CHEMICAL AND TOXICOLOGICAL IMPACT APPROACH OF ORGANIC CONTAMINANTS ASSOCIATED TO MICROPLASTICS IN URBAN WASTEWATERS FROM LAS PALMAS DE GRAN CANARIA (SPAIN) AND MAHDIA (TUNISIA).

Las Palmas de Gran Canaria (spain) and Mahdia (Tunisia): a comparative study approach of wastewaters contamination.

Hedi Ben Mansour[1,2], Ludovit Schreiber[1], Zoraida Sosa-Ferrera[1], José Juan Santana-Rodríguez[1]

[1] Instituto Universitario de Estudios Ambientales y Recursos Naturales (i-UNAT), Universidad de Las Palmas de Gran Canaria, 35017 Las Palmas de Gran Canaria, Spain
[2] Research Unit of Analysis and Process Applied on the Environment – APAE UR17ES32, Higher Institute of Applied Sciences and Technology, University of Monastir, Mahdia, Tunisia.

Abstract

It has been reported that microplastics (MPs) can adsorb various environmental chemical contaminants, thereby serving as a sink and source of these associated chemical contaminants and potentially changing their toxicity, bioavailability, and fate. Among these compounds different families of so called organic contaminants of emerging concern (OCECs) attract more and more attention in recent years. For this, we have prospected in this work to identify and quantify OCECs by using Ultra High Performance Liquid Chromatography coupled to mass spectromtetry in tandem detector (UHPLC-MS/MS) in the influents of wastewater treatment plants (WWTPs) from Las Palmas de Gran Canaria (Spain) and Mahdia (Tunisia). Results indicated the contamination of MPs isolated from influent LPA-WWTP by four OCECs and MPs isolated from influent Mahdia-WWTP by three of them. The simultaneous

occurrence of multivarious contaminants does not go with consequences on the environment and human health. In fact a battery of eucaryotic (comet and micronucleus assays) and procaryotic assay (SOS chromotest) were carried out and showing a high correlation between the level of contamination with OCECs and the level of the DNA damage.

Keywords: wastewater treatment plants; genotoxicity, organic contaminants of emerging concern; microplastics; Las Palmas de Gran Canaria; Mahdia.

INTRODUCTION

It is obvious that urban wastewater has been the major problem of environmental pollution for years, given the standards required by control bodies which are increasingly restrictive. Several chemical molecules can exist in wastewater and escape conventional treatment systems and reach the receiving environment, which is usually the sea. Organic contaminants of emerging concern (OCECs) represent an important group of these contaminants and for some time they have attracted the attention of environmental researchers and control authorities worldwide. In this way, and for example, pharmaceutical products have been the subject of many studies and have been detected with concentrations ranging from a few nanograms to several micrograms per liter in ~~the~~ municipal WWTPs (Afsa et al., 2020 ; Tahrani et al., 2017; Mohapatra et al., 2014 ; Kołecka et al., 2019). It's true that major concern has been given to the contamination of wastewater by pharmaceutical products and UV-Filters and many researchers work this. However these contanminanats could exist free in wastewaters but also adsorbed in the MPs. Until now, few studies have focused on OCECs associated with MPs despite the fact that some studies have shown that MPs have a significant capacity to fix emerging micropullants (Camacho et al.,2019, Magadini et al., 2020, Atugoda et al., 2021). In fact, microplastics are well known for vector transport of hydrophobic organic contaminants, and there are growing concerns regarding their potential adverse effects on ecosystems and human health (Atugoda et al., 2021). This concern is very important because contaminants can be released into the environment and cause serious disturbances to the ecosystem but also to human health.

In this research, different organic contaminants of emerging concern associated to MPs – influent samples from two urban wastewater treatment plants (WWTPs) in Las Palmas de Gran Canaria (Spain) (LPA-WWTP) and Mahdia Tunisia (Mahdia –WWTP), have been identified and quantified through an optimized method by using Ultra High Performance Liquid Chromatography coupled to MS/MS detector (UHPLC-MS/MS). On the other hand genotoxicity was performed with the prokaryotic SOS chromotest assay using *Escherichia coli* PQ37. Genotoxic effect was also assessed by two complementary *in vitro* eukaryotic tests : the comet test and the micronucleus test on human liver hepatocellular carcinoma (C3a) cells. All obtained results are compared and discussed according to the origin of WWTPs.

MATERIAL AND METHODS

The collection of water samples (2.5 L) was carried out in November 2023. The influent were collected from two wastewater treatment plants of Las Palmas de Gran Canaria (Spain) and Mahdia (Tunisia) using instant sampling and respecting the hydraulic retention time. All the aforementioned samples were filtered and the MPs were collected and kept in amber glass bottles at -20°C, until they were analysed. It should be noted that we did not find MPs in the effluents, which is why the study focused only on the influents.

Extraction of OCECs from MPs

We used Minitab® software to conduct an experimental design and evaluate the ultrasound extraction procedure for the analytes adsorbed on MPs. This allowed us to study the impact of various variables on the extraction process and examine their correlations. We investigated three variables at two levels. The complete experimental design was performed using three different solvents types (Methanol, Acetonitrile, Methanol/Acetonitrile 50:50, v/v), extraction volume (5 - 10 mL) and extraction time (10 - 50). The optimal extraction conditions were as follows: Methanol was used as an extractant, the solvent volume was 5 mL, and the extraction time was 30 min.

Ultrahigh-performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS)

Following extraction, an ultra high-performance liquid chromatography system coupled to a triple quadrupole mass spectrometer (UHPLC-MS/MS) was employed for the separation and detection of the analytes. This system consists of a quaternary pump, sample manager capable of injecting up to 96 samples, column oven and a triple quadrupole detector) with an electrospray interface (ESI). The UPLC system was controlled, and results were obtained using MassLynx Mass Spectrometry software. They were all supplied by Waters (Massachusetts, USA). The separation was performed on a Phenomenex Kinetex PS C18 analytical column (100 mm × 2.1 mm, 2.6 µm) from Phenomenex (California, USA). The chromatographic separation was carried out in gradient mode using a mobile phase consisting of water with 5 mM ammonium formate (pH adjusted to 3.2 with formic acid) (A) and methanol with 0.05% formic acid (v/v) (B). The gradient starts with 5% A: 95% B during the first minute, then it changes to 0% A: 100% B in 6 minutes, and after 4 minutes of cleaning step, gradient returns to the initial conditions. The total chromatogram time lasted 15 minutes. The injected extract volume was 5 µL. For mass spectrometry detection, the following ESI parameters were employed: a capillary voltage of 3 kV, a radio frequency lens voltage of 2.5 V, an extractor voltage of 3 V, and source and desolvation temperatures set at 150 °C and 500 °C, respectively. The cone gas flow was maintained at 50 L/h, while the desolvation gas flow was set to 600 L/h. Nitrogen served as the desolvation gas, and argon was utilized as the collision gas."

Gentoxicity investigation

For the genotoxic activities the samples of the PhACs extracted from MPs of influent samples from two urban wastewater treatment plants (WWTPs) in Las Palmas de Gran Canaria (Spain) (LPA-WWTP) and Mahdia (Tunisia) (Mahdia – WWTP) were prepared according to the technique described by Tahrani et al. (2017). Thus, the tested concentrations were 1 µg/assay, 10 µg/assay, and 50 µg/assay. The genotoxicity was carried out through three assays : The prokaryotic test (i) *SOS chromotest assay* using *E. coli* PQ37

was carried out according to the procedure described by Ben Mansour et al. (2007). The SOS-induction potency (SOSIP) was calculated from the linear part of the induction factor (IF) dose-response as a measure of genotoxicity. The eucaryotic assays (ii) Comet assay and Micronucleus test were peformed in *in vitro* culture cells of the human hepato-cellular liver carcinoma cells (C3a) as the method described by Tahrani et al. (2017).

Statistical analysis

All experiments were repeated independently at three times, and mean values were calculated. For results of SOS chromotest, the Univariate statistical analysis was performed using the SPSS v22 software (SPSS, Chicago, IL, USA). Differences between means were assessed using the one-way ANOVA test. Comparative analysis was performed by Tukey's HSD test. If we have the same letter the values of p was <0.05 and the difference was considered significant. For Comet and micronucleus assays : Statistical analysis of the results was performed using SPSS standard version 13.0 software. The significance of the differences between mean values was estimated using a non-parametric Kruskall-Wallis test. Significance was set at $p < 0.05$. The significance of differences between mean values was estimated using the nonparametric Kruskall-Wallis test. Asterisk indicates the significant differences ($p<0.01$).

RESULTS AND DISCUSSION

Chemical analysis

Some organic contaminants which extracted from MPs deriving from influents of wastewater samples collected in November 2023 from Gran Canaria and Tunisia WWTPs were identfied using UHPLC-MS/MS. In total four micopolluantants were detected at different concentrations. Indeed we detected antidepressants (O-desmethyl venlafaxine), antifungals (Clotrimazole) and UV-filters (Avobenzone and Octocrylene). We also noticed that the MPs collected from influential LPA-WWTP are significantly more loaded with organic contaminants than those from influential Mahdia-WWTP (Table 1). This is clearly observed in the case of organic UV -filters whose concentrations of Avobenzone (406 ng/l) and Oc-

tocrylene (504 ng/l) are of the order of 3 times the concentrations in the influential Mahdia-WWTP. The high concentration of the UV filters detected in the MPs is attributed to the capacity the ability of MPs to adsorb organic contaminants especially UV filters (O'Donovan et al., 2020). The very high concentration of O-desmethyl venlafaxine in the MPs of influent LPA-WWTP can be explained by the excessive consumption of antidepressants by the population of Las Palmas de Gran Canaria as a result of COVID-19 and its consequences (Moreno et al., 2023). On the othe hand, the presence of two UV filters like Avobenzone and Octocrylene in both studied WWTPs, from two turistic areas, can be explained by the common use of these compounds as components of sunscreen products. These results are agree with the study by Cadena-Aizaga et al. (2023) who studied the presence of UV filters in wastewater samples from LPA-WWTP.

Contaminant	Extracted contaminants from MPs of influent LPA-WWTP Extracted contaminants from MPs of influent LPA-WWTP		Extracted contaminants from MPs of influent Mahdia-WWTP	
	Concentration (ng/L)	RSD (%)	Concentration (ng/L)	RSD (%)
O-desmethyl venlafaxine	38 ± 09	23	Not detected	-
Clotrimazole	19 ± 05	26	05 ± 01	22
Avobenzone	406 ± 65	16	143 ± 27	19
Octocrylene	507 ± 05	1	162 ± 13	8

Table 1. Concentration of OCECs in MPs collected from LPA-WWTP and Mahdia-WWTP (*) n = 3

Gentoxicity analysis

In a series of SOS Chromotest assays on the MPs extracts from influent LPA-WWTP and influent Mahdia-WWTP had no effect on the viability the tester strain.

Overall, significant genotoxicity was observed when tested the samples of influents in the presence of the metabolic hepatic system (S9) given that the IF exceed significantly the value of 1.5. This genotoxic effects increase in a dose-dependent manner. We have

observed that samples of influent of LPA-WWTP induced strongly the genotoxicity when compared to the influent of Mahdia-WWTP in the presence all of the tested concentrations (Table 2a and 2b).

Table 2a. Genotoxic effect of OCECs adsorbed on the MPs from influents wastewater collected from WWTP of LPA and Mahdia and evaluated by the SOS chromotest with *E. coli PQ37* in the presence and in the absence of the metabolic activation system (S-9)

	Extracted contaminants from MPs of influent LPA-WWTP		Extracted contaminants from MPs of influent Mahdia-WWTP	
Dose	Induction factor (IF)			
	+S9	-S9	+S9	-S9
50 µg/assay	3.83±0.07^{c}	3.08±0.02^{e}	2.50±0.^{50a}	2.78±0.02^{d}
10 µg/assay	2.37±0.03^{d}	1.96±0.04^{e}	1.54±0.06^{d}	1.88±0.02^{b}
1 µg/assay	1.76±0.04^{c}	1.89±0.01^{c}	1.27±0.03^{b}	1.71±0.09^{a}

Table 2b. Genotoxic effect of positive controls by the SOS chromotest with *E. coli* PQ37 in the presence and in the absence of the metabolic activation system (S-9)

Positive control	+S9	-S9
Nifuroxazide (10µg/assay)	NA	6.31±0.09
Nitrofurantoin (5 µg/assay)	5.24±0.06	NA

NA : not applicable

We observed the presence of micronuclei after exposure to different concentrations of MPs extracts influents, which might have been the result of a chromosome laggard during anaphase. The genotoxic effects observed using a battery of prokaryotic and eukaryotic bioassays can be explained by the presence of UV-filetrs even at low concentrations. But it is true that the UV-filters are the main responsible for genotoxicity in the MPs of influent-WWTP (O'Donovan et al., 2020) as demonstrated already described results other contaminants could be cause perturbation in DNA.

Table 3. Micronuclei assay results determined in C3a cells treated by OCECs adsorbed on the MPs from influents wastewater collected from WWTP of LPA and Mahdia.

Dose	Binucleated cells with micronuclei			
	Extracted contaminants from MPs of influent LPA-WWTP	Extracted contaminants from MPs of influent Mahdia-WWTP	PC	NC
50 µg/assay	18,67±1.03**	9,33±0.07*	-	-
10 µg/assay	9,33±1.07**	6,66±1.04*	-	-
1 µg/assay	5,34±0.06**	4,67±0.03*	-	-
			19,61±1.09	2,40±0.60

NC: Negative control (unexposed cells); PC: positive control (MMS)

**p < 0.01; *p < 0.05

In the same way the same observations were noted with in vitro eukaryotic tests carried out on human hepatocellular liver carcinoma cells (C3a) (Table 4). Indeed, the MPs coming from influent LPA-WWTP remains the most genotoxic (to a lesser extent the influent deriving from MPs influent Mahdia-WWTP). We have performed the Comet assay, also known as single cell gel electrophoresis assay. It is a rapid and sensitive method for the detection of DNA damage in individual cells induced by a variety of genotoxic agents (Mustapha et al., 2013).

Table 4. Total DNA damage observed in human cell line C3A estimated by the alkaline Comet assay after 24 h of treatment by the extract contaminants from MPs of influents wastewater collected from LPA and Mahdia WWTPs

	% Tail DNA	
Dose	Extract contaminants from MPs of influent LPA-WWTP	Extract contaminants from MPs of influent Mahdia-WWTP
50 µg/assay	62,67±1.03**	42,33±3.07**
10 µg/assay	29,33±2.07**	16,66±2.04**
1 µg/assay	21,34±1.06*	14,67±1.03*

**p < 0.01; *p < 0.05

Acknowledgements

Dr. Hedi Ben Mansour thanks the Spanish government his Maria Zambrano grant. Dr Ludovit Schreiber thanks the European

Union his post-doc grant agreement No.101090291 (IMPACTAS project). Also, this work has been partially funded by Spanish Ministry of Science and Innovation through the Project HORPLANAT (PID2020-118966RJ-I00).

REFERENCES

1. Afsa S, Hamden K, Lara Martin PA, Mansour HB. Occurrence of 40 pharmaceutically active compounds in hospital and urban wastewaters and their contribution to Mahdia coastal seawater contamination. Environ Sci Pollut Res Int. 2020. 27:1941-1955.

2. Atugoda T, Vithanage M, Wijesekara H, Bolan N, Sarmah AK, Bank MS, You S, Ok YS. Interactions between microplastics, pharmaceuticals and personal care products: Implications for vector transport. Environ Int. 2021. 149:106367.

3. Ben Mansour H, Corroler D, Barillier D, Ghedira K, Chekir L, Mosrati R. Evaluation of genotoxicity and pro-oxidant effect of the azo dyes: Acids yellow 17, violet 7 and orange 52, and of their degradation products by Pseudomonas putida mt-2. Food Chem. Toxic. 2007. 45:1670–1677.

4. Cadena-Aizaga MI, Montesdeoca-Esponda S, Sosa-Ferrera Z, Santana-Rodríguez JJ. Corrigendum to Occurrence and environmental hazard of organic UV filters in seawater and wastewater from Gran Canaria Island (Canary Islands, Spain)'. Environ. Poll. 2023. 335: 22242

5. Camacho M, Herrera A, Gómez M, Acosta-Dacal A, Martínez I, Henríquez-Hernández LA, Luzardo OP. Organic pollutants in marine plastic debris from Canary Islands beaches. Sc. Total Environ. 2019. 662: 22–31

6. Debra L. Magadini, Joaquim I. Goes, Sarah Ortiz, John Lipscomb, Masha Pitiranggon , Beizhan Yan. Assessing the sorption of pharmaceuticals to microplastics through in situ experiments in New York City waterways. Sc. Total Environ. 2020. 729: 138766

7. Dellai A, Dridi D, Lemorvan V, Robert J, Cherif A, Mosrati R, Mansour HB. Decolorization does not always mean detoxification: case study of a newly isolated Pseudomonas peli for decolorization of textile wastewater. Environ Sci Pollut Res Int. 2013. 20:5790-6.

8. Golovko, O., et al., Occurrence and removal of chemicals of emerging concern in wastewater treatment plants and their impact on receiving water systems. Sc. Total Environ. 2021. 754: 142122.

9. Kołecka K, Gajewska M, Stepnowski P, Caban M. Spatial distribution of pharmaceuticals in conventional wastewater treatment plant with Sludge Treatment Reed Beds technology. Sci Total Environ. 2019. 647:149-157.

10. Mohapatra DP, Brar SK, Tyagi RD, Picard P, Surampalli RY. Analysis and advanced oxidation treatment of a persistent pharmaceutical compound in wastewater and waste-

water sludge-carbamazepine. Sci Total Environ. 2014: 470:58-75.

11. Mustapha N, Zouiten A, Dridi D, Tahrani L, Zouiten D, Mosrati R, Cherif A, Chekir-Ghedira L, Mansour HB. 2016. Comet assay with gill cells of Mytilus galloprovincialis end point tools for biomonitoring of water antibiotic contamination: Biological treatment is a reliable process for detoxification. Toxicol Ind Health. 32:686-93.

12. O'Donovan S, Mestre NC, Abel S, Fonseca TG, Carteny CC, Willems T, Prinsen E, Cormier B, Keiter SS, João Bebianno M. Effects of the UV filter, oxybenzone, adsorbed to microplastics in the clam Scrobicularia plana. Sci Total Environ. 2020. 733:139102.

13. Tahrani L, Van Loco J, Anthonissen R, Verschaeve L, Ben Mansour H, Reyns T. Identification and risk assessment of human and veterinary antibiotics in the wastewater treatment plants and the adjacent sea in Tunisia. Water Sci Technol. 2017. :3000-3021.

14. Huang W, Song B, Liang J, Niu Q, Zeng G, Shen M, Deng J, Luo Y, Wen X, Zhang Y. Microplastics and associated contaminants in the aquatic environment: A review on their ecotoxicological effects, trophic transfer, and potential impacts to human health. J Hazard Mater. 2021. 405:124187.