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RESEARCHES AND STUDIES REGARDING THE MICROBIAL INDICATORS OF WATER POLLUTION OF CASTAILOR CREEK, BISTRITA

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Abstract

The pollution of the aquatic ecosystem is an issue with great concern all over the world and due to this fact a national and international call of urgency has been made for a better management and better policies of water resources. In assessing the water quality, classical methods are no longer sufficient to express the complexity of the effects that pollutants have over the environment. Therefore, advanced researches have been made to acquire better methods and instruments for a cleaner environment. Utilizing microorganism and their metabolic activity for evaluating the water condition is not totally new but the research over these methods is improved by every study that is made bringing fresh perspectives in this area. In order to evaluate the quality of water and sediment, samples have been taken from three main areas of Castailor Creek (upstream of the city, industrial area and downstream of Bistrita city). Some groups of microorganisms, used as pollution indicators, have been assessed, and also the effect of pollutants on creek micro biota. The physical-chemical parameters of water from different sampling points were also determined. The quantitative enzyme activity has been assessed: actual and potential dehydrogenase activity, catalase and phosphatase activities. Based on the results, the bacterial and enzymatic indicators of water and sediment quality from Castailor Creek were also calculated. The main purpose of this research is to assess microbiological and enzymological indicators of pollution in the water and sediment of Castailor Creek, Bistrita.

Key words*: bacterial and enzymatic indicators, coliforms, water quality*

INTRODUCTION

Water is one of the essential resources for socio-economic development and for maintaining a healthy ecosystem (Pander and Geist, 2013). With the population growth and the development of human settlements increase, water consumption has also increased, thus pressures on water resources has intensified, resulting in water scarcity, quality deterioration and pressure over aquatic biodiversity (UN-Water, 2012; Ercin and Arjen, 2012).

Microorganisms, as recycling agents (Coelho et al., 2015), are responsible in maintaining the biosphere, take part in the degradation processes, transformation processes and recovery of the nutrients presents in water (National Research Council, 1993). Microbial flora that is found in water can be classified into two categories: natural microbial flora and microbial flora of contamination (Munteanu et. al., 2011). When in the environment appear new substances, some of these may inhibit the metabolic activity of certain organisms, and other substances can intensify it (Filimon et al., 2010).

Understanding the role of bacteria in ecosystems and biogeochemical circuit of the elements, knowing the link between growth, abundance and diversity of microorganisms in the environment, can be used as indicators of environmental quality and have been studied by other authors (Muntean, 1996; Carpa et al., 2009; Bodoczi et al., 2010; Farkas et al., 2013).

Microbiological indicators shows a generally seasonality, with higher rates in the warm season; hence, temperature affects the dynamics of bacteria in the environment (Janelidze et al., 2011).

In the sediments of rivers there are always a greater number of organisms than in the mass of the water, and enzymatic activity provides suggestive data on the processes occurring in the environment, in a shorter time than microbiological analyzes (Orban et al., 2010; Bodoczi et al., 2010).

Creek Castailor flows from North of the city, enter into the industrial area, crosses the city and flows in Bistrita River, accumulating pollutants on its way and transporting them to the main river of the city (BLOM Romania, 2011). There is no biological monitoring for this water flow; therefore we consider that this paper will make a great addition in information regarding the ecological status of water quality, and draws attention about pollutants effect over biotic environment.

MATERIALS AND METHODS

Site sampling. In autumn of 2012, samples of water and sediment were collected from 3 different points of Castailor Creek, Bistrita, in order to test the water quality. Therefore, three sampling points were chosen for study: 1-upstream (Castailor Valley before entering the industrial area), 2 -industrial area (the place where is intersected with Industry Rout and the railways), and 3 at the confluence with Bistrita River.

Physical parameters. To determine the actual acidity of water and sediment, the potentio-metric method was used for determination of pH values, which is based on measurements of the hydrogen ions activity, using a pH meter with a calomel electrode capable of reading the water or sediment reaction, directly on device monitor. For measuring the oxidation-reduction potential, E_h-meter was calibrated before each determination and the data was read from Mettler-Toledo device.

Microbial analyses. In order to analyze the water bacteriological status, the number of aerobe heterotrophic bacteria, was determined using the method of turning plates, by inoculating the sample or decimal dilutions, with bullion agarized medium incubated at 37°C for 48 hours (Dragan-Bularda, 2000; Carpa et al., 2014)

To determine the probable number of coliforms, the multiple tube method was used. The presence of the total coliforms was highlighted by the presumptive test, inoculating water and decimal dilutions in a number of vials and test-tubes with a liquid media; the positive reaction was evidenced by a confirmatory test on solid medium at 37°C after 24 hours. Based on the number of positive tubes confirmed, the probable number coliform bacteria was calculated utilizing the McCrady's table (Tillett, 1987; Carpa et al., 2014).

Enzymatic analyses. Among the enzymatic activities, the fallowing four have been sudied: actual and potential dehydrogenase (ADA and PDA) - activities expressed in mg formazan/g sediment (Casida, 1977); phosphatase (P)- activity expressed in gfenol/g sediment (Krámer and Erdei, 1959); catalase (C) – activity expresssed in mg splitted H_2O_2/g sediment(Gianfreda and Bollag, 1996).

RESULTS AND DISCUSSIONS

1. Physical-chemical characteristics

The actual acidity (pH) is very important because it influences physiological groups of bacteria (Qian et al., 2014); their activity is optimal at certain values of it (Ríos et al., 2017).

The redox potential (E_h, ORP) is used in water quality assessments because measures the capacity of chemical spices to aquire electrons and by that be reduced (Suslow, 2004). A high redox potential indicates a greater affinity of chemical species for electrons and a greater tendency to be reduced, therefore, an aerobic enviroment (Malschi, 2015). Redox potential is mesured in volts (V) or millivots (mV).

In all water sample actual acidity (pH) and redox potential (E_h) has been determined by potentiometric method using Mettler-Toledo pH-E_h meter.

As shown in Table 1, the lowest pH was recorded in the industrial area. This is due to the fact of pronounced pollution in this area. In the same time, it can be seen that in the same point E_h was the lowest too.

Table 1. Actual acidity values and oxidation-reduction potential of the Castailor Creek (autumn 2012)

Sampling points	pH	Reactions Classes	Eh (mV)
Upstream	6.8	Very weakly acidic	90
Industrial area	5.2	Acidic	61
At confluence	6.3	Weakly acidic	72

2. Microbial analyses

This study on the dynamics of bacterial population in the sediment and water from the Castailor Creek, Bistrita, is a necessity given that there is no data on the role, function and dynamics of bacteria in the investigated water flow.

The aerobic heterotrophs bacteria. (BHA) constituted the largest eco-physiological group of bacteria and it is used as a global indicator of microbiological water quality (WHO, 2008).

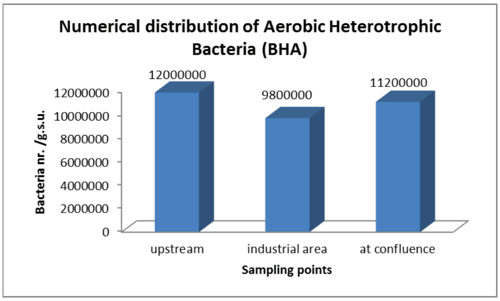

Figure 1. Distribution of the aerobic heterotrophic bacteria (BHA) from three sampling points of Castailor Creek, Bistrita

It can be seen from Figure 1 that the greatest abundance of BHA is upstream (point 1), which means that the water quality level in this area is a little higher than in the others. The sampling with the lowest distribution is the point of industrial area (2). This may be due to the presence of heavy metals or other inhibitors of micro-organisms proliferation (Gikas, 2008).

The coliform bacteria represents the most common bio indicator of fecal pollution, and *Escherichia coli* and other coliforms like *Klebsiella* or *Enterobacter* are methodical targeted (UNESCO/WHO/UNEP, 1996).

To determine the probable number of coliform bacteria the multiple tubes method was used. The results of *presumptive test* shows, using McCardy table, that per 1000 ml number of coliform bacteria was at falowing: in samplepoint 1 (upstreem) -120; in samplepoint 2 (industrial area) - 240; in samplepoint 3 (at the confluence with Bistrita River) – 310.

It can be said that the microbial load is not excessive, but there are sources of fecal pollution that affect this stream. The reduced number of the bacteria, may be due to the fact that the sampling was done in autumn, when the water temperature is lower, and the optimum of the development for these bacteria is between 22 and 37°C (UNESCO/WHO/UNEP, 1996).

The results of *confirmation test* was done on GEAM (gelose-lactose-eosin-methilen blue) culture media and are shown in Figure 2.

Figure 2. . Confirmation of bacterial colonies on GEAM culture medium

From Figure 2 it can be observed that in the analyzed samples the strains of *Escherichia coli* are predominant. They have a particular metallic sheen that makes them discernible. Other strain types, such as *Klebsiella* sp. or *Enterobacter* sp. were identified also. The species of this genus are routinely present in natural environments, being bio-recycling organisms (Allen et al., 2004), but when they are in large numbers they indicate a fecal pollution because they normally colonize human and animal intestine.

3. Enzymological characterization of samples from Castailor Creek

In the samples collected the following enzymatic activities were assessed: actual and potential dehydrogenase activities (ADA and PDA), phosphatase activity (PA) and catalase activity (CA). The results of the enzymological tests are presented in Table 2.

Table 2. Enzymatic activities in samples from *Castailor Creek* (autumn 2012)

Sampling points	Dehydrogenase activities		Phosphatase Activity mg phenol/g sedimet	Catalase activity (mg H_2O_2/g sediment)
	ADA	PDA		
Upstream	0.232	0.272	12.69	32.81
Industrial area	0.323	0.247	2.63	45.9
At confluence	0.314	0.752	13.64	54.06

Actual and potential dehydrogenases activities. Dehidrogenases activitiy reflects the respiratory potential of microbiota from water or sediment (Richardson, 2000), so these shows the current environemental activity (Fekete et al., 2013).
As it can be observed in Figure 3, the actual dehydrogenases activity was more intense in the samples taken from point 3 (at confluence with Bistrita River), and the lowest from the point 2 (industrial area).

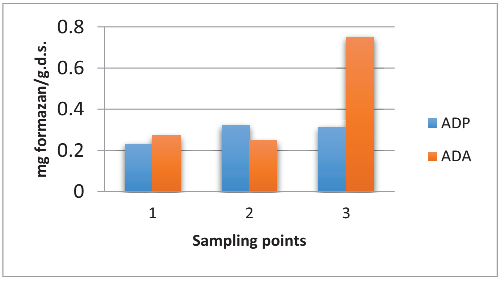

Figure 3. The intensity of actual and potential dehydrogenase activity (ADA and PDA) registred in Castailor Creek (autumn 2012)

The potential dehydrogenase activity (PDA) normally has higher values because of the carbon source (glucose) added.
As a whole it can be seen that the highest values are recorded in sample 3, at the confluence with Bistrita River, this is due to the environmental capacity of the auto-regulation, but probably there is a source of organic pollution also. The addition of glucose acts as a constant but low incentive, on dehydrogenase activity. These enzymes that catalyze the oxidation of many organic compounds by the exchange of protons and electrons are located only in intact living cells.
Phosphatase activity. The phosphatase activity was detected in all three sediment samples from Castailor Creek using Erdei and Kramer (1959) method and was expressed in mg phenol /g sediment.
The distribution of phosphatase activity values is shown in Figure 4. It can be seen that phosphatase activity was most intens in sample from upstream (1) (before entering in the city) and at the confluence with Bistrita River (3).
The lowest phosphatase activity was recorded in sample 2, the industrial zone. From this can be said the phosphatase activity of the bacteria show higher valence in areas outside the city, away from polluted areas.

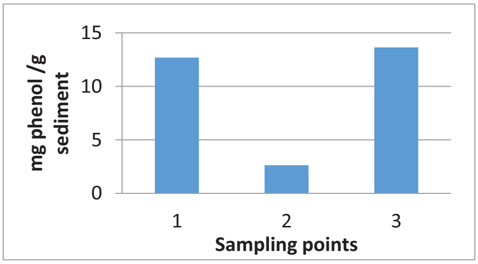

Figure 4. The phosphatase activity in samples taken from Castailor Creek (2012)

Compared with the other tests, it can be said that the phosphatase activity express the water quality of Castailor Creek (Matavulj et al., 1989).
Catalase activity. Catalase activity was very intense in sample from the upstream of the creek and less intense in the sample from the confluence with the Bistrita River, as it can be seen in Figure 5.
Catalase is an enzyme which accumulates and maintains its activity for a long time. The catalase is related to the amount of organic matter and the number of microorganisms presents in the environment. This enzyme participates in the degradation and/or incorporation of xenobiotic substances in the organic matter in the environment in which they are (Gianfreda and Bollag, 1996).

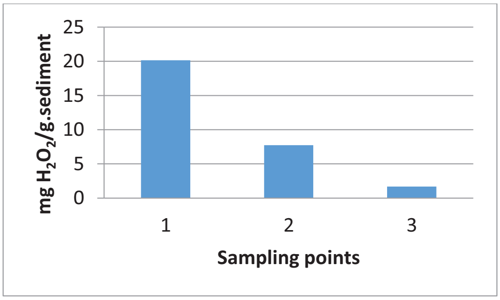

Figure 5. The catalase activity in the samples taken from Castailor Creek (2012)

Catalases along with dehydrogenases produce extensive transformation of the characteristics of contaminants, and they are even implicated in the pollutants complete conversion into innoxious inorganic end products (Ogbolosingha et al., 2015).

CONCLUSIONS

The physical analyzes showed that the lowest pH was recorded in the industrial area. This is because of the pollution from nearby factories, but also because of the fact that is a high traffic area for cars and trains. On the other hand, redox potential is higher in the sample 1 (upstream) showing a batter quality because Eh affects the affinity of the nutrients and the presence of aerobic bacteria.

Numerical distribution of aerobic heterotrophic bacteria shows higher values in the samples taken from upstream (1) and lowest in the samples from industrial zone (2).

In the sampling point 3, has been observed a higher intensity of the phosphatase and catalase activity, this may indicate a source of organic pollution, but implicates an auto-regulation ability of the environment, also.

The total number of coliforms bacteria is not exaggerated but suggests that there are still sources of fecal pollution that affect this watercourse

The microbial and enzymological activities represent indicators for evaluating the effect of the polluants on water microbiota.

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