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Trace metal concentrations in free-ranger, tube-dweller chironomid larvae and a weakly polluted fluvial sediment

by

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Abstract

The objective of this study was to analyze macroinvertebrate assemblages dominated by Chironomidae (Diptera) and to assess the protective nature of these midges' larval tubes against trace metals in the weakly polluted Bzura River. This low order lowland river flows through the Łódź City Municipal Area and is supplied by a large amount of organic matter from ecotones and a polluted roadside. Determination of metal content in sediments and chironomid tissue and tubes was conducted using atomic absorption spectroscopy. Our study has shown that the composition of chironomid assemblages was not determined by trace metals but rather by high organic enrichment, which caused the dominance of two species representing the same trophic group – gathering collectors – but differing in behavior. One of them belongs to free-rangers (*Prodiamesa olivacea*), while the other (*Chironomus riparius*) to tube-dweller larvae. Although the accumulation of certain trace metals in the tubes was found, the larvae of both types of behavior had a similar concentration of metals in their tissues, which proves effective metal detoxification in these insects.

Key words: river, benthos, tubes, larvae, imagines, *Chironomus riparius*, *Prodiamesa olivacea*, detoxication

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Introduction

Chironomidae (Diptera), called also non-biting midges, are a family of aquatic flies with worldwide distribution. These dipterans are common inhabitants of most freshwater habitats, and often dominate in aquatic macroinvertebrate assemblages, both in abundance and species richness (Armitage et al. 1995, Broza et al. 2000, Ferrington 2008). Many of chironomid species can tolerate and develop in heavily metal-polluted watercourses (Lindegaard 1995, Mousavi et al. 2003, Czerniawska-Kusza 2007, Tang et al. 2010). They were found to represent up to 80% of the total benthofauna, but less than 10% in an unpolluted section of the same stream (Winner et al. 1980). According to many studies, these dipterans appear to be less sensitive to metals than gastropods and crustaceans or other insects, i.e. ephemeropterans and trichopterans (Poulton et al. 1995). Usually, concentrations of metals such as copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), manganese (Mn) and nickel (Ni) were simultaneously measured in the sediments and in benthic macroinvertebrates. The most toxic of them, i.e. Cd, Pb and Ni, were listed as priority substances in the EU Water Framework Directive (EU WFD 2000).

Many of the chironomid larvae live in tubes in the soft benthic sediments of freshwater habitats and affect the water-sediment exchange in these ecosystems by stimulating biogeochemical and microbial processes occurring in river sediments. Although Chironomidae are less effective in reducing the sediment clogging compared to other common dwellers, i.e. Tubificidae (Oligochaeta), nonetheless they play a major role in bioturbation (water-sediment exchange) (Nogaro et al. 2006). However, the tube-dwelling behavior of chironomids is also very important for each organism. Originally it evolved as anti-predator adaptation against both invertebrates and vertebrates (Hershey & Dodson 1987) and/or as a protection from being washed away in rivers (Brennan & Mclachlan 1979). This adaptation was later modified in some chironomid groups to facilitate feeding and respiration (Walshe 1950).

In recent years, the question has arisen whether the tube of a benthic chironomid larva plays a role in protecting its dweller against chemical toxicants in sediment and/or from environmental disturbances (Halpern et al. 2002, Vedamanikam & Shazili 2009). We try to answer this question on the basis of taxa living in a weakly polluted river, contrary to previous investigations in which organisms with tubes inhabited more polluted ecosystems. The first aim of our study was to estimate the abundance of zoobenthos and the contribution of Chironomidae (Diptera) in these macrobenthic assemblages. We also want to compare the trace metal concentration in the chironomid larvae tissue of *Chironomus riparius* Meigen and *Prodiamesa olivacea* Meigen. These two dominant taxa in these macrobenthic assemblages settled the same stream section, represented the same functional feeding group (FFG), i.e. gathering collectors, but displayed different behavior. The first one belongs to tube-building species, while the other to free-rangers. Additionally, we analyzed the tissue of other chironomid taxa (*Paratanytarsus*), representing also filtering collectors. The other objective of our study was to compare the accumulation of selected metals in tubes built by several other species of Chironomidae.

Materials and methods

Study area

The small lowland Bzura River is a part of the Vistula drainage basin. The river arises at 235 m a.s.l., and is 166 km long. Its catchment area is 7787.5 km^2 and the slope is 2.4‰ in the upper course. The study site is located in the first order stream section which flows through the Łódź City Municipal Area (Fig. 1).

The source reach of the Bzura River flows through the largest urban forest in Europe (Łagiewniki). The riparian zone is dominated by *Alnus glutinosa* (L.) Gaertn., *Fraxinus* L. and *Salix alba* L., thus large amounts of allochthonous organic matter, especially tree leaves, covered the stream bed over the annual period of the study. The main characteristics of this upstream section were sampled monthly over the annual period and are presented in Table 1. This slowly-flowing reach receives pollutants from roadsides and surface run-off from meadows and the forest. It is commonly known that pollutants from roadsides also play a key role for soils located close to roads (Szyszlak-Bargłowicz et al. 2013).

Further details of the study area were given by

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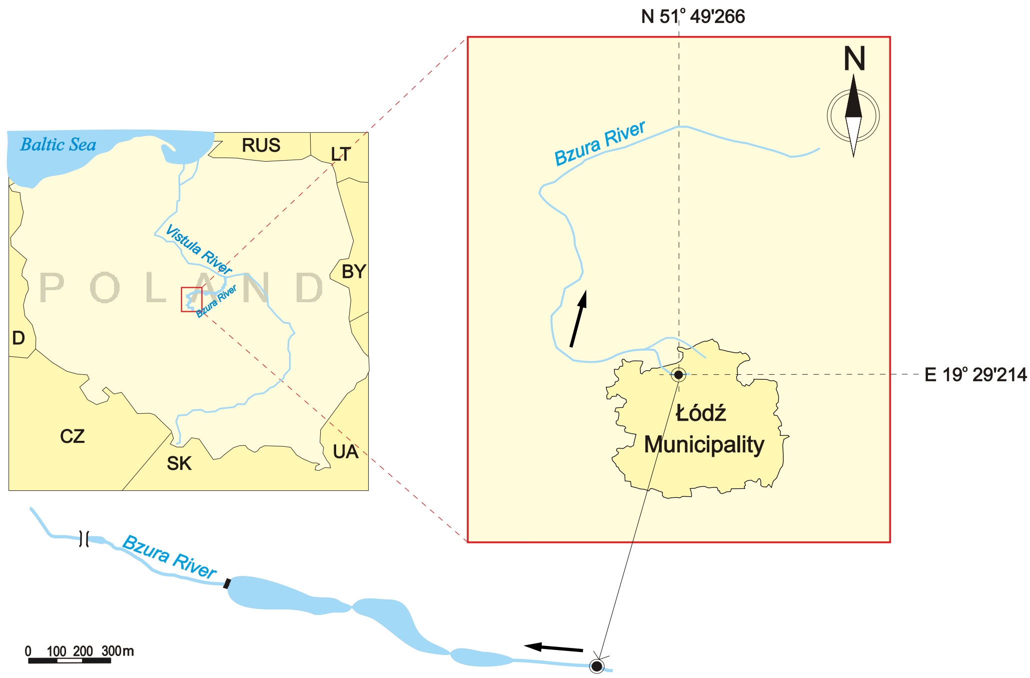

Figure 1

Study area of the Bzura River with the marked sampling site (after Grzybkowska et al. 2012, modified)

Grzybkowska (1995), Grzybkowska & Głowacki (2011) and Grzybkowska et al. (2012).

Sampling and preliminary analysis

Bottom sediment samples with macroinvertebrates from the Bzura River were collected monthly, from November 2009 to October 2010, because we wanted to investigate the macrobenthic assemblage at this site. Ten samples were collected randomly on each sampling occasion with a tubular sampler (25 cm^2 in area). The sampler was pushed into sediment to a depth of 15 cm. During the annual sampling cycle, depth, current velocity and dissolved oxygen were measured monthly.

The samples were also used to analyze the composition of particulate inorganic matter according to Cummins (1962). Field data on the particle-size distribution were transformed into the single substrate index (SI) by summing up the mid-point values of size classes weighted by their percentage cover (Quinn & Hickey 1990). These samples were also used to determine the organic matter content in the bottom sediments. Benthic organic matter (both coarse CPOM and fine FPOM fractions) was dried at 60°C for 72 h and weighed (Petersen et al. 1989).

Moreover, additional samples were collected in late winter to obtain chironomid larvae and sediments for the metal analysis. As it is known, physico-chemical parameters of sediments can significantly influence the availability and toxicity of many trace metals (Harding 2005). We studied metal concentrations in insects in late winter because this season reflects long-term exposure of

Table 1

Mean (for the period of November 2009-October 2010) value and range of selected parameters of the investigated site of the Bzura River; SI – Substrate Inorganic Index; BCPOM (coarse) and BFPOM (fine) particulate organic matter; TPOM – transported particulate organic matter

Parameter	Mean	Range
Discharge (m^3 s^{-1})	0.010	0.002-0.028
Water velocity (m s^{-1})	0.14	0.05-0.42
Width (m)	0.8	0.4-1.4
Depth (m)	0.08	0.02-0.11
SI (mm)	3.4	0.4-6.8
BCPOM (g m^{-2})	2021	1227-3454
BFPOM (g m^{-2})	5786	3822-8330
TPOM (g m^{-3})	82	1-194
Oxygen (mg l^{-1})	4.3	1.0-11.5

freshwater organisms to metal contamination. The detailed data concerning the period needed for the development of a generation (Cohort Production Interval) of each species were given by Grzybkowska (1995), Grzybkowska & Głowacki (2011) and Grzybkowska et al. (2012).

The last instar larval stage (4^{th}) of four dominant chironomid genera, tube-dwellers of *Chironomus*, *Paratendipes* and *Paratanytarsus* and chironomid free-ranger of *Prodiamesa olivacea*, were selected for further analysis of metal contents. The larvae were rinsed with deionized water, placed in acid washed polypropylene vials, and stored at -20°C until analysis. To obtain chironomid tubes and imagines, we organized a laboratory rearing. The larvae were located in crystallisers filled with river sediment and water at room temperature. After the tubes had been abandoned by pupae, both tubes and imagines were collected for further analysis.

Metal analysis

All the chironomid samples (larvae, tubes, imagines) and sediments were analyzed for the content of Cu, Zn, Cd, Pb, Mn and Ni. Determination of metal content in sediments, insects' tissue and tubes was conducted using the Atomic Absorption Spectroscopy (Spectra spectrophotometer 300/400, Varian) with flame atomization (acetylene – air mixture) and with non-flame atomization in a *GTA-96* graphite tube atomizer. The analysis was performed in the Laboratory of Computer and Analytic Techniques, Faculty of Biology and Environmental Protection, University of Łódź. The concentrations of the metals studied in both substrata were calculated on a dry weight (dw) basis and expressed as μg g^{-1}. A total of six replicates of the measurements were taken.

Preparation of samples, calibration and precision of the method for metal analysis were given by Dukowska et al. (2012).

In this study, the bioconcentration factor (BCF) was used to relate pollutant concentration in aquatic organisms to the pollutant concentration in the sediments (Janssen et al. 1997):

$$BCF = \frac{\text{[metal] organism}}{\text{[metal] sediment}}$$

[metal] organism – total metal concentration in a given organism (μg g^{-1} dw)
[metal] sediment – total metal concentration in the sediment (μg g^{-1} dw).

We used Student's *t*-test to compare the concentration of the analyzed metals in the sediment, tubes, larvae and imagines (StatSoft Inc. 2011).

Results

Macrobenthic assemblages

Chironomidae and Oligochaeta were the major groups of macroinvertebrate assemblages and their density was over 80% of the total macroinvertebrate density over the annual period (Fig. 2). Among other macroinvertebrates, high density was recorded for

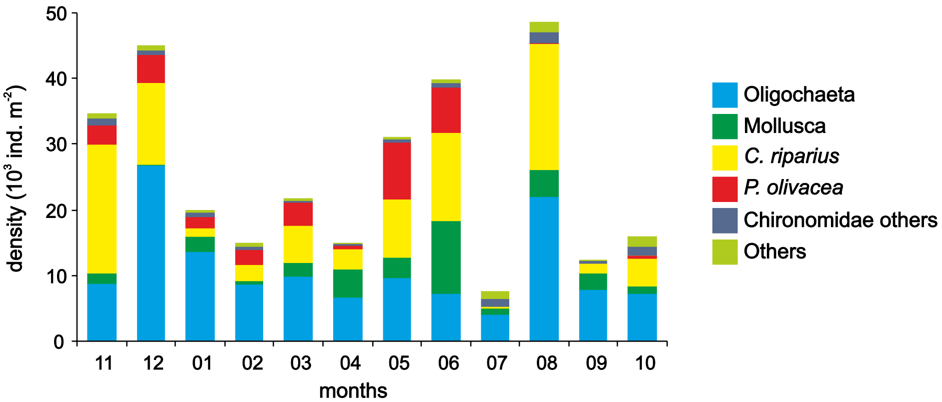

Figure 2

Seasonal dynamics of the main macroinvertebrate taxa from the sampling site of the Bzura River (after Grzybkowska et al. 2012, modified).

Mollusca (mainly Sphaeridae, over 10% of the total macroinvertebrate density, Fig. 2).

The macrobenthic taxa showed a seasonal pattern that mainly reflected the chironomid seasonal dynamics. The density of *C. riparius* represented almost 25% of the total macrobenthic density; the maximum values in November and August were close to 20 000 ind. m^{-2}. Another chironomid species, *P. olivacea*, a free ranger connected with leaf packs, was less abundant and represented nearly 10% of the total zoobenthos. The other tube-building larvae from the subfamily Chironominae, such as *Paratendipes* sp. (Chironomini) and *Paratanytarsus* sp. (Tanytarsini), were much less numerous. Very low density and species richness of further chironomid taxa, such as Orthocladiinae (scrapers) and Tanypodinae (predators), were also recorded at this site.

Tubes and tube-building

Many larvae of chironomid taxa build tubes using salivary secretions to bind small particles – principally of detritus, medium-sized particles – mainly of detritus and diatoms (very well visible in Fig. 3c), and a variety of other types of particles, including filamentous algae and sand grains into relatively cylindrical retreats. As you can see in the part a and b of Fig. 3, the time of construction and/or reconstruction of this tube is very short.

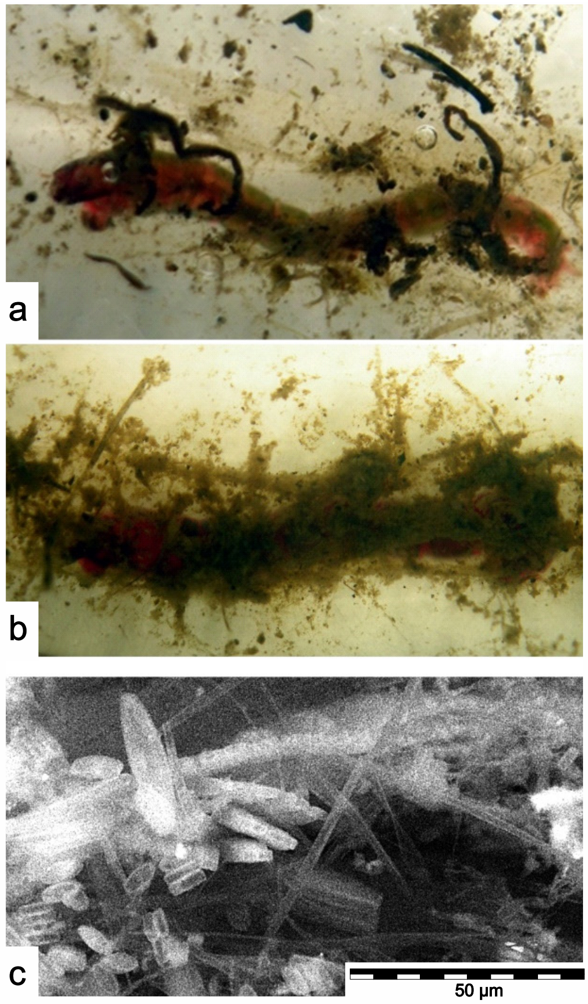

Figure 3

Chironomus larva a) after 15 min; b) after 90 min after placement in a crystallizer with particulate organic matter (detritus); c) part of a tube (2000x). Photo 3c was made using scanning microscope Vega Ts, at the Department of Solid State Physics, Faculty of Physics and Applied Informatics, University of Łódź.

Metals in sediments and in the inhabitants of the bottom

The concentration of toxic metals in the sediment of the Bzura River was not high; the highest values were determined for Zn and Mn, while the lowest for Cd, Ni and Pb (Fig. 4).

Larval tubes of Chironomini (*Chironomus* and *Paratendipes*) were very similar in regard to the concentration of these particular elements (Fig. 5). In turn, *Paratanytarsus* (Tanytarsini) significantly differs from the previously mentioned taxa in terms of the concentration of the studied metals (Student's *t*-test, df=10, p=0.000).

We also compared the content of trace metals in larvae representing the same trophic group, i.e. gathering collectors, but with different behavior. As evidenced by our results (Fig. 4), the metal concentration was higher in *C. riparius* larvae compared to *P. olivacea*, except for Cd, which occurred in the same amounts in the tissues of both species. On the other hand, these metals did not show such a uniform trend in imagines, i.e. their concentrations were higher in *C. riparius* (Zn, Cu) or in *P. olivacea* (Ni, Pb, Mn).

The bioconcentration factor (BCF) calculated for each metal varied between larvae, tubes and imagines, and reached the highest values for Cd (imagines and tubes of *Paratanytarsus*, tubes of

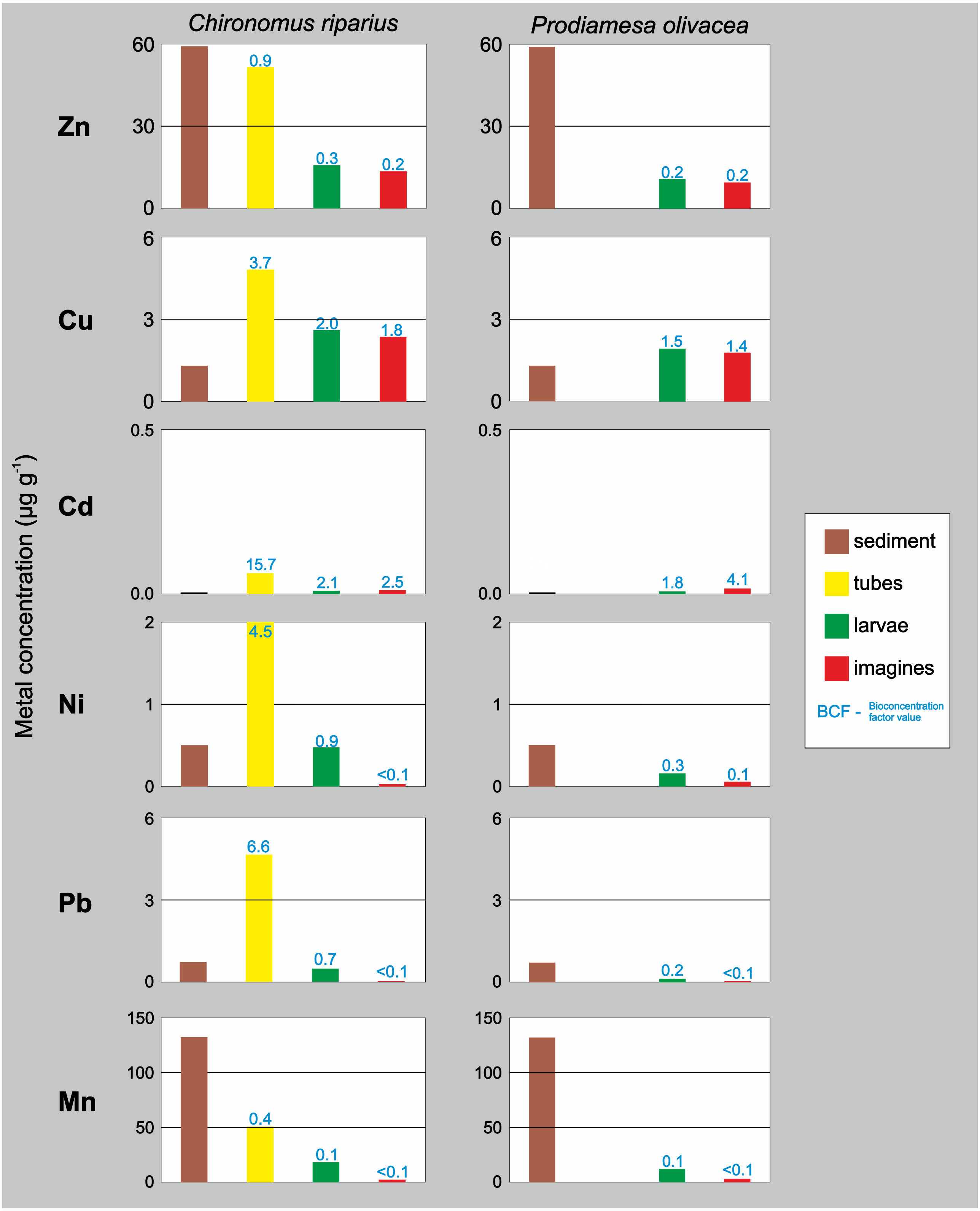

Figure 4

Metal concentrations in sediments and chironomid larvae, their tubes and imagines. Values of the bioconcentration factor are given above each histogram.

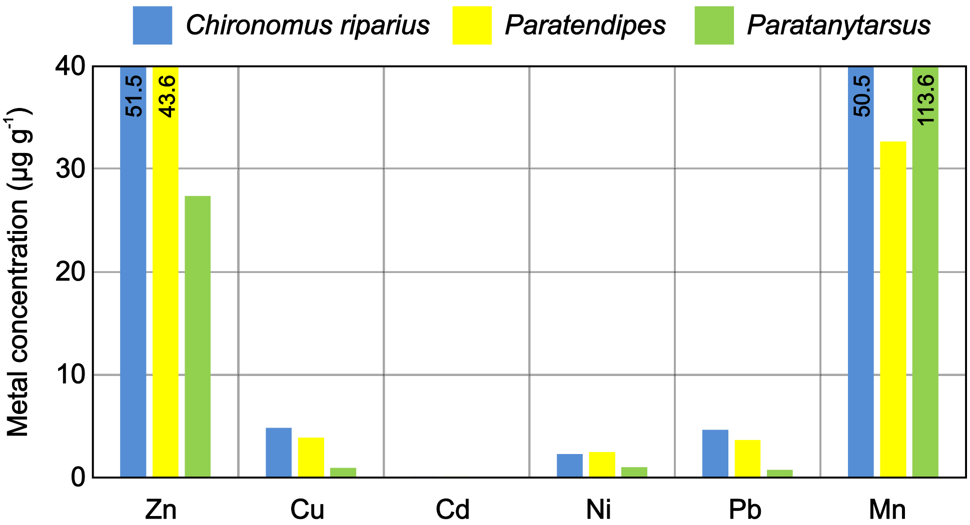

Figure 5

Metal concentration in chironomid tubes: Chironomini (*Chironomus, Paratendipes*) and Tanytarsini (*Paratanytarsus*)

Paratendipes, tubes and imagines of *C. riparius*), for Pb and Ni in tubes of *C. riparius* and *Paratendipes*. The majority of BCF values for other studied metals were slightly below or above 1.

Discussion

Habitat heterogeneity versus macroinvertebrates in rivers

Longitudinal zonation of lotic waters based on physical characteristics is reflected in biological characteristics (Vannote et al. 1980). Macroinvertebrates living on or in the bottom sediment of watercourses (onbenthos and inbenthos) are very essential for the functioning of their biotas. The amounts (both the number and biomass) as well as the diversity of this ecological group change along the river course, primarily according to the flow regime, the granulometry of mineral substrate (SI) and temperature. The last factor also regulates the availability of food to a large extent (the quality and quantity of food sources, both of allochthonous and autochthonous origin, including the amount of benthic particulate organic matter, BPOM (Minshall & Robinson 1998). Macroinvertebrates are crucial elements to the functioning of freshwater ecosystems, because they represent a link between detritus (both of allochthonous and autochthonous origin) and grazing food chains. The division of stream invertebrates into functional feeding groups is based on the mechanisms by which they acquire their food. Morphological and behavioral adaptations of organisms are helpful in obtaining different kinds of POM, including animal matter (Berg 1995, Cummins et al. 2005).

Chironomid larvae frequently dominate in the benthos, with the highest annual production/biomass (*P*/*B*) values being determined for aquatic metazoans (Benke 1993). Thus it is not surprising that hydrobiologists have began to search for indicator species among these dipterans, which have particular requirements for different kinds of stress, among them the types of pollutants, and the species may be used to characterize the quality of environments (Wiederholm 1984, Johnson et al. 1993, Lindegaard 1995, Lindegaard & Broderson 1995). On the basis of previous studies of chironomid assemblages, a pollution index was proposed. Winberg (1978) reported that polluted rivers were dominated by Tanypodinae, while Winner et al. (1980) found that Orthocladiinae dominate in this kind of ecosystems. Taking all the above searches into consideration, one genus of these midges, i.e. *Chironomus* (Chironomini), become the most extensively studied, in terms of various aspects of environmental stress, including pollutants. However, the discussion about midges as indicators of anthropogenic contaminations is still open. Tang et al. (2010) who analyzed the structure

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and species abundance of chironomids, as well as all macroinvertebrates in streams of varying degrees of contamination in Korea, reported that species richness of chironomids was almost stable at clean to intermediately polluted sites.

Our investigations showed that the chironomid assemblages of the Bzura River were not determined by trace metals but rather by high organic enrichment (both CPOM and FPOM), with a dominance of Prodiamesinae (*P. olivacea*) and Chironominae – Chironomini (*Chironomus*), but not Orthocladiinae and Tanypodinae. This pattern of assemblages has been characteristic for many years in this slow-flowing, sandy-bottom river, with high amounts of POM (Grzybkowska 1995, Grzybkowska et al. 2012), and comparable with other natural watercourses (De Bisthoven et al. 1992). It should be emphasized that in comparison with other studied polluted lotic ecosystems (Bervoets et al. 1997, 1998; Martinez et al. 2002; Smolders et al. 2003; Farag et al. 2007; Kucuksezgin et al. 2008), the Bzura River belongs to weakly trace metal polluted ecosystems.

Similarly to the uptake via the body surface and via food, and using different transport mechanisms, depending on each pollutant (Muscatello & Liber 2010), metals may also be excreted in different ways: feaces, exuviae, or through some detoxification processes (Rainbow 1996). Schaller et al. (2011) determined the accumulation of metals in situ under natural conditions within the food web mostly via invertebrate shredders (the key FFG in a low order stream section) which, however, was very small. This accumulation occurs due to reduction of the uptake of inessential elements in the presence of nutrient-rich food, even in habitats with higher contamination levels. On the basis of our study, this statement can be extended to the next trophic group, gathering collectors.

Some studies provide examples of adaptations enabling some chironomid taxa to cope with pollution, including increased capacity of oxygen storage and presence of huge amount of haemoglobin (Osmulski & Leyko 1986), capacity of long-term anaerobic metabolism, avoidance of polluted sediments, both active and passive (drift) behavior pattern, changing feeding behavior, modification of life history and recently emphasized – protection by tubes.

A free-ranger or tube-dweller chironomid larvae

The groups of tube-building organisms (mainly from the subfamily Chironominae: Chironomini and Tanytarsini) may reach high densities and alter the properties of the cohesive sediment, forming a matrix of tubes (Ferrington 1992). According to Chaloner & Wotton (1996), the tubes in soft sediments can reach several centimeters in depth and protrude a few millimeters above the sediment surface; openings of the tubes above the sediment, in which the organisms live, may effectively reduce the pressure on the bed (Ólafsson & Paterson 2004).

Some investigations have shown that generally both free-ranging chironomid larvae and tube-dwelling species, which spend a lot of time foraging outside the tube, are highly susceptible to predation (Tokeshi 1995). However, the advantage and disadvantage of being a free-ranger/tube dweller may alter the relative importance of predation and competition in the community varies in time and/or space. Susceptibility to predation depends on the foraging behavior of predators and the relative size ratio of predators to prey, for example young-of-the-year (*YOY*) fish eat mainly small chironomid larvae (Dukowska & Grzybkowska 2014), while older fish consume bigger, well-visible red prey owing to their high haemoglobin concentration, and due to the fact that they are large-sized and thus make higher energetic net gain (Dukowska et al. 2009).

The majority of chironomid pupae also live in tubes and the most dangerous episode for each individual is leaving the tubes and rising to the surface to eclose; during this movement they may be eaten in large numbers by predators (e.g. fish, Makino et al. 2001). So during the ontogenetic development, the period of staying in tubes is very important for their safety. It is not surprising that a damaged tube is quickly rebuilt by a larva, while a pupa lacking a tube accelerates its development in order to minimize the danger of been eaten (Mckie 2004).

As shown in the course of our studies, tubes built by closely relative taxa, such as *Chironomus* and *Paratendipes,* contain similar concentrations of trace metals, while *Paratanytarsus* is a less related genus and accumulates smaller amounts of metals. Thus, our finding confirmed data of Brennan & Mclachlan (1979) that both tube construction and accumulation of metals is, to a large extent, species-dependent.

Another hypothesis found in the literature, e.g. by Halpern et al. (2002), was that tubes may also play a key role in protecting their dwellers, as in the case of *C. luridus*, against chemical toxicants in the sediment (in addition to making feeding and respiration easier and serving as an anti-predator mechanism). This presumption was put forward by microbiologists on the basis of a high activity of non-specific esterase in midgut cells of these larvae.

Although the accumulation of certain trace metals in the tubes was determined, yet the larvae with both types of behavior (free-rangers and tube-builders) had a similar concentration of metals in their tissues, which proves the effective metal detoxification in these insects (Rainbow 1996).

Acknowledgements

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References

Armitage, P.D., Cranston, P.S. & Pinder, L.C.V. (1995). *The Chironomidae. The biology and ecology of non-biting midges*. London: Chapman & Hall.

Benke, A. (1993). Concepts and patterns of invertebrate production in running waters. *Verhandlungen des Internationalen Verein Limnologie* 25: 15-31.

Berg, M.B. (1995). Larval food and feeding behavior. In P.D. Armitage, P.S. Cranston & L.C.V. Pinder (Eds.), *The Chironomidae. Biology and ecology of non-biting midges* (pp. 136-168). London: Chapman & Hall.

Bervoets, L., Blust, R., De Wit, M. & Verheyen, R. (1997). Relationships between river sediment characteristics and trace metal concentrations in tubificid worms and chironomid larvae. *Environ. Pollut.* 95: 345-356. DOI: 10.1016/S0269-7491(96)00134-0.

Bervoets, L., Solis, D., Romero, A.M., Van Damme, P.A. & Ollevier, F. (1998). Trace metal levels in chironomid larvae and sediments from a Bolivian River: impact of mining activities. *Ecotox. Environ. Safe.* 41: 275-283. DOI: 10.1006/eesa.1998.1707.

Brennan, A. & Mclachan, A.J. (1979). Tubes and tube-building in a lotic chironomid (Diptera) community. *Hydrobiologia* 67: 173-178. DOI: 10.1007/BF00126716.

Broza, M., Halpern, M. & Inbar, M. (2000). Non-biting midges (Diptera; Chironomidae) in waste stabilization ponds: an intensifying nuisance in Israel. *Water Science & Technology* 42: 71-74.

Chaloner, D.T. & Wotton, R.S. (1996). Tube building by larvae of 3 species of midge (Diptera: Chironomidae). *J. N. Am. Benthol. Soc.* 15: 300-307. DOI: 10.2307/1467278.

Cummins, K.W. (1962). An evaluation of some techniques for the collection and analysis of benthic samples with special emphasis on lotic waters. *Am. Midl. Nat.* 67: 477-504. DOI: 10.2307/2422722.

Cummins, K.W., Matousek, J. & Shackelford, A. (2005). *Invertebrate functional group analysis for the freshwater creek watershed*. Arcata, USA: Institute for River Ecosystems, Humbold State University.

Czerniawska-Kusza, I. (2007). Exploitation of changes in the structure of benthic macroinvertebrate and of the occurrence of deformations in *Chironomus* sp. larvae for assessing the ecological state of lowland rivers. In C. Rosik-Dulewska & M. Głowacki (Eds.), *Environmental protection at university studies of nature* (pp. 229-239). Opole: University of Opole (In Polish).

De Bisthoven, L.J., Van Looy, E., Ceusters, R., Gullentrops, F. & Ollevier, F. (1992). Densities of *Prodiamesa olivacea* (Meigen) (Diptera: Chironomidae) in a second order stream, the Laan (Belgium): relation to river dynamics. *Neth. J. Aquatic Ecol.* 26: 485-490.

Dukowska, M. & Grzybkowska, M. (2014). Coexistence of fish species in a large lowland river: food niche partitioning between small-sized percids, cyprinids and sticklebacks in submersed macrophytes. *PLoS ONE* 9(11). DOI:10.1371/journal.pone.0109927.

Dukowska, M., Grzybkowska, M., Marszał, L. & Zięba, G. (2009). The food preferences of three-spined stickleback, *Gasterosteus aculeatus* L., downstream of a dam reservoir. *Oceanol. Hydrobiol. Stud.* 38(2): 39-50. DOI: 10.2478/v10009-009-0020-x.

Dukowska, M., Michałowicz, J. & Grzybkowska, M. (2012). Metal accumulation in sediments and insect larvae in weakly polluted small lowland river. *Pol. J. Ecol.* 60(2): 351-362.

EU FWD Directive 2000/60/EC of the European Parliament and of the council of 23 October 2000 establishing a framework for community action in the field of water policy. OJ L 327: 1-87.

Farag, A.M., Nimick, D.A., Kimball, B.A., Church, S.E., Harper, D.D. & Brumbaugh, W.G. (2007). Concentrations of metals in water, sediment, biofilm, benthic macroinvertebrates, and fish in the Boulder River Watershed, Montana, and the role of colloids in metal uptake. *Arch. Environ. Contam. Toxicol.*

52: 397-409. DOI: 10.1007/s00244-005-0021-z.

Ferrington, L.C.Jr. (1992). Habitat and sediment preferences of *Axarus festivus* larvae. *Neth. J. Aquatic Ecol.* 26: 347-354. DOI: 10.1007/BF02255261.

Ferrington, L.C.Jr (2008). Global diversity of non-biting midges (Chironomidae; Insecta-Diptera) in freshwater. *Hydrobiologia* 595: 447-445. DOI: 10.1007/978-1-4020-8259-7_45.

Grzybkowska, M. (1995). Impact of human-induced flow perturbation on the chironomid communities in the first order stream section of the Bzura River (Central Poland). In P. Cranston (Ed.), *Chironomids - from genes to ecosystems* (pp. 247-253). Melbourne, Australia: CSIRO Publications.

Grzybkowska, M. & Głowacki, Ł. (2011). Chironomidae (Diptera) diversity in lowland rivers of various orders and of different levels of human impact in central Poland. In X. Wang & W. Liu (Eds.), *Proceedings of the 17th International Symposium on Chironomidae - Contemporary chironomid studies* (pp. 282-295). Tianjin, China: Nankai University Press.

Grzybkowska, M., Kurzawski, M. & Dukowska, M. (2012). Response of Chironomidae (Diptera) to impoundments in small lowland rivers. In T. Ekrem, E. Stur & K. Agaard (Eds.), *Proceedings of the 18th International Symposium on Chironomidae - Fauna norvegica* 31 (pp. 25-33). Trondheim: NTNU. DOI: 10.5324/fn.v31i0.1379.

Halpern, M., Gasith, A. & Broza, M. (2002). Does the tube of a benthic chironomid larva play a role in protecting its dweller against chemical toxicants? *Hydrobiologia* 470: 49-55. DOI: 10.1023/A:1015665027535.

Harding, J.S. (2005). Impact of metals and mining on stream communities. In T.A. Moore, A. Black, J.A. Centeno, J.S. Harding & D.A. Trumm (Eds.), *Metal contaminants in New Zealand* (pp. 343-357). Christchurch: Resolutionz press.

Hershey, A.E. & Dodson, S.I. (1987). Predator avoidance by *Cricotopus:* cyclomorphopsis and the importance of being big and hairy. *Ecology* 68: 913-920. DOI: 10.2307/1938362.

Janssen, R.P.T., Posthuma, L., Baerselman, R., Den Hollander, H.A., Van Veen, R.P.M. & Peijnenburg, W.J.G.M. (1997). Equilibrium partitioning of heavy metals in dutch field soils. II. Prediction of metal accumulation in earthworms. *Environ. Toxicol. Chem.* 16: 2479-2488. DOI: 10.1002/etc.5620161207.

Johnson, R.K., Wiederholm, T. & Rosenberg, D.M. (1993). Freshwater biomonitoring using individual organisms, populations, and species assemblages of benthic macroinvertebrates. In D.M. Rosenberg & V.H. Resh (Eds.), *Freshwater biomonitoring and benthic macroinvertebrates* (pp. 40-125). London: Chapman & Hall.

Kucuksezgin, F., Uluturhan, H. & Batki, H. (2008). Distribution of heavy metals in water, particulate matter and sediments of Gediz River (Eastern Aegean). *Environ. Monit. Assess.* 141: 213-225. DOI: 10.1007/s10661-007-9889-6.

Lindegaard, C. (1995). Classification of water-bodies and pollution. In P.D. Armitage, P.S. Cranston & L.C.V. Pinder (Eds.), *The Chironomidae. Biology and ecology of non-biting midges* (pp. 385-404). London: Chapman & Hall.

Lindegaard, C. & Brodersen, K.P. (1995). Distribution of Chironomidae (Diptera) in the river continuum. In P. Cranston (Ed.), *Chironomids: from genes to ecosystems* (pp. 257-271). Melbourne, Australia: CSIRO Publications.

Makino, W., Kato, H., Takamura, N., Mizutani, H., Katano, N. & Mikami, H. (2001). Did chironomid emergence release *Daphnia* from fish predation and lead to *Daphnia*-driven clear-water phase in Lake Towada, Japan? *Hydrobiologia* 442: 309-317. DOI: 10.1023/A:1017532717135.

Martinez, E.A., Moore, B.C., Schaumloffel, J. & Dasgupta, N. (2002). The potential association between menta deformities and trace elements in Chironomidae (Diptera) taken from a heavy metal contaminated river. *Arch. Environ. Contam. Toxicol.* 42: 286-291. DOI: 10.1007/s00244-001-0190-0.

Mckie, B.G. (2004). Disturbance and investment: developmental responses of tropical lotic midges to repeated tube destruction in the juvenile stages. *Ecol. Entomol.* 29: 457-466. DOI: 10.1111/j.0307-6946.2004.00622.x.

Minshall, G.W. & Robinson, C.T. (1998). Macroinvertebrate community structure in relation to measures of lotic habitat heterogeneity. *Fundam. Appl. Limnol.* 141: 129-151.

Mousavi, S.K., Primicerio, R. & Amundsen, P. (2003). Diversity and structure of Chironomidae (Diptera) communities along a gradient of heavy metal contamination in a subarctic watercourse. *Sci. Total Environ.* 307: 93-110. DOI: 10.1016/S0048-9697(02)00465-5.

Muscatello, J.R. & Liber, K. (2010). Uranium uptake and depuration in aquatic invertebrate *Chironomus tentans*. *Environ. Pollut.* 158: 1696-1701. DOI: 10.1016/j.envpol.2009.11.032.

Nogaro, G., Mermillod-Blondin, F., Franc, F., Caillet, O., Gaudet, J.P., Lafont, M. & Gibert, J. (2006). Invertebrate bioturbation can reduce the clogging of sediment: an experimental study using infiltration sediment columns. *Freshwater Biol.* 51: 1458-1473. DOI: 10.1111/j.1365-2427.2006.01577.x.

Òlafsson, J.S. & Paterson, D.M. (2004). Alteration of biogenic structure and physical properties by tube-building chironomid larve in cohesive sediments. *Aquatic Ecol.* 38: 219-229. DOI: 10.1023/B:AECO.0000032050.10546.bb.

Osmulski, P. & Leyko, W. (1986). Structure, function and physiological role of *Chironomus* hemoglobin. *Comp. Biochem. Physiol. B: Comp. Biochem.* 85: 701-722. DOI: 10.1016/0305-0491(86)90166-5.

Petersen, R.C., Cummins, K.W. & Ward, G.M. (1989). Microbial and animal processing of detritus in a woodland stream. *Ecol. Monograph.* 59: 21-39. DOI: 10.2307/2937290.

Poulton, B.C., Monda, D.P., Woodward, D.F., Wildhaber, M.L. & Brumbaugh, W.G. (1995). Relations between benthic community structure and metals concentrations in aquatic macroinvertebrates: Clark Fork River, Montana. *J. Fresh. Ecol.* 10: 277-293. DOI: 10.1080/02705060.1995.9663447.

Quinn, J.M. & Hickey, C.W. (1990). Magnitude of effects of substrate particle size, recent flooding, and catchment development on benthic invertebrates in New Zealand rivers. *N. Z. J. Mar. Freshwat. Res.* 24: 387-409. DOI: 10.1080/00288330.1990.9516433.

Rainbow, P.S. (1996). Heavy metals in aquatic invertebrates. In W.N. Beyer, G.H. Heinz & A.M. Redmon-Norwood (Eds.), *Environmental contaminants in wildlife* (pp. 405-425). Boca Raton, USA: Lewis Publisher.

Schaller, J., Brackhage, C. & Dudel, E.G. (2011). Invertebrates minimize accumulation of metals and metalloids in contaminated environments. *Water Air Soil Pollut.* 218: 227-233. DOI: 10.1007/s11270-010-0637-0.

Smolders, A.J.P, Lock, R.A.C., Van der Velde, G., Hoyos, R.I.M. & Roelofs, J.G.M. (2003). Effects of mining activities on heavy metal concentrations in water, sediment, and macroinvertebrates in different reaches of the Pilcomayo River, South America. *Arch. Environ. Contam. Toxicol.* 44: 314-323. DOI: 10.1007/s00244-002-2042-1.

StatSoft Inc. (2011). STATISTICA (data analysis software system), version 10.

Szyszlak-Bargłowicz, J., Słowik, T., Zając, G. & Piekarski, W. (2013). The Content of Heavy Metals in the Drainage Ditches by Communication Routes. *Annual Set The Environment Protection* 15: 2309-2323 [English summary]. Retrieved: March 15, 2015, from http://www.ros.edu.pl/index.php/RO/article/view/182/174.

Tang, H., Song, M.Y., Cho, W.S., Park, Y.S. & Chon, T.S. (2010). Species abundance distribution of benthic chironomids and other macroinvertebrates across different levels of pollution in streams. *Ann. Limnol.- Int. J. Lim.* 46: 53-66. DOI: 10.1051/limn/2009031.

Tokeshi, M. (1995). Species interactions and community structure. In P.D. Armitage, P.S. Cranston & L.C.V. Pinder (Eds.), *The Chironomidae. Biology and ecology of non-biting midges* (pp. 297-335). London: Chapman & Hall.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedel, J.R. & Cushing, C.E. (1980). The river continuum concept. *Can. J. Fish. Aquat. Sci.* 37: 130-137. DOI: 10.1139/f80-017.

Vedamanikam, V.J. & Shazili, N.A.M. (2009). The chironomid larval tube, a mechanism to protect the organism from environmental disturbances? *Toxicol. Environ. Chem.* 91(1): 171-176. DOI: 10.1080/02772240802074934.

Walshe, B.M. (1950). The feeding habits of certain chironomid larvae (subfamily Tendipedinae). *Proceedings of the Zoological Society of London* 121: 63-79. DOI: 10.1111/j.1096-3642.1951.tb00738.x.

Wiederholm, T. (1984). Responses of aquatic insects to environmental pollution. In D.M. Rosenberg & V.H. Resh (Eds.), *The ecology of aquatic insects* (pp. 508-557). New York, USA: Praeger.

Winberg, G.G. (1978). Experimental application of various systems of biological indication of water pollution. In D.I. Mount (Ed.), *Proceeding of first and second USA – USSR symposium on effect of pollutants upon aquatic ecosystems* (pp. 14-149). Duluth, USA: Environmental Research Laboratory, US Environmental Protection Agency.

Winner, R.W., Boesel, M.V. & Farrell, M.P. (1980). Insect community structure as an index of heavy metal pollution in lotic ecosystems. *Can. J. Fish Aquat. Sci.* 37: 647-655. DOI: 10.1139/f80-081.