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Aquatic Worm Assemblages along the Danube: A Homogenization Warning

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Abstract: In this study, we analyzed the impacts of different environmental conditions on aquatic worm communities along the Danube River, based on two longitudinal surveys, the Joint Danube Surveys 2 and 3 (JDS; 2007 and 2013). We identified the most important environmental factors (among analyzed groups) that shape worm communities: hydromorphlogical alterations, flow velocity and substrate (HYMO group), dissolved oxygen, nitrates and nitrites (physico-chemical parameters), zinc and nickel (metals), monobutyltin cation, benzo(b) fluoranthene and benzo(k)fluoranthene, polychlorinated biphenyls PCB 77 and PCB 118 (selected chemical determinants—organotin compounds, Polycyclic aromatic hydrocarbons—PAHs and PCBs). A homogenization of species composition of Oligochaeta assemblages along the Danube was confirmed. As one of main factors related to biotic homogenization, hydromorphological alterations represented by similar changes in flow velocity and substrates along Danube's course could be singled out. Our results indicate that Oligochaeta could be used for the identification of the level of hydromorphological degradation in large rivers (homogenization), rather than for stressors classified as nutrient and organic pollutants. Our results provide additional evidence in risk assessment of the environment, contributing in water management and monitoring of the ecological status as proposed by the Water Framework Directive.

Keywords: Oligochaeta; large lowland rivers; longitudinal distribution; multiple stressors; pollution

1. Introduction

The Danube is a large water resource for more than 80 million people. As one of the longest rivers in Europe, it represents an important connecting factor for biodiversity conservation [1–4]. It is therefore essential to collect reliable and comparable data on the functioning of this river and to adequately translate the results from research to the management level. To this end, the examination of the relation between environmental parameters and structures of communities and the subsequent identification of the typology based on selected natural characteristics of water types must be undertaken. These activities will provide the foundation for effective water management and monitoring of the ecological status, as proposed by the Water Framework Directive (WFD) [5]. Grouping of similar rivers is a prerequisite for adhering to the river-type-specific approach, as defined in the WFD. Thus, the classification of river types as relatively homogeneous ecological systems is a prerequisite for an improved understanding and continual assessment of associated biological communities.

In the Danube, as in many European rivers, there are significant anthropogenic influences due to river flow regulation, navigation improvement, and in particular, as a result of river engineering. Many human-made alterations are responsible for changes in the cycling of matter and are responsible for qualitative and quantitative changes in the composition of biocenoses.

The nutrient loads and their consequences have been recognized as one of the most striking issues in the Danube catchment area, thus, comprehensive studies and projects were dedicated to this problem in recent decades [6–8]. The "nutrient pollution" was recognized as the second major cause that affects the risk of failure to achieve "good ecological status" of a high proportion of water bodies across the Danube River basin [8]. Based on the risk assessment approach used and based on the available data, in total 55% of the Danube River length and 49% of the Danube tributaries are "at risk" or "possibly at risk" due to nutrient pollution [9].

The Danube basin is characterized by large gradients of anthropogenic and natural indicators, which are important for affecting nutrient inputs into the river system. Agricultural activities are a main source for the diffuse nutrient emissions into the river system but data on land use and discharge into the Danube basin were not available from all Danube countries until comprehensive Joint Danube Surveys (JDS1, 2, and 3) were conducted.

The first Joint Danube Survey (JDS1) was carried out in 2001, providing for the first time comparable data about the entire course of the river which were used as an essential information source for the first analysis of the Danube River Basin according to WFD. The pollution of the river Danube by heavy metals, As, Cr, Cu, Pb, Hg, Ni, and Zn, was regarded as rather low. Elevated concentrations of Cd were found in the lower stretch of the river Danube beginning at the Iron Gate [10]. It was found that concentrations of these elements represent main constituents of sediments not subjected to anthropogenic changes. Lowest element concentrations were found in the Hungarian river stretch, while highest concentrations were determined in the Iron Gate Reservoir. Downstream concentrations of heavy metals remained stable with decreasing to the Danube Delta.

There is little information available, from previous investigations, on the occurrence of organic compounds and micropollutants in the sediment of the Danube. During JDS1, for the first time the identification of volatile organic hydrocarbons, polar pesticides, and pharmaceuticals provided information on direct pollution inputs and helped detect pollution hot-spots [6]. Navigation along the Danube is the main source of oil pollution. The polyaromatic hydrocarbons (PAHs) were determined as one of the most important group of Petroleumhydrocarbons with the highest values in sediment of the Middle Danube reach. The Lower Danuber was generally more contaminated than the Upper Danube. The contamination of the Danube with volatile organic hydrocarbons was very low. Only Atrazine of pesticides was found along the Danube in average concentrations with higher results in tributaries (the Sava River). Significant concentrations of harmful chemical pollutants (WFD List of Priority Pollutants) were found in bottom sediments with concentration up to more than 100 mg/kg in the Serbian section of the Danube caused by the use of alkylphenol-containing detergents in this region [6].

The second Joint Danube Survey (JDS2) has created a comprehensive and homogeneous database on the status of the aquatic ecosystem of the Danube and its major tributaries [7]. The survey confirmed a generally improving trend for water quality along the main Danube River and distinguished specific problems, in tributaries and downstream of large cities. Some of the specific objectives of third Joint Danube Survey (JDS3) [8] were identification and prioritization of specific substances, investigation of quality of sediments, and monitoring of priority substances.

As Titizer and Banning [2] stated, it has been over 40 years since the first comprehensive survey of the Danube and publication of the species list [1]. Changes in the structure of the macroinvertebrate community have been reported since then. Especially notable is the increase in the number of Oligochaeta species adapted to the organic load related to waste water inflow, as shown by previous investigations of the Danube and other large lowland rivers in Europe, where the highest diversity was displayed by the tubificins [11–16].

Many hydraulic constructions have been built on the Danube River and its tributaries, and about 70 reservoirs have been constructed by 1980 [17]. The upper course of the Danube (Germany and Austria) has more weirs than the middle course—44 impoundments from 2600 to 2000 Rkm and three more dams from 1950 Rkm downstream [2]. Many of these dams were built to improve the navigation conditions and were unable to regulate water flow, however, they contributed to a certain decrease in suspended sediment runoff [17]. Poff et al. [18] showed that natural flow regimes have been significantly reduced in regulated rivers, and that postdam homogenization is statistically significant, with a broad range of hydrology alterations caused by dams. The consequence of homogenization on aquatic systems is the replacement of regional biotas with cosmopolitan species [19].

Aquatic worms, as one of the main components of communities in large rivers, are dominated by widely distributed taxa. Therefore, the information collected from analyses of the Danube could be used for other large rivers, not only in Europe, but in Palearctic and Nearctic areas. The basic faunistic features of the oligochaetes assemblages and the distribution of Oligochaeta (Annelida) in the Danube River were discussed in Atanacković et al. [16], where it was pointed out that the structure of the bottom sediment is an important factor which influences species composition. The value of oligochaetes as indicators in Europe is well known and these organisms have been included in different monitoring programs [20–23]. In contrast to some other organisms such as Diptera, which are used in monitoring, oligochaetes are more suitable because they filter the mud. The sediment is not only a passive reservoir of different pollutants, but oligochaetes themselves through burial, substrate plowing, nutrition, and respiration, represent significant accumulators in aquatic ecosystems, especially as they play an important ecological role in sediments when occurring at high densities, as we recorded in the Danube. Pollutants indirectly become toxic to animals at a higher trophic level.

Further, hydrophobic organic micropollutants (such as organotins, PAHs, and PCBs) in aquatic ecosystems have increased towards the end of the 20th century, and several of these organic pollutants belong to the Water Framework Directive priority substances (EQS Directive, 2013/39/EU), and have hydrophobic properties. PAHs, PCBs, and organotin compounds are therefore usually associated with suspended particulate matter that settles in the bottom sediment, and an extensive database on aquatic oligochaetes has demonstrated their sensitivity to a range of chemicals (metals, pesticides, PAHs, etc.) [23].

The focus of this paper is on the relation of Oligochaeta assemblages and the available data on environmental variables, such as physico-chemical parameters and chemical determinants in water and sediment and the level of hydromorphological degradation. Since the information of concentrations of pollutants and the macroinvertebrate community have been published in the Joint Danube Survey Reports [7,8], the novelty of this paper is the detailed analysis of oligochaetes assemblages along the Danube River and the statistical analysis that was undertaken in order to find relationships between the chemical and hydromorphological environmental variables and biological data. The construction of dams and river regulation has led to the homogenization of habitats and thus to an increase in limno(rheo)philic taxa that prefer slow-flowing and lentic zones. As there is a lack of papers dealing with the homogenization (biotic and hydrological) of large European rivers, the present paper is a contribution to this field. We hypothesized that the Oligochaeta are suitable bioindicators of the deterioration of environmental conditions, and we recognized these organisms as typology descriptors (indicators for the homogenization of the river) for sectioning of the Danube.

2. Materials and Methods

2.1. Study Area and Selection of Sampling Points

Oligochaeta assemblages were collected in the frame of the Joint Danube Surveys JDS2 [7] and JDS3 [8], during 2007 and 2013, respectively. More than 2580 km of the Danube were investigated, including 96 sites during the JDS2, and 68 sites during the JDS3 (Figure 1).

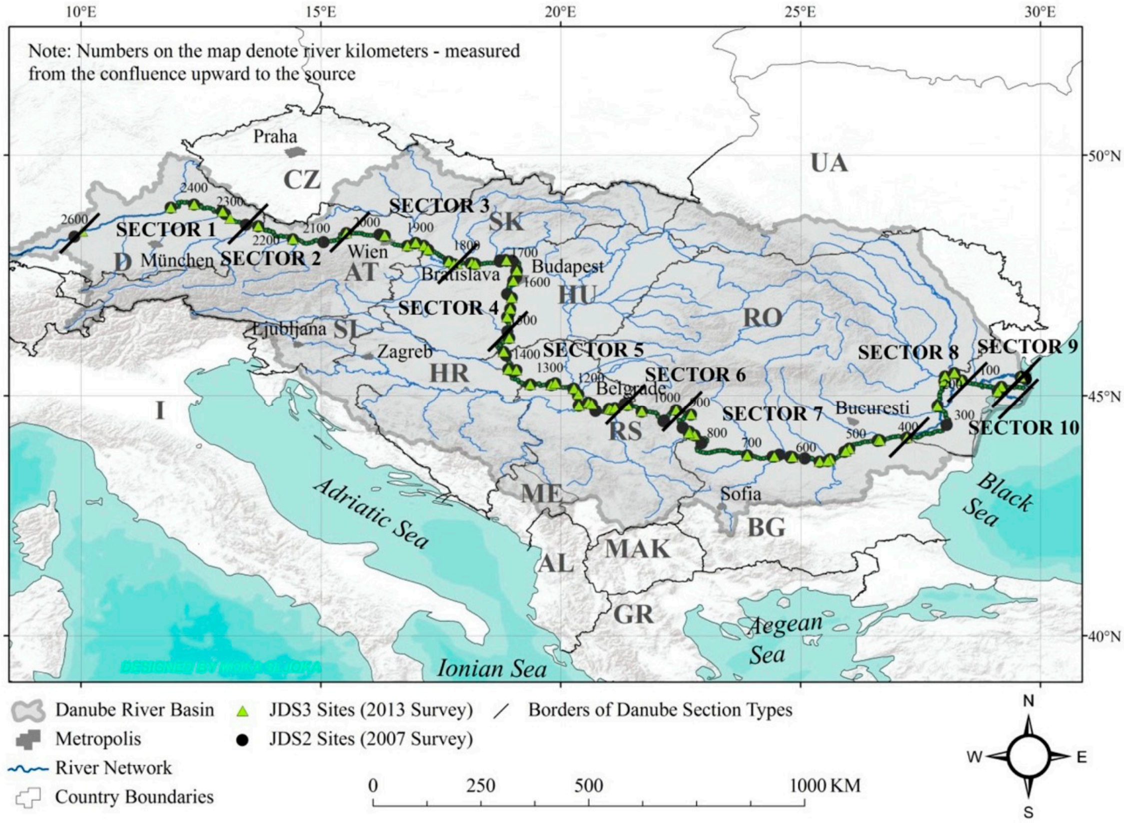


Figure 1. Sampling sites and sectors along the Danube during Joint Danube Surveys JDS2 and JDS3.

While keeping in mind the size of the watercourse and the significant number of analyzed parameters, an a priori classification of sectors was applied [24], which implies division of the river into 10, biologically meaningful section types, as follows: Sector 1—Upper Course of the Danube (sampling site 1 during JDS2); Sector 2—Western Alpine Foothills Danube (sites 2–5); Sector 3—Eastern Alpine Foothills Danube (sites 6–10; 6–8); Sector 4—Lower Alpine Foothills Danube (sites 11–19; 9–18); Sector 5—Hungarian Danube Bend (sites 20–37; 19–27); Sector 6—Pannonian Plain Danube (sites 38–58; 28–42); Sector 7—Iron Gate Danube (sites 59–62; 43–45); Sector 8—Western Pontic Danube (sites 63–86; 46–60); Sector 9—Eastern Wallachian Danube (sites 87–93; 61–65); Sector 10—Danube Delta (sites 94–96; 66–68) (Figure 1).

A discussion regarding the selection of sampling sites was presented in Liška et al. [7,8].

The overall descriptions of the hydromorphology during the surveys have remained comparable/essentially unchanged. The channel patterns have been significantly altered along the entire Danube by navigation and hydroelectric power plants, with only a very few sections retaining natural banks (in 6.45% of sites). The banks of the Danube have been entirely transformed in urban areas.

A significant stretch of the Danube (21.28% of the river course) is affected by hydromorphological changes (backwater/impoundment), especially in the upper reach in Germany and Austria [7,8]. About 77% of the river course belongs to a good hydromorphological class (free flowing). One percent of the investigated sites (Sector 1) exhibit a significantly reduced water flow. Studies during JDS3 [8] showed that 21% of the analyzed reach was slightly modified, 39% was moderately modified, and 40% fell into extensively and severely modified sites, while "near natural" sites could not be found at all.

The assessment results from the JDS3 have confirmed the main findings of the JDS2 in 2007. The bed material of the investigated sampling sites with strong impoundments is mainly composed of fine sediment. Fine, medium, and coarse gravel is present with a participation of 31.92%, while only 6% of the riverbed is covered with bedrock. These sites, which are mostly downstream of dams, are missing fine fractions. Some areas (9.57% of sites) have a significant amount of organic matter. In certain areas, the riverbed is covered by macrophyte vegetation (about 34% of the riverbed in the littoral zone). Significant changes in river processes (erosion/deposition) that are caused by various degrees of hydromorphological degradation along the Danube were observed, such as the decrease of suspended sediment concentration along the impounded sections, deposition areas upstream of the barrier, and the absence of sediments in the downstream direction, especially a deficit of fine sediments downstream of the Iron Gate [8].

2.2. Data Collection

The employed sampling methodology is explained in detail in Liška et al. [7,8]. The biological component was collected, identified, and analyzed by the authors. The following sampling methods during the surveys were used: air-lift samples were used in the study of the faunal composition of deep water habitats and a modified Multi-Habitat-Sampling (MHS) approach [25] was performed to highlight the importance of specific micro-habitats. A combination of techniques was used in both surveys: kick and sweep multihabitat sampling or K&S (FBA hand net, mesh size 500 μm) [26] and dredging.

The following environmental factors were analyzed during the surveys JDS2 and JDS3: hydrological alterations, flow velocity, substrate type, and bank modifications (HYMO group). The physical and chemical parameters were analyzed too. Data on physical and chemical parameters from both surveys are available in the ICPDR Water Quality Database (http://www.icpdr.org/wq-db/). We used information from the database for common parameters such as water temperature, pH, dissolved oxygen, conductivity, ammonia (NH_4^+), nitrites (NO_2^-), nitrates (NO_3^-), orthophosphates (PO_4^-) together with the concentrations of WFD priority substances: diethylhexyl phthalate (DEHP), PAHs, PCBs, organotin compounds, dioxins and dioxin-like compounds (polychlorinated dibenzo-p-dioxins—PCDDs, polychlorinated dibenzofurans—PCDFs, and dioxin-like polychlorinated biphenyls—PCB-DL), and metals (copper (Cu), arsenic (As), cadmium (Cd), chromium (Cr), nickel (Ni), and lead (Pb)) in the sediments. The measured concentrations of heavy metals were estimated in relation to target values evaluated for heavy metals [27]. It should be mentioned that there are currently no commonly accepted limit values for chemical pollutants in sediments in the European water legislation [1].

2.3. Data Analyses

The frequency of occurrence (F) for each species in oligochaetes assemblages was calculated using the formula: F = n/N, where n is the number of samples in which a taxon was found, and N is the total number of samples.

The percentage participation of species for each sector was calculated and is presented in Tables 1 and 2. Taxa that were not identified up to the species level are not shown in the tables.

In order to determine the species variability in different sectors along the Danube, the preliminary discriminant analysis (DA) [28,29] was applied. This analysis was used on species data to check whether specific sectors could be identified. An input matrix consists of 66 Oligochaeta taxa and 590 samples (collected using K&S, airlift and dredging, with 183 and 407 samples gathered during the JDS2 and JDS3, respectively). As the first step, standardization of the raw species data was performed (species percentages per sample/relative abundance) to avoid inconsistencies of different data formats obtained by different sampling techniques.

Table 1. Oligochaeta distribution recorded during JDS2. The distribution of Oligochaeta taxa along Danube sectors based on the percentage participation (the percentage of species in each sector) with a total frequency (F) in the Danube. The abbreviations/code for the names of taxa used in further analyses are provided in a separate column.

JDS2												
The Percentage Participation of Oligochaetes (%)												
Taksa	**Code**	**F**	**Sector 1**	**Sector 2**	**Sector 3**	**Sector 4**	**Sector 5**	**Sector 6**	**Sector 7**	**Sector 8**	**Sector 9**	**Sector 10**
Naidinae												
Dero obtusa d'Udekem, 1835	Dob	0.05					0.46		1.75	0.08		
Nais alpina Sperber, 1948	Nal	0.01					0.01					
Nais bretscheri Michaelsen, 1899	Nbr	0.02				1.47		0.06				
Nais christinae Kasprzak, 1973	Nch	0.01								0.09		
Nais communis Piguet, 1906	Nco	0.02						0.05	2.04			
Nais pardalis Piguet, 1906	Npa	0.02					0.18			1.19		
Nais pseudobtusa Piguet, 1906	Nps	0.01					0.04					
Nais simplex Piguet, 1906	Nsi	0.01						0.11				
Ophiodonais serpentina (Müller, 1773)	Ose	0.05				0.12	0.02		2.64	1.56		
Piguetiella blanci (Piguet, 1906)	Pbl	0.01			0.02							
Stylaria lacustris (Linnaeus, 1767)	Sla	0.04	9.46				1.07		1.15	1.56		
Tubificinae												
Aulodrilus japonicus Yamaguchi, 1953	Aja	0.02			0.02						12.98	
Aulodrilus pluriseta (Piguet, 1906)	Apl	0.05		0.79	0.27		0.54				0.02	
Aulodrilus limnobius Bretscher, 1899	Ali	0.05			3.11			0.87		0.74		
Bothrioneurum vejdovskyanum Štolc, 1888	Bve	0.01								0.13		
Branchiura sowerbiy Beddard, 1892	Bso	0.27				0.07	1.19	10.33	16.44	7.41		8.33
Embolocephalus velutinus (Grube, 1879)	Eve	0.03							0.55	0.45		0.13
Isochaetides michaelseni (Lastočkin, 1936)	Imi	0.47			2.41	0.51	0.90	19.30		24.75	17.23	29.86
Limnodrilus claparedeanus Ratzel, 1868	Lcl	0.41		0.79	18.18	10.65	15.93	16.49	14.88	8.99	2.50	10.73
Limnodrilus hoffmeisteri Claparède, 1862	Lho	0.59		23.35	29.23	7.98	7.42	11.69	18.45	3.23	2.52	11.24
Limnodrilus profundicola (Verrill, 1871)	Lpr	0.31		3.97	4.20	1.03	1.72	1.57	2.38	2.07	0.02	7.31
Limnodrilus udekemianus Claparède, 1862	Lud	0.37			0.53	0.71	4.45	5.82	1.64	2.34	14.23	8.33
Potamothrix bavaricus Oschmann, 1913)	Pba	0.01	2.74									
Potamothrix danubialis (Hrabě, 1941)	Pda	0.18			0.54	0.23	3.62	0.30	1.40	1.30	2.50	
Potamothrix hammoniensis (Michaelsen, 1901)	Pha	0.08			0.15		0.03	0.29	2.51			
Potamothrix isochaetus (Hrabě, 1941)	Pis	0.16			0.70	1.05	2.21	1.80	4.08	1.56		
Potamothrix moldaviensis Vejdovsky and Mrázek, 1902	Pmo	0.38		11.36	10.10	8.61	10.79	6.23		5.51	0.65	2.08
Potamothrix vejdovskyi (Hrabě, 1941)	Pve	0.12			1.35	4.15	0.54	0.23	5.05	0.57	0.62	
Psammoryctides albicola (Michaelsen, 1901)	Psl	0.22	1.24	0.40	0.35	1.84	1.64	2.33		1.64		
Psammoryctides barbatus (Grube, 1861)	Psb	0.38	1.65	5.24	4.81	6.11	3.91	3.26	4.08	0.94	2.81	
Psammoryctides moravicus (Hrabě, 1934)	Psm	0.16				0.41	0.55	0.32		1.51	1.63	

Table 1. *Cont.*

JDS2												
The Percentage Participation of Oligochaetes (%)												
Taksa	**Code**	**F**	**Sector 1**	**Sector 2**	**Sector 3**	**Sector 4**	**Sector 5**	**Sector 6**	**Sector 7**	**Sector 8**	**Sector 9**	**Sector 10**
Rhyacodrilus coccineus (Vejdovský, 1875)	Rco	0.01	22.26									
Tubifex ignotus (Štolc, 1886)	Tig	0.02				0.28	0.13					
Tubifex tubifex (Müller, 1774)	Ttu	0.22			0.56	4.74	0.47	0.94		3.73		
Enchytraeidae												
Enchytraeidae Gen. sp.		0.03			0.22	6.39						
Propappidae												
Propappus volki (Michaelsen, 1916)	Pvo	0.06		0.38		0.45				8.15		
Lumbriculidae												
Lumbriculus variegatus (Müller, 1774)	Lva	0.01	0.34									
Rhynchelmis limosella Hoffmeister, 1843	Rli	0.01	1.03									
Stylodrilus lemani Grube, 1879	Sle	0.01	0.68									
Stylodrilus heringianus Claparède, 1862	She	0.29	19.85	34.95	11.27	24.00	22.21	1.85		1.56		
Lumbricidae												
Eiseniella tetraedra (Savigny, 1826)	Eta	0.14	7.69	0.44		0.62	0.03	0.04		0.02		6.18
Criodrilidae												
Criodrilus lacuum Hoffmeister, 1845	Cla	0.35		1.59		0.18	1.23	0.77	1.10	2.07	14.33	1.22
Haplotaxidae												
Haplotaxis gordioides (Hartmann, 1821)	Hgo	0.05					1.68					
Number of taxa			**11**	**12**	**20**	**23**	**28**	**22**	**16**	**28**	**13**	**10**

Table 2. Oligochaeta distribution recorded during JDS3. The distribution of Oligochaeta taxa along Danube sectors based on the percentage participation (% of species in each sector) with a total frequency (F) in the Danube. The abbreviations/code for the names of taxa used in further analyses are provided in a separate column.

JDS3												
The Percentage Participation of Oligochaetes (%)												
Taxa	**Code**	**F**	**Sector 1**	**Sector 2**	**Sector 3**	**Sector 4**	**Sector 5**	**Sector 6**	**Sector 7**	**Sector 8**	**Sector 9**	**Sector 10**
Naididae												
Dero digitata Müller, 1773	Ddi	1.79						0.14		1.21	1.70	
Dero obtusa d'Udekem, 1835	Dob	1.52					0.38	0.01	0.05	0.32		
Nais alpina Sperber, 1948	Nal	0.28		0.02	0.36							
Nais barbata Müller, 1773	Nba	1.10		1.09	1.62	0.45	0.01		0.06			
Nais bretscheri Michaelsen, 1899	Nbr	6.76		0.59	1.88	4.99	0.28	0.03	0.03	1.03	0.13	
Nais christinae Kasprzak, 1973	Nch	4.28		0.04	2.52		0.12	0.02	0.40	4.53		
Nais communis Piguet, 1906	Nco	0.69						0.01		0.30		
Nais elinguis Müller, 1774	Nel	0.14				0.01						
Nais pardalis Piguet, 1906	Npa	3.03		0.63	4.95		0.15	0.02	0.15	0.63		
Ophiodonais serpentina (Müller, 1773)	Ose	2.76		0.12				0.05	12.93	1.49		
Paranais frici Hrabě, 1941	Pfr	0.14			0.46							
Piguetiella blanci (Piguet, 1906)	Pbl	0.14			4.09							
Specaria josinae (Vejdovksy, 1883)	Sjo	2.48				0.06	0.02	0.11	1.41	0.59	4.69	
Stylaria lacustris (Linnaeus, 1767)	Sla	7.03		0.89		1.37	0.03	0.54	9.30	5.05	0.11	
Uncinais uncinata (Orsted, 1842)	Uun	0.55							0.15			
Pristinidae												
Pristina aequiseta	Pae	0.28								0.21		
Pristina longiseta Ehrenberg, 1828	Plo	0.14										
Pristina rosea (Piguet, 1906)	Pro	0.14								0.29		
Tubificidae												
Aulodrilus japonicus Yamaguchi, 1953	Aja	1.24						0.10		0.03	0.14	0.26
Aulodrilus pluriseta (Piguet, 1906)	Apl	0.28		0.17								
Bothrioneurum vejdovskyanum Štolc, 1888	Bve	0.55		0.01				0.01	0.05		0.01	
Branchiura sowerbyi Beddard, 1892	Bso	6.07		0.03	0.04		0.02	0.92	0.50	0.07	0.10	1.59
Embolocephalus velutinus (Grube, 1879)	Eve	0.97					0.03		0.48	0.04		
Isochaetides michaelseni (Lastočkin, 1936)	Imi	16.55		0.10	0.57	0.42	5.67	1.36	0.03	27.42	50.64	1.22
Haber speciosus (Hrabě, 1931)	Hsp	0.41							0.53	0.59		
Limnodrilus claparedeanus Ratzel, 1868	Lcl	13.26		0.15	1.49	1.78	4.48	43.77	28.34	20.26	16.10	40.35
Limnodrilus hoffmeisteri Claparède, 1862	Lho	25.66		3.44	68.51	24.50	16.28	28.84	11.17	18.77	5.53	11.10
Limnodrilus profundicola (Verrill, 1871)	Lpr	0.69					0.62	6.22				
Limnodrilus udekemianus Claparède, 1862	Lud	7.59		0.02	0.15	0.52	0.47	6.67	0.12	0.03	0.25	0.78
Potamothrix bavaricus Oschmann, 1913)	Pba	0.14								0.01		
Potamothrix danubialis (Hrabě, 1941)	Pda	4.55			0.98		1.74	2.47		2.50	13.43	19.22

Table 2. *Cont.*

JDS3												
The Percentage Participation of Oligochaetes (%)												
Taxa	**Code**	**F**	**Sector 1**	**Sector 2**	**Sector 3**	**Sector 4**	**Sector 5**	**Sector 6**	**Sector 7**	**Sector 8**	**Sector 9**	**Sector 10**
Potamothrix hammoniensis (Michaelsen, 1901)	Pha	4.83		0.02	0.26	1.07	0.37	0.34	0.06	0.03		0.48
Potamothrix moldaviensis Vejdovsky and Mrázek, 1902	Pmo	13.39		54.70	0.65	6.57	40.38	2.39	7.33	3.21	3.85	16.39
Potamothrix vejdovskyi (Hrabě, 1941)	Pve	7.45		0.65	2.55	1.29	4.06	0.08	5.02	0.68		
Psammoryctides albicola (Michaelsen, 1901)	Psl	1.10			0.01		0.01	0.02		0.01		0.01
Psammoryctides barbatus (Grube, 1861)	Psb	10.62		4.20	2.54	1.24	1.45	0.11	1.88	1.25	0.03	0.01
Psammoryctides moravicus (Hrabě, 1934)	Psm	0.41								0.01		
Spirosperma ferox	Sfe	0.14		0.02								
Tubifex ignotus (Štolc, 1886)	Tig	0.83		0.19			0.22					
Tubifex newaensis (Michaelsen, 1903)	Tne	0.28								0.01		0.01
Tubifex tubifex (Müller, 1774)	Ttu	3.86		0.14			2.28	0.76	0.68	0.08	0.59	0.02
Enchytraeidae												
Enchytraeus sp.	Enc sp	0.14			0.01							
Henlea ventriculosa (d'Udekem, 1854)	Hve	0.14			0.11							
Propappidae												
Propappus volki (Michaelsen, 1916)	Pvo	0.97		0.43		0.02	0.01	0.01		0.03		
Lumbriculidae	Lrl											
Lumbriculus variegatus (Müller, 1774)	Lva	0.14										
Stylodrilus brachystylus Hrabě, 1929	Sbr	0.14							0.15			
Stylodrilus lemani Grube, 1879	Sle	0.55							0.77	0.03		
Stylodrilus heringianus Claparède, 1862	She	11.59		18.19	0.33	46.74	8.76	0.01	0.02	0.01		
Rhynchelmis limosella Hoffmeister, 1843	Rli	0.28		0.01		0.04		0.01				
Lumbricidae												
Eiseniella tetraedra (Savigny, 1826)	Ete	2.76		0.17		0.30	0.05			0.08		0.01
Criodrilidae												
Criodrilus lacuum Hoffmeister, 1845	Cla	1.10		0.06			0.13			0.01		
Number of taxa				**27**	**21**	**18**	**27**	**27**	**25**	**34**	**15**	**15**

Canonical correspondence analysis (CCA) [30] served to determine the relation between species distribution and environmental variables (six groups in total: hydromorphological alterations, physico-chemical parameters in water samples, and metal concentrations as well as organotins, PAHs, and PCBs in sediments). After preliminary testing of the analyzed variables, ammonium and dioxins (with a variance close to zero) were excluded from further analyses. The significance of CCA, with all environmental variables included, was tested to check the applicability of forward selection (FS) [31] of the environmental variables. The obtained results for the R correlation test were considered significant at $p < 0.05$. Forward selection was used to select environmental variables that explained most of the variations observed in the primary taxa matrix. The variables were processed by an automatic FS with a Monte Carlo permutation test [32] by using "Flora" Software (Version 2011) [33]. The same software was used to perform DA and CCA.

3. Results

3.1. Environmental Data

During both surveys the water temperatures varied from 16.9 to 26.6 °C.

Values of pH showed a slight increase in the upper section of the Danube (i.e., a slightly more alkaline environment) but as Liška et al. [8] observed, the pH and dissolved oxygen content demonstrated an overall good balance between primary production and decomposition of organic matter. Concerning the dissolved oxygen content, the longitudinal profile accompanied the pH profile with an increase in the upper Danube. Conductivity was relatively constant along the main watercourse, exhibiting several distinctive profiles, such as a significant decrease in the measured values in the Upper Danube during 2013 (from 566 µS/cm at Böfinger Halde, to 320 µS/cm at Oberloiben).

Along the river course, a markedly declining profile for ammonium was obtained during both years of investigation, excluding a few specific peaks in the upper course. In the majority of sampling sites, the concentration of ammonium was below the limit of detection. The spatial variations in nitrite and nitrate concentrations displayed a significant decrease profile in the upper course of the Danube. This was also observed along the middle stretch, while downstream, from the Iron Gate dams, a nitrate increase was determined (similar to the ammonium, nitrite, and dissolved oxygen).

Orthophosphate exhibited high spatial variability along the river. Decreasing concentration was noted in the upper course. In the middle course, we obtained a slightly ascending profile, especially in the backwaters of the Iron Gates reservoir, whereas in the lower course, an increase was observed.

According to Liška et al. [8], the total nitrogen and phosphorous contents were comparable in the two JDSs.

Significant variations in cadmium contents were observed downstream of the two major tributaries, Tisa and Sava. At 75.54% of sites, the concentration value was greater than 0.8 mg/kg (the standard value for the concentrations in sediment [27]). In the case of lead, the longitudinal profile indicated low concentrations along the Upper Danube, and high variation downstream from the confluence of the Tisa and Sava rivers. The sediment standard was exceeded (85 mg/kg) in 21.08% of the samples. During the JDS2 the concentration of nickel in bottom sediment at most sampling sites (86.41%) exceeded the 50 mg/kg (standard value for the concentration in sediment) and significantly increased downstream from the confluence of the Tisa and Sava rivers. Most of the results for arsenic were between 50 and 100 mg/kg, however, a very high concentration of 432.27 mg/kg was measured at sampling site JDS 60 (Iron Gate reservoir). The overall distributions of concentrations of copper, chromium, and zinc were similar to that of nickel.

In general, the concentrations of metals in the bottom sediments determined during the JDS3 were similar to those observed during the JDS2 [7]. The longitudinal concentration profiles for most metals in sediments of JDS3 declined compared to the JDS2 (especially for Cu, Ni, and Zn).

DEHP was present in most sediment samples, with a maximum concentration of 26 mg/kg at sampling site JDS 9 (Klosterneuburg). The sediment downstream from the confluence of the Velika

Morava was contaminated with 16.7 mg/kg DEHP. In JDS3, the concentrations of DEHP were higher in the middle part of the Danube compared to the observations during the JDS2. The presence of PAHs in the sediment was observed in more than 50% of sites, the maximum values ranging between 57 and 489 μg/kg.

Monobutyltin, dibutyltin, and triphenyltin were the most abundant organotin compounds in the sediment samples detected in both longitudinal surveys with similar concentrations. The different dioxins and dioxin-like compounds are converted into toxic equivalents (according to WHO 2005 Toxic Equivalence Factors—TEF) and summed up. Most PCDDs and PCDFs were quantified in all samples. These compounds, analyzed during the JDS3 survey, can be considered as emerging substances in sediment samples [8]. Comparison of the concentrations in the sediment revealed results similar to those obtained during the JDS2.

Due to the large amount of data collected during the surveys, the concentrations for most important environmental parameters are presented in Figure 2.

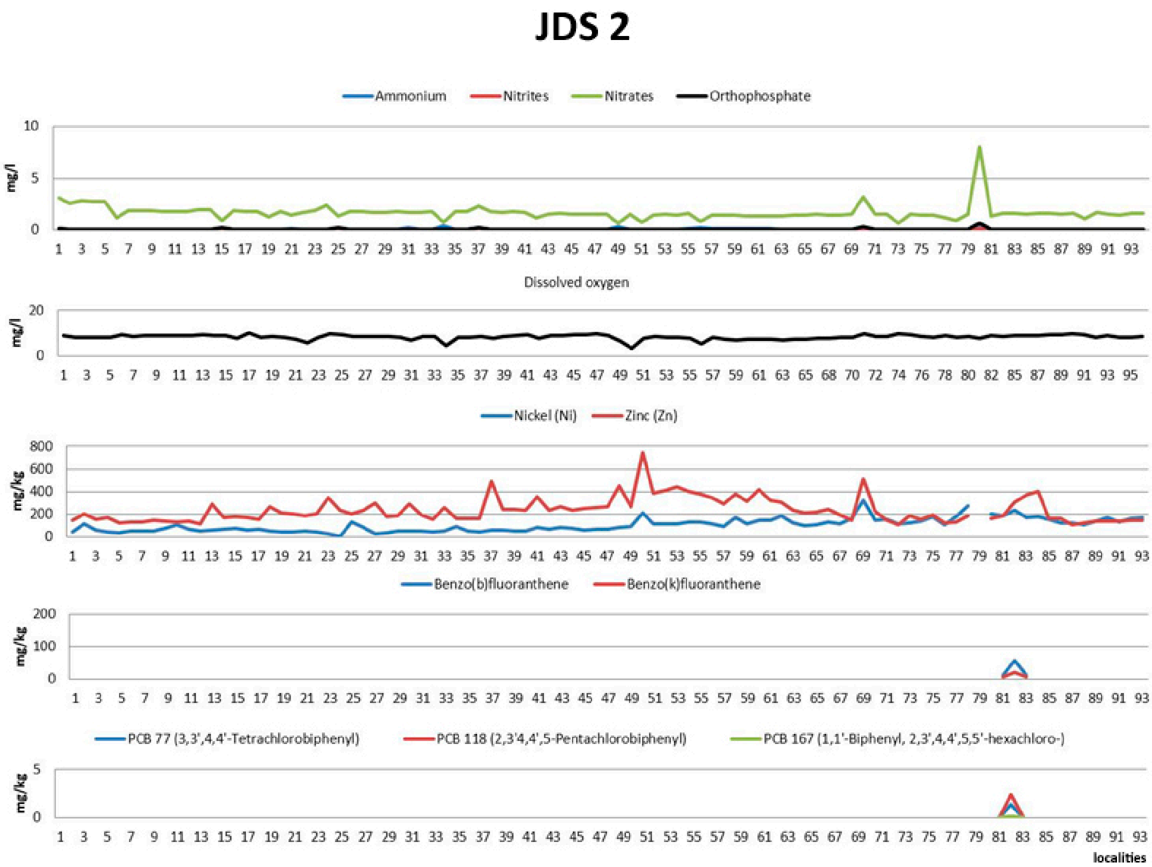


Figure 2. *Cont.*

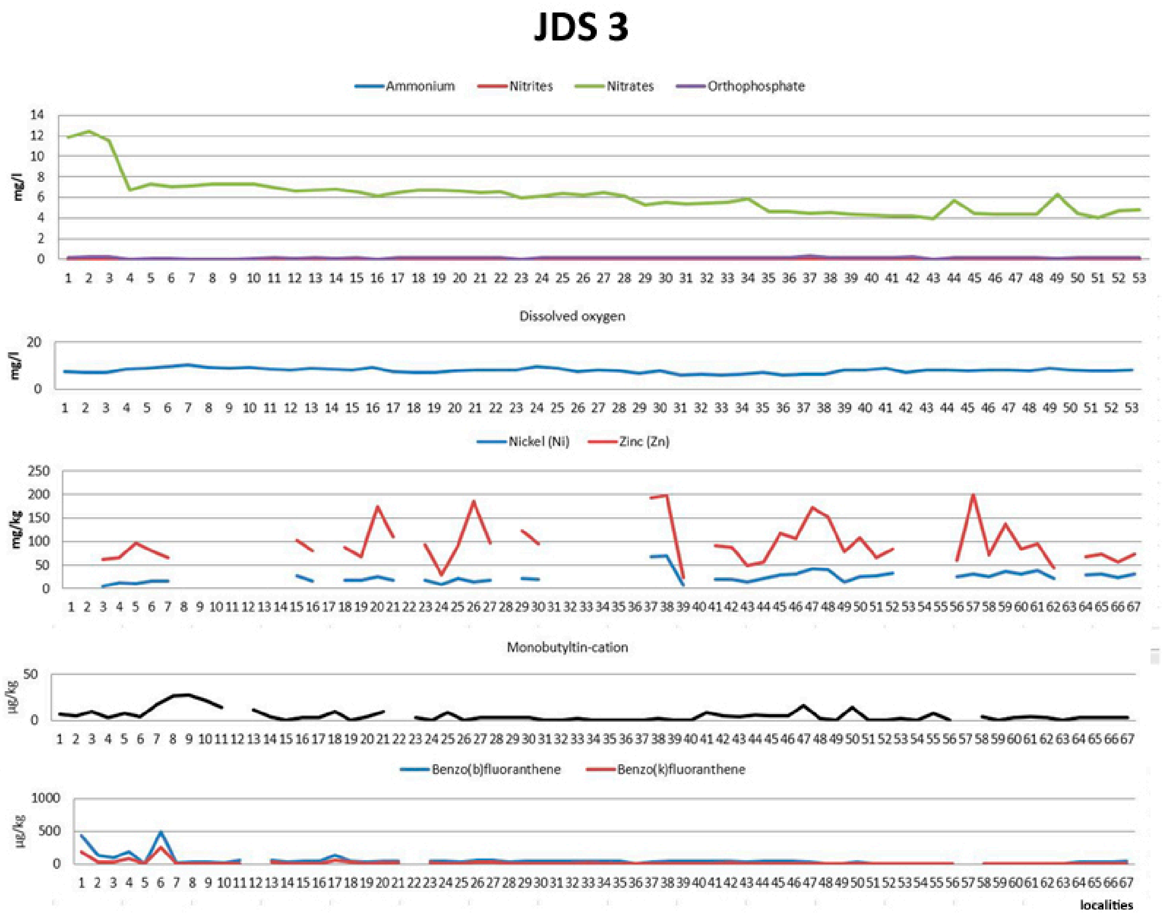


Figure 2. Concentration (*y*-axis) of the most important physical and chemical parameters along the Danube (*x*-axis).

3.2. Fauna

The distribution of Oligochaeta taxa along the Danube during JDS2 and JDS3 is presented in Tables 1 and 2, respectively. Oligochaeta with 66 recorded taxa, represent a significant component of the macroinvertebrate community of the Danube. The species richness was similar during both the JDS2 and JDS3.

In total, 43 taxa were recorded during JDS2, and 51 taxa were recorded during JDS3. In 2013, 15 new taxa were recoreded in oligochaetes assemblages (*Dero digitata*, *Nais barbata*, *Nais elinguis*, *Paranais frici*, *Pristina aequiseta*, *Pristina longiseta*, *Pristina rosea*, *Specaria josinae*, *Uncinais uncinata*, *Haber speciosus*, *Spirosperma ferox*, *Henlea ventriculosa*, *Stylodrilus brachystylus*, *Tubifex newaensis*, and *Enchytraeus* sp.), whereas six species were not observed (*Nais simplex*, *Nais pseudobtusa*, *Aulodrilus limnobius*, *Potamothrix isochaetus*, *Rhyacodrilus coccineus*, and *Haplotaxis gordioides*).

The most diverse family was Naididae, with 26 species of subfamily Tubificinae and 17 species of subfamily Naidinae, followed by families Lumbriculidae (five taxa) and Enchytraeidae (two taxa). The families Propappidae, Haplotaxidae, and Criodrilidae were represented by one species each, which is in accordance with their distribution in Europe, while the family Lumbricidae was not identified to the species level, except *Eiseniella tetraedra*.

During the JDS2, the highest number of aquatic worm taxa was recorded in the middle stretch of the Danube, the Hungarian Danube Bend, and the Western Pontic Danube (sectors 5 and 8, respectively). During the JDS3, the diversity of oligochaetes assemblages was uniform along the entire stretch. Lower numbers of species were in the upper course of the Danube (JDS2) and the Danube Delta (both JDSs).

The most widespread species was *Limnodrilus hoffmeisteri* (Tubificidae), exhibiting the highest frequency of occurrence during both surveys. Other tubificid species *Isochaetides michaelseni*, *L. claparedeanus*, *Potamothrix moldaviensis*, and *Psammoryctides barbatus* were frequent as well. Besides, *Criodrilus lacuum* (F = 35.00 in 2007) and *Stylodrilus heringianus* (F = 11.59 in 2013) stood out with a high frequency of occurrence.

3.3. Multivariate Analysis

During the first analysis stage, estimation of the DA for the community data (relative abundance) was performed in order to define the main community patterns along the longitudinal profile of the Danube. Based on the performed analysis, (DA diagram, Figure 3), the majority of the defined sectors [24] could not be separated, but a distinction between the samples from the lower Danube (sectors 7–10) and samples from all other sectors (upper and middle Danube) was observed.

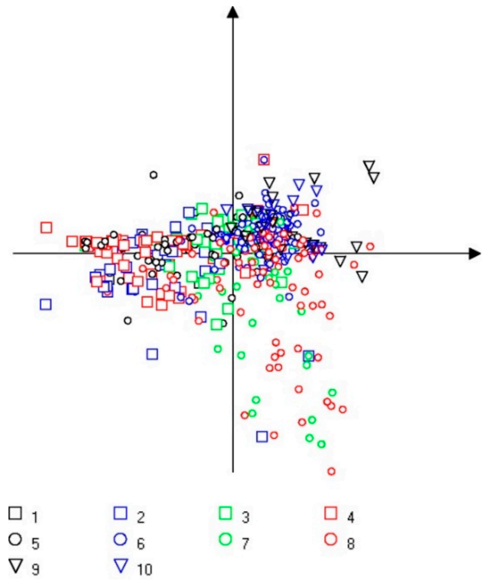


Figure 3. DA biplot based on Oligochaeta taxa from samples along the Danube River (input matrix 66 taxa × 590 samples). The bivariate space of the first two axes covers 61.2% of the total data variability (DA axis 1–44.8%, DA axis 2–16.4%). Defined sectors of the river are numbered (1–10) as in Figure 1.

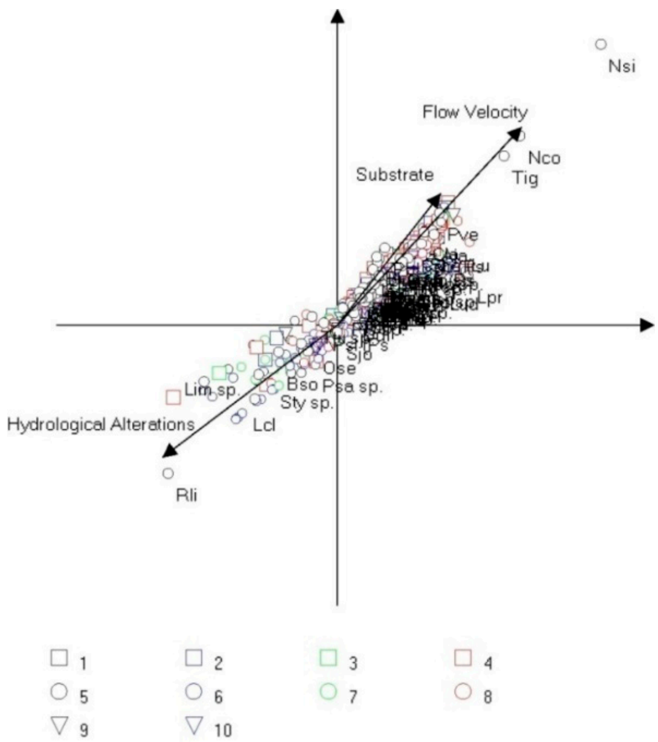
Especially, high heterogeneity between samples collected from the Hungarian and Serbian (sectors 5 and 6) and German stretch (sector 2) of the Danube was observed. Prominent species from the Upper Danube were *S. heringianus*, *E. tetraedra*, *N. bretscheri*, Lumbriculidae, *P. moldaviensis*, *P. barbatus*, and for the lower Danube: *I. michaelseni*, *N. christinae*, *O. serpentina*, *B. sowerbyi*.

CCA was used to analyze complex multivariate interactions between oligochaetes and the selected environmental variables. To assess whether the taxa matrix could be elucidated by the environmental matrix, a preliminary CCA (correlation coefficient (R) at $p < 0.05$) was performed for each group of environmental variables. Forward selection showed that the most important environmental variables that determine the taxa matrix/species distribution (from each environmental group) were as follows: hydrological alterations, flow velocity and substrate (HYMO group), dissolved oxygen, nitrates and nitrites (physico-chemical parameters), zinc and nickel (metals), monobutyltin cation (organotins group), benzo(b)fluoranthene and benzo(k)fluoranthene (PAHs), and PCB 77 and 118 (PCBs).

After CCA analysis of the selected environmental variables and taxa data, we obtained insight into the major environmental gradients. The results regarding the relations of the HYMO group and the Oligochaeta assemblages are presented in Figure 4a. The negative correlation between hydrological alterations on the one hand, and flow velocity and substrate on the other (more prominent alterations, lower flow and finer substrate, and vice versa) should be noted. Hydrological alterations were identified as the most important environmental variable overall, particularly along the first CCA axis; it was the main explainable variation in the taxa matrix/distribution. The vast majority of taxa seem to be unresponsive to these factors (the large taxa group situated near the coordinate center of the CCA diagram). Species that tolerate faster flow and a larger substrate (with more detritus/organic components) are on the right side of the diagram: *N. simplex*, *N. communis*, *T. ignotus*, and *P. vejdovskyi*. On the other hand, *R. limosela*, *Limnodrilus* sp., *L. claparedeanus*, *Stylodrilus* sp., and *B. sowerbyi* are taxa associated with more prominent hydrological alterations. Analysis of the relation of oligochaetes assemblages and these factors revealed that species that exhibit significant differences were the most numerous, as expected.

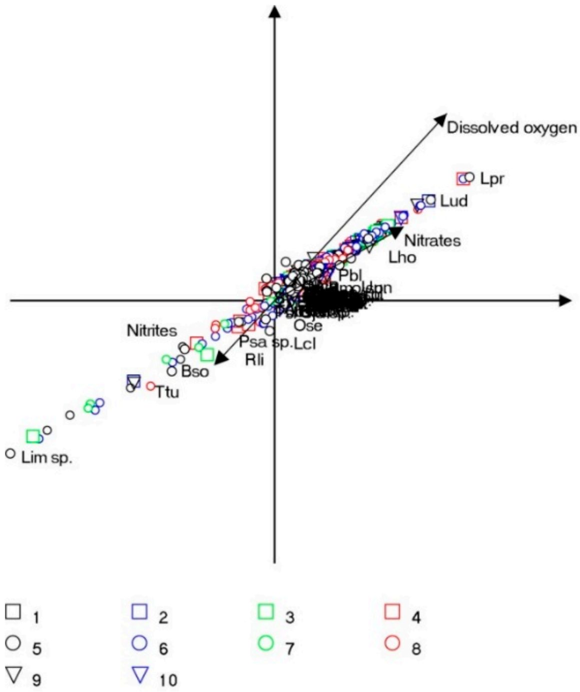
The impact of the main physico-chemical characteristic of the water on oligochaetes assemblages along the Danube is presented in Figure 4b. Dissolved oxygen was found to be the most important factor, and it is positively correlated with nitrates, whereas a negative correlation was noted with nitrites. Oligochaeta that favor more oxygenated water and tolerate higher concentrations of nitrates were *L. profundicola*, *L. udekemianus*, and *L. hoffmeisteri*. On the other side of the diagram, *Limnodrilus* sp., *T. tubifex*, and *B. sowerbyi* were associated with increased concentrations of nitrites and were more tolerant to lower concentrations of dissolved oxygen.

Analysis of the relations of metals in sediments and Oligochaeta revealed that zinc and nickel were the most important environmental variables. Oligochaeta assemblages in the multivariate space defined by selected factors are shown in Figure 4c. High concentrations of these metals were recorded during the JDS2 in the majority of sample sites belonging to sectors 6–10 (values over 400 and 100 mg/kg, respectively). The highest values for zinc and nickel were obtained at site 51, the confluence of the Sava River (745 and 210 mg/kg, respectively). Zinc and nickel are negatively correlated with the first CCA axis (69% of explained variance). The distinctive sample on the diagram was collected at Upstream Cernavoda (sector 9). Oligochaeta taxa tolerant to increased concentrations of these metals are as follows: *A. japonicus*, *P. volki*, *A. limnobius*, *N. simplex*, *N. communis*, *P. albicola*, and *P. isochaetus*. Among them *A. japonicus*, *A. limnobius*, and *P. volki* tolerated nickel (CCA axis 2), while *N. simplex*, *N. communis*, and *P. albicola* tolerated zinc.

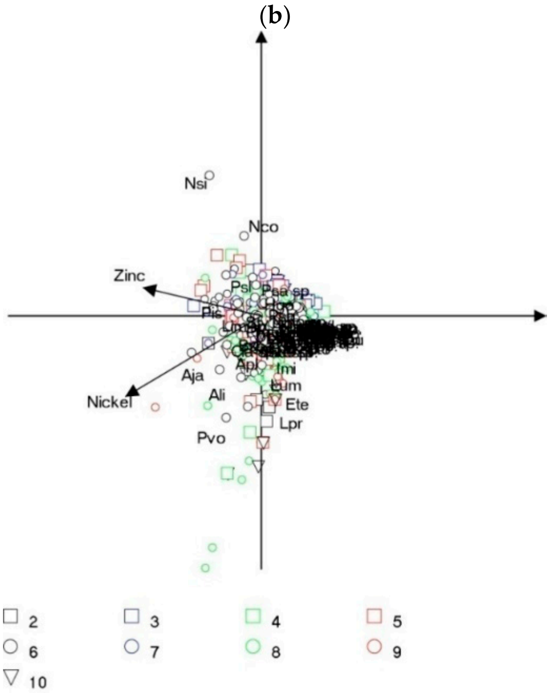


(**a**)

Figure 4. *Cont*.



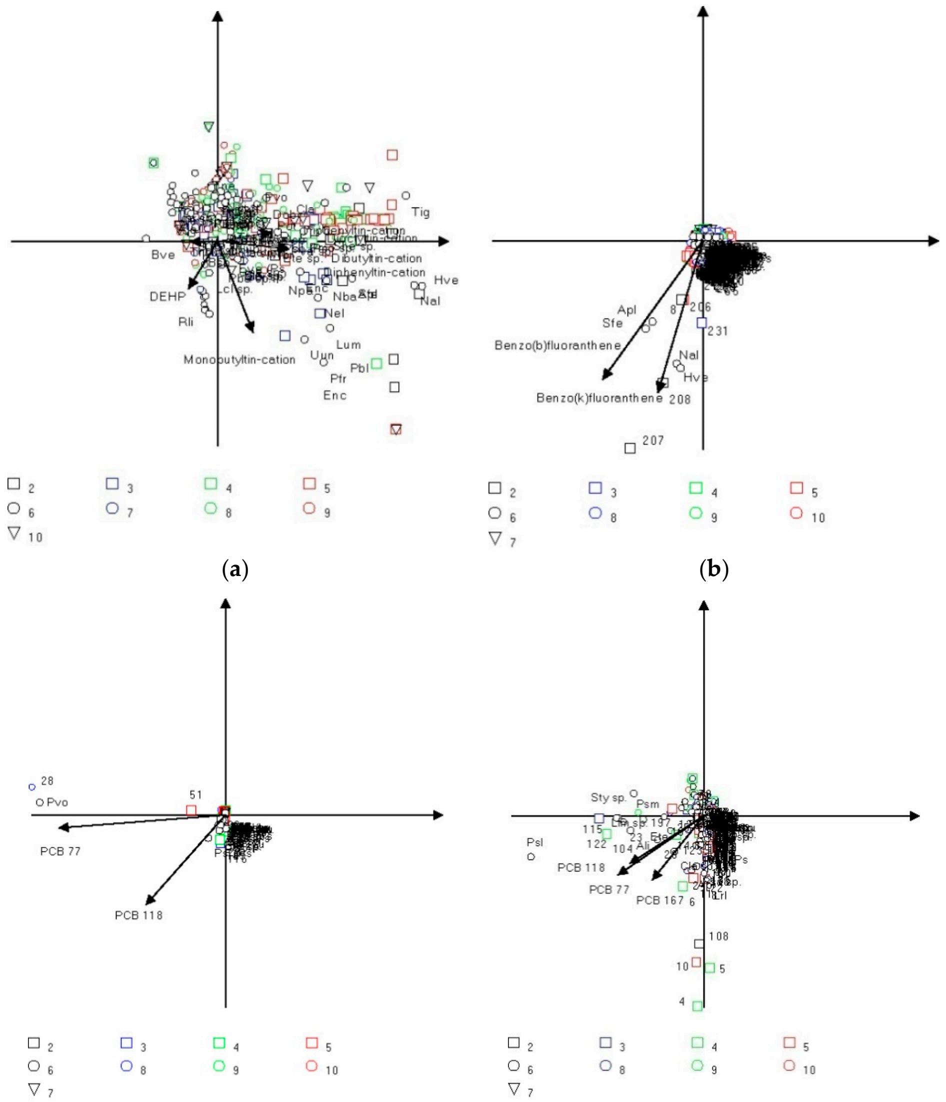
(**b**)



(**c**)

Figure 4. CCA triplot of oligochaetes samples relative to (**a**) FS hydro-morphological alterations along the Danube. Input matrices 66 × 590 and 3 × 590; weighting averages (WA), Monte Carlo test, least (canonical) squares (LCS). The bivariate space of the first two axes covers 83.3% of data variability (CCA axis 1–56.3%; CCA axis 2–27%). (**b**) FS physico-chemical parameters along the Danube. Input matrices 66 × 576 and 3 × 576; weighting averages (WA), Monte Carlo test, least (canonical) squares (LCS). The bivariate space of the first two axes covers 75% of data variability (CCA axis 1–63.6%; CCA axis 2–24%). (**c**) FS heavy metal concentrations in the sediment along the Danube. Input matrices 64 × 533 and 2 × 533; singular value decomposition (SVD), Monte Carlo test, least (canonical) squares (LCS). The bivariate space of the first two axes covers 100% of the data variability (CCA axis 1–69%; CCA axis 2–31%). Defined sectors of the river are numbered (1–10) as in Figure 1. Oligochaeta taxa are coded as in Tables 1 and 2.

Organotins, PAHs, and PCBs were tested to check whether their variability allowed for multivariate analyses and those with zero variance were excluded from further analyses. FS revealed that only one factor (monobutyltin cation) was important (p level 0.05) among organotins, thus in order to analyze these parameters in a multivariate space, CCA with all seven factors was used (Figure 5a). *P. frici*, *P. blanci*, *U. uncinata*, *R. limosella*, Enchytraeidae, and Lumbricidae were taxa that tolerated increased concentrations of monobutyltin cation. The highest concentrations of this pollutant were recorded during the JDS3 at the sampling sites from the Geisling power plant to Bratislava (sectors 3 and 4).



(**a**) (**b**) (**c**) (**d**)

Figure 5. CCA triplot diagram of oligochaetes samples along the Danube relative to (**a**) organotins (input matrices 54 × 397 and 7 × 397; singular value decomposition (SVD), Monte Carlo test, least (canonical) squares (LCS)) (fitted CCA axis 1–42%, CCA axis 2–22.5%); (**b**) FS PAHs (input matrices 62 × 429 and 2 × 429; singular value decomposition (SVD), Monte Carlo test, least (canonical) squares (LCS) (fitted CCA axis 1–77%, CCA axis 2–23%); (**c**) FS PCBs (input matrices 54 × 208 and 2 × 208; singular value decomposition (SVD), Monte Carlo test, least (canonical) squares (LCS)); fitted CCA axis 1–90%, CCA axis 2–10%); (**d**) excluded species *Propappus volki* and sample No. 28; FS PCBs (input matrices 53 × 207 and 3 × 207; singular value decomposition (SVD), Monte Carlo test, least (canonical) squares (LCS)). (CCA axis 1–86%, CCA axis 2–11.5%). Defined sectors of the river are numbered (1–10) as in Figure 1. Oligochaeta taxa are coded as in Tables 1 and 2.

Among PAHs, FS distinguished benzo(b)fluoranthene and benzo(k)fluoranthene as the most important environmental variables. The relations in the multivariate space among oligochaetes assemblages and selected variables are presented in Figure 5b. As it can be observed, these variables are negatively correlated with the CCA axes, particularly with the second axis. Taxa tolerant to increased concentrations of selected PAHs were as follows: *S. ferox*, *A. pluriseta*, *N. alpina*, and *H. ventriculosa*. Samples with higher concentrations of these PAHs are separated on the CCA diagram and mainly belong to the Danube sectors 2 and 3.

The FS of PCBs singled out PCB 77 and 118 as the most important PCBs. The connection between oligochaetes and these variables is shown in Figure 5c. *P. volki* is the only species showing variations of selected variables. Samples from Downstream Arges, Oltenita, and Upstream Olt (during JDS2) had the highest concentrations of these pollutants. In a sample from Oltenita, *P. volki* was the dominant member of the Oligochaeta community with the highest abundance. To better evaluate the correlation between other species and selected environmental variables, after excluding the species *P. volki* and the Oltenita sample, CCA with FS was performed again (Figure 5d). PCB 167 was added as the third most important PCB and it positively correlated with PCB 77 and 118, while all variables negatively correlated with the CCA axes. Several taxa from samples from different sectors (sites Downstream Pancevo, Stara Palanka-Ram, Sulina, Jochenstein, Klosterneuburg, Bratislava, and Braila) were found to be associated with increased concentrations of PCBs; these were *P. albicola*, *Stylodrilus* sp., *Limnodrilus* sp., *A. limnobius*, *E. tetraedra*, *P. moldaviensis*.

4. Discussion

The investigated sectors of the Danube exhibited high faunistic similarities due to the dominant influence of ubiquitous tubificins with wide ecological valences, such as *Limnodrilus*, *Potamothrix*, and *Psamoryctides* species. In regard to the higher level of sectioning (the large river stretches—Upper, Lower, and Middle Danube), separation of samples from the lower stretches of the Danube could be observed, which has been explained in detail by Atanacković et al. [16]. However, performed DA based on the Oligochaeta community showed that sectioning of the Danube according to Robert et al. [24] could not be observed. This occurrence indicates the high homogenization of the habitat and community as well. It is obvious that in a multistressed environment as it is in large rivers, with short environmental gradients and homogenous water quality along the entire river course, a few species with wide ecological valences could dominate along the entire stretch of the river, thus diminishing the potential of the entire community to react properly [34]; it was confirmed for the Chironomidae community in the Danube [34], and could be observed from the presented results for the Oligochaeta community. Tubificins, particularly *L. hoffmeisteri*, predominated by frequency and abundance in almost all investigated sectors. One of the main causes of biotic homogenization (related to Oligochaeta communities) of the entire river were the HYMO parameters. Actually, regarding the main HYMO factors, CCA showed high habitat variability within each individual sector, i.e., there are similar microhabitats in each sector as a result of hydromorphological alterations, changes of the water regime, and substrate structure, thus the different sectors show habitat similarities. In each sector, there are sites influenced by dams, with a backwater effect (a slowing down of the current and increased sedimentation), sites with bank modifications and with natural banks, sites with a high nutrient/organic load, so that each individual sector was characterized by the presence of all substrate types. These conditions led to the "potamalization" of upper and middle river stretches, followed by changes in faunal structure [35], transforming these sections of the river flow into reservoirs and causing a change from rhithral to potamal type of communities. The existence of similar types of microhabitats within each sector causes homogenization of the oligochaetes community of the entire Danube. This means that there is a similar oligochaetes community with dominance of tubificins in each sector along the entire Danube. The slowing down of the current which has a backwater effect, increased sedimentation, rising water temperature and accumulation of organic matter caused by dams in upper and middle river stretches have led to an increase in the number of limnorheophilic species (characteristic for the lower part of the River) instead of reophilic species (characteristic for upper and middle parts of the River). Dumnicka [11] pointed out that damming had a marked influence on oligochaetes fauna. In addition, the predominance of potammal species in the Danube, observed by Moog [35], is in accordance with our results which confirmed that the high participation of tubificins that was recorded in each sector reveals the existence of well-developed muddy zones.

Many authors have pointed out that the bottom structure, which is closely related to the river current, is the most important factor for Oligochaeta fauna in the Danube [12,36]. This is confirmed by our analyses, and to which we can add one more factor—hydrological alterations. Most of the recorded species prefer fine, muddy, and sandy substrates, with slow currents, while only three species, *N. simplex*, *N. communis*, and *T. ignotus*, can be distinguished by their preference for large substrates and faster currents. The importance of substrate type, urban pollution, and hydromorphological alterations (changes in current velocity, riverbed structure, etc.) in the Slovakian and Hungarian Danube stretch was confirmed [12,37,38].

Considering that the entire course of the Danube has been altered under significant HYMO pressure, and that only 6.45% of the investigated sites had natural banks, it can be concluded that a homogenous environment of the entire river stretch has been created (the upper river stretches no longer differ from the middle and lower river parts), enabling the spread of similar ecological groups of oligochaetes species in each individual sector, thus making the oligochaetes community of the Danube uniform along the entire watercourse. Species with wider ecological valences, such as tubificins, predominate, so no obvious distinction between defined sectors regarding species composition of oligochaetes assemblages could be observed.

As the Danube flows through a relatively densely populated area of Europe with several large cities and extensive/intensive agriculture, a high input of organic load from different sources (communal waters, agriculture etc.) is evident. Tubificid species are expected to be recorded with higher abundance and frequencies at sites with higher nutrient values. Indeed, such sampling sites were the most numerous in our investigation, and are characterized by muddy substrates, slow currents, and increased sedimentation. Our findings are in accordance with these observations, with a positive correlation of tubificids (*Limnodrilus* genera) with nitrates. Moreover, turbificid species *T. tubifex* and *B. sowerbyi* are positively correlated with nitrites.

The results of the influence of metals on the distribution of Oligochaeta revealed that zinc and nickel were the most influential factors. Species that displayed a positive correlation with metal concentrations in the sediment, as expected belong to the Tubificinae (*A. japonicus*, *A. limnobius*, *P. isochaetus*, and *P. albicola*), with Naidinae (*N. simplex* and *N. communis*), and Propapidae (*P. volki*), exhibiting greater tolerance. No oligochaetes species were found to be sensitive to increased metal concentrations. Ecotoxicological significance of any heavy metal does not solely depend on its concentrations, but also on the form that it is present in the environment [21] and it is not surprising that no oligochaetes species were found to be sensitive to increased metal concentrations.

Several taxa, such as *P. volki*, *P. albicola*, *Stylodrilus* sp., correlated with elevated concentrations of organic pollutants such as PAHs and PCBs. In the case of PCBs, this could be due to the higher tolerance and more effective system of excretion of the taxa; on the other hand, it could be because of the lower degree of bioaccumulation due to the specificity of the habitats (organic matter, depth, etc.).

Aquatic oligochaetes have a long history of use in aquatic pollution assessments [22], though they rarely were used directly in ecological risk assessment due to complicated species identification and traditional linking to organic pollution only. Moreover, the whole group of Oligocaheta has been considered 'pollution tolerant' where this perception of 'tolerance' has been incorrectly projected to chemical pollution [22].

Water quality assessment as a tool in water management should deal with at least the major environmental conditions and certainly not be restricted to the saprobic part [39]. In this respect, we confirmed the significance of oligochaetes as indicators of hydromorphological changes, not just as pollution tolerant species, as was thought in the past.

According to our results, physico-chemical and chemical determinants in water and sediment (organic pollution and nutrient load) are not the main factors that shape oligochaetes communities and influence their distribution. The complexity of this issue is pronounced considering the structure of microhabitats caused by hydromorphological changes. Our results indicate that Oligochaeta could be used for the identification of the level of hydromorphological degradation in large rivers (homogenization), rather than for stressors classified as nutrient, heavy metal, and organic pollutants. The presence of metals in tissues can serve as an indicator of contamination of an organism [23] and these data provide additional evidence in risk assessment of the environment, but there is no standard response of specimens within a taxon to increasing concentrations of metals. Furthermore, it would be preferable to assess the effects of different pollutants found in aquatic environments to the level of DNA damage of Oligochaeta species like it was done for *L. udekemianus* [40] indicating that this species is suitable model organism in ecogenotoxicology.

The traditional distinction between Danube sectors is not applicable if we consider Oligochaeta communities. The main reasons causing biotic homogenization are hydromorphological alterations that are represented by similar changes in flow velocity and substrates along its course. As Oligochaeta are fully aquatic organisms with a low mobility, they are a good indicator of changes in aquatic habitats, which has been confirmed in our study. Moreover, if we consider Oligochaeta communities, this large river could be considered as "one habitat–one community river". Smutz and Moog [41] found that hydromorphological alterations (particularly damming) could have negative effects on aquatic communities of the hyporheic river zone resulting in impoverished fauna, dominated by few species and reducing the ecological status of this lacustrine sections, in reservoirs downstream dams. To get a more complete picture regarding aquatic biota and its relations to environmental pressures along the Danube, incorporating additional biotic components is needed.

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References

1. Liepolt, R. *Limnologie der Donau*; Schweizerbart'sche Verlagsbuchhandlung: Stuttgart, Germany, 1967; p. 591.
2. Titizer, T.; Banning, M. Biological assessment in the Danube catchement area: Indications in shifts in species composition induced by human activities. *Eur. Water Manag.* **2000**, *3*, 35–45.
3. Sommerwerk, N.; Hein, T.; Schneider-Jakoby, M.; Baumgartner, C.; Ostojic, A.; Paunovic, M.; Bloesch, J.; Siber, R.; Tockner, K. The Danube River Basin, Part 3. In *Rivers of Europe*; Tockner, K., Uehlinger, U., Robinson, C.T., Eds.; Academic Press: San Diego, CA, USA, 2009; pp. 59–112.
4. Zavadsky, I.; Liska, I. A Comprehensive monitoring and assessment survey on the Danube. *Water Res. Manag.* **2015**, *5*, 3–13.

5. Directive:, W.F. Water framework directive—Directive 2000/60/ec of the European parliament and of the council establishing a framework for community action in the field of water policy. *Off. J. Eur. Commun.* **2000**, *22*, 2000.
6. JDS1. *Summary of the Final Report Joint Danube Survey ICPDR—International Commission for the Protection of the Danube River/Permanent Secretariat*; Vienna International Centre: Vienna, Austria, 2002; p. 30.
7. Liška, I.; Wagner, F.; Slobodnik, J. *Joint Danube Survey—Final Report*; ICPDR—International Commission for the Protection of the Danube River: Vienna, Austria, 2008; p. 235.
8. Liška, I.; Wagner, F.; Sengl, M.; Deutsch, K.; Slobodnik, J. *Joint Danube Survey 3—A Comprehensive Analysis of Danube Water Quality, Final Report*; ICPDR—International Commission for the Protection of the Danube River: Vienna, Austria, 2015; p. 369.
9. Schmedtje, U. (Ed.) *The Danube River Basin District—Part A Basin-Wide Overview, ICPDR WFD Roof Report 2004*; International Commission for the Protection of the Danube River in Cooperation with the Countries of the Danube River Basin District: Wien, Austria, 2005; p. 191.
10. Woitke, P.; Wellmitz, J.; Helm, D.; Kube, P.; Lepom, P.; Litheraty, P. Analysis and assessment of heavy metal pollution in suspended solids and sediments of the river Danube. *Chemosphere* **2003**, *51*, 633–642. [CrossRef]
11. Dumnicka, E. The effect of dam reservoirs on oligochaeta communities in the River Dunajec (Southern Poland). *Acta Hydrobiol.* **1987**, *29*, 25–34.
12. Šporka, F. The typology of floodplain water bodies of the Middle Danube (Slovakia) on the basis of the superficial polychaete and oligochaeta fauna. *Hydrobiologia* **1998**, *386*, 55–62. [CrossRef]
13. Moog, O.; Brunner, S.; Humpesch, U.H.; Schmidt-Kloiber, A. The distribution of benthic invertebrates along the Austrian stretch of the River Danube and its relevance as an indicator of zoogeographical and water quality patterns—Part 2. *Arch. Hydrobiol.* **2000**, *11*, 473–509. [CrossRef]
14. Timm, T.; Seire, A.; Pall, P. Half a century of oligochaeta research in Estonian running waters. *Hydrobiologia* **2001**, *463*, 223–234. [CrossRef]
15. Rîşnoveanu, G.; Vadineanu, A. Long term functional changes within the Oligochaeta communities within the Danube River Delta, Romania. *Hydrobiologia* **2003**, 399–405. [CrossRef]
16. Atanacković, A.; Šporka, F.; Csányi, B.; Vasiljević, B.; Tomović, J.; Paunović, M. Oligochaeta of the Danube River—A faunistical review. *Biologia* **2013**, *68*, 269–277. [CrossRef]
17. Levashova, E.A.; Mikhailov, V.N.; Mikhailova, M.V.; Morozov, V.N. Natural and Human-Induced Variations in Water and Sediment Runoff in the Danube River Mouth. *Water Resour.* **2004**, *31*, 235–246. [CrossRef]
18. Poff, N.L.; Olden, J.D.; Merritt, D.M.; Pepin, D.M. Homogenization of regional river dynamics by dams and global biodiversity implications. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 5732–5737. [CrossRef]
19. Rahel, F.J. Homogenization of Freshwater Faunas. *Annu. Rev. Ecol. Syst.* **2002**, *33*, 291–315. [CrossRef]
20. Lafont, M. Oligochaeta communities as biological descriptors of pollution in the fine sediments of rivers. *Hydrobiologia* **1984**, *115*, 127–129. [CrossRef]
21. Prygiel, J.; Rosso-Darmet, A.; Lafont, M.; Lesniak, C.; Durbec, A.; Ouddane, B. Use of oligochaeta communities for assessment of ecotoxicological risk in fine sediment of rivers and canals of the Artois-Picardie water basin (France). *Hydrobiologia* **2000**, *410*, 25–37. [CrossRef]
22. Chapman, P.M. Utility and relevance of aquatic oligochaetes in ecological risk assessment. *Hydrobiologia* **2001**, *463*, 149–169. [CrossRef]
23. Rodríguez, P.; Reynoldson, T.B. *The Pollution Biology of Aquatic Oligochaetes*; Springer: Dordrecht, The Netherlands, 2011; p. 265.
24. Robert, S.; Birk, S.; Somenhauser, M. Typology of the Danube River—part 1: Top-down approach. In *UNDP/GEF Danube Regional Project, Activity 1.1.6, Typology of Surface Waters and Definition of Reference Conditions for the Danube River—Final Report*; ICPDR—International Commission for the Protection of the Danube River: Vienna, Austria, 2003; pp. 51–59.
25. Aqem Consortium. Manual for the application of the AQEM system. In *A Comprehensive Method to Assess European Streams Using Benthic Macroinvertebrates, Developed for the Purpose of the Water Framework Directive, Version 1*; The AQEM consortium: Duisburg-Esen, Germany, 2002; pp. 619–630.
26. European Committee for Standardisation. *Water Quality—Methods for Biological Testing—Methods of Biological Sampling: Guidance on Handnet Sampling of Aquatic Benthic Macroinvertebrates*; EN 27828:1994, ISO 7828-1985; British Standards Institution: London, UK, 1994.

27. Van Lynden, G.W.J.; Mantel, S.; van Oostrumvan, A. *Guiding Principles for the Quantitative Assessment of Soil Degradation with a Focus on Salinization, Nutrient Decline and Soil Pollution*; International Soil Reference and Information Centre, Food and Agriculture Organization of the United Nations: Rome, Italy, 2004; p. 73.
28. Fisher, R.A. The use of multiple measurements in taxonomic problems. *Ann. Eugen.* **1936**, *7*, 179–188. [CrossRef]
29. Lachenbruch, P.A.; Goldstein, M. Discriminant Analysis. *Biom. Perspect. Biometry* **1979**, *35*, 69–85. [CrossRef]
30. Ter Braak, C.J. Canonical Correspondence Analysis: A New Eigenvector Technique for Multivariate Direct Gradient Analysis. *Ecology* **1986**, *67*, 1167–1179. [CrossRef]
31. Ter Braak, C.J.; Verdonschot, P.F.M. Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquat. Sci.* **1995**, *57*, 255–289. [CrossRef]
32. Hope, A.C.A. A Simplified Monte Carlo Significance Test Procedure. *J. R. Stat. Soc. Ser. B Stat. Methodol.* **1968**, *30*, 582–598. [CrossRef]
33. Karadžić, B. FLORA: A software package for statistical analysis of ecological data. *Water Res. Manag.* **2013**, *3*, 45–54.
34. Milosevic, D.; Mančev, D.; Čerba, D.; Piperac, M.S.; Popović, N.; Atanackovic, A.; Đuknić, J.; Simić, V.; Paunović, M. The potential of chironomid larvae-based metrics in the bioassessment of non-wadeable rivers. *Sci. Total. Environ.* **2018**, *616*, 472–479. [CrossRef]
35. Moog, O. *Fauna Aquatica Austriaca—Katalog zur Autecolologischen Einsfung*; Austrian Federal Ministry of Agriculture Forestry Environment and Water Management: Vienna, Austria, 2002.
36. Russev, B. Influence of some ecological factors on changes of the standing crop of zoobenthos of the Danube in the Bulgarian stretch. In Proceedings of the IBP-UNESCO Symposium on Productivity Problems of Freshwaters, Kazimierz Dolny, Poland, 6–12 May 1970; Kajak, Z., Hillbricht-Ilkowska, A., Eds.; PWN-Polish Scientific Publishers: Warsaw, Poland, 1972; pp. 813–826.
37. Šporka, F.; Nagy, Š. The macrozoobenthos of parapotamon-type side arms of the Danube River and its response to flowing conditions. *Biologia* **1998**, *53*, 633–643.
38. Elexová, E.; Némethová, D. The effect of abiotic environmental variables on the Danube macrozoobenthic communities. *Lirnnologica* **2003**, *33*, 340–354. [CrossRef]
39. Verdonschot, P.F.M. The role of oligochaetes in the management of waters. *Hydrobiologia* **1989**, *180*, 213–227. [CrossRef]
40. Kračun-Kolarević, M.; Kolarević, S.; Atanackovic, A.; Marković, V.; Gačić, Z.; Paunovic, M.; Vuković-Gačić, B. Effects of 5-Fluorouracil, Etoposide and CdCl2 in Aquatic Oligochaeta Limnodrilus udekemianus Claparede (Tubificidae) Measured by Comet Assay. *Water Air Soil Pollut.* **2015**, *226*, 242. [CrossRef]
41. Schmutz, S.; Moog, O. Dams: Ecological Impacts and Management. In *Riverine Ecosystem Management*; Aquatic Ecology Series 8; Schmutz, S., Sendzimir, J., Eds.; Springer Nature: Cham, Switzerland, 2018; pp. 111–127.