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# Entry of heavy metals from anthropogenic discharges into small streams: Where can they be found again? Viewing samples of water, sediment and Gammarus spp. (Crustacea: Amphipoda)

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# Abstract

The entry of heavy metals into rivers affects both water quality and aquatic biocoenosis. Discharged with wastewater, heavy metals are transported downstream, where they accumulate in sediments and are absorbed by aquatic organisms. This study investigates whether anthropogenic heavy metals can be detected in water, sediment and biota samples and which medium is best suited to assess different aspects of riverine contamination, including the role of gammarids as bioindicators. Several smaller rivers in Hesse and Bavaria (Germany) were examined in connection with wastewater discharge points, covering pollution scenarios such as contaminated sites, technical defects and sewage treatment plants. Thirty gammarid, 100 sediment and 30 water samples were collected from 50 m river sections. ICP-MS was used for lead, cadmium, total chromium, copper, nickel and zinc, while mercury was analysed via CV-AAS. Results show that sediment concentrations of heavy metals are about 10,000 times higher than in corresponding water samples, while concentrations in Gammarus spp., containing G. fossarum, G. pulex and G. roeselii, are approximately 1,000 times higher than in water. No linear relationship is observed between gammarids and sediment, refuting such a hypothesis. Gammarids inhabit the riverbed and banks, accumulating heavy metals through their diet and serving as reliable accumulation indicators due to their abundance. However, their collection is labor-intensive, requiring sufficient biomass and the separation of other macrozoobenthos species. Sediment sampling is less demanding, while water samples can be obtained rapidly and universally. Case studies illustrate different perspectives. In the Kössein and Röslau rivers (Bavaria), historically contaminated with mercury, concentrations decreased after dredging and securing the contaminated banks. In the Wieseck River (Hesse), untreated sewage discharged for one week resulted in sediment contamination still detectable a year later. At Rosbach (Taunus region), a combined sampling approach proved effective. Water analysis revealed elevated zinc downstream of a wastewater treatment plant, which was later confirmed in sediment further along the course. In conclusion, water sampling is particularly suited for short-term monitoring of acute contamination events, while sediment and gammarids provide valuable insights into long-term impacts and past discharges. Both matrices confirm increases in heavy metal contamination associated with wastewater inputs.


# Background

Watercourses are essential components of the Earth's ecosystem. They serve as habitats for plants and animals within and around these water bodies. For humans, water bodies are used for irrigation in agriculture, for cooling industrial processes, for drinking water production and as recreational areas.

However, human interventions also pose a threat to the natural balance of these water bodies. The input of various pollutants endangers both the water quality and the living conditions of aquatic flora and fauna. The impacts of pollutant contamination are evident in local examples in Germany, such as the mercury legacy of the Chemical Factory in Marktredwitz (1788 – 1985) (Nováková et al. 2022), wastewater discharge into the Wieseck River in 2020 (Bothe 2020) or fish mortality in the Oder River in 2022 (Free et al. 2023). The pollutant contamination in rivers and watercourses can originate from

natural sources (such as geogenic background pollution in soils) or anthropogenic sources (including atmospheric deposition and wastewater discharges).

The wastewater discharge examined in this work concerns so-called mixed water discharge points (in the following “waste water discharge point”). In Germany, a system is often used in which domestic and commercial wastewater and rainwater flow together in a canal to the sewage treatment plant. After heavy rain events, the amount of wastewater cannot always be contained by the canal, so the excess is discharged into nearby rivers in specially designed structures and there can contaminate the river (Abwassertechnische Vereinigung 1994).

In the case of point sources, the improved purification performance of multi-stage wastewater treatment plants, as well as legislative measures such as the implementation of regulations for indirect dischargers, result in a reduced input of pollutants (Fuchs et al. 2002).

The influence of diffuse pollutant inputs is difficult to assess and limit. Wastewater also contains substances such as heavy metals that cannot be completely removed in wastewater treatment plants. These substances either remain in the sewage sludge or enter receiving waters with the treated wastewater. Aquatic microorganisms are already harmed by traces of lead, cadmium, or copper (Hillenbrand et al. 2005). Moreover, heavy metals accumulate in aquatic organisms and can enter the human body through the food chain. This study focuses on the heavy metals lead, cadmium, chromium, copper, nickel, mercury and zinc, as these are typically found in municipal wastewater. Various target values exist for these metals to protect surface inland waters, for instance, set by the German Working Group of the Federal States on water issues (LAWA) (LAWA 1998a).

The Water Framework Directive 2000/60/EC (WFD) mandates the protection and improvement of aquatic ecosystems, aiming for a good ecological and chemical status for all surface water bodies by 2027 (EEA 2000). This requires suitable measures and management practices, monitored through assessment procedures. In Germany, surface waters are monitored and assessed according to the Surface Water Ordinance (OGewV). Environmental quality standards (EQS) for priority and river basin-relevant pollutants are listed in Annexes 6 and 8 of the OGewV (OGewV 2016). If the LAWA targets are achieved, significant adverse effects on the aquatic ecosystem can be largely ruled out (LAWA 1998b). In our study the influence of anthropogenic discharges on the heavy metal contents in sediment, water and gammarid samples will be compared. The latter are proposed as alternative monitoring organisms to fish in fish-poor or fish-free smaller watercourses. For this purpose, the species *Gammarus fossarum* (Figure 1), *G. pulex*, and *G. roeselii* were selected, as they represent the three most common species of freshwater amphipods in Central European running waters (Pöckl 1993; LANUV 2010).

**Figure 1:** Habitus of *Gammarus pulex* (© Ute Windisch)

Gammarids inhabit the bottom and bank of water bodies, where they reside among stones, aquatic plants, root weaves and leaf litter (Haeckel et al. 1973; Meijering 1982; Westheide et al. 1996; Schönborn

1999). They can move short distances by swimming or crawling sideways, provided there are enough bottom structures for cover (Meijering 1972; Brehm et al. 1996).

In small watercourses, *Gammarus* species are often the most abundant in terms of individuals and biomass. They hold an essential role within the ecosystem of a watercourse (Meijering et al. 1982; Foeckler et al. 1985; Pöckl 1993; Pöckl 2014). Firstly, they consume organic matter and thereby uptake heavy metals that are dissolved in water or absorbed through their food in habitats close to sediment (Maltby et al. 1994). Secondly, due to their high reproduction rate, they are considered one of the most significant food sources for fish and other predators (Meijering et al. 1982; Foeckler et al. 1985).

Furthermore, *Gammarus* species are relatively site-specific and have specific habitat requirements (Pöckl 1993; Schirling et al. 2005). Overall, due to these characteristics, they are suitable as bioindicators. Finally, gammarids reproduce quickly and in large numbers, making the collection of larger samples ecologically unproblematic (Pöckl 1993; Pöckl 2014).

In the context of this study, the hypothesis to be verified is whether localized wastewater discharges or contamination from legacy pollution sites lead to increased concentrations of heavy metals in the water body compartments water, sediment and gammarids.

### Sampling locations

The study area encompasses various small rivers in Hesse and Bavaria in Germany. Samples are collected from the respective watercourses, both near discharge points ("contaminated") and at relatively uncontaminated sections, to assess the impact of anthropogenic wastewater discharges on the heavy metal levels in the studied water compartments. The selection of sampling locations focused on the characteristics of known pollution cases in the Wieseck at Buseck/Hesse and the Kössein at Marktredwitz/Bavaria.

These river sections therefore are expected to be located in silicate-rich low mountain streams and rivers and exhibit good ecological conditions. Their surroundings should predominantly be agricultural but not urban. Additionally, the sampling locations should be accessible by foot. All water bodies should have fine-grained sediments including clay and sand (aimed but not examined particle size < 20 µm), hence rivers with a natural bed structure and moderately altered course were chosen. However, due to natural variations in the rivers, these requirements could not always be met and sediments with larger or not classifiable grain sizes were used in 55 out of 100 samples, for example gravel or organic sludge.

Furthermore, rivers were selected that include sections influenced by combined sewage or wastewater treatment plant discharges as well as sections that are as natural and minimally affected as possible (comparison sections).

For this work it was not possible to probe comparison sections on each river because they did not fit the requirements of our sampling methods e. g. the absence of grained sediments, the missing of gammarids or already anticipated pollution near the source. The mentioned data were sourced from

governmental specialized information systems, namely the 'WRRL-Viewer Hessen' (HLNUG 2022a), 'UmweltAtlas Bayern' (LfU 2022a) and 'Gewässerkundlicher Dienst Bayern' (LfU 2022b).

Ten rivers in Hesse and two rivers in Bavaria that have similar properties were chosen. The rivers Wieseck, Krebsbach, Wetter, Nidda and Eichelbach originate from the Vogelsberg volcano mountain in middle Hesse, the rivers Erlenbach, Seulbach, Eschbach, Fahrenbach and Rosbach are located southwest from the Vogelsberg at the Taunus mountain range but still in the middle of Hesse. The Kössein flows into the Röslau in the examined Area, the Fichtel Mountains in northeastern Bavaria.

In the following table, all sampling locations are numbered in an upstream order, and their proximity to a discharge point is indicated by a specific abbreviation.

Table 1: Sampling locations in Hesse and Bavaria

The abbreviation following the sampling location indicates the proximity to discharge points.

c: uncontaminated comparison section upstream of the following sampling locations

d: directly (≤ 0,2 km) downstream a wastewater discharge point

n: near (> 0,2 ≤ 2 km) downstream a wastewater discharge point

f: far (> 2 ≤ 12,2 km) downstream a wastewater discharge point

d*/n*: directly/near downstream a wastewater treatment plant

| district | location | watercourse | sampling locations |
|---|---|---|---|
| Gießen | Buseck-Großen-Buseck | Wieseck | WG1(d), WG2(d) |
| | Buseck-Trohe | | WT1(n), WT2(d), WT3(n), WT4(n), WT5(n), WT6(d) |
| Gießen | Buseck-Beuern | Krebsbach | KR1(c), KR2(n) |
| Gießen | Laubach<br>Laubach-Wetterfeld | Wetter | WE1(c)<br>WE2(d) |
| Vogelsberg | Schotten-Rudingshain | Nidda | N1(c) |
| | Schotten | | N2(d) |
| | Schotten-Rainrod | | N3(n) |
| Wetterau | Nidda-Eichelsdorf | | N4(d), N5(d) |
| Vogelsberg | Schotten-Eschenrod | Eichelbach | E1(f) |
| | -Wingershausen | | E2(d) |
| | -Eichelsachsen | | E3(d) |
| Wetterau | Nidda-Eichelsdorf | | E4(f) |
| Hochtaunus | Wehrheim-Obernhain | Erlenbach | ER1(c) |
| | Wehrheim | | ER2(n) |
| | Köppern | | ER3(f) |
| | Burgholzhausen | | ER4(n), ER5(d), ER6(n), ER7(d) |
| | Bad Homburg-Ober-Erlenbach | | ER8(d*) |
| Hochtaunus | Bad Homburg-Ober-Erlenbach | Seulbach | S1(n) |
| Hochtaunus | Oberursel-Oberstedten | Eschbach | ES1(c), ES2(d) |
| | Bad Homburg-Ober-Eschbach | | ES3(n*) |
| Frankfurt a. M. | Nieder-Eschbach | | ES4(n) |
| Wetterau | Rosbach v. d. H. | Fahrenbach | F1(c) |
| | | Rosbach | RO1(d*) |
| | Wöllstadt | | RO2(d) |
| Wunsiedel | Waldershof | Kössein | K1(f) |

| | | | |
|---|---|---|---|
| (Bavaria) | Marktredwitz | | K2(f), K3(n*) |
| | Arzberg | | K4(n) |
| | Schirnding-Fischern | Röslau | R1(n) |

The exact geographical locations of the sampling locations were taken roughly in the middle of the probed river section. They can be found in the following table 2.

Table 2: Geographical coordinates for all sampling locations of the respective rivers

* Sampling location for gammarids

[i] Sampling location for water

| river | geographical coordinates (WGS84) | | | |
|---|---|---|---|---|
| Wieseck | WG1*,i: | 50°36'12.1"N 8°48'00.9"E | WG2*,i: | 50°36'31.2"N 8°46'23.7"E |
| | WT1*,i: | 50°36'35.0"N 8°45'54.6"E | WT2: | 50°36'34.0"N 8°45'40.3"E |
| | WT3: | 50°36'34.1"N 8°45'38.1"E | WT4: | 50°36'33.9"N 8°45'35.0"E |
| | WT5: | 50°36'33.6"N 8°45'32.7"E | WT6*,i: | 50°36'32.4"N 8°45'18.9"E |
| Krebsbach | KR1*,i: | 50°38'09.5"N 8°49'40.3"E | KR2*,i: | 50°36'58.2"N 8°48'37.2"E |
| Wetter | WE1*,i: | 50°32'03.7"N 9°00'31.5"E | WE2*,i: | 50°32'54.8"N 8°56'52.0"E |
| Nidda | N1*,i: | 50°31'20.9"N 9°10'54.4"E | N2*,i: | 50°29'38.2"N 9°06'55.8"E |
| | N3*: | 50°27'52.3"N 9°04'13.8"E | N4*: | 50°27'26.9"N 9°03'25.1"E |
| | N5*,i: | 50°27'02.5"N 9°02'43.6"E | | |
| Eichelbach | E1*,i: | 50°29'02.3"N 9°09'48.6"E | E2: | 50°27'54.0"N 9°07'40.5"E |
| | E3i: | 50°27'28.8"N 9°07'07.6"E | E4*,i: | 50°27'03.1"N 9°03'39.3"E |
| Erlenbach | ER1*,i: | 50°17'13.0"N 8°32'00.5"E | ER2*,i: | 50°17'30.7"N 8°34'34.0"E |
| | ER3*,i: | 50°16'46.3"N 8°38'02.1"E | ER4*: | 50°15'41.1"N 8°40'16.2"E |
| | ER5*: | 50°15'28.2"N 8°40'25.1"E | ER6: | 50°15'15.5"N 8°40'21.4"E |
| | ER7*,i: | 50°14'53.5"N 8°40'29.3"E | ER8*,i: | 50°13'14.7"N 8°41'36.6"E |
| Seulbach | S1*: | 50°13'53.6"N 8°39'54.1"E | | |
| Eschbach | ES1*,i: | 50°13'49.8"N 8°32'56.8"E | ES2*,i: | 50°13'30.1"N 8°35'16.0"E |
| | ES3*,i: | 50°12'41.6"N 8°39'50.2"E | ES4*,i: | 50°11'30.1"N 8°40'43.7"E |
| Fahrenbach | F1i: | 50°17'42.7"N 8°40'13.6"E | | |
| Rosbach | RO1*,i: | 50°18'06.3"N 8°43'26.5"E | RO2*,i: | 50°18'06.3"N 8°43'26.5"E |
| Kössein | K1,i: | 49°59'03.2"N 12°04'34.6"E | K2,i: | 50°00'13.6"N 12°06'10.1"E |
| Röslau | K3: | 50°00'30.9"N 12°07'33.4"E | K4,i: | 50°01'43.2"N 12°09'27.1"E |
| | R1*,i: | 50°05'49.6"N 12°14'48.8"E | | |

# Methods

At the selected river sections, a total of 30 gammarid, 100 sediment and 30 water samples were collected in January 2022. Samples for gammarids and water were collected from a selection of 30 river sections. Whereas in several sections, two to three sediment samples were collected. Sections where gammarids were not present in sufficient numbers for sampling were excluded for collecting water and

gammarids samples. The sections were also limited to those considered particularly relevant for analysis, for example, directly downstream of a wastewater discharge point. The sampling sites for gammarids including water, gammarids without water and water only without gammarids can be found in Table 2.

At each sampling point, a 50-meter-long representative section was sampled against the direction of the flow to prevent possible contamination. Furthermore, all equipment and containers that came into contact with the samples were made of chemically inert material that does not interact with the heavy metals under investigation. The samples were transported to the laboratory under cooled conditions and stored at temperatures below -18 °C until analysis (DWA-M 517).

The water samples were collected in LDPE laboratory bottles directly from the watercourse according to DIN EN ISO 5667-6. A 50 mL aliquot of river water was collected to assess the current contamination level at the time of sampling (DIN EN ISO 5667-1). A significant portion of heavy metals in water samples is adsorbed on particles. Therefore, the water samples were analyzed unfiltered, including suspended particulate matter, to provide an overview of the entire heavy metal load. Additionally, for further preservation, the water samples were acidified with nitric acid to lower the sample's pH to below 2 (DIN EN ISO 5667-3).

Sediment sampling was performed using a stainless steel Van Veen grab sampler, aided by spoons made of stainless steel and plastic, along with a plastic funnel. To assess the current contamination situation (deposition from the water), sediment was exclusively taken from the uppermost centimetres of the surface layer using the Van Veen grab sampler (DWA-M 517). During sampling, multiple subsamples were taken in a uniform grid and combined to create a composite sample. Great care was taken to ensure that mainly fine-grained sediments (fraction < 20 µm) were sampled, as heavy metals tend to accumulate predominantly in the fine particle fraction (particle size effect) (LAWA 2002). Two or three composite samples were collected at the investigation sites according to DIN ISO 5667-12, as river sediments are vertically and horizontally heterogeneous (DIN ISO 5667-12). Approximately 100 g of sediment for each of the two or three samples per river section were placed in wide-mouth LDPE bottles for transport to the laboratory under cooled conditions (DIN ISO 5667-15).

For gammarid sampling, plastic containers, hand sieves, nets, stainless steel spoons and spring steel tweezers were used. Gammarids were sampled from various habitats within a sampling site following DIN EN 16150 standards (DIN EN 16150). Typical habitats of gammarids include root weavers, leaf accumulations and aquatic plants (Pöckl 2014). During the 'kick-sampling' method, the riverbed was agitated by kicks, and the stirred-up material was captured using a downstream-held net. Additionally, habitat structures located just below the water surface were shaken by hand, and the gammarids were captured using hand sieves. The amphipods were randomly identified as the following species using a binocular microscope and the key from (Eggers et al. 2001). Individuals of *Gammarus fossarum*, *G. pulex* and *G. roeselii* were combined into a composite sample (pool sample) for each sampling location after removing other macrozoobenthos species. Approximately 5 to 20 g of fresh weight was needed for

laboratory analysis to ensure the homogeneity of the samples. Excess river water was decanted from the sample bottles and without additional clean water purging the samples were frozen at -18 °C until laboratory analysis.

Before each individual sampling, a handwritten protocol was labelled based on a specially created form. It contains different information like the name of the river/-section, coordinates, date and weather. The heavy metal contents of the samples were determined qualitatively and quantitatively by the external laboratory 'SGS Analytics' using appropriate standard methods. All samples were analyzed as single determinations. Certified reference materials were used for quality assurance. For the elements lead, cadmium, chromium (total), copper, nickel and zinc, the inductively coupled plasma mass spectrometry (ICP-MS) method according to DIN EN ISO 17294-2 was employed, which, among other things, provides for the use of standard solutions and blank value solutions to ensure quality (DIN EN ISO 17294-2). Due to its specific chemical and physical properties, mercury was determined using the cold vapor atomic absorption spectrometry (CV-AAS) method as per DIN EN ISO 12846, which also provides for the use of standard solutions and blank value solutions to ensure quality of analyzis (DIN EN ISO 12846).

Both processes require that the laboratory environment, such as the room air and the laboratory equipment, be clean and uncontaminated (DIN EN ISO 17294-2, DIN EN ISO 12846).

For water sample analysis, the limit of quantification (LOQ) for the elements lead, chromium, copper, nickel and zinc was 0.001 mg/L, while for cadmium and mercury, it was 0.0001 mg/L. In sediment sample analysis, the LOQ for lead, chromium, copper, nickel and zinc was 3 mg/kg dry matter (DM), for cadmium, it was 0.3 mg/kg DM and for mercury, it was 0.05 mg/kg DM. In gammarid sample analysis, the LOQ was set at 0.08 mg/kg for lead, 0.01 mg/kg for cadmium, chromium, copper, nickel and zinc and 0.005 mg/kg for mercury.

For water sample analysis, the acidified water samples were directly used.

The sediment samples were dried at 40 °C before the heavy metal analysis, and the dry matter was determined following DIN EN 15934 (DIN EN 15934). The sediments were not sieved, but ground to < 45 µm for homogenization. From the sample, 1 g was mixed with 3 ml $HNO_3$ and 9 ml HCl, digested in the microwave at 160 °C for 20 minutes, filtered off after cooling, and made up to a final volume of 25 ml with distilled water (DIN EN 13657). For the ICP-MS measurement, the digestions were pre-diluted in a ratio 1:50; for the measurement of Hg only 1:10.

In the case of Gammarid samples, the liquid phase in the sample bottles was initially separated. The fresh gammarids were then crushed and homogenized using a titanium knife mill. 1.5 to 2.0 g of the homogenized gammarid sample was mixed with 4 ml $HNO_3$ and 1 ml $H_2O_2$ in a Teflon vessel, tightly sealed and heated at 190 °C for 30 min. After cooling, the clear solution was quantitatively transferred to a defined volume of 15 ml, and a 1:10 dilution was measured.

# Results and Discussion

### Variation among investigated water compartments

Significant differences in the average heavy metal concentrations are observed among the water compartments: sediment, gammarids and water (Figure 2).

**Figure 2:** Comparison of heavy metal concentrations as box plots in the sediment [mg/kg DM], in the gammarids [mg/kg] and in the water [mg/L]

The analysis of sediment samples reveals average concentrations ranging from 0.4 to 104 mg/kg DM, which is about 10,000 times higher than in the river water.

Consequently, this study confirms previous findings of a pronounced increase of heavy metal concentration in river sediments compared to river water. This is supported by the fact that the adsorption of heavy metals on fine-grained sediment and the sedimentation of suspended matter containing heavy metals have the greatest enrichment potential of all sample types examined (Förstner et al. 1974; Lichtfuß et al. 1981). Hence, sediments are considered as a 'long-term memory' for stream contaminants (Fritsche et al. 2002). In the sedimentation zones of water bodies, substantial proportions of released heavy metals from the surroundings are often stored (Macklin et al. 1997; Müller 1986). Additionally, the heavy metal content in river sediments affects the aquatic fauna since fine-grained sediment, due to its high biological productivity, serves as a feeding ground for many aquatic organisms (ARGE Elbe 1980).

Gammarid samples show average heavy metal concentrations ranging from 0.05 to 10.4 mg/kg. Consequently, the accumulation of heavy metals in gammarids results in concentrations that are, on average, approximately 1,000 times higher than those in water samples. Aquatic organisms accumulate heavy metals both through the surrounding water and contaminated organic food sources. Gammarids accumulate available heavy metals in their habitat over their lifetime due to their relatively site fidelity (Pöckl 1993, Schirling et al. 2005). Aquatic organisms intake heavy metals through the food chain, which could lead to increased heavy metal levels in higher trophic levels. For modelled marine food webs different transfer behaviour of trace metals has been shown (Sun et al. 2020): Biomagnification was found for lead, mercury and zinc, while no significant change in the concentration in the food webs occurred for cadmium and copper. In the above-mentioned work, biodilution with increasing trophy levels was demonstrated for the essential trace metal nickel. The results in this work show the comparatively highest heavy metal concentrations in gammarids for copper and zinc. This is consistent with a study in mangroves in China, where food web investigations found substantial accumulation of copper and zinc in crustaceans (Zheng et al. 2023). An increased copper concentration was also found in crustaceans in a study in an Australian seagrass ecosystem. However, the effect did not occur for the other heavy metals examined, including zinc (Barwick et al. 2003).

Thus, the heavy metal content in sampled fish would likely be higher, as indicated by the Hessian and Bavarian fish monitoring reports (HLNUG 2022c; LfU 2022b).

In the water samples, average heavy metal concentrations range only from 0.0001 to 0.008 mg/L. This sample type investigates both dissolved and particulate-bound heavy metals in the water, as the water samples were not filtered. However, the unfiltered river water samples do not exhibit significant contamination with heavy metals. The magnitude of the measured values for the investigated heavy metals aligns with the results from the monitoring program of Hessian waters and the river Rhine (IKSR 2022; HLNUG 2022b). River water as a sample material allows only a snapshot of the current pollution in the water body since present heavy metals are distributed by the flow. Events such as inputs from point sources or precipitation events lead to fluctuations in heavy metal concentrations.

**Suitability of sample types for assessing heavy metal contamination**

In this study, similar amounts of heavy metals exceeding the limit of quantification (LOQ) were detected in samples of *Gammarus* and sediment. Only about 14% (29 out of 210 measurements) of the recorded heavy metal values were below the LOQ in *Gammarus* samples, and around 19% (136 out of 700 measurements) were below the LOQ in sediment samples. In contrast, despite the low LOQ, a total of 61% (129 out of 210 measurements) of the measured heavy metal levels in river water were below the LOQ. Furthermore, only three out of seven heavy metals showed a sufficient number of measured concentrations above the LOQ. These are copper, nickel and zinc. Therefore, individual measurements of river water are not suitable for comparisons.

Next, the assessed heavy metal concentrations in *Gammarus* are evaluated to determine if they correlate with sediment sample measurements. For each heavy metal, a linear regression between the two sample types is calculated and the coefficient of determination ($R^2$) is computed (Table 2). The individual values of the 30 sampling points where gammarids were found are compared with the average values of the sediment samples from these water sections.

**Table 3:** Linear correlation between the heavy metal concentration of Gammarus and sediment at 30 sampling points, represented by the coefficient of determination ()

| heavy metal | lead | cadmium | chromium | copper | nickel | mercury | zinc |
|---|---|---|---|---|---|---|---|
| $R^2$ | 0.014 | 0.010 | 0.197 | 0.020 | 0.240 | 0.998 | 0.001 |

For the element mercury, a linear relationship is indicated ($R^2 = 0.998$). However, this strong correlation for mercury is less meaningful due to the proximity of the measurement values of both sample types to the LOQ. For the other heavy metals, either no or only a slight linear relationship exists. For instance, the coefficient of determination $R^2$ is 0.240 for nickel, and for the remaining five heavy metals, it is less than 0.200.

Possible reasons for the low correlation found between the heavy metal concentration of gammarids and sediment are that *Gammarus* spp. feed predominantly on organic litter, which may also have sediment particles adsorbed on it. On the other hand, the rivers examined differ, also in terms of the

magnitude of the measured values. In addition, other environmental influences not examined here, for example the geogenic background, fertilizers introduced into the soil or emissions from the chemical industry, can influence the heavy metal concentration of the sample types (Förstner et al. 1974). Therefore, the examination of heavy metals in sediment cannot be replaced by assessing heavy metals in gammarids.

As these organisms are part of the food chain, their consideration is also relevant with regard to compliance with other limit values. It allows an examination of the ecological impact and also reveals increased concentrations along the course of the water body.

German and European regulations indicate that the chemical status assessment based on biota investigations (fish, crustaceans, molluscs) is preferred (OGewV 2016; Directive 2013/39/EU 2013). In this study, gammarids were investigated as bioindicators because they play a key role in a water body as shredders and decomposers of organic substances and as prey organisms for predators. Therefore, the identified heavy metal contamination in gammarids is particularly relevant for the aquatic ecosystem. However, the time required for gammarid sampling was significantly higher than that for sediment and water, and gammarids were not present at every sampling site.

A study conducted by the University of Bonn demonstrates that heavy metals accumulate significantly in *Gammarus fossarum* during their relatively short lifespan of approximately 12 to 18 months. This finding is supported by the fact that the levels of heavy metals in the examined *Gammarus* samples were higher than in the leaf samples (their food source) (Hüsecken et al., 2013). Gammarids can also be used for assessing heavy metal pollution in small streams, as they are ubiquitous unlike fish and, in our experience, often in large numbers of individuals within the macrozoobenthos community.

In summary, for a separate analysis of heavy metal pollution in specific flowing waters and their anthropogenic discharge points, the measurements from water samples are inadequate since a large portion of the determined concentrations is below the limit of quantification (LOQ). The results from the examined *Gammarus* samples provide a comprehensive view of heavy metal pollution in the water sections, as the measurements, except for mercury, predominantly exceed the LOQ. However, not all sampling sites can be investigated using *Gammarus* samples due to the absence of freshwater amphipods at certain locations. Furthermore, only one pool sample could be taken from each study section for analysis, as the weight of the organisms found was only sufficient for one analysis sample if the necessary quantity was taken into account.

In contrast, during sediment sampling, multiple samples were collected at all locations. This allowed the assurance of the heavy metal content by averaging individual measurements and identifying any potential differences. Additionally, sediment analysis is a standard procedure in heavy metal investigations, providing data on sediment heavy metal contents in previously surveyed water bodies. In flowing waters with known pollution (e.g., Kössein due to mercury contamination) as well as in major rivers (e.g., the Rhine), the heavy metal content of water, suspended solids and sediment is analysed (Pedall et al., 2011; IKSR 2022; HLNUG 2022b).

The legal requirement for this comes from the German Surface Water Ordinance which requires trend tests for the elements lead, cadmium and mercury in biota, suspended solids or sediment, that have to be executed at least once or twice per year for lead and cadmium and, possibly less often for the ubiquitous mercury. (OGewV, 2016). However, implementation varies from state to state. For example, in North Rhine-Westphalia, several heavy metals are being examined in the sediment of a few different rivers, whereas sediment investigations in Thuringia as of 2020 were mainly carried out for mercury. (Ministry of the Environment NRW, 2020; Thuringian State Office for the Environment, 2020). Overall, the sediment heavy metal content in this study stands out as the most informative dataset. Hence, in the following sections, the sediment heavy metal contents will be utilized for assessing relevant heavy metal pollution.

### Temporal trend of mercury levels in the effluents of the hazardous waste site „Chemical Factory Marktredwitz“

In this chapter, the determined mercury levels in the effluents of the hazardous waste site "Chemical Factory Marktredwitz (CFM)" are compared with the measurement data from earlier investigations. The aim is to demonstrate to what extent the effects of remediation measures are reflected in the mercury concentrations in the sediments. Thus, Figure 3 compares the average mercury levels in the sediments of the rivers Kössein and Röslau in the years 2011 and 2022. The actual flow direction of the water is depicted from left to right by arranging the sampling points. The data from 2011 are derived from the study "Quecksilber im Zulauf zum Stausee Skalka” commissioned by the responsible water authority (Pedall et al. 2011).

**Figure 3:** Comparison of mercury levels in the sediments of the rivers Kössein (K) and Röslau (R) in 2011 (Pedall et al. 2011) and 2022 (see also Table 1)

The mercury levels in the surveyed sections of the watercourses decreased in the period from 2011 to 2022. However, despite this reduction, the LAWA target value of 0.8 mg/kg DM (LAWA 1998a) was exceeded at the four survey points both in 2011 and 2022. The mercury contamination at the surveyed sections decreases over time as increased water flow mobilizes sedimented heavy metal particles, carrying them downstream into the Skalka reservoir. The largest quantity of mercury is adsorbed by the finest fraction, which in turn exhibits slow settling rates (Dikau et al., 2019). According to Czech authorities, up to 50 kilograms of mercury enter the reservoir annually (Gschwendtner, 2021). Therefore, primarily oxbows, reservoirs and areas with low flow velocities are affected in these two waters.

The surveyed section of the Kössein at the former CFM site (K2) exhibits the lowest mercury concentration at 0.9 mg/kg DM, even though a mercury content of 227 mg/kg DM was measured in a regulatory examination in 1977 before the closure of the chemical factory (LfW, 1998). The investigated section underwent artificial restructuring due to decontamination and site remediation and contaminated river sediment was properly disposed of (Pedall et al., 2011; Hošek et al., 2020; Haussel, 2009). Additionally, increased flow velocities resulted from the construction of the riverbed and the straightening of the river, transporting contaminated sediment downstream. Furthermore, existing heavy

metal contents are diluted as uncontaminated fine material from the upper reaches deposits into the watercourse.

Similar to 2011, the section 2 km after the CFM at the 'Wölsauerhammer' weir (K3) holds the highest concentration of mercury. The sedimentation of heavy metals is favored by the weir structure and the confluence of a drainage ditch (Pedall et al., 2011). 5,5 km after the CFM, the confluence point of the Kössein into the Röslau (K4) shows a significant decrease in mercury concentration. In 2021, an upstream stretch of the riverbank was fortified due to heavy soil erosion (Bavarian State Parliament, 2022). The successful remediation of the contaminated section may have resulted in the decrease of mercury concentration approximately one kilometer away at the sampling site. The mercury content has also decreased at the sampling site of the Röslau before it merges into the Skalka reservoir (R1) about 21 km after the CFM. The high flow velocity of the watercourse, particularly following the previous flood event, leads to a relatively low mercury contamination in the river sediment.

### Effects of sewage discharge into the Wieseck River

In November 2020, due to a technical fault in a control valve of a storage canal (combined sewer overflow system), untreated wastewater was discharged into the Wieseck for approximately one week. This led to significant deposits and a fish mortality (Bothe 2020). Subsequently, within an observation program, the ecological condition of the affected sections of the watercourse was evaluated based on ecological and physicochemical quality components. The present study supplements this program by investigating the heavy metal contamination upstream and downstream of the discharge point. The average concentrations of heavy metals in the analyzed sediments from three sections of the watercourse upstream (WG1, WG2, WT1) and five sections downstream of (WT2 to WT6) the wastewater discharge point were calculated (Figure 4).

**Figure 4:** Heavy metal content in the sediment of the Wieseck River in watercourse sections upstream (WG1, WG2, WT1) and downstream (WT2 to WT6) of the wastewater discharge point

The analyzed sediment samples show an increase in the concentrations of the heavy metals chromium, copper, nickel and zinc after the faulty discharge point. Therefore, the uncontrolled wastewater discharge at the faulty control valve has an impact on downstream sections of the watercourse. Normally, the upstream section before the considered discharge point is already affected by six additional combined sewer overflow points with anthropogenic wastewater discharges. The highest percentage increase is observed in the elements zinc and copper, typically originating from municipal wastewater, thus confirming the heavy metal input through the faulty discharge point (Hillenbrand et al. 2005). Despite the elevation of some heavy metal concentrations, all environmental quality standards and targets are met.

The results demonstrate that even one year after the anthropogenic discharge, heavy metal contamination can still be observed in the analysed sediments ("long-term memory"). The cause of the high lead concentration upstream of the discharge point could not be determined. Various sources are possible, as lead has been used as chemical compounds in different products, for example, from

foundries, the chemical industry, fertilizer production, batteries, or from its past use as a gasoline additive (Förstner et al. 1974).

### Influence of wastewater discharges on heavy metal concentrations in river sediment using the example of the Rosbach

At the Rosbach River, with a sampling point near its source (F1), as well as a site immediately after a municipal wastewater treatment plant (RO1), and several kilometres downstream from it (RO2), the increase of heavy metal concentration in river sediment can be demonstrated (Figure 5).

**Figure 5:** Heavy metal contents in the sediment of the Rosbach in the near-source section (F1), downstream of a wastewater treatment plant (RO1) and downstream of several combined water discharge points (RO2)

Most of the heavy metal concentrations in section RO1, just downstream of the wastewater treatment plant, show only slight increases (lead, copper) or are not significantly elevated, except for zinc, which appears in concentrations about three times higher. In contrast, notably higher levels of heavy metals were measured in section RO2, located downstream of several combined sewer overflow outlets. Chromium and nickel concentrations, in particular, are notably higher in this section. Only trace amounts of mercury and cadmium are detected, with slight increases that are not visible in the diagram.

Regarding the parallel water analysis, it can be observed that zinc was found in higher concentration (0.0192 mg/L) only in RO1, directly after the wastewater treatment plant. Both F1 (0.0116 mg/L) and RO2 (0.0111 mg/L) show lower measurements at roughly the same level. The other heavy metal elements show similar concentrations. For example, the variation in copper from F1 (0.0010 mg/L) to RO1 (0.0011 mg/L) and to RO2 (0.0013 mg/L) is within the LOQ.

The discrepancy between sediment and water analyses, except for zinc, can be attributed to the flushing of heavy metals after rain discharge from the treatment plant or through combined sewer overflow outlets. These heavy metals are primarily transported with the river water and eventually settle in the later course of the water body as sediment. Therefore, in the water phase, they can only be detected during the exact period when such rain discharge occurs. Heavy metals discharged into the water during these events subsequently lead to a concentration increase in more distant sections, explaining the previously described trend towards RO2.

Zinc emerges from this study as an element commonly found in the water phase downstream of effluents. The already higher concentration of zinc in the water downstream the wastewater treatment plant could be the cause of the comparatively higher zinc levels in the sediment at RO1 compared to other elements. Zinc and copper are heavy metal elements, and based on the obtained analysis results, their sediment concentrations frequently increase after the discharge of municipal wastewater.

### Comparison of zinc and copper concentrations in the sediment of the sampling sites closer to the source with those of the subsequent watercourse sections

The hypothesis is put forth that the concentrations of heavy metals in the sediment increase along the watercourse. Various discharge points may be causative factors for this. The values of a sampling site closer to the source (comparison) are presented, which are compared to the measurement results of all examined sampling sites downstream (Figure 6).

Figure 6: Zinc and copper concentrations in the sediment of the sections closer to the source (comparison) and in the following river course

The values of each subsequent river course are derived from the average of all sampling sites located downstream from the comparison section.

In six out of ten watercourses (Krebsbach, Wieseck (Trohe), Nidda, Erlenbach, Eschbach and Rosbach), the concentrations of zinc and copper are higher below the comparison sections closer to the source. Conversely, the concentrations in the comparison sections of Wieseck (Buseck) and Kössein are higher. In the case of Wetter, the zinc concentration in the comparison section is significantly lower, and the copper content is not much higher than that of the subsequent river sections. For Eichelbach, the comparison concentration for zinc is only slightly higher. The comparison of zinc and copper elements shows overall analogies.

The comparison sections closer to the source are not all completely uncontaminated, as there may be a geogenic background contamination or diffuse or unknown inputs.

In summary, it can be observed that there has been an increase of heavy metal concentration in the sediment of the examined watercourses due to wastewater discharges.

# Conclusions

This study shows that the sample types examined – river water, sediments and gammarids – differ significantly in terms of their suitability for detecting heavy metal contamination. Due to dynamic processes, river water only provides a snapshot of the current situation, whereas gammarids accumulate metals from the surrounding water and contaminated food during their lifetime. Sediments, especially fine-grained fractions, show the highest accumulation and are the most reliable indicators of past sewage discharges. In certain small rivers that were heavily polluted with mercury in the past, a decline in mercury levels has been observed due to increased flow rates and successful remediation measures. In other watercourses, local anthropogenic discharges have led to an increase in chromium, copper, nickel and zinc, underscoring the long-term memory of sediments. Gammarids can serve as bioindicators, although the measurement data show only a weak correlation with those of sediments and are limited by the availability of species and sample size. Overall, sediment analysis remains the most reliable method for assessing historical contamination, while gammarids provide complementary information on ecological relevance and bioavailability. The results underscore the importance of site selection, reference sections and combined monitoring approaches for accurate assessment of heavy metal contamination in freshwater systems.

# Declarations

**Ethics approval and consent to participate**: Not applicable

**Consent for publication**: Not applicable

**Availability of data and material**: "The datasets supporting the conclusions of this article are included within the article

**Competing interests**: All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review for any other publication.

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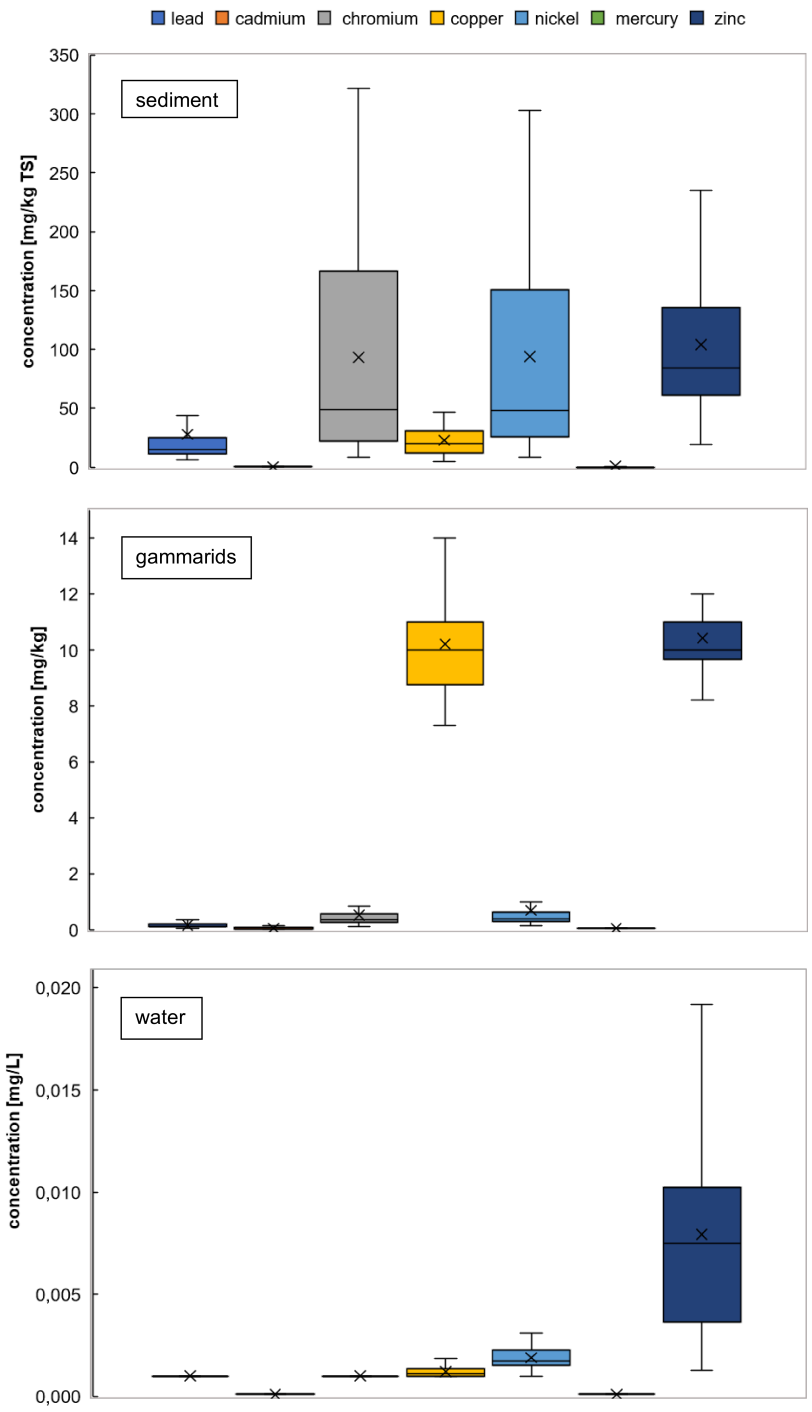
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# Figures



**Figure 1**

Habitus of *Gammarus pulex* (© Ute Windisch)




## Figure 2

Comparison of heavy metal concentrations as box plots in the sediment [mg/kg DM], in the gammarids [mg/kg] and in the water [mg/L]

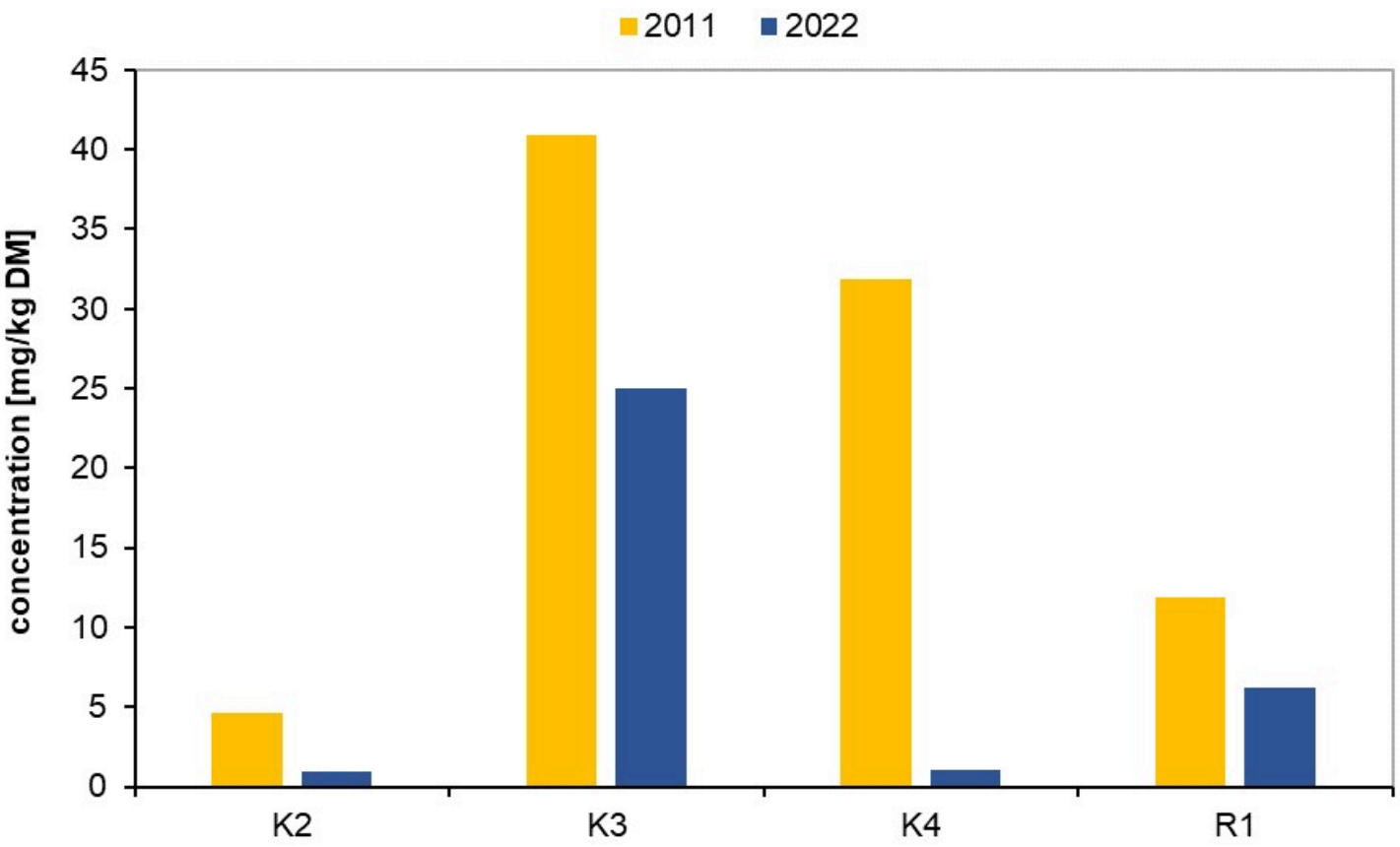



**Figure 3**

Comparison of mercury levels in the sediments of the rivers Kössein (K) and Röslau (R) in 2011 (Pedall et al. 2011) and 2022 (see also Table 1)

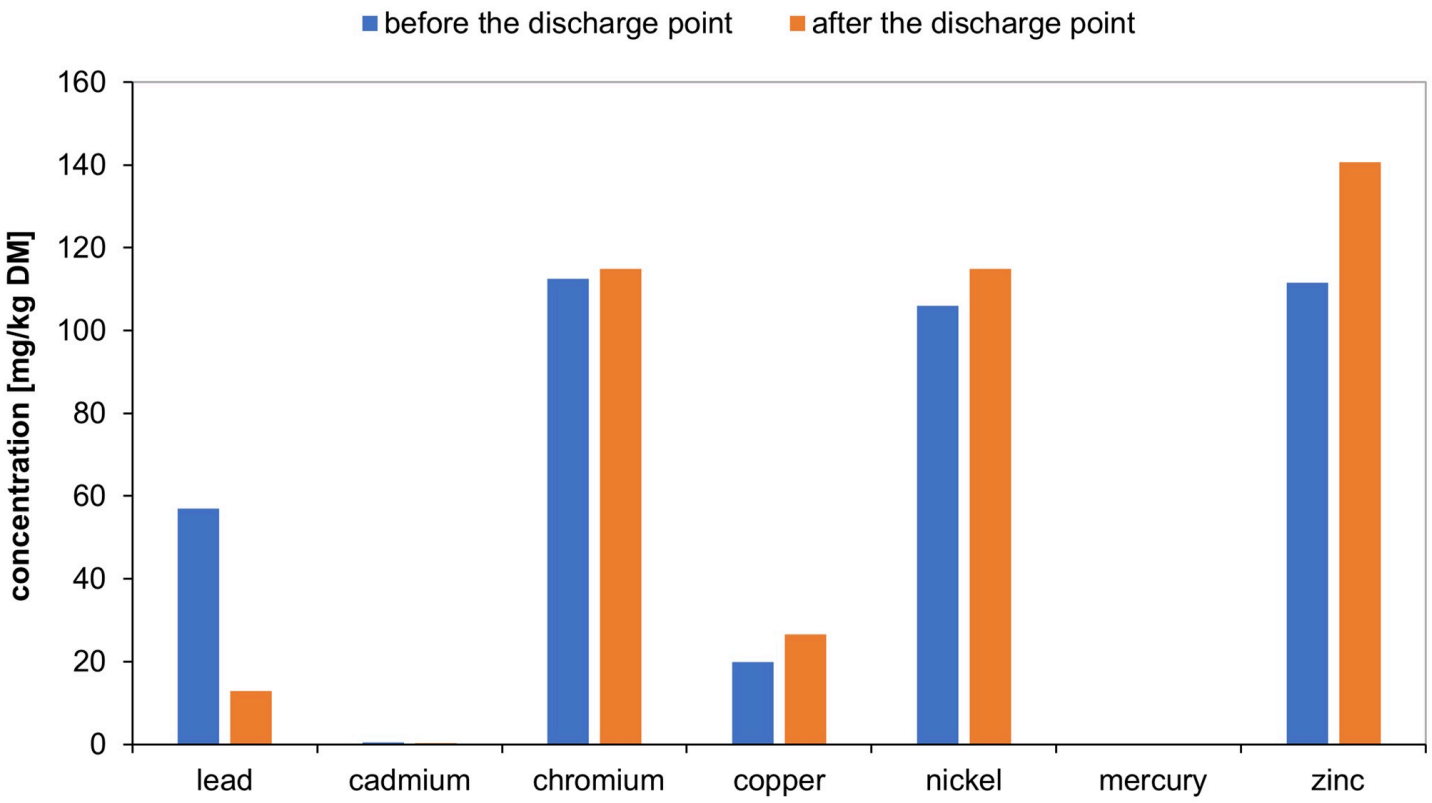



**Figure 4**

Heavy metal content in the sediment of the Wieseck River in watercourse sections upstream (WG1, WG2, WT1) and downstream (WT2 to WT6) of the wastewater discharge point

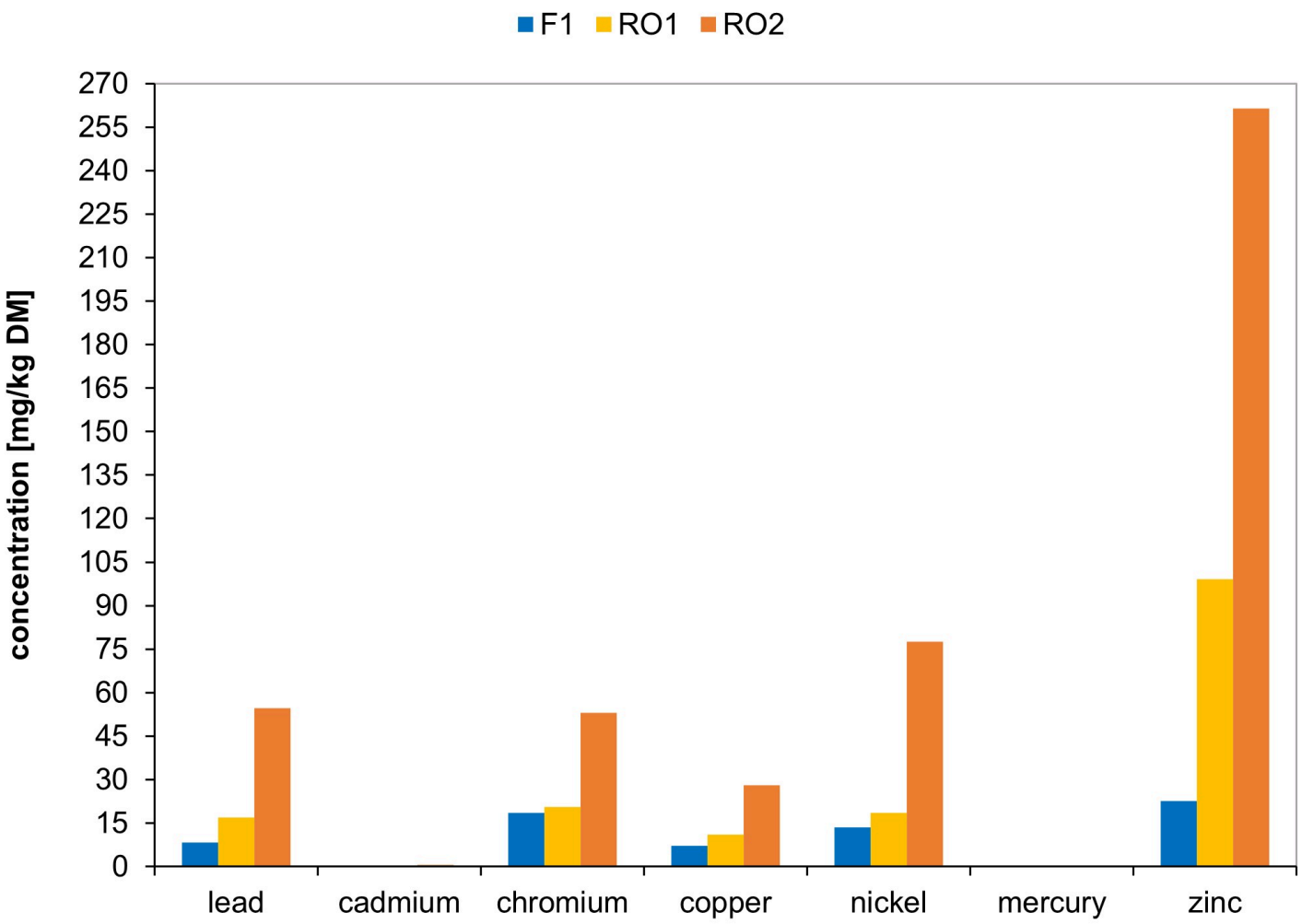
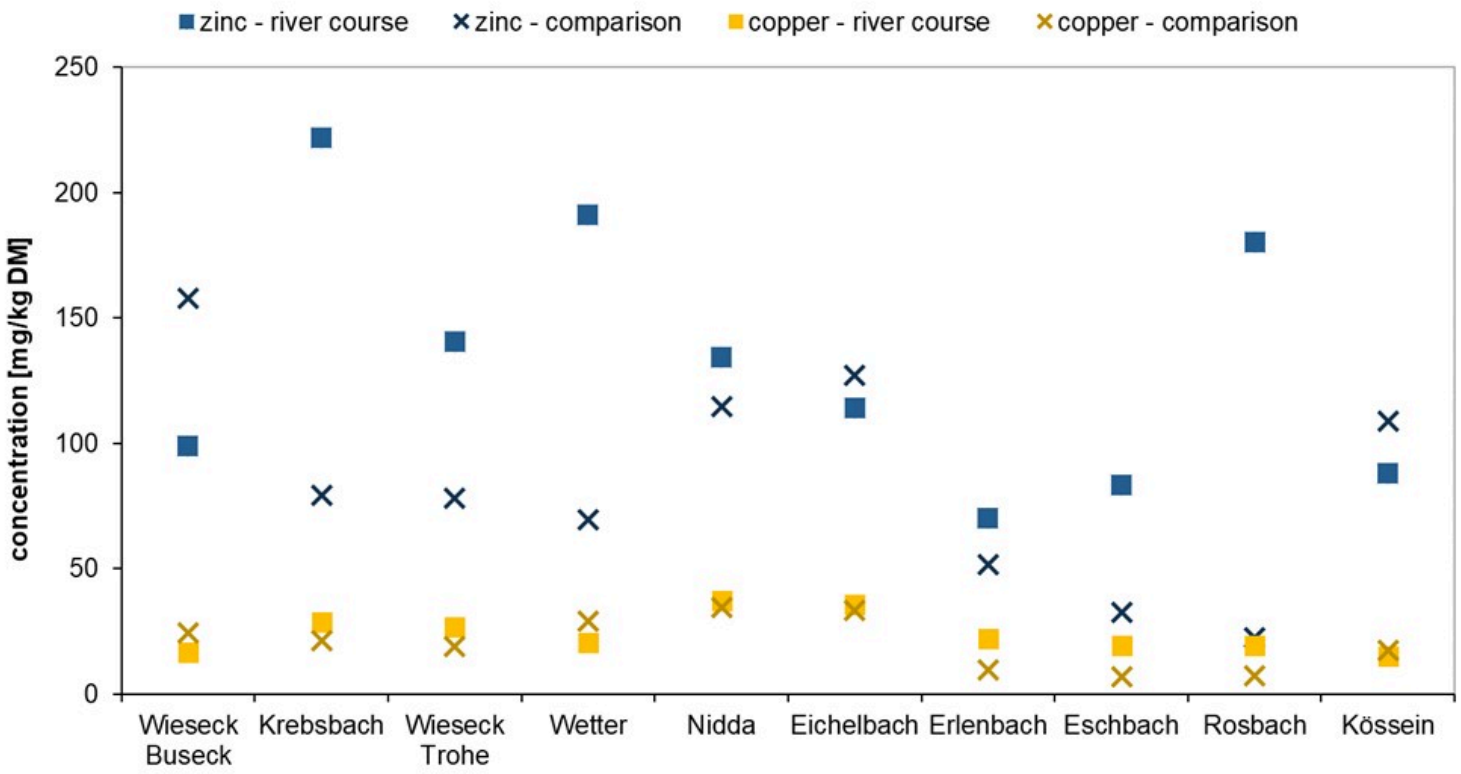



**Figure 5**

Heavy metal contents in the sediment of the Rosbach in the near-source section (F1), downstream of a wastewater treatment plant (RO1) and downstream of several combined water discharge points (RO2)




## Figure 6

Zinc and copper concentrations in the sediment of the sections closer to the source (comparison) and in the following river course