Freshwater amphipods (Gammarus pulex/fossarum) and brown trout as bioindicators for PFC contamination with regard to the aquatic ecological status of a small stream

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Abstract

Background: Per- and polyfluorinated chemicals (PFC) have been in use for more than 60 years. As a result of their high thermal and chemical stability, they have found numerous applications in industrial processes. However, PFC also commonly show disadvantageous properties such as persistence and ubiquitous presence in the environment. The issue of PFC contamination of surface water is addressed in this publication. One aim of this study was to find a potential bioindicator for PFC contamination of small streams, and a second aim was to determine the aquatic ecological quality of such a stream. Standardized methods were used including structural quality mapping of a watercourse, the PERLODES method and electrofishing in four study sections of the stream. PFC contamination was determined in freshwater amphipods (Gammarus pulex/fossarum) and brown trout.

Results: This study shows that PFC originating from water contamination can be detected both in amphipods and in internal organs of brown trout. The fingerprints in these two species differ considerably from one another. The highest concentrations of PFC were found in the liver and kidneys of brown trout. The methods used in this study also show that the four study sections of the small stream tested fail to achieve the “good” ecological status required by the Water Framework Directive. In particular, this is due to inadequate benthic invertebrates.

Conclusions: Even though it is not possible to determine a causal relationship between the ecological status of the small stream and the detection of PFC in aquatic organisms, appropriate measures must be developed and applied to reduce the spreading of PFC in the environment. In addition to the brown trout, freshwater amphipods proved to be useful as a bioindicator for PFC contamination of streams. In the future it will be necessary to observe whether the number of species in the benthic invertebrates continue to decline.

Keywords: Bioindication, Accumulation, Per- and polyfluorinated chemicals, Liver, Muscle, Kidney, Benthic invertebrates, Watercourse structure quality, PERLODES method, Aquatic ecological status

Background

The term per- and polyfluorinated compounds (PFC) designates anthropogenic chemicals that have been in use for more than 60 years in various industrial processes. This group of substances comprises more than 4730 individual compounds [1]. Due to their special technical properties (a number of them are water and...
dirt repellent, temperature resistant, surface active, chemically very stable and persistent), they find diverse applications [2–4]. PFC are used in a variety of ways, for example in the furniture, paper and textile industry, at fire departments, in agriculture and sports. Among these, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) constitute the two so-called lead substances that are toxicologically most important and the most widespread in the environment [5, 6]. Some PFC are enriched in the environment, in plants, humans and animals [7]. The half-life in humans has been shown to be 8.67 years for PFOS [8] and 4.37 years for PFOA [9]. In animal studies, both substances have been shown to be carcinogenic as well as toxic for the liver and for reproduction [10]. Environmental dispersion takes place by different pathways including the water route, since PFC up to a chain length of eight carbon atoms have comparatively good aqueous solubility [11, 12]. At the same time, PFC contamination can also be shown in soil and in the air. Paths of entry into the environment are, for example, wastewater, fertilizer, sewage sludge or the use of firefighting foams [13]. As a consequence, PFC derived from the soil and water are passed by plants and fed to animals [14, 15] and finally to humans [6, 16].

PFC have been a wide-ranging environmental topic in Germany since 2006 as a result of studies made on water samples taken from the drainage basin of the Ruhr and Möhne rivers in North Rhine-Westphalia (NRW). This contamination originated from surrounding farmlands on which a so-called “soil improver” that was highly contaminated with PFC had been spread. Farmers unwittingly applied this contaminated fertilizer on their fields over a period of years. It is assumed that use of these PFC-contaminated fertilizers began as early as 2003. The PFC were washed out of the soil by precipitation, reached adjoining streams and consequently found their way into the ground water via leaching [11, 12]. The Ministry of the Environment, Agriculture, Nature and Consumer Protection of the State of North Rhine-Westphalia (MUNLV NRW) subsequently informed the Hessian Ministry of the Environment, Climate Protection, Agriculture and Consumer Protection (HMUKLV) about the PFC contamination. It was then determined that contaminated fertilizer was spread on fields in Hesse from 2003 to 2006. The State of Hesse then undertook numerous studies on drinking water, surface waters, soil and foodstuffs as well as livestock feed [17, 18]. While fish are considered good bioindicators for PFC in large surface waters [19], no bioindicators for PFC are available for smaller water bodies with few or no fish. The objective of the present study was to find a suitable bioindicator for PFC contamination for small streams in which possibly no fish exist, and to assess the aquatic ecological status of such a small stream in the Hessian highlands using standardized methods. A stream was chosen with adjoining fields that had been treated with fertilizer containing PFC in the past [20].

**Methods**

**Survey site**

The survey site is an 8.4-km-long small stream in the Hessian highlands, belonging to the Weser river system and is an oligotrophic highland brook Type 5. The catchment basin of the stream covers an area of about 27 km² [21].

Four 50-m study sections for sampling were delineated along the course of the small stream (Fig. 1). Section 1 is located near the source of the stream and near a residential area. It is assumed that the PFC contamination hotspot lies to the west of this segment, as confirmed by PFC measurements in the stream water (Table 1). Section 2 is in a forested area. Sections 3 and 4 are near the mouth of the stream.

![Diagram of the sampled small stream including the four study sections](image-url)
Structure quality

The structural quality at the studied survey sites was assessed using the method proposed by the German Working Group on Water Issues of the Federal States and the Federal Government [22]. Analyzed stream stretches are assigned to quality classes ranging from 1 (not modified) to 7 (completely modified). The quality of the water structure plays an important role in the self-purification capacity as well as the biodiversity of a body of water. The benchmark for evaluation is the Current Potential Ecological Status of surface water (CPES). The CPES is a description of the state of a body of water that would occur after removal of all obstructions and uses and which would be evaluated as quality grade 1. The quality of water structure was recorded on-site for each of the four study sections with the help of assessment questionnaires, and was then subsequently evaluated.

Macroinvertebrate community and ecological status classes

A record of benthic macroinvertebrates is of major significance in the study of the biological components of a body of water [23]. PERLODES is the German standardized system for assessing the macroinvertebrate community. The actual species diversity in the analyzed stream is compared with the expected species diversity for the particular type of body of water and consequently evaluated, allowing conclusions about certain stress factors. This methodology is described in detail in Meier et al. [24]. Sampling was performed according to the PERLODES method for all four study sections. The invertebrates collected were classified in the laboratory according to Bauerfeind and Humpesch [25], LANUV [26], Nagel [27], Sundermann and Lohse [28] and Waringer and Graf [29]. Computer-assisted evaluation was performed using the software ASTERICS 4.0.4 [30].

Sampling of bioindicator organisms on-site

Electrofishing was used to obtain samples of trout. The basis for the procedure is the passive monitoring of fish as an indicator of accumulation VDI 4230 Blatt 4 [31]. Sampling of fish by electrofishing was performed in November of 2018 according to DIN EN 14011 [32]. The procedure was carried out by trained technical personnel with appropriate equipment. Fishing in the study sections was performed against the flow using direct current. Six brown trout (Salmo trutta fario) were collected from each of the study sections 2, 3 and 4. No fish were found in section 1. Freshwater amphipods (Gammarus pulex/fossarum) were also collected at each of the sections 1 to 4 using a landing net by kick sampling (Fig. 2). It was not possible to make an on-site classification between the two species of amphipod (Gammarus pulex/fossarum), because it is not possible to differentiate between these species with the unaided eye.

Sampling and sample analysis

After sample collection the freshwater amphipods were transferred to 96% ethanol and the brown trout placed in coolers with ice (−10 °C) for interim storage. Subsequent storage of the samples was at the THM (Technische Hochschule Mittelhessen). The amphipods were rinsed with demineralized water to remove the alcohol, weighed on an analytical balance and dried to constant weight in a drying cabinet by 40 °C for 36 h. Drying resulted in a weight loss of about 90% (Table 2). The amphipod samples were stored until analysis at room temperature in glass jars with snap-on plastic lids. The brown trout were stored frozen at −18 °C in plastic bags.

The amphipod and trout samples were sent to the Bavarian Agency for Health and Food Safety (LGL) for preparation and analysis. The individual species (or organs) were pooled, processed and subsequently

| Substance                        | Measuring point 69 (n = 6) Location near section 1 | Measuring point 77 (n = 6) Location near section 2 | Measuring point 119 (n = 6) Location near sections 3 and 4 |
|----------------------------------|-----------------------------------------------------|-----------------------------------------------------|-------------------------------------------------------------|
| Perfluoropentanoic acid (PFPeA)  | 2260 (2000–4200)                                    | 453 (210–740)                                       | 6 (1–12)                                                   |
| Perfluorohexanoic acid (PFHxA)   | 9933 (7000–13,000)                                  | 1238 (670–2000)                                    | 18 (11–32)                                                 |
| Perfluorohexanoic acid (PFHpA)   | 1850 (1300–2900)                                    | 150 (100–230)                                       | 4 (3–5)                                                    |
| Perfluorooctanoic acid (PFOA)    | 1500 (1100–2200)                                    | 171 (69–350)                                       | 8 (2–10)                                                   |
| Perfluorononanoic acid (PFNA)    | < LOQ                                               | < LOQ                                              | < LOQ                                                       |
| Perfluorodecanoic acid (PFDA)    | < LOQ                                               | < LOQ                                              | < LOQ                                                       |
| Perfluorobutane sulfonate (PFBS) | 41 (< LOQ–110)                                     | 8 (6–10)                                           | < LOQ                                                       |
| Perfluorohexane sulfonate (PFHxS)| 0.5 (< LOQ–2)                                      | < LOQ                                              | < LOQ                                                       |
| Perfluorooctane sulfonate (PFOS) | 0.5 (< LOQ–2)                                      | < LOQ                                              | < LOQ                                                       |

* Limit of quantification (LOQ) for all substances: 1 ng/l
The skin was removed from fish muscle tissue before processing. Homogenization of the tissue was performed with a table-top cutter (robot coupe R3-3000; Vincennes Cedex, France). Liver and kidney tissue was homogenized with a metal spatula. The amphipod samples were crushed and simultaneously homogenized in a ceramic mortar with ceramic pestle (Bauscher, Weiden, Germany). Sample weight for fish muscle, liver and kidney was 1 g and for the amphipods 0.25 g per sample. All samples underwent a single determination. Further processing of the samples was performed in the same manner: use of the Quick-Easy-Cheap-Effective-Rugged-Safe-method (QuEChERS) allows a cost-effective and rapid determination of a large number of samples with regard to pesticides and similar substances [33]. A modified variant of the QuEChERS method was applied. The following isotope-labeled standards were first added to the samples at a concentration of 50 μg/ml in methanol (±2.5 μg/ml): (perfluoro-n-[3,4,5-13C3]pentanoic acid, perfluoro-n-(1,2,3C4)hexanoic acid, perfluoro-n-[1,2,3,4-13C4]heptanoic acid, perfluoro-n-(1,2,3,4-13C4)octanoic acid, perfluoro-n-(1,2,3,4,5-13C5)nonanoic acid, perfluoro-n-(1,2,3,4C2)decanoic acid, perfluoro-n-(1,2,3,4C2)undecanoic acid, perfluoro-n-(1,2,3,4C2)dodecanoic acid, Sodium perfluoro-1-[2,3,4,5-13C4]-butanesulfonate, sodium perfluoro-1-hexane[18O2]sulfonate, sodium perfluoro-1-[1,2,3,4-13C4]-octanesulfonate, concentration (all standards obtained from Wellington Laboratories, Canada). A specific isotope-labeled standard was used for subsequent quantification of each analyte. Ten milliliter of ultra-pure water (Merck Millipore, Darmstadt, Deutschland) was then added to each sample, and in the case of the freshwater amphipods the samples were soaked to swell in an ultrasonic bath (Bandelin, Sonorex, Berlin, Germany) for 10 min. After addition of 10 ml acetonitrile (LC–MS Grade, Fisher Scientific, Schwerte, Germany) the samples were manually shaken for 1 min. The samples were then once again placed in the ultrasonic bath followed by treatment with unbuffered salt tube (Supel™ QuE, Sigma Aldrich, Steinheim, Germany), shaking and centrifugation (10 min at 1000 g) (Eppendorf Centrifuge 5810 R, Wesseling-Berzdorf, Germany). The aliquot acetonitrile phase was subsequently collected and 200 μl water added. The samples were then concentrated down to 200 μl in a stream of nitrogen (Barkey, Leopoldshöhe, Germany) at 40 °C.

### Table 2 Weight determination of amphipods (*Gammarus pulex/fossarum*) with comparison of wet and dry weights

| Section 1 | Section 2 | Section 3 | Section 4 |
|-----------|-----------|-----------|-----------|
| Wet weight (g) | 12.6 | 13.1 | 11.5 | 12.1 |
| Dry weight (g) | 1.12 | 1.25 | 1.02 | 1.17 |
| Weight loss (%) | 91.1 | 90.4 | 91.1 | 90.3 |

Fig. 2 Sampling of freshwater amphipods by kick sampling with a net (left) and brown trout caught by electrofishing (right)
An acetonitrile–methanol–mixture (v/v, 1:1) was added for a final volume of 400 µl (methanol hypergrade for LC–MS, Merck, Darmstadt, Germany). The samples were homogenized by shaking on a laboratory shaker (Vortex-Genie-2, Scientific Industries, Inc., Bohemia, New York, USA). Sample analysis was performed by LC–MS/MS (liquid chromatography–mass spectrometry/mass spectrometry) [19, 34] on an API 3000 (AB Sciex, Ontario, Canada). A mixture of water, acetonitrile and ammonium acetate served as eluent. For the LC–MS/MS a gradient-elution column: Gemini 3 µm C18 110A° (100 mm × 2 mm, Fa. Phenomenex, Aschaffenburg, Germany (reversed phase, 18 C-atoms in the side-chain) was used with a precolumn Gemini C18 (4 × 2 mm, Fa. Phenomenex) ESI (electrospray-ionization). The uncertainty of measurement was also determined, calculated from the coefficient of variation and the instrumental deviation. The coefficient of variation was determined by dividing the individual samples into 6 portions. The expanded measurement uncertainty was obtained by multiplying the coefficient of variation by a factor of 2. Since all values were 50% below the measurement uncertainty, in the interest of comparability of the values the analytical laboratory defined an expanded measurement uncertainty of 50%.

Results and discussion

Structural quality

Mapping of the structural qualities was performed on-site in May of 2018. None of the study segments of the small stream were in use for any activities such as sports. The width of the stream is between 1 and 5 m in all sections and the sections are all of the wide river system type. The results of mapping are shown in Table 3.

The results show that the water structure in three of four segments is in unaltered state and therefore supports the achievement of an ecologically “good” status according to the European Water Framework Directive. Study section 1 is the only one showing alterations. This is mostly the result of this section being in close proximity to a residential area, and therefore underwent minor straightening. As a consequence, this section does not display a “natural profile”, resulting in degradation in the overall evaluation [22].

Macroinvertebrate community and ecological status classes

The PERLODES method was carried out on-site from March 11 to March 13 in 2018. Substrate mapping showed that three of the four sections exhibit large substrate diversity. Section 1 showed the least substrate diversity of all. In this section sand and/or mineral sludge (Psammal/Psammopelal) dominate, together with submerged aquatic plants (macrophytes). Common substrates in the sections 2, 3 and 4 were fine to medium gravel (Akal) as well as stones of various sizes (macro-, meso-, microlithal). The substrates found correspond for the most part with the description of a stream Type 5 (coarse material rich siliceous highland stream) [35]. Straightening of the watercourse eliminated the possibility of forming a semi-natural embankment structure. In addition, the stream bed is deeper, resulting in reduced substrate diversity [36].

Three aspects (designated as “modules”) were considered in the evaluation of the ecological state indicated by macroinvertebrates (Table 4). The module with the lowest ranking determines the overall result (“worst-case principle”) [24]. The module “general degradation” presented the “worst case” for each of the four investigated

| Table 3 Results of structure quality mapping in all four investigation segments |
|-----------------------------------|-----------------|-----------------|-----------------|-----------------|
| Channel pattern                   | Weakly altered  | Weakly altered  | Weakly altered  | Unaltered       |
| Longitudinal profile              | Weakly altered  | Weakly altered  | Weakly altered  | Weakly altered  |
| Transverse profile                | Weakly altered  | Weakly altered  | Weakly altered  | Unaltered       |
| Streambed structure               | Moderately altered | Unaltered      | Unaltered       | Unaltered       |
| Embankment structure              | Moderately altered | Unaltered      | Unaltered       | Unaltered       |
| Water-body environment            | Weakly altered  | Unaltered       | Unaltered       | Weakly altered  |
| Overall evaluation                | Moderately altered/grade 3 | Unaltered/grade 1 | Unaltered/grade 1 | Unaltered/grade 1 |

| Table 4 Evaluation of the ecological status based on three modules |
|-------------------------------------------------------------------|
| Section 1 | Section 2 | Section 3 | Section 4 |
| Module saprobity | Good | Good | Good | Good |
| Module acidification | Very good | Very good | Very good | Very good |
| Module general degradation | Bad | Poor | Poor | Poor |
| Module ecological status | Bad | Poor | Poor | Poor |
sections. Thus section 1 was evaluated as “bad”, and segments 2 to 4 were evaluated as “poor” (Table 4).

The results of the module “saprobity”, “acidification” and “general degradation” are assured, meaning that indicator taxa for each of the four study sections were adequately abundant [37]. The module “saprobity” was evaluated as “good” for all four sections. This indicates that organic pollution in the water as well as the resulting oxygen depletion is minor [37]. The module “acidification” was evaluated as “very good” for all four sections. Streams of the Type 5 generally tend toward acidification. This circumstance counteracts the geological substrate in the regions examined. The rocks in the substrate are alkaline, and thus counter the acidification of the stream water [37]. The “general degradation” describes the impact of various stressors and consists of the following metrics (FI = fauna-index; HR = hyporhithral colonizers; EPT = Ephemeroptera, Plecoptera, Trichoptera; RI = rheo index). These metrics are influenced by the structural diversity, the composition of the habitat, flow conditions, sedimentation and the density of the microhabitats within the stream [37, 38]. Specific results of the metrics for each section are shown in Table 5.

The results in the module “general degradation” indicate structural deficits. In particular section 1 received a bad evaluation. Sections 2–4 are evaluated as poor. The main reason for this is the lack of indicator species such as, for example, mayflies (Ephemeroptera). This condition is an indication of a stream type with inadequate macrobenthic community [37, 38]. Section 4 showed the largest biodiversity with 23 species present. This species diversity could not positively affect the overall evaluation, however. Section 1 received the worst evaluation as a result of minimal substrate diversity and the poor rating in the module “general degradation”. Macrobenthic studies were undertaken on sections 2 and 4 by the Hessian State Office for Nature Conservation, Environment and Geology (HLNUG) in March 2005 and April 2007. The results of those studies are compared with the results from the present study in Table 6.

Although more than 10 years have passed, the values for the modules “saprobity” and “acidification” have remained the same. In contrast, however, differences are observed in the module “general degradation”. Whereas section 2 received an evaluation of “good” in the year 2007, section 4 received a rating of moderate, also showing deficits at that time. As seen in Table 4 as a result of the worst-case evaluation, the module “general degradation” is the determining factor in the overall evaluation. Whereas 34 Taxa were found in the year 2007, only 21 were seen in 2018. In 2005, a total of 41 taxa were registered in section 4; in 2018 there were only 23 [21]. The differences may be a result of new stressors such as climate change and agricultural pollutants [39]. As a further possible consequence, displacement of more sensitive by less-sensitive species could have taken place. Additionally, the PFC pollution, which presumably occurred between 2003 and 2006, could come into question as a cause of the loss of species diversity [10].

In addition to data on species diversity, the PERLODES method also comprises an estimation of abundance and can therefore serve as a basis for choosing an appropriate bioindicator. In all four study sections, the order Amphipoda (freshwater amphipods) make up the largest proportion of all taxa found (>50% of the total), as seen in Fig. 3, for example. This order comprised solely the species Gammarus pulex and Gammarus fossarum. Since these species make up the largest part of the biomass they prove to be suitable bioindicators for the accumulation of PFC contamination. This is also true for the sections

### Table 5 Results of the metrics for the module “general degradation”

|                | Section 1 | Section 2 | Section 3 | Section 4 |
|----------------|-----------|-----------|-----------|-----------|
| Fauna index    | Bad       | Poor      | Poor      | Poor      |
| Hyporhithral colonizers | Poor      | Poor      | Poor      | Poor      |
| Ephemeroptera, Plecoptera, Trichoptera | Bad       | Poor      | Poor      | Poor      |
| Rheo index    | Bad       | Moderate  | Moderate  | Poor      |
| Overall result | Bad       | 0.17      | 0.32      | 0.31      | 0.30      |

### Table 6 Comparison of ecological status class from the present study with results from the HLNUG studies from the year 2005 (section 4) and 2007 (section 2) [21]

| Time point of sampling | Section 2 | Section 4 | HLNUG 2007 | 2018 | HLNUG 2005 | 2018 |
|------------------------|-----------|-----------|------------|------|------------|------|
| Module saprobity       | Good      | Good      | Good       | Good | Good       | Good |
| Module general degradation | Good     | Unsatisfactory | Moderate | Unsatisfactory |
| Module acidification   | Very good | Very good | Very good | Very good | Very good |
| Ecological status      | Good      | Unsatisfactory | Moderate | Unsatisfactory |
evaluated as “poor” as well as those with an aquatic ecological status of “unsatisfactory”.

PFC concentrations in freshwater amphipods and brown trout

The results of the PFC analyses in freshwater amphipods (*Gammarus pulex/fossarum*) and brown trout are presented in Tables 7, 8, 9, 10. The results are organized according to study section, whereby no trout were found in section 1. This is possibly the result of the steep V-shaped slope, too shallow water and a relatively straight physical structure of the small stream, which is unattractive to trout because it does not provide shelter or resting areas. In addition, for spawning trout need loose gravel sediment in which to form their spawning pits. Fine bottom substrate, as found in section 1 is not suitable for spawning [40]. The PFC concentrations of the various measurements are graphically presented in Figs. 4, 5, 6, 7.

**Freshwater amphipods**

In all study sections, adequate numbers of freshwater amphipods could be obtained. The PFC concentrations measured in these amphipods are presented in Table 7.

In addition to the PFC substances listed in Table 7, the following substances were analyzed: perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluorononanoic acid (PFUnDA), perfluorododecanoic acid (PFDDA), perfluorobutanesulfonic acid (PFBS), perfluorohexane sulfonate (PFHxS), PFOA-surrogate (KDONA), 6:2 fluorotelomer sulfonic acid (6:2-FTS) and 8:2 fluorotelomer sulfonic acid (8:2-FTS). The concentrations of these substances, however, were below the limit of quantification. Figure 4 shows a graphic representation of the PFC concentrations in freshwater amphipods.

Of particular note is the high concentration of PFOA, especially in section 1, but also apparent in sections 2 and

**Table 7 Result of the PFC determinations in freshwater amphipods (Gammarus pulex/fossarum) in µg/kg**

| Substance                        | Section 1          | Section 2          | Section 3          | Section 4          |
|----------------------------------|--------------------|--------------------|--------------------|--------------------|
| Perfluorobutanoic acid (PFBA)    | <LOQ<sup>a</sup>   | <LOQ               | <LOQ               | <LOQ               |
| Perfluoropentanoic acid (PFPeA)  | 16.4±8.2           | 12.4±6.2           | <LOQ<sup>b</sup>   | <LOQ               |
| Perfluoroheptanoic acid (PFHpA)  | 41±21              | 23±12              | <LOQ<sup>c</sup>   | <LOQ               |
| Perfluorooctanoic acid (PFOA)    | 18.4±9.2           | 7.7±3.9            | 4.8±2.4            | <LOQ<sup>d</sup>   |
| Perfluorooctane sulfonate (PFOS) | 211±106            | 84±42              | 65.0±32.5          | 12.6±6.3           |

<sup>a</sup> Limit of quantification (LOQ) perfluorobutanoic acid: 14 µg/kg  
<sup>b</sup> Limit of quantification (LOQ) perfluoropentanoic acid: 12 µg/kg  
<sup>c</sup> Limit of quantification (LOQ) perfluoroheptanoic acid: 8 µg/kg  
<sup>d</sup> Limit of quantification (LOQ) perfluorooctanoic acid: 4 µg/kg  
<sup>e</sup> Limit of quantification (LOQ) perfluorooctane sulfonate: 2 µg/kg

**Table 8 Results of the PFC analysis of brown trout liver in µg/kg**

| Substance                        | Section 2          | Section 3          | Section 4          |
|----------------------------------|--------------------|--------------------|--------------------|
| Perfluoropentanoic acid (PFPeA)  | <LOQ<sup>a</sup>   | 4.4±2.2            | 8.5±4.3            |
| Perfluoroheptanoic acid (PFHpA)  | 2.0±1.0            | 3.1±1.6            | 5.0±2.5            |
| Perfluoroctanoic acid (PFOA)     | 59±30              | 53±27              | 101±51             |
| Perfluorononanoic acid (PFNA)    | 2.3±1.2            | 1.9±1.0            | 1.5±0.8            |
| Perfluorodecanoic acid (PFDA)    | 3.8±1.9            | 2.6±1.3            | 2.1±1.1            |
| Perfluorooctane sulfonate (PFOS) | 15.1±7.6           | 13.1±6.6           | 7.7±3.9            |

<sup>a</sup> Limit of quantification (LOQ) perfluoropentanoic acid: 2 µg/kg

![Fig. 3 Estimated number of individuals in each order per m² using section 3 as an example](image-url)
3. It is also quite evident that PFC concentrations decline from sections 1 to 4. This trend is evident for PFOA, as well as for PFHpA, PFHxA and total PFC concentration. This indicates that the greatest PFC concentration is section 1 and is thus in the area of the suspected PFC contamination. PFBA and PFPeA were only found in sections 1 and 2. PFOS was only determined in trace levels.

**Brown trout liver tissue**

Analysis was performed on brown trout that were caught in sections 2 to 4. The PFC concentrations in the livers are shown in Table 8. In addition to the PFC substances listed in the table the following substances were tested: perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDDA), perfluorobutanesulfonic acid (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS linear) PFOA-surrogate (KDONA), 6:2 fluorotelomer sulfonic acid (6:2-FTS) und 8:2 fluorotelomer sulfonic acid (8:2-FTS). The concentrations of these substances, however, were below the limit of quantification and are therefore not listed.

By comparison with the results in Fig. 4 (freshwater amphipods), it can be seen that higher concentrations of PFOA are found in the liver of brown trout. PFOS and PFHxA are present in low concentrations. PFPeA, PFHpA, as well as PFNA and PFDA are found in very low concentrations (Fig. 5).

### Table 9 Results of PFC determination in brown trout muscle in µg/kg

|                      | Section 2 | Section 3 | Section 4 |
|----------------------|-----------|-----------|-----------|
| Perfluorooctanoic acid (PFOA) | 5.4 ± 2.7 | 3.7 ± 1.9 | 10.9 ± 5.5 |
| Perfluorooctane sulfonate (PFOS) | 1.1 ± 0.6 | < LOQ | < LOQ |

* Limit of quantification (LOQ) perfluorosulfonic acid: 1 µg/kg

### Table 10 Results of PFC determination in brown trout (kidney) in µg/kg

|                      | Section 2 | Section 3 | Section 4 |
|----------------------|-----------|-----------|-----------|
| Perfluoropentanoic acid (PFPeA) | < LOQa | < LOQ | < LOQ |
| Perfluorohexanoic acid (PFHxA) | 5.6 ± 2.6 | 4.4 ± 2.2 | 5.7 ± 2.9 |
| Perfluoroheptanoic acid (PFHpA) | 2.3 ± 1.2 | 3.0 ± 1.5 | 4.1 ± 2.1 |
| Perfluorooctanoic acid (PFOA) | 59.0 ± 29.5 | 58 ± 29 | 84 ± 42 |
| Perfluorononanoic acid (PFNA) | 2.9 ± 1.5 | 2.4 ± 1.2 | < LOQb |
| Perfluorodecanoic acid (PFDA) | 5.0 ± 2.5 | 3.6 ± 1.8 | 2.3 ± 1.2 |
| Perfluorooctane sulfonate (PFOS) | 16.3 ± 8.2 | 14.6 ± 7.3 | 6.5 ± 3.3 |

* Limit of quantification (LOQ) perfluorononanoic acid: 1.5 µg/kg
|                      | Section 2 | Section 3 | Section 4 |
|----------------------|-----------|-----------|-----------|
| Perfluorooctanoic acid (PFOA) | 59.0 ± 29.5 | 58 ± 29 | 84 ± 42 |
| Perfluorononanoic acid (PFNA) | 2.9 ± 1.5 | 2.4 ± 1.2 | < LOQb |
| Perfluorodecanoic acid (PFDA) | 5.0 ± 2.5 | 3.6 ± 1.8 | 2.3 ± 1.2 |
| Perfluorooctane sulfonate (PFOS) | 16.3 ± 8.2 | 14.6 ± 7.3 | 6.5 ± 3.3 |

* Limit of quantification (LOQ) perfluoropentanoic acid: 1.5 µg/kg

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**Fig. 4** Graphic representation of the PFC concentrations in freshwater amphipods (*Gammarus pulex/fossarum*) in the four sections of the stream. The error bars represent the expanded measurement uncertainty.
Fig. 5 Graphic representation of the PFC concentrations in brown trout livers in the four sections of the stream. The error bars represent the expanded measurement uncertainty.

Fig. 6 Graphic representation of PFC concentrations in brown trout muscle in the four sections of the stream. The error bars represent the expanded measurement uncertainty.
In contrast to freshwater amphipods (Fig. 4), however, no clear concentration gradient was found along the four sections for the trout liver data. Nonetheless, just as with freshwater amphipods, the PFC sum concentration was greatest in section 4. The concentrations in sections 2 and 3 were similarly high. A total of seven individual PFC compounds were found in the liver of the brown trout, whereas only five were found in freshwater amphipods. This difference may be attributed to the accumulative property of the liver [19, 41, 42].

Brown trout muscle tissue
The PFC concentrations in the samples of trout muscle are shown in Table 9. PFC with concentrations under the limit of quantification were not shown for the sake of clarity. It is notable that in contrast to the liver only PFOA and PFOS were detectable in muscle tissue.

Figure 6 shows a graphic representation of the PFC concentrations expanded measurement uncertainty in muscle tissue of brown trout.

It can be seen that PFOA concentrations are low compared to the liver, which is reflected in the total PFAS contamination. PFOS was only detectable in muscle tissue from trout in section 2.

Brown trout kidney tissue
The PFC concentrations in the kidneys are shown in Table 10. Here again, PFC concentrations that are below the limit of quantification were not listed.

As with liver and muscle, PFOA was responsible for the main proportion of PFC in the kidney tissue (Fig. 7).

The results are quite similar to that from the liver (Fig. 5), whereby the PFC concentration in the liver is higher with regard to PFOA. Muscle tissue was found to have considerably lower concentrations of PFC than liver and kidney. This is in agreement with other studies of PFC contamination [41–44]. PFC accumulate in the kidneys, liver and gallbladder among other organs. In general, accumulation in muscle tissue is comparatively low and PFC are almost never found in fatty tissue [3]. As with the other brown trout organs, a concentration gradient along the sampled study sections was not apparent.

Figures 4, 5, 6, 7 show a clear correlation between the PFC concentrations, study sections and indicator species. Whereas the PFC concentrations in the freshwater amphipods consistently decrease from section 1 to section 4 (Fig. 4), there is no such tendency in brown trout, when measuring the concentrations in individual organs. The habitat and behavior of these two organisms differ greatly. Amphipods exhibit a wide dietary spectrum and compared to trout are much more sedentary, as a rule
only drifting or moving upstream a few meters per day within a given segment [45, 46]. They are euryoecious (relatively tolerant of environmental variation) reach an age of up to 2 years and are at the beginning of the food chain in streams [47, 48]. In contrast, brown trout may travel a number of kilometers per day when foraging and in spawning season. They mostly feed on insects and smaller fish, but also freshwater amphipods [49, 50]. They are stenococious, may reach an age of 15 years and as predators find their place at the top of the food chain in the stream [51]. These differences may influence the results of this study. Due to their migratory behaviour, uptake of substances by brown trout cannot be attributed to a particular region (here, individual study sections 2 to 4 of the stream). Instead, the fish visit different segments with possibly varying PFC concentrations (see Table 1), ingesting food that may also be more-or-less contaminated with PFC. Consequently, in direct comparison freshwater amphipods must be considered more site specific and as such are more meaningful indicators of PFC contamination within a defined segment of a stream. It must also be noted that the samples that were studied here were pooled. Therefore, no differentiation could be made depending upon age or size of the trout. The results show that of the two lead components in this study, PFOA makes up the largest part of the total PFC sum concentration in both the freshwater amphipods and in the liver, kidney and muscle of the brown trout. In other studies on liver and muscle of various organisms, PFOS was found at the highest concentrations; for instance in eel in European rivers (up to 498 µg/kg PFOS and up to 23 µg/kg PFOA in the liver; up to 18 µg/kg PFOS in muscle tissue) [44], roach (fish) in the Ems (up to 194 µg/kg PFOS and < 5 µg/kg PFOA in the liver; up to 40 µg/kg PFOS and <5 µg/kg PFOA in muscle tissue) [43], polar bears in the Arctic (up to 3868 ng/g PFOS and up to 17.6 ng/g PFOA in the liver) [52] or wild pigs in Hessen (up to 1780 µg/kg PFOS and up to 45 µg/kg PFOA in the liver; up to 28.6 µg/kg PFOS and up to 7.4 µg/kg PFOA in muscle tissue) [42]. In the present study we could not confirm these results. The highest concentrations of PFOS (16.3 µg/kg) were found in the kidneys of brown trout in section 2, an indication that trout accumulate PFOS significantly more strongly, particularly in the liver and kidneys, than do freshwater amphipods. It is also notable that total (sum) of PFC concentrations in brown trout are lower than in freshwater amphipods (Figs. 4, 5, 6, 7). Since brown trout are at the end of the food chain in the stream they should theoretically accumulate more PFC and therefore have higher concentrations of these substances than freshwater amphipods [3, 53]. The measurements of the freshwater amphipods, however, were made on pooled samples and the stomach contents were not removed. Consequently, this may represent a source of error, since the stomach contents of the freshwater amphipods may also be contaminated with PFC. The contamination of this stream is most likely the result of the use of contaminated fertilizer [20]. Whereas high PFOS concentrations are primarily the result of contamination resulting from the use of fire-fighting foam, from sewage and from electroplating sludge, high PFOA concentrations in streams are among other things, connected with the use of fertilizers [5, 54]. Since the section of the stream with the highest PFOA concentrations (study section 1) is in close proximity to agricultural lands this type of contamination is most likely the main source. The further downstream one goes the lower the individual PFC concentrations become. In the further course of the brook, individual PFC concentrations decrease. In addition, water analyses in the year 2011 showed that PFOA concentrations were higher than PFOS concentrations with an average of 8.9 µg/l for PFOA and <0.05 µg/l for PFOS [55]. PFOA is also more water soluble than PFOS [56]. It is not possible to establish a causal relationship because water testing only represents a snapshot at the moment of sampling. The remaining PFCs such as PFPeA, PFHxA, PFHpA, PFNA and PFDA are found only at low concentrations or are not detectable. These substances make up between 5 and 23% of the total PFC concentration (according to the organ) in the trout (Fig. 8) and between 13 and 40% of total PFC concentration in the freshwater amphipods.

PFPeA, PFHxA and PFHpA are short-chain compounds (<7 perfluorinated carbon atoms), which are more mobile and spread through ground water and the soil more rapidly than do long-chain PFC (>7 perfluorinated carbon atoms), however they are scarcely bioaccumulated. Long-chain compounds such as PFNA and PFDA are prone to bioaccumulation, however this occurs slowly and are especially found in organisms that have been in contact with these compounds for an extended period of time [2, 34]. Little is known about the short-chain compounds, beyond the fact that they are being used in industry as substitutes for long-chain PFC. There are, however, indications that these are possibly of no less relevance toxicologically than long-chain PFC. Short-chain PFC show a lower tendency to accumulate in organisms, however they are more mobile and can therefore more rapidly penetrate raw water, ground water and soil. Toxicological evaluation of short-chain PFC is not presently possible because current information on these substances is inadequate [57]. Limits exist, regulating the amount of PFC in fertilizers (the sum of PFOA + PFOS = 100 µg/kg TM) [13]. Guidelines or reference values have been recommended for drinking water (for example, for PFOS TWLW = 0.1 µg/l) [58]. So-called PNECaquatic exist for surface waters. These describe a
“predicted no effect concentration” (PNEC) in µg/l. Concentrations at which aquatic organisms are considered not to be adversely affected. The PNEC in surface waters for PFPeA is 320 µg/l. No PNEC aquatic exist for PFOA, PFOS, PFNA and PFDA; however, these compounds are listed as SVHC (substances of very high concern) in Annex XIV of the European REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) regulation. As such, threshold values cannot be defined since these substances represent a risk even in low concentrations due to their persistence [59]. The surface water directive stipulates, in form of an Environmental Quality standard (EQS) of natural biota, a maximal concentration for PFOS and its derivatives of 1 µg/kg in muscle tissue of fish. This concentration is exceeded in liver and kidneys of trout from stream sections 2 and 3. The biota EQS only refers to muscle tissue of fish. There is no biota EQS for kidney, liver or other organs of fish since these organs are not usually eaten. According to the environmental quality standard the protection target resource is primarily human health [60]. Under the REACH regulation PFOA was placed on the SVHC (substances of very high concern) list in the year 2013. There are no suitable measures for clean-up of surface PFC contamination, therefore protection, reduction and restriction measures must be followed [61, 62]. Most important, however, are measures that counter further introduction of PFC into the environment. This can primarily be ensured through preventative measures in the form of limits, guidelines, restrictions and bans.

Conclusions
The results presented show an insufficient hydro-ecological quality of the stream studied here. PFC contamination was detected, both in freshwater amphipods and in various organs of brown trout. The macroinvertebrate community should be analyzed tested in the future. Continual monitoring can provide an overview as to whether species numbers decline further. Especially, section 1 should be considered for restoration measures. It stands out among the other sections as a result of straightening and the lack of diversity. As a consequence, this section is in the greatest need of action. It must, however, also be noted that the other sections of the stream do not meet the requirements set by the Water Framework Directive for a “good” ecological condition. Freshwater amphipods would appear to potentially serve as bioindicators for PFC contamination in small streams. These organisms are ubiquitous in streams, even in those that are characterized by a lack of fish due to structural deficits. Freshwater amphipods are easier to obtain than fish samples since they do not require electrofishing. What is more, freshwater amphipods generally represent the majority of the biomass of benthic invertebrates, even in cases of poor ecological conditions. However, further studies will be required to validate these results. In addition, it will be necessary to show whether age (juvenile and adult) plays a role in the accumulation of PFC in the organism, and also whether there are species specific differences (Gammarus pulex und G. fossarum) in accumulation behavior. Since pooled samples were used it was not possible in this study to determine the age of the freshwater amphipods. This must be taken into account in future studies. Other species such as Gammarus roeseli and Gammarus tigrinus of freshwater amphipods should also be investigated regarding their PFC accumulation. Additionally, periodic on-site monitoring of PFC contamination should be undertaken, and dissemination of these substances in the environment should be counteracted. Furthermore, restorative measures must be developed. Surrounding streams, as well as contaminated agricultural areas are potential sources of PFC contamination and pose a potential risk for aquatic ecosystems. As a long-term approach, however, preventative measures alone come into question in order to eliminate the entry of these substances into the environment. Once PFC are introduced into the environment, their removal has proven to be extremely difficult.
**Abbreviations**

6:2-FTS: Fluorotelomer sulfonic acid; 8:2-FTS: 8:2 fluorotelomer sulfonic acid; CPES: Current Potential Ecological Status; EQS: Environmental quality standard; ESL: ElectrospRAY-ionization; HMUKS: Hessian Ministry of the Environment, Climate Protection, Agriculture and Consumer Protection; KODA: PFOA-surrogate; LAWAK: German Working Group on water issues of the Federal States and the Federal Government; Lc–MS/MS: Liquid chromatography–mass spectrometry/mass spectrometry; LGL: Bavarian Agency for Health and Food Safety; LoQ: Limit of quantification; MUNLV NRW: Ministry of the Environment, Agriculture, Nature and Consumer Protection of the State of North Rhine-Westphalia; NRW: North Rhine-Westphalia; PFC: Per- und polyfluorinated chemicals; PFDA: Perfluorododecanoic acid; PFHpA: Perfluorohexanoic acid; FFHxS: Perfluorohexane sulfonate; PFNA: Perfluorononanoic acid; PFOA: Perfluoroctanoic acid; PFOS: Perfluorooctane sulfonate; PFOA: Perfluorooctanoic acid; PFHxS: Perfluorohexane sulfonate; PFHxS: Perfluorohexane sulfonate; PFOA: Perfluorooctanoic acid; FFHxS: Perfluorohexane sulfonate; PN PNEC (water): Predicted No-Effect Concentration in the aquatic environment; QuEChERS: Quick-Easy-Cheap-Effective-Rugged-Safe; REACH: Registration, Evaluation, Authorization and Restriction of Chemicals; SVHC: Substances of very high concern; THM: Technische Hochschule Mittelhessen.

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