Greenpeace Research Laboratories Analytical Results 2019-01

A 'snapshot' survey of microplastics in surface waters of the Vltava and Labe (Elbe) Rivers in the Czech Republic

March 2019

Introduction

In early September 2018, a total of ten 1 litre samples of surface water were collected from locations on the Labe-Vltava river system in the Czech Republic, including six locations on the Vltava River as it flows through the city of Prague, and four locations on the Labe (Elbe) River to which the Vltava flows, including 3 samples close to the town of Ústí nad Labem and one at Hřensko, where the Labe crosses the Czech border into Germany. The sample set included waters collected upstream, adjacent to and downstream from the Praha Troja sewage treatment plant in Prague and similarly upstream, adjacent to and downstream from the Neštěmice sewage treatment plant in Ústí nad Labem. Although only a limited, 'snapshot' survey of microplastic contamination in surface waters at three key locations on the Vltava – Labe river system, to our knowledge this is nonetheless the first such survey conducted in the Czech Republic.

All samples were returned to the Greenpeace Research Laboratories at the University of Exeter (UK) for analysis for the presence and identify of microplastic fragments and fibres, using Fourier Transform Infra-Red (FT-IR) microscopy. Details of the samples received, including their GPS co-ordinates, are provided in Table 1. Approximate locations of the sample sites are shown in Figure 1.

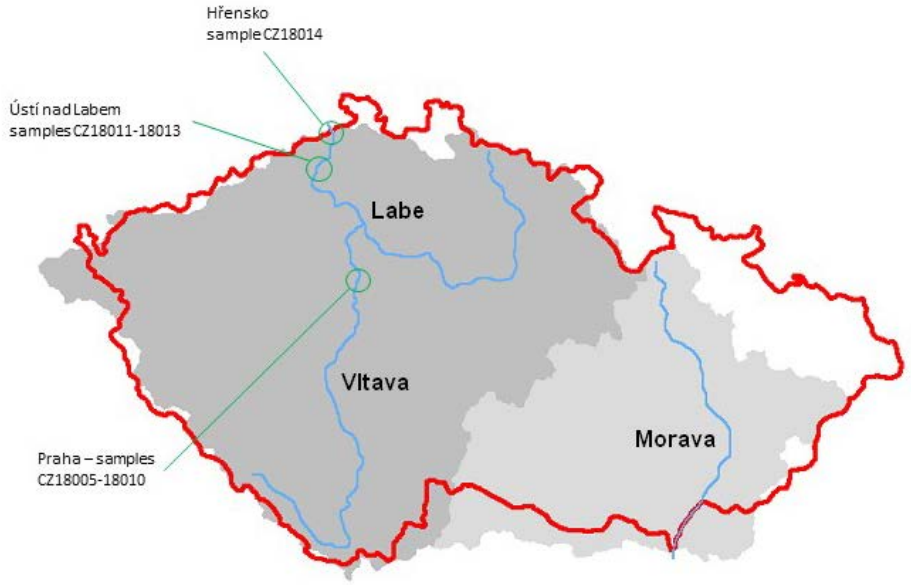

Figure 1: *approximate locations for the 10 surface water samples collected in September 2018*

Samples code	Sampling date	Sampling time	Location	Coordinates
CZ18005	05.09.18	10:43	Port Praha Smíchov	50.0541308N, 14.4114894E
CZ18006	05.09.18	11:11	Prague -Botič river inlet	50.0671014N, 14.4146139E
CZ18007	05.09.18	15:11	Port Praha Libeň, next to Rokytka river inlet	50.1077906N, 14.4665767E
CZ18008	05.09.18	15:31	Praha Troja, 100 m upstream from wastewater treatment plant (WWTP) outlet	50.1167950N, 14.3987006E
CZ18009	05.09.18	15:44	Praha Troja, next to WWTP outlet	50.1173678N, 14.3974400E
CZ18010	05.09.18	15:51	Praha Troja, 300 m downstream from WWTP	50.1300647N, 14.4006300E
CZ18011	06.09.18	11:15	Ústí nad Labem, 100 m upstream from Neštěmice WWTP	50.6671958N, 14.1069925E
CZ18012	06.09.18	11:30	Ústí nad Labem, next to Neštěmice WWTP	50.6692372N, 14.1110694E
CZ18013	06.09.18	11:45	Ústí nad Labem, 300 m downstream from Neštěmice WWTP	50.6745953N, 14.1183650E
CZ18014	06.09.18	14:12	Hřensko - Czech-German border	50.8876497N, 14.2335500E

Table 1: *Details of the ten (10) surface water samples collected in the Czech Republic, September 2018*

Materials and methods

Prior to sample collection, all sample bottles (1 litre Schott Duran bottles) were detergent washed, rinsed 3 times with deionised water and then a further 3 times with 5 µm-filtered deionised water in order to remove any plastic particles or fibres from the inner surfaces. All other glassware used subsequently in the handling, filtration, storage and analysis of the samples were also pre-cleaned using the same procedure, with the final three rinses being carried out immediately before use. Two additional bottles were prepared in the laboratory using this procedure and filled with 5 µm-filtered deionised water to act as procedural blanks. Cotton lab-coats were worn throughout glassware preparation and sample handling.

In the field, samples were collected directly from the river surface (at the locations listed above) into the pre-cleaned 1 litre glass bottles, filled on the up-current side of a small boat. Samples were recapped immediately and transferred to the cold and dark in a cool box, before being transferred to a

refrigerator for storage prior to sample shipment. All samples arrived at our laboratories on 26th September and were immediately transferred to a refrigerator for storage at 4°C prior to analysis.

Working in a pre-cleaned fume cabinet (turned off and with the sash closed to minimise airflow and dust deposition), each sample was shaken for approximately 20s, and then decanted into a 1 litre pre-cleaned glass measuring cylinder to record volume, before filtering the entire sample onto a 47 mm diameter silver-coated membrane filter (pore size 5 µm, Sterlitech), held under vacuum in a pre-cleaned glass vacuum filtration assembly. The filter was removed using forceps when superficially dry and placed into a 50 mm glass petri-dish for storage. Both the silver filters and petri dishes had been inspected before use using a dissecting stereomicroscope under both low and high magnification in order to verify that they were completely free from fibres and fragments.

Filters were bathed in 10 ml of 100 vol. (30%) hydrogen peroxide at 60°C for 4 hours in order to breakdown some of the organic matter that might otherwise obscure any microplastics captured on the filters. Each filter in turn was then place on the cleaned vacuum filter assembly once again and the bathing solution pipetted from the petri dish to pass through the filter, thereby recapturing any fragments or fibres that may have been displaced during the peroxide digest. The peroxide was rinsed through the filters using a further 20 ml of 5 µm-filtered deionised water which had first been used to rinse the corresponding petri dishes.

Each filter was then placed back in its petri dish and inspected immediately under the dissecting stereomicroscope in order to identify candidate microplastics (fibres and fragments) for FT-IR analysis, marking the location of each candidate using a dissecting needle to scratch the silver surface of the filter and number each candidate with Roman numerals. All filters were then dried at 40°C for 18 hours to remove all traces of water before being stored at room temperature in a sealed container prior to analysis.

Individual candidate materials (fibres and fragments) retained on each of the silver filters were subsequently examined using a PerkinElmer Spotlight 400 FT-IR Imaging System (MCT detector, KBr window) operating in reflectance mode across a wavenumber range from 4000 to 750 cm^{-1} and with a resolution of 4 cm^{-1}. A total of 16 scans were collected for at least two sections of each candidate fibre or fragment. The infrared spectra were acquired, processed and analysed using PerkinElmer Spectrum software (version 10.5.4.738), with polymers being identified by automated matching combined with expert judgment against commercially available spectral libraries (including polymers, additives, solvents, etc.) and an additional custom spectral library prepared in our laboratory using a range of polymer standards and potential contaminating materials (e.g. tissues, gloves, laboratory coats). Only match qualities greater than 70% were accepted for identification purposes. Any fibres or fragments appearing on the filters other than those previously marked were excluded.

Because of interference from remaining organic material on the filters which may otherwise have obscured the presence of microplastics, three of the ten sample filters (CZ18007, 18010 and 18013) were digested in hydrogen peroxide (as described above) for a second time, before being filtered once again, dried as before and then re-analysed.

Laboratory code	Sample location	Synthetic materials found	Detailed description of synthetic materials found
CZ18005	Port Praha Smíchov	2 fibres	1 x black polyacrylate fibre 1 x black modified cellulose fibre
CZ18006	Prague -Botič river inlet	2 fibres 1 fragment	2 x transparent modified cellulose fibres 1 x transparent EVA copolymer fragment
CZ18007	Port Praha Libeň, next to Rokytka river inlet	2 fibres 2 fragments	1 x blue modified cellulose fibre 1 x transparent formaldehyde-resin impregnated fibre (possibly chipboard) 1 x transparent/white PVA fragment 1 x transparent/white polynorbornene rubber fragment
CZ18008	Praha Troja, 100 m upstream from wastewater treatment plant (WWTP) outlet	4 fibres	1 x red polyester fibre 1 x white polyester fibre 1 x transparent modified cellulose fibres 1 x blue modified cellulose fibre
CZ18009	Praha Troja, next to WWTP outlet	1 fibre	1 x black polyacrylate fibre
CZ18010	Praha Troja, 300 m downstream from WWTP	no synthetic fragments or fibres	n/a
CZ18011	Ústí nad Labem, 100 m upstream from Neštěmice WWTP	1 fragment	1 x black chlorinated polyethylene fragment
CZ18012	Ústí nad Labem, next to Neštěmice WWTP	10 fibres 8 fragments	1 x transparent formaldehyde-resin impregnated fibre (possibly chipboard) 1 x transparent PTFE fibre 1 x blue nylon fibre 1 x transparent nylon fibre 1 x transparent modified cellulose fibre 2 x blue modified cellulose fibres 1 x black modified cellulose fibre 1 x transparent glass fibre 1 x blue polyester fibre 1 x blue epoxy fragment 2 x red fragments/particles (possibly a urea-fomaldehyde-based copolymer mix) 1 x transparent fragment/film (unidentified polymer film) 3 x white fragments/particles (unidentified polymer/ copolymer) 1 x white fragment/tube (possibly chlorinated rubber)
CZ18013	Ústí nad Labem, 300 m downstream from Neštěmice WWTP	1 fibre 1 fragment	1 x black polyester fibre 1 x white fragment (unidentified fluoropolymer)
CZ18014	Hřensko - Czech-German border	1 fibre 1 fragment	1 x transparent glass fibre 1 x white polypropylene fragment

Table 2: *details of microplastics and other synthetic fibres isolated from the ten river water samples*

Results and Discussion

Of the ten 1 litre surface water samples collected, nine contained at least one confirmed microplastic fibre or fragment (see Table 2), the exception being sample CZ18010 (collected 300 m downstream from the Praha Troja WWTP). Neither of the filtered procedural blanks contained any synthetic fibres or fragments.

In most cases, samples were found to contain between 1 and 4 synthetic fibres or fragments per litre of surface water, with around half those being microplastics, the remainder being made up by cellulosic fibres that were often brightly coloured and fairly uniform in cross-section, indicating that they had been modified through an industrial process rather than simply being natural plant-derived fibres. Fibres varied in diameter between approximately 15 and 35 µm, and ranged from approximately 350 and 4500 µm in length. Fragments fell in a broad size range from approximately 40 x 40 µm to approximately 2500 x 2000 µm. Some examples of the fragments and fibres identified are shown in Figure 2, while Figure 3 shows representative Fourier-transform infra-red (FT-IR) spectra for a number of the different microplastic types identified.

The diversity of fibre and fragment types identified in these samples is illustrated in Figure 4, along with their relative abundances across all samples combined. A total of 12 types of polymer were represented among the microplastic fibres and fragments identified in these samples, as well as a small number of glass fibres, formaldehyde-resin impregnated fibres (possible from fibre-board materials) and a total of 8 cellulosic fibres of uniform cross-section. A further 4 fragments and one piece of transparent film showed many characteristics in their FT-IR spectra typical of common plastics (polyethylene, polypropylene and PVC), the quality of the spectra was not sufficient to give a firm identification.

By far the highest concentration and most diverse mix of fragments and fibres (a total of 18 per litre) was found in surface water sampled adjacent to the Neštěmice WWTP, downstream from the town of Ústí nad Labem (sample CZ18012). Of those 18 fibres and fragments, 5 were identified as modified cellulosic fibres, one a glass fibre and the remainder were microplastic fibres or fragments. This finding suggests that the Neštěmice WWTP was acting as a significant point source of microplastics to the Labe River at the time of sampling.

Taking the sample set overall, however, there appeared to be no consistent relationship between sampling location and either the numbers of fragments or fibres found per litre or the types of material from which they were constituted. For example, unlike the Neštěmice WWTP sample described above, an equivalent sample collected adjacent to the Praha Troja WWTP contained only one identifiable microplastic fibre at the time of sampling, whereas a sample collected 100m upstream from that location contained four identifiable synthetic fibres, two of which were confirmed to be polyester.

Although this may initially seem counter-intuitive, it is important to keep in mind that, within the time and resource constraints of this study, it was possible to collect only single, 1 litre samples from each location, samples which therefore give a ‘snapshot’ of the contaminant levels in the water at that moment of sampling but which cannot be assumed to provide a representative picture of the levels of microplastic contamination integrated over time at each location. This is especially the case with microplastics and other synthetic fibres because, in contrast to dissolved or dispersed chemical contaminants, they are by their very nature discrete contaminants which may therefore be expected to be distributed heterogeneously within any water body and to vary considerably in their concentration over both time and space within any particular water body.

It must also be remembered that, within any urban environment, there will be many potential sources of microplastics to freshwaters, including direct surface run-off and atmospheric deposition, as well as discharges from storm drains, river traffic and from other wastewater treatment plants upstream. In order to estimate average microplastics loadings at different locations along the river system, it would be necessary to collect a number of samples repeatedly from each location at different times, and even then it is likely that the variation between those samples would remain high because of the inherent heterogeneity of microplastics distributions in the environment. Furthermore, in order to investigate the potential contributions from various point sources (including the respective WWTPs) it would be necessary to collect samples specifically from the wastewater discharges themselves, rather than from adjacent receiving waters, as the latter will inevitably be influenced both by the discharge and by other sources upstream.

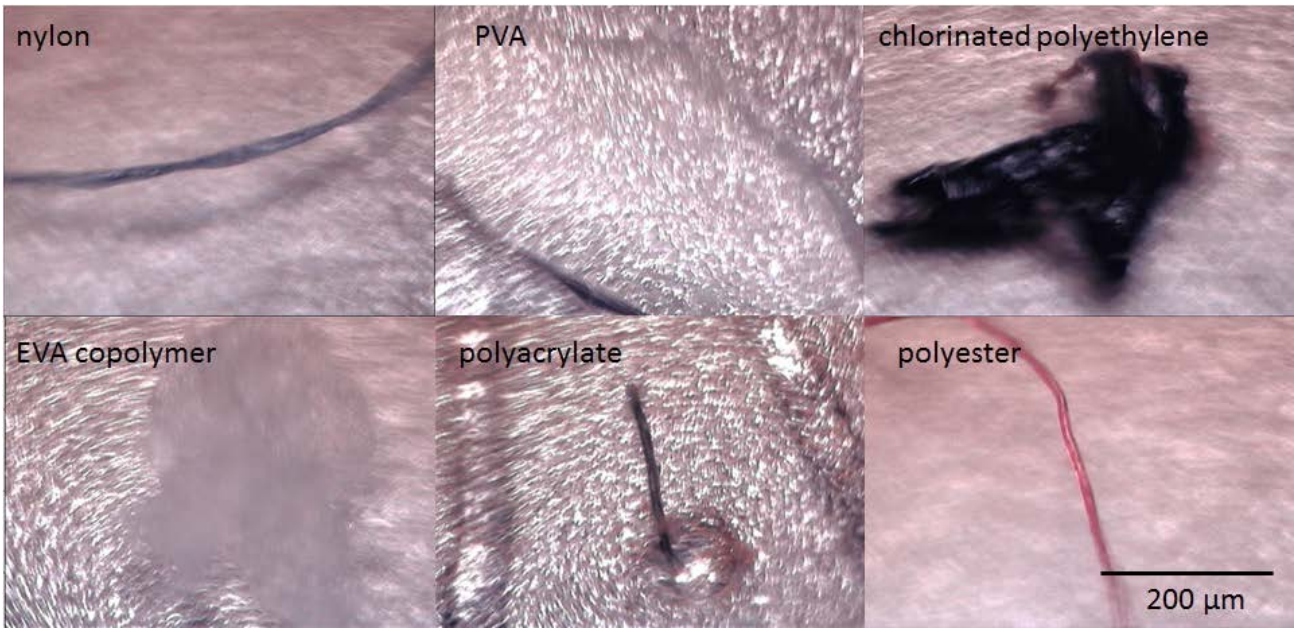

Figure 2: *some examples of the microplastic fibres and fragments found in surface water samples from the Vltava-Labe river system, and their identities as determined by FT-IR microscopy.*

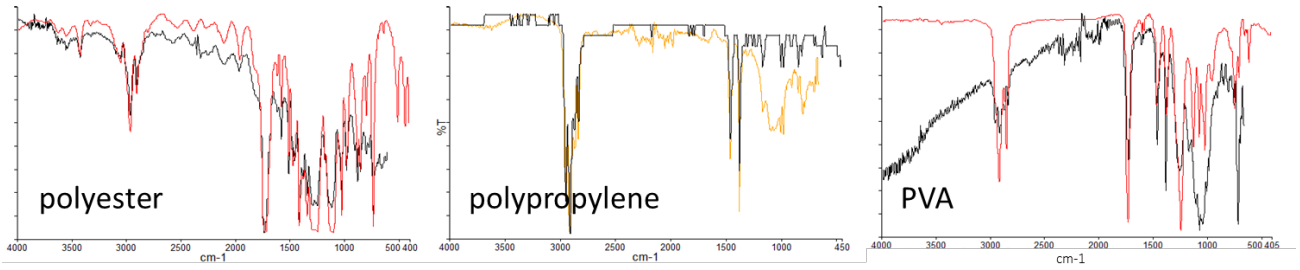

Figure 3: *typical micro FT-IR spectra for microplastic fibres and fragments found in the surface water samples*

Notwithstanding these limitations, it is still possible to consider the results from the ten samples collected in this study as a combined data set, and an indication that, although concentrations did vary considerably from sample to sample, overall the results indicate that microplastics were found in the majority of surface water samples collected from the Vltava-Labe river system at the time of sampling.

The concentrations recorded in this study may appear relatively low, with a mean of 3.7 particles (combined fibres and fragments) per litre (standard deviation 5.2 particles per litre, median 2 particles per litre, range 0-18 particles per litre), but in fact are of a similar order to the concentrations reported for surface waters in some of the few other studies so far available. For example, Su *et al.* (2014) reported average concentrations ranging from 3.4 to 25.8 particles per litre for 5 µm-filtered surface waters collected in Taihu Lake in Jiangsu Province (China), and within a similar particle size range (from 5 - 5000 µm). Zhao *et al.* (2014) reported an average concentration of 4.165 ± 2.460 particles per litre for estuarine waters of the Yangtze River (range 0.5-10.2 per litre), based on collection of water samples at 1 metre depth filtered through a 32 µm steel mesh. More recently, Di & Wang (2018) recorded concentrations ranging from 1.597 - 12.611 particles per litre, with an average of 4.703 ± 2.186 particles per litre, for the waters of the reservoir behind the 3 Gorges Dam, using a similar collection technique but filtering through a 48 µm steel mesh. It should also be noted that, although a few microplastics per litre may sound like a low level of contamination in absolute terms, considering the overall surface area and volume flow rate of the Vltava-Labe river system (averaging around 300 000 litres per second at Ústí nad Labem), even small numbers per litre can add up to high cumulative microplastic loadings flowing downstream.

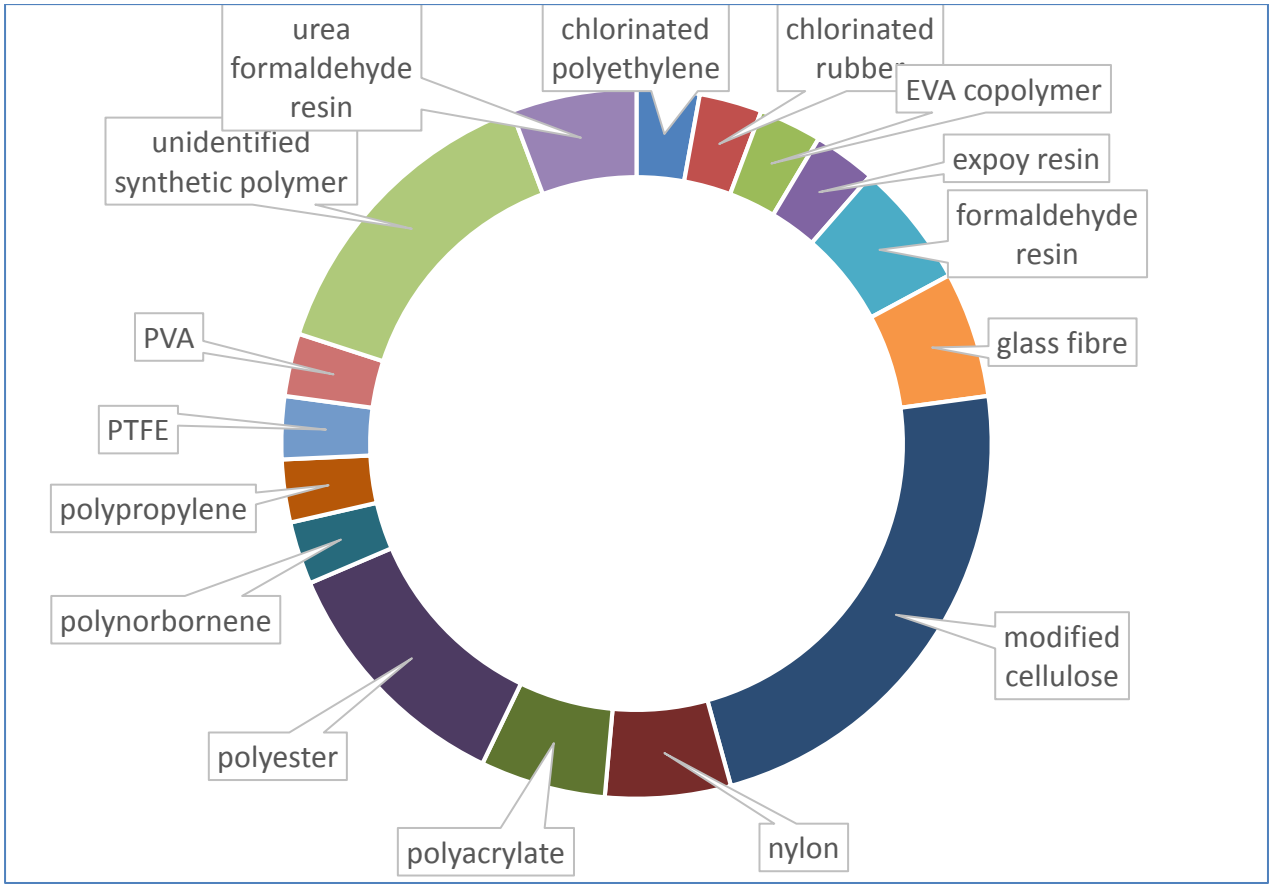

Figure 4: *the diversity of synthetic fibres and fragments, including microplastics, found across all samples combined, with an indication of their relative abundances.*

To date, the majority of studies carried out without European waterways (rivers and lakes) have relied on the use of manta nets or plankton nets with a relatively large mesh size (commonly 330 µm or larger), through which the majority of the microplastics identified in our study would have passed without being retained. Hence the average concentrations reported for surface waters are commonly far lower than those found in our study. For example, using a net with a mesh size of 500 µm, Lechner *et al.* (2014) reported a two year average concentration of only 0.317 particles per m^3 for surface waters of the River Danube, more than 1000 times lower than those we report for smaller size range particles. For the River Rhine, Mani *et al.* (2015) recorded an average of 0.937 particles per m^3 for samples collected in 2010, and less than one tenth of that in 2012, using a manta trawl with a net mesh size of 300 µm, while Faure *et al.* (2015) found an average of 7.0 microplastics per m^3 across a number of Swiss rivers using a similar trawl, with the highest values found in the Venoge (64 particles per m^3), a tributary of the Rhône, during a rain event.

In the River Seine as it flows through Paris, Dris *et al.* (2015) similarly recorded between 0.28 and 0.47 particles per m^3 using a 330 µm mesh manta net, but reported higher levels of between 3 and 108 particles per m^3 when using a plankton net with a mesh size of 80 µm. By filtering whole water samples of treated effluent, the same authors reported between 14-50 microplastic particles per litre, far higher again and even slightly above the range of concentrations we report for the Vltava-Labe system in our study.

Overall, these various studies illustrate that, notwithstanding differences in methods applied, microplastics are contaminants that are common to almost all the surface freshwater systems and samples investigated to date. They also demonstrate that the quantitative results obtained depend very heavily on the specifics of the sampling methodology used, with smaller mesh sizes retaining and revealing the presence of disproportionately higher concentrations of smaller microplastics (<300 µm) compared to larger sizes (>300 µm), perhaps in part as a result of ongoing physical break-up of microplastics into smaller and smaller fragments over time.

For this reason, studies that are able to investigate microplastics in even smaller size ranges, through the application of Raman spectroscopy and electron microscopy for example, have reported very high concentrations of microplastics in water samples, including in wastewaters and in drinking waters. For example, while Pivokonsky *et al.* (2018) report the presence of between 338 and 628 microplastic particles per litre in three samples of treated drinking water from the Czech Republic, the authors note that the majority of those particles (up to 95%) fell in the size range between 1 and 10 µm. Relatively high concentrations of microplastics in these smaller size ranges have also been documented for bottled water samples (e.g. Oßmann *et al.* 2018), though in such cases it is possible that plastic fragments from the filtration and bottling processes themselves may make a substantial contribution.

Such particles are, in any case, below the size range for good resolution and identification with infra-red spectroscopy and would therefore not have been quantifiable in our study. Furthermore, at those very small particle sizes (<10 µm), accurate identification and confirmation of material becomes increasingly difficult and uncertain, such that there may be an increased risk of false positive identifications for microplastics amongst the smallest particle sizes.

Summary

Although only a relatively small, 'snapshot' survey of surface waters on the Vltava-Labe river system, this study has shown that microplastics, sometimes along with other synthetic fibres, could be detected at 9 out of 10 of the locations sampled, illustrating the widespread nature of these contaminants, even if present in most cases at concentrations of only a few fibres or fragments per litre. Across the sample set as a whole, 12 different polymers were represented, in addition to a number of cellulosic fibres whose colours and/or uniform cross sections strongly indicate an industrial/manufactured source rather than a natural source.

Although results for these individual samples cannot be assumed to be representative in themselves of the typical or average concentrations at each sample location, given the discrete and variable nature of microplastics as pollutants and the low absolute numbers captured in the samples, the average concentration calculated for the entire sample set taken together (mean 3.7 particles per litre, median 2 particles per litre) is in the range reported for microplastics of similar size ranges in the few studies so far available on rivers and lakes in other parts of the world, including in China. As far as we have been able to establish, this is the first survey so far of microplastics present in the surface waters of rivers in the Czech Republic.

With more time and resources, it would be possible to conduct a much more detailed survey of microplastic contaminant levels in the Vltava-Labe system, including monitoring the variability in concentrations at individual locations over time and investigating the significance of WWTP discharge and other specific point sources. It should also be noted that our investigation has included only those microplastics present in the top few cm of the river at each location – other sampling strategies would be necessary in order to investigate the abundance of microplastics in deeper waters and in the sediments. Furthermore, although it is not possible to draw any conclusions from our data regarding the potential impact of microplastics on the Vltava-Labe system and its wildlife, the mere presence of these contaminants is an illustration of the complexity of the plastics problem and the difficulties inherent in controlling that problem other than by preventing, as far as possible, their discharge, emissions and losses at source.

References

Di, M. & Wang, J. (2018). Microplastics in surface waters and sediments of the Three Gorges Reservoir, China. Science of the Total Environment 616-617: 1620-1627. DOI: 10.1016/j.scitotenv.2017.10.150

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N. & Tassin, B. (2015). Microplastic contamination in an urban area: a case study in Greater Paris. Environmental Chemistry 12(5): 592-599. DOI: 10.1071/EN14167

Faure, F., Demars, C., Wieser, O., Kunz, M. & de Alencastro, L.F. (2015). Plastic pollution in Swiss surface waters: nature and concentrations, interaction with pollutants. Environmental Chemistry 2(5): 582-591. DOI: 10.1071/EN14218

Lechner, A., Keckeis, H., Lumesberger-Loisl, F., Zens, B.,Krusch, R., Tritthart, M., Glas, B. & Schludermann, E. (2014). The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. Environmental Pollution 188: 177-181. DOI: 10.1016/j.envpol/2014.02.006

Mani, T., Hauk, A., Walter, U. & Burhardt-Holm, P. (2015). Microplastics profile along the Rhine River. Scientific Reports 5: 17988, 7 pp. DOI: 10.1038/srep17988

Oßmann, B.E., Sarau, G., Holtmannspötter, H., Pischetsrieder, M., Christiansen, S.H. & Dicke, W. (2018). Small-sized microplastics and pigmented particles in bottled mineral water. Water Research 141: 307-316. DOI: 10.1016/j.watres.2018.05.027

Pivokonsky, M., Cermakova, L., Novotna, K., Peer, P., Cajthaml, T. & Janda, V. (2018). Occurrence of microplastics in raw and treated drinking water. Science of the Total Environment 643: 1644-1651 DOI: 10.1016/j.scitotenv.2018.08.102

Su, L., Xue, Y., Li, L., Yang, D., Kolandhasamy, P., Li, D. & Shi, H. (2016). Microplastics in Taihu Lake, China. Environmental Pollution 216: 711-719 DOI: 10.1016/j.envpol.2016.06.036

Zhao, S., Zhu, L., Wang, T. & Li, D. (2014). Suspended microplastics in the surface water of the Yangtze Estuary System, China: first observations on occurrence, distribution. Marine Pollution Bulletin 86: 562-568. DOI: 10.1016/j.marpolbul.2014.06.032

For more information please contact:

David Santillo