring. Finally, further studies are indeed warranted to explore the effects of these hazardous wastes on the biodiversity of aquatic creatures, which is crucial for public health.

Data availability

'The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request'.

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References

1. Gautam, P. et al. Prevalence and predictors of waterpipe smoking cessation among young adults in the US: Findings from a population-based study. *Addctn. Behav.* **135**, 107457 (2022).
2. Organization, W.H., *Tobacco-Key Facts.* . https://www.who.int/news-room/fact-sheets/detail/tobacco., (2020)
3. Rezaei, S. et al. Economic burden of smoking: A systematic review of direct and indirect costs. *Med. J. Islamic Repub. Iran* **30**, 397 (2016).
4. Stroud, L. et al. Waterpipe (hookah) tobacco use in pregnancy: Use, preferences and perceptions of flavours. *Tob. Control.* **29**(Suppl 2), s62–s71 (2020).
5. Jawad, M. et al. The prevalence and trends of waterpipe tobacco smoking: A systematic review. *Plus One* **13**(2), e0192191 (2018).
6. Masjedi, M. R. et al. Post-consumption waterpipe tobacco waste as an unrecognized source of toxic metal (loid) s leachates into aquatic environments. *Sci. Total Environ.* **879**, 163207 (2023).
7. Dehvari, M. & Babaei, A. Research, Analysis of heavy metals and PAHs in the waste resulting from hookah consumption: Ahvaz City, Iran. *Environ. Sci. Pollut. Res.* https://doi.org/10.1007/s11356-021-17910-8 (2022).
8. Masjedi, M. R. et al. First report on BTEX leaching from waterpipe tobacco wastes (WTWs) into aquatic environment. *Heliyon* https://doi.org/10.1016/j.heliyon.2023.e21946 (2023).

9. Hauser, C. D. et al. Waterpipe tobacco smoke toxicity: the impact of waterpipe size. *Tob. Control* https://doi.org/10.1136/tobaccocontrol-2019-054960 (2019).
10. Schubert, J. et al. Waterpipe smoking: The role of humectants in the release of toxic carbonyls. *Arch. Toxicol.* **86**, 1309–1316 (2012).
11 Montazeri, Z. et al. Waterpipe smoking and cancer: systematic review and meta-analysis. *Tob. Control* https://doi.org/10.1136/tobaccocontrol-2015-052758 (2016).
12 Maziak, W. et al. The global epidemiology of waterpipe smoking. In *Principles of Addiction* 891–900 (Elsevier, 2014).
13. Qasim, H. et al. The effects of hookah/waterpipe smoking on general health and the cardiovascular system. *Environ. Health Prev. Med.* **24**(1), 1–17 (2019).
14 Kassem, N. O. et al. Waterpipe device cleaning practices and disposal of waste associated with waterpipe tobacco smoking in homes in the USA. *Tob. Control* https://doi.org/10.1136/tobaccocontrol-2019-054959 (2019).
15 Jafari, A. J. et al. Determination of metals and BTEX in different components of waterpipe: charcoal, tobacco, smoke and water. *J Environ Health Sci Engineer.* **18**, 243–251 (2020).
16 Heydari, G. et al. Load characteristics and inhalation risk assessment of benzene series (BTEX) pollutant in indoor air of Ghalyan and/or cigarette cafes compared to smoking-free cafes. *Environ. Pollut. Bioavailab.* **32**(1), 26–35 (2020).
17. Masjedi, M. R. et al. Air pollutants associated with smoking in indoor/outdoor of waterpipe cafés in Tehran, Iran: concentrations, affecting factors and health risk assessment. *Sci. Rep.* **9**(1), 3110 (2019).
18 Masjedi, M. R. et al. Post-consumption waterpipe tobacco waste as an unrecognized source of toxic metal(loid)s leachates into aquatic environments. *Sci. Total Environ.* **879**, 163207 (2023).
19. Organization, W.H., *Tobacco and its environmental impact: an overview*: World Health Organization (2017)
20. Torkashvand, J. et al. Effect of cigarette butt on concentration of heavy metals in landfill leachate: Health and ecological risk assessment. *J. Environ. Health Sci. Eng.* **19**, 483–490 (2021).
21 Dobaradaran, S. et al. Polycyclic aromatic hydrocarbons (PAHs) leachates from cigarette butts into water. *Environ. Pollut.* **259**, 113916 (2020).
22. Dobaradaran, S. et al. Environmental fate of cigarette butts and their toxicity in aquatic organisms: A comprehensive systematic review. *Environ. Res.* **195**, 110881 (2021).
23. Soleimani, F. et al. Toxic effect of cigarette butts leachates on blood markers of Periophthalmus waltoni species from the Persian Gulf region. *Chemosphere* **319**, 138036 (2023).
24. Soleimani, F. et al. Chemical contents and toxicity of cigarette butts leachates in aquatic environment: A case study from the Persian Gulf region. *Chemosphere* **311**, 137049 (2023).
25 Slaughter, E. et al. Toxicity of cigarette butts, and their chemical components, to marine and freshwater fish. *Tob Control.* **20**(Suppl 1), i25 (2011).
26. Osuala, F. et al. Relative toxicity of cigarette butts leachate and usefulness of antioxidant biomarker activity in Nile tilapia Oreochromis niloticus (Trewavas, 1983). *Ethiop. J. Env Stud. Manag.* **10**(1), 75–88 (2017).
27. Lawal, M. S. & Ologundudu, S. O. Toxicity of cigarette filter leachates on Hymenochirus curtipes and Clarias gariepinus in Nigeria. *J. Environ. Ext.* **11**, 7–14 (2013).
28 Santos-Echeandía, J. et al. The role of cigarette butts as vectors of metals in the marine environment: Could it cause bioaccumulation in oysters?. *J. Hazard. Mater.* **416**, 125816 (2021).
29 Richardot, W. H. et al. Leached compounds from smoked cigarettes and their potential for bioaccumulation in rainbow trout (Oncorhynchus mykiss). *Chem. Res. Toxicol.* **36**(11), 1703–1710 (2023).
30 Masjedi, M. R. et al. Polycyclic aromatic hydrocarbon (PAH) leachates from post-consumption waterpipe tobacco waste (PWTW) into aquatic environment-a primary study. *Environ. Pollut.* **327**, 121500 (2023).
31. Boehm, P. D. Polycyclic aromatic hydrocarbons (PAHs). In *Environmental forensics* 313–337 (Elsevier, 1964).
32. Choi, H., et al., *Polycyclic aromatic hydrocarbons*, in *WHO guidelines for indoor air quality: selected pollutants*. World Health Organization (2010)
33. Hoh, E. et al. Environmental tobacco smoke as a source of polycyclic aromatic hydrocarbons in settled household dust. *Environ. Sci. Technol.* **46**(7), 4174–4183 (2012).
34. Recabarren-Villalón, T. et al. Seasonal distribution pattern and bioaccumulation of Polycyclic aromatic hydrocarbons (PAHs) in four bioindicator coastal fishes of Argentina. *Environ. Pollut.* **291**, 118125 (2021).
35 Abdel-Shafy, H. I. & Mansour, M. S. M. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egypt. J. Petrol.* **25**(1), 107–123 (2016).
36 Baali, A. & Yahyaoui, A. Polycyclic aromatic hydrocarbons (PAHs) and their influence to some aquatic species. In *Biochemical Toxicology - Heavy Metals and Nanomaterials* (eds Ince, M. et al.) (IntechOpen, 2019).
37 Mizukami, I. et al. Diversity and distribution of air-breathing sea slug genus Peronia Fleming, 1822 (Gastropoda: Onchidiidae) in southern Japanese waters. *PeerJ.* **10**, e13720 (2022).
38 Dayrat, B. et al. Systematic revision of the genus peronia fleming, 1822 (Gastropoda, euthyneura, pulmonata, onchidiidae). *ZooKeys* **972**, 1 (2020).
39. Fariman, G. A. & Moosavipoor, Y. Phylogenetic study of Peronia peronii species (Mollusca Gastropod: sea slug) in inter-tidal Chabahar coast, based on 18s-rDNA sequence. *New Cell. Mol. Biotechnol. J.* **4**(15), 29–34 (2014).
40 Arast, Y. et al. Toxicity of Persian Gulf shell-less marine mollusc (Peronia peronii) methanolic extract on melanoma tumor mitochondria. *Cutan. Ocul. Toxicol.* **42**(1), 12–18 (2023).
41. Dobaradaran, S. et al. Polycyclic aromatic hydrocarbons (PAHs) leachates from cigarette butts into water. *Environ. Pollut.* **259**, 113916 (2020).
42. Xiang, N. et al. Occurrence and distribution of Polycyclic aromatic hydrocarbons (PAHs) in seawater, sediments and corals from Hainan Island China. *Ecotoxicol. Environ. Saf.* **152**, 8–15 (2018).
43 Ripp, J. *Analytical detection limit guidance & laboratory guide for determining method detection limits* (Wisconsin Department of Natural Resources. Laboratory Certification Program, 1996).
44. (EC), E.C.R., *European Commission Regulation (EC) No 2015/1125 of 10 July 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in Katsuobushi (dried bonito) and certain smoked Baltic herring.* Official Journal of the European Union, (2015)
45 Bignal, K. L., Langridge, S. & Zhou, J. L. Release of polycyclic aromatic hydrocarbons, carbon monoxide and particulate matter from biomass combustion in a wood-fired boiler under varying boiler conditions. *Atmospheric Environ.* **42**(39), 8863–8871 (2008).
46. Arfaeinia, L. et al. Bioaccessibility-based monitoring and risk assessment of indoor dust-bound PAHs collected from housing and public buildings: effect of influencing factors. *Environ. Res.* **204**, 112039 (2022).
47. Ali, M. U. et al. Emission sources and full spectrum of health impacts of black carbon associated polycyclic aromatic hydrocarbons (PAHs) in urban environment: A review. *Crit. Rev. Environ. Sci. Technol.* **51**(9), 857–896 (2021).
48 Kermani, M. et al. Ambient air PM2. 5-bound PAHs in low traffic, high traffic, and industrial areas along Tehran Iran. *Human Ecol. Risk Assess.: Int. J.* **27**(1), 134–151 (2021).
49 Gomes, A. et al. Effect of fat content, casing type and smoking procedures on PAHs contents of Portuguese traditional dry fermented sausages. *Food Chem. Toxicol.* **58**, 369–374 (2013).
50 Polachova, A. et al. Estimation of human exposure to polycyclic aromatic hydrocarbons (PAHs) based on the dietary and outdoor atmospheric monitoring in the Czech Republic. *Environ. Res.* **182**, 108977 (2020).

51. Monzer, B. et al. Charcoal emissions as a source of CO and carcinogenic PAH in mainstream narghile waterpipe smoke. *Food Chem. Toxicol.* **46**(9), 2991–2995 (2008).
52. Hansen, B. H. et al. Partitioning of PAHs between crude oil microdroplets, water, and copepod biomass in oil-in-seawater dispersions of different crude oils. *Environ. Sci. Technol.* **52**(24), 14436–14444 (2018).
53 Brandt, H. C. A. & De Groot, P. C. Aqueous leaching of polycyclic aromatic hydrocarbons from bitumen and asphalt. *Water Res.* **35**(17), 4200–4207 (2001).
54 Andersson, T. & Förlin, L. Regulation of the cytochrome P450 enzyme system in fish. *Aquat. Toxicol.* **24**(1–2), 1–19 (1992).
55. Meador, J. et al. Comparative bioaccumulation of polycyclic aromatic hydrocarbons from sediment by two infaunal invertebrates. *Mar. Ecol. Prog. Ser.* **123**, 107–124 (1995).
56. Patel, A. B. et al. Polycyclic aromatic hydrocarbons: sources, toxicity, and remediation approaches. *Front. Microbiol.* **11**, 562813 (2020).
57. Stanley, G., M. Britz, and S. Boonchan, *Degradation of polycyclic aromatic hydrocarbons by microorganisms*, Google Patents.(2004)
58. Wang, Z. et al. Spatiotemporal distribution, bioaccumulation, and ecological and human health risks of polycyclic aromatic hydrocarbons in surface water: A comprehensive review. *Sustainability* **16**(23), 10346 (2024).
59. Sanou, A. et al. Assessment of heavy metal contamination of fish from a fish farm by bioconcentration and bioaccumulation factors. *Egypt. J. Aquat. Biol. Fish.* **25**(1), 821–841 (2021).
60. Balcıoğlu, E. Potential effects of polycyclic aromatic hydrocarbons (PAHs) in marine foods on human health: A critical review. *Toxin Rev.* **35**(3–4), 98–105 (2016).
61 Bao, Y. et al. Distribution of 31 endocrine-disrupting compounds in the Taihu Lake and application of the fish plasma model. *Environ. Sci. Eur.* **32**(1), 1–16 (2020).
62 Kwok, C. et al. Biota–sediment accumulation factor (BSAF), bioaccumulation factor (BAF), and contaminant levels in prey fish to indicate the extent of PAHs and OCPs contamination in eggs of waterbirds. *Environ. Sci. Pollut. Res.* **20**, 8425–8434 (2013).
63. Arias, A. H. et al. Bioaccumulation of PAHs in marine zooplankton: an experimental study in the copepod Pseudodiaptomus marinus. *Environ. Earth Sci.* **75**, 1–9 (2016).
64. El Bahgy, H. E. et al. Heavy metals bioaccumulation in marine cultured fish and its probabilistic health hazard. *Environ. Sci. Pollut. Res.* **28**, 41431–41438 (2021).
65. Agency, E.C., *Guidance on Information Requirements and Chemical Safety Assessment: Chapter R.11: PBT Assessment (Version 1.1).* (2012).
66 Han, M. et al. Polycyclic aromatic hydrocarbons (PAHs) in corals of the South China Sea: occurrence, distribution, bioaccumulation, and considerable role of coral mucus. *Environ. Manag.* **384**, 121299 (2020).
67 Alegbeleye, O. O., Opeolu, B. O. & Jackson, V. A. Polycyclic aromatic hydrocarbons: A critical review of environmental occurrence and bioremediation. *Environ. Manag.* **60**, 758–783 (2017).
68. Froehner, S., Maceno, M. & Machado, K. S. Predicting bioaccumulation of PAHs in the trophic chain in the estuary region of Paranagua. *Brazil. Environ. Monit. Assess.* **174**(1–4), 135–145 (2011).
69. Tolosa, I. et al. Aliphatic and aromatic hydrocarbons in marine biota and coastal sediments from the Gulf and the Gulf of Oman. *Mar. Pollut. Bull.* **50**(12), 1619–1633 (2005).
70 Baali, A. et al. Bile metabolites of polycyclic aromatic hydrocarbons (PAHs) in three species of fish from Morocco. *Environ. Sci. Eur.* **28**, 1–6 (2016).
71. Christian Wild, M. R. Ursula Werner, Ulrich Franke, Ron Johnstone, Markus Huettel, Degradation and mineralization of coral mucus in reef environments. *Mar. Ecol. Prog. Ser.* **267**, 159–171 (2004).

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Author contributions

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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