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Analytical note

Determination of trace elements in macrozoobenthos samples by total-reflection X-ray fluorescence analysis[☆]

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Abstract

The paper describes the analysis of a set of metals in macrozoobenthos samples from a river in Western Austria by using total reflection X-ray fluorescence analysis (TXRF). Metal concentrations in aquatic insect larvae from an industrially contaminated site are significantly higher than in larvae from a reference site. Furthermore, species-specific differences in metal accumulation were found. TXRF allows multi-element analysis of very low metal concentrations in very small sample masses (e.g. single aquatic insect larvae with a dry weight of only a few milligrams). Due to its multi-element capability and high sensitivity total reflection X-ray fluorescence analysis is a valuable tool for biomonitoring studies of metal contamination in aquatic ecosystems. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: TXRF; Biomonitoring; Aquatic insects; Bioindicator; Trace elements

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1. Introduction

A widespread set of analytical methods is used to control the fate of toxic metals in aquatic ecosystems. However, monitoring of water and sediments by metal analysis is not sufficient because of fluctuating metal concentrations in the water and delayed responses of sediments [1,2,5,6]. It also provides little information about metal bioavailability in aquatic ecosystems. Therefore, aquatic organisms are increasingly used for the biomonitoring [7,8,10] of the actual metal load of aquatic biota. Aquatic insect larvae satisfy some important criteria established for bioindicators, they are thus valuable biomonitoring organisms of metal contamination in freshwater ecosystems.

Monitoring the trace metal content of macrozoobenthos samples is an efficient way of obtaining information on the actual metal load of aquatic systems and of surveying temporal trends of metal pollution. In many ecological studies, the determination of a great number of elements is of growing interest. Due to the low mobility of many benthic insect species it is possible to detect even minimal pollution differences between sampling sites.

The aim of the present study was the investigation of the metal contents of several benthic invertebrate species from an Austrian river by

using total reflection X-ray fluorescence spectrometry (TXRF).

2. Experimental

2.1. Apparatus

The TXRF spectrometer that was used consists of an Extra II A module (Atomika, Oberschleißheim) with double beam excitation (molybdenum and tungsten tubes), an X-ray generator, a Si(Li) detector, an automatic sample changer and a computer-controlled multichannel analyzer system. Both Mo-tube and W-tube excitation were used for analysis with tube settings of 50 kV and 10–38 mA. Measuring time was uniformly 1000 s for Mo-excitation and 2000 s for W-excitation.

2.2. Sampling

Caddisfly larvae (Trichoptera, *Allogamus auricollis*, Fig. 1) and mayfly larvae (Ephemeroptera, *Baetidae* and *Heptageniidae*) were sampled with a kick sampler at a reference site and a station contaminated by industrial waste water emissions in a western Austrian river. Gammarids (*Gammarus pulex*) were collected in an unpolluted upper Austrian river by using a small dip net.

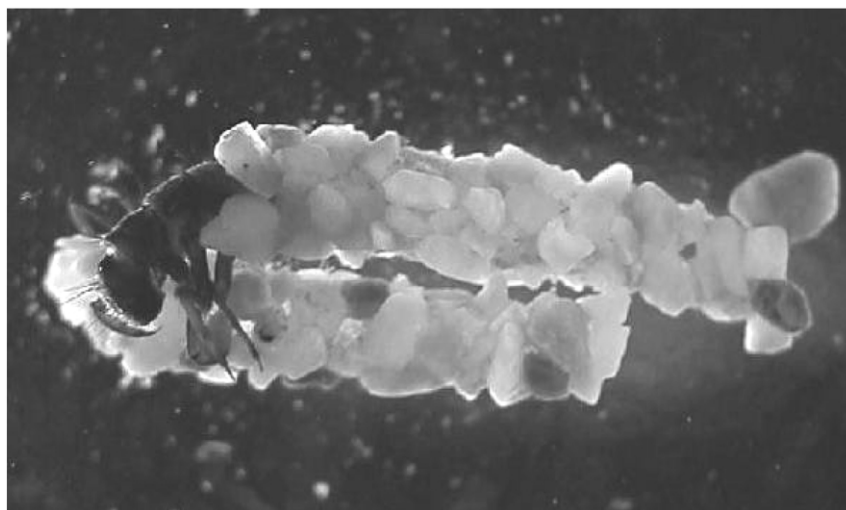


Fig. 1. Caddisfly larvae (*Allogamus auricollis*; 15 mm length).

2.3. Sample preparation

The animals were sorted by hand (cases of caddisfly larvae were removed) and washed in triple-distilled water. For most invertebrate species, four subsamples (containing 15 individuals each) were collected and stored in acid-washed polypropylene tubes. After drying at 60°C to constant weight samples were digested with HNO₃ (Merk Suprapur) and H₂O₂ in a microwave oven (Microwave Laboratory Systems MLS 1200).

Gammarids were dissected under a stereomicroscope and the eggs were transferred with a pair of tweezers directly onto the sample carrier.

2.4. Measurements

TXRF analysis was performed according to Miesbauer [1]. In short, 7 µl of the sample solution was transferred onto a silica sample carrier. Gallium was added as internal standard (for the *Gammarus pulex* eggs Yttrium was used as internal standard), then the sample was dried on a hot plate at 60°C.

Quality assurance protocols for determination of metal concentrations in invertebrate samples included analysis of National Research Council Canada standard reference materials (TORT-1 lobster hepatopancreas, DORM-1 dogfish mus-

cle), procedural blanks, and triplicate determination. In all cases, metal concentrations in procedural blanks were below detection limits.

For control purposes, the Cd and Pb concentrations of a few invertebrate samples were counterchecked by graphite furnace atomic absorption spectrophotometry (GFAAS; Hitachi Z-8200, Zeeman background correction). No significant differences were found between the two methods.

Metal data are given on a dry weight base. Statistical analysis of the data was performed with Student's *t*-test. Statistical significance was assigned at $P \leq 0.05$.

3. Results and discussion

Determination of metals in caddisfly (Fig. 1) larvae from the western Austrian river reveals clear differences between the sampling sites. Concentrations of Mn, Cu, Zn, As, Ba and Pb in caddisfly larvae from the sampling site influenced by industrial waste water emissions are significantly higher than in larvae from the control site (Fig. 2). Concentrations of Pb and Cu in caddisfly larvae from the reference site were comparable to those found previously by Köck [3,4] in *Allogamus auricollis* larvae from an unpolluted sampling site located at the same river in western Austria.

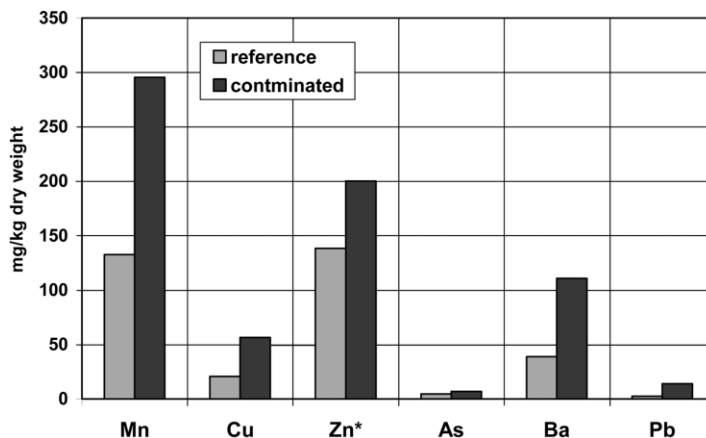


Fig. 2. Concentrations of trace elements in caddisfly larvae (*Trichoptera*, *Allogamus auricollis*) from a reference and an industrial contaminated sample site in a western Austrian river (*Zn is shown reduced by factor 10).

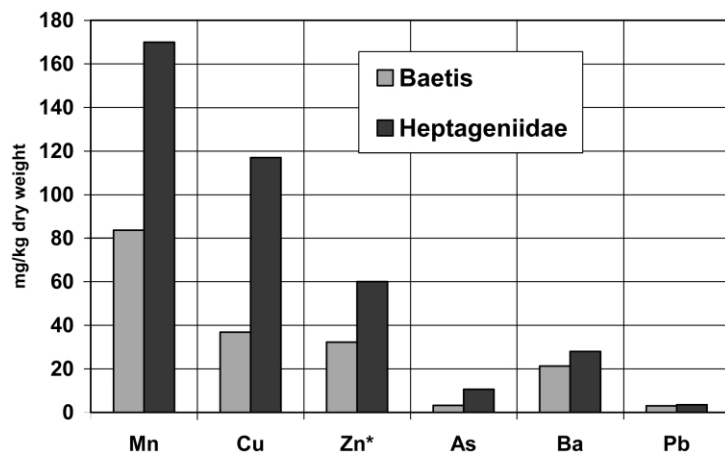


Fig. 3. Concentrations of trace elements in mayfly larvae (Ephemeroptera, *Baetis* and Ephemeroptera, *Heptageniidae*) from an industrial contaminated site (*Zn is shown reduced by factor 10).

Thus, it is assumed that the background concentrations for Pb and Cu for *Allogamus auricollis* larvae are within a range of approximately 3.5–4.5 mg Pb/kg (dry wt.) and approximately 18–22 mg Cu/kg (dry wt.), respectively.

Our results show clear species-specific differences between the insect larvae studied (Fig. 3). Metal concentrations in mayfly larvae from the family *Heptageniidae* are significantly higher than

in *Baetidae*. Furthermore, when compared to caddisfly larvae from the same site, concentrations of Mn, Zn, Ba and Pb are significantly lower in *Baetidae*, whereas *Heptageniidae* exhibit higher Cu concentrations than caddisfly larvae. The differences in metal accumulation are most likely a consequence of species-specific feeding behavior and thus different uptake of metals from their diet.

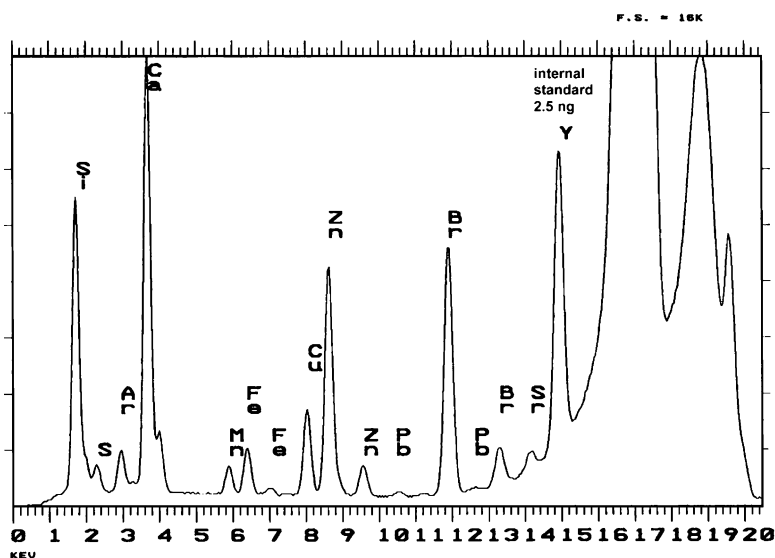


Fig. 4. TXRF spectrum of a *Gammarus pulex* egg (0.4 mm diameter).

In consequence, aquatic insect species to be employed as bioindicators should be carefully chosen with respect to comparability and purpose of the biomonitoring study.

One of the fundamentals in biomonitoring studies is to estimate the population variance of the examined species. If too few specimens are taken for analysis, the confidence in the resulting measurements may be unacceptable for the intended purpose of the study. However, the sampling of too many individuals can waste the resources by increasing the costs of the investigation [9]. The population variance is the decisive factor for optimizing the sample size, and thus, improvement of the cost/value ratio of a scientific project. However, estimation of the population variance requires the analysis of metal concentration in single individuals of aquatic insect larvae and is often hindered by inadequate detection limits of many analytical methods.

One additional possibility is to expose only selected parts of benthic Insects. Our study shows that the simple preparation techniques for TXRF allows accurate multi-element analysis even in very small sample masses (less than 1 mg) such as a single egg (0.4 mm diameter) of *Gammarus pulex* (Fig. 4). The variance for the values of five *Gammarus pulex* eggs, taken from one individual, was approximately 10%.

Metal concentrations of some samples were counterchecked by GFAAS, allowing the comparison of the length of time necessary for determination of a set of metals by using both instruments. TXRF provides results for a widespread set of metals in a single shot, whereas GFAAS requires time-consuming repeated measurements to gain the same results.

4. Conclusions

To summarize, our results indicate TXRF measurements to be a highly efficient method of analyzing the metal contamination of macrozoobenthos samples. The method allows multi-element analysis in very small sample masses (e.g. single aquatic insect larvae with a dry weight of only a few milligrams). Due to its multi-element capability and high sensitivity TXRF is a valuable tool for biomonitoring studies of metal pollution in aquatic ecosystems.

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