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WATER QUALITY ASSESSMENT USING BIOTIC INDICES BASED ON BENTHIC INVERTEBRATES IN THE CARAȘ CATCHMENT AREA

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SUMMARY. The present paper represents a study on benthic invertebrate communities from 9 sampling sites located in the Caraș River catchment area. The data were used to calculate different biotic indices and subsequently to assess the water quality in the area. The following biotic indices were considered: the Extended Biotic Index (E.B.I.), the Biological Monitoring Working Party (B.M.W.P.) and the Average Score Per Taxon (A.S.P.T.), already in use in different European countries. A total number of 75 taxa was found in three sampling seasons in 2012. The water quality from the Caraș River catchment area ranged from very good to moderate (classes I to III).

Keywords: A.S.P.T., B.M.W.P., diversity, E.B.I., similarity

Introduction

The Water Framework Directive 2000/60/EC of the European Parliament stipulates that water quality assessment should rely firstly on biotic communities, with physical and chemical parameters used to complete these data. In the last few years, numerous studies on water quality assessment based on invertebrates were performed in Romania, using European indices (Cîmpean, 2004; Avram *et al*., 2009).

The Caraș is a left tributary of the Danube River that flows in Romania and Serbia. There are numerous protected areas in its catchment area: the National Park Cheile Carașului – Semenic, the scientific reserves Cheile Carașului and Izvoarele Carașului on its upper course, together with one site of community importance (SCI) ROSCI0226 Semenic – Cheile Carașului, and one Special Protected Area (SPA) ROSPA0086 Munţii Semenic – Cheile Carașului (Brînzan, 2013).

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The only previous studies on benthic invertebrates from the Caraș catchment area are Petrucean *et al*. (2009) and Eftenoiu *et al*. (2011), where only five sampling sites were considered. Thus, a systematic study was required in the area, due to its importance and uniqueness.

The major objectives of the present study are: (1) to provide a list of aquatic invertebrates from the investigated area; (2) to assess the water quality using benthic invertebrate communities; and (3) to identify the most drastic human impacts in the Caraș catchment area.

Materials and methods

The study area is represented by the Caraș catchment area. The river has its source in the western part of the Semenicului Mountains at 700 m a.s.l. The total drainage basin area is 1,118 km² and its total length is 110 km, out of which 85 km are in Romania. It flows through three different relief regions: (1) the upper one, located at an altitude ranging from 900 to 400 m a.s.l.; (2) the second one, a calcareous sector 28.5 km long, where the river forms a gorge; and (3) the lower part, a large alluvial plain. The river tributaries are relatively small, but symmetrical (Ujvari, 1972).

Nine sampling sites were considered, out of which three were located on the main river course (C1 – The Caraș River – source; C2 – The Caraș River – gorge; C3 – The Caraș River – Vrani) (Table 1; Fig. 1). C1 was located in the ”Izbucul Caraşului”, right at the river source, and represented the first benthic invertebrate sampling location so far. C2 was situated inside the river gorge, near the river exit, while C3 was chosen in the proximity of the Serbian border, in the lowlands.

Table 1.

The nine sampling sites with the codes used for the present paper

Sampling site name	Sampling site code
The Caraș River – source	C1
The Caraș River – gorge	C2
The Nermed River	Ne
The Natra River	Na
The Lișava River – upstream the mine	L1
The Lișava River – downstream the mine	L2
The Lișava River – upstream the junction with the Caraș River	L3
The Ciornovăț River	Ci
The Caraș River – Vrani	C3

One sampling site was located on the Natra River (Na), but only spring samples were collected because in the other two seasons access was not allowed. This sampling location was upstream the uranium mine drained by the river.

Three locations were considered on the Lișava River (L1 – The Lișava River – upstream the mine; L2 – The Lișava River – downstream the mine; L3 – The Lișava River – upstream the junction with the Caraș River) (Fig. 1). L1 replaced the site from the Natra River. L2 was located near the Lișava and Natra Rivers's junction, downstream of the abandoned uranium mine, where the river waters became reddish in color, similar to the substrate.

Two other rivers were sampled, with one sampling location on each one: The Nermed River (Ne) and the Ciornovăț River (Ci) (Table 1; Fig. 1). The samplings from the Nermed River took place downstream of the homonym village, where the riverbed included a high percentage of bricks, tiles and other domestic wastes. The sampling site from the Ciornovăț River was situated downstream the five localities crossed by the river, before its junction with the Caraș River.

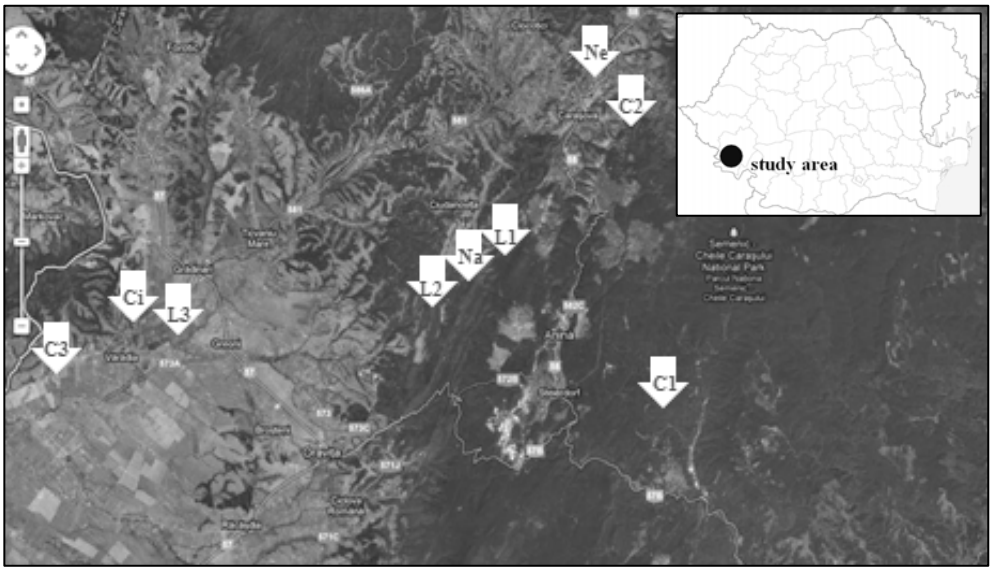

Figure 1. Location of the nine sampling sites from the Caraș catchment area (C1 – The Caraș River – source; C2 – The Caraș River – gorge; Ne – The Nermed River; L1 – The Lișava River – upstream the mine; Na – The Natra River; L2 – The Lișava River – downstream the mine; L3 – The Lișava River – upstream the junction with the Caraș River; Ci – The Ciornovăț River; C3 – The Caraș River – Vrani) (source: GoogleEarth)

Qualitative samples of benthic invertebrates were collected in 2012, in spring (1.05.2012), summer (6.08.2012) and autumn (28.10.2013). The samples were collected with a 250 μm mesh net and in 4% formaldehyde. Invertebrate identifications were made to the genus level for Plecoptera, Ephemeroptera, Turbellaria and Hirudinea; and to the family level for Oligochaeta, Copepoda, Amphipoda, Trichoptera, Diptera, Coleoptera, Odonata and Heteroptera. Based on these identifications, the following biotic indices were calculated: the Extended Biotic Index (E.B.I.), the Biological

Monitoring Working Party (B.M.W.P.) and the Average Score Per Taxon (A.S.P.T.). The E.B.I. (Ghetti, 1997) is used in Italy and it is included in the Italian environmental legislation. The B.M.W.P. was first developed in U.K. (Walley and Hawkes, 1996; 1997) and it was subsequently adapted for Poland. The A.S.P.T. represents the ratio between the B.M.W.P. and the total number of families in the sample. Table 2 presents the GPS coordinates of the nine sampling sites and their most important characteristics (river width and depth).

Table 2.

Location of the nine sampling sites from the Caraș catchment area, with their major characteristics (sp – spring 2012; su – summer 2012; au – autumn 2012)

Sampling site	Altitude (m a.s.l.)	GPS coordinates	Riverbed width (m)			Maximum depth (cm)		
			sp	su	au	sp	su	au
C1	760	N 45º04'40.16" E 21º54'56.52"	6	4.7	5.2	50	50	50
C2	235	N 45º12'03.11" E 21º52'22.30"	13	10.3	11	65	60	60
Ne	229	N 45º13'20.95" E 21º51'07.03"	5	1.8	4	50	30	35
Na	338	N 45º05'45.56" E 21º46'04.12"	2.5	-	-	30	-	-
L1	331	N 45º06'20.69" E 21º46'42.32"	-	2.8	2.5	-	25	20
L2	312	N 45º06'21.71" E 21º45'57.34"	2.7	1.5	1.6	20	15	15
L3	97	N 45º04'54.41" E 21º33'01.93"	6	5.6	5.1	60	40	35
Ci	101	N 45º05'33.01" E 21º33'01.16"	8	7.4	7	50	45	40
C3	88	N 45º02'57.47" E 21º28'49.42"	32	31	30	130	110	120

Results and discussion

Water temperature variation in the nine sampling sites and three seasons is characteristic to rivers having a longitudinal gradient from the water source to the mouth. The river source has a relatively constant water temperature throughout the year, with a few degrees variation, as for the C1 sampling site (The Caraș River – source) (Fig. 2). The water temperature increases from headwaters to mouth, with higher variations during summer (Fig. 2).

Tables 3 and 4 present the list of invertebrate taxa, identified to family or genus level, in accordance with the requirements for the biotic index calculation. The structure of benthic communities is a complex one, including 75 taxa belonging to different groups.

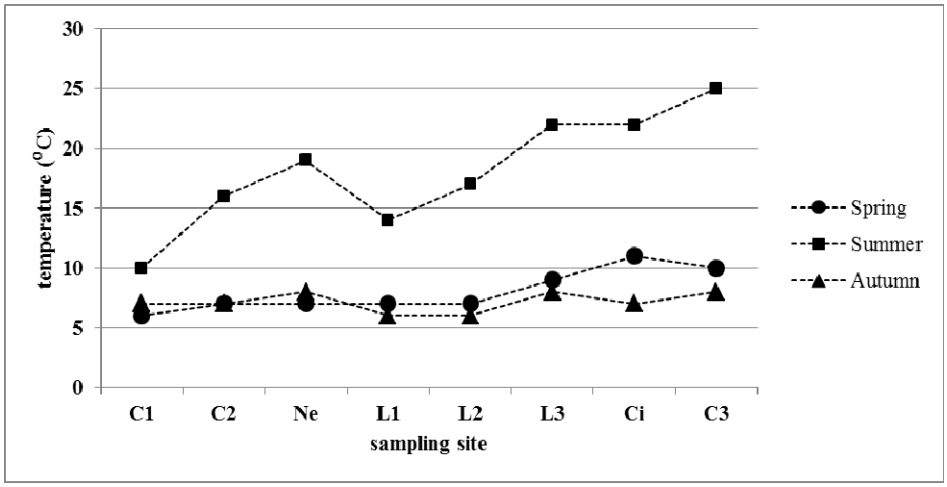

Figure 2. Variation of water temperature (°C) in three seasons during 2012, at the following sampling sites: C1 – The Caraș River – source; C2 – The Caraș River – gorge; Ne – The Nermed River; L1 – The Lișava River – upstream the mine; L2 – The Lișava River – downstream the mine; L3 – The Lișava River – upstream the junction with the Caraș River; Ci – The Ciornovăț River; C3 – The Caraș River – Vrani

Aquatic earthworms (Oligochaeta) appear in all sampling sites, with Family Naididae having the highest frequency of appearance in the sampling locations. Side-swimmers (Amphipoda) are present in all sampling sites, but not in all sampling seasons (Table 3; Table 4). Mayflies (Ephemeroptera) were identified in all sampling sites, but the genera diversity is higher in the headwaters of the Caraș River and its tributaries. At the Caraș River – gorge (C2) seven Mayfly genera were found (*Baetis, Caenis, Ephemera, Habroleptoides, Habrophlebia, Rithrogena* and *Torleya*), besides *Ecdyonurus*, the only genus found by Eftenoiu (2011) (Table 3). In the Nermed River (Ne), the same genus found by Eftenoiu (2011), *Ecdyonurus*, was joined by five others (*Baetis, Caenis, Ephemera, Habroleptoides* and *Serratella*). Similarly, at L1 – The Lișava River – upstream the mine 3 other genera (*Baetis, Habroleptoides* and *Rithrogena*) were identified besides *Ecdyonurus* and *Ephemera*, found by Eftenoiu (2011) (Table 4). The higher number of taxa in the present study is caused by systematic sampling, covering three seasons, which enabled us to find taxa in stages of their life cycles that are impossible to see in only one sampling occasion.

Stoneflies (Plecoptera) were present only in the headwaters: in the Caraș River (C1 – source and C2 – river gorge) and in the The Lișava River (L1 and L2 – upstream and downstream of the mine). Caddisflies include a high number of families in C1, C2 and L1, while in the rest of the sampling sites, no more than three Trichoptera families were present (Table 3; Table 4).

Table 3.

List of benthic invertebrate taxa identified in the three sampling sites located on the main river course (C1 – The Caraș River – source; C2 – The Caraș River – gorge; C3 – The Caraș River – Vrani; sp – spring 2012; su – summer 2012; au – autumn 2012)

Sites; seasons → **Taxa↓**	**C1 sp**	**C1 su**	**C1 au**	**C2 sp**	**C2 su**	**C2 au**	**C3 sp**	**C3 su**	**C3 au**
Nematoda						+			+
Oligochaeta									
Lumbricidae					+			+	+
Lumbriculidae	+	+	+		+	+			
Naididae		+			+		+	+	+
Copepoda									
Cyclopidae							+		
Ostracoda									+
Amphipoda									
Gammaridae	+	+	+	+	+	+		+	
Hydrachnidia		+	+		+	+			
Ephemeroptera									
Baetis	+	+	+	+	+	+	+	+	+
Caenis					+	+	+	+	+
Ecdyonurus	+	+	+		+	+			
Ephemera				+	+	+	+	+	+
Habroleptoides					+				
Habrophlebia				+	+	+			
Paraleptoflebia							+		
Rhithrogena				+	+				
Torleya					+	+			
Plecoptera									
Isoperla	+	+	+	+					
Leuctra			+		+	+			
Nemoura					+	+			
Trichoptera									
Beraeidae			+		+	+	+		+
Glossosomatidae			+		+				
Hydropsychidae				+	+				
Hydroptilidae			+		+				
Odontoceridae						+			
Philopotamidae	+		+	+	+	+			
Phryganeidae			+		+				
Rhyacophilidae	+	+	+						
Sericostomatidae			+			+			
Diptera									
Athericidae		+	+					+	+
Ceratopogonidae			+	+		+	+		+

Table 3. continued

Chironomidae	+	+	+	+	+	+	+	+	+
Empididae	+		+		+	+			
Limoniidae	+		+	+	+	+			
Psychodidae			+			+			
Ptychopteridae	+	+	+						
Tabanidae					+		+	+	+
Tipulidae									
Coleoptera									
Dryopidae	+		+	+	+	+			
Dytiscidae					+				
Elminthidae	+	+	+	+	+	+			
Others									
Helobdella						+			
Dugesia	+	+	+	+	+	+			
Gastropoda	+					+	+		+
Bivalvia					+	+			+
Asellidae									+
Corixidae					+	+	+	+	
Aeshnidae							+		+
Gomphidae				+		+	+		+
Lestidae					+				
Libellulidae								+	
Megaloptera						+			

Twelve two-winged fly families (Diptera) were identified in the sampling locations, with Family Chironomidae present in all sites and all seasons. Beetles (Coleoptera) recorded a high frequency of appearance in the sampling sites, missing from only one location: C3 – The Caraș River – Vrani. Similarly, the only site with no dragonfly representatives (Odonata) was The Caraș River source (C1).

Nine copepod species were identified in four sampling sites (Table 3; Table 4). Most copepod species are common to benthic and hyporheic habitats: *Acanthocyclops robustus* Sars 1863; *Diacyclops bisetosus* (Rehberg 1880); *Eucyclops serrulatus proximus* (Lilljeborg 1901) or *Paracyclops fimbriatus* (Fischer 1853) (Dole-Olivier *et al*., 2000). Some are benthic crawlers, like *Macrocyclops albidus* (Jurine 1820) or *Macrocyclops fuscus* (Jurine 1820). However, there are cosmopolitan species too, found in all kinds of waters: *Megacyclops viridis* (Jurine 1820) and *Canthocamptus staphylinus* (Jurine 1820). *Diacyclops bicuspidatus* (Claus 1857) is characteristic to springs.

Table 4.

List of benthic invertebrate taxa identified in the main tributaries of the Caraș River (Ne – The Nermed River; Na – The Natra River; L1 – The Lișava River – upstream the mine; L2 – The Lișava River – downstream the mine; L3 – The Lișava River – upstream the junction with the Caraș River; Ci – The Ciornovăț River; sp – spring 2012; su – summer 2012; au – autumn 2012)

Sites; seasons → **Taxa↓**	**Ne sp**	**Ne su**	**Ne au**	**Na sp**	**L1 su**	**L1 au**	**L2 sp**	**L2 su**	**L2 au**	**L3 sp**	**L3 su**	**L3 au**	**Ci sp**	**Ci su**	**Ci au**
Nematoda	+		+						+			+			
Oligochaeta															
Haplotaxidae															+
Lumbricidae			+	+		+							+	+	+
Lumbriculidae	+	+	+	+	+										
Naididae		+	+	+			+	+	+	+	+	+	+	+	+
Copepoda															
Cyclopidae			+									+	+		+
Canthocamptidae			+												
Ostracoda			+												
Amphipoda															
Gammaridae		+		+	+	+	+	+	+	+	+	+	+		
Hydrachnidia			+	+	+	+	+	+	+	+		+		+	
Ephemeroptera															
Baetis	+		+		+	+	+		+		+	+	+		
Caenis		+	+							+	+	+	+	+	
Cloeon													+	+	+
Ecdyonurus	+					+	+	+	+						
Ephemera	+	+			+	+		+		+	+	+	+	+	
Habroleptoides	+			+	+	+									
Habrophlebia													+		
Rhithrogena					+		+	+							
Seratella	+														
Plecoptera															
Leuctra					+	+									
Nemoura				+	+	+	+	+	+						
Perla				+											
Siphonoperla				+											
Trichoptera															
Beraeidae	+	+	+		+	+					+	+	+	+	
Glossosomatidae			+			+									
Hydropsychidae				+			+								
Odontoceridae					+										
Philopotamidae				+	+	+		+	+			+			
Phryganeidae						+									
Rhyacophilidae					+	+	+								
Sericostomatidae				+	+	+									

Table 4. continued

Diptera															
Athericidae					+	+			+		+				
Blephariceridae					+										
Ceratopogonidae			+	+			+		+	+		+	+	+	+
Chironomidae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Empididae	+					+	+		+	+		+			
Limoniidae			+	+	+	+	+	+				+		+	
Psychodidae			+	+		+									+
Simuliidae			+												
Stratiomidae	+			+		+									
Tabanidae			+									+	+	+	+
Tipulidae			+	+								+			+
Coleoptera															
Dryopidae					+	+			+						
Dytiscidae	+											+			
Elminthidae			+	+	+	+	+	+	+	+		+	+	+	+
Hydraenidae			+												
Others															
Helobdella														+	+
Piscicola													+	+	
Dugesia				+	+	+									
Gastropoda			+	+									+		+
Bivalvia								+	+					+	+
Ligiidae							+								
Trachelopodidae														+	
Corixidae		+								+	+	+		+	
Aeshnidae		+									+	+	+	+	
Calopterygidae							+								
Coenagrionidae														+	
Cordulegasterida										+			+		
Gomphidae	+		+	+		+	+		+	+	+	+			+
Lestidae								+							
Plactynemididae			+						+			+			
Megaloptera							+	+		+					

The total number of taxa varies depending on the sampling site and sampling season, with higher values in autumn 2012. The maximum number of taxa was found in the Caraș River gorge (C2), reaching 32 taxa in summer 2012 (Fig. 3).

Similarity analysis based on the Jaccard index showed the existence of two clusters. The first one includes the benthic communities from L1 and C1 sampling sites (that are 60% similar), together with the biota from C2 (Fig. 4). All these three locations withstand low or no human impacts, because they are included in protected areas (The National Park Cheile Carașului – Semenic, ROSCI0226 Semenic – Cheile Caraşului, ROSPA0086 Munţii Semenic – Cheile Caraşului).

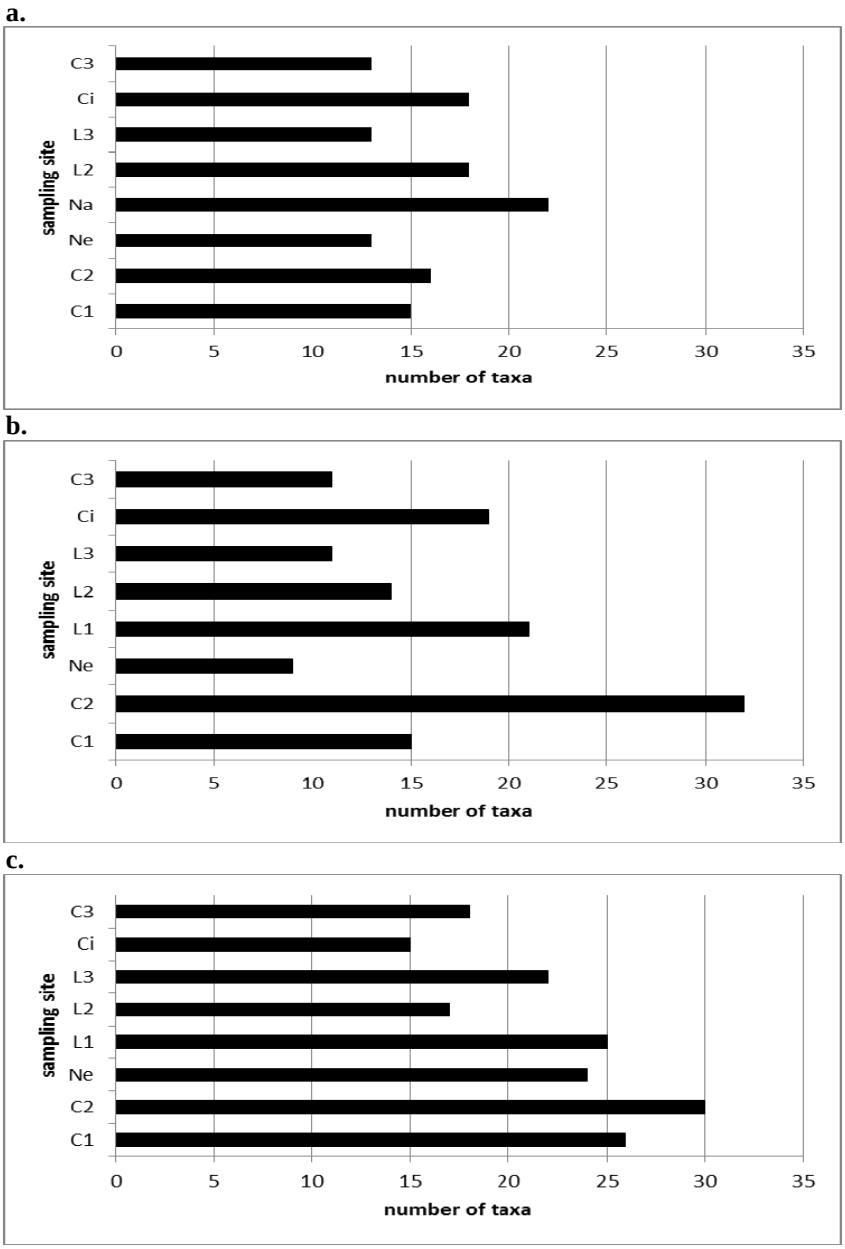

Figure 3. Number of taxa present at the sampling sites from the Caraș catchment area in spring (a), summer (b) and autumn (c)(C1 – The Caraș River – source; C2 – The Caraș River – gorge; Ne – The Nermed River; L1 – The Lișava River – upstream the mine; Na – The Natra River; L2 – The Lișava River – downstream the mine; L3 – The Lișava River – upstream the junction with the Caraș River; Ci – The Ciornovăț River; C3 – The Caraș River – Vrani)

Benthic communities from The Nermed River (Ne), The Lișava River – upstream the junction with the Caraș River (L3) and The Ciornovăț River (Ci) form the second cluster (50% similarity) probably due to similar human impacts, caused by the presence of human settlements along the rivers. The rest of the three sampling sites differ from the ones presented above: C3, The Caraș River – Vrani, accumulates all the negative effects from the river catchment area, because it is located downstream, near the Serbian border; L2, The Lișava River – downstream the abandoned uranium mine, where the river water has a reddish colour; and finally, Na, The Natra River, where only spring samples were collected (Fig 4.).

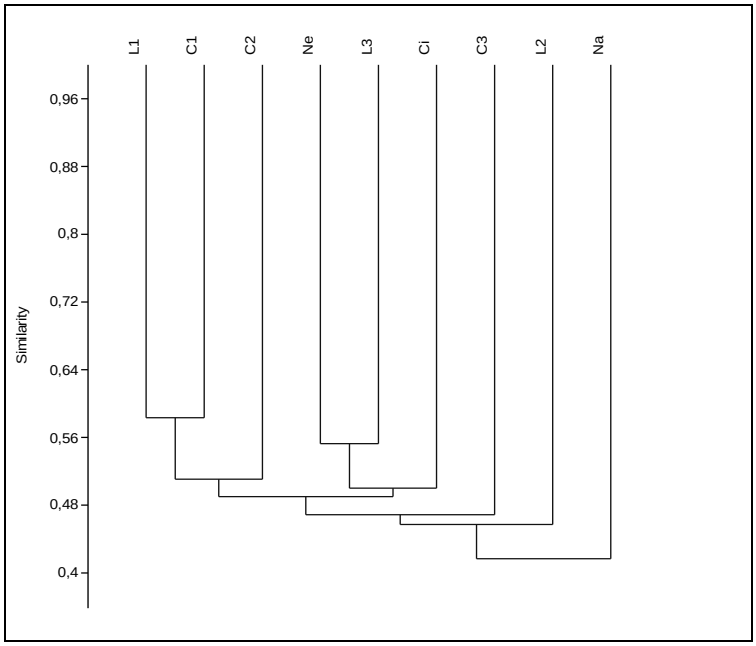

Figure 4. Similarity of benthic communities from the nine sampling sites (C1 – The Caraș River – source; C2 – The Caraș River – gorge; Ne – The Nermed River; L1 – The Lișava River – upstream the mine; Na – The Natra River; L2 – The Lișava River – downstream the mine; L3 – The Lișava River – upstream the junction with the Caraș River; Ci – The Ciornovăț River; C3 – The Caraș River – Vrani) based on the Jaccard index

In order to assess the water quality from the Caraș catchment area, quality classes were estimated based on three biotic indices: E.B.I., B.M.W.P. and A.S.P.T. Since different indices indicated different quality classes in several occasions, only one class was accepted, based on expert judgment (Table 5, Fig. 5).

At the Caraș River source (C1), with no human impacts, the water quality class was II, and not I, as expected. This is due to a series of limiting factors, like constant low water temperatures, variable volume of water depending on precipitations

etc. Biotic indices consider the sensitivity of invertebrates to environmental factors, and spring habitats differ from ”true” lotic ones. In case of the Caraș River, Plecoptera and Ephemeroptera genera, known to be sensitive to pollution, are poorly represented in the headwaters, but they were identified downstream. They were replaced by side-swimmers (Amphipoda), considered to be more tolerant to pollution.

Good and very good water quality was found in the Caraș River gorge (C2) and in the Lișava River, upstream the mine (L1), because of the high number of taxa sensitive to pollution. Even if in the Lișava River, downstream the abandoned uranium mine (L2) the water quality decreased (biotic indices showed moderate quality, class III), a clear improvement was seen in L3 (The Lișava River – upstream the junction with the Caraș River) due to the natural cleaning processes, in spite of the agricultural lands and human settlements along the river. The Natra River had a good water quality in spring 2012 (class II); in the other two seasons no samples were collected due to lack of access in the area.

The sampling site from the Nermed River was located downstream of the homonym locality and the good-moderate quality of the water (class II-III) reflected the human impacts caused by the village. Similarly, the water quality from the Ciornovăț River was only moderate (class III) due to intensive farming and the impact of the five rural communities located in the river catchment area of 124 km^2. Finally, the last sampling site, the Caraș River – Vrani (C3), located near the Serbian border, reflected the synergic effects of all impact factors from the river catchment area (agriculture, uranium mine, domestic wastes from human settlements), reaching only the moderate water quality (class III).

Table 5.

The quality classes calculated according to the three biotic indices and accepted quality classes, for the sampling sites considered in the Caraș catchment area (E.B.I. – Extended Biotic Index, B.M.W.P. – Biological Monitoring Working Party, A.S.P.T. – Average Score Per Taxon, C1 – The Caraș River – source; C2 – The Caraș River – gorge; Ne – The Nermed River; L1 – The Lișava River – upstream the mine; Na – The Natra River; L2 – The Lișava River – downstream the mine; L3 – The Lișava River – upstream the junction with the Caraș River; Ci – The Ciornovăț River; C3 – The Caraș River – Vrani)

Sites	**E.B.I. quality classes**	**B.M.W.P. quality classes**	**A.S.P.T. quality classes**	**Accepted quality classes**
C1	I-II	III	I-II	II
C2	I	I-II	II	I-II
Ne	II	III-IV	II-III	II-III
Na	I	III	III	II
L1	I	II	I-II	I-II
L2	II	III	III	III
L3	I-II	III-IV	II-III	II-III
Ci	II-III	III	III	III
C3	II	III-IV	III	III

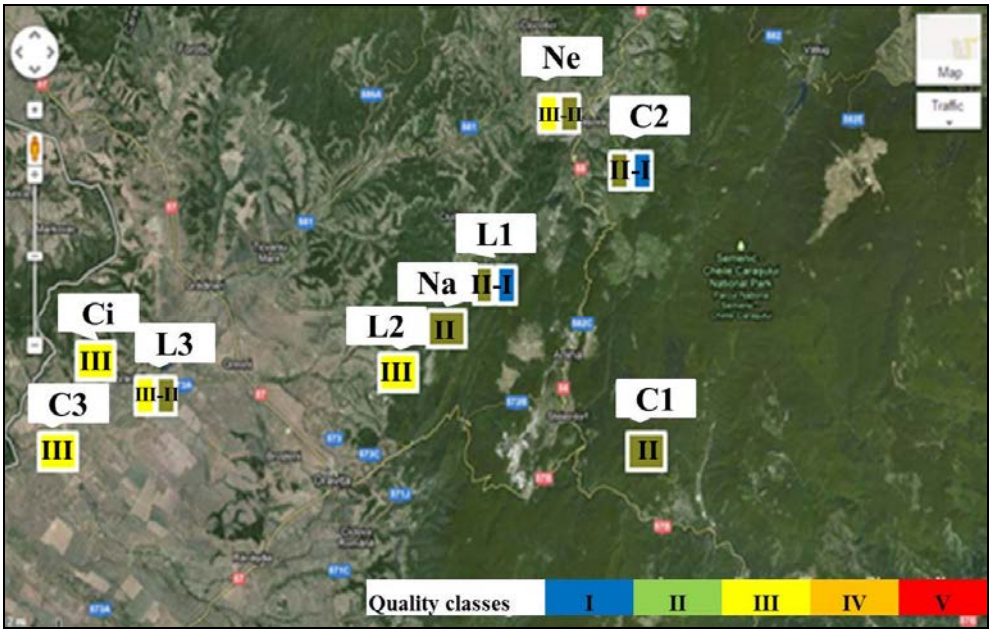

Figure 5. The accepted quality classes according to the considered biotic indices for the nine sampling sites in the Caraș catchment area (C1 – The Caraș River – source; C2 – The Caraș River – gorge; Ne – The Nermed River; L1 – The Lișava River – upstream the mine; Na – The Natra River; L2 – The Lișava River – downstream the mine; L3 – The Lișava River – upstream the junction with the Caraș River; Ci – The Ciornovăț River; C3 – The Caraș River – Vrani)

Conclusions

A total number of 75 taxa was found in the Caraș River catchment area, with the highest number of taxa identified at the Caraș River gorge. Jaccard similarity clearly differentiated the benthic communities characteristic to clean habitats (living in the sampling sites located in the headwaters for example) and those located in impacted environments, like the one from downstream of the abandoned uranium mine or the one situated in the Caraș lower course.

The water quality from the Caraș River catchment area ranged from very good to moderate (classes I to III), however no poor or bad water quality classes (IV and V) were depicted by the biotic indices in the nine sampling sites. This fact could be caused by the lack of major human settlements in the area and the lack of heavy industrial areas. However, the water quality decreased from the headwaters to mouth, since several human impacts were present on the river course and on its tributaries: domestic wastes, intensive agriculture, or the uranium mine.

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