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Mayfly larvae (*Baetis rhodani* and *B. vernus*) as biomonitors of trace metal pollution in streams of a catchment draining a zinc and lead mining area of Upper Silesia, Poland

Wojciech Fialkowski[a,*], Malgorzata Klonowska-Olejnik[a], Brian D. Smith[b], Philip S. Rainbow[b]

[a]*Department of Hydrobiology, Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 3, 30-387 Krakow, Poland*
[b]*Department of Zoology, Natural History Museum, Cromwell Road, London SW7 5BD, UK*

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"Capsule": *Mayfly larvae are suitable bioindicators of water pollution in Central Europe.*

Abstract

Larvae of two *Baetis* species were used to investigate spatial and temporal variability in the bioavailabilities of cadmium, copper, lead, zinc and iron in the river Biala Przemsza and its tributaries draining an area of lead and zinc mining in Upper Silesia, Poland. Accumulated metal concentrations were measured in April, May, August and November 2000. Both species indicated significant local geographical variability in availabilities of zinc, iron, lead and cadmium, but not copper. Accumulated concentrations of lead, zinc and cadmium confirmed the high general contamination of the Biala Przemsza system by these three trace metals. Larvae showed little seasonal variation in concentrations of cadmium, copper, lead and iron. Accumulated zinc concentrations were low in *Baetis rhodani* in August, perhaps as a result of insufficient time for high concentrations to accumulate since hatching of the larvae. Samples collected in August most nearly matched criteria of the greatest availability of larvae for collection and their size homogeneity, minimising the possibilities of any effect of differential larval size and/or age on accumulated metal concentrations. Mayfly larvae are members of a suite of potential stream biomonitors in Central Europe, which together can provide information on the different sources of bioavailable trace metals present in aquatic ecosystems. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Bioaccumulation; Biomonitoring; Mayfly; Streams; Trace metals

1. Introduction

The catchment of the river Biala Przemsza is situated in Upper Silesia in south-west Poland. Upper Silesia has been a centre of coal and ore mining for centuries, and has been affected greatly by the adverse impact of heavy industry and urbanisation. In the Biala Przemsza basin itself, the main industries are lead and zinc mines and smelters, with coal mines and limestone quarries also present. Thus, heavy loads of industrial and municipal pollution enter the local watercourses via wastewater and atmospheric fallout (Dudka et al., 1995; Suschka et al., 1994; Ullrich et al., 1999).

Trace metals, even essential ones, are toxic when present above threshold availabilities, and may pose a significant threat to the biota of streams affected by mining and industrial run-off (Rehfeldt and Söchtig, 1991; Gower et al., 1994). Emphasis in studies of the pathways and fates of metals in the environment has shifted towards their accumulation in biota, since not all metal present may be available for uptake by fauna and flora, and it is the bioavailable fraction that is of direct ecotoxicological relevance and concern. The use of aquatic organisms, particularly benthic invertebrates, as biomonitors of the local availabilities of potentially toxic trace metals has become increasingly widespread (Cain et al., 1992; Hare, 1992; Phillips and Rainbow, 1993), not least in the form of Mussel Watch Programmes in coastal waters (e.g. Cantillo, 1998).

Bioaccumulation of heavy metals by aquatic insect larvae including mayflies is comparatively well studied

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* Corresponding author. Tel.: +48-12-264-2520×148; fax: +48-12-269-0927.
E-mail address: wfialkow@if.uj.edu.pl (W. Fialkowski).

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(Hare et al., 1991; Hare, 1992; Goodyear and McNeill, 1999), and larvae have been employed in biomonitoring studies of fresh waters (Cain et al., 1992; Hare, 1992). In a review of metal bioaccumulation by freshwater invertebrates of different feeding guilds, Goodyear and McNeill (1999) concluded that concentrations of cadmium, copper, lead and zinc in mayfly larvae are directly proportional to those in sediments, reflecting their ability to reflect ambient metal availabilities in accumulated body concentrations. In a study specifically involving larvae of *Baetis rhodani*, one of the most common mayflies in running waters of central European uplands, Rehfeldt and Söchtig (1991) also found that concentrations of cadmium, copper, lead and zinc in larvae were correlated with those in river sediments in Lower Saxony, Germany. They also showed that the benthic macroinvertebrate community of metal-contaminated rivers was characterised by an absence of gammarid amphipods and a reduced number of species and larval individuals of the insect families Heptageniidae, Leuctridae, Hydropsychidae and Chironomidae. *B. rhodani* was the dominant benthic macroinvertebrate in metal-rich streams as a result of its high metal tolerance combined with reduced interspecific competition (Rehfeldt and Söchtig, 1991). Interestingly, in a study of streams in south-west England affected by past metal mining, Gower et al. (1994) found that mayfly larvae were absent from streams with the highest copper concentrations, while triclad flatworms and chironomids (especially Orthocladiinae) were dominant. The mayfly species *B. rhodani* and *Baetis vernus* were, however, considerably more metal-tolerant than *Baetis muticus*. It is also likely that physico-chemical conditions other than metal concentrations of the two stream systems studied were different, and had different effects on local metal bioavailabilities. Thus, copper may have been more available in the most copper-rich streams studied by Gower et al. (1994) than in the streams investigated by Rehfeldt and Söchtig (1991).

This paper describes the results of a survey of spatial and temporal variation in the bioavailabilities of zinc, lead, copper, cadmium and iron in the catchment of the Biala Przemsza river, Upper Silesia, using larvae of two locally common mayfly species, *B. rhodani* and *B. vernus* as biomonitoring subjects. Accumulated concentrations of metals were measured in the bodies of the larvae from four sites along the Biala Przemsza river itself, and from one site in each of two of its tributaries (the Biala and Sztola rivers). It is almost impossible to distinguish larvae of the two species in the field, and we were interested in the consequences of failure to identify the species correctly on conclusions drawn from the biomonitoring survey. Since the life cycles of mayflies result in the presence of larvae of different ages and therefore sizes at different times of year, we also investigated the effect of season on our data.

2. Material and methods

2.1. Study area

The study area (Fig. 1) has been described in some detail by Fialkowski et al. (in press), who drew on information on metal inputs presented by Labus (1999). The Biala Przemsza originates from springs south of Wolbrom, flows to the west-south-west and after 64 km joins the Czarna Przemsza near Myslowice to form the River Przemsza, a tributary of the Vistula. The Biala Przemsza receives water from nine main tributaries and a network of ditches and canals that carry wastewater from mines and industrial plants in the south-west of the catchment.

The catchment of the Biala Przemsza can be subdivided into smaller hydrological units (Labus, 1999; Fialkowski et al., in press). In the uppermost section there appear to be no watercourses carrying significant amounts of heavy metals. In the middle part of the basin (Fig. 1), trace metals enter the upper section of the river Biala via a canal carrying wastewater from mine drainage and ore flotation processes ([a] in Fig. 1). In the lower part of the catchment, the Biala Przemsza receives water from ore processing plants via the Warwas stream ([c] in Fig. 1), and from mines that drain into the Sztola river ([b] in Fig. 1).

Four sampling sites were selected along the course of the Biala Przemsza (Fig. 1, Table 1):

(P1) Klucze—station located just upstream of the city, considered to be upstream of any metal-contaminated water source, and therefore receiving any metal inputs from atmospheric deposition (Labus, 1999; Fialkowski et al., in press).

(P2) Lipowka—station located downstream of the confluence of the Biala Przemsza and the Biala rivers. Before reaching this station, the Biala Przemsza receives pollutants carried by the Biala as well as from Klucze.

(P3) Slawkow-Burki upstream of the confluence with the Sztola River—station established to enable the influence of heavily polluted waters from ore processing plants entering the Biala Przemsza through the Warwas stream upstream of the site to be distinguished from contamination coming through the Sztola river.

(P4) Maczki—station located close to the disused intake of potable water for the city of Sosnowiec, downstream of the confluence of the Sztola river (carrying mine drainage) with the Biala Przemsza.

In addition to the above, two sites were located one on each of two tributaries of the Biala Przemsza (Table 1, Fig. 1):

(B1) Biala—site located about 200 m upstream of the discharge of the river Biala which carries water from mines.

(S1) Sztola—site located about a 100 m upstream of the confluence of the river Sztola, which also carries considerable amounts of water from mines, and the Biala Przemsza river.

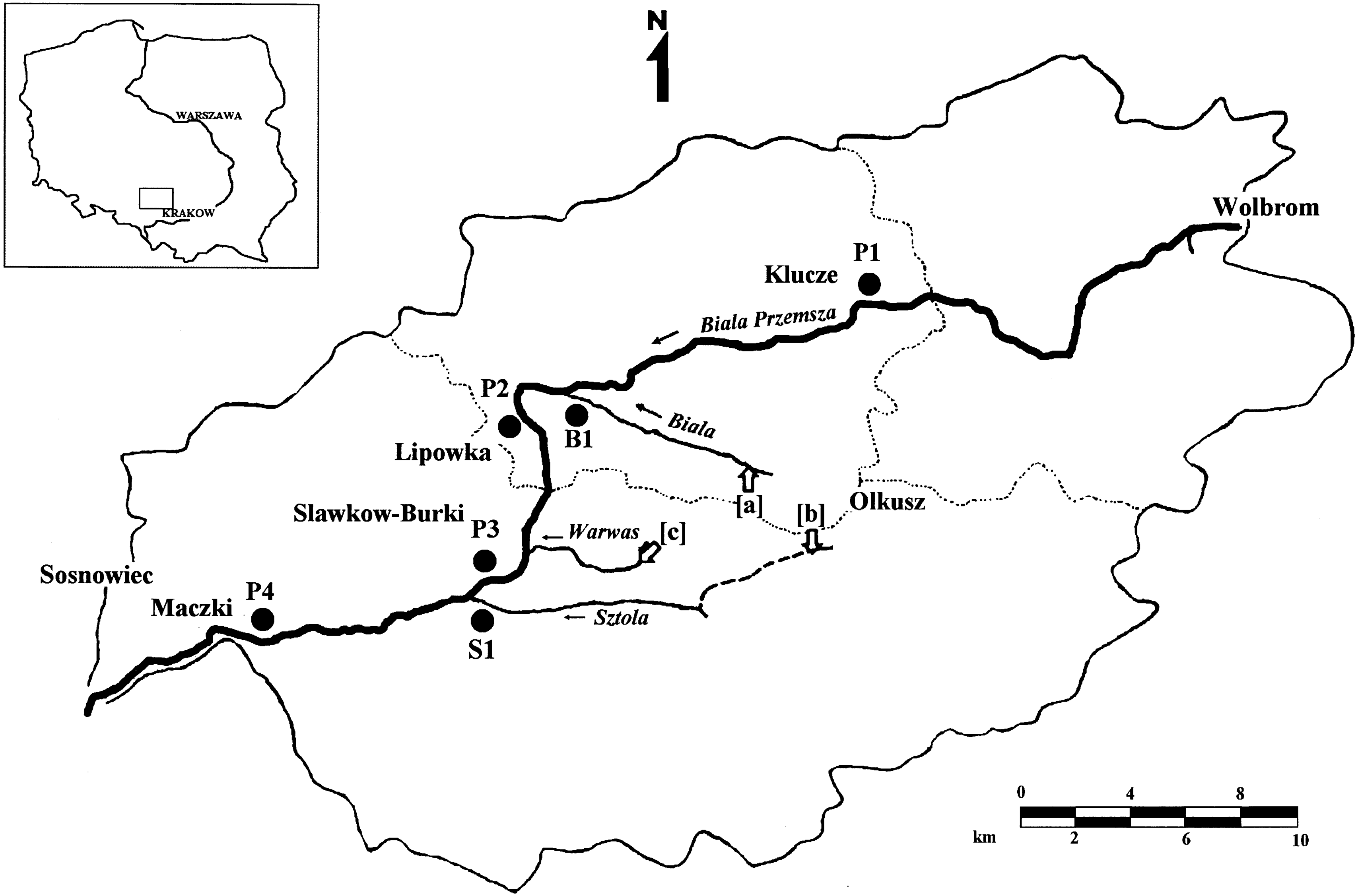

Fig. 1. Location of the investigated watercourses within the catchment basin of the Biala Przemsza river, Upper Silesia, Poland. Sampling stations are marked with filled circles; open arrows indicate main inputs of wastewater from the mining area; broken line represents a channel carrying wastewater from the mining area to the river Sztola; dotted lines mark subdivisions of the catchment basin. See text for further explanations.

Table 1
Baetis spp.: dates of collection, numbers of pooled samples and range of mean individual dry weights (mg) of mayfly larvae in pooled replicate samples from six sites in Upper Silesia, Poland

Site	Position and altitude	Date	Species	Number of pooled samples	Range of mean individual dry weight (mg)
B1 River Biala at its discharge into the Biala Przemsza		11 April 00	*B. rhodani*	5	1.24–1.52
			B. vernus	0	
	50° 19′ 54″ N	31 May 00	*B. rhodani*	3	2.61–2.94
	19° 24′ 50″ E		*B. vernus*	3	1.64–1.96
	309 m	8 Aug 00	*B. rhodani*	5	0.72–1.35
			B. vernus	5	0.52 -0.83
		2 Nov 00	*B. rhodani*	1	2.00
			B. vernus	1	1.60
P1 The Biala Przemsza at Klucze		11 April 00	*B. rhodani*	5	1.76–4.82
			B. vernus	1	1.10
	50° 21′ 19″ N	31 May 00	*B. rhodani*	2	1.39–1.62
	19° 33′ 57″ E		*B. vernus*	4	1.08–1.26
	315 m	8 Aug 00	*B. rhodani*	5	1.00–1.20
			B. vernus	3	0.55–0.97
		2 Nov 00	*B. rhodani*	3	0.33–5.00
			B. vernus	2	0.21–1.26
P2 The Biala Przemsza at Lipowka		11 April 00	*B. rhodani*	2	4.65–4.76
			B. vernus	1	1.40
	50° 19′ 42″ N	31 May 00	*B. rhodani*	4	2.04–3.29
	19° 23′ 21″ E		*B. vernus*	1	2.48
	270 m	8 Aug 00	*B. rhodani*	6	0.57–1.07
			B. vernus	3	0.57–0.83
		2 Nov 00	*B. rhodani*	2	1.10–2.53
			B. vernus	0	
P3 The Biala Przemsza at Slawkow-Burki upstream of mouth of the Sztola		11 April 00	*B. rhodani*	0	
			B. vernus	0	
	50° 15′ 57″ N	31 May 00	*B. rhodani*	4	1.02–1.25
	19° 21′ 26″ E		*B. vernus*	1	1.04
	270 m	8 Aug 00	*B. rhodani*	3	1.15–1.44
			B. vernus	2	0.55–0.86
		2 Nov 00	*B. rhodani*	0	
			B. vernus	1	1.46
P4 The Biala Przemsza at Maczki		11 April 00	*B. rhodani*	8	2.45–5.89
			B. vernus	0	
	50° 15′ 27″ N	31 May 00	*B. rhodani*	4	0.42–0.65
	19° 16′ 36″ E		*B. vernus*	2	0.40–0.55
	252 m	8 Aug 00	*B. rhodani*	5	0.61–0.87
			B. vernus	3	0.53–0.80
		2 Nov 00	*B. rhodani*	2	0.38–1.77
			B. vernus	2	0.23–1.70
S1 River Sztola at its discharge into the Biala Przemsza		11 April 00	*B. rhodani*	5	2.19–2.95
			B. vernus	1	1.04
	50° 15′ 55″ N	31 May 00	*B. rhodani*	3	0.95–1.05
	19° 21′ 30″ E		*B. vernus*	2	0.58–0.69
	270 m	8 Aug 00	*B. rhodani*	5	0.33–0.73
			B. vernus	2	0.23–0.27
		2 Nov 00	*B. rhodani*	4	0.19–2.54
			B. vernus	0	

Altitudes (m) are amsl (above mean sea level).

2.2. Sampling and analysis

The two species in question, particularly *B. rhodani*, are known to have very changeable life cycles, which can vary between seasons and locations. Generally they are bivoltine, with overwintering nymphs emerging as adults early in spring and producing a fast-growing summer generation that may mature in autumn (Humpesch, 1979; Clifford, 1982; Soldán et al., 1998) and oviposit early enough for its offspring to complete early stages of larval development before winter. The summer generation is frequently missing, however, resulting in univoltinism, especially in *B. rhodani* (Clifford, 1982; Elliott et al., 1988).

We sampled twice in spring 2000: in April when we were fairly certain to have only overwintering individuals, and in May to collect the first larvae large enough to be analysed, which may have hatched from eggs laid earlier by newly emerged females. We sampled next in August 2000 when there was no trace of the generation which had begun life the previous year. We could also assume that virtually all larvae present so late in the summer would overwinter and emerge the next year. As a check, we sampled finally in November 2000.

As the two *Baetis* species could not be distinguished at the time of sampling, as many larvae as could be obtained in about one hour (up to 64 larvae) were collected alive at each site from samples taken with a kick net, and returned to the laboratory in a cool box. On the day of collection, individual nymphs were identified to species under a stereomicroscope, rinsed briefly in distilled water, and pooled by species (up to 12 individuals per pooled sample) to give replicates of larvae of an approximately equal size range in each sample (Table 1).

The question arose as to whether larvae should be maintained alive in the laboratory to allow depuration of gut contents. Brooke et al. (1996), for example, recommend that mayfly larvae should be held for 12–24 h in clean water in the laboratory to clear their guts. Hare et al. (1989), however, found that individual larvae of the mayfly *Hexagenia limbata* vary substantially in the rate at which they egest gut contents, and that 48 h is not enough to ensure a complete emptying of the gut. They also showed that it is possible to compensate for the weight of gut contents in larvae analysed without depuration or dissection by applying a percentage reduction factor, and concluded that this method offers simplicity and time saving while providing reasonable accuracy, dependent on the goals of the study (Hare et al., 1989). We decided not to introduce any extra variability that might be caused by attempts to promote gut depuration of mayfly larvae in the laboratory, and also decided not to calculate and apply a reduction percentage factor, for this would have no effect on the conclusions to be drawn from the statistical analysis used.

Pooled samples were dried to constant weight at 60 °C in individual preweighed acid-washed polyethylene vials, digested in Aristar concentrated HNO_3 (BDH. Ltd) at 100 °C, and made up to 2 ml with double distilled water for analysis of concentrations of trace metals (Cd, Cu, Pb, Zn, Fe) on an International Laboratory IL-157 atomic absorption spectrophotometer (AAS). Most samples were analysed for most metals, but occasionally metal concentrations in the digests were beneath detection limits, or insufficient digest was available to complete the series. Samples of Tort-2 Lobster Hepatopancreas certified reference material (National Research Council, Canada) were analysed simultaneously (Table 2), and agreement is considered satisfactory.

Many invertebrates show an effect of body size on accumulated trace metal concentrations, the relationship between metal concentration (y) and body dry weight (x) being modelled by the power function $y = ax^b$ (Rainbow and Moore, 1986; Rainbow et al., 1989, 1998, 2000; Fialkowski et al., 2000). Data, therefore, were transformed logarithmically to ensure fit to normal distributions and to transform the power function to a linear relationship for comparison of best fit regression lines by analysis of covariance (ANCOVA). ANCOVA can be used to test for significant differences in the transformed trace metal concentrations after allowance for differences in the transformed dry weights, with the precondition that there is no significant difference between the regression coefficients (slopes).

All data sets were examined for size effects on accumulated trace metal concentrations in larvae. Correlation and regression analyses of the relationships between metal concentration and individual dry weight (logged data) were carried out on the complete data set for each metal including all larvae of a species analysed for that metal irrespective of site and season, and on each data set for each metal at each site and season. For each metal there were significant ($P < 0.05$) correlations and regressions in at least one data set to be compared for location or for seasonal differences. To account for the significant effect of size, ANCOVA was used to compare accumulated metal concentrations in larvae between sites and seasons. It was also confirmed that for each metal there was no significant ($P > 0.05$) difference between the regression coefficients of the relationships that were being compared. It was therefore possible to compare the accumulated trace metal concentrations by comparing the elevations of the regression lines. The Tukey HSD test was used for post hoc comparisons of concentrations of a metal in mayfly larvae (between sites on one date, or between dates at one site as appropriate) where ANCOVA had indicated significant differences among samples.

Table 2
Comparisons of mean (±95% CL, $n = 4$) measured and certified (±95% Tolerance Limits) trace metal concentrations (μg g^{-1}) in Certified Reference Material [TORT-2 Lobster Hepatopancreas (TORT-1 for Pb); NRC, Canada]

	Measured concentration	Certified concentration
Cadmium	29.5 ±2.5	26.7±0.6
Copper	115 ±9.0	106±10
Lead	9.4 ±0.6	10.4±2.0
Zinc	191 ±14	180±6
Iron	117 ±11	105±13

3. Results

Examination of the mean individual larval dry weights of both species showed that in April the Biala Przemsza populations consisted primarily of large larvae that had overwintered from the previous year. This was particularly distinct in the case of *B. rhodani*, which was noticeably bigger than *B. vernus* (Fig. 2). Mean dry weights of all the larvae studied were 1.36 and 0.80 mg respectively for the former and the latter species. This difference was significant ($F_{1,124} = 16.063$, $P < 0.01$).

In May, individuals of the next generation started to appear, with some noticeable delay in the river Biala and in the Biala Przemsza at Lipowka. In August we found only young instars, the offspring of the summer generation. These nymphs had grown by November (Fig. 2), and at some stations were associated with smaller larvae, which probably had taken more time to hatch.

3.1. Site differences

Fig. 3 presents a summary of the results for the concentrations of the five metals in mayfly larvae (separated into species or combined) at the six sites on the four sampling occasions. Data are expressed as the particular metal concentration in a mayfly larva of mean individual dry weight for the whole data set (the weight-adjusted mean), estimated from the double log regression of the data for the site and occasion. Because most metals were analysed in nearly all pooled samples of mayfly larvae, the mean individual dry weights of larvae analysed for each metal were extremely similar. Grand mean (±S.D.) individual dry weights for all samples were 1.75 (±1.26) mg for *B. rhodani*, and 0.94 (±0.52) mg for *B. vernus*. The leftmost site in Fig. 3 (B1) is on the river Biala which enters the Przemsza between Klucze (P1) and Lipowka (P2), the first and second of the four stations located along the Przemsza (Fig. 1). The rightmost site in the graphs (S1) is on the river Sztola which enters the Przemsza between Slawkow-Burki (P3) and Maczki (P4) (compare Figs. 1 and 3). The graphs indicate geographical differences in the concentrations of each metal accumulated by mayfly larvae on each occasion.

ANCOVA allowed comparison between accumulated concentrations in mayfly larvae at each site on each occasion (Table 3). Metal concentrations in mayfly larvae from sites sharing a common letter for a metal in the left-hand column under each date (for an individual species or for the two species combined) do not differ significantly ($P > 0.05$).

Data for the two species separately and for the species combined are all consistent with each other (Fig. 3, Table 3). For cadmium the highest accumulated concentrations were usually present in mayfly larvae from Slawkow-Burki, with slightly decreased concentrations further downstream at Maczki except in November larvae; concentrations in the Sztola were always lower than at Slawkow-Burki and Maczki (Fig. 3, Table 3). There was some evidence that larvae from the river Biala had higher cadmium concentrations than those at Klucze, suggesting that the entry of the Biala into the Przemsza is associated with the raising of concentrations at Lipowka above those at Klucze (Fig. 3, Table 3). Copper concentrations showed the least geographical variation of the five metals and no consistent geographic pattern (Fig. 3, Table 3). Lead concentrations were high in mayflies from the Biala, Lipowka and Slawkow-Burki, lowest in those from Klucze; lead concentrations in Sztola mayflies were below those at the downstream Przemsza sites (Fig. 3, Table 3). Zinc concentrations were lowest at Klucze, raised in the Biala and at Lipowka, and generally high in the Sztola and at two downstream Przemsza sites (Fig. 3, Table 3). Iron concentrations were usually high in mayfly larvae from the Biala river and from Lipowka below the mouth of the Biala (Fig. 3, Table 3). Mayflies from the downstream Przemsza sites also had high iron concentrations, higher than those in the incoming Sztola river, while mayflies from Klucze, upstream on the Przemsza, typically had the lowest iron concentrations.

3.2. Seasonal changes

Fig. 4 summarises seasonal differences in accumulated trace metal concentrations of mayfly larvae collected on four occasions between April and November 2000 at each of the six sites. Table 3 provides information on the statistical comparisons by ANCOVA. Metal concentrations in mayflies sharing a common number in the row alongside each site (for an individual species or for the two species combined) do not differ significantly ($P > 0.05$).

The seasonal data set for *B. rhodani* is more extensive than that for *B. vernus*, but where sufficient data were available for the latter, patterns were similar for the two species. There were no clear patterns of seasonal change in the concentrations of cadmium, copper and lead in either species (Fig. 4, Table 3). In the case of zinc in *B. rhodani* larvae, August concentrations at most sites were significantly lower than at other times of the year, a feature also apparent in *B. vernus* at the one site (Maczki) showing significant variation over time (Fig. 3, Table 3). Iron concentrations in *B. rhodani* larvae only showed significant change with time at one site, Klucze, where August concentrations were highest (Fig. 4, Table 3).

4. Discussion

Local geographical variations in the input of trace metals from the mines of the Olkusz region into the Biala Przemsza river are reflected not only in changes in

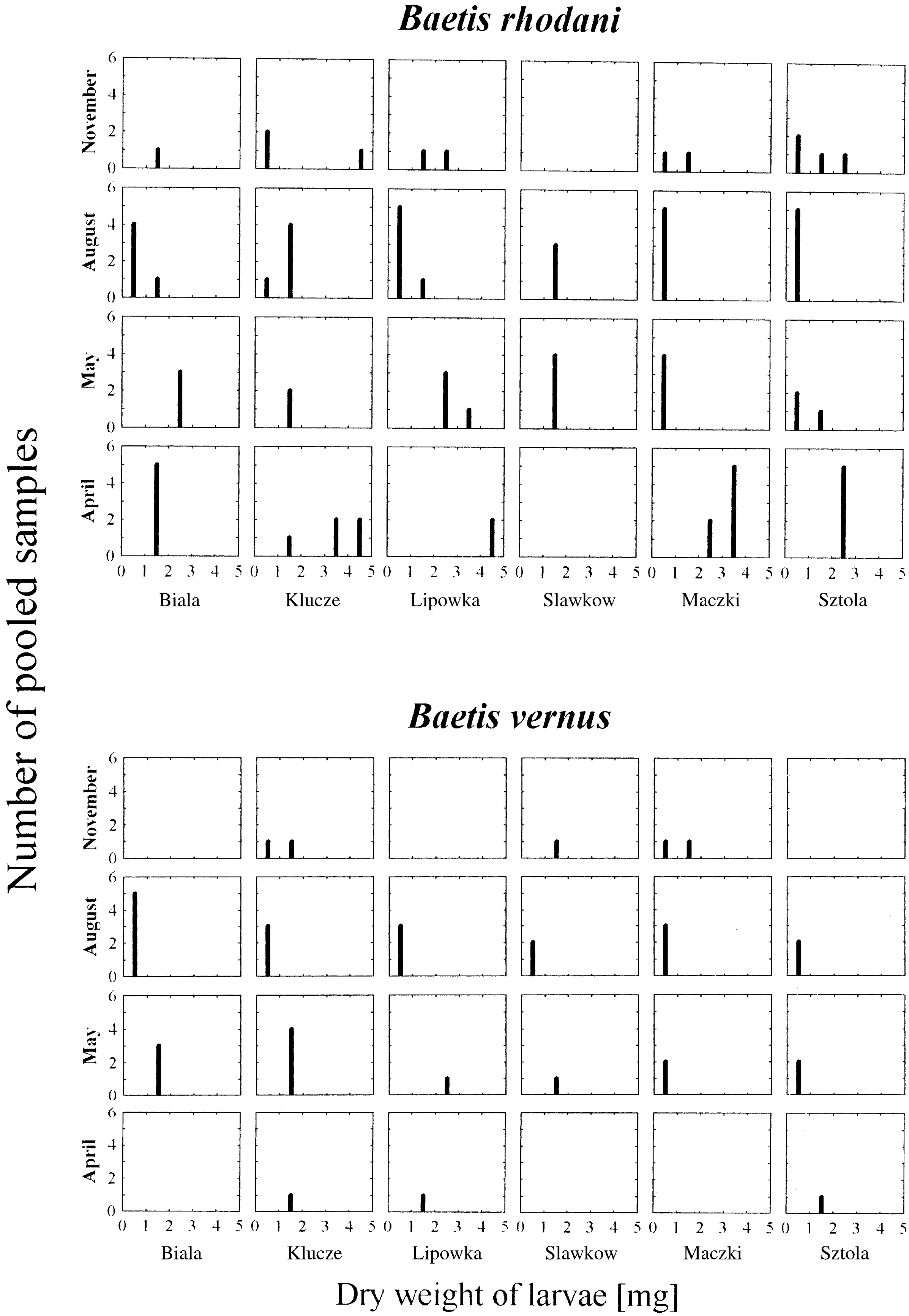

Fig. 2. *Baetis* spp.: frequency distributions of mean individual dry weights of pooled larvae of each species on each sampling occasion at each site.

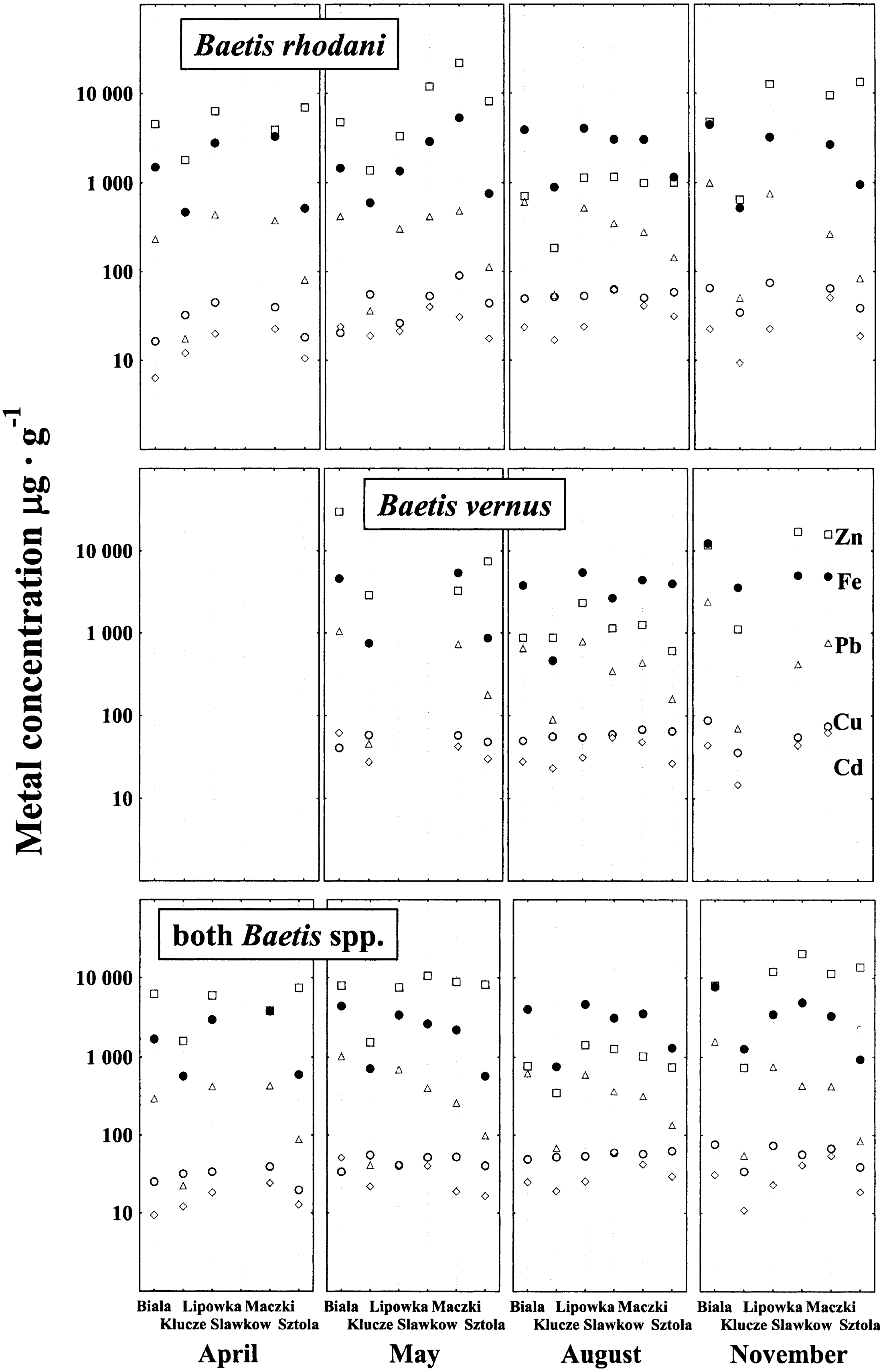

Fig. 3. *Baetis* spp.: weight-adjusted mean trace metal concentrations (µg g^{-1}) in mayfly larvae from six sites (see Table 1) arranged to reflect the actual disposition of sites on the Biala, the Biala Przemsza and the Sztola rivers, on 4 sampling occasions 2000. See text for details.

Table 3
Summary of analysis of covariance of accumulated metal concentrations in *B. rhodani*, *B. vernus* and both species combined at six sites on four sampling occasions

	April 2000		May 2000		August 2000		November 2000	
Cadmium								
Baetis rhodani								
Biala	AC	**1**	B	**1**	CD	**1**	AB	**1**
Klucze	B	**1**	B	**1**	D	**1**	B	**1**
Lipowka	BC	**2**	B	**1,2**	CD	**3**	AB	**1,3**
Slawkow-Burki			A	**2**	A	**1**		
Maczki	A	**1**	A	**1**	AB	**1**	A	**1**
Sztola	ABC	**3**	B	**2**	BC	**1**	B	**3**
Baetis vernus								
Biala			A	**1**	A	**1**	A	
Klucze			A	**1**	A	**1**	A	**1**
Lipowka					A			
Slawkow-Burki					A		A	
Maczki			A	**1,2**	A	**2**	A	**1**
Sztola			A	**1**	A	**1**		
Baetis spp.								
Biala	AB	**1**	A	**1**	CD	**1**	AB	**1**
Klucze	B	**3**	A	**1**	D	**1,2**	B	**2,3**
Lipowka	AB	**1**	A	**1**	CD	**1**	B	**1**
Slawkow-Burki			BC	**2**	A	**1**	AB	
Maczki	A	**2**	B	**1**	AB	**1**	A	**1**
Sztola	AB	**1**	AC	**1**	BC	**1**	B	**1**
Copper								
Baetis rhodani								
Biala	C	**2,3**	A	**3**	A	**1**	AB	**1,2**
Klucze	ABC	**1**	A	**1**	A	**1**	B	**1**
Lipowka	ABC	**2**	A	**2**	A	**1**	A	**1**
Slawkow-Burki			A	**1**	A	**1**		
Maczki	AC	**1**	A	**1**	A	**1**	A	**1**
Sztola	B	**1**	A	**1**	A	**1**	B	**1**
Baetis vernus								
Biala			A	**1**	A	**1**	A	
Klucze			A	**1**	A	**1**	A	**1**
Lipowka					A			
Slawkow-Burki					A		A	
Maczki			A	**1**	A	**1**	A	**1**
Sztola			A	**1**	A	**1**		
Baetis spp.								
Biala	AB	**1**	C	**1**	A	**2**	A	**2**
Klucze	AB	**1,3**	A	**1,3**	A	**2**	B	**2,3**
Lipowka	AB	**3**	BC	**3**	A	**2**	A	**1**
Slawkow-Burki			A	**1**	A	**1**	AB	
Maczki	A	**1**	A	**1**	A	**1**	A	**1**
Sztola	B	**1**	AB	**1**	A	**1**	AB	**1**
Lead								
Baetis rhodani								
Biala	B	**1**	A	**1**	A	**1**	A	**1**
Klucze	D	**2**	C	**1**	C	**1**	C	**1**
Lipowka	A	**1**	AB	**1**	A	**1**	A	**1**
Slawkow-Burki			A	**1**	AB	**1**		
Maczki	A	**1**	A	**1**	AB	**1**	AB	**1**
Sztola	C	**1**	BC	**1**	B	**1**	BC	**1**
Baetis vernus								
Biala			A	**1**	A	**1**	A	
Klucze			B	**1**	C	**1**	A	**1**
Lipowka					A			

(*continued on next page*)

Table 3 (*continued*)

	April 2000		May 2000		August 2000		November 2000	
Slawkow-Burki					AB		A	
Maczki			A	**1**	AB	**1**	A	**1**
Sztola			A	**1**	B	**1**		
Baetis spp.								
Biala	B	**1**	A	**1**	A	**1**	A	**1**
Klucze	D	**1**	C	**1**	D	**1**	B	**1**
Lipowka	AB	**1**	A	**1**	A	**1**	A	**1**
Slawkow-Burki			A	**1**	AB	**1**	AB	
Maczki	A	**1**	A	**1**	B	**1**	A	**1**
Sztola	C	**1**	B	**1**	C	**1**	B	**1**
Zinc								
Baetis rhodani								
Biala	A	**1**	AB	**1**	A	**2**	A	**1**
Klucze	C	**1**	C	**1**	B	**3**	B	**2**
Lipowka	AB	**1,2**	B	**1,2**	A	**2**	A	**1**
Slawkow-Burki			AB	**1**	A	**2**		
Maczki	B	**2**	A	**1**	A	**3**	A	**1**
Sztola	A	**2**	AB	**2**	A	**3**	A	**1**
Baetis vernus								
Biala			AB	**1**	A	**1**	A	
Klucze			B	**1**	A	**1**	A	**1**
Lipowka					A			
Slawkow-Burki					A		A	
Maczki			A	**1**	A	**2**	A	**1**
Sztola			A	**1**	A	**1**		
Baetis spp.								
Biala	A	**1**	A	**1**	AB	**2**	A	**1**
Klucze	C	**1**	B	**1**	B	**2**	B	**1,2**
Lipowka	AB	**1**	A	**1**	A	**1**	A	**1**
Slawkow-Burki			A	**1**	A	**2**	A	
Maczki	B	**2**	A	**1**	A	**3**	A	**1**
Sztola	A	**1**	A	**1**	A	**2**	A	**1**
Iron								
Baetis rhodani								
Biala	A	**1**	AB	**1**	A	**1**	AB	**1**
Klucze	B	**2**	B	**1,2**	B	**1**	B	**1,2**
Lipowka	A	**1**	AB	**1**	A	**1**	A	**1**
Slawkow-Burki			A	**1**	A	**1**		
Maczki	A	**1**	A	**1**	A	**1**	AB	**1**
Sztola	B	**1**	B	**1**	B	**1**	AB	**1**
Baetis vernus								
Biala			A	**1**	A	**1**	A	
Klucze			B	**2**	B	**2**	A	**1**
Lipowka					A			
Slawkow-Burki					A		A	
Maczki			A	**1**	A	**1**	A	**1**
Sztola			B	**2**	A	**1**		
Baetis spp.								
Biala	A	**1**	A	**1**	A	**1**	A	**1**
Klucze	B	**1**	B	**1**	B	**1**	A	**1**
Lipowka	A	**1**	A	**1**	A	**1**	A	**1**
Slawkow-Burki			A	**1**	A	**1**	A	
Maczki	A	**1**	A	**1**	A	**1**	A	**1**
Sztola	B	**1**	B	**1**	B	**1**	A	**1**

Metal concentrations from sites sharing any common letter for one metal in the left-hand column under each date do not differ significantly ($P > 0.05$) between sites. Metal concentrations sharing any common number in the row against each site for each metal do not differ significantly ($P > 0.05$) between sampling occasions.

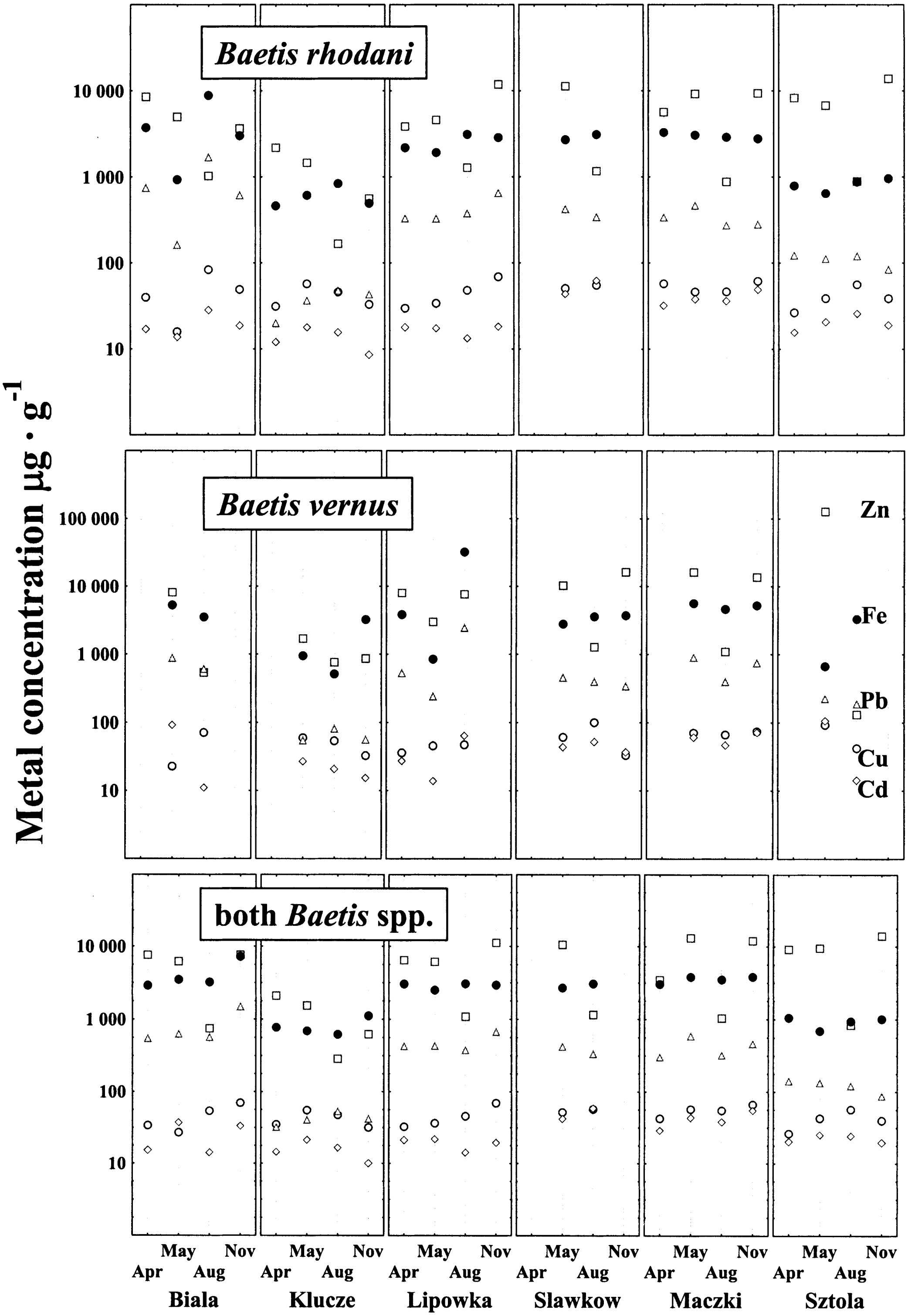

Fig. 4. *Baetis* spp.: weight-adjusted mean trace metal concentrations (µg g^{-1}) in mayfly larvae showing temporal variation at each of 6 sites.

their concentrations in sediments, water column and suspended matter reported by other authors (Suschka et al., 1994; Ciszewski, 1998; Labus, 1999), but also in their bioavailabilities to mayfly larvae as reflected in their accumulated metal concentrations (Figs. 3 and 4, Table 3). This is also the case for the amphipod crustacean *Gammarus fossarum* (Fialkowski et al., in press).

The study provides some evidence of the Biala river contributing to raised cadmium bioavailabilities to the mayfly larvae in the Przemsza river, but there is no contribution from the Sztola river further downstream. The raised cadmium bioavailability at Slawkow-Burki, usually decreasing slightly downstream at Maczki, can be attributed to another source, probably the Warwas stream (Fialkowski et al., in press). Little and inconsistent variability in copper bioavailabilities (Figs. 3 and 4, Table 3) suggests that there are no major point sources of copper input in this part of the Biala Przemsza catchment. Copper concentrations in *B. rhodani* and *B. vernus* are therefore indicative of background copper contamination.

Lead bioavailabilities, on the other hand, as reflected in the accumulated concentrations in mayfly larvae, showed significant local variation indicating point inputs of anthropogenic metal. Lead levels are high in the Biala river, and input from the Biala causes raised bioavailability at Lipowka, well above that at Klucze upstream of the confluence with the Biala (Fig. 3, Table 3). The effect of the Biala on lead bioavailability is still apparent but decreasing at Slawkow-Burki and Maczki. The Sztola river is not, however, a source of bioavailable lead to the Przemsza. In contrast, zinc bioavailabilities are raised in both the Biala and Sztola rivers, with consequent effects on zinc bioavailabilities at the sites downstream of their confluences with the Przemsza river (Fig. 3, Table 3). Moreover, the zinc data do not rule out another source of bioavailable zinc upstream of Slawkow-Burki (Fig. 3), probably the Warwas stream. The pattern of iron bioavailabilities indicates high bioavailability in the Biala but not the Sztola, although there seems to be additional input upstream of Slawkow-Burki, perhaps again through the Warwas stream (Fig. 3, Table 3).

Thus, it can be concluded that the Biala is a source of bioavailable lead, zinc, iron and sometimes also cadmium to the Przemsza river, while the Sztola river is a source of bioavailable zinc. The mayfly data also indicate an additional input of cadmium, iron, and possibly zinc upstream of Slawkow-Burki. The Warwas stream is the most probable source of these inputs (cf. Labus, 1999).

Larvae of the two mayfly species are almost impossible to distinguish in the field. Analysis of the data for the two species separately and combined has shown, however, that the same conclusions can be drawn from the different data sets. Thus, no error would have been introduced if the larvae of the two *Baetis* species had not been separated after identification. Nevertheless, it is important not to conclude that specific identification is never necessary in the design of biomonitoring programmes. The two congeneric mussel species *Mytilus edulis* and *M. trossulus* (Lobel et al., 1990) and the two barnacle species *Balanus amphitrite* and *B. uliginosus* (Rainbow et al., 1993) show significant differences in accumulated metal concentrations when collected from the same sites. Thus failure to identify collected material to specific level would in these cases have introduced errors into analysis and conclusions of biomonitoring programmes using mussels and/or barnacles (Phillips and Rainbow, 1993; Rainbow and Phillips, 1993; Rainbow, 1995).

Fialkowski et al. (in press) also used the amphipod crustacean *G. fossarum* as a biomonitor in the Biala Przemsza system. Similar conclusions on local trace metal bioavailabilities were reached with the amphipod as with the mayfly larvae, indicating that the sources of bioavailable trace metals were similar to both. There are, however, some noteworthy differences between the picture based upon mayfly data and that produced by the crustaceans.

The increase in body concentrations of lead and zinc, attributed to the influence of the Biala river, were generally more pronounced in both species of *Baetis* than in *G. fossarum*. As far as the influence of the wastewater carried by the Warwas stream is concerned, both biomonitors showed an increase of cadmium body content, but only in the mayflies did we notice elevated levels of zinc (compare Fig. 2 in Fialkowski et al., in press, with Fig. 3 here). The drop in metal bioavailabilities observed along the stretch of the Biala Przemsza between Slawkow-Burki and Maczki, was much smaller in *Baetis*, particularly *B. vernus,* than in *G. fossarum*. (compare Fig. 2 in Fialkowski et al., in press, with Fig. 3 here). The exception here was zinc, the level of which did not fall in the amphipod on most occasions (Fig. 2 in Fialkowski et al., in press).

The above-mentioned dissimilarities in the sensitivity of the biomonitors stem primarily from two sources. One source is the different patterns in physiology of trace metal accumulation in amphipods and mayfly larvae (Rainbow, 1998; Goodyear and McNeill, 1999). Thus, at the stations where they co-occurred, baetids were stronger bioaccumulators of iron and zinc, while *G. fossarum* had higher accumulated concentrations of copper. The other source of differential sensitivity is likely to be the dissimilar size spectrum of food particles constituting the main portion of the diets of the animals in question. The major bioavailable source of metals might be organically-rich detritus, either in suspension or deposited as sediment, that constitute the diet of both. However, *Baetis* larvae ingest predominantly smaller particles than *Gammarus*, which prefer coarse detritus (Cummins, 1973; Cummins et al., 1989). The fact that the mayfly larvae seemed more sensitive to influxes of trace metals (see also Rehfeldt and Söchtig, 1991), and at the same time their metal concentrations were dropping more slowly in the less contaminated stretches of the Biala Przemsza, suggests that finer ingested particles may contain greater amounts of bioavailable metals, perhaps as a result of their larger surface-to-volume ratio.

Rehfeldt and Söchtig (1991) quote concentrations for accumulated trace metals in larvae of *B. rhodani* from mine-affected streams in Lower Saxony, Germany (April–May 1985) that can be compared with concentrations recorded here, in order to gain insight into the relative levels of bioavailable metal contamination of the two stream systems. The highest mean zinc concentration (± S.D.) quoted by Rehfeldt and Söchtig (1991) is 9398±1764 μg g^{-1} dry weight in larvae from the river Grane, compared to a weight-adjusted mean of 21,920 μg Zn g^{-1} dry weight in *B. rhodani* larvae collected at Maczki in May in this study. The highest mean lead concentration in the Lower Saxony study is 694±79.2 μg g^{-1} dry weight in mayfly larvae from the river Laute, whereas those from the November samples from the river Biala had a weight-adjusted mean of 997 μg Pb g^{-1}. In contrast to zinc and lead, cadmium concentrations in *B. rhodani* were higher in Germany, a mean concentration of 120±26.1 μg g^{-1} dry weight in larvae from the river Grane (Rehfeldt and Söchtig, 1991) compared to a weight-adjusted mean of 50.1 μg Cd g^{-1} in larvae collected in November at Maczki in Upper Silesia. Given the absence of point sources of entry of anthropogenic copper into the Przemsza river system, it is not surprising that concentrations of that metal in Lower Saxony mayflies also exceed those in this study—a mean concentration of 226±26.1 μg Cu g^{-1} dry weight from the river Oker near Schladen (Rehfeldt and Söchtig, 1991) against a weight-adjusted mean of 90.2 μg Cu g^{-1} dry weight at Maczki in May. Thus the Upper Silesia stream system studied here appears to be more contaminated by zinc and lead, but less contaminated by cadmium and copper than the stream system draining the northernmost slopes of the Harz Mountains investigated by Rehfeldt and Söchtig (1991). Iron concentrations were not measured by Rehfeldt and Söchtig (1991).

Comparative trace metal concentrations for larvae of *B. vernus* have been presented by Jop (1991), also from southern Poland, but from a lowland stream in the Niepolomice Forest near Krakow. The stream was also influenced by trace metal pollution, the bulk of it coming via atmospheric fallout (Jop, 1981, 1991; Grodzinski et al., 1984). The highest mean concentrations of cadmium (8.6±0.9 μg g^{-1}), lead (66.2±7.5 μg g^{-1}) and zinc (1521±395 μg g^{-1}), but not copper (66.2±7.5 μg g^{-1}), in different larval stages of *B. vernus* were well below equivalent concentrations measured here (see Fig. 2), highlighting the high general contamination of the Biala Przemsza river system by these three trace metals.

The extremely high concentrations of lead and zinc in mayfly larvae in our study are worthy of comment. Potentially toxic trace metals accumulated to this degree require detoxification, usually in insoluble form (see Phillips and Rainbow, 1993), and the mechanisms of detoxified storage have been reviewed with respect to insect larvae by Hare (1992). In larvae of the mayfly *B. thermicus* inhabiting a metal-polluted river in Yokohama, Japan, accumulated copper and iron were localised in midgut epithelial cells whereas zinc was more generally distributed through the tissues including the midgut epithelium, fat bodies and muscle (Sumi et al., 1991).

A probable cause of seasonal variation in accumulated metal concentrations in *Baetis* larvae was the presence of larval stages of different age and therefore size. In spite of the variation being wide, relatively few seasonal changes in accumulated metal concentrations were statistically significant (Table 3, Fig. 4). The low accumulated zinc concentrations in small *B. rhodani* collected in August may be a consequence of their relatively young age, and therefore insufficient time for the build-up of a large store of detoxified accumulated zinc. In contrast, the high iron concentration in small *B. rhodani* larvae collected in August at Klucze may result from relatively more surface-adsorbed metal, for iron is a surface active metal and small larvae would have a relatively large surface area to volume (and therefore dry weight) ratio. Jop (1991) did not measure iron concentrations in *B. vernus* larvae of different stage and size but found that concentrations of cadmium, copper, lead and zinc all decreased with increasing larval size and age in the lowland stream near Krakow. Such a pattern may be typical of sites with less severe metal contamination. At more heavily metal-contaminated sites, such as in the Biala Przemsza river system in Upper Silesia, accumulated metal stored internally by mayfly larvae may counteract any growth dilution of larval body metal concentration.

Hare and Campbell (1992) reviewed temporal variation of trace metal concentrations in aquatic insects and noted that although temporal fluctuations occur, they do so inconsistently. Typically, they are more apparent in the case of the non-essential metal cadmium than for the essential ones copper and zinc. For that reason, such fluctuations can sometimes be ignored in biomonitoring studies, depending on the aims of a particular project, (Hare and Campbell, 1992). In our study, local geographical variation in trace metal bioavailabilities was identifiable in each of the four collections made. The time of sampling for biomonitoring purposes requires adequate numbers of larvae to be present, and their size-range to be relatively homogeneous in order to minimise possible effects of size and/or age on accumulated metal concentrations. In the river system studied here, the August (late summer) samples most nearly matched these ideal criteria.

Larvae of the mayflies *B. rhodani* and *B. vernus* (whether considered separately or together) proved to be excellent biomonitors of trace metals in the streams of Upper Silesia. Like the amphipod crustacean *G. fossarum*, the larvae can be used as members of a suite of stream biomonitors in Central Europe, which together can provide information on different sources of bioavailable trace metals present in the system under investigation.

We would like to stress that the use of a suite of biomonitors is preferable to use of a single one, because levels of bioaccumulation differ among species of insects and crustaceans, and several species therefore provide a more comprehensive picture of metal pollution in the system under investigation.

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