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Occurrence and seasonal variation of plasticizers in sediments and biota from the coast of Mahdia, Tunisia

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Abstract :

Plasticizers are compounds often involved in the manufacturing of plastic products. Nevertheless, the ageing of the latter generates plasticizers that generally end up in the marine environment. In fact, marine pollution by phthalate acid esters (PAEs) and their alternatives has become an environmental and health issue of serious concern, as they are largely and ubiquitously present in the environment and aquatic organisms. In the present study, four PAEs, such as diethyl phthalate (DEP), diisobutyl phthalate (DiBP), dibutyl phthalate (DBP), and di(2-ethylhexyl) phthalate (DEHP), and one non-phthalate plasticizer (NPP), namely di-2-ethylhexyl terephthalate (DEHT), are wanted in different marine compartments from the coast of Mahdia in Tunisia such as sediment, seagrass, and mussel. The most abundant and frequently detected congener was DEHT at the concentrations reached 1.181 mg/kg in the sediment, 1.121 mg/kg in the seagrass, and 1.86 mg/kg in the mussel. This result indicates that the DEHT could emerge through the food chain and therefore bioaccumulate in marine compartments. In addition, we noticed that the seasonal variations of plasticizers were seriously affected by environmental factors including industrial and urban discharges.

Keywords : Monitoring, Phthalic acid esters, Non-phthalate plasticizer, Bioaccumulation Sediment, M. galloprovincialis, P. oceanica

INTRODUCTION

Anthropogenic dispersal of chemical contaminants has the potential to pose one of the greatest environmental threats to humanity. Without appropriate treatment, these contaminants and related transformation products end up drained into the aquatic environment where they degrade the quality of receiving water (**Chavoshani et al. 2020; Methneni et al. 2020, 2021**). In recent decades, plastic pollution has become a major marine pollution origin (**Bonanno and Orlando-Bonaca 2020**). Indeed, plasticizers are everywhere and are continuously being used as the material of choice. Their annual global production accounts for several million tons **(Guo and Kannan 2011).** Phthalate acid esters (PAEs) are the plasticizers most commonly used globally. In fact, about 11 billion pounds are annually produced **(CSTEE 2004)** of which the bis 2-ethylhexyl phthalate (DEHP) is one of the major compounds and represents above one-third of the global plasticizer market **(Huang et al. 2018).** Because of their flexibility, durability, and softness, PAEs have been applied as polyvinyl chloride (PVC) additives since 1926 **(Ferguson et al. 1946)** and are widely being used in a variety of applications including medical and electronic devices, building materials, packaging, and childcare articles **(Plastic Europe 2008).** Considering that PAEs are not chemically bonded but only physically attached to polymeric raw materials, over time they can be easily leached out from the products during the ageing of the packaging and especially if these packaging are shipped in the environment **(Cadogan et al. 1994; Chan and Meek 1994).** Several research studies highlighted ubiquitous pollution by PAEs in the air, water, soil, sediments, and biota, as well as humans **(Staples et al. 1997; Fromme et al. 2002; Abtahi et al. 2019; Zhao et al. 2020a).**

The ecological and human health effects of plasticizers are mainly related to interfering with hormones and the endocrine systems of living beings. Indeed, several PAEs have been identified

as reproductive and developmental toxicants acting as endocrine-disrupting chemicals **(Net et al. 2015b, Poopal et al. 2017; Tian et al. 2018; Karabulut et al. 2018; Wang et al. 2022; Mandal et al. 2022; Jebara et al. 2022).** For instance, studies revealed that n-butyl benzyl phthalate (BBP) may have endocrine-disrupting effects in fish and birds, and mammals could suffer such impacts through food chain exposures **(Tickner et al. 2001; UBA 2007; Wargo et al. 2008).** As endocrine disruptors (EDs), most PAEs can cause adverse developmental, behavioral, metabolic, neurological, and reproductive disorders **(Abtahi et al. 2019).**

Consequently, six PAEs namely, dibutyl phthalate (DBP), diisobutyl phthalate (DiBP), DEHP, BBP, di-isodecyl phthalate (DiDP) and diisononyl phthalate (DiNP) have been placed on the priority pollutant list of the United States Environmental Protection Agency (U.S. EPA) and the European Union (EU) and the PAEs concentrations have been regulated for water consumption **(European Commission 2007; CPSIA 2008; USEPA 2014; Commission Regulation 2018)**. The use of PAEs is now subject to stricter control and some of them have been prohibited or their reduction in numerous plasticized materials has been recommended **(EUR-Lex 2005; CPSIA 2008).**

In the past few decades, a number of substances have been identified and employed as alternative plasticizers such as Dioctyl terephthalate (DEHT). Because of their lower migration frequency and the absence of use restriction, these non-phthalate plasticizers (NPPs) are increasingly used in various industrial products **(Dugo et al. 2011; Di Bella et al. 2014; Calafat et al. 2015; Lo Turco et al. 2016, 2020).**

Although they showed no carcinogenicity, mutagenicity, and reproductive toxicity effects, significant exposure may lead to adverse health effects. Indeed, similarly to PAEs, NPPs are not chemically tied to the polymer and are subjected to leaching out of products **(Bui et al. 2016).**

Hence, deeper investigations, such as developmental toxicity or endocrine disruption effects, should be addressed.

Furthermore, previous studies suggest that NPPs could act as a "pseudo-persistent" pollutant due to their continuous production and diffusion into habitat and surroundings, thus leading to continuous environmental and human exposure **(Eliason et al. 2011; Mackay et al. 2014; Bui et al. 2016; Harmon et al. 2018; Roggeman et al. 2022).**

Aligned with the economic development and urbanisation expansion, wastewater discharge from industries and domestic outlets coupled with flow dynamics of seawater were found to be the primary sources of plasticizers **(Chakraborty et al. 2019).** The lack of sophisticated sewage treatment technologies and systematic wastewater discharge plans, the people's low environmental awareness, as well as untreated or poorly treated domestic sewage, discharged into urban seawater, lead to a rapid accumulation of pollutants and extinction of aquatic organisms, and therefore, pose detrimental threats **(Wang and Zhao 2014; Zhang et al. 2018b).**

Data related to environmental biomonitoring of PAEs and their alternatives are relatively great, with a major focus on freshwater ecosystems **(Keil et al. 2011; Sánchez-Avila et al. 2012; Chen et al. 2013; Li et al. 2016, 2017; Chen et al. 2018; Jiang et al. 2018 ; Bartsch et al. 2019 ; Lee et al. 2019a).** In Tunisia, although multiple research studies have monitored the occurrence of PAEs and NPPs in food, cosmetic and medicinal products **(Beltifa et al. 2017, 2018, 2021),** few studies have assessed the fate of these pollutants in marine compartments **(Gugliandolo et al. 2020; Jebara et al. 2021)**. Therefore, understanding the distribution and accumulation of plasticizers in marine matrices is necessary to protect and manage the aquatic environment. In this context, the objective of this paper aimed to improve knowledge about the accumulation profiles and seasonal variation of several plasticizers (diethyl phthalate (DEP), DiBP, DBP, DEHP and

DEHT) in three different marine samples: sediments, seagrass plants (*Posidonia oceanica L. Delile),* and mussels (*Mytilus galloprovincialis)* in the Rejiche seacoast (Government of Mahdia, Tunisia). This region is a probable pollutant-release area, being an industrial and urban zone as well as possessing a sewage treatment plant nearby its coast sea. *P. oceanica* is an aquatic plant, being an interesting bioindicator of the coastal sea environmental quality **(Richir et al. 2010).** The *M. galloprovincialis* is the most common species found in the Rejiche coast and are frequently consumed by the local population. In addition, mussels have been commonly used as sentinel organisms for assessing environmental pollution in coastal and estuarine ecosystems **(Taze et al. 2016; Bråte et al. 2018)** due to their extensive distribution, abundance, easy sampling, low mobility, their filtration of large volumes of water for nutrition, and economic and ecological interests **(Chiesa et al. 2018).**

The findings of this scientific report can be used to provide important data for developing sediment, mussel, and seagrass plant quality criteria of PAEs and NPPs in Tunisia.

MATERIAL AND METHODS

Study area and sample collection

Rejiche, is a municipality in the governorate of Mahdia (Tunisia) lying on the coast of Tunisia and facing the Mediterranean Sea. This coastal site is characterised by high marine biodiversity. Nevertheless, the environmental quality of this area is impacted by the huge volumes of wastewater discharged by a nearby sewage treatment plant and by industrial activities including medical, tourism and aquaculture **(Fig. 1).**

Sampling was conducted during the wet (January-February-March) and dry season (June-July-August) in 2020. Surface sediment samples were gathered at a depth of 4 meters using Niskin

bottles with a Van Veen bucket with three different spots at the sampling point and immediately transferred to glass bottles. The collected sediments were freeze-dried and sieved with screen mesh (250 μm), and then the meshed samples were stored at -20°C until further processing. Seagrass samples were collected at a depth of 4 meters and wild mussels were caught by local fishermen from the bottom of the sea at a depth ≈10 meters.Then, they were immediately transported to the laboratory where they were stored at -20°C until analysis.

A total number of 54 of sediment samples for two seasons and 252 for seagrass samples and equally for mussels were collected for plasticizers analysis. All samples were performed in three replicates.

Chemicals and reagents

In total, four PAEs, including, DEP, DiBP, DBP and DEHP, and one NPP (DEHT) were detected in this study and their standard solutions were obtained from Supelco (Bellefonte, PA, USA, certified purity ≥ 96%). Internal standards (ISs) (including labelled DBP-d4 and DEHP-d4) were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Solvents used in this study (Acetone, n-hexane, ethyl acetate, diethyl ether) were SupraSolv grade and were furnished from Merck (Darmstadt, Germany). Solid sorbent such as magnesium silicate (Florisil, 60-100 mesh, coarse powder) was furnished from Fluka (Sigma-Aldrich, AG, Switzerland); whereas C18 cartridges (Supelclean C18, 3 mL, 500 mg sorbent) were from Supelco (Bellefonte, PA, United States). Sodium sulphate was heated for 4 h at 140°C and, after cooling, kept in a tightly sealed glass vial. Glassware and stainless instruments, used for sample manipulation, were previously washed with acetone, rinsed with hexane and fried at 120°C for 4h. During sample preparation and analysis, plastic item usage was prevented.

Sample preparation

Sediments were freeze-dried for 48 h (Martin Christ Alpha 1-2/LD Plus, Germany), homogenised and sieved (2 mm). Then, 5 g of samples were spiked with 0.001mg of DBP-d4 and 0.001 mg of DEHP-d4. Then, a centrifuge glass tube was prepared, containing the spiked sample with anhydrous sodium sulphate and 30 mL of n-hexane: acetone solution (1:1, v/v). The obtained mix was ultrasonically extracted for 10 min and then was centrifuged at 3000 rpm during 10 min (Awel MF 20-R centrifuge, Awel SAS, France) to separate the organic supernatant from the bottom layer. The obtained supernatant was further extracted three times, according to the same procedure **(Mackintosh et al. 2006).** The final extract was completely dried using rotavapor (BUCHI Labortechnik AG, Switzerland) and re-suspended in 1 mL of hexane. The subsequent solid phase extraction (SPE) was executed by a glass column (30 cm × 10 mm) packed with 5 g of Florisil (previously activated at 140°C for 16 h) and 1 g of anhydrous sodium sulphate, which catches water molecules and prevents its passage to the extract. Eluate was processed with 60 mL of a diethyl ether: n-hexane solution (1:1, v/v) and evaporated to dryness **(Mackintosh et al. 2006).**

Mussel (*M. galloprovincialis*) samples were freeze-dried for 72h, and then grinded into consistent powder. Afterwards, samples were spiked as described above and subjected to a matrix solid phase dispersion (MSPD). A mix was prepared, consisting of 0.1 g of sample, 0.2 g of anhydrous sodium sulphate and 0.4 g of solid sorbent (Florisil), which were homogenised, all together, using mortar and pestle. The obtained mix was transferred into a glass Pasteur pipette, pre-filled with 0.1 g of Florisil and provided with a small amount of glass wool at the bottom. Targeted plasticizers were eluted with n-hexane: acetone (1:1, v/v) and dehydrated using rotavapor **(Kremer et al. 2005)**. For seagrass (*P. oceanica*) analysis, all samples were dried then subjected to homogenization and are weighted to around 0.05 g each. Then, they were spiked by ISs at a concentration level of 5 mg/L

and were, subsequently, extracted by n-hexane, 5 mL. All samples were transferred to a 15 mL centrifuge tube prior packaged with 900 mg Magnesium sulphate ($MgSO_4$) and 150 mg PSA for disperse SPE and then was centrifuged at 4500 rpm for 10 min. The obtained extracts were filtered through Sodium sulphate (Na_2SO_4) and concentrated to about 0.5 mL by evaporation. Each sample set extraction has a corresponding relative blank, contains only solvents and reagents. Blanks are used for quality assurance.

GC-MS analysis

Analysis was determined by a gas chromatography system (GC-2010, Shimadzu, Japan) equipped with an autosampler (HT300A, HTA, Italy) and combined to a single quadrupole mass spectrometer (QP-2010 Plus, Shimadzu, Japan). Chromatographic separations were carried out on a SPB-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness, Supelco, USA). The oven temperature program was: from 60°C to 190°C at 8°C/min (5 min hold), from 190°C to 240°C at 8°C/ min (5 min hold), and from 240°C to 315°C at 8°C/ min. The injection port was at 260°C and was provided with a narrow inlet liner (0.75 mm ID, Agilent Technologies). Sample injection occurred in splitless mode, with sampling time of 60 s, then split ratio 1:15. Injection volume was 1 μL.

Carrier gas (He, 210.0 KPa, pressure control mode) was operated at a linear velocity of 30 cm/s. As for the MS setup, the temperature of the EI source was set at 200°C, ionisation energy and emission current were 70 eV and 250 μA, respectively; while interface temperature and electron multiplier voltage were, respectively, equal to 300°C and 1.0 kV.

Data acquisition was executed both in full scan (mass range: 40-400 m/z) and selected ion monitoring (SIM) by monitoring three characteristic mass fragments for every analyte. Data

acquisition and processing occurred by GC-MS solution software. Identification of plasticizers were performed by comparison of their retention times and mass spectra with those of corresponding commercial standards. The quantitative analysis was realised in SIM mode, taking into account the relative base peak ions and exploiting the internal standard normalisation. In the supplementary table 1, one target ion (T) and the two qualitative ions (Q1 and Q2) used for each analyte are shown.

Statistical analysis

Descriptive data analysis, including mean and median minimum and maximum concentrations were carried out. Spearman correlation was applied to assess the relationship between phthalate levels in sediment, seagrass and mussel samples, using SPSS version 17.0 (SPSS Inc., Chicago. IL. USA). A statistical comparison of every plasticizer level among different marine samples and during different months was carried out by one-way ANOVA, followed by Tukey's honestly significant difference (HSD) post hoc test in order to investigate seasonal variations. Statistical significance was defined as $p < 0.05$.

RESULTS

Analytical performance and method validation

Each standard solution was injected five times for the calibration curves construction. For DEP, DiBP and DBP compounds, the normalization was processed against the peak area of characteristic fragment (m/z 153) of DBP-d4, while DEHP-d4 (m/z 153) was applied for DEHP and DEHT. Linearity showed a reliable coefficient of determination (R^2), varied between 0.9802 and 0.999 **(Table 1).**

The limit of detection (LOD (mg/kg) = 3 × (relative standard deviation percentage) RSD % × concentration) and the limit of the quantification (LOQ (mg/kg) = 10 × RSD % × concentration) for each compound were determined from the RSD percentage of six replicate injection at the lowest detectable concentration (with a signal-to-noise ratio < 3). LOD values ranged from 0.01 to 0.08 mg/kg while LOQ varied from 0.03 to 0.241 mg/kg **(Table 1).** The repeatability was determined by the RSD percentage values of peak area measurements (n = 6) detected at the lowest detectable concentration of each analyte. Our results showed values always greater than 5.31 %. For recovery investigation, the appropriate known quantity of each standard compound was added to a sample previously analysed. After 24 hours, the spiked sample was exposed to the pre-treatment procedures previously outlined. Recovery values were determined on the average of three replicate analyses and the values were between 94.4% and 108.6 % **(Table 1).**

Occurrence of PAEs and NPP in sediments and biota

Four PAE congeners (DEP, DiBP, DBP and DEHP) and one NPP (DEHT) were analyzed in sediment, seagrass and mussel samples and the obtained results have been presented in **Fig. 2** and **Table 2.**

PAEs and DEHT were detected in all marine samples with obvious quantity differences. **The fig. 2** showed a significantly greater Σ_4 PAEs content in sediments compared to the concentrations detected in the seagrass and the mussels. The concentrations of Σ_4 PAEs in the analysed sediment samples ranged between 0.839 and 3.88 mg/kg, dry weight (dw), with a median and mean of 4.732 and 2.423 mg/kg, respectively. Among the four analyzed PAEs, DEHP exhibited the highest concentration (mean: 1.275 mg/kg, median: 2.208 mg/kg, dw), followed by DiBP (mean: 0.909 mg/kg, median: 1.707 mg/kg). DiBP and DEHP showed the highest detection rate 98% and 97.1%, respectively in all investigated sediment samples.

In the present study, the concentrations of PAEs detected in the marine matrices were compared to those reported from other areas of the world **(table 3)**.

For the DEHT compound, the concentration rate in the analysed sediment samples ranged between 0.674 and 2 mg/kg, with a mean and median of 1.181 mg/kg and 2.008 mg/kg, respectively **(Table 2).**

Concerning the biota compartment, the total plasticizer content in seagrass plants ranged between 0.511- 4.059 mg/kg a mean equal to 1.713 mg/kg and median equal to 1.414 mg/kg **(Table 2).** Among PAEs (mean = 0.592 mg/kg; median = 0.414 mg/kg), DiBP was the predominant pollutant (range: 0.050 - 1.216 mg/kg; mean level: 0.355 mg/kg), followed by DEHP (range: 0.082 - 0.15 mg/kg; mean level: 0.101 mg/kg). Higher concentration was attributed to DEHT (mean = 1.121 mg/kg, median = 1 mg/kg). *M. galloprovincialis* revealed a comparative load of total plasticizer to the value found in sediment samples **(Table 2)**. Considering PAEs, DiBP was clearly predominant (mean =0.727 mg/kg, median = 0.473 mg/kg) followed by DEHP (mean =0.65 mg/kg, median = 0.521 mg/kg).

Correlation analysis

The results of the Pearson correlation analysis of the PAEs and DEHT concentration data in the analysed marine samples are shown in **Table 4.**

Positive correlations have been recorded between the Σ_4PAE among the three studied marine samples. The greatest correlation was observed in seagrass samples between DiBP and DBP levels ($r = 0.83$, $p < 0.05$). The lowest correlation was found in mussels between DBP and DEP levels ($r = 0.03$). In seagrass samples, DiBP and DBP displayed positive correlations. While in sediment samples, only DiBP exhibits positive correlations with other congeners. Overall, DEHT was correlated with Σ_4PAE with the exception of DBP.

Seasonal variation

The seasonal variation of PAEs and DEHT in every marine sample were shown in **Fig. 3.**

For the analysed sediment samples, although all four PAEs single and DEHT average concentrations in the wet season increased compared with those in the dry season, this seasonal variation was not significant.

On the other hand, for the seagrass *P. oceanica*, DEP and DEHT concentrations were significantly higher in the wet season (0.089 mg/kg, 1.608 mg/kg, respectively, $p < 0.05$). The other PAEs average concentrations were not significantly different among dry and wet seasons ($p > 0.05$).

Considering the *M. galloprovincialis*, only DEHT showed a significant content increase during the wet season (0.961 mg/kg). The seasonal distribution of the PAEs in mussel samples was not significant ($p > 0.05$).

DISCUSSION

In Tunisia, the coastline of Mahdia is one of the most important aquatic ecosystems occupying a very strategic geographical territory with approximately 75 km of coastline, presenting a preponderant fishing, heritage, and agricultural resources. However, the coast of Mahdia is subject to several sources of urban and industrial pollution (**Archiplan-dgat 2019**). Once discharged into the environment, numerous micropollutants can cause environmental disturbances and threaten the maintenance of aquatic ecosystems and public health, such as phthalates and their alternatives. As a matter of fact, PAEs are the plasticizers most employed globally. This has created a serious concern given the rising levels of plastic pollution and PAEs presence in the marine environment, therefore a reliable monitoring is essential to study and compare their dispersion pattern. In our study, i) we monitored the traces of four PAEs congeners (namely, DEP, DBP, DiBP, and DEHP)

and one NNP, namely DEHT, in sediments, *P. Oceania* and *M. galloprovincialis* collected from Rejiche coast in Mahdia and ii) we compared their seasonal distributions.

The analytical screening by GC-MS revealed that PAEs and DEHT were detected in all marine samples, suggesting their ubiquitous distribution in the marine compartments of the Rejiche coast. DEHP and DiBP are the PAE compounds that showed the maximum level in sediments. From the obtained results, it can be stated that, despite the strictly regulated use, DEHP and DiBP remain still the most abundant PAEs detected in the environment (**Li et al. 2016; Chen et al. 2018; Zhang et al. 2018, Lo Turco et al. 2016, 2020)**. **VAN WEZEL et al.** (**2000**) set a guideline for environmental risk limits (URLs) and established a limit of 0.7 mg/kg for DEHP in marine sediments. Based on the scientific literature of the last 15 years (**Table3)**, the collected results in this study were in accordance with the previous studies reporting dominance of DEHP and DiBP in sediments in Tunisia (**Jebara et al. 2021),** Germany **(Fromme et al. 2002),** China **(Yuan et al. 2002; Zhang et al. 2018a; Zhao et al. 2020b)**, South Africa **(Fatoki et al. 2010)** and France-Belgium **(Net et al. 2015a)**. However, the concentrations of PAEs in sediment samples from Rejiche coast were greater than other marine regions previously studied in the Korean bays (0.82 10^{-3} - 0.46 mg/kg dw) **(Kim et al. 2020)**, the Asan Lake of Korea (0.52 10^{-3}- 0.38 mg/kg dw) **(Lee et al. 2019a)** and the coastal areas of the False Creek Harbor (Vancouver, Canada, USA) (0.004 - 2.9 mg/kg dw) **(Mackintosh et al. 2006),** with exception of Kaohsiung Harbor (Taiwan), and Dutch North sea (Netherlands) which showed higher DBP (0.29mg/kg) and DEHP levels (0.17-3.39mg/kg), respectively **(Klamer et al. 2005; Chen et al. 2013)**. High concentrations of DEHP and DiBP in sediments may indicate their lower degradation rates and stronger sorption capacities. Moreover, the abundance of DEHP and DiBP in sediments could be attributed to the routine discharge of industrial effluents into the coastline of Mahdia. Further hypothesis could be

attributed to the physicochemical properties of sediments, such as the total organic carbon (TOC) and the particle size, which plays an important factor in PAEs dissemination **(Li et al. 2016, 2017; Jebara et al. 2021).**

Concerning the contamination by phthalates alternatives, only one recent work monitored DEHT in sediments from Korean semi-enclosed bays and coast showed considerably lower DEHT content (semi-enclosed bays: 0.0043 mg/Kg, coast: 0.0097 mg/Kg, dw) with respect to the coastal sediments from this work (**Kim et al. 2020; Lee et al. 2020**).

P. oceanica samples were studied for plasticizers, and DiBP was established as the most abundant compound among PAEs. With respect to previous literature, only one study was conducted in Mahdia Coasts (in 2018-2019) reported lower concentrations of DiBP (0.355 mg/kg > 0.101 mg/kg, dw), however, DBP and DEHP were higher at levels of 0.389 mg/kg, 0.729 mg/kg, respectively **(Jebara et al. 2021).** These results suggest that seagrass plants, such as *P. Oceanica*, could act as potential "adsorbents" of emerging chemicals in the marine ecosystem **(Photiou et al. 2021).** Indeed, it has shown its effectiveness in adsorbing organic and inorganic pollutants (**Ben Douissa et al. 2016; Boubakri et al. 2017; Elmorsi et al. 2019**).

Similarly to the seagrass plant, DiBP was found to be the highest PAE compound detected in *M. galloprovincialis*. Whereas, with comparison to previous studies **(Table 3),** the collected results for DEP, DBP and DEHP revealed lower concentrations in mussels than those detected in Spanish aquaculture (3.20-6 mg/kg, 6-32 mg/kg, 2-12 mg/kg, respectively) **(Rios-Fuster et al. 2021).** Further Spanish research on wild raw *M. galloprovincialis* demonstrated higher DBP and DEHP contents (0.68 mg/kg, 2.65 mg/kg, respectively) with the exception of DEP which displayed less content level (0.05 mg/kg) **(Cañadas et al. 2021).** An English survey examining the ability of mussels bioaccumulation showed greater concentrations of DiBP and DEHP (4.4 mg/kg, 4.1

mg/Kg, respectively) **(Brown and Thompson 1982).** The relatively high lipid content in marine organisms, fish and mussels may boost the uptake of such pollutants and facilitate their bioaccumulation **(Huckins et al. 1993; Shahid et al. 2018).** Variability in PAEs concentrations observed in mussel species may be due to variation in lipid content that can lead to differences in body burden of contaminants **(Kandie et al. 2020).** In fact, mussels appeared to be excellent toxicants bio-monitoring markers due to their tolerance to variations in the water environment, as well as their accumulation capability of various contaminants, especially plasticizers with low polarity index **(Liu et al. 2011; Stankovic et al. 2012; Suárez et al. 2013; Salgueiro-González et al. 2016; Mata et al. 2022).** Hence, the high bioaccumulation potential of the PAEs congeners indicates that these compounds may reach high concentrations in aquatic organisms. To the knowledge of the authors, there are no comparative studies on alternative phthalates DEHT from marine biota that can be inferred from previous papers.

Comparison of the incidence of PAEs and NPP in different environmental compartments of Mahdia Coast: PAEs and DEHT were detected in all marine samples. Although these chemicals were present in all matrices, their concentrations in the individual matrix were quite different. For example, DiBP and DEHP compounds were among the compounds frequently detected at high levels in sediment and mussel samples, whereas this was not the case in seagrass samples. This finding is probably due to a quite complex bioaccumulation regime involving different uptake pathways **(Contardo-Jara et al. 2011)** and high small-scale variance of exposure **(Kandie et al. 2020).** In general, given that the seagrass *P. oceanica* represents a lower trophic level as a primary producer species compared with consumer species (like mussels), it would be expected for it to bioaccumulate less of these contaminants in its tissues **(LeBlanc 1995; Agawin et al. 2022).**

Furthermore, the application patterns, emission events, and industrial, agricultural or urban runoff during rain events could influence the presence of a contaminant in the aquatic environment **(Meyer et al. 2011; Stehle et al. 2013; Inostroza et al. 2017)**.

A Pearson correlation analysis was performed to evaluate the strength of relationships between concentrations of single PAEs and NPPs. PAEs congeners were positively correlated in the three marine samples, this correlation may be explained by the continuous and simultaneous consumption of both plasticizers and their alternatives **(Lee et al. 2019a; Kim et al. 2020).** DEHT and DEHP shared a high positive correlation in sediment, seagrass and mussel samples ($r = 0.6$, 0.49, and 0.3, respectively). This finding may support the hypothesis of the growing usage of DEHT coherently with DEHP with the evidence that NPPs are increasingly replacing conventional PAEs in the industrial sector (**Tickner 2001**; **Lee et al. 2019b**) and to their similar usage profile and geochemical behavior in the marine ecosystem **(Lee et al. 2019a),** determining the strength and direction of the monotonic relationship between both compounds.

Considering the seasonal variations, DEP, DiBP, DBP, DEHP and DEHT displayed a comparable temporal distribution in sediments. Although no significant differences have been recorded, PAEs were slightly higher in wet season. The decrease of PAEs levels in the dry season could be partly due to high photolytic activity, microbial degradation, and oxidation **(Tran et al. 2015; Boll et al. 2020).**

In addition, the seasonal variations of such compounds could be explained by weather-related factors, such as rainfall, stormwater flooding, and atmospheric fluctuations **(Zeng et al. 2019).** In fact, the concentrated precipitation may cause large amounts of land-sourced pollutants from multiple sources to be carried by surface runoff into the seacoast (**Chen et al. 2013**). Some papers have also stated that atmospheric deposition and rainfall runoff are the main factors affecting

changes in the concentration of pollutants in marine sediments (**Zeng et al. 2008, Lin et al. 2009; Li et al. 2016; Lee et al. 2019a).**

PAEs congeners were also detected during dry season (from June to August). This could be related to tourism activities particularly because DEHP level increased during summer (**Gugliandolo et al. 2020**). On the other hand, the extent of industrial and urban activities as well as sewage treatment stations, generate preferentially inputs of plasticizers in the coastal seawater. Furthermore, the governorate of Mahdia benefits from a wealthy fishing harbor and intense maritime traffic that are predominant during summertime.

Findings in *P. Oceanica* and *M. galloprovincialis* showed comparable seasonal PEAs profiles with the exception of DEP which exhibited significantly higher levels in the wet season in seagrass samples. While DEHT was recorded to be significantly higher during wet season in both matrices. Although the seasonal variation of PAEs and their alternatives in marine biota has not been yet investigated with greater focus, it may be stated that the seasonal characteristics of rainfall and behavior of runoffs and streams may play a leading role in the transmission and accumulation of anthropogenic pollutants in the aquatic environment, affecting the exposure risk of marine organisms **(Lin et al. 2021)**.

Taking all results into consideration, we could speculate that sediments do not only act as plasticizers final sink and reservoir, but they may contribute to their routes as an intermediate in plasticizers transport from environmental media to biological organisms in an aquatic ecosystem **(Mackintosh et al. 2004; Lee et al. 2019a; Jebara et al. 2021).** In fact, PAEs in sediments may have low to moderate potential risks of aquatic organisms (**Lin et al. 2021).** In summary, the discharge of industrial and domestic wastewater, rainfalls and surface runoffs may transport a large

amount of PAEs in the drainage basin of the Rejiche seacoast causing pollutants accumulation in sediments, which may pose a risk of harm to aquatic organisms.

Conclusion

A comprehensive investigation of the occurrence, source, and seasonal trends of four PAE congeners (DEP, DBP, DiBP and DEHP) and one NPP (DEHT) in sediment, seagrass and mussel samples from Rejiche seacoast in Mahdia governorate (Tunisia), was performed in this study. Among the investigated PAEs, DEHP and DiBP were the most abundant and frequently detected congeners in every marine compartment. However, the coast of Rejiche was more polluted by DEHT than Σ_4PAEs being coherent, in terms of routes, with conventional phthalates. ΣPAEs with DEHT screening in sediments was alike in mussels, suggesting that these contaminants could bioaccumulate through the food chain. Whereas *P. oceanica* showed lower loads of these pollutants, probably due to its low capacity to uptake and bioaccumulate plasticizers from sediment. The PAEs concentrations in this present study were at a medium level, compared to previous research. Our results suggest that the detected plasticizers in the studied area might be related to the discharge of domestic sewage as well as touristic and industrial inputs. The seasonal changes might affect their temporal distribution probably due to anthropogenic activities and weather-related factors.

Supplementary materials

The supplementary table can be found online

Statements & Declarations

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Table 1 Linearity, sensitivity, repeatability and recovery of total phthalates esters (Σ_4PAEs) + DEHT

Compound	R^2	LOD (mg/kg)	LOQ (mg/kg)	RSD (%)	Recovery (%)		
					Sediment	Seagrass	Mussel
DEP	0.9911	0.012	0.040	3.25	108.6	110.4	101.3
DiBP	0.9933	0.021	0.065	4.22	98.4	95.4	94.4
DBP	0.9941	0.010	0.030	5.31	101.4	99.3	102.3
DEHP	0.9999	0.010	0.031	2.27	102.1	103.3	105.8
DEHT	0.9802	0.080	0.241	2.46	105.4	103.1	103.7

DEP : Diethyl phthalate; DiBP: Diisobutyl phthalate; DBP: dibutyl phthalate; DEHP: di (2-ethylhexyl) phthalate; DEHT: di-2-ethylhexyl terephthalate; R^2: coefficient of determination; LOD: limit of detection; LOQ: limit of quantification, RSD: relative standard deviation.

Table 2 Concentrations (range, mean and median) of Σ_4PAEs + DEHT content (expressed in mg/Kg) and their detection rate in the single marine matrix from Rejiche coastal sea (Mahdia governorate, Tunisia)

	Compound	Range	Mean	Median	Detection rate (%)
Sediment (mg/kg, dw)	**DEP**	0.016-0.047	0.027	0.022	90.0
	DiBP	0.379-1.734	0.909	1.707	98.0
	DBP	0.130-0.326	0.214	0.413	96.0
	DEHP	0.314-1.773	1.274	2.590	97.1
	DEHT	0.674-2.000	1.181	2.008	
	Σ_4 PAEs	0.839-3.880	2.423	4.732	95.0
	Total	1.513-5.880	3.670	6.250	
Seagrass (mg/kg, dw)	**DEP**	0.037-0.098	0.068	0.209	100
	DiBP	0.050- 1.216	0.355	0.077	100
	DBP	0.027-0.054	0.068	0.039	100
	DEHP	0.082-0.150	0.101	0.090	100
	DEHT	0.315-2.541	1.121	1.000	
	Σ_4 PAEs	0.196-1.518	0.592	0.414	100
	Total	0.511-4.059	1.713	1.414	
Mussel (mg/kg, dw)	**DEP**	0.071-0.169	0.110	0.097	100
	DiBP	0.363-1.961	0.727	0.473	100
	DBP	0.119-0.219	0.178	0.176	100
	DEHP	0.454-1.223	0.650	0.521	100
	DEHT	0.578-5.682	1.860	0.997	
	Σ_4 PAEs	1.007-3.572	1.665	1.267	100
	Total	1.585-9.254	3.525	2.264	

DEP : Diethyl phthalate; DiBP: Diisobutyl phthalate; DBP: dibutyl phthalate; DEHP: di (2-ethylhexyl) phthalate; DEHT: di-2-ethylhexyl terephthalate; PAE: phthalates acid esters; dw: dry weight

Table 3 Comparison between concentrations of 4 PAEs (expressed in mg/Kg) in different matrices from marine environments, in the present study and in other studies throughout the world.

	Study area	DEP	DiBP	DBP	DEHP	Reference
Sediment (mg/kg, dw)	The present study	0.0266	0.9085	0.214	1.274	
	Mahdia 5 seacoasts	0.095	0.219	0.055	4.594	**(Jebara et al. 2021)**
	Korean bays	0.82×10^{-3}	0.011	0.003	0.460	**(Kim et al. 2020)**
	Korean coast	0.52×10^{-3}	0.009	0.001	0.380	**(Lee et al. 2019a)**
	Kaohsiung Harbor, Taiwan	ND	-	0.29	0.29	**(Chen et al. 2013)**
	False Creek Harbor, Vancouver	0.021	0.004	0.103	2.90	**(Mackintosh et al. 2006)**
	Dutch North Sea	< 0.01	-	< 0.08	0.17-3.39	**(Klamer et al. 2005)**
Seagrass (mg/kg, dw)	The present study	0.068	0.355	0.068	0.101	
	Mahdia 5 seacoasts	0.0592	0.101	0.726	0.726	**(Jebara et al. 2021)**
Mussel (mg/kg, dw)	The present study	0.11	0.727	0.178	0.65	
	Large-scale laboratory exposed Mussels	-	4.4	-	4.1	**(Brown and Thompson 1982)**
	Gulf of Thermaikos (Thessaloniki, Greece)	-	-	-	0.053	**(Tsochatzis et al. 2019)**
	Galician Rias (Spain)	0.05	-	0.68	2.65	**(Cañadas et al. 2021)**
	An aquaculture in Spain	3.20-6.00	-	6.00-32.00	2.0-12.0	**(Rios-Fuster et al. 2021)**

DEP: Diethyl phthalate; DiBP: Diisobutyl phthalate; DBP: dibutyl phthalate; DEHP: di (2-ethylhexyl) phthalate; DEHT: di-2-ethylhexyl terephthalate; PAE: phthalates acid esters; dw: dry weight; ND: Not Detected

Table 4 Spearman Correlation analysis of total phthalates esters (Σ4PAEs) + DEHT in sediment, seagrass and mussel samples

	Compounds	DEP	DiBP	DBP	DEHP	DEHT
	DEP	1				
	DiBP	0.6	1			
Sediment	**DBP**	-0.14	0.37	1		
	DEHP	-0.37	0.26	-0.03	1	
	DEHT	0.3	0.3	-0.26	0.6	1
	DEP	1				
	DiBP	-0.6	1			
Seagrass	**DBP**	-0.43	**0.83***	1		
	DEHP	0.12	0.41	0.75	1	
	DEHT	0.37	0.09	0.08	0.49	1
	DEP	1				
	DiBP	0.54	1			
Mussel	**DBP**	0.03	-0.38	1		
	DEHP	-0.09	0.09	0.41	1	
	DEHT	0.6	0.6	-0.41	0.37	1

DEP: Diethyl phthalate; DiBP: Diisobutyl phthalate; DBP: dibutyl phthalate; DEHP: di (2-ethylhexyl) phthalate; DEHT: di-2-ethylhexyl terephthalate; PAE: phthalates acid esters. * Significant at 0.05 level.

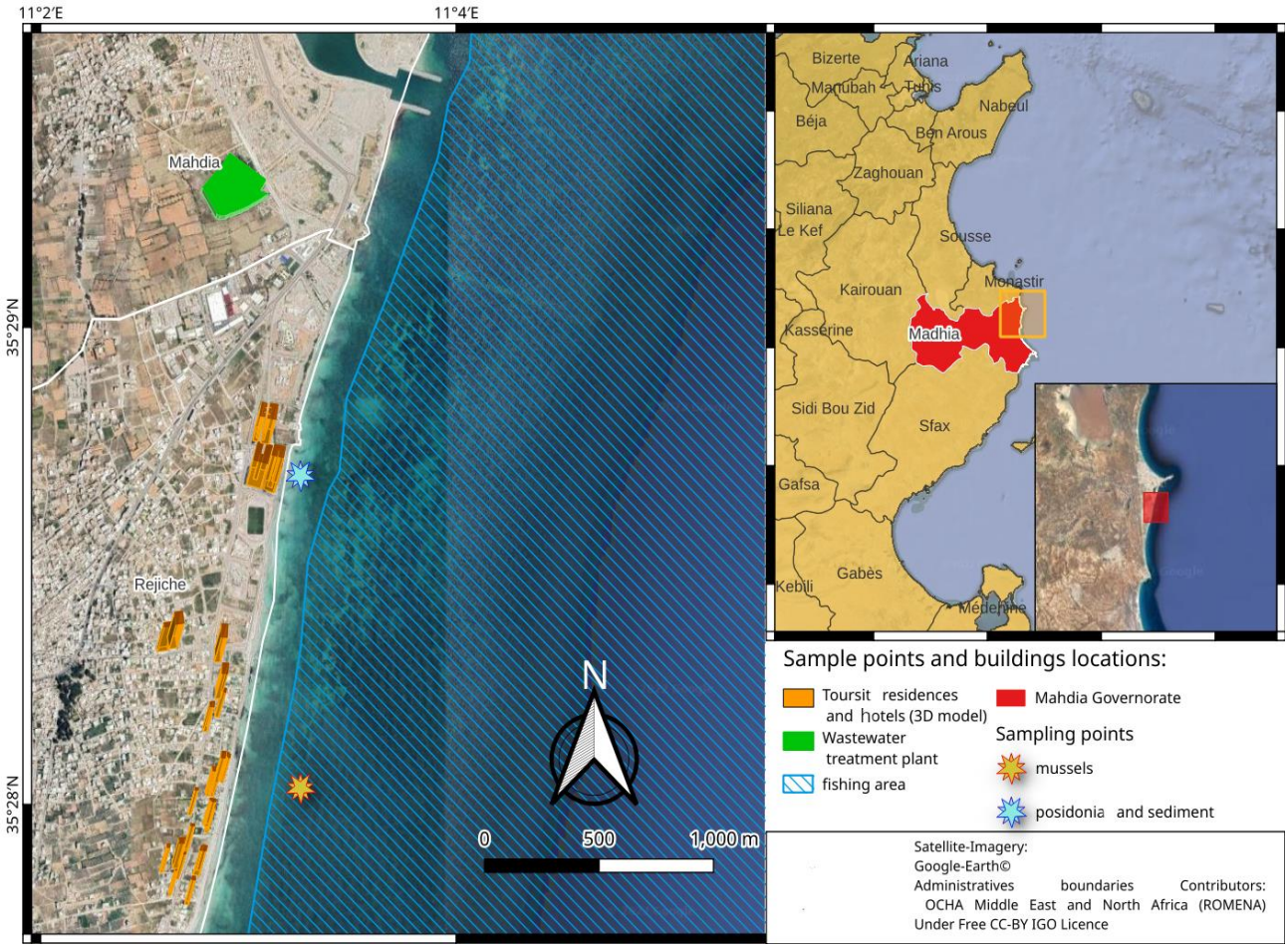


Fig.1 Map illustrating the geographical location of Rejiche in Mahdia governorate (Tunisia) and the sampling sites of sediment, seagrass (*P. oceanica)* and mussels (*M. galloprovincialis*)

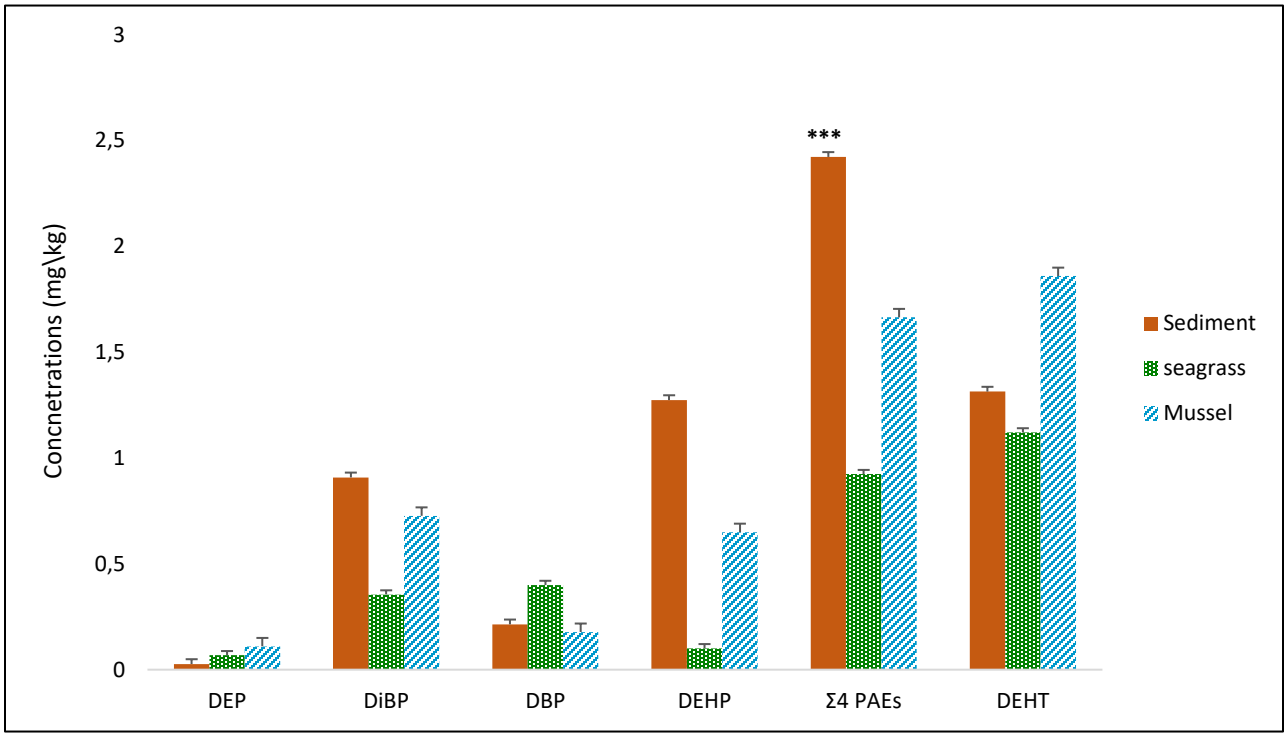
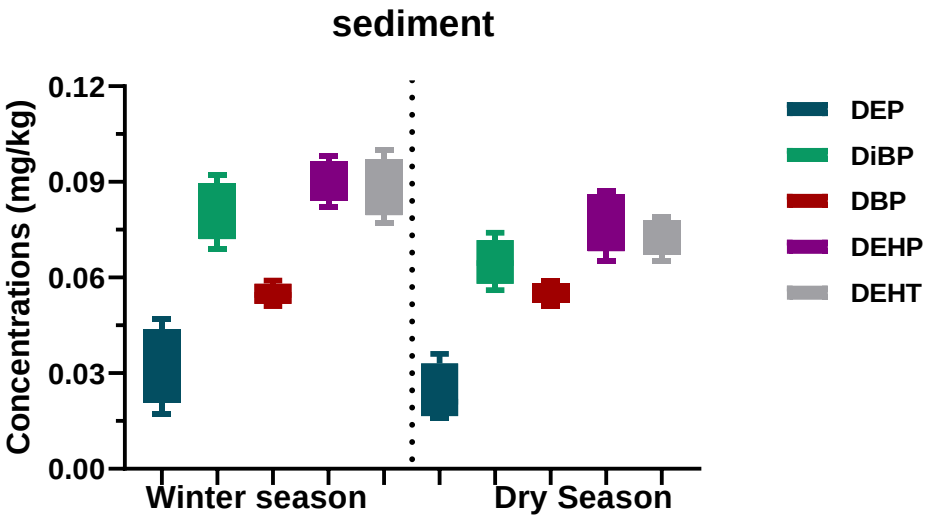
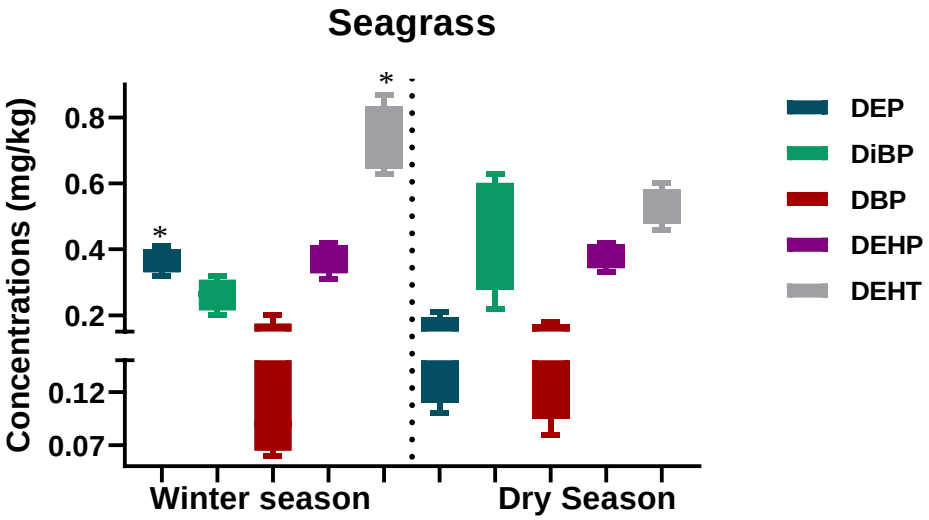


Fig.2 Concentration of single PAE congener, total phthalates esters (Σ4PAEs), and DEHT in sediment, seagrass and mussel samples

(DEP: Diethyl phthalate; DiBP: Diisobutyl phthalate; DBP: dibutyl phthalate; DEHP: di (2-ethylhexyl) phthalate; PAE: phthalates acid esters; DEHT: di-2-ethylhexyl terephthalate. *** Significant at 0.001 level).


sediment
Concentrations (mg/kg)
0.12
0.09
0.06
0.03
0.00
Winter season
Dry Season
DEP
DiBP
DBP
DEHP
DEHT


Seagrass
Concentrations (mg/kg)
0.8
0.6
0.4
0.2
0.12
0.07
*
*
Winter season
Dry Season
DEP
DiBP
DBP
DEHP
DEHT

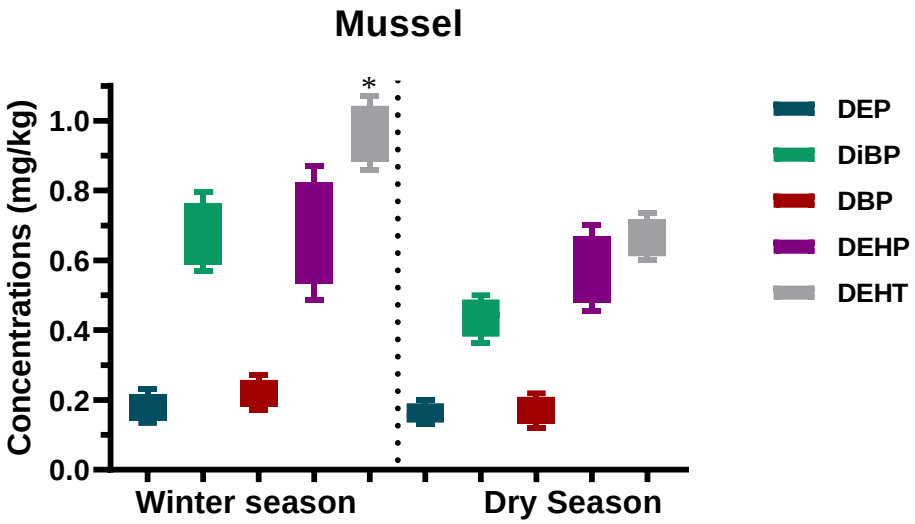


Fig.3 Seasonal variation of single PAE (phthalates esters) congeners, and DEHT in sediment, seagrass and mussel samples collected from Rejiche coastal sea (Mahdia governorate, Tunisia) during wet and dry seasons

(DEP: Diethyl phthalate; DiBP: Diisobutyl phthalate; DBP: dibutyl phthalate; DEHP: di (2-ethylhexyl) phthalate; DEHT: di-2-ethylhexyl terephthalate. * Significant at 0.05 level)