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The Impact of Pollution on Diversity and Density of Benthic Macroinvertebrates in Mountain and Upland Rivers

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Abstract: This article summarizes the studies concerning the impact of pollutants on benthic macroinvertebrate communities in the mountain and upland rivers of southern Poland. The Carpathian Raba River, which in the 1960s retained its natural character and had good water quality, was considered as a reference in terms of benthic macroinvertebrate communities. The other two analyzed rivers were polluted to different degrees. The Carpathian Dunajec River was contaminated mainly by sewage from small towns and treatment plant, while the upland Vistula River mainly by sewage from the Upper Silesian Industrial Region and saline waters from coal mines. In studied ecosystems in response to pollutions, a rapid increase in density of fauna caused mainly by the massive development of Oligochaeta was found. In the mountain river, the impact of contamination on macroinvertebrate diversity was negligible. There, taxa considered as indicators of clean water (Ephemeroptera, Plecoptera, and Trichoptera) were abundant and their diversity was similar to that of an uncontaminated river. In the heavily polluted upland Vistula River, the sites with a muddy bottom were dominated by Oligochaeta (99.4–99.9%), while at sites with stony bottoms, apart from Oligochaeta, there were also Chironomidae, Gastropoda, and Hirudinea. In comparison to the 1950s, all Ephemeroptera, Plecoptera, Odonata, Trichoptera, and Megaloptera were extinct.

Keywords: Oligochaeta; Ephemeroptera; pollution; biomarker

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1. Introduction

Development of industry and urban agglomeration in the XIX century has caused huge amounts of water consumption and pollution. The protection of human health has made it necessary to develop methods of controlling pollution in the aquatic environment. It has been pointed out that water pollution alters water biocenoses. Kolkwitz and Marson [1,2] introduced the concept of biological indicators of environmental condition. They developed a saprobic system based on species specific to a given water quality class. The system has been modified several times over the years [3–5]. Benthic invertebrates are very good indicators of organic pollution and hydro-morphological deficits at the micro-habitat scale [5–9]. It has been found that Tubificidae (Oligochaeta) are indicators of heavy pollution of freshwaters, Chironomidae (Diptera) are indicators of average contamination, and Ephemeroptera, Plecoptera, Trichoptera, and Odonata are indicators of clean waters [10].

In parallel, studies on the zonal distribution of biocenoses along the river courses have been carried out. The first studies dealt with fish zones in rivers [11,12], and later with zonal distribution of benthic fauna communities [13,14].

The aim of this paper was to indicate how the different degrees of contamination affect the diversity and density of benthic macroinvertebrates in different river types and zones. To realize this aim, we analyzed the results of hydrochemical and hydrobiological studies carried out in the Vistula River (1982–1983) and its Carpathian tributaries: the Raba (1966–1970) and Dunajec (1992–2000) rivers in southern Poland. The mountain Raba River was selected as a reference, because in the 1960s it retained its natural character and had good water quality, in terms of physico-chemical parameters. The mountain Dunajec River in the section between the towns Nowy Targ and Nowy Sącz was contaminated in the 1990s with municipal sewages below small towns. The Upper Vistula River has been heavily polluted with municipal and industrial sewage from the Upper Silesian Industrial Region since the 1960s. Two groups of benthic macroinvertebrates, Oligochaeta and Ephemeroptera, are discussed as the indexes of polluted and clean waters, respectively.

2. Materials and Methods

2.1. Study Area

The Raba River and Dunajec River are the right-side tributaries of the Vistula River (southern Poland). The Raba River has a length of 137.4 km and a catchment basin of 1528 km^2 with draining waters from the Western Beskid Mountains (Western Carpathians), Carpathian foothills, and Sandomierska Basin and flows into the Vistula River (180 m asl). The highest springs within the river basin lie at 1240 m asl (northern slopes of Mt Turbacz) and give origin to the Olszowy Potok. Most of the catchment basin lies at the altitude interval of 300–500 m above sea level; the average gradient amounted to 4.5‰ (the upper course 23.6–4.1‰, middle 2.7–1.7‰, and lower course 0.6‰) [15]. A considerable part (86%) of the river basin is located in the Carpathians region, with only the mouth part being located in the lowlands [16]. The river was regarded as relatively uncontaminated in the 1960s, although in its basin, five small towns (Rabka, Mszana, Myślenice, Dobczyce, and Bochnia) without sufficient sewage system were located. This research was carried out along the entire length of the river [15,17] (Table 1).

Table 1. Description of the sampling sites at Raba, Dunajec and Vistula rivers [18–21].

Site	Site	Altitude (m a.s.l.)	Maximum Wide (m)	Maximum Depth (m)	Riverbed
	Raba River				
1	Olszowy stream	1180	0.4	0.1	flat stones of smaller size
2	Olszowy stream	990	1.2	0.2	stones
3	Olszowy stream	780	1.5	0.3	stones
4	Olszowy stream	580	4.0	0.4	stones
5	Porębianka stream	460	8.0	0.6	stones
6	Raba River	370	15.0	0.8	stones
7	Raba River	315	20.0	0.8	stones
8	Raba River	250	25.0	1.0	stones
9	Raba River	210	30.0	1.2	smaller stones, thick gravel, sand, mud
10	Raba River	185	30.0	1.5	smaller stones, thick gravel, sand, mud
	Dunajec River				
1	Harklowa	638			stones
2	Sromowce Niżne	487			stones
3	Szczawnica	430			stones
4	Kadcza	320	120		stones
5	Nowy Sącz	280	100		stony, sandy
	Vistula River				
1	Okleśna	215.6	80	1.2	sticky black mud
2	Łączany reservoir	215.5	2000	up to 1.5	mud
3	Łączany—below the dam	209	100	up to 0.7	mostly mud
4	Czernichów	206.1	80	10–70	mud, sand
5	Wołowice	205.1	100	20–70	stones of different size, gravel, sand
6	Jeziorzany	203.5	100	70	stones, mud

The Dunajec River (a length of 246 km, catchment area of 6798 km^2) originates in the Tatra Mts in the Western Carpathians of southern Poland (1540 m asl). It flows through a variety of geographical regions differing in geology, geomorphology, and climate [18].

This research was carried out on the sub-mountain section of the river between the towns of Nowy Targ and Nowy Sącz, characterized with a stony bottom as well as fast and turbulent current (Table 1). Until the mid-1990s, this river section had a natural character. It was contaminated by municipal wastewaters from towns (Nowy Targ, Szczawnica, Krościenko, and Nowy Sącz—sewage from the treatment plant), wastewaters from villages, and contaminants from agricultural lands [22].

The Vistula River is the longest river in Poland (1047 km). In the upper course, until the Goczałkowice Reservoir (located 62 km), the river has a mountain and sub-mountain character, while downstream the dam sits in the lowlands. The tributaries of the Vistula, the rivers Przemsza and Biała, carry sewage from the Upper Silesia Industrial Region (USIR) (numerous foundries, Zn-Pb mines, collieries, and large towns), the Bielsko-Biała Industrial Region (plants of textile, chemical, and heavy industry, deep water from a coal mine), and the town Oświęcim (municipal sewage, chemical industry) [20].

This research was carried out in the section of the Vistula River below the inflow of the Przemsza River and Krakow (128–153 km of the river course). The Łączany barrage (dam height 6 m, average water exchange 16 h, located ~133 km) was built with a navigable-energetic function in 1961. About 20 m^3 s^{-1} of water was diverted from the channel to the Skawina electric power station, heated, and discharged back to the Vistula below the studied section [23]. Below the Łączany barrage, the river had largely natural hydrological characteristics [20].

2.2. Sampling Sites

In the Raba River, studies of benthic macroinvertebrates were carried out at 10 sites situated from the sources to the mouth in 1969–1970 [15,17], while hydrochemical studies at sites 6–10 were conducted in 1966–1967 [19] (Tables 1 and 2, Figure 1).

Table 2. Mean and range values of physico-chemical parameters in the waters of Raba (1966–1967) [19], Dunajec (1990 and 2000 *, data from Provincial Inspectorate for Environmental Protection in Kraków), and Vistula (1982–1983) [24,25] rivers.

Parameter	Unit	River		
		Raba	Dunajec	Vistula
Temperature	°C	13.9	9.1	14.0
		7.0–22.9	0.2–20.2	3.9–24.0
pH		7.6–8.5	7.7–8.9	6.0–7.6
Dissoved oxygen	mg O_2 dm^{-3}	10.7	11.6 *	8.5
		8.2–13.4	7.8–14.2	1.1–13.1
Oxygen saturation	%	102.1	104.4 *	-
		90.7–123.0	76–136	-
Conductivity	µS cm^{-1}	289.1	330.8 *	4295 **
		179–509	233–409	
Cl^-	mg dm^{-3}	6.8	13.5	837.1
		3.8–14.0	6–23	242–1438
SO_4^{2-}	mg dm^{-3}	25.6	33.5	234.5
		18.9–35.8	14–56	78.0–435
Ca^{2+}	mg dm^{-3}	49.2	45.6 *	103.8
		31.4–62.9	32–70.4	57.2–142.6
Mg^{2+}	mg dm^{-3}	10.5	9.3 *	48.0
		7.6–14.3	5.5–15.5	17.3–74.8
Na^+	mg dm^{-3}	6.9	8.1 *	469.0
		2.8–13.2	4.0–17.0	102.4–710.0
K^+	mg dm^{-3}	2.3	2.3 *	20.8
		1.3–3.7	1.5–3.5	5.4–30.0
BOD5	mg O_2 dm^{-3}	1.8	3.5	16.1
		0.8–2.7	1.3–5.8	4.0–50.8
COD	mg O_2 dm^{-3}	2.4	4.0	15.8
		1.5–4.0	1.6–8.4	5.4–28.1

Table 2. *Cont.*

Parameter	Unit	River		
N-NO_3	mg dm^{-3}	0.8	1.6	0.59
		0.3–1.2	0.5–2.7	0.10–2.18
N-NH_4	mg dm^{-3}	0.107	0.29	3.59
		0.072–0.210	0.06–0.71	1.30–8.0
P-PO_4	mg dm^{-3}	0.006	0.053	0.052
		nd-0.024	0.016–0.098	0.021–0.107
P-tot	mg dm^{-3}	-	0.047 *	1.35
		-	0.010–0.12	0.62–14.2

** according to [24].

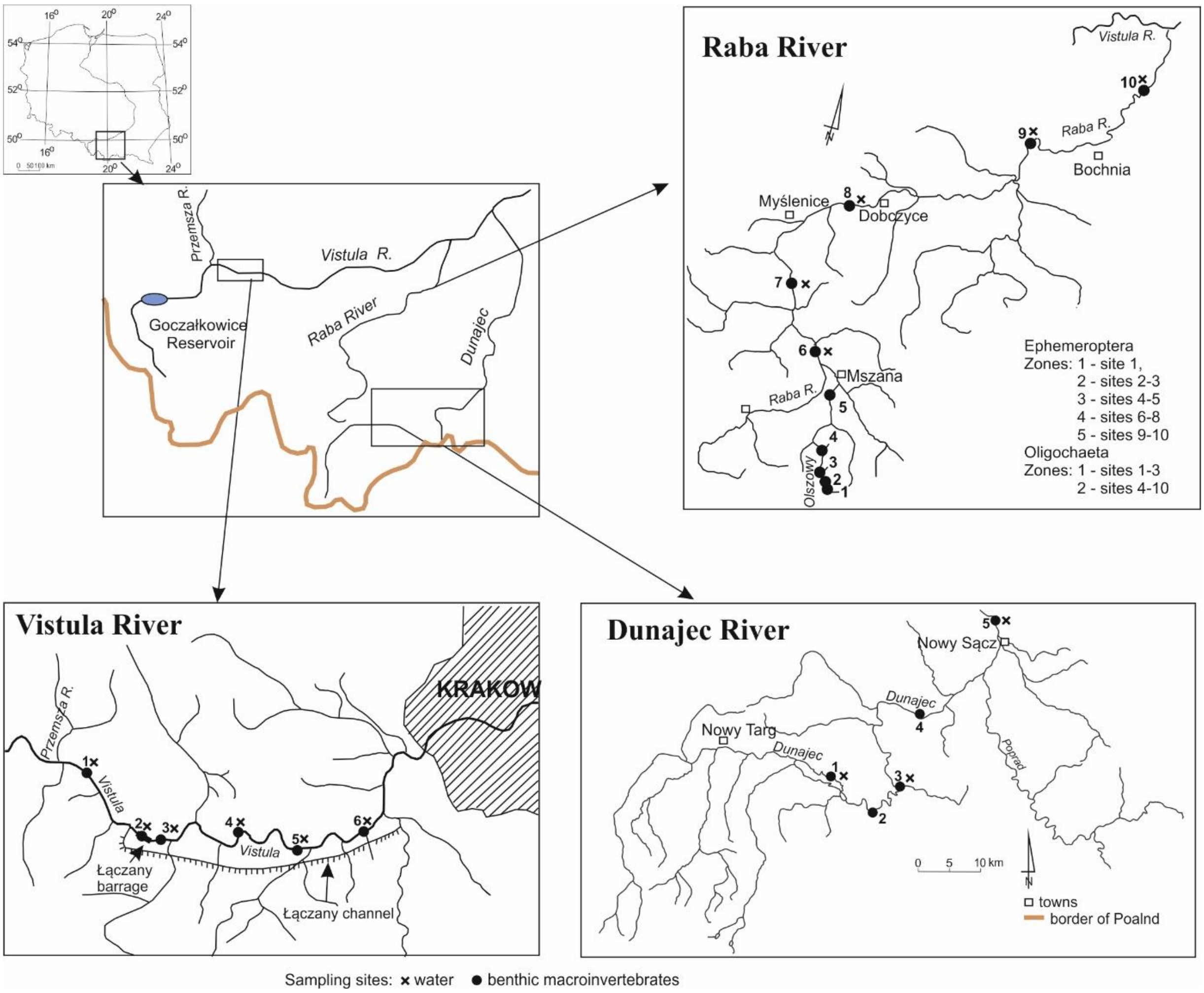


Figure 1. Sampling sites in the Raba, Dunajec, and Vistula rivers.

The studied section of the Dunajec River (between Nowy Targ and Nowy Sącz) included 5 sampling sites (Table 1, Figure 1). The studies of benthic macroinvertebrates at sites 1–3 were carried out four times per year in 1992–1993 [26] and at sites 4–5 in April and August 1999 [18]. The hydrochemical studies were carried out at sites 1, 3, and 5 every month in 1990 and 2000 (sites 1 and 3: January–December; site 5: February–November).

In the Upper Vistula River, the studies of benthic macroinvertebrates and water chemistry were carried out at 6 sites monthly from December 1982 to December 1983 [25,27] (Tables 1 and 2, Figure 1). Site 1 was situated above the Łączany barrage and had regulated banks, site 2 was in the Łączany barrage near the dam, and site 3 was ca. 200–300 m below the Łączany barrage. Until site 4, the Vistula remains a lowland river with a gradient of

0.3‰, while at sites 5 and 6 it resembles a sub-mountain river. It flows across the calcareous area of the Kraków Upland, has stone overgrown by algae as a bottom, and forms numerous rapids [20].

2.3. Methods

Physico-chemical analysis of the river waters were carried out according to Just and Hermanowicz [28] and Standard Method [29]. Data of the physico-chemical parameters of the Dunajec River water were received from the Provincial Inspectorate for Environmental Protection (WIOŚ) in Kraków.

From each site, 10 subsamples of benthic macroinvertebrates from different microhabitats were collected. From the stony bottom of the rivers, Raba and Dunajec, the samples were collected with a Surber sampler of size 20 × 20 cm (mesh size 0.3 mm). From the muddy bottom of the Vistula River, the samples were taken with a tube grab from an area of about 80 cm^2 (2 subsamples of 40 cm^2 each), while on the stony bottom they were taken with a Surber sampler. The obtained material was preserved with 4% formalin. In the laboratory, all macroinvertebrates were picked up under 10× magnification, and as far as was possible, identified to the species level. The results are shown as density (ind/m^2), number of taxa, and percentage of dominant Ephemeroptera and Oligochaeta species. The first dominant was the species with the highest percentage at particular site. The percentage of the first dominant and other dominant species ranged from 10 to 100% at a given site.

3. Results

3.1. Physico-Chemical Characteristics of the Rivers

The description of water chemistry of the Raba River (sites 6–10) is based on Bombówna [19], the Dunajec River on data obtained from WIOŚ (for 1990, 2000) and Kownacki [18,30], and the Vistula River based on Kasza [25].

3.1.1. Raba and Dunajec Rivers

The water chemistry of the Raba River and Dunajec River were typical for Carpathian rivers. The water temperature of the Raba River ranged from 7.0 °C to 22.9 °C (for spring, summer and autumn) and the Dunajec River from 0.2 °C to 20.2 °C (all year) (Table 2). Usually, the rivers were covered with ice for 1–3 months during winter, depending on the year. Generally, the river waters had high contents of dissolved oxygen (8.2–13.4 mg O_2 dm^{-3} and 7.8–14.2 mg O_2 dm^{-3}; oxygen saturation 90.7–123.0% and 76–136%, respectively) and from neutral to alkaline pH (7.6–8.5 and 7.7–8.9, respectively). The Dunajec River at sites 1 and 2 had higher oxygen concentrations than at site 5 (mean 11.7, 12.7, and 9.0 mg O_2 dm^{-3}, respectively). The water oxygenation resulted from the primary production of attached algae overgrowing the stones as well as rapid turbulent current (ensuring constant mechanical water oxygenation).

Conductivity values in the waters of the Raba River (179–509 µS cm^{-1}) and the Dunajec River (233–409 µS cm^{-1}) were related to both natural and anthropogenic factors. An increase in conductivity and some ion (especially Cl^-, Na^+, and/or SO_4^{2-}) concentrations in the Dunajec River was observed at low flow near towns (sites 1 and 5). The ionic composition of the waters were dominated by HCO_3^- and Ca^{2+} (Table 2), which included the waters to the calcium-carbonate type.

In the studied rivers, the concentrations of nutrients were N-NO_3 0.3–2.7 mg dm^{-3}, N-NH_4 0.06–0.71 mg dm^{-3}, and P-PO_4 nd (not detectable)–0.098 mg dm^{-3}, and the indices of the organic matter content were BOD5 0.8–5.8 mg dm^{-3} and COD 1.5–8.4 mg dm^{-3} (Table 2). In the Dunajec River, the above parameters were higher (N-NO_3, N-NH_4, BOD5, COD 1.7–2.7 times, P-PO_4 8.8 times) compared to those in the Raba River. In the Dunajec River, larger increases in N-NH_4 concentrations and COD values were observed below towns (sites 1 and 3) at low flow, indicating the input of municipal sewages. Additionally, in the Raba River, a slight increase in BOD values (similarly like Cl^-, Na^+) at low flow was periodically observed at some sites (6, 8, 10).

3.1.2. Vistula River

The water chemistry of the Vistula River differed considerably from that found in the Raba and Dunajec rivers (Table 2). The water had from slightly acidic to neutral pH (6.0–7.6). Mean content of dissolved oxygen was 2-times lower at sites 1 and 2 (mean ~4.4 mg dm^{-3}) than at sites 3–6 (mean 8.4–9.2 mg dm^{-3}). Below the Łączany barrage (site 3), it ranged from 1.1 up to 11.7 mg dm^{-3}, mainly due to mechanical water oxygenation. At sites 5 and 6, the waters were well oxygenated (up to 13.1 and 12.2 mg O_2 dm^{-3}) due to the rapid current on a stony bottom which caused turbulent water oxygenation.

In the water high concentrations of Cl^-, SO_4^{2-}, and Na^+ were found (Table 2). The concentrations of ions Cl^-, SO_4^{2-}, Na^+, and K^+ (up to 1438, 435, 710, and 30 mg dm^{-3}) exceeded from several to several hundred times (123, 9.2, 68, and 9, respectively) those in the Raba River and changed slightly along the river course (Table 2). The water belonged to the Cl-Na type.

The water (sites 1–6) was rich in N–NH_4 and P-tot as well as in organic compounds (mean: BOD5 16.1 mg O_2 dm^{-3}, COD 15.8 mg O_2 dm^{-3}). The contents of N–NH_4 were several dozen times higher than those in the Raba (~35 times) and Dunajec rivers (~12 times). Moreover, N–NH_4 dominated (~6 times higher) over N–NO_3, inversely to the Carpathian rivers. The highest P–tot content and BOD5 value were found below the Łączany barrage (site 3, mean annual 9.15 and 21.5 mg dm^{-3}, respectively). The slight decrease in BOD5 and COD values occurred at the Łączany barrage (site 2) due to the slow flow, which favors the organic matter sedimentation, as well as at sites 4–6, where a slight improvement in water purity was associated with better water oxygenation. However, the process of self-purification of the Vistula water was very slow. This was explained by the formation of new, slow-decaying organic compounds, similar to humus substances, during organic matter decomposition, which were transported over a long distance.

3.2. Benthic Macroinvertebrates in the Raba, Dunajec and Vistula Rivers

The detailed descriptions of Ephemeroptera (Table S1) and Oligochaeta (Table S2) of the Raba River are based on Sowa [15] and Kasprzak and Szczęsny [17], the Dunajec River based on Szczęsny [26] and Kownacki et al. [18,30], and the Vistula River based on Dumnicka and Kownacki [27,31].

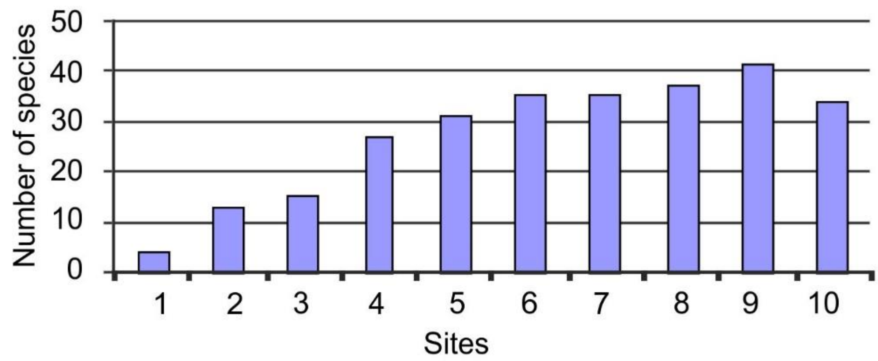
3.2.1. Benthic Macroinvertebrates in the Raba River (Reference Data)

The insect larvae were the main components of the bottom fauna in the Raba River. The most important in terms of abundance and diversity were the Ephemeroptera (66 species in total) [15], Plecoptera (56 species) [32], Trichoptera (73 species) [21], and Oligochaeta (58 species) [17]. Diptera larvae were also very important, especially those from the Chironomidae (55 taxa) and Simuliidae families [33]. Odonata, which inhabited the middle and lower course of the Raba River, were represented by four species [34] and Heteroptera by two species [35]. Mollusca were very rare, and found only as single specimens [36]. Apart from Ostracoda [37] and Hydrachnidia [38], other groups (Nematoda, Hirudinea, and Turbellaria) were not studied. The Raba River belongs to one of the best-known rivers in Europe from a faunistic point of view.

In the Raba River, five zones with characteristic zoocenoses may be distinguished. The zone boundaries vary slightly for particular macroinvertebrate groups. Zonation was clearly shown for the Ephemeroptera communities (Figure 2). In zone 1 (site 1), including the stream section lying near the spring, Ephemeroptera communities were poor and limited to four species *Baetis alpinus* (Pictet, 1843), *Ecdyonurus subalpinus* Klapalek, 1907, *Rhithrogena iridina* (Kolenati, 1839), and *R. loyolaea* Navàs, 1922. In zone 2 (sites 2 and 3), the diversity of Ephemeroptera increased (13–15 species). The larvae of *Baetis alpinus*, *Rhithrogena iridina*, and *Ameletus inopinatus* Eaton, 1887 dominated. By contrast, *Ecdyonurus subalpinus* was no longer present. In zone 3 (sites 4 and 5), biodiversity increased (27–31 species). The first dominant was the *Baetis rhodani* (Pictet, 1843) larvae. *Rhithrogena carpatoalpina* Klonowska & Olechowska & Sartori & Weichselbaumer, 1987 and *Baetis lutheri* Muller-

Liebenau, 1967 as well as *Baetis alpinus*, were numerous in the upper part of this zone. In zone 4 (sites 6–8), biodiversity was high (35–37 species). The first dominant was *Baetis fuscatus* (Linnaeus, 1761), although *Baetis vardarensis* Ikonomov, 1962 was also abundant. The species that were characteristic for zones 1–3 were not found. In zone 5, biodiversity ranged from 41 species at site 9 to 34 species at site 10. The first dominant was *Baetis vardarensis*.

A



B

Species	Zones I	II	II	III	III	IV	IV	IV	V	V
Altitude (m asl)	1180	990	780	580	460	370	315	250	210	185
Sites	1	2	3	4	5	6	7	8	9	10
Baetis alpinus (Pictet, 1843)	52	41	31	27	1.9	0.2				
Ecdyonurus subalpinus Klapalek, 1907	28	0.9								
Rhithrogena iridina (Kolenati, 1839)	15	11	34	1.8						
Rhithrogena loyolaea Navàs, 1922	5	17	0.2	x						
Ameletus inopinatus Eaton, 1887		22	11	0.1	x					
Baetis rhodani (Pictet, 1843)		1.2	2.2	34	37	19	5.1	2.0	0.7	0.6
*Rhithrogena carpatoalpina**			x	13	9	0.6	0.4			
Baetis lutheri Muller-Liebenau, 1967				4.6	16	6.7	11	2.7	0.3	0.1
Baetis fuscatus (Linnaeus, 1761)					1.2	23	26	33	12	5.4
Baetis vardarensis Ikonomov, 1962					2.3	12	15	15	22	32
Serratella ignita (Poda, 1761)				1.5	1.0	4.2	3.8	6.1	5.9	11
Caenis pseudorivulorum Keffermuller, 1960					0.3	0.4	0.6	14	11	18
Oligoneuriella rhenana (Imhoff, 1852)					0.1	2.2	19	2.9	10	0.7

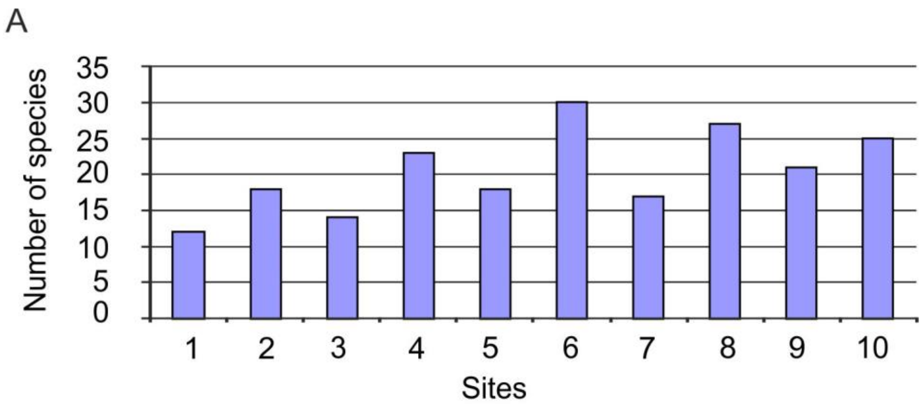
* *Rhithrogena carpatoalpina* (Klonowska & Olechowska & Sartori & Weichselbaumer 1987 (= *R. ferruginea* Navas)

First dominant (the highest percentage at the site) Dominant (10–100%) x below 0.1%

Figure 2. The number of species (**A**) and percentage of dominant species (**B**) of Ephemeroptera in the Raba River in 1969–1970 (according to [15], modified).

Less clearly, zonation showed Oligochaeta communities in the Raba River (Figure 3). Generally, two large Oligochaeta communities can be distinguished. The first community inhabited sites 1–3 and had relatively low diversity (12–18 species), while the second group occurred at sites 4–10 and had higher diversity (17–31 species). Within these large groups, some differentiation of Oligochaeta communities based on the dominant species was observed. At site 1, the first dominant was *Mesenchytraeus armatus* (Levinsen, 1884), while at sites 2–3, species of the genus *Cernosvitoviella* dominated. In contrast, in the second group, at sites 4–8, *Nais bretscheri* Michaelsen, 1899 and *Nais alpina* Sperber, 1948 dominated, and at sites 9–10, *Propappus volki* Michaelsen, 1916. *Nais communis* Piguet, 1906 was the only taxon that occurred at all sites, but usually in low density.

A



B

Species	Zones									
	I	II	II	III	III	IV	IV	IV	V	V
	Altitude (m asl)									
	1180	990	780	580	460	370	315	250	210	185
	Sites									
	1	2	3	4	5	6	7	8	9	10
	Lotic conditions									
Mesenchytraeus armatus (Levinsen, 1884)	26	16	9.4	0.3		x				x
Nais communis Piguet, 1906	10	2.6	3.7	3.9	0.6	1.3	0.1	6.1	0.5	8.8
Cernosvitoviella spp.	6.2	55	25	1.0	0.2	x	0.1			
Fridericia spp.	3.1		26	0.2						
Nais bretscheri Michaelsen, 1899		1.5		23	58	63	69	19	0.6	44
Nais alpina Sperber, 1948				34	20	7.2	2.2	18	2	12
Nais pardalis Piguet, 1906				16	16	7.4	9.9	0.9	0.2	1.8
Propappus volki Michaelsen, 1916	5.2			20	1.9	0.9	4.7	17	86	16
Nais elinguis Muller, 1774				0.1	1.3	13	10	34	0.1	13
	Lenitic conditions									
Mesenchytraeus armatus (Levinsen, 1884)	26	15	9.6	x		x				
Cernosvitoviella spp.	6	64	56	0.1						
Nais pardalis Piguet, 1906			12	33	16	3	5	0.2	0.4	0.4
Nais bretscheri Michaelsen, 1899			3	42	59	39	82	16	1	1.1
Nais alpina Sperber, 1948				14	15	3.1	5.3	12	2.5	3.8
Nais elinguis Muller, 1774					0.3	45	1	18	0.6	1.5
Amphichaeta leydigi Tauber, 1879								36		
Propappus volki Michaelsen, 1916				1.4	2.3	0.7	3.5	1.8	64	43
Nais communis Piguet, 1906		1.8	0.6	0.8	0.4	1.7	0.7	2	3.2	22

First dominant (the highest percentage at the site) Dominant (10–100%) x below 0.1%

Figure 3. The number of species (**A**) and percentage of dominant species (**B**) of Oligochaeta in the Raba River in lotic and lenitic conditions in 1969–1970 (according to [17], modified).

3.2.2. Benthic Macroinvertebrates in the Dunajec River

In the studied section of the Dunajec River, 174 taxa of benthic macroinvertebrates were identified. Like in the Raba River, the insect larvae were the main component of the fauna. The most important groups were Chironomidae (68 species), Ephemeroptera (28 species), Trichoptera (12 species), Plecoptera (9 species), and Oligochaeta (37 species). The remaining groups of insects were identified to the families Coleoptera—three families, and Diptera—seven families. Moreover, at some sites, single specimens of Hirudinea, Gastropoda, Bivalvia, Amphipoda, and Isopoda were found.

The density of benthic macroinvertebrates was the highest at sites 1 and 5, below towns Nowy Targ and Nowy Sącz, (93,000 ind/m^2 and 105,000 ind/m^2, respectively), a lower one at site 4 (45,000 ind/m^2), and the lowest densities at sites 2 and 3 (22,000 ind/m^2 and 16,000 ind/m^2, respectively) (Figure 4). At sites 1 and 5, Oligochaeta dominated (65% and 90%, respectively), which indicates river contamination. At sites 2 and 3, the dominant group was Chironomidae (~60%), the Ephemeroptera, Plecoptera, and Trichoptera (EPT) consisted of 15–20%, while the share of Oligochaeta dropped to 20%. This indicates the improvement in water quality in comparison to site 1. At site 4, the dominance structures were similar to those at sites 2 and 3. The number of benthic invertebrate taxa was similar in all of the studied sites of the Dunajec River (sites 1and 4: 96 taxa; sites 2, 3, and 5: 109, 108, and 117 taxa) (Figure 4).

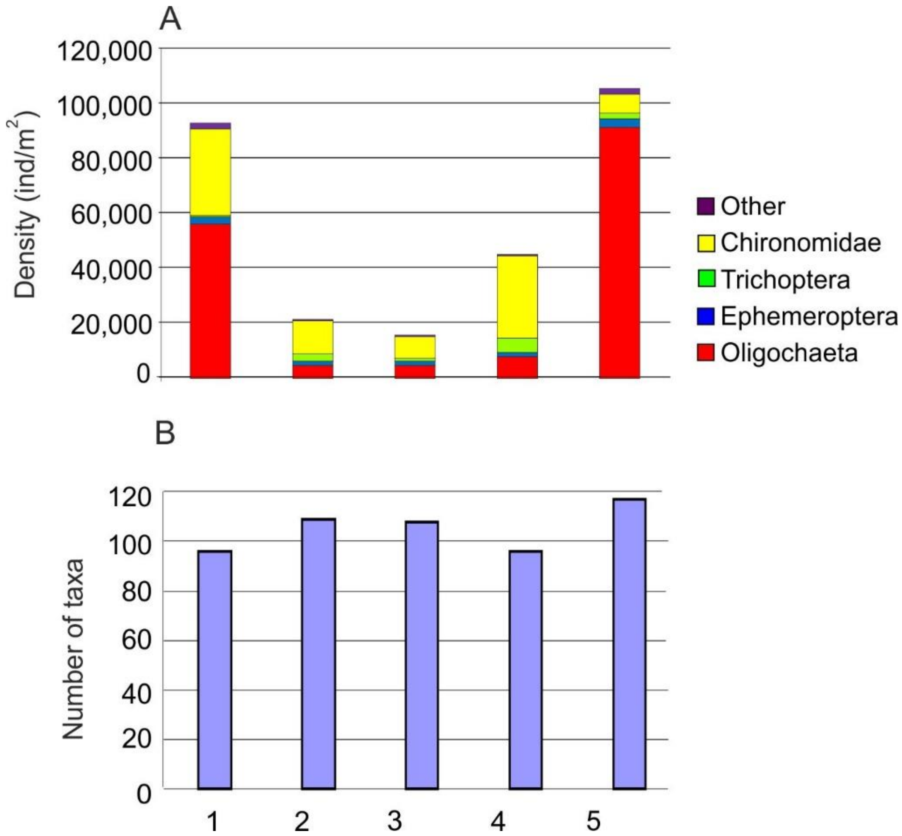
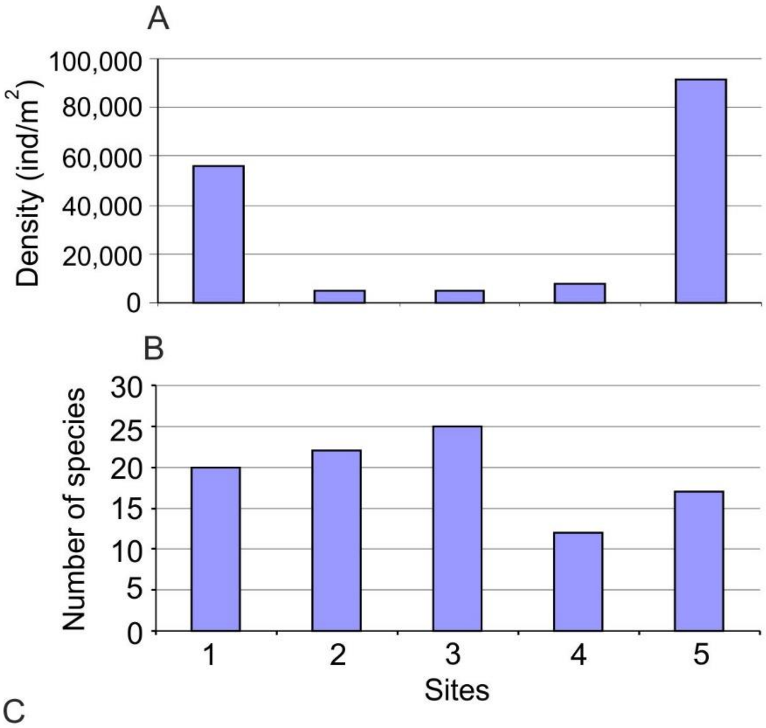


Figure 4. The density (**A**) and number of taxa (**B**) of benthic macroinvertebrate groups in the Dunajec River in 1992–1993 (sites 1–3) and 1999 (sites 4–5) (according to [18,26], modified).

The density of Oligochaeta was much higher at sites 1 and 5 below the towns (55,927 ind/m^2 and 91,723 ind/m^2, respectively) than the other sites (sites 2 and 3: less than 5000 ind/m^2; site 4: 7500 ind/m^2) (Figure 5). In the studied river section, a total of 37 Oligochaeta species were found. The number of species varied from 12 at site 4 to 25 at site 3 (Figure 5). The Oligochaeta were mainly represented by the family Naididae (21 species), which included the dominant species. At sites 1 and 5, *Nais barbata* Müller, 1774 was the first dominant (48% and 44% of all Oligochaeta, respectively), while at the other sites, its percentage was below 10%. At all sites where *Nais bretscheri* (first dominant at sites 2 and 4) and *Nais elinguis* Müller, 1774 (first dominant at site 4) were present, *Nais alpina* and *Nais christinae* Kasprzak, 1973 were accompanying species. The indicators of polluted water such as *Tubifex tubifex* Müller, 1774 or *Limnodrilus hoffmeisteri* Claparède, 1862 were present as single individuals. The remaining species of Oligochaeta were represented by a small number of individuals and only found in some localities.



C

Species	Sites 1	2	3	4	5
Nais barbata Muller, 1774	48	9.1	5.5	3.9	44
Nais bretscheri Michaelsen, 1899	18	38	24	61	0.4
Nais elinguis Muller, 1774	18	18	36		31
Nais alpina Sperber, 1948	0.3	15	2	9.2	x
Nais christinae Kasprzak, 1973	0.1	0.5	0.1		10

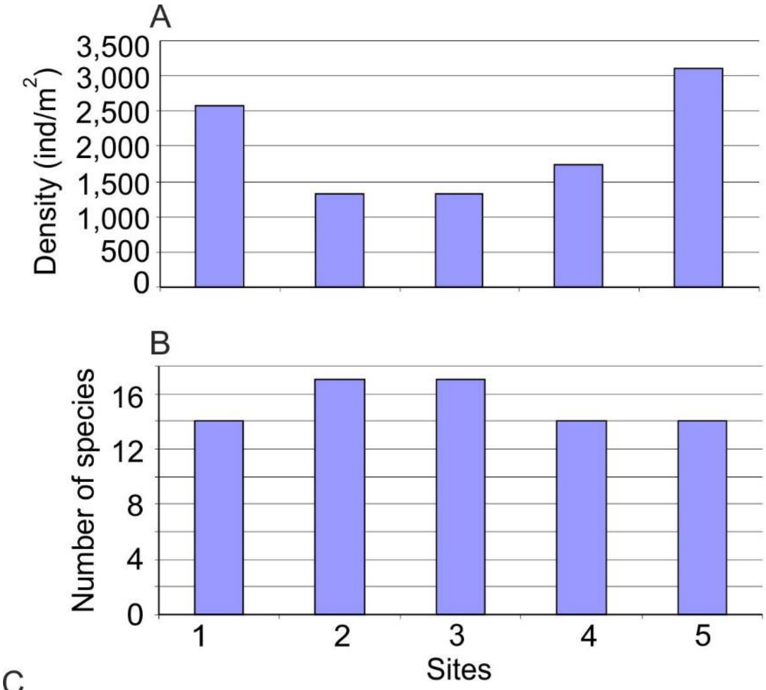
First dominant (the highest percentage at the site)
Dominant (10–100%)
x below 0.1%

Figure 5. The density (**A**), number of species (**B**), and percentage of dominant species (**C**) of Oligochaeta in the Dunajec River in 1992–1993 (sites 1–3) and 1999 (sites 4–5) (according to [18,26], modified).

Ephemeroptera were present at all sites of the river section. Their density was higher at the more contaminated sites like site 1 (2720 ind/m^2) and 5 (3112 ind/m^2) and lower at the remaining sites (1320–1740 ind/m^2) (Figure 6). The number of species varied slightly between sites (sites 2 and 3 had 17 species; sites 1, 4, and 5 had 14 species) (Figure 6). *Baetis lutheri* was present at all sites, and dominated at sites 1–4. *Baetis fuscatus* was dominant at sites 1–3 and was probably abundant at sites 4 and 5 where juvenile *Baetis* sp. were found in large numbers. An associated species, which was quite abundant at all sites, was *Serratella ignita* (Poda, 1761). Other species were less abundant and occurred at some sites.

3.2.3. Benthic Macroinvertebrates in the Vistula River

In the 1980s, a section of the Vistula River from the inflow of the Przemsza River to Kraków was the most polluted along the entire river length. A total of 43 taxa of benthic macroinvertebrates were identified. The fauna was chiefly represented by Oligochaeta (20 species) and Chironomidae (12 taxa). Additionally, single specimens of Hirudinea (three species), Gastropoda (three species), Amphipoda (one species), Diptera (families Simuliidae, Chaoboridae, and Psychodidae) and Coleoptera were found at sites 4–6.



C

Species	Sites				
	1	2	3	4	5
Baetis fuscatus (Linnaeus, 1761)	44	33	46		
Baetis lutheri Muller-Liebenau, 1967	38	47	30	17	2.9
Serratella ignita (Poda, 1761)	2.9	12	10	8.5	5
Baetis spp.				48	73
Heptageniidae (juv.)				8.0	11

First dominant (the highest percentage at the site)
Dominant (10–100%)
x below 0.1%

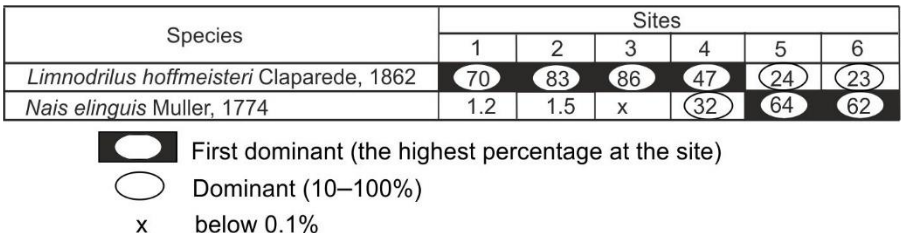
Figure 6. The density (**A**), number of species (**B**), and percentage of dominant species (**C**) of Ephemeroptera in the Dunajec River in 1992–1993 (sites 1–3) and 1999 (sites 4–5) (according to [18,26], modified).

Among benthic macroinvertebrates, Oligochaeta were the most abundant and their density was high at all sites (1–6). Nevertheless, marked differences in the density and dominance structure of Oligochaeta were observed between the sites. The density was extremely high at sites 2 (the Łączany barrage; 290,482 ind/m^2) and 3 (below the Łączany dam; 275,953 ind/m^2) and lower at the remaining sites (30,018–77,058 ind/m^2). Densities of other groups were low: Chironomidae (183–381 ind/m^2 at sites 1–3, 1027–16,339 ind/m^2 at sites 4–6), Hirudinea (206–440 ind/m^2) and Gastropada (245–694 ind/m^2) (Table 3).

The percentage share of Oligochaeta was 99.4–99.9% at sites 1–3, 95.7% at site 4, and 91.2% at site 6 (Table 3). Only at site 5, with a rocky bottom and better water oxygenation, the percentage of Oligochaeta decreased to 63.6%, while that of Chironomidae increased to 34.6%. *Limnodrilus hoffmeisteri* was dominant at all sites, although its percentage was higher at sites 1–3 (71–86%) than at sites 4–6 (21–47%). At sites 4–6, the dominant species was also *Nais elinguis* (32–64%) (Figure 7).

Table 3. Density (individual/m^2) of benthic macroinvertebrate groups and their percentage in the Vistula River in 1982–1983 (according to [27]).

Group of Macrofauna	Sites					
	1	**2**	**3**	**4**	**5**	**6**
Density						
Oligochaeta	64,035	290,482	275,953	42,646	30,018	77,058
Chironomidae	391	229	183	1027	16,339	6745
Hirudinea				206	264	440
Gastropoda				694	561	245
Percentage (%)						
Oligochaeta	99.4	99.9	99.9	95.7	63.3	91.2
Chironomidae	0.6	0.1	0.1	2.3	34.6	8.0
Hirudinea				0.5	0.6	0.5
Gastropoda				1.5	1.2	0.3



Species	Sites					
	1	2	3	4	5	6
Limnodrilus hoffmeisteri Claparede, 1862	70	83	86	47	24	23
Nais elinguis Muller, 1774	1.2	1.5	x	32	64	62

Figure 7. Percentage of dominant Oligochaeta species in the Vistula River in 1982–1983 (according to [27], modified).

In the 1980s, Ephemeroptera were no longer present in the studied section of the Vistula River.

4. Discussion

The right-bank tributaries of the Upper Vistula River originate from springs located on the northern slopes of Carpathians. In the upper and middle sections, they have the characteristics of mountain streams and rivers with rocky bottom, fast turbulent flow, and good water oxygenation. Only in the lower section they have the characteristics of lowland rivers, with laminar flow and bottoms covered by gravel, sand, and silt. Until the 1950s, these rivers had a natural character. Currently, most rivers are regulated, and several dam reservoirs/weirs have been built on them, significantly changing the natural characteristics of biocoenosis, including benthic macroinvertebrate communities [30,39]. Comparison of the Raba River water chemistry in the 1960s with the classification of water quality presented in the Regulation of the Minister of Marine Economy and Inland Navigation in Poland [40] indicated generally good water quality (Class I). A slight increase in nutrient and organic matter indices has periodically been observed at low flow at some sites (6, 8, 10). However, contaminated water was purified in relatively short sections, indicating an excellent ability to self-purify. Therefore, comprehensive studies of the benthic fauna communities along the Raba River course carried out in the 1960s [15,17,21,32–38] showed their natural changes and gave the reference material for the assessment of the human pressure or climate change in the Carpathian rivers.

The Dunajec River between the towns Nowy Targ and Nowy Sącz was also uncontaminated until the mid-1960s. In subsequent years, there was an increase in river contamination, mainly by nutrient and organic matter, especially below the towns [18,22,41]. In 1990, the river water had increased values of the following parameters: BOD5 and $N\text{-}NO_3$ (sites 1, 3, 5, below Class II, below good water quality), $N\text{-}NH_4$ (sites 1 and 5 below Class II; site 3 Class II, good water quality), $P\text{-}PO_4$ (sites 1, 3, 5 Class II) [40]. As a consequence, the benthic macroinvertebrate communities also changed. At sites 1 and 5, the density of benthic macroinvertebrates increased considerably due to the massive development of Oligochaeta, which were the dominant group, while the benthic fauna diversity only slightly changed. The dominant species of Ephemeroptera were the same in the Raba and Dunajec rivers. In the Dunajec River, *Beatis fuscatus*, *B. lutheri*, and *Serratella ignita* dominated. In the Raba River *B. lutheri* dominated in zones III and IV, *B. fuscatus* in zone IV, while *Serratella ignita* was numerous in zones III and IV and dominated in zone V. At more contaminated sites 1 and 5 of the Dunajec River, the density of Ephemeroptera was ~2 times higher, while the number of taxa was slightly lower in comparison to the remaining sites (especially 2 and 3). Similarly, the Oligochaeta *Nais bretscheri* and *N. elinguis* were dominant in both the Raba River (zones III, IV) and Dunajec River. *Nais barbata* was found in large numbers only in the Dunajec River below the sewage discharge from the small towns (sites 1 and 5), but in Raba and Vistula it was found sporadically. *Limnodrilus hoffmeisteri* (Tubificide) is a common species in various types of aquatic habitats, especially in those contaminated with sewages, as it feeds on organic-rich fine-grained particles [42]. In the Carpathian rivers, single specimens of *L. hoffmeisteri* were found.

Until the 1950s, the Upper Vistula River had similar benthic macroinvertebrate communities to the Raba River in the lower section. There were Ephemeroptera (*Ecdyonurus venosus* (Fabricius, 1775), *E. dispar* (Curtis, 1834), *E. insignis* (Eaton, 1870), *Serratella ignita*, *Baetis fuscatus*, *Caenis macrura* Stephens, 1835, *Heptagenia sulphurea* Müller, 1776, *H. flava* Rostock, 1878, *H. coerulans* Rostock, 1878, *Paraleptophlebia submarginata* (Stephens, 1835), and *Cloeon dipterum* (Linnaeus, 1761)), Porifera, Plecoptera, Trichoptera, and Odonata [31]. From the 1960s to the 1990s, the Upper Vistula between the Przemsza River mouth and the city of Kraków was found to be the most polluted section of this river [25,43]. The main sources of pollution were sewage from the Upper Silesian Industrial Region and saline waters from coal mines. According to the classification of water quality [40], the river water at all sites was of poor quality (below Class II) in terms of BOD5, N-NH_4, and P-tot as well as at sites 1 and 2 in term of dissolved oxygen content. High conductivity values (4295 µS cm^{-1}; [24]) indicated poor water quality (below Class II) with respect to water salinity. In this period (1983), the sediment of the Vistula River was also heavily polluted by heavy metals. The concentrations of metals at site 1 (fraction < 60 µm, in µg g^{-1}: Cd 138.1, Pb 665, Cu 500, Zn 5287, Ni 112, Cr 370; [44]) exceeded the severe effect level (SEL: Cd 10, Pb 250, Zn 820 µg g^{-1}; [45]); therefore, toxic effects of metals in the sediment on benthic macroinvertebrates were expected. Such high metal concentrations are typical for freshwater sediments in industrial areas or areas exposed to pollution from mining of metal ores [46–48].

Such severe pollution has completely changed the benthic invertebrate communities. Oligochaeta dominated, mainly *L. hoffmeisteri*, which is an indicator of polysaprobic waters and has a wide range of salinity tolerances [49]. Oligochaeta (as a whole) in various types of classifications are regarded as being the most tolerant to pollution [50,51]. However, in contaminated mountain and partially lowland rivers (Raba and Dunajec), the main dominant species were from the genus *Nais*, which are found in alpha- and beta-mesosaprobic waters [6,10], as well as in uncontaminated rivers. Species of the genus *Nais* mainly inhabit stones overgrown by algae, which serve as a hiding place and a nutrition source [17]. *Propappus volki* did not occur in the polluted Vistula River, although it dominated in the lower sections of the Raba River (sites 9–10). This species is typical to clean, well-oxygenated waters of lowland rivers with low concentrations of phosphates and organic matter, with sandy bottoms and strong or moderate flow [52,53].

Since the beginning of the 1990s, as a result of the reduction of heavy industries and mining in Silesia, a gradual decrease in salinity and ammonia in Vistula River water has been observed [49]. In 2014, at site 4, concentrations of K^+, Na^+, Cl^-, and SO_4^{2-} in the water were 1.6–2.6 times lower compared to 1983; Ephemeroptera (Beatidae, Caenidae) and Trichoptera were again found. Moreover, for the first time, Chironomidae were the most abundant group, where previously Oligochaeta dominated [49].

5. Conclusions

In the Carpathian Raba River, with low human activity, the diversity of benthic macroinvetebrates was high and the density relatively low. On the basis of Ephemeroptera communities, five river zones were distinguished. In the sub-mountain section of the Dunajec River, with a stony bottom, fast current, and good water oxygenation, an increase in contamination caused an increase in density and changes in the percentage of individual benthic macroinvertebrate groups. The increase in density of benthic macroinvertebrates was due to the massive growth of Oligochaeta. The diversity of Oligochaeta and Ephemeroptera species changed slightly and was similar to that in the Raba River. Conversely, in the heavily polluted lowland Vistula River, with a laminar flow and a silty-sand substrate, the density was high and caused the massive growth of Oligochaeta. *Limnodrilus hoffmeisteri* dominated, with this species commonly being recognized as the indicator of the polysaprobial zone. The other groups of benthic macroinvertebrates were found sporadically or became completely extinct, such as Ephemeroptera.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14091349/s1, Table S1: List of Ephemeroptera species in the Raba River in 1966–1967 [15], Dunajec River in 1992–1993 (sites 1–3) and 1999 (sites 4–5) [18,26] and Vistula River in 1982–1983 [27] (+ <1%; ++ 1–10%; +++ >10%; X—extinct species, present in the 1940's [31]). Table S2: List of Oligochaeta species in the Raba River in 1966–1967 (+ <100, ++ 100–1000, +++ >1000 specimens) [17] as well as in the Dunajec River in 1992–1993 (sites 1–3) and 1999 (sites 4–5) [18,26] and Vistula River in 1982–1983 [27] (+ <1%; ++ 1–10%; +++ >10%).

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