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Detection of Microplastic Types and Polycyclic Aromatic Hydrocarbon Levels: Evaluating the Depuration Effect on Accumulation in Two Freshwater Invertebrate Species in the Nile Delta, Egypt

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ABSTRACT

The River Nile is the main life artery in Egypt. Recently, concerns have been raised about accumulation of microplastics and polycyclic aromatic hydrocarbons (PAHs) in water, sediments, and aquatic animals' tissue, especially that there are few studies on these points in freshwater. The present study aimed to assess the accumulation of microplastics and polycyclic aromatic hydrocarbons in two freshwater invertebrates with two different modes of feeding (*Procambarus clarkii* and *Anodonta anatine*), and evaluate the effect of depuration on them. Samples were collected in summer from the El- Gharbiya region in the Egyptian Delta. The obtained results indicated the occurrence of some microplastics with the abundance of Polyethylene terephthalate (PET). Concerning PAHs, their composition pattern in sediment samples is mainly dominated by four-ring PAHs, whereas low molecular weight PAHs dominated water and invertebrate samples. PAHs levels in invertebrates were significantly higher than those in sediments and water. The highest bioaccumulation was detected in *Anodonta anatine*. Anthracene, phenanthrene and pyrene accumulation were significantly increased in *Procambarus clarkia*. However, significant declines were observed in fluorene and naphthalene levels. While, a highly significant decrease at all PAHs levels were observed in *Anodonta* sp. except for fluorene. Overall, the study detected various microplastic types in the Nile River, including polyamide (PA, nylon), polystyrene (PS), polypropylene (PP), polyethylene terephthalate (PET), high-density polyethylene (HDPE), and cellophane. Additionally, twelve polycyclic aromatic hydrocarbon (PAH) compounds were recorded for accumulation in two invertebrate models. Unfortunately, depuration did not have a significant effect on microplastic accumulation in both bivalves and crayfish, and only a slight effect on some types of polycyclic aromatic hydrocarbons. Urgent and radical solutions are needed to preserve the Nile water, especially since pollutants can be transferred to humans through drinking water or the food chain.

INTRODUCTION

Among water resources in Egypt, the Nile River is the primary source of freshwater. Egyptians depend on the Nile River for all their water needs **(Abd Ellah, 2020)**. However, it was found to be highly contaminated with PAHs **(Badawy & Embaby, 2010; Haiba, 2019**).

Human activities, driven by rapid population growth and the expansion of industrial, agricultural, and animal husbandry sectors, have significantly contributed to the increase in pollution. Escalating levels and continuous discharge and occurrence of microplastics or polycyclic aromatic hydrocarbons (PAHs) pose serious threats to the survival and health of both organisms and humans. Thus, immediate solutions are needed for environmental protection **(Bagheri *et al*., 2020; Kongpran *et al*., 2021)**.

In aquatic ecosystems, bottom sediments are the main sink of contaminants. Upon discharge, microplastics become suspended in the water column or attach to the sediments according to their size and shape **(Cole *et al*., 2011; Pittura *et al*., 2018)**. Microplastic (MP) ingestion by organisms can lead to a range of impacts, from physical damage due to accumulation in the digestive tract to minor disruptions in physiological functions. Adverse effects, such as pseudo-satiation, can decrease food intake and may ultimately result in mortality **(Hui *et al.,* 2020)**. Moreover, the ingestion and accumulation of MPs can also enhance increased exposure to other pollutants, which have deleterious impacts on physiological processes responsible for nutrition, growth, and survival **(Van Cauwenberghe & Janssen, 2014; Sussarellu *et al*., 2016)**. Furthermore, the organic compounds and trace elements adsorbed onto MPs pose a potential health threat to organisms **(Zhu *et al*., 2019)**. On the other hand, PAHs tend to accumulate and persist on sediments due to their hydrophobic and lipophilic nature **(Hussain *et al*., 2015; Younis *et al*., 2023)**. Aquatic biota is vulnerable to PAHs' toxicity either directly from the water column or indirectly via their diet. Furthermore, high levels of PAHs in waterbodies or their accumulation on sediments trigger subsequent bioaccumulation in organisms, thereby contamination of food chains and eventually biomagnification at higher trophic levels, posing a health risk to humans **(Yang *et al*., 2014; Miller *et al*., 2020; Malhat *et al*., 2021; Uddin & Xu, 2024)**.

Polycyclic aromatic hydrocarbons (PAHs) comprise an important category of persistent organic pollutants ubiquitous in aquatic ecosystems **(Barakat *et al*., 2011; Cox & Clements, 2013; Awe *et al*., 2020)**. Generally, they are organic compounds comprised of two or more fused aromatic rings. They are released from natural sources such as forest fires and volcanic emissions or anthropogenic processes through the incomplete combustion of fossil fuel **(Awe *et al*., 2020; Malhat *et al*., 2021)**.

Likewise, plastic pollution is a top emerging ecological hazard due to its persistence, ubiquity, and danger posed to biota **(Iannilli *et al*., 2019)**. Plastic debris has become one of the most pervasive forms of pollution worldwide. Despite being versatile, resistant, and cheap material **(El- Naggar *et al.,* 2024)**, the increased demand and production of plastics have caused widespread contamination of ecosystems. Every year, an average of 10 million tons of plastic debris enter the ocean, with a significant amount originating from rivers (between 1.15 and 2.41 million tons). Microplastics, in particular, can act as sponges,

absorbing persistent organic contaminants from the surroundings, and recently they have been proven to be perfect transmitters of PAHs to aquatic biota **(Pittura *et al*., 2018; Vo & Pham, 2021)**.

Most of bivalve mollusks are filter feeders, and consciously they are directly exposed to the MP in the water column **(Van Cauwenberghe & Janssen, 2014)**. Mussels are one of the most vulnerable species to contamination. They are a direct intermediary for contaminants to reach humans through the food chain as they are an important source of animal nutrition **(Yozukmaz, 2021)**. According to **Vandermeersch *et al*. (2015)**, bivalves are excellent indicators of environmental health because of their widespread abundance, sessile nature, ease of sampling, tolerance to salinity fluctuations, and stress resistance. Additionally, their tendency to accumulate different contaminants makes them ideal for studying the behavior of pollutants in aquatic ecosystems, including their distribution, accumulation, and induced toxicity through both laboratory experiments and *in-situ* monitoring **(Azizi *et al*., 2018)**. Additionally, according to **Aly *et al.* (2020)**, freshwater crayfish *Procambarus clarkia* are common in Egyptian freshwater. Although this species is not a filter feeder, it is a valuable bioindicator due to its wide ecological tolerance. *P. clarkia* can thrive in various conditions. Therefore, it has been accepted as a test organism in crustacean toxicological studies **(Younis *et al*., 2022)**.

Depuration was validated as an effective tool to remove many organic chemicals **(FAO, 2008)** by reducing the concentration of pollutants within the gastrointestinal tract (GIT); depuration can influence factors such as toxicity, the transfer of additives and chemicals, and the possibility of trophic transfer (the movement of contaminants through a food chain) **(Dawson *et al*., 2018; Bour *et al*., 2020b)**. Subsequently, an enhanced understanding of MPs and PAHs' ingestion and depuration of aquatic biota may help throw light on risks posed by these contaminants **(Piazza *et al*., 2016; Santana *et al*., 2022)**.

Unfortunately, limited research has investigated the occurrence of microplastic in the Nile River. **Khan *et al.* (2020)** and **Khallaf *et al.* (2023)** were the first researchers that recorded microplastics in the Nile River by using only fish samples. The current study aimed to detect the MPs and polycyclic aromatic hydrocarbon compounds in the Nile River and their presence and accumulation in two endemic invertebrate models *Anodonta* sp. and *Procambarus clarkia* as indicators, noting the difference in the nature of nutrition in each of the two species chosen for this study. In addition, this study was organized to test the efficacy of freshwater depuration in decreasing the levels of contaminants in the studied samples.

MATERIALS AND METHODS

1. Samples collection

Samples of the freshwater crayfish *Procambarus clarkii* and freshwater *Anodonta anatine* (duck mussel) were collected in the summer season from El- Gharbiya region in the Egyptian Delta, specifically Kafr El- Zayat (30.824722°N 30.815278°E). Samples were

collected in the summer season to avoid reproduction periods in spring and dormancy in winter. Thirty samples from each species were collected, cleaned, and stored in plastic bags in a cooler before being transferred to the Invertebrates Laboratory at the Faculty of Science, Tanta University. In addition, water and sediments samples were collected from the same site and transported to the laboratory.

2. Sampling depuration and storage

In the laboratory, mussels were scrubbed to remove biofouling. About 15 samples of mussels and crayfish were kept frozen at −80°C as non-depurated controls. The remaining 15 individuals of each species were placed in a 300L glass aquarium system containing dechlorinated tap water, without water exchange, and equipped with dry-wet filters and skimmer devices. They were maintained for 93 hours without feeding to allow for the depuration of stomach contents **(Lee *et al.*, 2008)**. The freshwater used for depuration was filtered and dechlorinated tap water. Dead mussels were promptly removed to prevent contamination, and depuration continued for approximately three weeks.

3. Samples preparation

After the depuration period, samples of crayfish and mussels (both depurated and non-depurated) were prepared for FTIR measurement. Mussels' soft tissues were extracted from their shells using a scalpel, sealed in aluminum foil, and stored at -80°C. Crayfish samples were dissected to extract the gills and digestive gland, then foil-sealed and kept at -80°C. To minimize contamination, samples were handled with latex gloves.

Next, the samples were ground to create a fine, homogeneous powder with particle sizes ideally below 100µm, using a mortar and pestle. Since moisture content could interfere with the analysis, drying was essential. The powdered samples were dried under controlled temperatures to avoid thermal degradation.

4. FTIR measurement of powder samples using Bruker FTIR instrument

Suspected MPs were analyzed with FTIR spectrometer (INVENIO FT-IR Spectrometer -Bruker) to identify the MPs polymer constituents. FTIR absorption spectra were collected by averaging 64 scans in the mid-infrared range of 4000 to 400cm^{-1}, with a resolution of 4 per cm. The resulting spectra were compared to reference spectra in a library to identify the type of polymer present **(Salem *et al*., 2023)**.

5. Determination of polycyclic aromatic hydrocarbons (PAHs)

Twelve polycyclic aromatic hydrocarbons (PAHs) were identified in sediment, water, and tissue samples using HPLC, following the methodology described by **Kumar *et al.* (2014)**. The PAHs included anthracene, benzo(a)pyrene, benzo[b]fluoranthene,

benzo[g,h,i]perylene, benzo[k]fluoranthene, chrysene, dibenzo[a,h]anthracene, fluorene, fluoranthene, phenanthrene, naphthalene, and pyrene.

6. Statistical analysis

Data were presented as mean ± SD to detect differences in concentrations of different PAHs between sediment, water and invertebrates' samples (*Procambarus clarkia* and *Anodonta* sp.). One-way ANOVA was employed to analyze the data, and Tukey's method for multiple comparisons was used to detect pairwise differences. A paired t-test was conducted to assess statistical differences in PAH concentrations between *Procambarus clarkii* and *Anodonta* sp. before and after depuration. Principal component analysis (PCA) was performed to reveal the relationships between the distribution of different PAHs in sediment, water, and both invertebrate species (depurated and undepurated) in an ordination plot, using PAST version 4.08 **(Hammer *et al.*, 2001)**. All data analyses were carried out using the Minitab software package version 19.0 and Microsoft Excel 365.

RESULTS

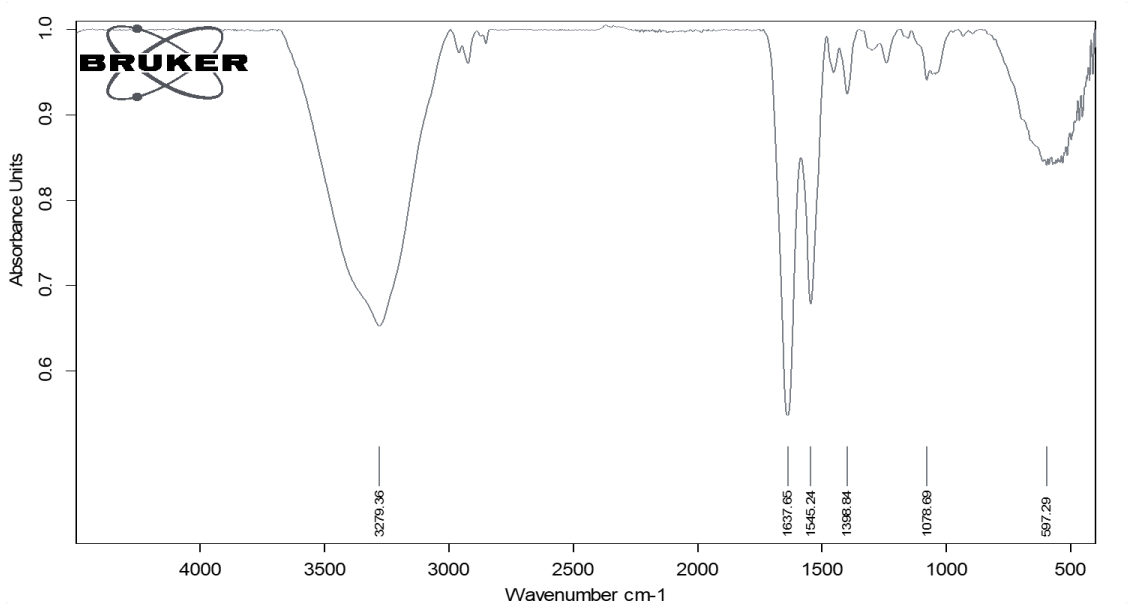

Fig. 1. FTIR analysis on *Anodonta* sp. before depuration

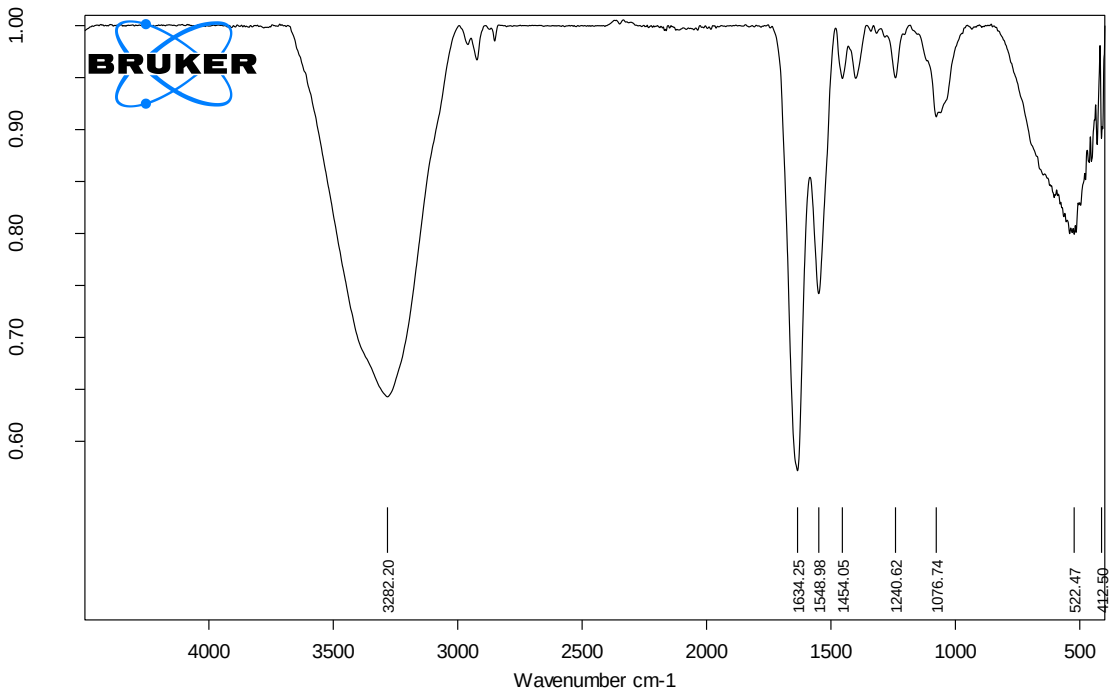

Fig. 2. FTIR analysis on *Anodonta* sp. after depuration

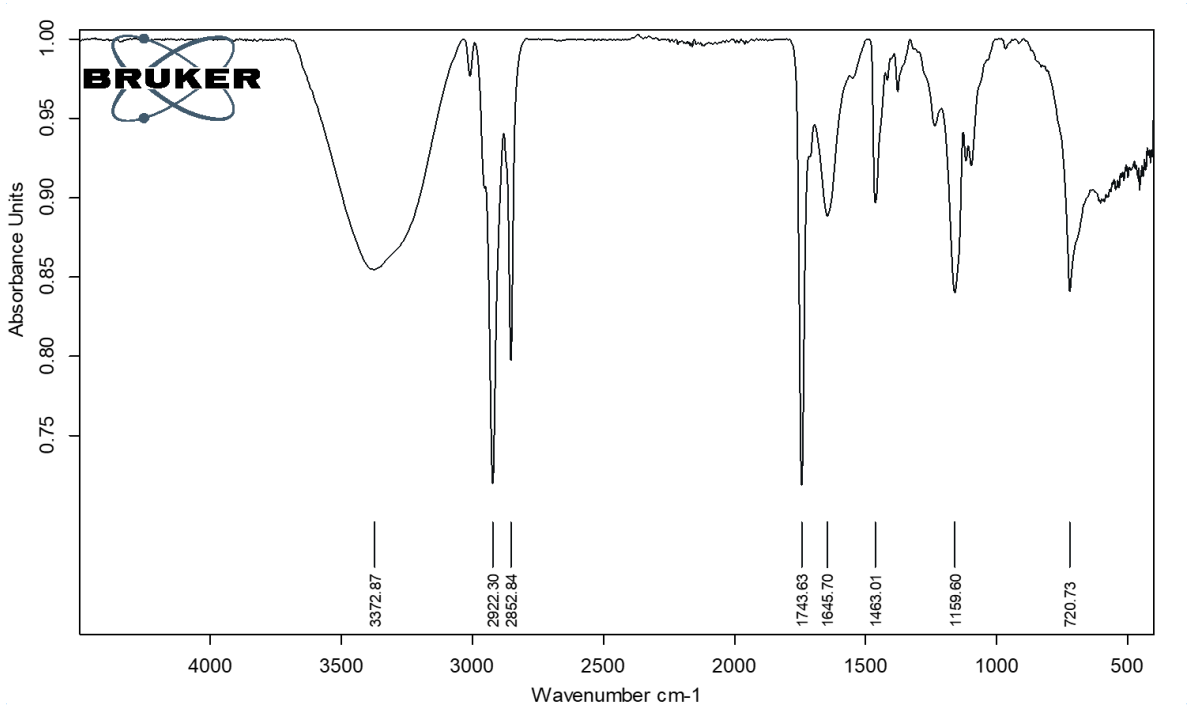

Fig. 3. FTIR analysis on hepatopancreas of crayfish before depuration

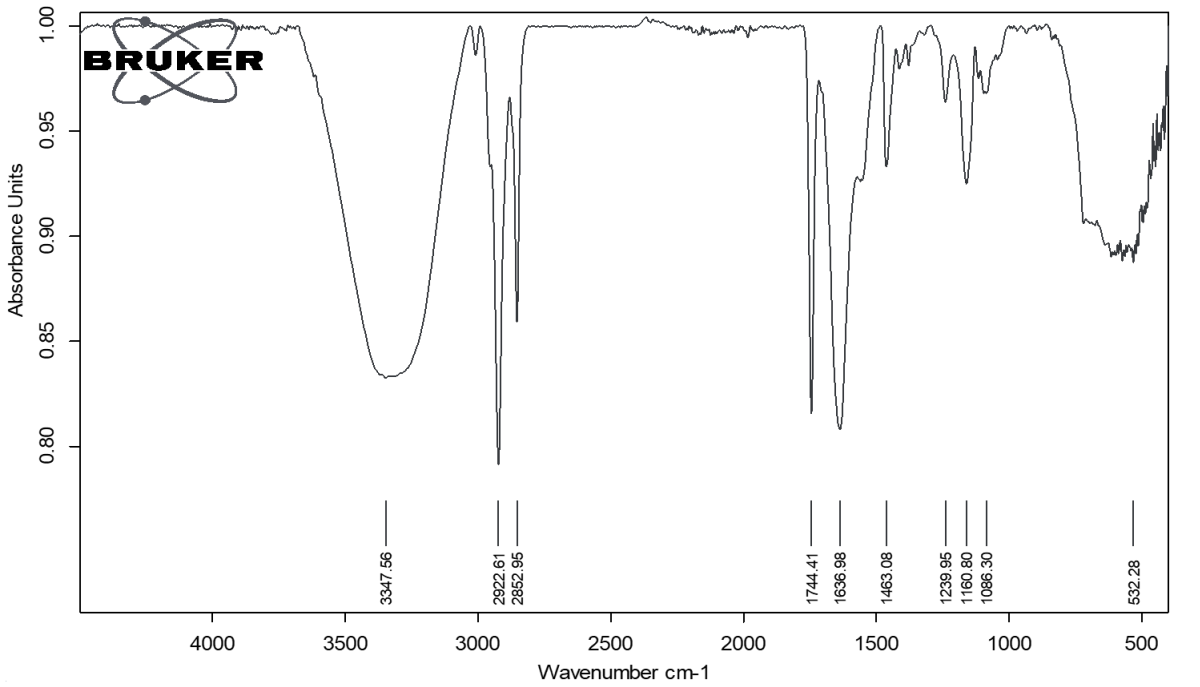

Fig. 4. FTIR analysis on hepatopancreas of crayfish after depuration

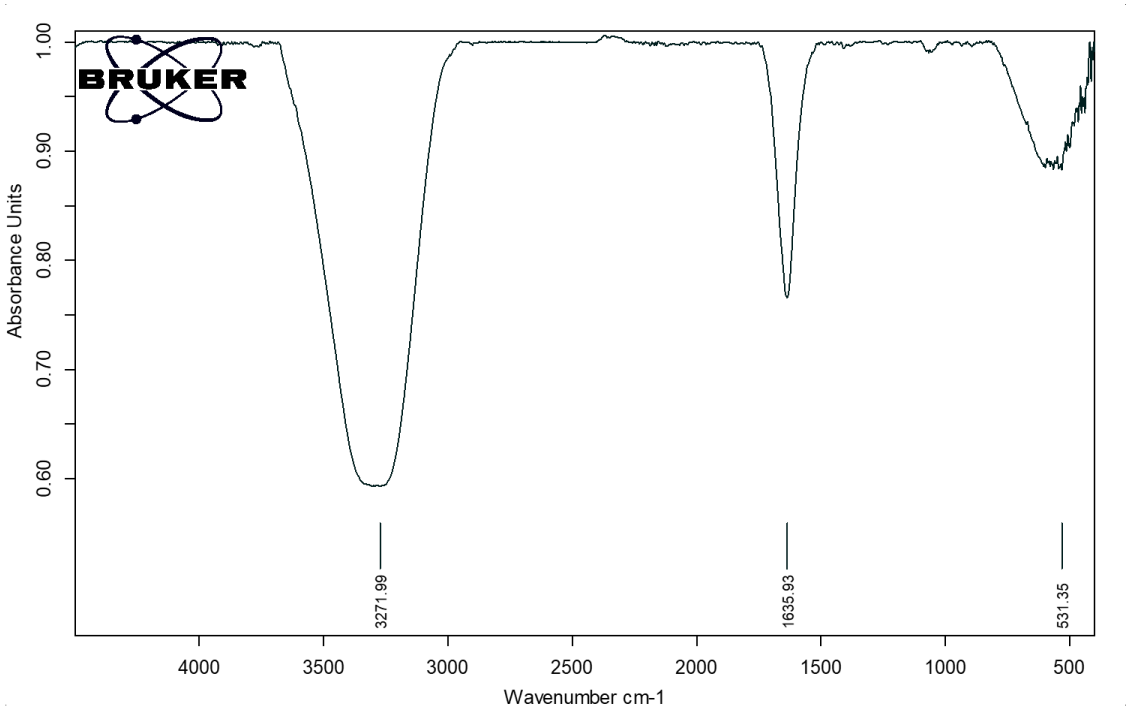

Fig. 5. FTIR analysis on water

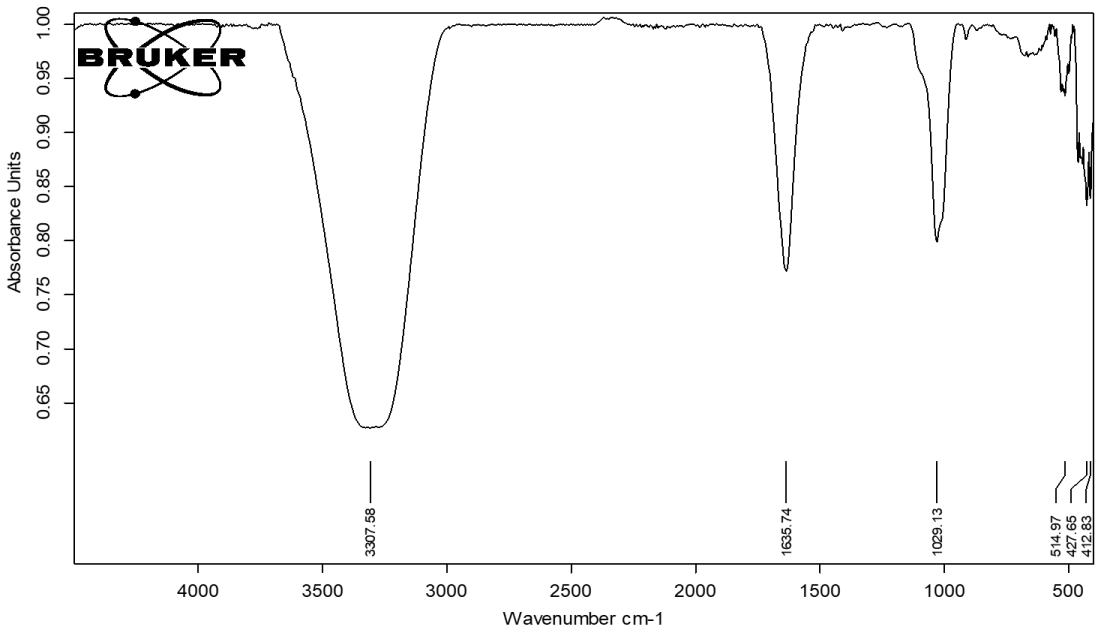

Fig. 6. FTIR analysis on sediment

1. FTIR analysis

The results revealed that all study samples had suspected microplastics, including *Anadonta anatina* (duck mussels), *Procambarus clarkia* (crayfish), water and sediments samples, as shown in Table (1) and Figs. (1, 2, 3, 4, 5, and 6).

Un-depurated tissue of *Anodonta* sp. mussel showed five different frequencies with five different functional groups. Depurated mussels also showed the same functional groups except for O-H bond (Carboxylic acid) that was shown in un-depurated tissue and wasn't in depurated tissues, on contrary of NH_2 bend (Alkanes) that was only in depurated tissues.

Referring to hepatopancreas of crayfish, it was found that FTIR analysis showed seven different functional groups referring to possibility of microplastics in un-depurated tissues. On the other hand, the same seven groups existed in the depurate tissues in addition to ether functional groups that wasn't shown in un-depurated tissues.

According to water analysis, only 2 functional groups were found (O-H stretch (Hydroxyl group), C=O stretch (Amides)), while in sediment analysis, the same two groups of water analysis were found in addition to ether group.

In mussel tissues, it was found that nylon dominated the microplastic types, while in crayfish, the most type found accumulated in its tissues was polyethylene terephthalate, followed by polyethylene. It was remarkably noted that depurated hepatopancreas of crayfish showed an appearance of cellophane and polyester that were not found in un-depurated samples.

The results showed that crayfish had more types of microplastics than duck mussels. Water and sediments samples showed the least accumulation of microplastics types with only two groups, cellophane and polyethylene terephthalate.

2. Polycyclic aromatic hydrocarbons (PAHs)

PAHs levels in sediment, water, and benthic invertebrates *Procambarus clarkia* and *Anodonta* sp. are exhibited in Table (1). The number of detected PAHs varied from 5 to 9 then to 11 in collected invertebrates' samples, water and sediments, respectively. Among detected PAHs benzo[b]fluoranthene, benzo[k]fluoranthene (5- ring PAHs) in addition to chrysene and fluoranthene (4- ring PAHs) were only detected in water and sediment. Benzo[a]pyrene and dibenz[a,h]antheracene (5- ring PAHs) were recorded only in sediment, whereas pyrene (4- ring PAH), anthracene, fluorene and phenanthrene (3- ring PAHs), as well as naphthalene (2- ring PAH) appeared in all tested samples (Fig. 1). PAH levels showed significant differences between tested samples. Generally, the highest concentrations were observed in invertebrates, i.e., in *Anodonta* sp. followed by *Procambarus clarkia*, whereas the lowest concentrations were recorded in water.

Regarding benthic invertebrates, in the case of *Procambarus clarkia*, depuration significantly increased anthracene, phenanthrene and pyrene (Fig. 2a). However, significant declines were observed in fluorene and naphthalene levels. On the contrary, in the case of *Anodonta* sp., depuration significantly decreased all PAH levels except for fluorene, where significant increases were recorded after depuration (Fig. 2b).

PCA biplot (Fig. 3) definitely showed that the two principal components (PC1 and PC2) explained 97.13% of total variation in PAH levels in tested samples, and showed clear partitioning of water and sediment from invertebrate species, particularly before depuration along the PC1. In addition, data revealed a positive correlation between anthracene, naphthalene, phenanthrene and pyrene.

Table 1. Frequency and functional groups found in different study groups in FTIR analysis

Study group	Frequency range	Functional group	Suspected microplastic types
Mussel un-depurated	3279	N-H stretch (Amides)	Nylon
	1637	C=O stretch (Amides)	Nylon
	1545	C=C	Polyesterene (PS)
	1398	O-H bond (Carboxylic acid)	Polypropylene (PP)
	1078	C-O-C stretch (ether)	Polyethylene terephthalate (PET)
Mussel depurated	3282	N-H stretch (Amides)	Nylon
	1634	C=O stretch (Amides)	Nylon
	1548	C=C	Polyesterene (PS)
	1454	NH_2 bend (Alkanes)	Nylon
	1076	C-O-C stretch (ether)	Polyethylene terephthalate (PET)
Hepatopancreas crayfish un-depurated	3372	N-H stretch (Amines)	Nylon
	2922	C-H aliphatic	Polyethylene (HDPE)
	2852	-CH_3 (Methyl)	Polyethylene (HDPE)
	1743	C=O (Esters)	Polyethylene terephthalate (PET)
	1645	C=O	Polyethylene terephthalate (PET)
	1463	CH2 bending	Polyethylene (HDPE)
	1159	C-O-C stretch (ether)	Polyethylene terephthalate (PET)
Hepatopancreas crayfish depurated	3347	O-H stretch (Hydroxyl group)	Cellophane
	2922	C-H aliphatic (aldehydes)	Polyethylene (HDPE)
	2852	-CH_3 (Methyl)	Polyethylene (HDPE)
	1744	C=O (Esters)	Polyethylene terephthalate (PET)
	1636	C=O stretch (Amides)	Polyethylene terephthalate (PET)
	1463	CH2 bending	Polyethylene (HDPE)
	1239	C-O-C stretch (ether)	Polyethylene terephthalate (PET)
	1160	C-O-C stretch (ester)	Polyester (PES)
	1086	C-O-C stretch (ether)	Polyethylene terephthalate (PET)
Water	3271	O-H stretch (Hydroxyl group)	Cellophane
	1635	C=O stretch (Amides)	Polyethylene terephthalate (PET)
Sediment	3307	O-H stretch (Hydroxyl group)	Cellophane
	1635	C=O stretch (Amides)	Polyethylene terephthalate (PET)
	1029	C-O-C stretch (ether)	Polyethylene terephthalate (PET)

Table 2. Concentrations of polycyclic aromatic hydrocarbons (PAHs) (mean ± SD) measured in sediment, water and two invertebrate species (*Procambarus clarkia* and *Anodonta* sp.) before depuration (Concentrations in water are in µg/L, concentrations in sediment and invertebrates are in µg/Kg DW)

Compound	Sediment	Water	*Procambarus clarkia*	*Anodonta* sp.	*P*-value
Anthracene	0.071±0.01^{b}	0.03±0.01^{b}	0.43±0.09^{b}	2.099±0.42^{a}	<0.001*
Benzo[a]Pyrene	0.105±0.02	-	-	-	-
Benzo[b]Fluoranthene	0.465±0.04	0.02±0.00	-	-	0.014*
Benzo[g,h,i]Perylene	-	-	-	-	-
Benzo[k]Fluoranthene	0.125±0.03	0.013±0.00	-	-	0.016*
Chrysene	0.553±0.11	0.069±0.01	-	-	0.017*
Dibenz[a,h]Antheracene	0.263±0.05	-	-	-	-
Fluorene	0.02±0.00^{c}	0.002±0.00^{c}	1.05±0.21^{b}	1.713±0.34^{a}	<0.001*
Fluoranthene	0.74±0.15	0.065±0.01	-	-	0.016*
Naphthalene	0.284±0.06^{b}	0.314±0.06^{b}	3.66±0.73^{a}	4.61±0.99^{a}	<0.001*
Phenanthrene	0.363±0.07^{b}	0.029±0.01^{b}	0.45±0.09^{b}	2.168±0.43^{a}	<0.001*
Pyrene	0.612±0.12^{b}	0.087±0.02^{c}	0.56±0.11^{b}	1.27±0.25^{a}	<0.001*

For all statistical tests *P*-value > 0.05 is considered not-significant. Significant *P*-values are marked with *.

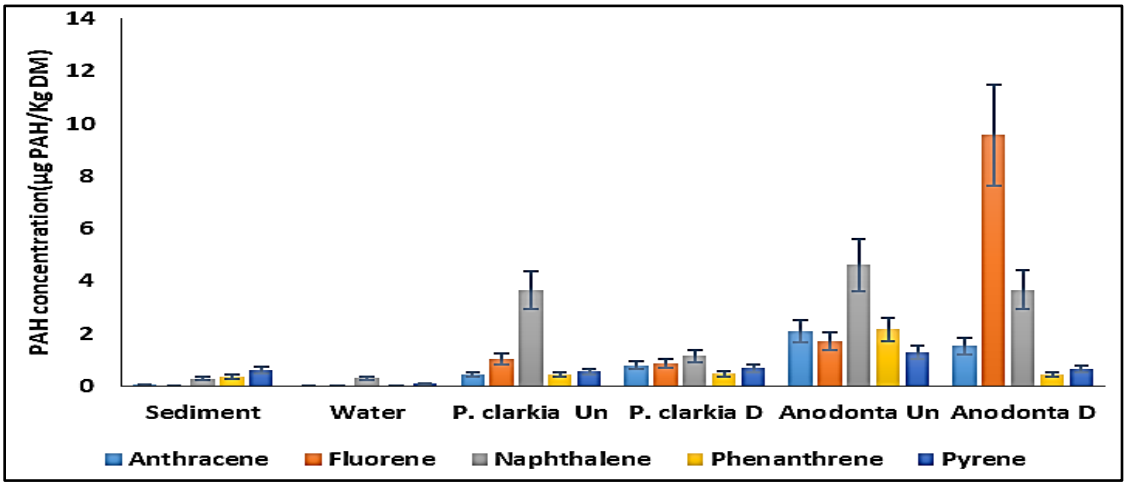

Fig. 7. Concentrations of the most common polycyclic aromatic hydrocarbons (mean ± SD) detected in sediment, water and both invertebrate species (*Procambarus clarkia* and *Anodonta sp.*) before (Un) and after (D)depuration. (µg PAH /L in water and µg PAH/Kg DM in sediment and invertebrates)

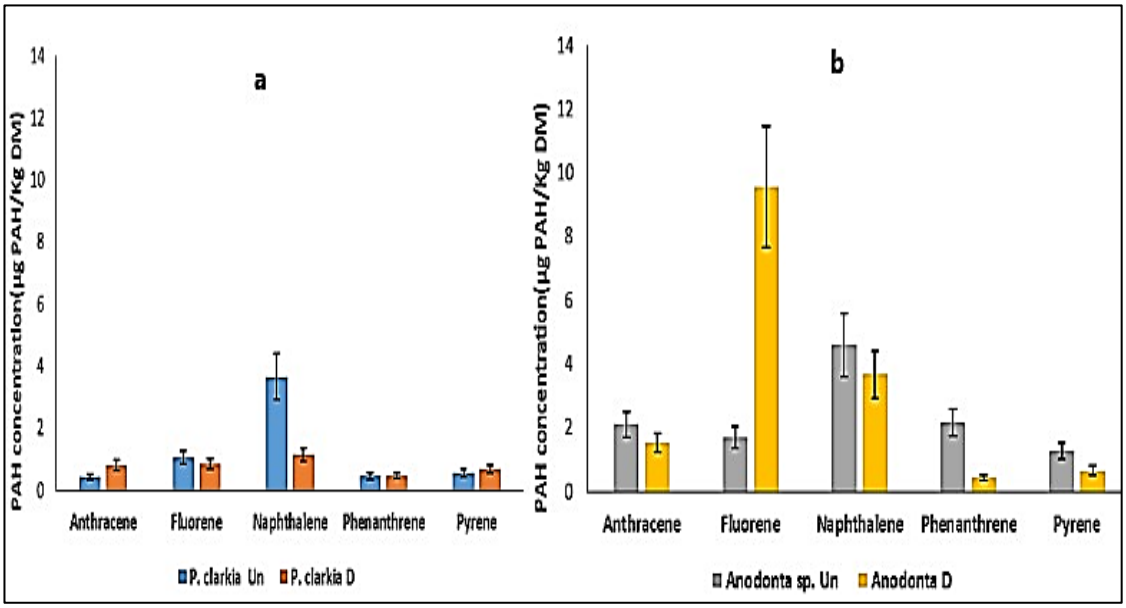

Fig. 8. Concentrations of polycyclic aromatic hydrocarbons (mean ± SD) (µg PAH/Kg DM) measured in **(a)** *Procambarus clarkia*, and **(b)** *Anodonta* sp. before (Un) and after (D) depuration

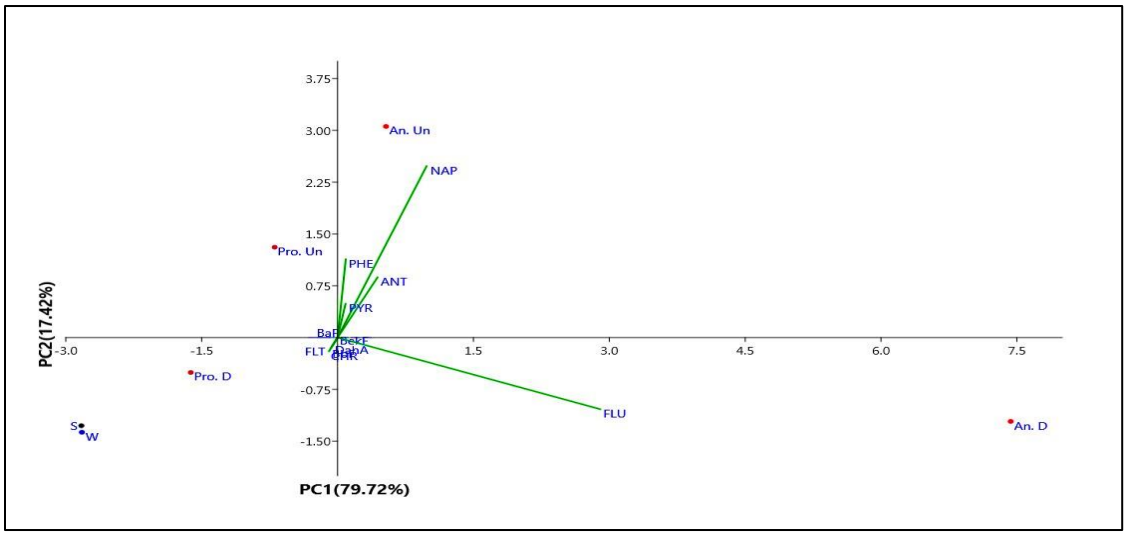

Fig. 9. Biplot of the principal component analysis (PCA) for PAHs distribution (ANT: anthracene, BAP: benzo[a]Pyrene, BbF: benzo[b]Fluoranthene, BkF: benzo[k]Fluoranthene, CHR: chrysene, DahA: Dibenz[a,h]Antheracene, FLU: Fluorene, FLT: Fluoranthene, NAP: Naphthalene, PHE: Phenanthrene and PYR: Pyrene)in S: sediment, W: water and invertebrate species (Pro. Un and Pro. D: *Procambarus clarkia* before and after depuration, respectively., and An. Un and An. DL *Anodonta* sp. before and after depuration, respectively)

DISCUSSION

The current study was conducted to assess the presence of microplastics and PAHs in tissues of *Anadonta anatina* (duck mussels), digestive gland of *Procambarus clarkia* (crayfish), water and sediments samples from Delta of the River Nile, Egypt. Moreover, the effect of depuration was evaluated whether if it has a positive or negative impact on the accumulation of microplastics and PAHs in both invertebrate models with different modes of food. Micro-FTIR imaging has emerged as a preferred method for microplastic (MP) analysis. Its ability to provide molecular-level information on MPs within short measurement times and its capability to examine large sample areas make it a highly efficient tool. This technique has gained significant popularity in the context of microplastic research **(Vinay Kumar *et al*., 2021)**.

The results revealed that all studied samples had microplastics. The types found in the samples were nylon, polyesterene (PS), polypropylene, polyethylene terephthalate (PET), polyethylene (HDPE), and cellophane. The most abundant type was polyethylene terephthalate (PET) that was found in all studied groups. Nylon and polyesterene (PS) were found in mussels, while polyethylene (HDPE) was strongly reveled in the hepatopancreas of crayfish.

The polypropylene (PP) was only found in un-depurated tissues of duck mussels, while cellophane and polyester (PES) were only found in depurated group of the crayfish's two groups.

Water and sediment showed only two MPs types; cellophane and polyethylene terephthalate (PET), but sediment samples showed an extra peak on FTIR analysis for PET than water samples. Microplastics have been recorded in different aquatic environments, including sediments, water columns, and coastal regions. Their widespread distribution emphasizes the ubiquitous nature of plastic pollution in waterways **(Derraik 2002; Browne *et al*., 2011)**.

Similar results were found by **Vinay Kumar *et al*. (2021)**, who found that the most abundant microplastics in mussels was PET. Moreover, **Birnstiel *et al*. (2019)**, **Bagheri *et al*. (2020)** and **Zhu *et al.* (2020)** found different types of microplastics in benthic organisms, such as edible oysters and farmed mussels.

The Nile River flows through Egypt from Upper Egypt downstream to the Nile Delta, where it empties into the Mediterranean Sea. The collection site was Kafr El-Zyat in El-Gharbiya Governorate, located within the Nile Delta. This area is characterized by urbanization and increased industrial activities, including numerous chemicals, textile, and clothing manufacturing plants.

Literature suggests that the location of study sites can significantly influence the levels of microplastics (MPs) found in organisms. **Luo *et al.* (2019)** indicated that higher levels of

MPs are often detected in freshwater environments compared to coastal waters. Additionally, the prevalence of MPs in rivers tends to increase from upstream to downstream, with the highest levels of contamination typically found near municipal centers. This pattern suggests that human activities, such as wastewater discharge and urban runoff, contribute to MP pollution in freshwater ecosystems, aligning with our findings.

Wardlaw and Prosser (2020) found that polypropylene is the main dominant polymer in mussels *Lasmigona costata*, which is employed in different industrial and domestic applications **(Andrady & Neal, 2009; American Chemistry Council, 2019)**. Furthermore, **Zbyszewski and Corcoran (2011)** found that polyethylene, polypropylene, and polyethylene terephthalate polymers; which were detected in our study; were the main types of MPs in aquatic environments. They have low densities that facilitate their transportation by running water. In addition, their non-degradable nature gives them long persistence in the environment (**Erni-Cassola *et al.*, 2019**).

Nylon (Polyamide PA) was found in both un-depurate and depurated mussel samples and in un-depurated crayfish samples in this study. The occurrence of nylon in aquatic ecosystems is possibly attributed to the huge quantity of nylon netting used in commercial fishing equipment **(Lusher *et al.*, 2017b)**. It is worth mentioning that freshwater fishing in the River Nile is one of the main business conducted in Egypt. Additionally,, according to **Bharath *et al.* (2021)**, the presence of nylon can be assigned to the release of bristles from toothbrushes, automotive parts, textiles, and fishing lines.

In the obtained results, polyamides dominated the microplastic types in un-depurated and depurated mussels' samples. These results agree with those of **Birnstiel *et al.* (2019)**, who identified a predominance of polyamide (PA) fibers in mussels, suggesting that these organisms may preferentially ingest denser microplastics (MPs) from the water, as PA is generally denser than both polyethylene (PE) and polypropylene (PP).

As filter feeders, mussels actively ingest microplastics from the surrounding water. Freshwater mussels exhibit selectivity in the particles they consume, with a specialized filtration system that includes papillae around the inhalant aperture acting as a coarse filter. Particles that pass through this initial filtering process are captured by the gills and then transported to the labial palps by ciliary action. The labial palps, located near the mouth, perform the final sorting of edible and nonedible particles, determining which material will enter the digestive tract **(Haag, 2012)**.

In oysters, gills filter water and facilitate the passage of materials and organic matter to other tissues (**Zhu *et al.*, 2020)**. This indicates that gills constitute a major pathway for MPs to enter oysters. In the current study, sampling area was previously identified as a highly polluted site **(Younis *et al.*, 2024)**, consequently mussels are

continuously exposed to MPs from various sources, including primary and secondary sources. i.e., residences, industries, and degraded debris from beaches, rivers, and fishing nets, respectively. While mussels have the ability to eliminate some of the ingested MPs, the ongoing exposure to these contaminants poses a significant threat to their health and the ecosystems they inhabit **(Woods *et al.,* 2018)**. They will continually ingest particles, including microplastics, leading to bioaccumulation of these contaminants in their tissues **(Mathalon & Hill, 2014; Gandara e Silva *et al.,* 2016)**.

The results also showed appearance of microplastics in crayfish digestive glands, even more than mussels. Similar results were shown by **Iannilli *et al.* (2019)**, who found microplastics in *Gammarus setosus*. This could be explained by the different way of feeding, as crayfish belongs to crustacean, which are considered as carnivores. **Iannilli *et al.* (2019)** demonstrated that the studied species can gather and ingest MPs when its habitat is contaminated. Possibly, these particles are mistaken for food, as amphipods may not be able to distinguish between plastic and organic matter. Moreover, indirect ingestion may happen when contaminated preys are used as a food source.

Our findings regarding freshwater benthos are consistent with those of **Bagheri *et al.* (2020)**. The elevated levels of microplastics (MPs) in aquatic environments are not the sole concern. The potential for MPs to interact with other pollutants, such as heavy metals and organic contaminants, poses additional risks. Microplastics can act as carriers, concentrating and transporting chemical pollutants to aquatic biota **(Cole *et al.,* 2011)**. It is suggested that microplastic particles may resemble plankton, the primary food source for planktivorous species such as mussels and shrimp **(Boerger *et al*., 2010)**. This similarity could explain why these organisms are drawn to microplastics.

FTIR analysis indicated that the primary types of polymers detected in the studied samples were polypropylene (PP), polyethylene (PE), nylon, and polyethylene terephthalate (PET). **Bagheri *et al.,* (2020)** indicated the prevalence of PP and PE polymers in the environment due to their widespread use in urban, industrial, agricultural, and other applications.

According to this study's results, crayfish accumulated more types of microplastics than duck mussels. This may be due to its nature of feeding. Since mussels are filter feeders while crayfishes are omnivorous feeding on smaller preys and planktons, and as we previously mentioned regarding that microplastics may resemble the look of planktons, it is possible to clarify why crayfishes may accumulate more microplastics than filter feeding mussels.

Only cellophane and polyethylene terephthalate (PET) were detected in water and sediment samples. Microplastics (MPs) possess hydrophobic properties, enabling them to adsorb various organic pollutants, including persistent organic pollutants (POPs) and heavy

metals **(Mato *et al.,* 2001; Fossi *et al*., 2014; Avio *et al.,* 2015; Barboza & Gimenez, 2015)**. These hydrophobic surfaces also facilitate biofilm formation, allowing microorganisms to colonize MPs and increase their density, leading to sinking and incorporation into sediments **(Zettler *et al*., 2013)**. Exposure to high-density polyethylene (PE) microplastics has been shown to negatively impact the stability of lysosomal membranes in oysters, highlighting the potential health risks associated with MP ingestion **(Moos *et al.,* 2012)**.

In this study, depuration was conducted to study its effect on the studied samples and whether detected microplastics would disappear under depuration effect or not. It was carried out for three weeks and results showed that types of microplastics didn't disappear in depurated samples, and since this study was qualitative not quantitative, we can't assure that the concentrations of microplastics decreased or not. However, **von Moos *et al.* (2012)** stated that many particles were still detected after depuration in *Mytilus* sp., indicating the possibility that the depuration time was insufficient for the complete elimination of microplastics, or that MPs may have been translocated to other tissue., or even to the circulatory system according to **Browne *et al.* (2008)**. Despite an extended depuration process, **Ribeiro *et al.* (2017)** still detected microplastics in *Scrobicularia plana*, indicating that prolonged depuration may not be sufficient for bivalves to fully recover from microplastic exposure. In addition, microplastics were not effectively depurated in *Potamocorbula* or *M. edulis* **(Cauwenberghe & Janssen, 2014)**. It is observed during the depuration process that there is an increase in the types of microplastics detected in models tissues after performing the depuration process, which is a dangerous indicator in the study because the water used in the purification process is drinking water treated with purification filters, which means that the purification filters used for drinking water are not sufficient to prevent the passage of these types of microplastics, which makes the human body vulnerable to these types, even when using home filters to purify water.

Microplastics constitute a significant hazard in the environment, having the ability to act as vectors for more toxic pollutants. These pollutants, which may not otherwise be able to enter the bodies of aquatic organisms, can attach to microplastics and be transported into the food chain **(Brennecke *et al.,* 2016; Hartmann *et al.,* 2017)**. Toxic materials adhering to microplastics can enter the hemolymph of invertebrates, allowing them to be transported throughout the organism and potentially affecting other organs. Being primary consumers, through consuming algae, bivalves play an important role in the aquatic food chain.

Literature has indicated that MPs can be transferred through the food chain, posing a threat to organisms at its top **(Farrell & Nelson, 2013; Nelms *et al*., 2018)**. For instance, accumulation of MPs has been detected in seafood, which raises concerns about the potential health consequences for its consumers from humans **(Smith *et al*., 2018; Cox *et al.,* 2019)**. Similarly, presence of PAHs in aquatic environments can induce potential

hazards **(Hussain *et al*., 2018)**. Therefore, quantifying levels of different PAHs in sediment, water, and inside tissues of benthic invertebrates was of central priority in our study. Regarding the composition of individual PAHs, high molecular weight PAHs (> 4 rings) were detected mostly in sediments followed by water samples. Moreover, the composition pattern of PAHs in sediment samples is mainly dominated by four-ring PAHs. Our results are consistent with **Bahroz *et al*. (2009)** and **Nasr *et al*. (2010)**. As a result of their hydrophobicity and recalcitrant nature, high molecular weight PAHs reach sediments and have long persistence. In sediments, degradation rates are generally slow due to low oxygen levels necessary to initiate ring cleavage (**Nasr *et al*., 2010; Kafilzadeh, 2015; Awe *et al*., 2020**). On the contrary, low molecular weight PAHs dominated water and invertebrate samples. **Nasr *et al.* (2010)** reported similar results in water samples. However, in our study, anthracene, fluoranthene, and pyrene levels in water exceeded the water quality guideline threshold, recommended by CCME for the protection of aquatic life **(CCME, 1999; Oko & Odoh, 2017)**.

Concerning invertebrate species, only anthracene, fluorine, naphthalene, phenanthrene and pyrene were detected. The dominance of low molecular weight PAHs in invertebrates was also reported in the study of **Ololade *et al.* (2021)**. Low molecular weight PAHs are less hydrophobic, with little adsorption to sediments, have a strong affinity to dissolved organic matter suspended in water column and are consequently more bioavailable to organisms **(Skic *et al*., 2023)**. Additionally, PAH levels were significantly higher than those in sediments and water.

Accumulation of PAHs in organisms despite their low levels in sediments was reported in previous studies, such as **Baumard *et al*. (1998)**. Our results agree with **Nasr *et al*. (2010)**, where higher concentrations of PAHs were detected in fish-tissue samples than in water samples. PAHs have a lipophilic nature; therefore, they tend to accumulate in fatty tissues of organisms, ultimately reaching toxic levels **(Nasr *et al*., 2010; Lawson *et al*., 2021)**. Moreover, higher PAH levels are commonly detected in invertebrate species compared to vertebrates **(Lawson *et al*., 2021)**. Nevertheless, PAHs accumulation was significantly higher in *Anodonta* sp. than in *Procamabrus clarkia*. The extent of accumulation differs among species, because of their trophic levels and disparities of physiological and biochemical processes that determine contaminants' uptake, distribution and elimination **(Tomza-Marciniak & Witczak, 2009)**. Mussels, including *Anodonta* sp., are filter feeders. They absorb PAHs from the filtration of particulates in water, then accumulate them in lipid tissues with a restricted metabolic transfer, which could explain their high degrees of bioaccumulation **(Skic *et al*., 2023)**. On the other hand, *Procamabrus clarkia* is an opportunistic omnivore, feeding on a variety of aquatic organisms, including submerged macrophytes, algae, invertebrates, and detritus **(Alcorlo *et al*., 2004)**. Furthermore, as reported above, mussels may tackle the denser fraction of microplastics, which may explain the higher bioaccumulation of PAHs.

The total quantity of polycyclic aromatic hydrocarbons (PAHs) in crayfish declined after depuration, primarily due to a significant decrease in naphthalene levels. Similar declines in naphthalene levels in crayfish post-depuration have been reported by **Tarshis (1981)**. In contrast, depuration did not reduce the total PAH quantity in clams, as there were significant increases in fluorene levels. This suggests that the depuration period may have been insufficient in our study. **Tanacredi and Cardenas (1991)** indicated that a three-week depuration period is inadequate for bivalve mollusks regarding PAH removal. Furthermore, PAHs are highly persistent in invertebrates, which often lack the ability to effectively depurate themselves even when placed in clean water. These organisms exhibit very slow depuration capabilities **(Boehm & Quinn, 1977; Takeuchi *et al*., 2009)**.

CONCLUSION

The current study confirmed the presence of microplastics in the tissues of the examined mussels and crayfish. Unfortunately, depuration negatively affected the reduction of microplastic types in the tissues of these models. Polycyclic aromatic hydrocarbons (PAHs) were detected in sediment, water, and invertebrate tissue samples, with sediments primarily dominated by four-ring PAHs. In contrast, water and invertebrate samples were dominated by low molecular weight PAHs. The highest levels of bioaccumulation were found in *Anodonta anatina*.

The duration of the depuration period was insufficient for the elimination of PAHs. We recommend serious measures to reduce the accumulation of microplastics and hydrocarbons through stricter government regulations, reduced plastic use, and investments in alternative materials. Future investigations should focus on quantitatively determining microplastics and exploring further methods for eliminating and reducing both microplastics and PAHs in water and animal tissues.

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