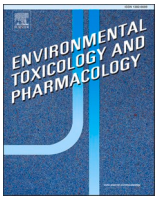
Environmental Toxicology and Pharmacology 100 (2023) 104166

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap



Screening of phthalate and non-phthalate plasticizers and bisphenols in Sicilian women's blood

Giuseppa Di Bella [a,*], Miriam Porretti [b], Mirea Cafarelli [b], Federica Litrenta [a], Angela Giorgia Potortì [a], Vincenzo Lo Turco [a], Ambrogina Albergamo [a], Monica Xhilari [c], Caterina Faggio [b,*]

[a] Department of Biomedical, Dental and Morphological and Functional Imaging Sciences of the University of Messina, Messina, Italy
[b] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences of the University of Messina, Messina, Italy
[c] Laboratory analysis Aesculapius, Patti, ME, Italy

ARTICLE INFO

Edited by Dr. M.D. Coleman

Keywords:
Phthalates
Non-phthalate plasticizers
Bisphenol
Blood
Women
Endocrine disruptor

ABSTRACT

The plastic accumulation and its degradation into microplastics is an environmental issue not only for their ubiquity, but also for the release of intrinsic chemicals, such as phthalates (PAEs), non-phthalate plasticizers (NPPs), and bisphenols (BPs), which may reach body organs and tissues, and act as endocrine disruptors. Monitoring plastic additives in biological matrices, such as blood, may help in deriving relationships between human exposure and health outcomes. In this work, the profile of PAEs, NPPs and BPs was determined in Sicilian women's blood with different ages (20–60 years) and interpreted by chemometrics. PAEs (DiBP and DEPH), NPPs (DEHT and DEHA), BPA and BPS were at higher frequencies and greater levels in women's blood and varied in relation to age. According to statistical analysis, younger females' blood had higher contents of plasticizers than older women, probably due to a more frequent use of higher quantities of plastic products in daily life.

1. Introduction

As a matter of fact, plastic-based waste is one of the biggest issues worldwide (Danzl et al., 2009; Aliko et al., 2022; Schmidt et al., 2017), and, accordingly, microplastics (MPs) with sizes varying between 1 and 5 mm, derived from the accidental degradation and fragmentation processes of plastic materials over time (Zhang et al., 2021), represent a major environmental threat in practically all ecosystems (Silva et al., 2018; Shahul Hamid et al., 2018; Alimba et al., 2021; Pastorino et al., 2023).

Humans may be exposed to MPs not only through the ingestion of contaminated food and water (EFSA Authority, 2016), but also by inhalation (both indoor and outdoor air), and direct dermal contact with personal care products, textiles, or indoor dust (Prata et al., 2020; Revel et al., 2018). The exposure may lead to their accumulation into tissues and organs and, eventually, to diverse health adverse effects, such as inflammation, cytotoxicity, and oxidative stress (Prokić et al., 2021; Philips et al., 2020; Jiang et al., 2020). Furthermore, MPs may release components of the matrix itself, such as chemicals added to the plastic during its manufacturing process, which have already demonstrated the ability to interfere with endogenous hormones (Cole et al., 2011).

Among the chemical additives, phthalates (PAEs) are used as

Abbreviations: PAE, phthalate; NPP, non-phthalate plasticizer; BP, bisphenol; MPs, microplastics; PVC, polyvinyl chloride; PC, polycarbonate; DMP, dimethyl phthalate; DEP, di-ethyl phthalate; DBP, di-butyl phthalate; DiBP, di-iso-butyl phthalate; DEHP, bis(2-ethylhexyl) phthalate; ISs, internal standards; BPA, bisphenol A; BPS, bisphenol S; BPF, bisphenol F; BPE, bisphenol E; BPB, bisphenol B; BPAF, bisphenol AF; BPAP, bisphenol AP; BPZ, bisphenol Z; BPP, bisphenol P; BPC, bisphenol C; BPG, bisphenol G; BPM, bisphenol M; BPFL, bisphenol FL; BPBP, bisphenol BP; FSH, follicle-stimulating hormone; SHBG, hormone-binding globulin; DEHA, bis(2-ethylhexyl) adipate; DMA, di-methyl adipate; DEA, di-ethyl adipate; DiBA, di-isobutyl adipate; DBA, di-n-butyl adipate; DPrP, di-propyl phthalate; BB, benzyl benzoate; BBP, benzyl butyl phthalate; DiHepP, diisoheptyl phthalate; DcHexP, di-cyclohexyl phthalate; DPhP, di-phenyl phthalate; DiNP, di-isononyl phthalate; DiDP, di-isodecyl phthalate; EDTA, ethylenediamonotetraacetic acid; LOD, limit of detection; ESI, electrospray ionisation; SIM, selected ion monitoring; PCA, Principal Component Analysis.

* Corresponding authors.
E-mail addresses: giuseppa.dibella@unime.it (G. Di Bella), miriam.porretti@unime.it (M. Porretti), mirea.c@virgilio.it (M. Cafarelli), federica.litrenta@unime.it (F. Litrenta), angela.potorti@unime.it (A.G. Potortì), vincenzo.loturco@unime.it (V.L. Turco), ambrogina.albergamo@unime.it (A. Albergamo), mxhilari@alice.it (M. Xhilari), cfaggio@unime.it (C. Faggio).

https://doi.org/10.1016/j.etap.2023.104166
Received 26 January 2023; Received in revised form 27 April 2023; Accepted 30 May 2023
Available online 1 June 2023

plasticizers for giving flexibility and duration in a variety of polyvinyl chloride (PVC) based products, like toys, childcare and body care products, food packaging, construction products and medicine equipment. They are classified into low-weight molecules, for example dimethyl phthalate (DMP), di-ethyl phthalate (DEP), di-butyl phthalate (DBP), and di-iso-butyl phthalate (DiBP), and into high weight molecules, including bis(2-ethylhexyl) phthalate (DEHP), and diisononyl phthalate (DiNP) (Beltifa et al., 2017). PAEs are notoriously identified as endocrine disruptors which, by interfering with cellular receptors deputed to the binding of steroid hormones, alter the proper functioning of the reproductive system (European Chemical Agency, 2018). Indeed, DBP has already demonstrated to have a relevant estrogenic activity, due to the ability to bind to estrogenic receptors (Chebbi et al., 2022; Beltifa et al., 2018a). A recent review of Green et al. (2021) reported that DEHP and its metabolites were associated with decreased antral follicle counts, lower oocyte production, lower pregnancy, and live birth rates, decreased serum follicle-stimulating hormone (FSH) and increased hormone-binding globulin (SHBG).

To accommodate the growing health issues, and the increased production of plastic products, non-phthalate plasticizers (NPPs), such as adipates, sebacates, and terephthalates, have been introduced and widely used in the production of PVC, food films, lubricants, and cosmetics, thanks to the fact that they have fewer migration rates and, to date, no use restrictions (Jebara et al., 2021). NPPs lack standard toxic effects such as carcinogenicity, mutagenicity and reproductive toxicity, but the link between these chemicals and other non-standard effects, such as early developmental toxicity or endocrine disruption, has not yet been tested, and based on the huge production volumes of such chemicals, it should be addressed at the earliest (Bui et al., 2016).

Bisphenols (BPs) are another relevant group of plastic additives used in polycarbonate (PC) products and present in the market for more than 50 years (Rios-Fuster et al., 2022), of which bisphenol A (BPA) has been mainly employed to produce plastics for various purposes, such as bottles, plastic tableware (plates and cups) and food containers. BPA is also used to make epoxy resins used in films and coatings of various food and beverage products. BPA is a typical endocrine-disrupting chemical, and it has been associated with many human diseases, such as diabetes, obesity, cardiovascular, chronic respiratory and kidney diseases, breast cancer, behavioural and reproductive disorders in both female and male individuals (Edaes and de Souza, 2022). As a result, one of the major issues is the safe replacement of this chemical with safer alternatives, such as BP analogues. Bisphenol S (BPS) and bisphenol F (BPF) are nowadays the most used BPA substitutes, predominantly in the manufacturing of epoxy resins and PC plastics. However, BPA-like effects can be hypothesized also for such BPs because of their very similar chemical structure (Eladak et al., 2015; Maćczak et al., 2017).

A literature review pointed out that PAEs, NPPs and BPs are readily distributed and accumulated in many marine matrices, including seawater, algae, sediments, and fish (Avellan et al., 2022; Balbi et al., 2016; Sambolino et al., 2022; Gugliandolo et al., 2020; Jebara et al., 2021; Alimba, Faggio, 2019; Burgos-Aceves et al., 2021; Savoca et al., 2019; Strungaru et al., 2019; Gholamhosseini et al., 2023), as well as pharmaceuticals, cosmetics and medical products (Gao and Kannan, 2020; Beltifa et al., 2021), and, not least, in bottled waters (Amiridou and Voutsa, 2011) and many foods (Di Bella et al., 2018; Edwards et al., 2022; Dugo et al., 2011; Lo Turco et al., 2015; Lo Turco et al., 2020; Beltifa et al., 2017; Beltifa et al., 2018b; Saitta et al., 2017; Di Bella et al., 2021; Di Bella et al., 2014; Beltifa et al., 2018a), thus, confirming the detrimental presence of plastics, with its MPs, in the total environment.

Over the past two decades, there has been an increase in studies on the detection and quantification of plastic additives in biological matrices, such as urine (He et al., 2009; Koch et al., 2007; Jala et al., 2022), breast milk (Iribarne-Durán et al., 2022; Main et al., 2006), hair (He et al., 2018), cord blood (Sunman et al., 2019) and, not least, blood (Colón et al., 2000; Lee et al., 2008), as they be considered as valid exposure biomarkers for health-related environmental monitoring of vulnerable population groups (Högberg et al., 2008; Katsikantami et al., 2016). Such biological matrices have in-depth investigated and differentially indicated for the study of short- or long-term exposure of humans to such contaminants. Also, potential links between plasticizers and human toxicity have also been highlighted, thanks to their usefulness in monitoring studies (Katsikantami et al., 2016).

Being considered as a matrix that carries most valuable information about short-term exposure due to its contact with all body cells and tissues (Owczarek et al., 2018), human blood, in particular, has already been in-depth studied for the presence of xenobiotic compounds (Bissinger et al., 2013; Eberhard et al., 2010; Briglia et al., 2015), including environmental contaminants, such as perfluoroalkyl substances (Hanssen et al., 2013; Jin et al., 2016), polycyclic aromatic hydrocarbons (Rengarajan et al., 2015) and heavy metals (Al-Saleh et al., 2011; Gil et al., 2011).

Moreover, the presence of diverse endocrine disruptors in blood has been already established, especially with reference to PAEs or BPs (Owczarek et al., 2018; Wan et al., 2013; Genuis et al., 2012; Latini et al., 2003; Lee et al., 2008; Colón et al., 2000; Yang et al., 2009; Cobellis et al., 2009). With a particular focus on females, the presence of blood PAEs was evaluated in relation to the pregnancy status and the potential transfer in fetal blood (Latini et al., 2003; Lee et al., 2008), as well in young girls with premature thelarche (Colón et al., 2000), and in patients with breast cancer (Yang et al., 2009) or endometriosis (Cobellis et al., 2009). However, a careful literature review pointed out that the comprehensive monitoring of endocrine disruptors, such as PAEs, NPPs and BPs, in female blood has not yet been performed and the relation between the content of such xenobiotics and the female's age has not yet been evaluated. In fact, certain age ranges correspond to the phases of female's reproductive life, entailing considerable changes in the physiological, anatomical, and biochemical attributes of body, which may have implications for a different exposure and susceptibility to environmental pollutants.

Within this background, aim of the study was to study PAEs, NPPs and BPs in blood from healthy females aged between 20 and 60 years and living in Sicily (Italy). Also, a statistical interpretation, based on univariate and multivariate tools, was performed to find a potential relationship between the blood content of such compounds and the variable "age". Hopefully, this study will provide insights on the issue of plasticizers in female blood, and it will lay also the groundwork for a better understanding of the early or late onset of those diseases linked to the presence of these compounds in biological matrices at given age groups.

2. Materials and methods

2.1. Chemicals

Standards of di-methyl adipate (DMA), di-ethyl adipate (DEA), di methyl phthalate (DMP), di-ethyl phthalate (DEP), di-isobutyl adipate (DiBA), di-n-butyl adipate (DBA), di-propyl phthalate (DPrP), benzyl benzoate (BB), di-isobutyl phthalate (DiBP), di-butyl-phthalate (DBP), benzyl butyl phthalate (BBP), bis-(2-ethylhexyl) adipate (DEHA), diisoheptyl phthalate (DiHepP), di-cyclohexyl phthalate (DcHexP), bis-(2-ethylhexyl) phthalate (DEHP), di-phenyl phthalate (DPhP), bis-(2 ethylhexyl) terephthalate (DEHT), di-isononyl phthalate (DiNP), di-isodecyl phthalate (DiDP), all of certified purity $>$ 99 %, were purchased from Aldrich Chemical (Chicago, IL, USA). DBPd4 and DEHP-d4 (100 ng/μL) in nonane, used as internal standards (ISs), were bought from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Single BP commercial standards, namely BPA, BPS, BPF, BPE, BPB, BPAF, BPAP, BPZ and BPP) were purchased by Sigma-Aldrich (Bornem, Belgium), and the IS 13C12-BPA was obtained from Cambridge Isotope Laboratories.

Solvents for analysis (i.e., acetonitrile, methanol, water, and hexane, LiChrosolv and SupraSolv grade) were purchased from Merck

(Darmstadt, Germany). Overall, the contact of laboratory equipment and solvents with samples, the sample preparation time, and the solvent volumes were mandatorily minimized to significantly reduce the background contamination caused by solvents and laboratory materials. Glassware and stainless-steel instruments were washed with acetone, rinsed with hexane, dried at 400 °C for at least 4 h, and finally wrapped with aluminium foils until analysis. All solvents were tested before use, and due to the negligible and not quantifiable levels of background contamination, they were employed throughout the analytical procedures without no further purification.

2.2. Samples

During the period May 2021–October 2021, many female volunteers were asked to complete a questionnaire regarding their gender, age, residence, and general health status with particular attention to the presence of infertility issues. As a result, n = 75 healthy females living in the province of Messina (Sicily, Italy) and aged between 20 and 60 years were selected for the present study. For each volunteer, informed consent was obtained, and 5 mL of whole venous blood was collected in sterilized glass tubes imbibed with 30 μL of 0.5 M ethylenediamonotetraacetic acid (EDTA) at pH 8.0 and stored at − 20 °C until analysis. Then, blood samples were subdivided into 3 cohorts according to women's age, the first group being between 20 and 44 years old (reproductive women, n = 25), the second between 45 and 50 years old (pre-menopausal women, n = 25), and finally 51–60 years old (menopausal women, n = 25) (Table 1).

To minimize contamination, in addition to the glass tubes properly capped with aluminium foil, glass syringes were employed throughout the sampling procedure to avoid a potential contamination by the most common PAEs or BPs present in medical equipment.

2.3. Plasticizers

The extraction of PAEs and NPPs was performed according to the method of Haishima et al. (2004). Briefly, 120 μL of every blood sample was transferred into screw-capped glass tubes, which were filled with distilled water up to the level of 1 mL. Sodium chloride (10 mg) was added to every sample and incubated for 30 min at room temperature. Then, hexane (2 mL) was added, and the obtained mixture was stirred for 20 min at room temperature, and the organic phase was collected and evaporated to dryness. Before GC-MS analysis, all dried extracts were re-suspended in 500 μL of n-hexane and spiked with 500 μL of each internal standard. For analytical validation purposes, the described procedures were also applied to additional and representative blank samples which were previously analysed by the GC-MS protocol reported ahead and revealed no plasticizer contamination.

Plasticizers were determined in all samples by a gas chromatography system (GC-2010, Shimadzu, Japan) equipped with an autosampler (HT300A, HTA, Italy) and coupled to a single quadrupole mass spectrometer (QP-2010 Plus, Shimadzu, Japan). Chromatographic separations occurred 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 8 °C/min.

Table 1
Information about the female participants in the study.

Age group	Age range	Age expressed as mean ± sd	Provenance
Reproductive women	20–44	32.67 ± 6.34	Sicily (province of Messina)
Pre-menopausal women	45–50	47.25 ± 1.87	
Menopausal women	51–60	56.83 ± 2.96	

The injection port was at 260 °C and was provided with a narrow inlet liner (0.75 mm ID, Agilent Technologies). Sample injection took a place 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 used at a linear velocity of 30 cm/s. The MS setup was: EI source temperature; 200 °C; ionization energy and emission current, 70 eV and 250 μA; interface temperature and electron multiplier voltage, 300 °C and 1.0 kV. Acquisition was performed both in full scan (mass range 40–400 *m/z*) and selected ion monitoring (SIM) by monitoring three characteristic mass fragments for every analyte (Table S1). Data acquisition and processing were performed by GCMS solution software. Identification of plasticizers occurred by comparison of their retention times and mass spectra with those of corresponding commercial standards. The quantitative procedure was carried out in SIM mode, considering the relative base peak ions (Table S1) and exploiting the internal standard normalization. The main figures of merit of analytical validation are reported in Table S2. Triplicate measurements were conducted for every sample, alternated with analytical blanks.

2.4. BPs

The BPs extraction was carried out according to a protocol already reported by Li et al. (2022). To every blood sample, 6 mL of acetonitrile were added and stirred at 250 rpm for 30 min, followed by an ultrasonic treatment for 40 min. Then, the mixture was centrifugated at 4000 rpm for 10 min, and the supernatant was transferred to a clean tube. The residue was extracted again by repeating the procedure. All extracts were evaporated under a nitrogen stream and diluted with 5 mL of water containing 0.5 mM ammonium acetate. A further purification was conducted by passing the extract through an Envi-C18 cartridge (Sigma-Aldrich, Oakville, ON, Canada), which was previously conditioned with 5 mL of methanol and 5 mL of water (containing 2.5 mM ammonium acetate). After the sample loading, the cartridge was rinsed with 5 mL of 10 % methanol in water, and target analytes were eluted with 5 mL of methanol containing 0.1 % ammonia. The eluent was evaporated under a gentle nitrogen stream, and the final volume was adjusted to 50 μL with methanol/water (50:50) before instrumental injection. BP residues were determined in every extract by an HPLC system (Shimadzu, Kyoto, Japan), consisting of an LC-20ADXR binary pump, a SIL-20AXR autosampler, and temperature-controlled column operator. The detector was a LCMS-8040 triple quadrupole mass spectrometer with an electrospray ionisation (ESI) source. Labsolution software was used for data control and analysis. Chromatographic separation was performed on an Agilent Zorbax SB-C18 column (5microm 4.6 ×250 mm). The flow rate was 0.7 mL/min. Mobile phases A and B were ultrapure water and acetonitrile, respectively. The following linear gradient was used: 0 min, 20 %B; 7 min, 40 %B; 25 min, 90 %B; 35 min, 20 %B. The injection volume was 20 μL and the column temperature was set at 40 °C. The MS was operated in negative ESI mode under the following specific conditions: nebulizer gas flow 3.0 L/min, nebulizer gas pressure 770 Kpa, drying gas flow 15.0 L/min, DL temperature 250 °C, CID gas 230 Kpa. The dwell time was set to 500 ms. Data were acquired in MRM mode and the resulting ion transitions were used for the identification and quantification (internal standard method) of BPs. MRM transitions and the main figures of merit of analytical validation for every target analyte are reported in Table S3. Every sample was monitored in triplicate, along with analytical blanks.

2.5. Statistical analysis

Statistical analysis was carried out using the SPSS 13.0 software package for Windows (SPSS Inc., Chicago, IL). Initially, the non-parametric Kruskal Wallis's test was applied on log-transformed data to assess differences between blood samples, with a statistical significance at $p < 0.05$. Subsequently, a Principal Component Analysis (PCA) was conducted on a starting data matrix where the cases (75) were the

analyzed blood samples and the variables (9) were the plasticizers and BPs residues reliably determined in blood samples (Škrbić et al., 2010). To this purpose, the data set was normalized to achieve independence of the different variables scale factors, outliers were removed and a PCA was performed to (i) evaluate the differentiation of blood samples in relation to the women's age, and (ii) reduce data dimensionality, while identifying those combinations of variables, which provide the largest contribution to sample variability, commonly known as principal components (PCs). Accounting for a major portion of the variance in the data set, and having the largest eigenvalues, the first PCs are typically used for sample differentiation, as already reported in previous studies (Albergamo et al., 2018).

3. Results

3.1. Plasticizers

Table 2 show the concentrations of plasticizers determined in every blood sample divided into 3 groups according to the age of the subjects. The results of GC-MS/MS analysis demonstrated the presence of PAEs and NPPs in every sample. Specifically, of the 19 plasticizers analysed, 5 PAEs and 2 NNPs were detected in all samples, while the other 12 compounds were below the relative limit of detection (LOD) (Supplementary table 1).

PAEs were determined in the three sample groups according to the concentration order: DEHP ≈ DiBP > DBP > DMP > DEP. DEHP and DiBP were the only PAEs detected in 100 % samples and resulted significantly different between the investigated sample groups ($p < 0.05$). In fact, they were least abundant in menopausal females (respectively, 0.59 mg/L and 0.62 mg/L) and had greater contents in premenopausal and (respectively, 0.75 mg/L and 0.65 mg/L) and reproductive (respectively, 0.62 mg/L and 0.64 mg/L) females.

DBP, DMP and DEP shared the same trend of detection frequency, as they were less detected in menopausal women (respectively, 83 %, 67 % and 44 %) than premenopausal (respectively, 86 %, 71 % and 48 %) and reproductive (respectively, 84 %, 79 % and 57 %) females. However, DMP ranged between 0.35 and 0.40 mg/L ($p < 0.001$), with the same concentration trend described above, while DBP and DEP were not statistically significant between investigated samples (respectively 0.40–0.42 mg/L, $p > 0.05$ and 0.27–0.30 mg/L, $p > 0.05$).

Considering NPPs, DEHT and DEHA were reliably quantified respectively in 94.3 % and 100 % samples. Interestingly, DEHT was least detected in menopausal females (83 %). However, in these blood samples such NPP was at the highest level (0.89 mg/L, $p < 0.001$), thus, resulting less abundant in reproductive and premenopausal women (0.57 mg/L and 0.38 mg/L, $p < 0.001$). Conversely, DEHA was non statistically different between the examined groups (0.39–0.41 mg/L, $p > 0.05$).

On this basis, the total mean content of plasticizers, intended as sum of PAEs and NPPs, ranged from 3.29 mg/L (in 86.4 % premenopausal females under study) to 3.56 mg/L (in 82.4 % menopausal females), being non significantly different between the age groups ($p > 0.05$), while the total content of PAEs spanned from 2.28 mg/L (in 78.8 % of menopausal females under study) to 2.52 mg/L (in 81 % of premenopausal females), being significantly different in relation to age ($p < 0.001$).

3.2. BP residues

Table 3 shows the BPs determined in Sicilian females' blood. The HPLC-MS/MS analysis revealed the presence of 2 of the 9 BPs investigated in every blood sample. Indeed, only BPA and BPS were successfully determined in most blood samples, the other BPs (i.e., BPF, BPE,

Table 3
Profile of BP residues (μg/L) and total content of BPs (ΣBPs, μg/L) revealed in Sicilian females' blood aged 20–44 years (reproductive females), 45–50 years (premenopausal females), and 51–60 years (menopausal females). Results are expressed as mean concentration (μg/L) and standard deviation of $n = 25$ subjects selected for each group. Statistics from Kruskal-Wallis's test is also reported.

Blood Samples		BPA	BPS	ΣBPs
Reproductive Females	mean ± sd	2.19 ± 0.20[a]	1.04 ± 0.08[a]	3.23 ± 0.23[a]
	% positive samples	100	98	99
Premenopausal Females	mean ± sd	1.86 ± 0.10[b]	0.80 ± 0.06[b]	2.66 ± 0.11[b]
	% positive samples	87	82	84.5
Menopausal Females	mean ± sd	1.56 ± 0.07[c]	0.58 ± 0.07[c]	2.14 ± 0.09[c]
	% positive samples	93	85	89
F Statistics		58.69	63.09	63.14
P-Value		**< 0.001**	**< 0.001**	**< 0.001**

[a–c] indicates homogeneous sample groups at $\alpha = 0.05$ and blood samples which do not differ from each other are designated by same letter. Bold p-values showed significant differences at $p < 0.05$ between different sample groups.

Table 2
Profile of PAEs (mg/L) and NPPs (mg/L) and total content of plasticizers (ΣPAEs+NPPs, mg/L) and phthalates (ΣPAEs, mg/L) revealed in Sicilian females' blood aged 20–44 years (reproductive females), 45–50 years (premenopausal females), and 51–60 years (menopausal females). Results are expressed as mean concentration and standard deviation of $n = 25$ subjects selected for each group. Statistics from Kruskal-Wallis's test is also reported.

Blood samples		PAEs					NPPs		ΣPAEs+NPPs	ΣPAEs
		DMP	DEP	DiBP	DEHP	DBP	DEHT	DEHA		
Reproductive females	mean ± sd	0.40 ± 0.02[a]	0.27 ± 0.03	0.62 ± 0.09[a]	0.64 ± 0.08[a]	0.40 ± 0.06	0.57 ± 0.12[a]	0.41 ± 0.03	3.34 ± 0.38	2.36 ± 0.15[a]
	% positive samples	84	79	100	100	57	100	100	88.6	84
Premenopausal females	mean ± sd	0.39 ± 0.00[a]	0.30 ± 0.06	0.75 ± 0.12[b]	0.65 ± 0.05[a]	0.42 ± 0.05	0.38 ± 0.15[b]	0.40 ± 0.07	3.29 ± 0.18	2.52 ± 0.19[b]
	% positive samples	86	71	100	100	48	100	100	86.4	81
Menopausal females	mean ± sd	0.35 ± 0.02[b]	0.29 ± 0.05	0.59 ± 0.10[a]	0.62 ± 0.10[b]	0.40 ± 0.07	0.89 ± 0.29[c]	0.39 ± 0.06	3.56 ± 0.62	2.28 ± 0.11[c]
	% positive samples	83	67	100	100	44	83	100	82.4	78.8
F statistics		31.89	0.24	19.05	7.12	0.34	23.60	1.51	0.16	17.25
p-value		**< 0.001**	0.89	**< 0.001**	**0.03**	0.84	**< 0.001**	0.47	0.93	**< 0.001**

a–c indicates homogeneous sample groups at $\alpha = 0.05$ and blood samples which do not differ from each other are designated by same letter. Bold p-values showed significant differences at $p < 0.05$ between different sample groups.

BPB, BPAF, BPAP, BPZ and BPP) being lower than relative LODs (Supplementary table 1). Interestingly, both BPA, BPS and ΣBPs were detected at higher frequencies in premenopausal and reproductive women's blood than in samples from menopausal females characterized by lower frequencies.

Similar conclusions can be done also for the concentrations of BPs, since they were at the lowest and highest levels respectively in menopausal and reproductive females. Specifically, BPA was detected in 93 % samples (100 % of positive reproductive females and 87–93 % of positive pre- and menopausal females) and was in the range 1.56–2.19 μg/L ($p < 0.001$), while BPS had lower detection frequencies (98 % of positive reproductive females and 82–85 % of positive pre- and menopausal females) and varied between 0.58 and 1.04 μg/L ($p < 0.001$). Accordingly, ΣBPs significantly decreased from reproductive to menopausal women (i.e., 3.23–2.14 μg/L, $p < 0.001$).

3.3. PCA

The PCA analysis was performed by using only those variables resulted to significantly different between the sample groups and the normalized data set (Marengo and Aceto, 2003). The suitability of the data set was pre-checked. The Kaiser–Meyer–Olkin measure revealed a value of 0.745, greater than 0.600 (Sola-Larrañaga and Navarro-Blasco, 2009), and the Bartlett's test of sphericity showed a Chi-squared value equal to 716.326 (at p level below 0.001), thus, supporting the suitability of the correlation matrix. According to the Kaiser Criterion, only those principal components (PCs) with eigenvalues greater than unity were retained (Di Bella et al., 2015). Accordingly, three PCs with respective eigenvalues of 3.45, 1.73, and 1.16, were extracted and they explained up to 79.21 % of the total variance (43.065 %, 21.645 % and 14.50 %, respectively). The three PCs and the relative communalities (h^2), namely the total amount of variance a variable share with all other variables, are reported in Table 4. Variables with low saturation in each component were not identified and all communalities were ≥ 0.620, therefore the extracted PCs were able to satisfactorily reproduce all original variables. Specifically, PC1 showed the highest positive correlation with BPA, BPS and ΣBPs phthalates, while a negative correlation could be observed for DMP. PAEs, such as DiBP, to a lesser extent DEHP, and ΣPAEs, had positive correlations with PC2, while the dominant variable in PC3 was DEHT.

As shown in the bidimensional score plots of Fig. 1, groups of reproductive and menopausal females are clearly separated into two distinct clusters. However, the cluster of perimenopausal women covered the central part of the plot, while partially overlapping with that of reproductive females. The role of PC1 in separating the different female groups is quite evident and the female blood is ranked according to a decreasing score order, namely samples from fertile, perimenopausal and menopausal females. In such order, BPs exhibited the best discrimination power.

4. Discussion

The research of MPs in the human body is rapidly evolving, with more studies expected to emerge in the near future. Despite available studies are still limited to a few world regions, MPs have been already detected in a variety of human tissues and biological matrices, including blood (Kutralam-Muniasamy et al., 2022; Leslie et al., 2022). In blood, moreover, MPs are inevitably throughout body organs via the circulation system (Leslie et al., 2022).

MPs contain several chemicals that are added during the manufacturing process, including plasticisers with known endocrine disrupting activity, such as PAEs, NPPs and BPs.Such intrinsic chemicals were reported to have a constant movement along the concentration gradient, as plastics kept being fragmented into MPs, and they may be translocated when MPs come into contact with body surfaces, organs or tissues (Browne et al., 2011), thus, interfering with endogenous hormones, even at low concentrations (Cole et al., 2011). In the last two decades, the monitoring of plasticizers in human blood was carried out (Owczarek et al., 2018; Wan et al., 2013; Genuis et al., 2012; Latini et al., 2003; Lee et al., 2008; Colón et al., 2000; Yang et al., 2009; Cobellis et al., 2009), also in references to vulnerable female groups from Italian population (Latini et al., 2003; Cobellis et al., 2009).

In accordance with previous literature and with the ubiquitous nature of these compounds, the present study confirmed the widespread exposure to diverse PAE and BP residues also in Sicilian women's blood with different ages, while NPPs, such as DEHT and DEHA, were detected in this study for the first time. Regardless of the age group, the most abundant residues found in female blood were DiBP, DEHP, DEHT and BPA. Between them, DEHP is still the most common plasticizer employed in plastic products despite the European Union has restricted its use in many commercial products (i.e., toys and childcare products) in the past twenty years (European Commission, 2006), DEHT has been emerging as one of the main replacement plasticizers in PVC materials (ChemSec, 2019), and BPA is the main plasticizer employed in the manufacturing of most common PC products. Moreover, their abundancy in biological matrices may be explained by a relatively high molecular weight, lower water solubility and, consequently, higher accumulation ability of such compounds in body tissues (Cheng et al., 2013; Torres-García et al., 2022).

Based on the obtained data, higher concentration ranges of PAEs were alarmingly displayed in this study with respect to previous literature (Colón et al., 2000; Högberg et al., 2008; Wan et al., 2013), thus, correlating with the drastic increase of plastic production volumes observed in the last decades. To facilitate this comparison, Table 5 summarizes the data from this study and compares them to those reported in the literature.

Colón and co-workers (2000) focused between 1994 and 1998 on very young (from 6 months to 8 years old) Puerto Rican females with premature breast development, determining in such subjects higher detection frequencies (68 %) and levels of PAEs (DBP 0.019–0.067 mg/L; DEP 0.002–0.009 mg/L; and DEHP 0.016–0.513 mg/L) than control females, where only DEHP was determined in 14 % of cases and at lower levels. However, the concentrations of PAEs discussed by Colón et al. (2000) were in any case lower than those obtained for healthy Sicilian females. In 2007, Högberg et al. (2008), screened the milk and blood serum of Swedish women and lower frequencies and concentrations of DEP (80.5 % of samples with a mean concentration of 0.0003 mg/L), DBP (70 % of samples with a mean concentration of 0.001 mg/L) and DEHP (47 % of samples with a mean concentration of 0.006 mg/L) were found out with respect to the present study.

Wan and co-workers (2013) monitored between 2010 and 2011 PAEs in both males' and females' blood from the Hong Kong population based on two age groups (16–39 years and 40–63 years) and they pointed out that older individuals had slightly higher total PAEs than the younger ones (0.043 mg/L vz. 0.041 mg/L). Accordingly, they revealed single congeners slightly most abundant in subjects aged 40–63 years than 16–30 years. For example, DiBP amounted to 0.006 mg/L and 0.005 mg/L in older and younger study participants, and DBP varied non significantly between 0.005 and 0.004 mg/L in the two age groups.

Table 4
Extracted PCs and relative variables communalities (h^2).

Compound	PC1	PC2	PC3	h^2
DMP	**-0.663**	-0.160	-0.410	0.633
DiBP	-0.342	**0.736**	-0.431	0.845
DEHP	-0.340	**0.568**	0.427	0.620
DEHT	-0.228	0.202	**0.769**	0.684
∑PAEs	-0.436	**0.771**	-0.130	0.802
BPA	**0.902**	0.240	-0.001	0.872
BPS	**0.905**	0.279	-0.117	0.911
∑BPs	**0.947**	0.266	-0.051	0.971

Bold values indicate the dominant variables in each PC.

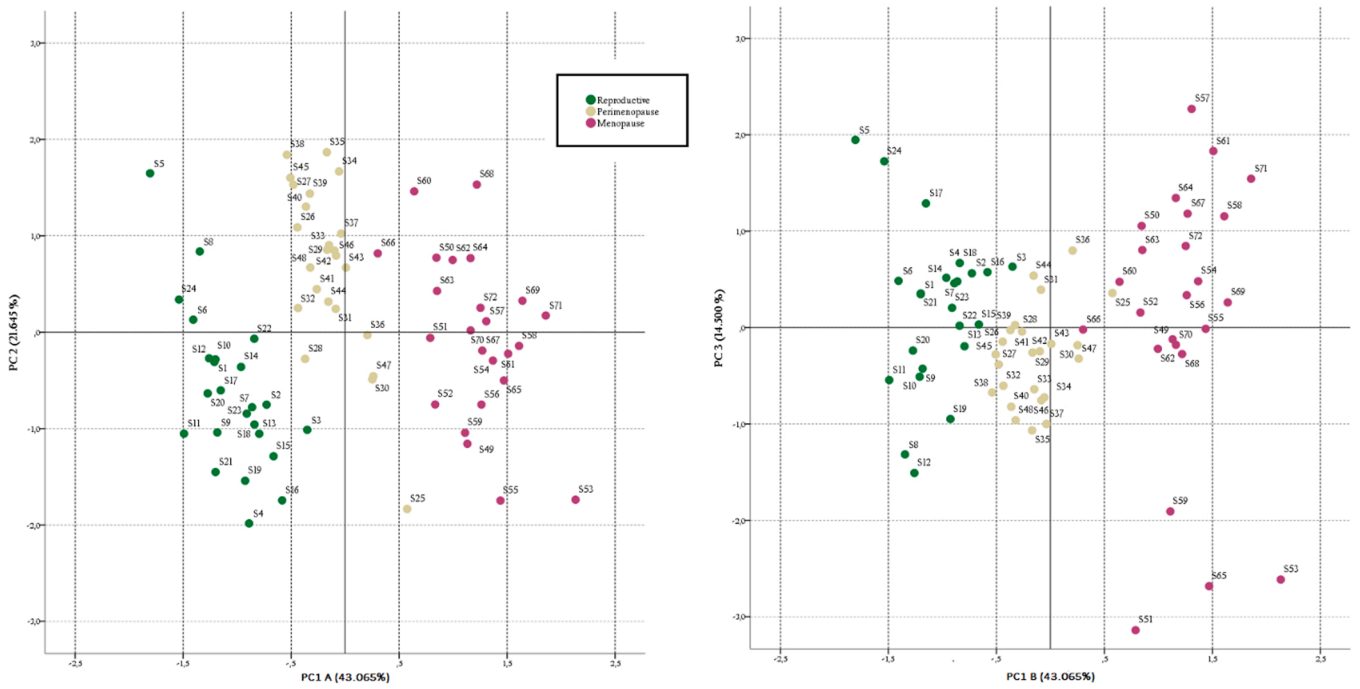


Fig. 1. Bidimensional score plots obtained by plotting the three main PCs, namely PC1viz. PC2 (A) and PC1viz. PC3 (B). The definition of three clusters, corresponding to the blood samples from the three investigated female groups, is most evident in plot (A), explaining a greater cumulative variance.

Table 5
Comparison between the level of plasticizers found in blood from this study and from previous literature.

Compound	Concentration range		Reference
	this study	Previous studies	
DMP	0.35–0.40 mg/L	–	
DEP	0.27–0.30 mg/L	0.002–0.009 mg/L 0.0003 mg/L	Colón et al. (2000) Högberg et al. (2008)
DiBP	0.59–0.75 mg/L	0.006–0.005 mg/L	Wan et al. (2013)
DEHP	0.62–0.65 mg/L	0.016–0.513 mg/L 1.15–2.05 mg/L	Colón et al. (2000) Latini et al. (2003)
DBP	0.40–0.42 mg/L; 0.38–0.89 mg/L; 0.39–0.41 mg/L	0.019–0.067 mg/L 0.006 mg/L 0.005–0.004 mg/L - -	Colón et al. (2000) Högberg et al. (2008) Wan et al. (2013)
DEHT			
DEHA			
BPA	1.56–2.19 μg/L; 0.58–1.04 μg/L;	<LOD-2.84 μg/L <LOD-66.46 μg/L, <LOD-8.86 μg/L 2.91 μg/L 0.12 μg/L 1.69 μg/L 5.15 μg/L 1.14 μg/L	He et al. (2009) Lee et al. (2008) Cobellis et al. (2009) Owczarek et al. (2018) Yang et al. (2009) Cobellis et al. (2009) Owczarek et al. (2018)
BPS			

However, DEHP had an opposite trend, being significantly more accumulated in younger volunteers (i.e., 0.011 mg/L vz. 0.010 mg/L). However, the blood contamination by PAEs studied in the Hong Kong's population was still lower than that found in the Sicilian females' blood.

In this framework, only Latini and colleagues (2003), focusing on pregnant women from the Campanian region (Italy), revealed a DEHP content both in maternal (71 % of samples with a mean content of 1.15 mg/L) and cord (44 % of samples with a mean level of 2.05 mg/L) blood up to 3 times higher than data from our study. According to the contents of the article, a weak analytical approach that did not include adequate measures of sample contamination prevention may be responsible for such results.

Differently from PAEs, BPA has been more thoroughly investigated in human blood (Yang et al., 2009; Cobellis et al., 2009, Lee, 2008; Owczarek et al., 2018; Genuis et al., 2012; He et al., 2009; Huang et al., 2019). Nevertheless, the evaluation of human exposure to BPs analogues other than BPA, is still scarce (Owczarek et al., 2018; Cobellis et al., 2009). Overall, previous literature highlighted that the contents of BPA and BPS in womens' blood were basically in agreement with those showed in this study.

For example, He and colleagues (2009) monitored serum BPA in a set of male and female subjects with different ages from the Shanghai's population. BPA was detected in 17 % of serum samples, and its content varied from <LOD up to 2.84 μg/L, thus, being in the same concentration range of the present work. In line with the present study, moreover, greater detection rates and levels of BPA in younger people (<40 years) than the older ones (>40 years) were highlighted.

BPA was also found in the serum of Korean pregnant women but also in the umbilical cords of their fetus, where it ranged from <LOD to 66.46 μg/L and <LOD to 8.86 μg/L respectively, thus, being much higher than BPA from our study. However, results from this study demonstrated how BPA can accumulate in the mother's body and reasonably migrate towards the fetus (Lee et al., 2008).

In a study of Cobellis et al. (2009), BPA and BPB were determined in Italian endometriotic women respectively with mean contents of 2.91 μg/L and 5.15 μg/L, thus, BPA resulting in line with the levels found in Sicilian females' blood of. Additionally, a correlation was made between the presence of BPA and BPB and the occurrence of endometriosis, with a 63.8 % occurrence rate in women aged 21–42 years.

To the best knowledge, only Owczarek and colleagues (2018)

comprehensively monitored 10 BP analogues (BPC, BPE, BPF, BPG, BPM, BPP, BPS, BPZ, BPFL and BPBP), other than BPA, in healthy Poland women aged 18–40 years. Differently from the present studies, all investigated analogues were detected although with lower frequencies (from 23.5 % for BPFL to 72 % for BPS and 91.4 % for BPA). Moreover, they showed variable contents, ranging from median value of 0.07 μg/L for BPFL to 1.14 μg/L for BPS. Surprisingly, BPA showed a lower content (0.12 μg/L), thus, resulting most similar to the other BP analogues. On this basis, BPS from Poland woman was comparable with the corresponding analogue determined in Sicilian menopausal female; while BPA resulted was more than 10 times less concentrated than the BPA found in this study.

Apart from the reliable determination of PAEs, NPPs and BPs, this study proposed for the first time a valid statistical approach able to display how certain compounds varied significantly based on the females' age. In fact, premenopausal and reproductive females shared greater contents of PAEs, such as DEHP and DiBP, and BPA and BPS than older menopausal women.

Based on the evidence that human blood contains information about short-term exposure to xenobiotics (Owczarek et al., 2018), and that plasticizers have a relatively short half-life as they are rapidly metabolized in human body (Koch et al., 2004; Wittassek, Angerer, 2008; Vandenberg et al., 2010), the greater contents of DEHP and DiBP in reproductive and premenopausal woman correlates well with younger age groups, who are prone to use at higher quantities a variety of PVC and PC products containing such common plasticizers, such as toys, cosmetics, baby care and body care products, food wraps, bottles, food containers etc. Additionally, although the content of plasticizers, such as DEP and DBP, showed no discrimination power in relation to the females' age, they were still characterized by different detection frequencies between the investigated age groups, which may underline a more frequent use of PVC products by younger than older females.

5. Conclusions

Results from this study indicate that the environmental abundance of plastics with their MPs, constantly exposes also the modest portion of female participants in this study to the wide spectrum of plasticizers, and that a correlation between the women's age and the profile of PAEs, NPPs and BPs outlined in blood, can be reasonably defined. However, the lack of information regarding dietary habits, education, lifestyle, employment etc. of participating subjects, prevented us from discovering the exposure routes in the study population, as well as correlating the obtained data with clear environmental sources of plasticizers. As a result, the sources of non-occupational/occupational exposure to such xenobiotics should be in-depth explored.

Undoubtedly, there is a need for a radical change in approaching the study of contaminants in human health, as more emphasis should be first placed on monitoring programs, population-based data, and epidemiological studies to derive reliable relationships between human exposure and health outcomes. By doing so, better predictive models of human response to toxicants may be established.

CRediT authorship contribution statement

Caterina Faggio: Conceptualization, Writing – review & editing, Supervision. **Giuseppa Di Bella:** Conceptualization, Writing – original draft, Visualization, Supervision. **Miriam Porretti:** Methodology, Formal analysis, Writing – original draft. **Angela Giorgia Portortì:** Software, Data curation. **Vincenzo Lo Turco:** Validation. **Ambrogina Albergamo:** Validation, Writing – review & editing. **Federica Litrenta:** Formal analysis, All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The authors do not have permission to share data.

Acknowledgments

Special thanks to the Aesculapius analysis laboratory for providing the samples.

Statement

The study was conducted in accordance with the Declaration of Helsinki, anonymously and with written consent.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.etap.2023.104166.

References

Albergamo, A., Mottese, A.F., Bua, G.D., Caridi, F., Sabatino, G., Barrega, L., Dugo, G., 2018. Discrimination of the Sicilian prickly pear (Opuntia ficus-indica L., cv. Muscaredda) according to the provenance by testing unsupervised and supervised chemometrics. J. Food Sci. 83 (12), 2933–2942. https://doi.org/10.1111/1750-3841.14382.

Aliko, V., Beqiraj, E.G., Qirjo, M., Cani, M., Rama, A., Bego, K., Reka, A., Faggio, C., 2022. Plastic invasion tolling: first evaluation of microplastics in water and two crab species from the nature reserve lagoonary complex of Kune-Vain, Albania. Sci. Total Environ. 849, 157799 https://doi.org/10.1016/j.scitotenv.2022.157799.

Alimba, C.G., Faggio, C., 2019. Microplastics in the marine environment: current trends in environmental pollution and mechanisms of toxicological profile. Environ. Toxicol. Pharmacol. 68, 61–74. https://doi.org/10.1016/j.etap.2019.03.001.

Alimba, C.G., Faggio, C., Sivanesan, S., Ogunkanmi, A.L., Krishnamurthi, K., 2021. Micro (nano)-plastics in the environment and risk of carcinogenesis: insight into possible mechanisms. J. Hazard. Mater. 416, 126143 https://doi.org/10.1016/j.jhazmat.2021.126143.

Al-Saleh, I., Shinwari, N., Mashhour, A., Mohamed, G.E.D., Rabah, A., 2011. Heavy metals (lead, cadmium and mercury) in maternal, cord blood and placenta of healthy women. Int. J. Hyg. Environ. Health 214 (2), 79–101. https://doi.org/10.1016/j.ijheh.2010.10.001.

Amiridou, D., Voutsa, D., 2011. Alkylphenols and phthalates in bottled waters. J. Hazard. Mater. 185 (1), 281–286. https://doi.org/10.1016/j.jhazmat.2010.09.031.

Avellan, A., Duarte, A., Rocha-Santos, T., 2022. Organic contaminants in marine sediments and seawater: a review for drawing environmental diagnostics and searching for informative predictors. Sci. Total Environ. 808, 152012 https://doi.org/10.1016/j.scitotenv.2021.152012.

Balbi, T., Franzellitti, S., Fabbri, R., Montagna, M., Fabbri, E., Canesi, L., 2016. Impact of bisphenol A (BPA) on early embryo development in the marine mussel mytilus galloprovincialis: effects on gene transcription. Environ. Pollut. 218, 996–1004. https://doi.org/10.1016/j.envpol.2016.08.050.

Beltifa, A., Feriani, A., Machreki, M., Ghorbel, A., Ghazouani, L., Di Bella, G., Van Loco, J., Reyns, T., Mansour, H.B., 2017. Plasticizers and Bisphenol A, in packaged foods sold in the Tunisian markets: study of their acute in vivo toxicity and their environmental fate. Environ. Sci. Pollut. Res. 24, 22382–22392. https://doi.org/10.1007/s11356-017-9861-0.

Beltifa, A., Belaid, A., Lo Turco, V., Machreki, M., Ben Mansour, H., Di Bella, G., 2018a. Preliminary evaluation of plasticizer and BPA in Tunisian cosmetics and investigation of hazards on human skin cells. Int. J. Environ. Health Res. 28, 491–501. https://doi.org/10.1080/09603123.2018.1489528.

Beltifa, A., Feriani, A., Macherki, M., Ghorbel, A., Ghazouani, L., Di Bella, G., Sire, O., Van Loco, J., Reyns, T., Mansour, H.B., 2018b. Persistent plasticizers and bisphenol in the cheese of Tunisian markets induced biochemical and histopathological alterations in male BALB/c mice. Environ. Sci. Pollut. Res. 25, 6545–6557. https://doi.org/10.1007/s11356-017-0857-6.

Beltifa, A., Alibi, S., Turco, V.L., Mansour, H.B., Di Bella, G., 2021. Identification and quantification of plasticizers, bisphenol, and environmental toxic mineral elements residues in medicines from Tunisian markets. Environ. Sci. Pollut. Res. 28, 50462–50470. https://doi.org/10.1007/s11356-021-14221-w.

Bissinger, R., Modicano, P., Frauenfeld, L., Lang, E., Jacobi, J., Faggio, C., Lang, F., 2013. Estramustine-induced suicidal erythrocyte death. Cell Physiol. Biochem. 32 (5), 1426–1436. https://doi.org/10.1159/000356580.

Briglia, M., Fazio, A., Faggio, C., Laufer, S., Alzoubi, K., Lang, F., 2015. Triggering of suicidal erythrocyte death by ruxolitinib. Cell Physiol. Biochem. 37 (2), 768–778. https://doi.org/10.1159/000430394.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines worldwide: sources and sinks. Environ. Sci. Technol. 45 (21), 9175–9179. https://doi.org/10.1021/es201811s.

Bui, T.T., Giovanoulis, G., Cousins, A.P., Magnér, J., Cousins, I.T., de Wit, C.A., 2016. Human exposure, hazard and risk of alternative plasticizers to phthalate esters. Sci. Total Environ. 541, 451–467. https://doi.org/10.1016/j.scitotenv.2015.09.036.

Burgos-Aceves, M.A., Abo-Al-Ela, H.G., Faggio, C., 2021. Impact of phthalates and bisphenols plasticizers on haemocyte immune function of aquatic invertebrates: a review on physiological, biochemical, and genomic aspects. J. Hazard. Mater. 419, 126426 https://doi.org/10.1016/j.jhazmat.2021.126426.

Chebbi, M., Beltifa, A., Alibi, S., Di Bella, G., Loturco, V., Sire, O., Ben Mansour, H., Le Tilly, V., 2022. Estrogenic hazards of short chain phthalates and bisphenols found in cosmetic products. Int. J. Environ. Health Res. 32, 252–263. https://doi.org/10.1080/09603123.2020.1749573.

ChemSec- International Chemical Secretariat, 2019. Replacing Phthalates. Available at: ⟨https://chemsec.org/publication/endocrine-disruptors,substitution/replacing-phthalates/⟩ (Accessed on 13 January 2023).

Cheng, Z., Nie, X.P., Wang, H.S., Wong, M.H., 2013. Risk assessments of human exposure to bioaccessible phthalate esters through market fish consumption. Environ. Int. 57, 75–80. https://doi.org/10.1016/j.envint.2013.04.005.

Cobellis, L., Colacurci, N., Trabucco, E., Carpentiero, C., Grumetto, L., 2009. Measurement of bisphenol a and bi-sphenol b levels in human blood sera from healthy and endometriotic women. Biomed. Chromatogr. 23, 1186–1190. https://doi.org/10.1002/bmc.1241.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62 (12), 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025.

Colón, I., Caro, D., Bourdony, C.J., Rosario, O., 2000. Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. Environ. Health Perspect. 108 (9), 895–900. https://doi.org/10.1289/ehp.108-2556932.

Danzl, E., Sei, K., Soda, S., Ike, M., Fujita, M., 2009. Biodegradation of bisphenol A, bisphenol F and bisphenol S in seawater. Int. J. Environ. Res. Public Health 6 (4), 1472–1484. https://doi.org/10.3390/ijerph6041472.

Di Bella, G., Potortì, A.G., Lo Turco, V., Saitta, M., Dugo, G., 2014. Plasticizer residues by HRGC–MS in espresso coffees from capsules, pods and moka pots. Food Control 41, 185–192. https://doi.org/10.1016/j.foodcont.2014.01.026.

Di Bella, G., Lo Turco, V., Potortì, A.G., Bua, G.D., Fede, M.R., Dugo, G., 2015. Geographical discrimination of italian honey by multi-element analysis with a chemometric approach. J. Food Compos. Anal. 44, 25–35. https://doi.org/10.1016/j.jfca.2015.05.003.

Di Bella, G., Ben Mansour, H., Ben Tekaya, A., Beltifa, A., Potortì, A.G., Saiya, E., Bartolomeo, G., Dugo, G., Lo Turco, V., 2018. Plasticizers and BPA residues in Tunisian and Italian culinary herbs and spices. J. Food Sci. 83 (6), 1769–1774. https://doi.org/10.1111/1750-3841.14171.

Di Bella, G., Potortì, A.G., Beltifa, A., Ben Mansour, H., Nava, V., Lo Turco, V., 2021. Discrimination of Tunisian honey by mineral and trace element chemometrics profiling. Foods 10, 724. https://doi.org/10.3390/foods10040724.

Dugo, G., Fotia, V., Lo Turco, V., Maisano, R., Potortì, A.G., Salvo, A., Di Bella, G., 2011. Phthalate, adipate and sebacate residues by HRGC-MS in olive oils from sicily and molise (Italy). Food Control 22, 982–988. https://doi.org/10.1016/j.foodcont.2010.12.006.

Eberhard, M., Ferlinz, K., Alizzi, K., Cacciato, P.M., Faggio, C., Föller, M., Lang, F., 2010. FTY720-induced suicidal erythrocyte death. Cell Physiol. Biochem. 26 (4–5), 761–766. https://doi.org/10.1159/000322343.

Edaes, F.S., de Souza, C.B., 2022. BPS and BPF are as Carcinogenic as BPA and are Not Viable Alternatives for its Replacement. Endocr Metab. Immune Disord. Drug Targets (Former. Curr. Drug Targets-Immune, Endocr. Metab. Disord.) 22 (9), 927–934. https://doi.org/10.2174/1871530322666220316141032.

Edwards, L., McCray, N.L., VanNoy, B.N., Yau, A., Geller, R.J., Adamkiewicz, G., Zota, A. R., 2022. Phthalate and novel plasticizer concentrations in food items from U.S. fast food chains: a preliminary analysis. J. Expo. Sci. Environ. Epidemiol. 32, 366–373. https://doi.org/10.1038/s41370-021-00392-8.

EFSA Authority, 2016. Presence of Microplastics and Nanoplastics in Food, with Particular Focus on Seafood. 14, e04501. ⟨https://doi.org/10.2903/j.efsa.2016.4501⟩.

Eladak, S., Grisin, T., Moison, D., Guerquin, M.J., N'Tumba-Byn, T., Pozzi-Gaudin, S., Benachi, A., Livera, G., Rouiller-Fabre, V., Habert, R., 2015. A new chapter in the bisphenol A story: bisphenol S and bisphenol F are not safe alternatives to this compound. Fertil. Steril 103 (1), 11–21. https://doi.org/10.1016/j.fertnstert.2014.11.005.

European Chemical Agency (ECHA) and European Food Safety Authority (EFSA) with the technical support of the Joint Research Centre (JRC); Andersson, N.; Arena, M.; Auteri, D.; Barmaz, S.; Grignard, E.; Kienzler, A.; Lepper, P.; Lostia, A.M.; Munn, S.; et al., 2018. Guidance for the Identification of Endocrine Disruptors in the Context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. EFSA J., 16, e05311. ⟨https://doi.org/10.2903/j.efsa.2018.5311⟩.

European Commission, 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the registration, evaluation, authorisation and restriction of chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. Official Journal of the European Union, 396, 1–849.

Gao, C.-J., Kannan, K., 2020. Phthalates, bisphenols, parabens, and triclocarban in feminine hygiene products from the United States and their implications for human exposure. Environ. Int. 136, 105465 https://doi.org/10.1016/j.envint.2020.105465.

Genuis, S.J., Beesoon, S., Birkholz, D., Lobo, R.A., 2012. Human excretion of bisphenol A: blood, urine, and sweat (BUS) study. J. Environ. Public Health 2012. https://doi.org/10.1155/2012/185731.

Gholamhosseini, A., Banaee, M., Sureda, A., Timar, N., Zeidi, A., Faggio, C., 2023. Physiological response of freshwater crayfish, *Astacus leptodactylus* exposed to polyethylene microplastics at different temperature. Comp. Biochem. Physiol. Part C 267, 109581.

Gil, F., Hernández, A.F., Márquez, C., Femia, P., Olmedo, P., López-Guarnido, O., Pla, A., 2011. Biomonitorization of cadmium, chromium, manganese, nickel and lead in whole blood, urine, axillary hair and saliva in an occupationally exposed population. Sci. Total Environ. 409 (6), 1172–1180. https://doi.org/10.1016/j.scitotenv.2010.11.033.

Green, M.P., Harvey, A.J., Finger, B.J., Tarulli, G.A., 2021. Endocrine disrupting chemicals: impacts on human fertility and fecundity during the peri-conception period. Environ. Res. 194, 110694 https://doi.org/10.1016/j.envres.2020.110694.

Gugliandolo, E., Licata, P., Crupi, R., Albergamo, A., Jebara, A., Lo Turco, V., Potortì, A. G., Mansour, H.B., Cuzzocrea, S., Di Bella, G., 2020. Plasticizers as microplastics tracers in tunisian marine environment. Front. Mar. Sci. 7, 589398 https://doi.org/10.3389/fmars.2020.589398.

Haishima, Y., Matsuda, R., Hayashi, Y., Hasegawa, C., Yagami, T., Tsuchiya, T., 2004. Risk assessment of Di(2-Ethylhexyl)phthalate released from PVC blood circuits during hemodialysis and pump–oxygenation therapy. Int. J. Pharm. 274, 119–129. https://doi.org/10.1016/j.ijpharm.2004.01.009.

Hanssen, L., Dudarev, A.A., Huber, S., Odland, J.Ø., Nieboer, E., Sandanger, T.M., 2013. Partition of perfluoroalkyl substances (PFASs) in whole blood and plasma, assessed in maternal and umbilical cord samples from inhabitants of arctic Russia and Uzbekistan. Sci. Total Environ. 447, 430–437. https://doi.org/10.1016/j.scitotenv.2013.01.029.

He, M.J., Lu, J.F., Ma, J.Y., Wang, H., Du, X.F., 2018. Organophosphate esters and phthalate esters in human hair from rural and urban areas, Chongqing, China: concentrations, composition profiles and sources in comparison to street dust. Environ. Pollut. 237, 143–153. https://doi.org/10.1016/j.envpol.2018.02.040.

He, Y., Miao, M., Herrinton, L.J., Wu, C., Yuan, W., Zhou, Z., Li, D.-K., 2009. Bisphenol A levels in blood and urine in a Chinese population and the personal factors affecting the levels. Environ. Res. 109, 629–633. https://doi.org/10.1016/j.envres.2009.04.003.

Högberg, J., Hanberg, A., Berglund, M., Skerfving, S., Remberger, M., Calafat, A.M., Filipsson, A., F., Jansson, B., Johansson, N., Appelgren, M., Håkansson, H., 2008. Phthalate diesters and their metabolites in human breast milk, blood or serum, and urine as biomarkers of exposure in vulnerable populations. Environ. Health Perspect. 116 (3), 334–339. https://doi.org/10.1289/ehp.10788.

Huang, S., Li, J., Xu, S., Zhao, H., Li, Y., Zhou, Y., Fang, J., Liao, J., Cai, Z., Xia, W., Bisphenol, A., Bisphenol, S., 2019. Exposures during pregnancy and gestational age – a longitudinal study in China. Chemosphere 237, 124426. https://doi.org/10.1016/j.chemosphere.2019.124426.

Iribarne-Durán, L.M., Serrano, L., Peinado, F.M., Peña-Caballero, M., Hurtado, J.A., Vela-Soria, F., Fernández, M.F., Freire, C., Artacho-Cordón, F., Olea, N., 2022. Biomonitoring bisphenols, parabens, and benzophenones in breast milk from a human milk bank in Southern Spain. Sci. Total Environ. 830, 154737 https://doi.org/10.1016/j.scitotenv.2022.154737.

Jala, A., Varghese, B., Dutta, R., Adela, R., Borkar, R.M., 2022. Levels of parabens and bisphenols in personal care products and urinary concentrations in Indian young adult women: implications for human exposure and health risk assessment. Chemosphere 297, 134028. https://doi.org/10.1016/j.chemosphere.2022.134028.

Jebara, A., Albergamo, A., Rando, R., Potortì, A.G., Lo Turco, V., Mansour, H.B., Di Bella, G., 2021. Phthalates and non-phthalate plasticizers in Tunisian marine samples: occurrence, spatial distribution and seasonal variation. Mar. Pollut. Bull. 163, 111967 https://doi.org/10.1016/j.marpolbul.2021.111967.

Jiang, Y., Li, J., Xu, S., Zhou, Y., Zhao, H., Li, Y., Xiong, C., Sun, X., Liu, H., Liu, W., et al., 2020. Prenatal Exposure to Bisphenol A and Its Alternatives and Child Neurodevelopment at 2 Years. J. Hazard. Mater. 388, 121774 https://doi.org/10.1016/j.jhazmat.2019.121774.

Jin, H., Zhang, Y., Jiang, W., Zhu, L., Martin, J.W., 2016. Isomer–specific distribution of perfluoroalkyl substances in blood. Environ. Sci. Technol. 50 (14), 7808–7815. https://doi.org/10.1021/acs.est.6b01698.

Katsikantami, I., Sifakis, S., Tzatzarakis, M.N., Vakonaki, E., Kalantzi, O.I., Tsatsakis, A. M., Rizos, A.K., 2016. A global assessment of phthalates burden and related links to health effects. Environ. Int. 97, 212–236.

Koch, H.M., Bolt, H.M., Angerer, J., 2004. Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. Arch. Toxicol. 78, 123–130. https://doi.org/10.1007/s00204-003-0522-3.

Koch, H.M., Müller, J., Angerer, J., 2007. Determination of secondary, oxidised di-iso-nonylphthalate (DINP) metabolites in human urine representative for the exposure to commercial DINP Plasticizers. J. Chromatogr. B 847, 114–125. https://doi.org/10.1016/j.jchromb.2006.09.044.

Kutralam-Muniasamy, G., Shruti, V.C., Pérez-Guevara, F., Roy, P.D., 2022. Microplastic diagnostics in humans:"The 3Ps" Progress, problems, and prospects. Sci. Total Environ., 159164 https://doi.org/10.1016/j.scitotenv.2022.159164.

Latini, G., Felice, De, Presta, C., Del Vecchio, G., Paris, A., Ruggieri, I., Mazzeo, F., 2003. P. Exposure to Di(2-Ethylhexyl)phthalate in humans during pregnancy. Neonatology 83, 22–24. https://doi.org/10.1159/000067012.

Lee, Y.J., Ryu, H.-Y., Kim, H.-K., Min, C.S., Lee, J.H., Kim, E., Nam, B.H., Park, J.H., Jung, J.Y., Jang, D.D., et al., 2008. Maternal and fetal exposure to bisphenol A in Korea. Reprod. Toxicol. 25, 413–419. https://doi.org/10.1016/j.reprotox.2008.05.058.

Leslie, H.A., Van Velzen, M.J., Brandsma, S.H., Vethaak, A.D., Garcia-Vallejo, J.J., Lamoree, M.H., 2022. Discovery and quantification of plastic particle pollution in human blood. Environ. Int. 163, 107199 https://doi.org/10.1016/j.envint.2022.107199.

Li, Z., Mao, W., Yao, L., Zhao, N., Zhang, Y., Zhao, M., Jin, H., 2022. First report on occurrence of bisphenol A iso-mers in human serum and whole blood. J. Hazard. Mater. 424, 127549 https://doi.org/10.1016/j.jhazmat.2021.127549.

Lo Turco, V., Di Bella, G., Potortì, A.G., Fede, M.R., Dugo, G., 2015. Determination of plasticizer residues in tea by solid phase extraction–gas chromatography–mass spectrometry. Eur. Food Res. Technol. 240 (2), 451–458. https://doi.org/10.1007/s00217-014-2344-3.

Lo Turco, V., Potortì, A.G., Ben Mansour, H., Dugo, G., Di Bella, G., 2020. Plasticizers and BPA in spices and aromatic herbs of Mediterranean areas. Nat. Prod. Res. 34 (1), 87–92. https://doi.org/10.1080/14786419.2019.1591403.

Maćczak, A., Cyrkler, M., Bukowska, B., Michałowicz, J., 2017. Bisphenol A, bisphenol S, bisphenol F and bisphenol AF induce different oxidative stress and damage in human red blood cells (in vitro study). Toxicol. Vitr. 41, 143–149. https://doi.org/10.1016/j.tiv.2017.02.018.

Main, K.M., Mortensen, G.K., Kaleva, M.M., Boisen, K.A., Damgaard, I.N., Chellakooty, M., Schmidt, I.D., Suomi, A., Virtanen, H.E., Petersen, J.H., Andersson, A., Toppari, J., Skakkebæk, N.E., 2006. Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in infants three months of age. Environ. Health Perspect. 114 (2), 270–276. https://doi.org/10.1289/ehp.8075.

Marengo, E., Aceto, M., 2003. Statistical investigation of the differences in the distribution of metals in Nebbiolo-based wines. Food Chem. 81, 621–630. https://doi.org/10.1016/S0308-8146(02)00564-2.

Owczarek, K., Kubica, P., Kudłak, B., Rutkowska, A., Konieczna, A., Rachoń, D., Namieśnik, J., Wasik, A., 2018. Determination of trace levels of eleven bisphenol A analogues in human blood serum by high performance liquid chromatography–tandem mass spectrometry. Sci. Total Environ. 628, 1362–1368. https://doi.org/10.1016/j.scitotenv.2018.02.148.

Pastorino, P., Anselmi, S., Zanoli, A., Esposito, G., Bondavalli, F., Dondo, A., Pucci, A., Pizzul, E., Faggio, C., Barcelo, D., Renzi, M., Prearo, M., 2023. The invasive red swamp crayfish (*Procambarus clarkii*) as a bioindicator of microplastic pollution: insights from lake Candia (Northwestern Italy). Ecol. Indic. 150, 110200.

Philips, E.M., Santos, S., Steegers, E.A., Asimakopoulos, A.G., Kannan, K., Trasande, L., Jaddoe, V.W., 2020. Maternal bisphenol and phthalate urine concentrations and weight gain during pregnancy. Environ. Int. 135, 105342 https://doi.org/10.1016/j.envint.2019.105342.

Prata, J.C., da Costa, J.P., Lopes, I., Duarte, A.C., Rocha-Santos, T., 2020. Environmental exposure to microplastics: an overview on possible human health effects. Sci. Total Environ. 702, 134455 https://doi.org/10.1016/j.scitotenv.2019.134455.

Prokić, M.D., Gavrilović, B.R., Radovanović, T.B., Gavrić, J.P., Petrović, T.G., Despotović, S.G., Faggio, C., 2021. Studying microplastics: lessons from evaluated literature on animal model organisms and experimental approaches. J. Hazard. Mater. 414, 125476 https://doi.org/10.1016/j.jhazmat.2021.125476.

Rengarajan, T., Rajendran, P., Nandakumar, N., Lokeshkumar, B., Rajendran, P., Nishigaki, I., 2015. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asian Pac. J. Trop. Biomed. 5 (3), 182–189. https://doi.org/10.1016/S2221-1691(15)30003-4.

Revel, M., Châtel, A., Mouneyrac, C., 2018. Micro (nano) plastics: a threat to human health? Curr. Opin. Environ. Sci. Health 1, 17–23. https://doi.org/10.1016/j.coesh.2017.10.003.

Rios-Fuster, B., Alomar, C., González, G.P., Martínez, R.M.G., Rojas, D.L.S., Hernando, P. F., Deudero, S., 2022. Assessing microplastic ingestion and occurrence of bisphenols and phthalates in bivalves, fish and holothurians from a Mediterranean marine protected area. Environ. Res. 214, 114034 https://doi.org/10.1016/j.envres.2022.114034.

Saitta, M., Di Bella, G., Fede, M.R., Lo Turco, V., Potortì, A.G., Rando, R., Russo, M.T., Dugo, G., 2017. Gas chromatography-tandem mass spectrometry multi-residual analysis of contaminants in Italian honey samples. Food Addit. Contam. Part A 34, 800–808. https://doi.org/10.1080/19440049.2017.1292054.

Sambolino, A., Ortega-Zamora, C., González-Sálamo, J., Dinis, A., Cordeiro, N., Canning-Clode, J., Hernández-Borges, J., 2022. Determination of phthalic acid esters and Di (2-Ethylhexyl) adipate in fish and squid using the ammonium formate version of the QuEChERS method combined with gas chromatography mass spectrometry. Food Chem. 380, 132174 https://doi.org/10.1016/j.foodchem.2022.132174.

Savoca, S., Capillo, G., Mancuso, M., Bottari, T., Crupi, R., Branca, C., Romano, V., Faggio, C., D'Angelo, G., Spanò, N., 2019. Microplastics occurrence in the tyrrhenian waters and in the gastrointestinal tract of two congener species of seabreams. Environ. Toxicol. Pharmacol. 67, 35–41. https://doi.org/10.1016/j.etap.2019.01.011.

Schmidt, C., Krauth, T., Wagner, S., 2017. Export of plastic debris by rivers into the sea. Environ. Sci. Technol. 51, 12246–12253. https://doi.org/10.1021/acs.est.7b02368.

Shahul Hamid, F., Bhatti, M.S., Anuar, N., Anuar, N., Mohan, P., Periathamby, A., 2018. Worldwide distribution and abundance of microplastic: how dire is the situation? Waste Manag. Res. 36 (10), 873–897. https://doi.org/10.1177/0734242X18785730.

Silva, A.B., Bastos, A.S., Justino, C.I., da Costa, J.P., Duarte, A.C., Rocha-Santos, T.A., 2018. Microplastics in the environment: Challenges in analytical chemistry-a review. Anal. Chim. Acta 1017, 1–19. https://doi.org/10.1016/j.aca.2018.02.043.

Škrbić, B., Szyrwińska, K., urišić-Mladenović, N., Nowicki, P., Lulek, J., 2010. Principal component analysis of indicator PCB profiles in breast milk from Poland. Environ. Int. 36, 862–872. https://doi.org/10.1016/j.envint.2009.04.008.

Sola-Larrañaga, C., Navarro-Blasco, I., 2009. Chemometric analysis of minerals and trace elements in raw cow milk from the community of Navarra, Spain. Food Chem. 112 (1), 189–196. https://doi.org/10.1016/j.foodchem.2008.05.062.

Strungaru, S.-A., Jijie, R., Nicoara, M., Plavan, G., Faggio, C., 2019. Micro- (nano) plastics in freshwater ecosystems: abundance, toxicological impact and quantification methodology. TrAC Trends Anal. Chem. 110, 116–128. https://doi.org/10.1016/j.trac.2018.10.025.

Sunman, B., Yurdakök, K., Kocer-Gumusel, B., Özyüncü, Ö., Akbıyık, F., Balcı, A., Özkemahlı, G., Erkekoğlu, P., Yurdakök, M., 2019. Prenatal bisphenol a and phthalate exposure are risk factors for male reproductive system development and cord blood sex hormone levels. Reprod. Toxicol. 87, 146–155.

Torres-García, J.L., Ahuactzin-Pérez, M., Fernández, F.J., Cortés-Espinosa, D.V., 2022. Bisphenol A in the environment and recent advances in biodegradation by fungi. Chemosphere, 134940. https://doi.org/10.1016/j.chemosphere.2022.134940.

Vandenberg, L.N., Chahoud, I., Heindel, J.J., Padmanabhan, V., Paumgartten, F.J., Schoenfelder, G., 2010. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ. Health Perspect. 118 (8), 1055–1070. https://doi.org/10.1289/ehp.0901716.

Wan, H.T., Leung, P.Y., Zhao, Y.G., Wei, X., Wong, M.H., Wong, C.K., 2013. Blood plasma concentrations of endocrine disrupting chemicals in Hong Kong populations. J. Hazard. Mater. 261, 763–769. https://doi.org/10.1016/j.jhazmat.2013.01.034.

Wittassek, M., Angerer, J., 2008. Phthalates: metabolism and exposure. Int J. Androl. 31 (2), 131–138. https://doi.org/10.1111/j.1365-2605.2007.00837.x.

Yang, M., Ryu, J.H., Jeon, R., Kang, D., Yoo, K.Y., 2009. Effects of bisphenol A on breast cancer and its risk factors. Arch. Toxicol. 83 (3), 281–285. https://doi.org/10.1007/s00204-008-0364-0.

Zhang, K., Hamidian, A.H., Tubić, A., Zhang, Y., Fang, J.K., Wu, C., Lam, P.K., 2021. Understanding plastic degradation and microplastic formation in the environment: a review. Environ. Pollut. 274, 116554 https://doi.org/10.1016/j.envpol.2021.116554.