A Focus on HORIBA European Network Activity around Microplastics

マイクロプラスチックの課題に対する欧州でのHORIBAの取り組み

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Current research on microplastics, ranging from discovering or confirming their presence in various environments, to quantifying their induced pollution in food matrices, to understanding their impact on wildlife, ecosystems and even human health has become a hot topic worldwide. Europe has always been attentive to environmental issues and prompt to sponsor coordinated programs at the academic level, but also to regulate the different industries whose activities may generate microplastics. This featured article will detail a few initiatives from HORIBA Scientific in Europe to contribute to the harmonization of analytical methods, including sample collection and preparation, as well as to the development and validation of standard reference samples in the field of microplastics research.

近年，環境マイクロプラスチックの研究は世界中で注目を集めている。その研究範囲はさまざまな環境下におけるマイクロプラスチックの存在を確認するところから始まり，食品への混入量，野生生物や生態系，さらには人間の健康に及ぼす影響評価にまで及んでいる。ヨーロッパでは環境問題に対する関心が非常に高く，産学連携という学術レベルでの連携プログラム構築の推進にとどまらず，マイクロプラスチックの発生源になると想定される多くの産業に対し規制をかけてゆく可能性が非常に高いと想定される。本項では，環境マイクロプラスチック研究で必要な標準試料の開発・検証だけでなく，サンプリング，前処理を含む分析方法の標準化に力を入れてきた，欧州HORIBAグループ科学セグメント(HORIBA Scientific in Europe)の取り組みを紹介する。

Introduction

Microplastics (MPs) are present in every environmental compartment, including in the remotest places on earth, and have gained recent interest as a major environmental pollutant. In 2015, the European Union (EU) produced 25 million tons of plastic waste, with 60% still originating from packaging, representing an average of 31 kg per person per year. Worth noting is the fact that the majority of the MPs released in the ocean originate from synthetic textiles, tire dust or city dust.

“Plastic” is not a well-defined term, but rather encompasses a set of synthetic polymeric materials having a wide range of high molecular weight, and whose particle dimensions span 6 orders of magnitude in size, from the nanometer up to 5 mm. MPs present a large variety of chemical compositions: (co)polymers, residual monomers, chemical additives, catalysts or fillers, and can even be contaminated by non-intentionally added substances. While naturally occurring polymers exist, such as rubber or cotton, plastic pollution mostly originates from a few synthetic polymeric families like Polystyrene (PS), polyethylene (PE), polypropylene (PP), polyvinylchloride (PVC) or polyethylene terephthalate (PET).

This diversity gave rise to a search of a variety of methodologies to answer the burning questions in MPs research and to support plastic pollution monitoring and mitigation policies under consideration by state and non-state actors. The existence of various definitions for different regulatory sectors and regions also complicates understanding and implementation of legislation.

Moreover, no validated and harmonized standard methods are currently available for the analysis of MPs and many analytical protocols and techniques are used. There is still no consensus on the reporting format, in terms of number of particles, mass of size fractions, and an absence of certified reference materials to investigate analytical proficiencies.

Those points were highlighted during the Global Summit on Regulatory Science (GSRS) 2019 Nanotechnology and Nanoplastics which took place in Ispra (Italy) in September 2019,[1] organized by the European Commission Joint Research Center (JRC), whose mission is to provide scientific advice and support to the European Union policy.

All this explains why open interlaboratory studies were recently set up in order to address those shortcomings.

Evidence of microplastics in food

HORIBA Scientific is proud to count world-leading research teams among his customers of Raman spectrometers. Their affiliations reveal the wide range of fields where Raman microscopy is used to study MPs: environment institutes, health and food safety authorities, oceanology and hydrology departments, marine biology agencies, ecotoxicology laboratories, but also water treatment and distribution entities or bottled water companies.

Clearly, the most pressing question on the scientific community agenda is whether or not MPs pose a threat to human health, especially through seafood consumption. To that extend, the European Food Safety Authority (EFSA) Panel for Contaminants in the Food Chain (CONTAM) was asked, following a request from the German Federal Institute for Risk Assessment (BfR), to deliver a statement on the presence of MP (but also nanoplastics) in food, with particular focus on seafood.[2]

This bibliographic review confirmed that MPs can be ingested by many marine invertebrates and have the potential to be transferred between trophic levels, as illustrated in Figure 1. Indeed, the presence of plastic debris, indicated as anthropogenic debris, in the gastrointestinal tract of fished on sale for human consumption was sampled from markets in several countries.

Following on this statement, a team of researchers from IFREMER (French Research Institute for Exploitation of the Sea) and ANSES (French Agency for Food, Environmental and Occupational Health & Safety) has developed a protocol to extract and characterize MPs from seafood tissues, which should be implemented to assure the relevance and comparison of further studies or assess seafood product quality, notably to follow recommendation from the OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic,

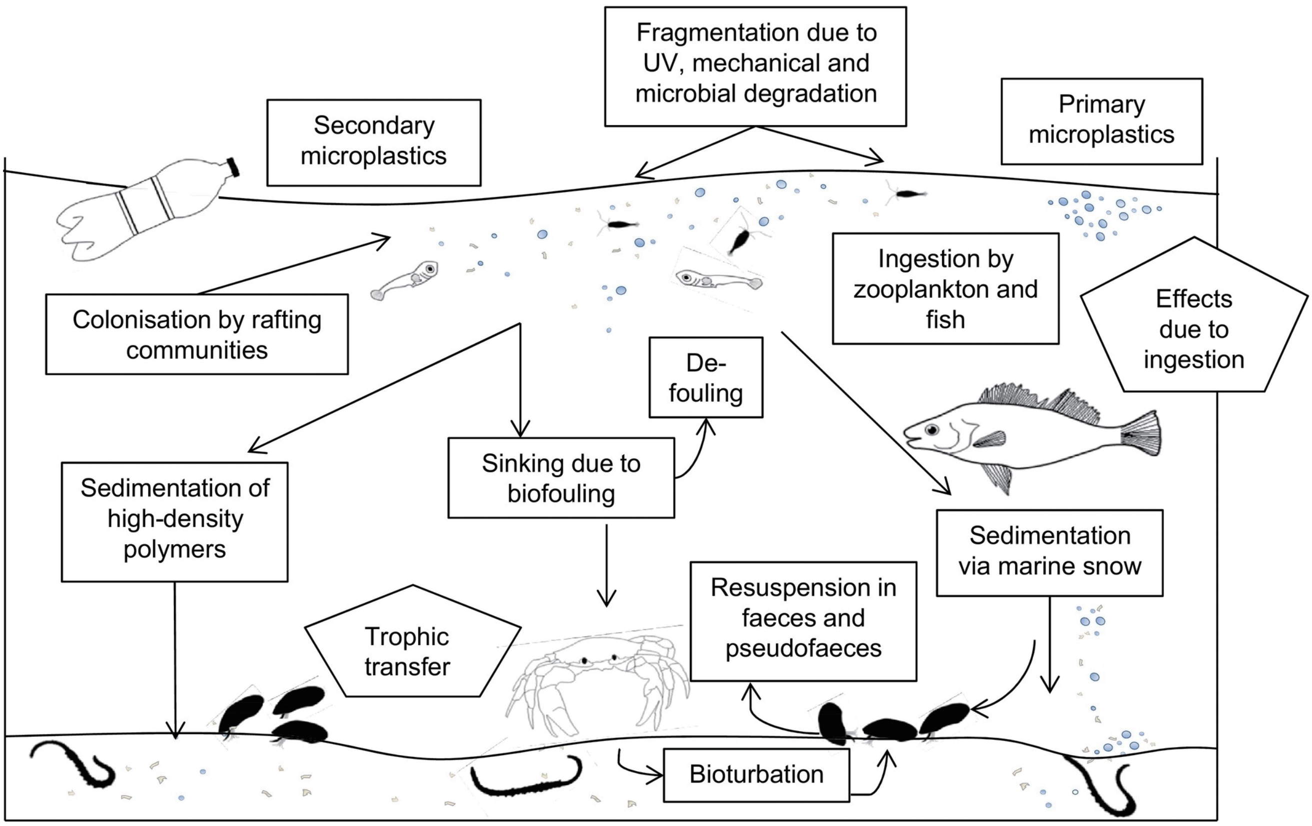

Figure 1 Potential pathways for the transport of microplastics and their biological interactions.[1]

ratified by 15 EU countries in relation with the Marine Strategy Framework Directive.

Plastic integrity and composition was evaluated through microscopic inspection and the use of HORIBA LabRAM Raman spectrometer, before and after digestion by KOH 10% solution with 24 h incubation at 60°C.[3]

Again, related to fish meal pollution, HORIBA Scientific recently participated in a study which demonstrated that cultured (farmed) organisms could be exposed to high levels of MPs via contaminated fish/shellfish used in fish meal production by the aquaculture industry.[4] The most abundant isolated plastic polymer was PE (63.0%) followed by PP (27.8%) and PET (8.8%), while the average size of the particles was found to be 855 µm.

Another publication coauthored by HORIBA Scientific in Scientific Reports[5] made the headlines when it revealed the presence of MPs even in commercial sea and lake salts originating from 8 different countries (Figure 2). This study also raised concerns over the possible transfer of other contaminants associated with MPs into salt, such as pigment fragments, some of them being toxic.

Another milestone article investigated the presence of MPs in mineral waters from different bottle types.[6] Led by scientists from the Bavarian Health and Food Safety Authority (LGL) in Germany, the team focused on small particles (below 5 µm) posing higher toxicological risks as they have the potential to translocate into body tissues and are more likely to penetrate deeply into organs. Using a HORIBA XploRA PLUS system to locate and identify particles down to a size of 1 µm on specially prepared aluminum coated polycarbonatemembrane filters, evidence of higher amounts of MPs in reusable bottles (PET as well as glass) was found compared to single use bottles.

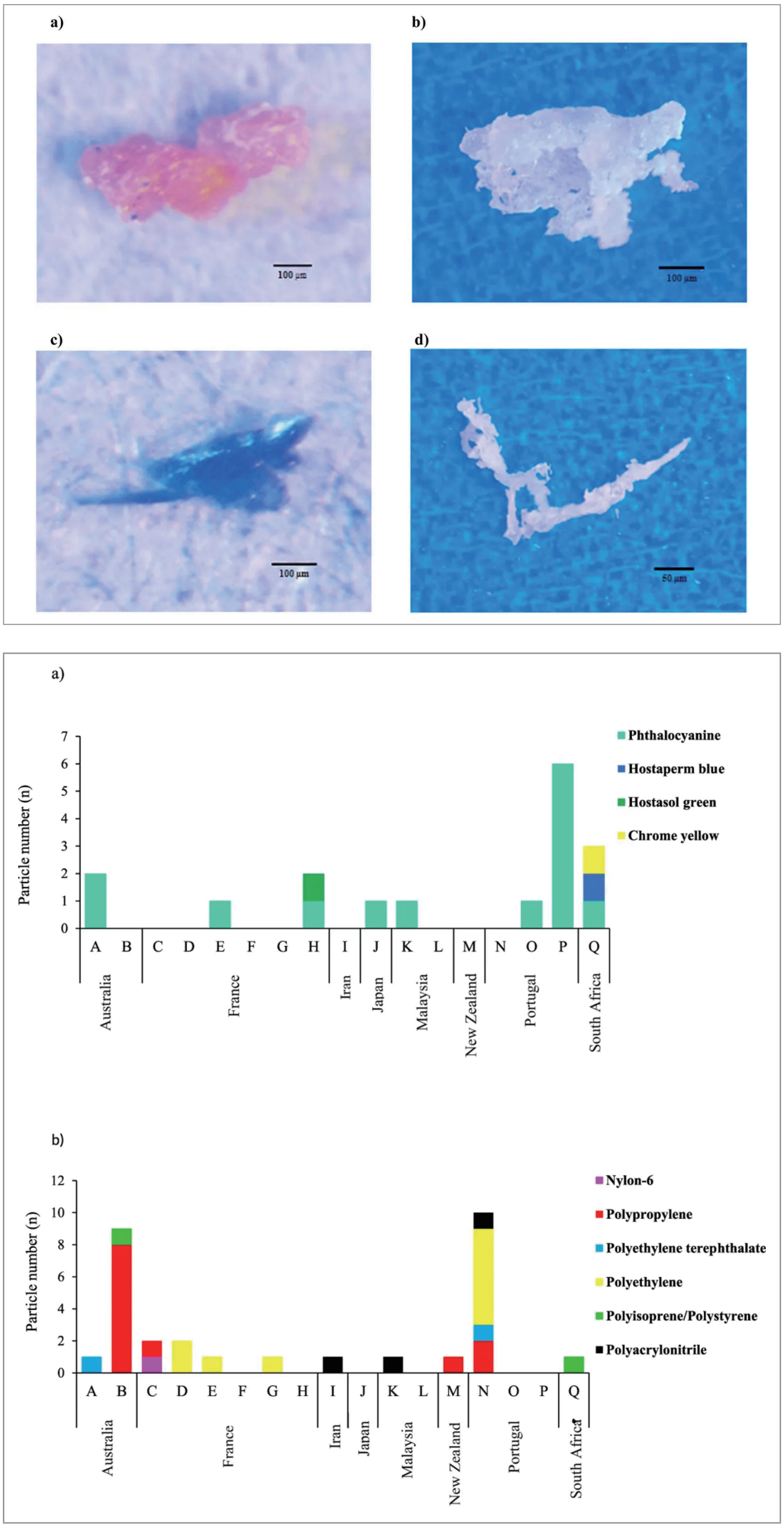

Figure 2 Top: Microscopic images of some of the extracted particles. (a) polyisoprene/polystyrene, (b) polyethylene, and (c) pigment (phthalocyanine) fragment. Image (d) is a nylon-6 filament.
Bottom: Stacked bar chart of the number of (a) plastic polymer and (b) pigment particles isolated from different salt brands.[5]

Open interlaboratory studies

A recent meeting hosted by the Group of Chief Scientific Advisors of the European Commission,[7] supported by the evidence review of the SAPEA Consortium (Science

Advice for Policy by European Academies),[8] concluded on the lack of harmonized methodologies in order to generate standardized data. On this basis, several organizations initiated collaborative programs with the aim of validating internal laboratories quality assessment and competence, supporting environmental data, providing data for national and international stakeholders or supporting accreditation.

One of the initiatives in which HORIBA took part was set up by the Vrije Universiteit Amsterdam (VUA), the Norwegian Institute for Water Research (NIVA) and the WEPAL (Wageningen Evaluating Programmes for Analytical Laboratories) organization based in the Netherland and recognized by the Dutch Accreditation Council (RvA).

This international interlaboratory study on MPs, called QUASIMEME for “Quality Assurance of Information in Marine Environmental monitoring”, saw 34 laboratories participating to analyze the test materials between May in August 2019, using several instrumental and quantification methods, with the objective of counting the particles and identifying their chemical family.

Test samples were prepared at NIVA, to enable the analysis by a broad variety of analytical methods and techniques: visual, hyperspectral imaging, Fourier transform Infrared Spectroscopy (FT-IR), Raman and Mass Spectrometry ; and consisted of 6 preproduction pellets, 5 tablets containing microplastics fragments (obtained after filtration of PET, PVC and PS powder) of fibres and 1 blank tablet. The fibres were created by washing polyester blankets in a typical domestic washing machine. While the majority of the participating laboratories used ATR-FT-IR (Attenuated Total Reflection FT-IR) or µ-FT-IR, we employed Raman microscopy (Figure 3), which is favorable for small size particles, typically below 20 µm.

Table 1 shows an example of reported table by the participants for one of the tablet sample. Although some polymer misidentification occurred in some cases, the polymer type was correctly assigned for both larger preproduction pellets (2-4 mm) and particles or fibres added to the tablets (150-300 µm). However, the reported number of particles varied considerably (up to 78% standard deviation), and the standard deviations of the determination of the polymer type in the tablets varied from 29% (for PET) to 99% (for PS).

Overall, the results of this first round indicate that polymer identification and quantification of the number of plastics particles in a sample (especially in smaller size fractions) is not simple of straightforward. Yet, HORIBA’s Applications Laboratory was able to demonstrate analytical results on par with recognized European facilities.

This round of the QUASIMEME study will be followed by exercises with increasing complexity and difficulty of samples, including MPs extracted from complex matrices (e.g. sediments and fishes). After several study rounds, the analytical methodologies for MPs are expected to be better comparable and will be included in a routine profi-

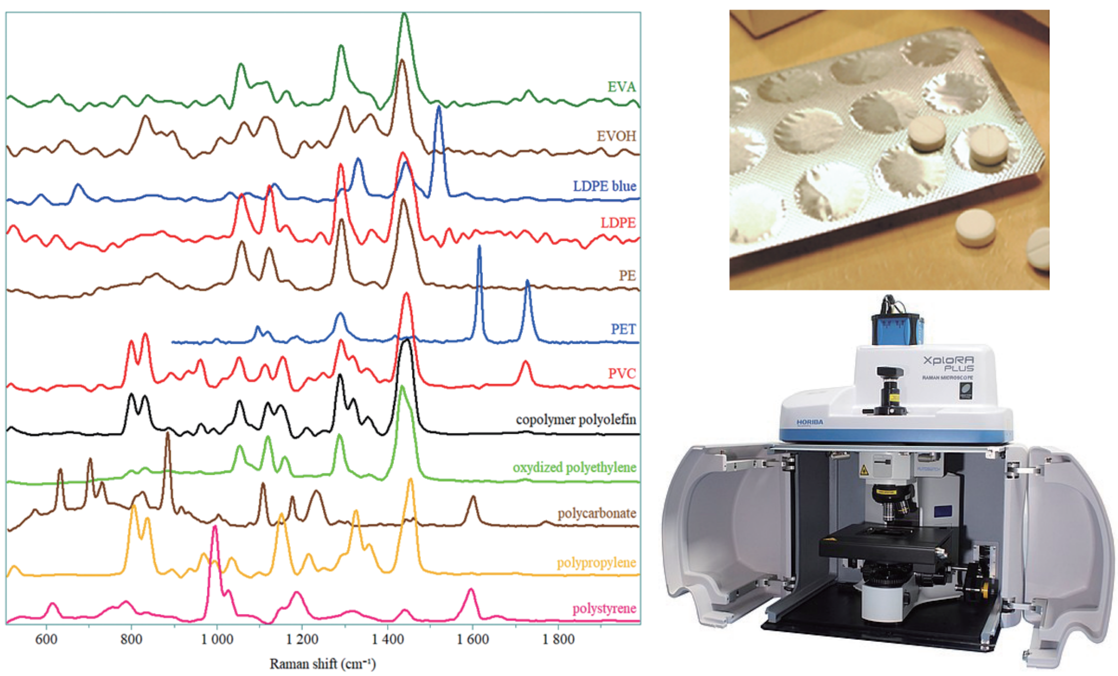

Figure 3 Left: Raman spectra recorded on different polymer families.
Top right: aluminum strip pellet containing 12 tablets sent to participants, that were to be dissolved in analytical grade water to control background contamination.
Bottom right: HORIBA Xplora PLUS Raman microscope with class I laser enclosure used for this study.

Table 1 Type and number of plastic particles reported for table in position no. 10 in the strip pellet shown in Figure 3, by all the participating laboratories of the QUASIMEME study.

Laboratory	acrylonitrile butadiene styrene	Black fiber	Blue fiber	Cellulose	Cellulose fiber black	Cellulose fiber white	Crystaline particles	Grey fiber	Grey piece	High density polyethylene	Low-density polyethylene	polymethylmethacrylate	Polyamide	polybutylmethacrylate	Polycarbonate	Polyester	Polyethylene	polyethylene terephthalate	Polypropylene	Polystyrene	Polytetrafluoroethylene	Polyurethane	Polyvinylchloride	red fiber	TiO2	Unknown	Total particles
H221																44											44
Q101				8														5	< 3	8			14				35
Q104					1	85												9		13			18				126
Q110																		3								4	7
Q114																		2								28	30
Q134											1							7	1	26			26				61
Q152																							34			3	37
Q153																										48	48
Q871																		7	2	37							46
Q968		2						1	1																		4
Q3175			3			6														10				2			21
Q3231																8				13						19	40
Q3239																											
Q3872																	8	11		33			21				73
Q3873	1											1					30		8	39							79
Q3876																				5			10				15
Q3877																											
Q3878																		31		22							53
Q3879																		5	5	6			17				33
Q3882																				11			30				41
Q3883																8				17			19				44
Q3884																				28			27				55
Q3885																		1		6			20				27
Q3887																		7		23			26				56
Q3888	1										8			1				10	6	51	2	1	10				90
Q3889							17			7																	24
Q3890													1					3		29			32				65
Q3891															30			6		12			14		3		65
Q3892																		3		2			11				16
Q3894																											
No. of reporting labs	2	1	1	1	1	2	1	1	1	1	2	1	1	1	1	3	2	15	6	20	1	1	16	1	1	5	27
Average	1	2	3	8	1	46	17	1	1	7	4.5	1	1	1	30	20	19	5.5	4.4	17.1	2	1	20.2	2	3	18.5	42.0
Standard deviation						56					4.9					21	16	4.0	3.9	13.3			9.25			20.9	24.3

ciency testing scheme.

HORIBA Scientific also recently responded to a call to enter an exploratory study organized by the JRC, with support from the German Federal Institute for Materials Research and Testing (BAM).[9] The aim of this proficiency test study on MPs in water in sediments is to help in the identification of possible method candidates for future validation and standardization.

In practice, reference samples employed to benchmark laboratories were developed and qualified beforehand. Those samples were sent to the different laboratories to be prepared on site through a reconstitution protocol, from vials containing a NaCl-carrier with embedded PET particles, a surfactant solution (triton X-100), and deionized water. Participants are to report the number of particles or mass of particles above 30 µm, the particles identified as PET, particles identified as plastic (including PET) and particles of any kind, with a report of the measurement uncertainty.

A workshop will take place during the summer of 2020 to discuss the results and conclusions once the participants report their findings.

Finally, HORIBA France is actively involved in a group of experts within the French Standardization Association (AFNOR) currently working on establishing a regulation on the analysis of MPs in drinking water, through spectroscopic techniques (µFT-IR and Raman). This group, part of the T91M "*Organic micropollutants*" Commission, gathers various governmental, academic and industry organizations, including the Standardization Bureau for Plastics and Plastics Engineering (BNPP), with the objective of drawing up a new norm for the first half of 2021.
This work was presented at the last ISO (International Organization for Standardization) meeting held by the Technical Committee TC 147 on Water Quality in Tokyo, with the purpose of reaching a global consensus in the near future.

Challenge and perspectives

With the improvement of the robustness of analytical techniques, researchers working in the field of MPs will more easily be able to trust their results and compare their studies.

The most pressing question to answer, as little is known at this point, concerns the toxicity of MPs on human health. In particular, an important aspect revolves on the fact that MPs both absorb and give off toxic chemicals and harmful pollutants, which may build up over time and stay in the environment.

There is also a clear lack of knowledge on nanoplastics (particles smaller than 0.1 µm), which may represent a greater risk to the environment and health. However, their characterization is currently hindered by technical limitations and will require new instrumental developments.

To conclude, it is worth mentioning that the microplastics scientific community will gather at MICRO 2020[10] in Arrecife (Spain), the major biannual international conference focusing on the fate and impacts of microplastics.

HORIBA Scientific will be present !

* Editorial note: This content is based on HORIBA's investigation at the year of issue unless otherwise stated.

References

[1] https://ec.europa.eu/jrc/en/event/conference/gsrs19-global-summit-regulatory-science-2019-nanotechnology-and-nanoplastics

[2] Presence of microplastics and nanoplastics in food, with particular focus on seafood. *EFSA Journal*, **14**(6), 4501 (2016).

[3] Alexandre Dehaut, Anne-Laure Cassone, Laura Frère, Ludovic Hermabessiere, Charlotte Himber, Emmanuel Rinnert, Gilles Rivière, Christophe Lambert, Philippe Soudant, Arnaud Huvet, Guillaume Duflos, Ika Paul-Pont. Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environmental Pollution*, **215**, 223-233 (2016).

[4] Samaneh Karbalaei, Abolfazl Golieskardi, Dorothy Uning Watt, Mathieu Boiret, Parichehr Hanachi, Tony R. Walker Ali Karami. Analysis and inorganic composition of microplastics in commercial Malaysian fish meals. *Marine Pollution Bulletin*,**150**, 110687 (2020).

[5] Ali Karami, Abolfazl Golieskardi, Cheng Keong Choo, Vincent Larat, Tamara S. Galloway & Babak Salamatinia. The presence of microplastics in commercial salts from different countries. *Scientific Reports*, **7**, 46173 (2017).

[6] Barbara E. Oßmann, George Sarau, Heinrich Holtmannspötter, Monika Pischetsrieder, Silke H. Christiansen, Wilhelm Dicke. Small-sized microplastics and pigmented particles in bottled mineral water. *Water Research*, **141**, 307-316 (2018).

[7] Environmental and Health Risks of Microplastic Pollution. doi:10.2777/54199

[8] SAPEA, Science Advice for Policy by European Academies. (2019). A Scientific Perspective on Microplastics in Nature and Society. https://doi.org/10.26356/microplastics

[9] https://ec.europa.eu/jrc/en/science-update/call-laboratories-participate-proficiency-tests-microplastics-drinking-water-and-sediments

[10] https://micro2020.sciencesconf.org/

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