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Research Article:

PAHs and Pb heavy metal absorption by *Dreissena polymorpha* (Pallas, 1771) bivalve under oil pollution

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Abstract

The main purpose of this study was to find the absorption of Pb as heavy metal and cyclic aromatic hydrocarbons (PAHs) by *Dreissena polymorpha* under oil pollution. Totally, 130 samples with an average length range of 3.5±0.7 cm and average weight range of 25.5±3 g were collected from the Tajan River estuary in Mazandaran province and transferred to the laboratory. Three stations were considered (Station 1: Oil Company Wastewater (OCW), Station 2: Car Oil Change Wastewater (COCW), Station 3: Agricultural Pumping Canal (APC)). The amounts of PAHs and lead metal uptake by bivalves were measured during warm and cold water seasons. Tissue mass was isolated to measure PAHs at each station. In the cold season, the maximum concentration of lead was in the OCW station (0.762±0.01 mg/L) and the lowest concentration was observed in APC station (0.367±0.06 mg/L) ($p<0.05$). In the warm season, the highest concentration of Pb was observed in COCW station (0.558±0.02 mg/L) and the lowest was in APC station (0.376±0.01 mg/L). There was no significant difference between OCW and COCW stations in the cold season ($p>0.05$). Also, in the warm season, the highest concentration of Pb was observed in COCW station and the lowest concentration was in APC station ($p<0.05$). During the experiment period, the highest absorbed concentration of bivalves was recorded at OCW station (328.39 ±1.33 ng/dw) and the lowest concentration was in APC station (62.85±8.9 ng/dw). Moreover, there was a significant difference between PAHs concentration of OCW station with other stations ($p<0.05$). The concentration of the compounds measured in February was higher than in August due to the precipitation and flooding of the route to the bivalve stations. Heavy compounds were also more concentrated than lighter compounds. Finally, it can be concluded that this bivalve can be used as an appropriate biomarker for monitoring oil compounds in the environment.

Keywords: Biosorption, PAHs, Lead, *Dreissena polymorpha*, Oil pollution

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Introduction

The petroleum industry has specific importance due to its role in supplying energy for other industries and producing many raw materials (Santos *et al.,* 2017). It is also one of the polluting industries in the environment (Han *et al.,* 2009). The use of oil resources is steadily increasing on a large scale. This problem is one of the major reasons for pollution in the petrochemical, oil, gas and refinery industries alongside sensitive bio-resources and human communities (Sabet Eghlidi *et al.,* 2013). The Oil Products Distribution Company has several repositories of various products with environmental issues. The most important environmental problems in these reservoirs are the presence of petroleum effluents and residues or sludge on the bottom of the reservoirs.

Due to the presence of water (brine) in crude oil, at all stages of extraction, as well as pre-treatment and purification of the oil, there is some water associated with the products that try to reduce it by various techniques (Jafarzadeh *et al.,* 2009). Among the pollutants caused by effluents from petroleum reservoirs, hydrocarbons, and heavy metals require more attention and control due to their rapid expansion into the environment, toxic effects and various diseases (Karamalidis *et al.,* 2007). Polycyclic aromatic hydrocarbons (PAHs) are also important pollutants in the environment (Perra *et al.,* 2011). Aromatic compounds have a strong tendency to bond with organic matter due to their high hydrophobicity and water solubility. As a result, it accumulates in soil and sediment (Di Gennaro *et al.,* 2008; D'costa *et al.,* 2018). These compounds have high toxicity, carcinogenic properties, and mutagenicity. For this reason, there is a special environmental sensitivity to PAHs. PAHs are classified by the Environmental Protection Agency (EPA) as one of the environmental pollutants (Zhao *et al.,* 2009).

Properties of petroleum sludge from the Tehran Petroleum Refinery showed that the total amount of petroleum hydrocarbons, aromatic hydrocarbons, and heavy metals (nickel, lead, cadmium, and zinc) in the sludge discharged to the environment which was higher than the western standard (Heidarzadeh *et al.,* 2010). In the study of (Kriipsalu *et al*. 2008) to identify the oil sludge of an oil refinery in Sweden, high levels of petroleum hydrocarbons such as benzene, toluene and also heavy metals such as lead, copper, mercury, and nickel were identified. Heavy metal emissions are considered as one of the most important and dangerous non-biodegradable sustainable pollutants and are a growing global problem mainly due to human activities such as textile, dyeing, plasticization, mining and is metallurgy (Fathy *et al.,* 2012).

Different approaches have been suggested in order to eliminate oil pollution and its consequences. In recent decades bioremediation has been preferred over the other methods due to low cost and high efficiency (Shahriari

Moghadam *et al.,* 2014). Bivalves, such as zebra mussels, are suitable organisms for the biodegradation of persistent pollutants such as trace metals and organic pollutants in the marine environments (Bayat *et al*., 2016; Hassanshahian, 2017). The main purposes of this study were to evaluate the variations in the concentration and type of PAHs and Pb as heavy metal in the Oil Company Wastewater (OCW), Car Oil Change Wastewater (COCW), and Agricultural Pumping Canal (APC) during warm and cold seasons in Gorgan City, Golestan Province (Fig. 1).

Materials and methods

In December 2018, 130 samples of *Dreissena polymorpha* (Pallas, 1771) bivalve with an average length of 3.5±0.7 cm and average weight of 25.5±3 g were collected and transferred to the laboratory from the Tajan River estuary (in Samskandeh area, Sari), Mazandaran province (36° 48´ 46´´ N; 53° 6´ 57´´ E). Three stations were determined along the effluent outlet channel route (Station 1: Oil Company Wastewater, Station 2: Car Oil Change Wastewater, Station 3: W Agricultural Pumping Canal). Bivalve tissue samples were taken from the natural environment before deployment at the stations. Thirty bivalves per station were placed in lattice baskets. The stations and bivalves were assayed during the 14th and 28th days of February and August.

PAHs bioaccumulation protocol

The samples were placed in aluminum foil. The labeled samples were transferred to the laboratory and stored in the freezer until preparation for chemical analysis. At the preparation stage, the samples should be completely dried due to the hydrophobic interference in PAH analysis (Pena, 2006). For this purpose, the specimens were kept in freeze drier at -50 °C for 72 hours under vacuum to be dried completely. The extraction step was performed using the microwave method (Pena, 2006). Then, 0.2 g of each dried sample was poured into the microwave cell. 4 ml of saturated potassium hydroxide solution and 10 ml of n-hexane alcohol were added and the cell lid was closed. After placing the cells in the microwave, the extraction was performed at 129°C for 20 minutes.

After cooling the solution-containing cell, 6 ml of the organic phase was transferred to the tube and centrifuged at 3000 rpm for 3 minutes. The extract was evaporated and concentrated by a rotary evaporator to a volume of 0.5 ml. Then, filtered using silica sheets that were activated by 4 ml of hexane-dichloro methane (1: 1 volume ratio). The concentrated tissue samples of bivalves were injected to GC-MS. The concentration of 16-aromatic hydrocarbon derivatives (16 PAH) was calculated using CRM (Certified Reference Material).

Determination of bioaccumulation levels of lead metal

The collected bivalve specimens were weighed to obtain wet weight. Then their shells were separated and tissue mass was picked. In order to obtain dry samples, tissue masses were placed in the oven at 75°C for 48 hours. The dry tissue masses were digested in 75% nitric acid for 24 h. They were then heated for 30 min to evaporate nitric acid. The obtained dry mass was suspended in 0.5% nitric acid and kept at 4°C until the metal content was measured (Raftopoulou and Dimitriadis, 2011). The digested solutions were analyzed using Flame Atomic Absorption Spectrophotometer (AA-300 puls) (Moloukhia and Sleem, 2011).

Sampling was done in two warm and cold seasons. In the cold season, in addition to the canal entrance, atmospheric rainfalls are entered as floods in the canal which are containing produced heavy aromatic compounds by cars and industries.The normality of data and homogeneity of variances were considered by Kolmogorov-Smirnov and Levene's tests, respectively. One-way ANOVA was used to compare differences between PAHs and lead metal accumulated in different bivalve tissues at the 5% level. Duncan's test was used to compare the means (Zar, 2010). Statistical tests were done in SPSS V. 23 and Microsoft Office Excel 2013.

Results

The results showed that the maximum concentration of lead was observed in OCW with a concentration of 0.762 ±0.01 mg/L. It also showed the highest amount (0.914 mg/L) in OCW station during the first month (February). The lowest concentrations were in APC station with the levels of 0.367±0.06 and 0.231 mg/L in the first and second months, respectively. In warm season, the highest concentration of lead was observed in the COCW station with a concentration of 0.582±0.02 mg/L. The lowest concentration of Pb was observed in the APC station with 0.376±0.01 mg/L. The results showed that in cold season, the concentrations of lead in stations were higher than warm season (Fig. 2).

The results showed that there was no significant difference between the OCW station and the COCW station in the cold season ($p>0.05$). Nevertheless, OCW station and APC station had a significant difference ($p<0.05$). Similarly, in the warm season, in contrast to the cold season, the highest concentration of Pb was observed in the COCW station ($p<0.05$). The lowest concentration also was observed in the APC station (Fig. 3).

All 16 PAHs compounds were observed in bivalve tissue mass in the COCW station in warm season; nonetheless the concentration of dibenzo anthracene (dBAn) was very low and was not detected in cold season. Also, in cold season, the PAHs content was higher than the warm season (Fig. 4).

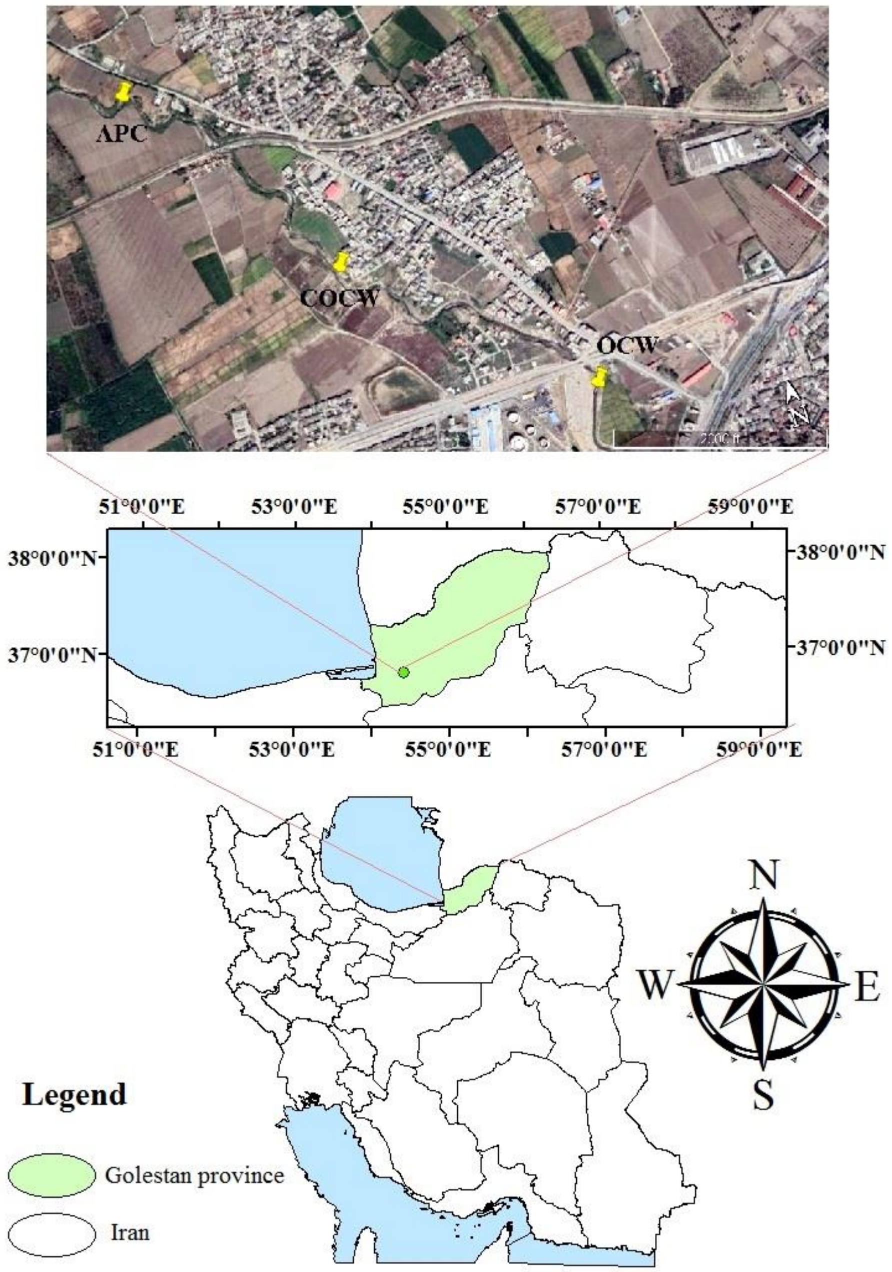

Figure 1: The geographical points of stations during the experimental period in Gorgan City, Golestan Provinces.

In the APC station, the concentration of di-benzo anthracene (dBAn) was lower than the detection range at the warm season, and indenopyrene and benzo-perylene compounds had the highest concentrations. In the cold season, naphthalene and benzo anthracene compounds were lower than the detection range. Benzo pyrene had the highest concentration among 16 aromatic compounds (Fig. 5).

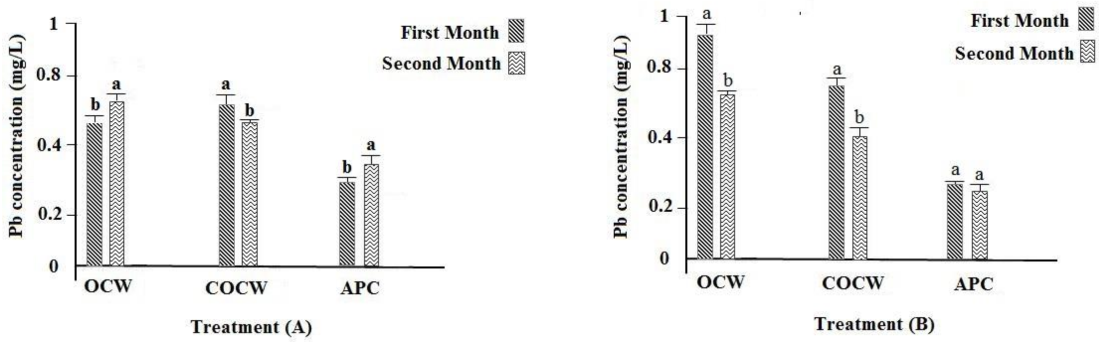

Figure 2: Different lead concentrations in cold (A) and warm (B) seasons.

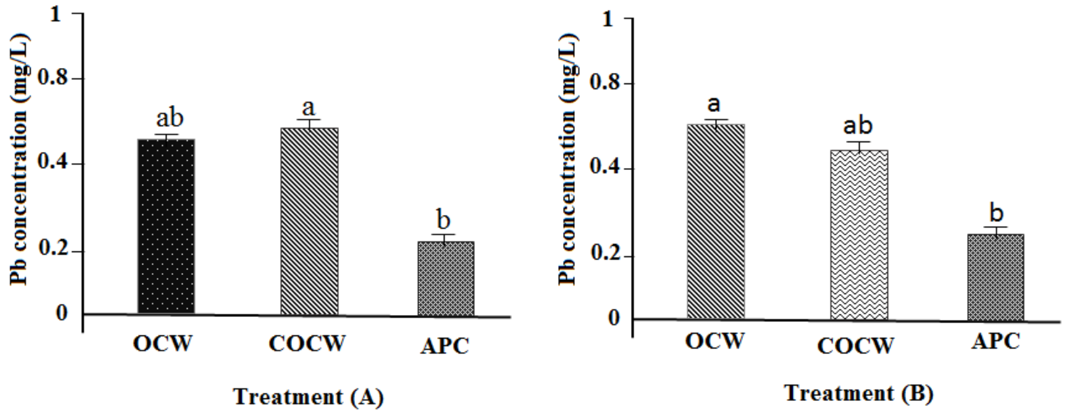

Figure 3: Average lead concentration in cold (A) and warm (B)seasons.

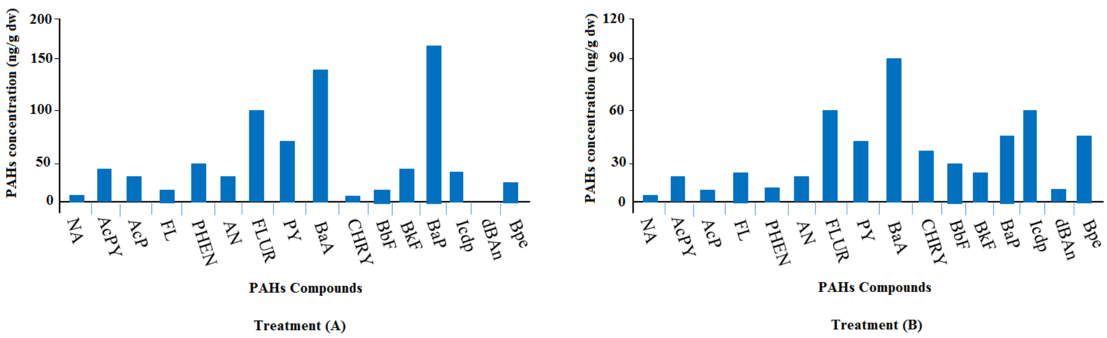

Figure 4: PAHs compounds in COCW station during the cold (A) and warm (B) seasons.

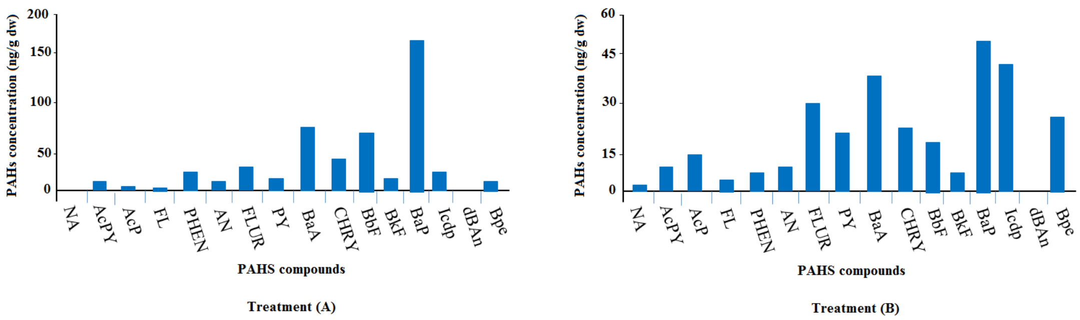

Figure 5: PAHs compounds in APC station during the cold (A) and warm (B) seasons.

The results showed that the concentrations of di-benzo anthracene, indonopyrene, and asphenylene in warn season were lower than the detection limit in OCW station. 16 PAHs were not found in the compound. The highest concentrations were also related to benzo pyrene and benzofluoranthene in warm season. In cold season, the concentration of naphthalene was the lowest and the concentrations of benzo pyrenes and benzo anthracene were higher than the other compounds (Fig. 6).

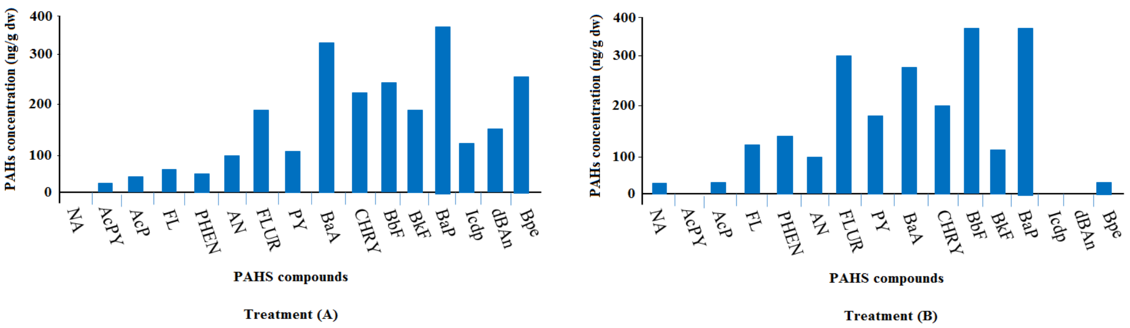

Figure 6: PAHs compounds in OCW station during the cold (A) and warm (B) seasons.

A comparison of the mean concentration of PAHs in the tissue mass in cold and warm seasons between different stations showed that the highest concentration of poly aromatic compounds was recorded in the OCW station. Also, the poly aromatics concentrations in the cold season were higher than the warm season (Fig. 7).

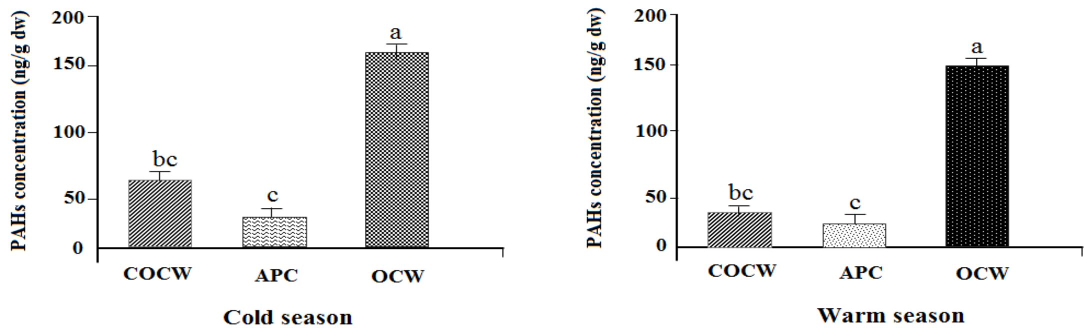

Figure 7: The average concentration of PAHs in tissue mass during the cold (A) and warm (B) seasons.

Discussion

In this study, concentrations of Pb and 16 important PAHs in the soft tissue mass of *D. polymorpha* were measured in selected stations in cold and warm seasons (February and August). The predominance PAHs in the bivalve soft tissue included phenanthrene, anthracene, benzopyrene, and fluoranthene in cold and warm seasons. The lowest percentages of naphthalene, acenaphthene, and acenaphthylene were observed. Totally, high molecular weight compounds containing 4, 5 and 6 cyclic compounds were predominant at all stations. This indicates that the mussel received pyrolytic contamination. In cold season, the concentration of naphthalene was lower than the detection range, and the concentrations of benzo pyrene and benzo anthracene were higher than the other compounds. Thestudy of Kumar *et al*. (2008) on poly aromatic hydrocarbons showed that edible clams have the ability to bioaccumulation of high molecular weight of PAH compounds. Generally, filter feeder bivalves tend to accumulate PAHs with lower molecular weight and higher solubility compare to PAHs with high molecular weight. However, the source of the present compounds in the environment also is effective. Accordingly, if most of the contaminants in the environment originate from pyrolytic sources, higher amounts of them are available than petrogenic hydrocarbons. The ability of bivalves and other organisms to metabolize low molecular weight compounds is also greater than high molecular weight aromatic compounds (Thompson *et al.*, 2000; Thorsen *et al.*, 2004). Due to dominance of high molecular weight aromatic hydrocarbons in bivalve tissues, the concentration of contamination has petrogenic origin in the high region. Therefore, most of the absorbed contamination originated from petrogenic sources. Bihari *et al*. (2007) measured cyclic aromatic hydrocarbons in *Mytilus galloprovincialis* of Rijeka Bay that two and three cyclic compounds were predominat. Economic facilities such as petrochemicals, electricity generating plants, and shipping activities on the Gulf coast were the source of aromatics compounds. Vavalanidis *et al*. (2008) identified 9 poly aromatic compounds in *Mytilus galloprovincialis* in the Gulf of Sarnikos.

In summer (warm season), there was a significant difference between the tPAHs concentrations in soft tissue of *D. polymorpha*. The highest amount of tPAHs concentration was 160.53±74 ng/gdw in the OCW station and the lowest was 24.46±11 ng/gdw in the APC station. In winter (cold season), the highest and lowest concentrations were in the OCW station with the amounts of 167.86 ±59 and 38.49±34 ng/gdw, respectively. Likewise, the highest concentration of bivalves absorbed was in the OCW station (328.39±1.33 ng/gdw) and the lowest concentration was in the APC station (62.85±8.9 ng/gdw) during the study period. Notar *et al*. (2001) studied the concentration of PAHs in the *Crassostrea virginica* in the Gulf of Mexico which reported PAHs in bivalve tissue was between 2470-42500 ng/g during the six-month study. The dominance of low to medium molecular weight of poly aromatic compounds also indicated the petrogenic source of pollution carried by the river. Gaspare *et al*. (2009) examined cyclic hydrocarbon levels in *Saccostera cucullata* and sediments in the intertidal zone which measured 16 PAHs were 78-25000 ng/gdw in the sediments and 170-650 ng/gdw in the bivalve. The results showed that the maximum amount of lead absorbed by bivalves was at the OCW station (0.762±0.01 mg/L) which was higher than the warm season. In cold season, the absorb rate was highest in the first month (December) in OCW station (0.914 mg/L).

The lowest absorption rate was observed at the APC station (0.367±0.06 mg/L). The lowest absorption was recorded in the APC station in the second month (0.223 mg/L) among the cold months. In the warm season, the highest concentration of absorbed lead was detected in COCW station with a concentration of 0.558±0.02 mg/L. The lowest concentration also was observed in APC station with 0.376±0.01 mg/L. The results showed that the concentration of absorbed lead in cold season by bivalve was higher than the warm season. In addition, the results showed that there was no significant difference between the amount of lead in the bivalve tissue mass in COCW and OCW stations in cold season. There was a significant difference between OCW and APC stations. Also, in warm season, the highest concentration of Pb was observed in COCW station and the lowest concentration was observed in APC station. Numerous studies on the bioaccumulation of heavy metals in mollusks and bivalves have shown that these organisms have the potential to accumulate large amounts of heavy metals (Fukunaga and Anderson, 2011). The results showed that with increasing exposure time, the amount of bivalve absorption had a decreasing trend. Shi and Wang (2004) showed that bivalve exposure to copper as heavy metal resulted in a significant decrease in filtration and adsorption rates, which is consistent with the present study. Finally, the presence of petroleum and petroleum derivatives such as heavy metals and aromatic compounds in the environment can have devastating effects on aquatic organisms and can lead to a variety of cancers and mutations in humans (Hamerlad *et al.*, 1990; Chikwe and Ogbole, 2019). The results showed that the concentration of the compounds measured in February was higher than in August due to precipitation and flooding of the route to bivalve station. Heavy compounds were also more concentrated than the lighter compounds. They are soluble in water as suspended small droplets and fat spots, and accumulate in bivalve body tissue when passing through siphons and gills. Finally, it can be concluded that this bivalve can be used as a suitable biomarker for monitoring oil compounds in the environment.

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