



Does the subfossil Chironomidae in sediments of small ponds reflect changes in wastewater discharges from a Zn–Pb mine?



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ABSTRACT

This study was aimed to show whether subfossil Chironomidae (Diptera) may be useful tool for assessing toxicity of heavy metals in the aquatic environment. Investigations were carried out in subsidence ponds affected by the activity of metal mining: (1) the older ones formed before mining activity and (2) the younger one formed after the mine was closed. Waters of ponds were analyzed for physico-chemical parameters, whereas sediments were studied for metals (Cd, Pb, and Zn), pH, organic matter, nutrients (TOC, N-tot, and P-tot), and subfossil Chironomidae. High concentrations of Cd 6.7–612 $\mu\text{g g}^{-1}$, Pb 0.1–10.2 mg g^{-1} , and Zn 0.5–23.1 mg g^{-1} were found in the seven analyzed sediment cores. In total, 374 head capsules of Chironomidae larvae, belonging to four subfamilies Chironominae, Orthocladiinae, Tanytopodinae, and Prodiamesinae, were determined. Both the diversity and density of Chironomidae change in particular sediment cores and layers. However, these changes were not related to metal concentrations, as shown in the statistical calculations (dendrogram of similarities, Mann-Whitney test, Spearman correlations). The only exception was the negative correlations between Cd, Pb, and Zn concentrations and the density of head capsules of *Polypedilum* sp. We found that organic matter and nutrient contents were important factors that control the spatial distribution of subfossil Chironomidae, mainly Orthocladiinae. Other factors which may influence subfossil Chironomidae distribution in sediment cores are discussed. To sum up, despite very high Cd, Pb, and Zn concentrations, the Chironomidae community was not clearly altered, therefore subfossil Chironomidae taxa appeared to be not useful for reconstructing dozen-years scale changes in the toxicity of the aquatic environment.

1. Introduction

Mining of metal ores has been recognized among many industrial activities as a one of the largest sources of heavy metal contaminants in fluvial systems. Whereas, mine effluents can deteriorate aquatic ecosystems during mining activity, naturally drained shafts, tailings and leachates from spoil heaps are a source of harmful substances in receiving streams long after the mining has stopped (Byrne et al., 2012; Ciszewski et al., 2012, 2013).

Most of metal contaminants accumulate in bottom sediments reaching values that can cause adverse effects on benthic macroinvertebrate community (Simpson and Batley, 2007). Larvae Chironomidae (Diptera), a major component of benthic macroinvertebrates in terms of their diversity and abundance (Kownacki, 2011), are often

used to study this effect (Smith and Cranston, 1995; Smolders et al., 2003; Janssens de Bisthoven et al., 2005; Michailova et al., 2012; Wright and Ryan, 2016; Pedrosa et al., 2017). Chironomidae are considered as a good indicator of surface water quality due to high species richness, occurrence in a broad range of habitats, sensitivity to environmental changes and a wide spectrum of ecological preferences with a narrow tolerance of many species to environmental factors (Brooks et al., 2007). The larvae are not noticeably mobile and inhabit the sediment subsurface, where they are exposed to toxicants in the pore water and from deposited particulates following ingestion (Al-Shami et al., 2013). Direct contact of Chironomidae larvae with contaminants, their accumulation in tissues and overall high sensitivity to contamination – all these features are favorable and give significant advantages to chironomids in comparison with other aquatic organisms

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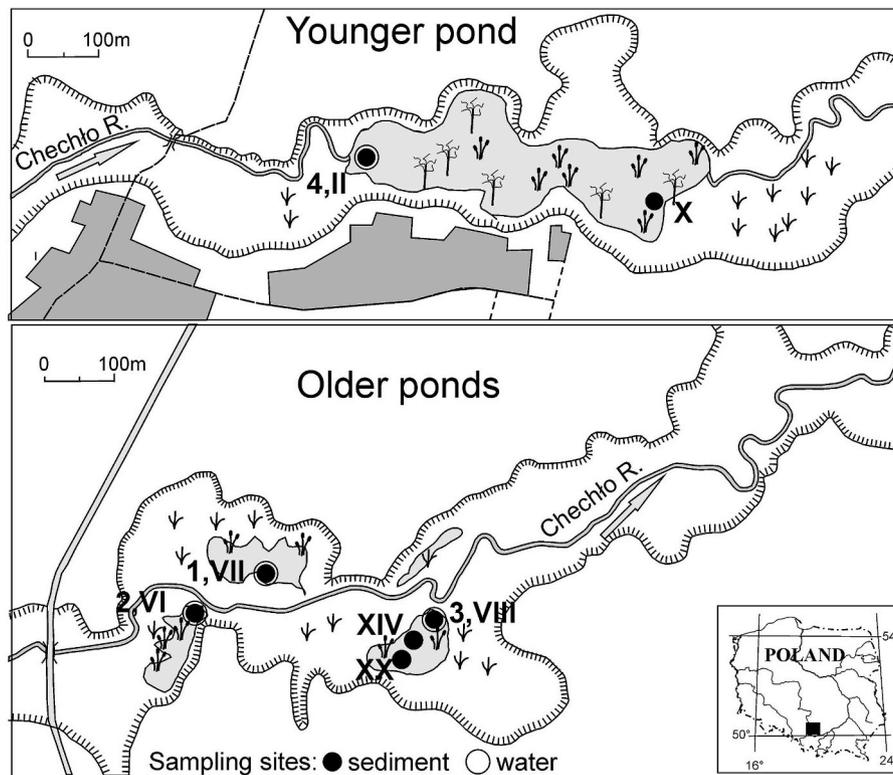


Fig. 1. Map of the study area and location of the sampling sites on small ponds on the Chechło River floodplain (sampling sites for sediment: II, X, VI, VII, VIII, XIV, XX; for water: 1, 2, 3, 4).

as biological indicators. Therefore, Chironomidae larvae have been applied as bioindicators of metals and other anthropogenic stressors (municipal sewages) of various water ecosystems and used successfully in genotoxicological studies as they are sensitive to chronic levels of contamination and other changes in water quality (Kownacki et al., 2002; Kownacki, 2011; Michailova et al., 2012; Al-Shami et al., 2013). They are included in the Extended Biotic Index (De Pauw et al., 1992) and the Annex V.1.2.6 of the E. C. Water Framework Directive, 2000.

Toxic effects of metals are often reflected in reduction of abundance and taxa richness of benthic invertebrates (Watanabe et al., 2000; Deacon et al., 2001; Swansburg et al., 2002). Exposure of Chironomidae larvae to metals induces mouthpart deformities (Martinez et al., 2002, 2004; Al-Shami et al., 2010; Di Veroli et al., 2012, 2014; Arimoroa et al., 2018), DNA strand breaks (Michailova et al., 2009; Al-Shami et al., 2013) and genome alterations (Michailova et al., 2015, 2018; Ilkova et al., 2016, 2018). Toxic effects of metals on chironomids may be also reflected in the reduction of larval growth (Timmermans et al., 1992; Gillis et al., 2002; Martinez et al., 2004) and biomass (Watanabe et al., 2000).

However, some authors (Canfield et al., 1994; Michailova et al., 2012) indicated a lack of impact of high metal concentrations in sediments on the biodiversity of Chironomidae communities at sites influenced by mining activities. This was supposed to be associated with higher tolerance of some Chironomidae species to pollutants (Dermott, 1991; Martinez et al., 2002; Janssens de Bisthoven et al., 2005) and adaptation of populations inhabiting metal-impacted freshwater systems to high metal concentrations (Pedrosa et al., 2017). Variable response of organisms to metals may be also related to different metal bioavailability. It depends, among others, on pH and redox potential in the water–sediment system, sediment properties such as the presence of acid-volatile sulfides (AVS), organic matter, texture (clay, silt or sand), Fe–Mn oxides and binding forms of metals in sediment (Calmano et al., 2005; Simpson and Batley, 2007; Zhang et al., 2014) as well as antagonistic or synergistic interactions between metals (Fargašová, 2001).

Moreover, biodiversity of benthic fauna community may be influenced by the presence of the other contaminants than metals (Kownacki et al., 2002; Kownacki, 2011).

Because head capsules of Chironomidae larvae are usually well-preserved in sediments, they are used in paleolimnological studies to track local climate change, and assessment of anthropogenic and environmental stressors (Brooks and Birks, 2004; Bitušik et al., 2009; Al-Shami et al., 2010; Hamerlík et al., 2016; Stoklasa et al., 2017; Yang et al., 2017). However, previous studies suggested that subfossil Chironomidae may be useful in tracking negative impact of metals on biota in water ecosystems impacted by metal mining activities (Ilyashuk et al., 2003; Brooks et al., 2005; Bitušik et al., 2018), it seems that a disturbance can be reflected most of all in shift toward domination of less sensitive organisms or even total deterioration during peak of contamination followed by subsequent changes after a mining cessation.

The aim of this study was to reconstruct heavy metal toxicity for Chironomidae communities recorded in sediments of the pond system affected by mine waters from the Zn–Pb mine. Moreover, an evaluation of subfossil Chironomidae as a tool for assessing the metal toxicity for water ecosystems was attempted. We achieved these by analyzing the density of the head capsule of Chironomidae larvae in sediment cores of the subsidence ponds on the floodplain of the Chechło River in southern Poland. Observed changes were related to changes in contamination with heavy metals, as well as to organic matter and nutrient contents in the sediment.

2. Material and methods

2.1. Study area

The Chechło River (26 km in length) is a left tributary of the Vistula River in southern Poland. The relatively small Chechło River, with 1.5 m³/s of average discharge received 0.2–0.5 m³/s of mine waters

from the Zn–Pb mine in Trzebinia until its closure in 2009. The river water quality was affected also by industrial and municipal sewage effluents from two towns (Trzebinia and Chrzanów) (Ciszewski, 1997). Currently, most of effluents from these towns are treated biologically and the river is much less polluted than in the 1990s. In the lower reach of the river, on its floodplain, small subsidence ponds appeared as a result of local ground subsidence. First ponds (sites 1, 2, 3) were formed at the end of 1980s in the period of intensive exploitation of Zn–Pb ores, while a younger one (site 4) was formed ca. 20 years later at the end of the mining (Fig. 1). The pond surface range from 0.5 to ca. 5 ha, with average depth about 1–2 m. The bottom of the ponds is muddy, but older ponds are largely overgrown with macrophytes (Fig. 1). The Chechło River flows through the younger subsidence pond, whereas the older ponds are supplied with the river water via short ditches and during floods.

2.2. Sample collection

In April 2016, four samples of surface waters (younger pond, site 4; older ponds sites: 1, 2 and 3) and seven sediment cores (younger pond cores: II and X; older pond cores: VI, VIII, XIV, VII, XX) from subsidence ponds were collected (Fig. 1). In the water samples, conductivity, pH, concentrations of ions Cl^- , SO_4^{2-} , NO_3^- , PO_4^{3-} , and NH_4^+ , as well as Cd, Pb, and Zn concentrations, in total and dissolved phases, were determined. In all core layers, concentrations of Cd, Pb, and Zn, density of head capsule, and Chironomidae taxa were determined. Additionally, in cores X (younger pond) and XX (older pond), the contents of organic matter, total organic carbon (TOC), total nitrogen (N-tot), and total phosphorus (P-tot) were analyzed. Influence of local variability of sedimentary conditions and post-depositional sediment mixing on the relationship between sediment toxicity and Chironomidae community was greatly reduced by the raw core sectioning where 10 cm-long core sections dominated.

2.3. Water

Conductivity and pH were measured using a WTW (Multi 340 and SET 2) apparatus, while for concentrations of ions Cl^- , SO_4^{2-} , NO_3^- , PO_4^{3-} , and NH_4^+ , ion chromatography was used (DIONEX, IC25 and ICS-1000, Dionex Corporation, Sunnyvale, USA). The Cd, Pb, and Zn concentrations, in total and dissolved phases (after filtration through 0.45- μm filter), were measured by atomic absorption spectroscopy (AAS), using an apparatus Varian Spectra AA-20 equipped with a Graphite Furnace (Varian 20, Varian Techtron PTY Limited, Mulgrave, Victoria, Australia). Standard reference materials for water SPS-SW1 Batch 12, National Institute of Standards and Technology (USA), were used to determine the accuracy of metal analyses in the water samples.

2.4. Sediment

The sediment samples were collected using a Multisampler piston corer with a diameter of 4.5 cm (Eijkelkamp, Giesbeek, Netherlands). In the field, cores were sectioned into 3 to 6 layers (Table 1, Fig. 2),

Table 1
Sectioning of sediment cores from the subsidence ponds on the Chechło River floodplain.

Sections	Layers (cm)						
	Core II	Core X	Core VI	Core VII	Core VIII	Core XIV	Core XX
1	0–10	0–5	0–10	0–5	0–10	0–15	0–5
2	10–20	5–10	10–20	5–10	10–20	15–30	5–10
3	20–30	10–15	20–30	10–20	20–30	30–45	10–15
4	30–40	15–20	30–40	20–30	30–40		15–17.5
5		20–25	40–50	30–40			17.5–20
6			50–60	40–50			

depending on sediment lithology reflected in macroscopic changes in color or grain-size of sediments, whereas profiles with no distinct layers were divided into 10 cm long subsamples. The sediments were usually composed of unstratified muds, sometimes with visible plant remnants (leaves, roots). The layers were homogenized by mixing (Pociecha et al., 2019). Analyses of chironomid head capsules were made in 2 cm³ of fresh sediment subsamples taken from each layer and stored at 4 °C.

For analysis of organic matter, TOC, N-tot and P-tot sediment samples were air dried. Organic matter content was estimated by loss on ignition (LOI) at 550 °C for 2 h. Total nitrogen was determined by the Elementar Vario MAX cube CNS analyzer. Total organic carbon was analyzed by the Tiurin method. Total P content was determined by Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Elan 6100, PerkinElmer) (Smykla et al., 2015). For metal (Cd, Pb, Zn) analysis, sediment samples were wet sieved through a 0.063 mm sieve and then dried at 105 °C and extracted in an aliquot of 10 cm³ of 65% HNO₃ and 2 cm³ of 30% H₂O₂ using a microwave digestion technique and filtered (Ciszewski et al., 2013). The Cd, Pb and Zn concentrations were determined using a flame atomic absorption spectrometer (F-AAS). Metal analyses were performed according to (standard certified) analytical quality control procedures.

The analysis of head capsules of Chironomidae larvae included deflocculating of the 2 cm⁻³ fresh homogenized samples for 30 min in 10% KOH heated to 75 °C on a magnetic stirrer, washing through a 90 μm sieve, elimination of carbonates by acidification with 10% HCl, and sieving on 212 and 90 μm mesh sieves (Lang et al., 2003). The head capsules were picked out under a binocular microscope with magnification 25x. For determination of Chironomidae taxa the keys of and Orendt and Spies (2012 a,b) were used. A substantial part of damaged head capsules or juvenile stages were identified as belonging to subfamilies Tanytopodinae, Orthocladiinae, tribes Chironomini and Tanytarsini, and even to the family Chironomidae. The density of head capsule is given as its number per 2 cm³ of wet sediment.

2.5. Statistical analysis

The earlier studies (Ciszewski and Łokas, 2019) documented that, basing on metal concentrations variability, pond sediments can be associated with pre-mining conditions of low pollution, with the period of high pollution during the mine operation and with post-mining period of moderate pollution. This general discrimination was a guide for the selection of the present data analyses. To determine the relationship between density of head capsule of Chironomidae and the values of physico-chemical parameters (contents of organic matter, TOC, N-tot, P-tot, Cd, Pb, Zn) (data were taken together) for cores X and XX, as well as for concentrations of Cd, Pb, and Zn in all cores, we calculated the Spearman linear correlation coefficient. The differences in the values of studied environmental parameters between cores X and XX were determined using Mann-Whitney test.

To estimate the impact of various metal concentrations in sediment on the Chironomidae taxa the core layers were classified according to hierarchical cluster analysis. Normalized data of Cd, Pb, and Zn were used for the calculations. The Euclidean distance and within-groups linkage were used as a grouping method. As a result, dendrograms of similarities were obtained, on the basis of which, groups (clusters) of sediment layers were separated. The above-mentioned cluster approach was used to check whether different sediment contamination with metals affects the Chironomidae communities and density of head capsules. The differences in metal concentrations and density of head capsule of Chironomidae larvae between selected groups were analyzed using a Mann-Whitney test.

Hierarchical cluster analysis was used to determine the differences in the Chironomidae subfamily (percentage share) between studied cores.

Table 2
Water chemistry of the subsidence ponds on the Chechło River floodplain in April 2016.

Parameter	Unit	Older ponds			Younger pond
		Site 1	Site 2	Site 3	Site 4
pH		7.1	7.5	7.4	7.9
Conductivity	$\mu\text{S cm}^{-1}$	535	588	460	773
HCO_3^-	mg dm^{-3}	217	241	200	236
SO_4^{2-}	mg dm^{-3}	82.4	81.6	50.3	147.1
Cl^-	mg dm^{-3}	39.8	46.0	31.0	65.1
NH_4^+	mg dm^{-3}	1.6	2.3	0.4	6.8
NO_3^-	mg dm^{-3}	11.2	2.2	2.7	6.8
PO_4^{3-}	mg dm^{-3}	0.166	0.124	0.056	0.224
Cd total	$\mu\text{g dm}^{-3}$	0.73	< 0.01	< 0.01	0.62
Cd dissolved	$\mu\text{g dm}^{-3}$	0.07	< 0.01	< 0.01	0.06
Pb total	$\mu\text{g dm}^{-3}$	2.6	1.2	3.0	3.8
Pb dissolved	$\mu\text{g dm}^{-3}$	1.6	0.2	1.8	1.0
Zn total	$\mu\text{g dm}^{-3}$	37.0	27.0	20.0	86.0
Zn dissolved	$\mu\text{g dm}^{-3}$	24.0	< 1	< 1	27.0

3. Results

3.1. Water

Waters had neutral (older ponds, sites 1–3) and slightly alkaline pH (younger pond, site 4) (Table 2). Water of the younger pond, through which the Chechło River flowed, was characterized by higher values of conductivity, contents of ions SO_4^{2-} , Cl^- , and nutrients (NH_4^+ , PO_4^{3-}) when compared to those in older ponds (Table 2). The concentrations of Cd, Pb, and Zn, in total and in dissolved phases in water, were low (Table 2).

3.2. Sediment

3.2.1. Organic matter and nutrients in cores X and XX

Core sediments X had pH 6.9–7.1, and contents of organic matter ranged 5.7–18.4%, TOC 2.8–9.3%, N-tot 0.2–0.6%, P-tot 0.4–2.4 mg g^{-1} . In core XX from a pond heavily overgrown by macrophytes, the values of pH (5.8–5.9) were lower, but contents of organic matter ranged 33.3–39.8%, and TOC 15.1–17.3%, N-tot 0.9–1.4%, P-tot 2.0–4.1 mg g^{-1} were 1.8–6.5 times higher (Fig. 2). The mentioned differences were statistically significant (Table 3). Ratio C: N ranged 12.1–15.8 in core X and 12.0–17.7 in core XX.

3.2.2. Cd, Pb and Zn in studied cores

Concentrations of Cd, Pb, and Zn in all the cores varied in a wide range: Cd 6.7–612 $\mu\text{g g}^{-1}$, Pb 0.1–10.2 mg g^{-1} , and Zn 0.5–23.1 mg g^{-1} (Fig. 3). Taking into consideration all cores the Mann-Whitney test showed no differences in Pb and Zn concentrations between younger and older ponds and significantly higher Cd

Table 3
The significance of differences (Mann–Whitney test) in the values of physico-chemical parameters in the sediment between core X from younger pond and core XX from older pond (N = 10). Only significant differences are given.

Parameter	Z	p - value
pH	-2.51	0.012
Organic matter	2.51	0.012
TOC	2.51	0.012
N-tot	2.51	0.012
P-tot	2.09	0.037
Fe	2.51	0.012
Ni	2.51	0.012

p – significance level, TOC – total organic carbon, Z – value of statistics.

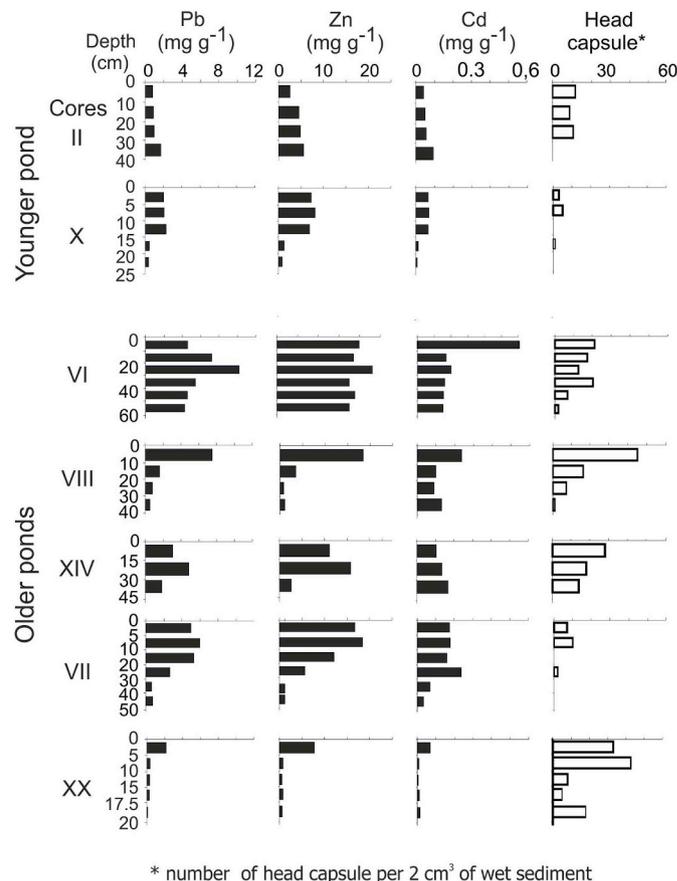


Fig. 3. Heavy metal concentrations and density of head capsule of Chironomidae larvae in all the sediment cores.

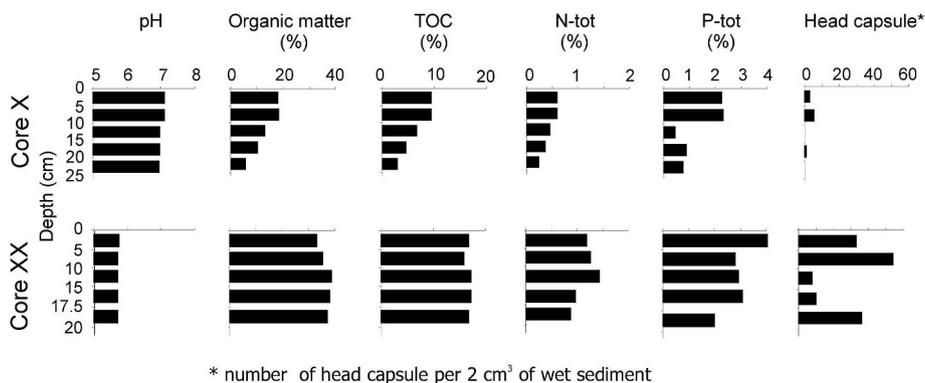


Fig. 2. Chemistry of core X from younger pond and core XX from older pond on the Chechło River floodplain.

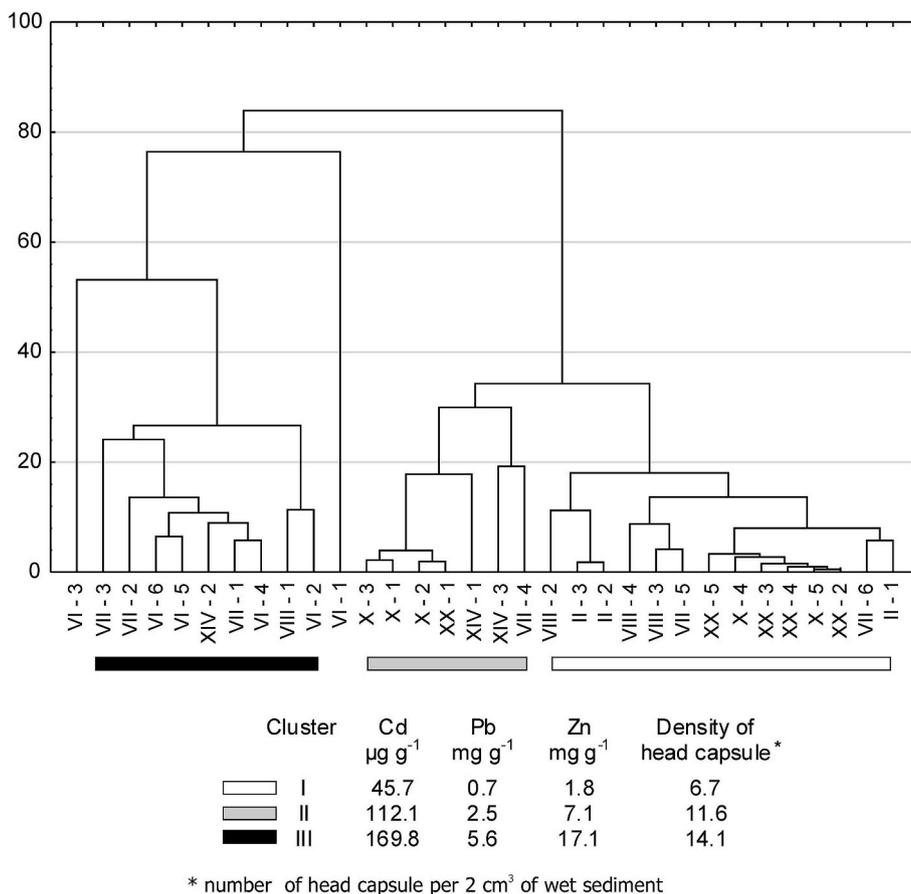


Fig. 4. Hierarchical cluster analysis based on concentrations of Cd, Pb and Zn in sediments of subsidence ponds on the Chechło River. The mean metal concentrations and density of head capsule in different clusters are given.

concentrations in older ponds (N1 = 8, older N2 = 24, Z = -2.63, p = 0.008). Sediment core II was characterized by relatively uniform metal concentrations, while the remaining cores showed an irregular distribution pattern. In older ponds, in cores VIII and XX, maximum metal concentrations occurred in the top layer. In cores VI, VII, and XIV, maximum Pb and Zn concentrations were found in the middle of the core, and their values decreased toward the surface (Fig. 3).

The dendrogram of similarities (Fig. 4, Tables 4 and 5) enabled identification of three Clusters of sediments significantly different in terms of Cd, Pb, and Zn concentrations. Cluster I, which included core II, most layers of cores VIII (10–40 cm) and XX (5–20 cm), and lower layers of cores VII (30–50 cm) and X (15–25 cm), had the lowest Cd, Pb, and Zn concentrations (Table 4). Cluster II included layers of cores: VII (20–30 cm), XIV (0–15, 30–45 cm), X (0–5, 5–10, 10–15), and XX (0–5 cm) with mean metal concentrations. Cluster III consisted of layers of cores; VI (10–60 cm), VII (0–20 cm), VIII (0–10 cm), XIV (15–30 cm) located in the older ponds contained the highest metal concentrations. The differences in the metal concentrations among the clusters were significant (Table 5), despite similar Cd concentrations in Cluster II and

Table 4
Mean Cd, Pb, and Zn concentrations and density of head capsule of Chironomidae in the sediment core layers of the ponds on the Chechło River floodplain in the studied clusters.

Cluster	N	Cd µg g ⁻¹	Pb mg g ⁻¹	Zn mg g ⁻¹	Density of head capsule ^a
I	14	45.7	0.7	1.8	6.7
II	7	112.1	2.5	7.1	11.6
III	11	169.8	5.6	17.1	14.1

^a Number of head capsule per 2 cm³ of wet sediment.

Table 5
Significant differences in the Cd, Pb, and Zn concentrations in sediments between an identified three groups of clusters (Mann–Whitney test).

Metals	Clusters					
	I–II (N ₁ = 14, N ₂ = 7)		I–III (N ₁ = 14, N ₃ = 9)		II–III (N ₂ = 7, N ₃ = 9)	
	Z	p - value	Z	p - value	Z	p - value
Cd	-2.50	0.012	-3.94	0.000	ns	ns
Pb	-3.62	0.000	-3.94	0.000	-3.28	0.001
Zn	-3.39	0.001	-3.94	0.000	-3.28	0.001

ns – not significant.

III. Core VI, layers 0–10 cm and 20–30 cm, were not taking into consideration.

3.2.3. Chironomidae in cores

In total, 374 Chironomidae head capsules in all sediment cores belonging to four subfamilies—Chironominae, Orthoclaadiinae, Tanypodinae, and Prodiamesinae were determined (Figs. 5–7). As the dendrogram of similarities shows, cores II and X from younger pond were clearly different from the other cores and also differed from each other (Fig. 7). In the core II, the subfamily Chironominae (41%) dominated, represented mainly by *Chironomus* sp. and *Micropsectra* sp., followed by Orthoclaadiinae (31%) and Tanypodinae (3%). In the core X, the lowest diversity of Chironomidae was found, and they were represented by Orthoclaadiinae (45%) and Prodiamesinae (22%). In older ponds, the taxonomic composition of Chironomidae recorded in the sediment showed greater similarity. In all cores from older ponds, the dominant group was Orthoclaadiinae (51–80%), followed by

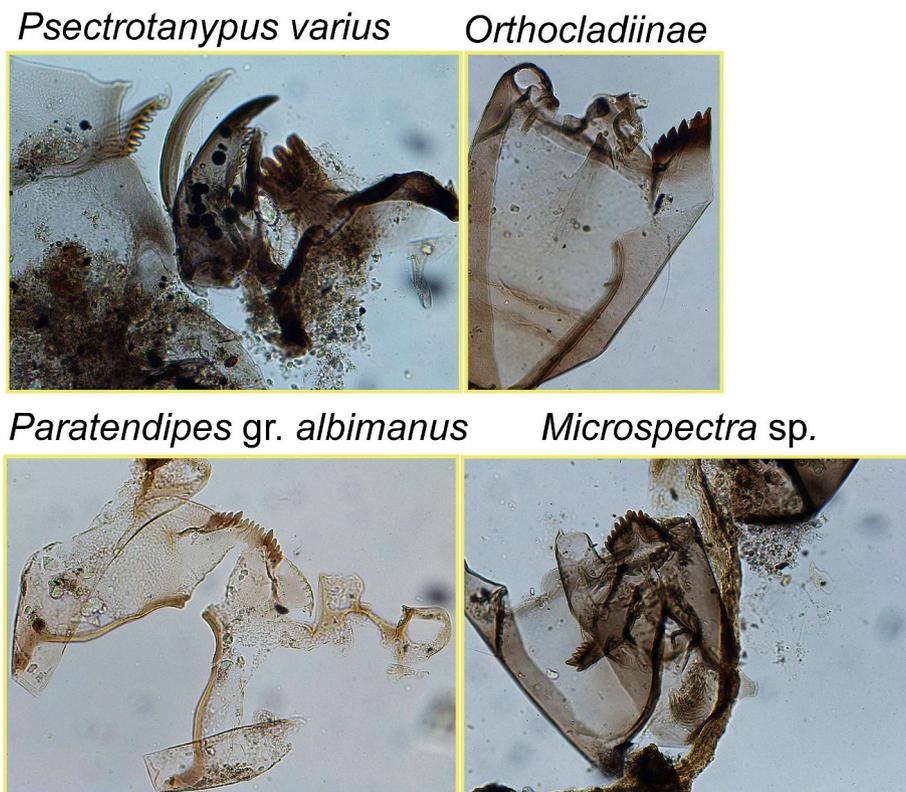


Fig. 5. Examples of head capsule of Chironomidae larvae collected in sediment cores.

Chironominae (9–24%) and Tanypodinae (4–13%, with the exception of core VII). Prodiamesinae (5%) was found only in core VI. In most cores, Orthocladiinae was represented by *Cricotopus (Isocladius)* sp. and occasionally by *Brillia* sp., *Cricotopus* sp., and *Psectrocladius* gr. *sordidellus* (Fig. 6). *Geothocladius*, *Limnophyes* sp., *Corynoneura* sp., and *Heterotrissocladius* gr. *marcidus* were found only in core XX, while *Metriccnemus* sp. was only found in core VII. Chironominae was represented by *Chironomus* sp. and occasionally by *Paratendipes* gr. *albimanus* and *Microspectra* sp., and Tanypodinae was represented by

Psectrotanypus varius, *Thienemannimyia-reihe*, and *Zavrelimyia* sp.

3.2.4. Distribution of head capsule in relations to Cd, Pb and Zn concentrations in cores

The distribution of the head capsules density varied greatly among the cores and layers (Fig. 3). In the cores II and X from the younger pond with lower metal concentrations, the density of head capsules of Chironomidae was lower and uniform throughout the core. The lower density, or even lack of head capsules, was found in core VII. The

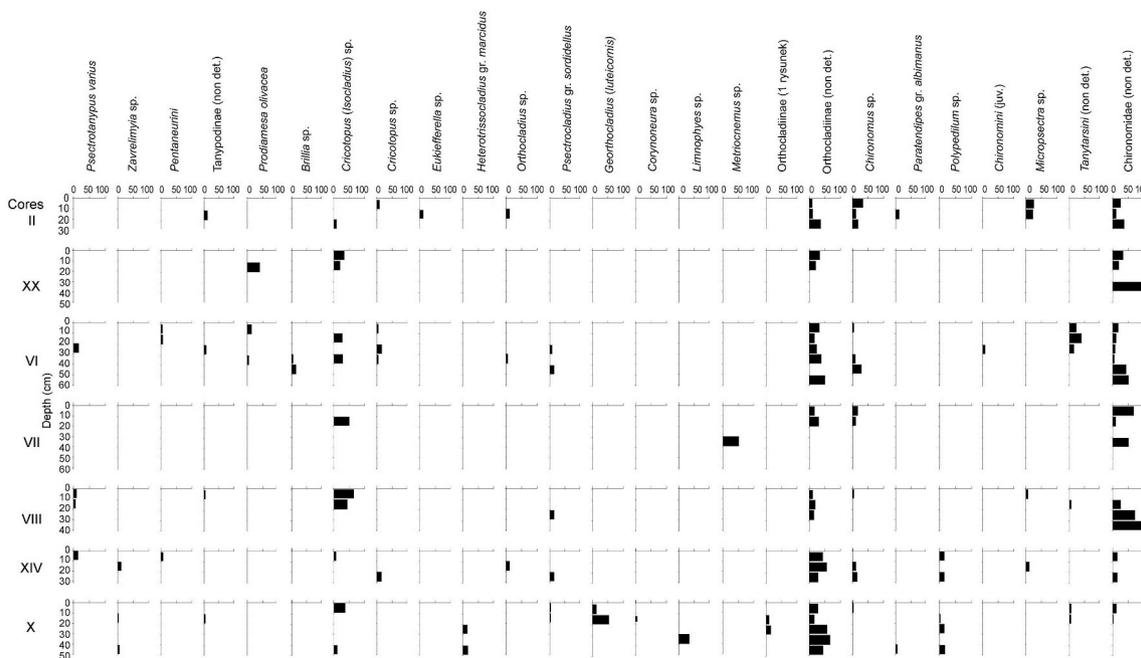


Fig. 6. The percentage share of Chironomidae taxa in the sediment cores from small ponds on the Chechło River floodplain.

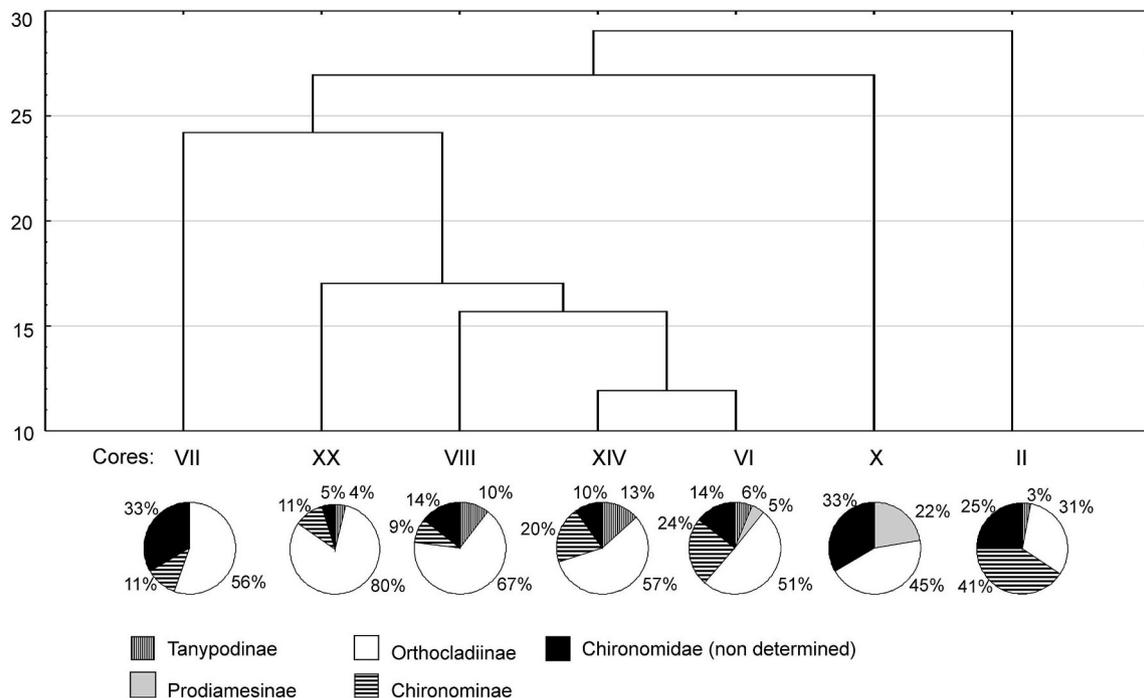


Fig. 7. Hierarchical cluster analysis and the percentage share of head capsules of Chironomidae in the cores.

presence or absence of head capsules of Chironomidae in the sediments were not correlated with high or low metal concentrations. In core VI, with the very high Cd, Pb, and Zn concentrations, we observed much higher density of head capsules from the surface to the depth of 40 cm (13–22 of head capsules per 2 cm³ of wet sediment) than in the lowest layers, where the density dropped to the minimum values 2–7 head capsules per 2 cm³ of wet sediment. The density of head capsule in cores VIII and XIV increased in younger layers irrespective of metal concentrations in the sediment. In the 0–10 cm layer of core VIII, it was the highest (45 head capsules) despite the strong heavy metals sediment contamination. In core XX with the lowest metal concentrations we found high density of head capsule, especially at the depth of 0–5 and 5–10 cm (33 and 43 head capsules, respectively).

Statistical calculation (Mann-Whitney test) did not show differences in the density of the head capsules of the Chironomidae (in total and for particular taxa) between the younger and older ponds of varied age, as well as between sediments with varied Cd, Pb, and Zn concentrations (Clusters I, II, and III) (Fig. 4). The density of head capsule of Orthoclaadiinae was positively correlated with the concentrations of organic matter, TOC, T-tot, and N-tot in cores X and XX (Table 6), and it was significantly higher (Mann-Whitney test; N = 10, Z = 2.50, p = 0.012) in core XX with their higher contents. Density of other taxa did not show differences between cores X and XX and the correlations with studied parameters. For all cores, Chironomidae taxa did not show negative correlation with the Cd, Pb, and Zn concentrations in the sediments, with the exception of *Polypedilum* sp. (Tables 6 and 7).

Table 6

The values of Spearman coefficient correlations for the environmental parameters and density of head capsule of Chironomidae taxa in core X (younger pond) and core XX (older pond) (data were taken together, N = 10); Only significant correlations are given, (p < 0.05).

Taxa	Organic matter	TOC	N-tot	P-tot	Cd	Pb	Zn
<i>Cricotopus (Isocladius) sp.</i>	ns	ns	ns	ns	0.70	0.70	0.68
Orthoclaadiinae (non det.)	0.76	0.79	0.84	0.76	ns	ns	ns
<i>Tanytarsini</i>	ns	ns	ns	ns	ns	ns	ns
<i>Polypedilum</i> sp.	ns	ns	ns	ns	ns	-0.67	-0.79
Chironomidae (non det.)	ns	ns	ns	ns	ns	0.72	ns

ns – not significant.

Table 7

The Spearman coefficient correlations between Cd, Pb, and Zn concentrations and density of head capsule of Chironomidae taxa in all the studied cores (data were taken together, N = 33). Only significant correlations are given, (p < 0.05).

Taxa	Zn	Cd	Pb
<i>Pentaneurini</i>	0.38	0.35	ns
<i>Cricotopus (Isocladius) sp.</i>	0.39	ns	0.46
<i>Chironomus</i> sp.	0.39	ns	0.36
<i>Polypedilum</i> sp.	-0.42	-0.36	-0.41

ns – not significant.

4. Discussion

All the sediment cores from subsidence ponds on the floodplain of the Chechlo River were heavily contaminated with Cd, Pb, and Zn, taking into consideration the sediment quality guidelines (SQGs) (MacDonald et al., 2000). Concentrations of Cd, Pb, and Zn exceeded the probable effect level (PEL, 3.53, 91.3, 315 μg g⁻¹, Smith et al., 1996) for all the core samples and the severe effect level (SEL: 10, 250, 820 μg g⁻¹, Persuad et al., 1993) for 94, 93, 91%, respectively, of the core samples from the ponds. Therefore, adverse effects of metals on the majority of dwelling organisms were expected. Such high metal concentrations are characteristic of aquatic sediments in areas affected by active or “historical” mining of metal ores (Byrne et al., 2012; Ciszewski et al., 2013). Large differences in distribution patterns of Pb, Zn, and Cd

concentrations in studied sediment cores, even located closely to each other, may have some reasons. High metal concentrations in the middle and top layers of sediment cores in older ponds (dendrogram of similarities, Cluster III) correspond to the maximum production of Zn–Pb mine in Trzebinia in the 1990s. Elevated metal concentrations in core X (layers 0–15 cm) from a younger pond (Cluster II) indicate the deposition site of sediment-associated metals transported by the Chechło River. As follows, decreased sediment contamination with Cd, Pb, and Zn of the top of the core II of the younger pond (Cluster I) and cores VII and XIV of older ponds can be related to the period of the sediment deposition after the mine closure and the cessation of wastewater discharge. The lowest metal concentrations in cores VIII and XX (Cluster I) are related to the sediment deposition by a small tributary stream flowing from the valley side. Distribution of Cd, Pb, and Zn in the sediments of X and XX cores were not related to an organic matter content (Spearman correlations).

We did not find (hierarchical cluster analysis, Spearman correlation, Mann–Whitney test) a clear direct negative impact of high Cd, Pb, and Zn concentrations on the density of head capsules and Chironomidae taxa (with the exception of *Polypedilum* sp.) in the contaminated sediments of the studied ponds. A lack of negative impact of high concentrations of As, Cd, Cu, Pb, Mn, and Zn in sediments on total abundance of Chironomidae was also found in the Milltown Reservoir in the USA, contaminated by past mining activities (Canfield et al., 1994). Our earlier studies (Michailova et al., 2012; Szarek-Gwiazda et al., 2013) also did not indicate the negative, detrimental impact of high Cd, Pb and Zn concentrations on Chironomidae in other water ecosystems affected by Zn and Pb mines.

The obtained results (Spearman correlations, Mann–Whitney test) indicate an important role of organic matter and nutrients in the distribution of Orthoclaadiinae, which prefers sites with higher metal (Cd, Pb, Zn) contents. In all the cores from older ponds, larvae of Orthoclaadiinae dominated, especially genus *Cricotopus*, which are commonly associated with aquatic macrophytes (Cranston, 2010). Organic matter of both cores contain a similar share of remains of vascular and non-vascular plants as the value of the C: N ratio indicates (12.0–17.7). The lower ratio C:N (4–10) indicates the presence of organic matter rich in proteins (algae), while the higher one (above 20) means organic matter rich in cellulose, the source of which are macrophytes and higher plants (Calvert, 2004). Similarly Brooks et al. (2005) found that changes in the chironomid fauna in sediment cores from ponds in the area of copper smelting in Russia were independent on the industrial activity but were driven by trophic change. In core II collected from the upper part of younger pond, which is not so densely overgrown with macrophytes and with muddy bottom, taxa of *Chironomus* sp. and *Microspectra* sp. dominated. *Chironomus* and *Cricotopus* were recognized as metal tolerant taxa and had already been representative of heavily polluted sites (Canfield et al., 1994). This species also dominated in the stream Matylda (southern Poland), whose sediments were heavily contaminated with Cd, Pb, Cu, and Zn due to wastewater discharges from the zinc and lead ore mine (Szarek-Gwiazda et al., 2013). The sediment pH in range 5.8–7.1 in the studied core did not influence the density of head capsule of Chironomidae (lack of correlation), although it is a factor that can negatively affect Chironomidae communities in various water ecosystems (Brooks and Birks, 2004; Hamerlík et al., 2016).

It is well known that water pH, contents of nutrients, DOC (dissolved organic carbon) or dissolved oxygen may influence the composition of some midge assemblages (Brooks and Birks, 2004; Janssens de Bisthoven et al., 2005; Hamerlík et al., 2016). The elevated values of conductivity and contents of ions SO_4^{2-} , Cl^- , Na^+ , and nutrients (NH_4^+ , PO_4^{3-}) in the studied pond waters in comparison to natural waters indicated their anthropogenic contamination. Water contamination (especially nutrients) might potentially influence Chironomidae communities, particularly in the younger pond, where the worst water quality and low density of head capsules were found (site 1,

core II). The older ponds are partially supplied with clean groundwater, but the inflow of the polluted river waters takes place during raised water flow and affects water quality as well. In core II, taxa of *Chironomus* sp., which often appear in eutrophic waters, dominated. The occurrence of *Psectrotanytus varius* larvae in some layers of cores VI, VIII, and XIV from older ponds indicated highly eutrophic waters during water discharges from the active mine. The pond waters show low metal concentrations. An increase in the Cd, Pb, and Zn concentrations in the pond water in post-mining area can be expected as a result of “internal loading” (Szarek-Gwiazda and Ciszewski, 2017) or during high river flow when metals are washed from the catchment area during heavy rains.

Moreover, other factors (sediment grain size, hydrology) may also influence taxonomic composition and distribution of head capsule within cores. Differences between cores II and X and others may be related to the flow of the Chechło River through the younger pond. A low number of the Chironomidae remaining in the bottom layers of some cores (VII, X) could be connected with the first phases of the pond's existence. *Limnophyes*, *Georthocladius*, and *Corynoneura*, found only in the core XX, usually occur along the shore of a lake, semi-terrestrial, and in stream habitats.

The results confirmed that Chironomidae larvae can live in sediments that are extremely contaminated with heavy metals, because, as it was found by Bahrndorff et al. (2006), most species are subject to adaptation processes to altered environmental conditions. Physiological processes, such as regulation of the heavy metals transport by membranes, sequestration or excretion (Rainbow, 2007), may be also responsible for these adaptations. Metallothioneins (Toušová et al., 2016) and glutathione (Nair et al., 2013) play an important role in the physiological processes. Nevertheless, the genome of the Chironomidae species is very sensitive to high metal concentrations (Michailova et al., 2012; Szarek-Gwiazda et al., 2013; Ilkova et al., 2016, 2018). Two types of chromosome rearrangements can appear in the genome: somatic, which are rare, occur in chromosomes in few cells in the salivary gland with a frequency less than 1%. They are good biomarkers of stress agents in aquatic ecosystem. Other aberrations, inherited, occur at a higher frequency and are associated with adaptations for living in specific environmental conditions (Michailova et al., 2012). The studies in the Chechło River and its ponds have shown the toxic effects of Cd, Pb, and Zn on the genome of the *Chironomus annularius* (Ilkova et al., 2016; Michailova et al., 2018). Moreover, the study of Pedrosa et al. (2017) shows genetically inherited tolerance to Cd in *C. riparius* populations inhabiting metal contaminated sites, despite no evidences of genetic erosion due to long-term metal contamination.

5. Conclusions

The obtained results indicate the lack of a clear negative impact of high concentrations of Cd, Pb, and Zn in the sediment of small subsidence ponds on Chironomidae communities. Therefore, remnants of Chironomidae taxa found in the pond sediments appear from the present study not to be a useful tool for reconstructing dozen-years scale changes in the toxicity of an aquatic environment. We found that the changes in the density of Chironomidae head capsules were associated with changes in the contents of organic matter and nutrients rather than changes in the contents of heavy metal. The difference in macrophyte cover at particular ponds also affected species composition. As a result, Orthoclaadiinae dominated in the older ponds, which were densely overgrown with macrophytes. In the core II from the younger ponds, Chironominae were dominated mainly by *Chironomus* and *Microspectra*, which is typical for muddy substrate. An increase of the number of Chironomidae remains, observed in the top strata of most of the profiles, seems to be the combination of increased nutrient conditions, development of more organic-rich sediments, and the macrophytes cover. A low number of the remains in the bottom samples of several profiles could be connected with the first phases of the pond's existence.

Declaration of competing interest

The authors declare no conflict of interest.

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