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Macrozoobenthic invertebrate assemblages in rivers Șușița and Sohodol (Gorj County, Romania) and their indicator value for the water quality characterization

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Abstract. The present research evaluates the macrozoobenthic invertebrate assemblages from two rivers situated in the Jiu River basin. The invertebrates were collected from three sample sites on Sohodol River and five sample sites on Șușița River. The sample sites were situated along the two rivers which flow almost along their entire length through a forested area. In the case of Sohodol River we have encountered anthropic activities related to seasonal tourism, but no localities or industrial activities were observed in the area. The aim of the study was to establish besides the structure of the macrozoobenthic invertebrate assemblages, the water quality using BMWP score and also to evaluate the human impact on the macrozoobenthos in Sohodol River and the differences between the macrozoobenthic assemblages in the two rivers caused by natural or anthropic factors.

Keywords: macrozoobenthic invertebrates, diversity indexes, water quality, feeding categories.

Introduction

Macrozoobentic invertebrates form assemblages with a high importance in the ecological characterisation of the ecological status of the surface waters wordlwide (Azrina *et al,* 2005; Opreanu, 2010; Varnosfaderany *et al.,* 2010; Moya *et al*., 2011; Sinitean and Petrovici, 2012; Mesa *et al*., 2013; Macedo *et al*., 2016; Young *et al*., 2014). They are sensitive to the variations of the physico-chemical paramethers of the water due to natural (Longing *et al*., 2010; Nguyen *et al*., 2014; Macedo *et al*., 2016) or anthropogenic causes (Varnosfaderany *et al.,* 2010; Cuffney *et al.,* 2010; Buss and Vitorino 2010; Sharifinia *et al.,* 2012; Ortiz *et al.*, 2013). Macrozoobenthic invertebrate assemblages reflect the disturbances produced by the human activities on river environments (Azrina *et al.,* 2005; Heino *et al.*, 2007; Fishar and Williams, 2008; Harrgrave, 2010; Li *et al.,* 2012). Nowadays at global level the rivers are among the most affected ecosystems by various anthropic pressures such as pollution, hydrotechnical constructions, sand and gravel extraction, (Faria *et al.,* 2006; Cuffney, *et al.,* 2010; Alvial *et al.,* 2012). In Romania the most important pollutant sources of the surface waters are represented by the domestic untreated or insufficient treated waste water effluents, the sand and gravel extraction, hydrotehnical floodind protection constructions, or microhydropower systems constructions (Opreanu, 201; Șerban and Ionuș, 2011; Sinitean and Petrovici, 2012; Truță, 2014; Cismașiu *et al.,* 2017; Zanfir *et al.,* 2019). These phenomena affect the structure of the macrozoobenthic invertebarate assemblages in terms of density, species structure, the ratio of different feeding groups, the ratio of different tolerant taxonomic groups. (Pîrvu and Pacioglu, 2012; Mititelu *et al.,* 2012; Cioboiu *et al.* 2017). The ratio of different feeding groups is influenced by the food resource availability in the riverbed. The increased nutrient content will allow a higher number of autotrophic groups development and this will modify the ratio of different feeding groups (Mesa *et al.,* 2013; Marcovic *et al.,* 2015). Physico-chemical paramethers disturbances can also be reflected in the structure of the macrozoobenthic invertebrate assemblages (Sharifinia *et al.,* 2012; Hedrick *et al.,* 2013; Young *et al.,* 2014; Chang *et al.,* 2014; Marcovic *et al.,* 2015). The water quality influence the number of the pollution tolerant groups, the most sensitive groups density in the water will decrease as the water quality diminish (Varnosfaderany *et al.,* 2010; Li *et al,* 2012; Sidagyte *et al.*, 2013; Chang *et al.,* 2014).

There are several researches upon the macrozoobenthic invertebrate assemblages and the effect of pollution or of different anthropic activities in the Jiu River Basin (Onciu *et al.,* 2007; Cupșa *et al.,* 2010; Dumbravă-Doboacă and Petrovici, 2010; Șerban and Ionuș, 2011; Mititelu *et al.*, 2012; Cioboiu and Cismașiu, 2016; Cioboiu *et al.,* 2017; Cismasiu *et al.,* 2017; Cioboiu and Cismașiu,2018; Zanfir *et al*., 2019) as well as in other rivers from Romania (Momeu *et al.,* 2009; Badea *et al,* 2010; Răescu *et al.,* 2011; Marin *et al.,* 2011; Benedek *et al.,* 2013; Stoica *et al.,* 2014; Popescu *et al.,* 2016; Marin *et al.,* 2018).

The aim of our research was to investigate the structure of the macrozoobenthic invertebrates assemblages from two rivers in the Jiu River basin: Șușița Seacă and Sohodol. These rivers have a series of similar hydrological characteristics: riverbed width and depht, flow value, substrate, physico-chemical parameters, structure of the neibouring ecosystems, but different human impact.

Șușița Seacă is a tributary of Jiu River, it flows through a beech forest, an area with no human activities, there are no localities in the investigated sector, only a forest road along the riverbanks. Sohodol River is a tributary of Tismana, in it's upstream sector it flows through a beech forest where a touristic camp is found, in the lower sector it passes an open landscape, than a gorge area. Along this river portion during summer we observed touristic activities especially one day visitors and a few tent campers.

Because the two rivers pass through almost the same type of environments but they are subject to different human activities we wanted to investigate how the macroozoobenthic invertebrates assemblages are structured and if they show differences caused by the natural or anthropic factors.

Materials and methods

Study area

Șușița seacă is formed by the confluence of Straja and Măcriș rivulets at an altitude of 1400 m and flows into the Jiu at an altitude of 178 m. The total lenght of the river is 37 km and a surface of the river cathment of 234 km^2 (Administrația Națională Apelor Române - Cadastrul Apelor – București). It is situated in an area with no human activities, no localities. The river flows along a forest road used especially for forest exploitation in a beech forest ecosystem. The forest ensures a covering of the riverbed of 75-90% in the upstream area. The width of the river is between 5-10 m and a depht of 10 - 50 cm, in some places upstream with 1m deep pools. The riverbed is hard formed mainly by rocks and boulders in the upstream sectors and rocks, peebles and sand in downstream portions. The vegetation in the riverbed is absent except the first sample site where some groups of *Fontinalis* moss on the rocks. The bioderm is lacking on the upstream sites or it is very scrace downstream, in the sites with slower flow of the river small quantities of sediment deposits made up mainly by tree leafs from the nearby forest. In some portions antierozional concrete sills can be found in the riverbed.

Sohodol river originates form Vâlcan Mountains from an altitude of 680 m and it flows in River Tismana close to the Godinești village at an altitude of 180 m. The lenght of the river is 14 km with an area of the catchment system of 66 km^2 (Administrația Națională Apelor Române - Cadastrul Apelor – București). It passes through an area of upstream course covered by beech forests and in the lower sector it passes a more open area where nearby small pastures are present. Upstream there is a camp area frequently visited by turists and the middle and downstream sectors are also visited by tourists for the spectacular gorges situated here. The width of the river is between 5-6 m upstream and 10-12 m downstream. In the upper sector it has a turbulent flow and it has antierozional concrete sills in the riverbed. The riverbed in this portion is covered by stones and pebbles. In the downstream sector the width of the riverbed is greater, hard bottomed, covered with a small amount of bioderm.

Water and macroinvertebrate sampling

The samples were collected from five sample sites on the river Șușița and 3 sample sites from river Sohodol, during 2019 summer, at low water flow period.

Water samples were taken from each sampling site. The following paramethers were determined on the field using a portable WTW 410i multiparameter: water temperature, conductivity, pH, dissolved oxygen content, total dissolved solids (TDS).

From each sample site three samples were taken. The samples were collected with a Surber sampler with a sampling surface of 0,1 m^2, and a 250 µm mesh size. The samples were fixed in the field in 4% formalin in plastic bags, labeled and transported in laboratory. In the lab the samples were sorted using a 10x – 40x magnifying stereomicroscope. The sorted samples were stored in 80% ethanol and determined to family, genus or species level using specific keys (Aubert, 1959; Ujhelyi, 1959; Steinmann, 1968; Elliot *et al*, 1988; Solem and Gullefors, 1996; Bouchard, 2004).

Data analysis

The commonly used non-parametric community structure indices were calculated: number of individuals (N), number of taxa (S), the abundance of taxa (% taxon), the proportion of Ephemeroptera, Plecoptera and Trichoptera (% EPT), the proportion of Chironomida (% Chironomida), EPT/Ch value, diversity indexes (Margalef, Berger-Parker, Simpson, Shannon-Wiener, Pielou).

The value of BMWP scoring for each sample sites was calculated by adding the individual scores of the families (Armitage *et al*., 1983).

The degree of similarity between the macroinvertebrate assemblages was tested on the basis of Jaccard simmilarity, Bray-Curtis simmilarity, one way analysis of variance (ANOVA). Kruskal-Wallis test for equal medians and Mann Whitney test were conducted to test significant difference of assemblages between sites. All statistical analysis was performed using the SPSS software (version 10).

Results

The values of the physico-chemical paramethers

The physico-chemical parameters from the analyzed rivers sample sites were relatively constant in values. The pH had alkaline values in both rivers in each sample site. In the case of Sohodol the pH values were more constant than in Șușița (Tab. 1).

The temperatures were relatively low different from the air temperatures situated between 28-32^{0}C during sampling. The lowest temperature was 13.5^{0}C on Șușița at sample site Su4, after the confluence with a rivulet; the highest temperature in Șușița river was registered in the last sample site from downstream Su5 - 21^{0}C (Tab. 1). In Sohodol river the lowest temperature was recorded in the first sample site from upstream So1 – 14.7^{0}C and the highest downstream at So3 - 16^{0}C (Tab. 1).

The amount of the dissolved oxygen was lower in Șușița (8.46 mg/L - Su 1) in the upstream sites than in Sohodol (9.42 mg/L – So1) (Tab. 1). The measured conductivity values were lower in Șușița (85-134 µS/cm) than in Sohodol (200-204 µS/cm) (Tab. 1). The values of total dissolved solids (TDS) were also lower in Șușița (43-68 mg/L) than in Sohodol (92-110 mg/L) (Tab. 1).

The river is narrower in the case of Șușița (6.5 m – 7.5 m) than in Sohodol (5.5 m – 11 m) and deeper in Șușița (20 cm – 55 cm) compared with Sohodol (20 cm – 25 cm) (Tab. 1).

Table 1. The mean values of the physico-chemical parameters at the investigated sites.

Sample sites	pH	T (^{0} C)	Dissolved O_2 (mg/L)	Conduc tivity (µS/cm)	TDS (mg/L)	River width (m)	Water depth (cm)
Su 1	7.93	14.3	8.46	85	43	6.5	55
Su 2	7.83	14.5	8.89	87	46	5.5	30
Su 3	7.91	14.4	8.96	95	48	6.5	20
Su 4	8.12	13.5	9.43	133	65	7.5	25
Su 5	8.66	21	9.01	134	68	3.5	30
So 1	8.35	14.7	9.42	200	92	11	20
So 2	8.50	15	8.75	202	97	5.5	15
So 3	8.47	16	8.65	204	110	11	25

The macrozoobenthic invertebrate assemblages

We have collected a number of 34 taxonomic groups from river Susita and 30 from Sohodol. The maximal number on sample sites was 24 (Su4) in Susita and 26 (So3) in Sohodol, and the minimal number 21 (So2) in Sohodol and 13 (Su1) in Susita (Tab. 2).

The most of the groups were represented by aquatic insect larva (Ephemeroptera, Odonata, Plecoptera, Trichoptera, Diptera) or adults (Coleoptera) in both investigated rivers. Beside the insect groups we have found also Turbelariata, Oligochaeta, Gastropoda, Gammarida, species in each river and in some sample sites from Șușița River also Nematomorpha specimens in small numbers.

The total number of collected specimens was 2812 (1450 from Sohodol and 1362 from Șușița). The number of specimens was between 41 (Su1) - 384 (Su4) in the case of Șușița river and 390 (So2) – 562 (So1) in Sohodol river (Tab. 2).

The percent of the taxa, was calculated as the ratio between the number of taxa found in one sample site and the number of taxa found in the whole investigated river section. The obtained values were between 38.24 (Su1) and 70.59 (Su4) in the case of Șușița river and between 70 (So2) and 86,67 (So3) in Sohodol river. The percent of Ephemeroptera, Plecoptera and Trichoptera (% EPT) was situated between 60.16% (Su4) and 69.28% (Su5) and the percent of Chironomida larva (%Ch) between 10.24 (Su5) and 26.83 (Su1) so the EPT/Ch ratio varied between 2.36 (Su1) and 6.77 (Su5) (Tab. 2).

In Sohodol river % EPT varied between 62.10 (So1) and 73.59 (So2), the % Ch between 2.05 (So2) and 7.83 (So3) so the EPT/Ch ratio has much higher values in Sohodol between 8.9 (So3) and 35.9 (So2) than in Șușița (Tab. 2).

The biodiversity indexes have very close values in the two investigated water courses and also between the sample sites in each river. Margalef index was between the maximal values of 3.87 (Su4) and 4.03 (So3) and minimal values of 3.22 (Su3) and 3.35 (So2) (Tab. 2). The Berger-Parker index was maximal 0.4 at Su3 and 0.37 So 3, and minimal 0.17 at Su2 and 0.28 So1 respectively (Tab. 2). The Simpson index has the maximal values 0.21 in Su3 and 0.19 in So3, and the minimal values 0.09 Su2 respectively 0.14 So1. The Simpson diversity was the highest 0.96 at Su2 and 0.86 So 2 and the lowest 0.79 in Su3 and 0.81 in So3 (Tab. 2). The Shannon-Wiener index was situated between 2.7 at Su2 and 2.18 at So 1, and 2.09 at Su3 and 2.12 at So2. The evenness (Pielou index) has also very uniform values between the sample sites and the two investigated rivers with maximal values of 0.87 at Su2 and 0.77 So3 and minimal values of 0.7 at Su3 and So2 (Tab. 2).

The feeding groups were present in different proportions in both rivers except the parasites which were represented by Nematomorpha specimens found only in Șușița River. From all feeding groups we have highlighted the proportion of collectors/gatherers with higher proportions in Sohodol between 57.47% (So1) and 66.67% (So2) and lower in Șușița between 19.51% (Su1) and 52.41% (Su5) (Tab. 2). The proportion of shredders was between 1.41% (So3) and 3.33% (So2), and between 0 (Su1) and 8.79 (Su2) in the case of Șușița River (Tab. 2).

The values of the BMWP score were higher in Sohodol 112 (So1) and 140 (So3) and much more variable in Șușița between 50 (Su1) and 114 (Su4) (Tab. 2). The values of the BMWP score were obtained especially from the presence of the sensitive groups of Ephemeroptera, Plecoptera and Trichoptera in almost all investigated sample sites.

Table 2. The values of the number of individuals (N), taxa (S), the abundance of taxa (% taxon, S/total number of taza from the investigated river*100), the proportion of Ephemeroptera, Plecoptera and Trichoptera (% EPT), the proportion of Chironomida (% Chironomida), EPT/Ch value, diversity indexes (Margalef, Berger-Parker, Simpson, Shannon, Pielou) % of collector/gatherer and % of shredder feeding groups, value of BMWP scoring for each sample site

Indexes	Su 1	Su 2	Su 3	Su 4	Su 5	So 1	So 2	So 3
N	41	239	366	384	332	562	390	498
S	13	22	20	24	23	23	21	26
% taxa	38.24	64.71	58.82	70.59	67.65	76.67	70	86.67
% EPT	63.41	66.53	65.85	60.16	69.28	62.10	73.59	69.68
% Ch	26.83	15.06	10.93	21.88	10.24	2.31	2.05	7.83
EPT/Ch	2.36	4.42	6.02	2.75	6.77	26.88	35.90	8.90
Margalef	3.23	3.83	3.22	3.87	3.79	3.47	3.35	4.03
Berger Parker	0.27	0.17	0.40	0.28	0.32	0.28	0.32	0.37
Simpson index	0.15	0.09	0.21	0.15	0.14	0.14	0.18	0.19
Simpson diversity	0.85	0.91	0.79	0.85	0.86	0.86	0.82	0.81
Shannon	2.18	2.70	2.09	2.31	2.45	2.38	2.12	2.25
Pielou	0.85	0.87	0.70	0.73	0.78	0.76	0.70	0.77
% collectors /gatherers	19.51	38.91	31.69	30.47	52.41	57.47	66.67	64.46
% shredders	0	8.79	1.09	0.78	4.52	2.49	3.33	1.41
BMWP score	50	104	96	114	104	112	116	140

CCA between the physico-chemical parameters and the diversity indexes in the sample sites of the two investigated rivers showed a weak negative correlation between pH and number of taxa, Shannon-Wiener and Pielou indexes and a weak positive correlation with the number of individuals (Fig. 1).

The conductivity is strong negatively correlated with the number of individuals and weak positive correlation with number of taxa, Shannon-Wiener and Pielou indexes, the dissolved oxygen values are weak positively correlated with all indexes, total dissolved solids (TDS) are strongly positive correlated with the number of individuals and weak with number of taxa, Shannon-Wiener and Pielou indexes, the riverbed width is weak positive correlated with the number of individuals, strong negative correlated with the number of taxa and Pielou index and weak negatively correlated with Shannon_Wiener index (Fig. 1). The riverbed depth is strong positively correlated with the number of individuals, taxa and Pielou index and weak positive correlation with the Shannon-Wiener index (Fig. 1).

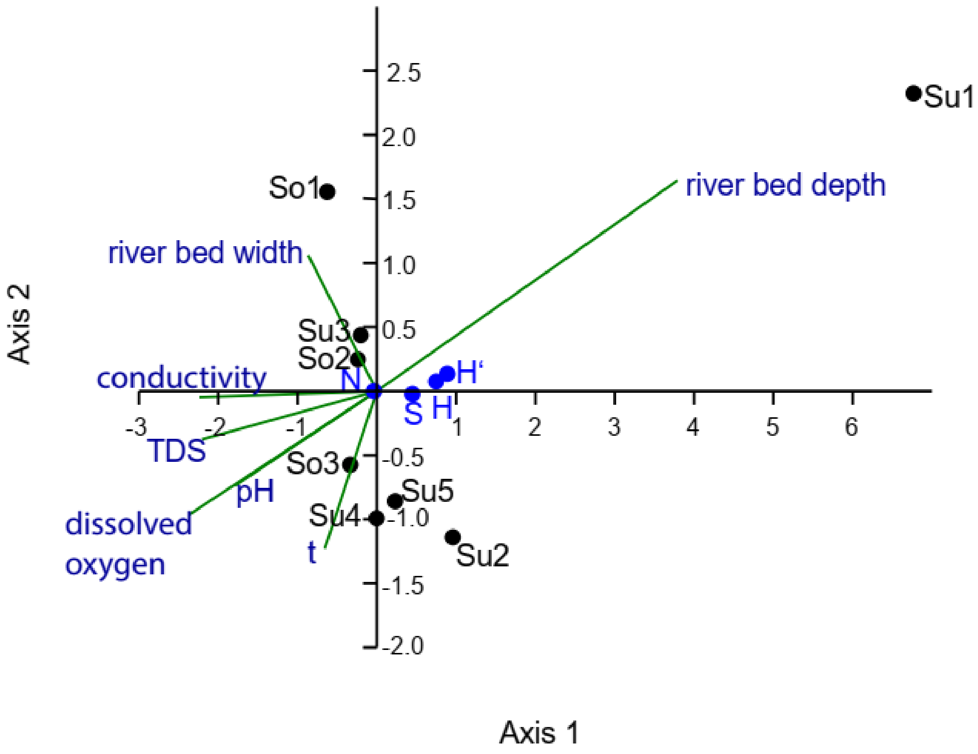
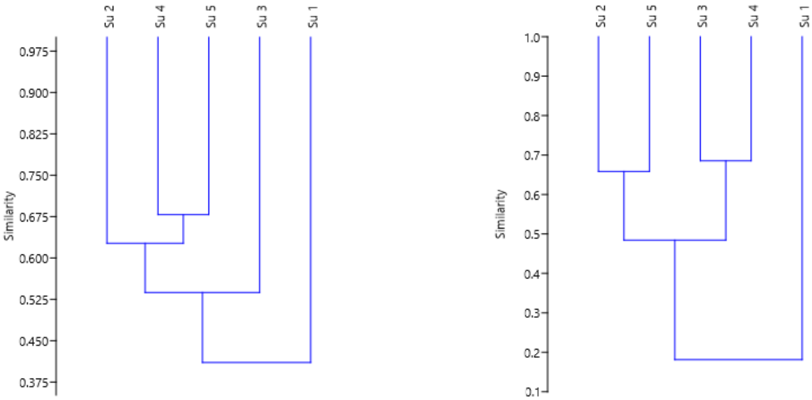


Figure 1. The result of the canonical correspondence analysis (CCA) based on the diversity indexes values with respect to environmental variables. Green lines represent the environmental variables, the blue circles the indexes and the black circles the sample sites. N= number of individuals, S= number of taxa H= Shannon-Wiener index, H`= Pielou index.

The Jaccard correlation index between macrozoobenthic invertebrate communities in river Şuşiţa was the highest between sample sites Su4 and Su5, 0.68 respectively 0.67, and with Su2 (0.49 and 0.66). The lowest value of the Jaccard index was between the Su1 sample site and the rest of the sites (Fig. 2A).

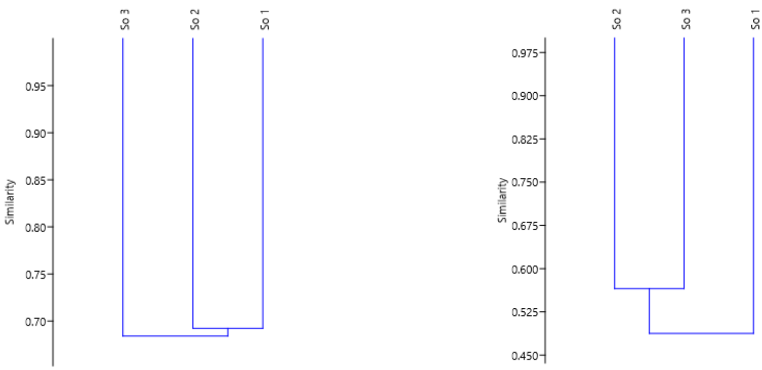
The Bray Curtis index shows the lowest similarities between the Su2 and Su5 0.66 macrozoobenthic communities on one hand and Su3 and Su4 0.68 on the other hand. These two communities form a separate branch of the cladogram on one side outstanding from the community of Su1 sample site which form another branch of the cladogram, results which look very alike as those obtained from the Jaccard index (Fig. 2B).



A **B**

Figure 2. Jaccard (A) and Bray-Curtis (B) similarity indexes between the macrozoobenthic communities along Șușița River

Between the macrozoobenthic communities along Sohodol river the Jaccard similarity index was the highest between So1 and So2 0.69 and lower with So3, but above 0.67 so a much higher similarity than in the case of Șușița river communities. According to the Bray-Curtis similarity the communities from So2 and So3 where less similar between each-other 0.57 and more with So1 0.48-0.50 (Fig. 3).



A B

Figure 3. Jaccard (A) and Bray-Curtis (B) similarity indexes between the sample sites along Sohodol River

One way ANOVA was calculated in case of macrozoobenthic invertebrate groups between the sample sites and showed no significannt differences between the communities from Șușița (p=0.169), from Sohodol (p=0.797) and from both rivers (p=0.266). Kruskal-Wallis test for equal medians have shown significant differences between the medians of the samples in Șușița (p=0.0115) and between the median of all samples (p=0.0255).

Table 3. The values of Mann-Whittney test for all communities from the sample sites on the two investigated rivers.

Sample sites	Su 1	Su 2	Su 3	Su 4	Su 5	So 1	So 2	So 3
Su 1		**0.004**	**0.023**	**0.002**	**0.002**	**0.002**	**0.019**	**0.001**
Su 2	**0.004**		0.620	0.876	0.851	0.594	0.645	0.519
Su 3	**0.023**	0.620		0.520	0.501	0.342	0.965	0.276
Su 4	**0.002**	0.876	0.520		0.992	0.773	0.497	0.679
Su 5	**0.002**	0.851	0.501	0.992		0.748	0.506	0.655
So 1	**0.002**	0.594	0.342	0.773	0.748		0.361	0.890
So 2	**0.019**	0.645	0.965	0.497	0.506	0.361		0.272
So 3	**0.001**	0.519	0.278	0.679	0.655	0.890	0.272	

The values of Mann Whitney test between the sample sites showed significant differences between the communities Su1 from Șușița river and the other macrozoobenthic invertebrate communities from the two investigated rivers ($p<0.05$). The rest of the sample sites from both rivers do not show significant differences in their macrozoobenthic communities according to this test $p>0.05$ (Tab. 3).

Discussion

The pH values of the two investigated rivers are alkalinne which is common for the unpolluted rivers and streams in temperate climate (Challar *et al*., 2011; Sharifinia *et al*., 2012; Li, *et al*., 2012; Nguyen *et al*., 2014). The lower pH in Șușița river in the upstream sample sites is due to probably to the higher content of SO_4 measured in these sample sites. The SO_4 ions combine with water and increase acidity of the environment. The SO_4 content is higher in Șușița than in Sohodol river probably due to a different lithoological substrate, considering that the river flows very close to the Rovinari mining basin. In the area there are lignite resources and this type of coal have a high content in sulfur compounds (Cristesco and Sandor, 1929; Iamandei and Diaconu, 2013). We asume that the SO_4 comes from natural sources because the riverbed in the investigated area is hard with very low amount of organic deposits and content, the temperature is low, the disolved oxygen has a high value and the water has a rapid flow, so the bacterial decomposing activities which can increase the SO_4 content (Ruuskanen et al., 2018) are almost absent.

The temperatures have low values at the sample sites situated in the forrested regions, where the water surface is shadowed by the forrest and the water remains cool. Downstream in the sample sites which are exposed to the sunlight the water tempertaure is higher, but compared to the air temperature is lower. In these areas the insolation period is short during the day because both rivers flow through a hilly region with high slopes close to the riverbed. These slopes shadow the river for the most part of the day.

The oxygen content of the two rivers corresponds to the normal values for a hilly water course, on a hard substrate and slightly or not affected by human activities (Chadar *et al.,* 2011; Sharifina *et al.,* 2012; Pîrvu and Pacioglu, 2013; Nguyen *et al.,* 2014).

The values of the water conductivity are lower in Șușița River than in Sohodol based on the different ions content of the two water courses. Both rivers have values which are easily tolerated by the macrozoobenthic invertebrates and similar to those of other rivers which flow on silicious substrates (Benetti *et al.,* 2012; Marcovic *et al.,* 2015).

The higher values of TDS in Sohodol can result in part from the diffuse organic pollution produced by human activities in the area and by the allohtone substances washed by the high level waters from the river banks (Chadar *et al.,* 2011; Chang *et al.*, 2013)

The number of taxonomic groups was slightly higher (34) number in Șușița River than 30 în Sohodol. In both rivers we have found the important groups of macrozoobenthic invertebrates characteristic for this type of water courses (Turbelariata, Oligochaeta, Gastropoda, Gammarida, Ephemeroptera, Odonata, Plecoptera, Coleoptera, Trichoptera, Diptera). In Șușița we have also find Nematomorpha specimens, which are aquatic only in the adult period of their life and can be find in the rivers only during summer months by chance due to their short adult stage and their low density (Hanelt and Janovy, 1999). In Șușița we have found one specimen of Heteroptera in the last sample site from the downstream (Su5) and 4 specimens of Gomphidae (Odonata) at sample site Su3. The Odonata specimes were found in a sample site where that water flow is slower and these insect larvae can find an adequate environment for their development (Kalkman et al., 2008; Dijkstra and Kalkman, 2012). Also here they have enough preys to be able to ensure their food necessities. The Heteroptera was found in sample site Su5 which is situated closer to other water bodies and to localities, they usual install in perturbed water bodies (Tchakonté *et al.,* 2015).

We observed also some differences concerning the presence of different families from order Ephemeroptera and Trichoptera, for example in Șușița we have found Leptophlebia specimens and in Sohodol Ephemera specimens. Leptophlebia species species which have a tolerance value of 10 according to BMWP scoring so they are characteristic to very good quality waters (Azrina *et al.,* 2005; Li *et al.,* 2012). Ephemera species are burrowing larvae so they need soft sediments at least near to the riverbanks to be able to settle in a stream (Lee *et al*., 2008).

The smallest number of taxonomic groups (13) and also specimens (41) were found in the first sample site from upstream of Șușița River. At this sample site the water flow is turbulent, with high velocity, the riverbed is covered by boulders and big stones which have no bioderm on their surface. These conditions do not allow a high number of species to settle in that type of riverbed because they haven't got enough food resources and they are exposed to downstream drift during high water levels after heavy precipitations (Li *et al.,* 2012).

The number of specimens/sample sites vary between normal values for this type of rivers and substrate (Momeu *et al.* 2009; Răescu *et al.,* 2011; Benedek *et al.,* 2013). The hard bottom of the rivers and the low amount of the sediments allow a small number of specimens to develop in the benthos due to a relatively scarce trophic offer.

The % of taxa on sample sites was high between 70%-86,67%, these values indicate a high stability of the environmental conditions, stability which ensure the same structure of the macrozoobenthic assemblages. (Hedrick *et al.,* 2010; Benetti *et al.,* 2012). The only sample site with a lower % of taxa is the first site from upstream Șușița 38,24%. Here the assemblage of the macroinvertebrates is less stable due to the caracteristics of the river in this portion mentioned above.

The % EPT was high in every sample site above 60% which reflect together with the low %Ch and high EPT/Ch ratio the fact that the water has a good quality in both rivers in each sample sites (Dumbravă-Dodoacă and Petrovici, 2010; Mititelu *et al*., 2012; Sinitean and Petrovici, 2012; Chang *et al.,* 2013). Although the Ephemeroptera species are considered to be very sensitive species to disturbances and Chironomids are considered as organic pollution indicators, there are species in the case of both groups which do not respect these general rules (Li *et al.,* 2012; Nguyen *et al.* 2014). There are several Ephemeroptera species which can tolerate moderate pollution (Moja *et al.,* 2011; Li *et al.,* 2012; Chang *et al.,* 2013) and also Chironomid species which are very sensitive to the water quality (Moja *et al.,* 2011).

The value of the Margalef index was relatively constant between the sample sites and slightly lower in Șușița compared to Sohodol River. The values of the index are obtained based on the total number of species and total number of individuals/sample. Due to this fact there is a linear relationship between the species richness (S) and the value of Margalef index (Gamito, 2010) (Tab. 2).

The Berger-Parker index have the highest values in sample sites So3 and Su3. This index has high values in sites dominated by the most common species (Olszewski, 2007) and it's increasing values can be a measure of an increasing disturbance (Caruso *et al.,*2007). In the sample sites which have the highest value of the index the assemblage is dominated by common species for this river sector such as Gammaridae, Baetidae and Hydropsychidae in Șușița and Gammarida and Baetida in Sohodol.

Simpson index and Simpson diversity shows high diversity in each sample site but because this index is not so sensitive to the less abundant species in the sample their value do not reflect the complete picture of the biodiversity from a sample site (Keylock, 2005). The Shannon-Wiener index has values between 2,7 and 2,09 showing a high diversity in each sample sites for both rivers at numerical values which are common to this type of river sectors (Azrina *et al.,* 2005; Sharifinia *et al.,* 2012; Mesa *et al.,* 2013; Benedek *et al.,* 2013).

The evenness (Pielou index) also have high values in each sample site which reflects together with Shannon-Wiener diversity index the existence of well-established benthic invertebrate assemblages in undisturbed or slightly disturbed environments (Azrina *et al.,* 2005; Mesa *et al.,* 2013; Benedek *et al.,* 2013).

The proportion of different feeding groups can be a measure of the perturbance of the river environment (Mesa *et al.*, 2013). Collectors/gatherers and shredders are the major primary consumers in forest streams which ensure the link between the organic input and the predator groups (Cheshire *et al.*, 2005). Shredders prefer leaf detritus colonized by microbial and fungal organisms which usually occur in river sectors from forest areas and collectors/ gatherers prefer fine detritus especially sediment related detritus but in some extent also transported detritus (Cummins, 1979). In wooded streams the ratio between shredders and collectors/gatherers during summer season was 0,125 and the annual mean ratio was 0,6 (Cummins, 1979). In our study the ratio between these two feeding groups varied between 0,02 and 0,04 for Sohodol showing a scarce resource of leaf litter in the water although it flows through a forested area. Despite this the leaf litter can't accumulate in the substrate because the drift of the vegetal detritus during high water flows in the spring period. In Șușița River the ratio between the two feeding groups was between 0,23 and 0. The Su2 sample site had the highest ratios due to the particularities of the riverbed. It is covered by stones and pebbles, between these the leaf litter can remain captive even at high flowing rates and allow the shredders to install. At normal flows the water has a laminar flow which allows the fallen leaves to sink to the substrate easily that in the first sample site. At the first sample site from upstream Su1, the riverbed is covered by boulders and stones, the water has a turbulent flow and it washes away the leaves fallen from the trees near the riverbanks, that is why we did't find any shredder specimens.

The small proportion of shredders is also frequent in tropical streams but the cause of their scarcity is the low quality of the leaf litter which contain a lot of tanin which makes it difficult to decompose (Oliveira and Nessimian, 2010; Mesa *et al.,* 2013).

The water quality was asses using BMWP score. The water quality is very good in each sample site except Su1 where the water quality is medium corresponding to a BMWP score 50. This site is situated in an unimppacted area, but because of the hydrological caracteristics, the macrozoobenthic assemblage is not very stable and diverse, beeing frequently washed out by the turbulent water flow especially after heavy precipitations. So the apparently lower water quality is given in fact by the unstable environment which cause a perturtance in the assemblage. Other authors (Moja *et al.,* 2011) also showed the fact that the BMWP score do not reflect the absolute quality of the water because it is based on family level scoring of the tolerance values. The tolerance values at family level can represent intermediate values of the species tolerance (Armitage *et al.,* 1983) so the indices at family level can over or underestimate the tolerance level of the species (Varnosfaderany *et al*., 2010). However the indces at family level is used because the species identification is a very time consuming procedure and sometimes taxonomic experts for each group are not available. The value of the BMWP score give enough accuracy to be largely used for water quality assessment in several countries worldwide both in Europe: UK (Armitage *et al.,* 1983), Greece (Artemiadou and Lazaridou, 2005), Italy (Solimini *et al.,* 2000), Poland (Czerniawska-Kusza, 2005), Spain (Zamora-Munoz *et al.,* 1995), Portugal (Faria *et al.,* 2006), in Asian countries: Thailand (Mustow, 2002), Malaysia (Azrina *et al.,* 2006), Hindu and Kush-Himalaya region (Ofenböck *et al.,* 2008), African countries: Egipt (Fishar and Williams*,* 2008), South American countries: Brazil (Silveira *et al.,* 2005), North-American and (Ruiz Picos *et al*., 2017), Central American ones: Costa Rica (Sedeño-Díaz et al., 2012)

The weak negative correlation between pH and number of taxa, Shannon-Wiener and Pielou indexes shows that the pH value is an important limitative factor for the macroinvertebrate assemblages structure (Marcovic *et al.,* 2015). They are sensitive to the variation of the pH values especially when it exceed the tolerance limits for some species. In the investigated rivers the pH tend to have a more alkalinic value due to the natural mineralization of the water and very low microbiological activity. The pH and TDS shows also a weak positive correlation with the number of individuals which increase together with the decrease of the species number according to the area-species curve theory (Williams, 1964).

The conductivity has a weak positive correlation with number of taxa because conductivity usually is positively correlated with the majority of the nutrients in the water (Chalar *et al.,* 2011). The nutrients to some extent are necessary for community development in a water body especially in the case of the autotrophic species which represent trophic base for benthic invertebrates.

The weak positive correlation between the riverbed width and the number of individuals as well as the strong positively correlation between the riverbed depth and the number of individuals is according to the area-species curve theory (Williams, 1964). Especially is the river width comes also with a great diversity of microhabitats, it creates favorable environmental conditions for a great diversity of invertebrates. The depth in the investigated rivers remain below 1 m, so it not represents a limiting factor for the development of the macrozoobenthic invertebrates.

The riverbed width is negative correlated with Pielou and Shannon_ Wiener index because the multitude of microhabitat encountered with the increasing value of river width decrease the evenness in the river sector. These two diversity indexes are positively correlated with the river depth because in the investigated rivers the depth variance is not very high (Tab. 1) so this factor do not create as much microhabitat types as the variation of the river width.

Jaccard similarity index shows the highest similarity between sample sites 4 and 5 from Șușița River which are the last two sites from downstream, they have the most similar hydrological parameters so their assemblages look very much alike. The next site with high similarity with the above mentioned is Su2 characterized by similar hydrology. The Su3 site situated between Su2 and Su 4 has a more turbulent flow and shallower water. These characteristics determine the establishment of a more different macrozoobenthic assemblage than in the above mentioned sites. The first site from upstream Su1 is less similar with the others because has the most turbulent flow, deeper water and a substrate dominated by boulders, here the assemblage is made up by a very few number of species and individuals due to frequent disturbances caused by the water volume variation determined by the rainfall volume.

In Sohodol River the most similar are the sample sites So1 and So2 situated closer to each other than So3 to So1. This similarity is according to the gradual variation of the macrozoobenthic assemblages along a water course (Răescu *et al.,* 2011; Benedek *et al.,* 2013). The first two sample sites from upstream even if they suffer some anthropic impact due to the camping site near So1, are more similar, because they have almost the same width, the river flows through a forested area in these sectors, unlike So3 which flows through an open sector, which is more exposed to the sun during daytime.

The Bray Curtis index shows two different clusters of sites with high difference between their assemblages structure in Șușița River Su2 and Su5 one hand and Su3 with Su4 one the other hand. These two groups of sites differ a lot in their hydrological characteristics that's why they develop slightly different macroinvertebrate assemblages. Su1 sample site forms another branch of the cladogram also according to this index due to the reasons mentioned above.

In Sohodol River the Bray Curtis index revealed the same similarities between the sample sites as Jaccard index, namely less similar are sample sites So2 and So3 than So1 and So2.

The one-way ANOVA showed no significant differences between the sample sites, those are situated in the same hydrological basin, the Jiu River basin, Șușița being a first rank tributary and Sohodol a second rank tributary of this river. They flow through similar habitats represented by deciduous beach forests, with minimal human impact in the case of Șușița River and a slight human impact generated by seasonal touristic activities in the case of Sohodol River. In this situation the macroinvertebrate assemblages along the river do not differ very much, because the river do not change very much its flowing characteristics and the total length of the two rivers is not very high. The only significant differences between the macrozoobenthic invertebrate assemblages were shown by the medians of the samples revealed by the Kuskall-Wallis test which emphasize the gradual differences between the sample sites along the river continuum (Sedell *et al.,* 1989; Yates *et al.,* 2018). Also a significant difference between sample sites was revealed by Mann Whitney test between sample site S1 and the other sample sites from the two investigated rivers. This significant difference is due to the very different hydrological and habitat characteristics of this site on Șușița River as it was mentioned above, which lead to a different assemblage structure in this site.

Conclusions

Our study revealed the fact the two analysed rivers have a very good water quality except Su1 sample site mainly because natural causes determined by the hydrological characteristics of the water as we mentioned above. In Sohodol the water quality was very good in every sample site, so the tourism in nearby the river do not affect the water quality for the moment.

The macrozoobenthic invertebrate assemblages in the two rivers are very similar, due to the similar dimensions, hydrological and physico-chemical characteristics of the investigated water courses. The only one site which has a very different assemblage is Su1 where the natural disturbances are high caused especially by the waterflow differences between periods with heavy precipitation and periods with drought.

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