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SCIENTIFIC PAPER

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APPLICATION OF THE MODEL OF CYLINDRICAL REACTOR FOR SELF-PURIFICATION BY INDIGENOUS MICROORGANISMS

Article Highlights

- Kinetic model of the autotrophic biofilm reactor is researched through its efficiency in the water
- The level of auto-purification is proven higher with the help of the kinetic model
- Decrease in NSAID concentrations is proven, which poses a significant ecological restoration aspect
- Study presents an excellent theory to better regional development and ecological sustainability

Abstract

Pharmaceutically active compounds (PhACs), in particular, nonsteroidal anti-inflammatory drugs (NSAIDs) are in increasingly wider usage, and as such are more and more frequently part of the organic matter of recipient rivers, especially in their lower courses. To indicate their significance as pollutants, as well as the significant role that the presence of autochthonous microflora plays in solving this issue, we undertook to perform this experiment. The experiment, titled "Application of the model of cylindrical reactor in self-purification by indigenous microorganisms", was conducted during a one-year period at the location of Vukovci, in the lower course of the Morača river. Assuming that the concentration of NSAIDs and PhACs in water can be reduced through self-purification, it has been proven that such processes result in a modification of phenotype in the indigenous microbiological population. Having the above-mentioned premise in mind, we constructed the experiment model, which entails kineticism of water, whereas the defined volume flow rate per unit time was 0.005 m/s, through the known distance of 432 m. Over one year of application of the model of the cylindrical reactor for enhancing self-purification capacity by indigenous microorganisms, auto-purification increased by 28.05%, the phenotype of the indigenous microorganisms changed by 24.62%, whereas the total concentration of particular PhACs, micropollutants, and NSAIDs decreased by 4.19%.

Keywords: pharmaceutically active compounds (PhACs), nonsteroidal anti-inflammatory drugs (NSAIDs); self-purification, water kineticism, indigenous microorganism phenotype.

Within natural aquatic environments, various physical, chemical, and biological processes occur which affect the content, transformation and fluctuation of the water constituents [1]. In most cases, river and lake waters are contaminated by waste, sewage, and pharmaceuticals, including nonsteroidal anti-inflammatory drugs (NSAIDs) and their degradation products [2-5]. Montenegro as a country, especially its central region, along with the mountain range surrounding it, is considered a hydrologically dense and rich area. An estimated average amount of 614 m³/s flows through its surface, which amounts to 19.3

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km³ per year, with an average module of 44.4 l/s/km³. Waterflows of equal or greater capacity than this appear on less than 3% of the Earth's surface. The longest and largest river in Montenegro is Morača, with a flow length of 110 km [6].

The Morača river, with an annual intake of 4,898,300 m^3 of treated water is mostly polluted due to the continuous human impact caused by large-scale urban, industrial, and agricultural activities, which affect this water source and the ecosystem it sustains [7]. Compounds that are frequently studied in the aquatic environment are analgesics and anti-inflammatories (such as diclofenac, ibuprofen, naproxen, acetylsalicylic acid, and paracetamol) [8].

However, out of the existing and theoretically feasible surface water purification methods, there are none currently implemented in Montenegro. At present, river water purification technologies can be categorized into physical, chemical, biological, and ecological methods. Physical methods include aeration and sediment dredging [9]. Chemical methods include chemical precipitation [9] and the application of chemical algaecide [10]. Biological methods include bioremediation [11], biofilms [12], contact oxidation [13], and membrane bioreactor technology [14]. Ecological methods include ecological ponds [15], plant purification treatment [16], ecological floating beds [17], and constructed wetlands [18]. They also offer ecological benefits: they have been demonstrated to be an economical and efficient sewage treatment and management method [19], and they have become a preferred ecological method to improve the water quality of rivers in cities around the world.

As a result of the above-stated information, developing an appropriate water purification method that uses the known property of microorganisms to use pollutants in their nutrition as the basic source of carbon, whereby the properties of the microorganisms are altered, was our primary objective. These microorganisms are categorized as natural purificators – self-purificators. Self-purification, in the ecological sense, represents the process of the ecosystem itself adapting and learning to face all changes and occurrences accordingly. It can also be seen as the basis for self-support that the system provides in case its growth and development are disturbed.

Auto-purification or self-purification is a process by which the system adequately faces all the changes and occurrences almost independently. The relationship between facultative oligotrophic and heterotrophic (index FO/H) represents one of the most significant microorganism parameters used to estimate water properties from an ecological aspect [20,21] and is a very good indicator of water self-purification capacity [22]. An idea for categorization of water self-purification capacity based on the relationship between FO/H [23], describes self-purification capacity of water as low (<1), sufficient (>1), and if >10 as good capacity of self-purification.

Therefore, we ran a pilot project in which we tested the applicability of the developed cylindrical reactor mathematical model.

By definition, a cylindrical reactor is a chemical reactor and an open system. However, the principles developed for chemical reactors can be applied to most, if not all, chemically reacting systems (*e.g.*, atmospheric chemistry, metabolic processes in living organisms, etc.) [24]. It is important to highlight the significance of its approximate ideal drift, which means there is no fusion inside the moving reactionary compound in the course of the flow (axial direction). The reactor resembles a line of elementary volumes of the reactionary compound (reaction compound inside the differential volume of that sort is homogenous), and the volumes passing through the reactor do not mix with the reactionary mass (Figure 1a).

On its way from the entrance to the exit, a hypothetical elementary (differential) volume spends a certain amount of time in the reactor, during which the composition of the reactionary mass changes [25]. According to that, the values of the dependent variables are the position functions on the z-axis.

Because of its simple cylindrical design (Figure 1b), free of any additional mixing devices, it is often used. Setting the component A balance (indigenous microorganisms) as the differential volume of the reactor, results in the basic equation of multiplication of component A (indigenous microorganisms):

$$FA - (FA + d\,FA) - r\,A\,d\,V = 0$$
$$-d\,FA - r\,A\,d\,V = 0$$

FA - molar flow rate for indigenous microorganisms in the cylinder; *dFA* - molar velocity differential element for indigenous microorganisms; *dV* - differential volume element; *rA* - the numerical value of indigenous microorganisms.

Therefore, the development of optimization functions of the model means that the number of replicas of indigenous microorganisms that would be self-supporting to the whole system should be met, which was our goal at the beginning.

However, the system also has its flaws. The main shortcoming is the size of the system itself, as it needs a large surface, and must be kept in the state of kinetic energy at all times. Also, an adequate flow

rate through the cylindric pipe must be determined, lasting no longer than 24h, to lower the temperature inside the reactor and at the same time keep the active oxygen that creates aerobic conditions. This paper describes a trial construction system of the cylindrical reactor for the self-purification of indigenous microorganisms. These results serve as support for the promotion and application of this technology.

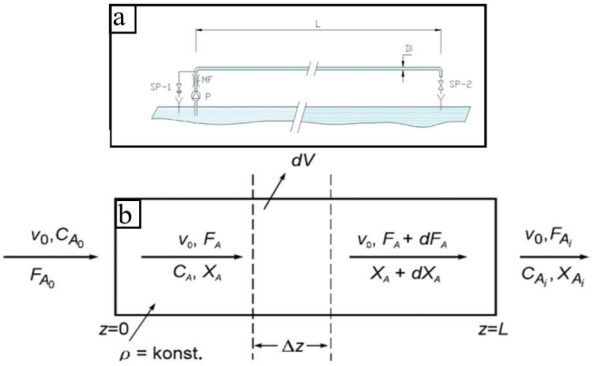

Figure 1. a) An example of the "cylindrical reactor for the purpose of self-purification by indigenous microorganisms" SP-1 water sampling site before entering the reactor; MF flow meter; P-pump; L-length of hose-cylindrical reactor; Di-section / diameter of the reactor hose; SP-2 designation for the point at the outlet of the hose / reactor where a water sample is taken; b) cylindrical reactor for enhancing self-purification capacity by indigenous microorganisms: v_0-volume flow (flow rate at the beginning), C_{A0}-concentration of reactant (in our case the number of indigenous microorganisms at the beginning); F_{A0}-molar flow rate of indigenous microorganisms at the beginning; FA-molar flow rate for indigenous microorganisms in the cylinder; CA-concentration of reactant (in our case indigenous microorganisms in the cylinder); XA-conversion of reactant reaction in our case of indigenous microorganisms; dV-differential volume element; z-axial direction; ρ-density; dFA-differential element of molar velocity for indigenous microorganisms; dX_A-differential element of conversion of the reactant reaction, in our case of indigenous microorganisms; L-length of reactor; F_{Ai}-molar flow of indigenous microorganisms at the outlet; C_{Ai}-concentration of indigenous microorganisms at the outlet; X_{Ai}-conversion of reactant reactions at the outlet (in our case of indigenous microorganisms).

EXPERIMENTAL

Model design

The model design of the cylindrical reactor for the self-purification of indigenous microorganisms is shown in Figure 1b. It consists of a built-in pump (P), flow meter (MF), rotameter, specifically with the ability to regulate the rate of the flow, and a long pipe (L) that represents a cylindrical reactor. Hypothetically, we have different flow rates in the pipe. The highest velocity, both theoretically and practically, is during laminar flow in the middle of the pipe's (*Di*) axis, which is parabolically arranged so that its outermost radius amounts to zero.

Average velocity is calculated by a continuity equation, flow Q = const, the maximum velocity of 0.005 m/s is adopted (*Di*), considering that in the worst case, the pipe can reach laminar flow that, compared to the turbulent velocity, has a higher velocity in the axis. Based on that, we can calculate the length of the cylindrical reactor:

$$L = Vmax \times 3600\text{s} \times 24\text{h} = 0.005\ E \times 3600\text{s} \times 24 = \\ = 432\text{m}$$

Samples were taken from localities SP1 and SP2. The water sample taken from locality SP1 was taken before the water entered the cylindrical reactor, and it is a so-called "native sample". The sample from locality SP2 was taken from the cylindrical reactor's faucet (water sample is taken from the kinetic reactor of indigenous microorganisms after a reaction lasting 24 h). The objective of the experiment has been to prove that under these conditions, facultative oligotrophs can increase their number and can affect modifications in the concentration of certain pharmaceuticals in water; accordingly, it would provide the grounds for the assumption that the same applies to other microorganisms.

Study area

The river Morača has its source at the topographic point of 975 m above the springs which flow down the Javorje and Zebalci slopes, with the largest of them being Javor and Rzav creeks. Morača is 113 km long, which makes it the longest river in Montenegro. We tested the locality Vukovci, situated at an altitude of 49 m.

The coordinates of the research location are 42°20′02″ N, 19°11′60″ E. The average yearly temperature at the locality Vukovci is 23.25 °C, and the average annual precipitation is 116.92 mm. The highest temperature recorded during the summer was 42.0 °C, and the lowest temperature recorded during the winter was -2.1 °C.

Additionally, this is the most densely populated area in Montenegro and is part of the central region. In 1948, the population of this region reached 128,872 which was equal to 34.2% of the total population, while in 2011, the central region accounted for 47.3% of the total population.

The river flows into the Skadar lake, the largest still body of freshwater in Montenegro. The main hydrological parameters of the river Morača are Q abs-

olute minimum: 5.5 m^3/s; *Q* average: 140 m^3/s; *Q* absolute maximum: 900 m^3/s. The annual average flow of the river Morača on locality Vukovci is 104,125 m^3/s.

Data collection and analysis

The quality of the water samples was monitored over a one-year period from September 2017, to December 2018. Samples were taken monthly, on the 5th day of each month, in the morning.

Samples taken for chemical analysis were collected using glass bottles with a capacity of 1 liter. To determine the concentrations of dissolved oxygen in water, Winkler's bottles with coded stoppers were used, with oxygen being immediately fixed by adding 1 ml KI and 1 mL $MgCl_2$ [26].

Sampling for microbiological analysis was performed in pre-sterilized glass bottles. Sampling bottles were washed and dried, then sterilized for one hour at 190 °C in a dry sterilizer. The sampling included the following procedures: the sampling for microbiological analysis was done by quickly submerging prepared bottles, to avoid contamination of the bottle. Samples were transported to the laboratory in a portable fridge. Microbiological analysis of the samples was performed at the Hydrobiological Institute of Montenegro, Department of Biology [27].

Samples intended for saprobiological analysis were taken using a 25 μm mesh plankton net, and they were analyzed without being fixated, or fixated using 96% alcohol.

Community-level physiological profiling of the microbial community (CLPP) is based on the BIOLOG microbial identification system in the Microbiological lab at Hemomont d.o.o, Podgorica, Montenegro. The BIOLOG system functions by identifying microorganisms on the phenotype level. It is especially important because every living community can react with 95 different sources of carbon on a microtiter plate. Prokaryote communities represent functional units that have the metabolic characteristics of bacteria, hence CLPP is used as a sensitive and fast method to identify the potential diversity of microbial communities. By degrading the sources of carbon, we reduce one out of 95 microbial mediums found in cupolas (not the one in the position (1,1) as it represents negative control), and we notice changes in the shades of orange. Those color changes manifest through the optical distance measurements (OD), and the change of the shade itself can identify the microbial community through the average metabolic response (AMR).

Average metabolic response (*AMR*) is by definition the average respiration of carbon sources used by microbial communities, and is predictable, measurable, and can be compared between communities

$$AMR = \Sigma\left(O.D.well - O.D.neg\right) / 95$$

where (*O.D.well*-*O.D.neg*) is the relation between measured values of optical density and negative control [28].

Analysis of PhACs (NSAID) in water samples

River water (100 mL) was collected to determine the presence of PhACs (NSAIDs) in the aqueous phase. River samples were filtered through 1 μm glass fiber filters followed by 0.45 μm nylon membrane filters and kept at -20 °C until analysis. Water samples were analyzed for PhACs according to [29]. Ultrapure water (100 mL) was spiked with a mixture of the target analytes and subsequently subjected to the extraction method. Briefly, 3mL of EDTA 1M (4 vol.%) was added to the water samples. A Baker vacuum system (J. T. Baker, The Netherlands) was used to pre-concentrate the samples in Oasis HLB cartridges (60 mg, 3 mL). They were loaded with 100 mL of water samples and eluted with 6 mL of methanol. The extracts were evaporated under a gentle nitrogen stream and reconstituted with 1 mL of the methanol-water mixture (volume ratio for PhACs), and 10 mL of IS mixture (1 mg/L) was added to the final extract. Method detection limits (*MDL*) and method quantification limits (*MQL*) were set as the minimum detectable amount of analyte with a signal-to-noise of 3 and 10, respectively. *MDL* and *MQL*s have been calculated as the average of those estimated in real samples and in the spiked samples. Chromatographic separations were carried out with a Waters Acquity Ultra-Performance™ liquid chromatography system, equipped with two binary pumps systems (Milford, MA, USA) using an Acquity HSS T_3 column (50 mm×2.1 mm i.d., 1.8 μm particle size) for the compounds analyzed under positive electrospray ionization (PI) and an Acquity BEH C18 column (50 mm×2.1 mm i.d., 1.7 μm particle size) for the ones analyzed under negative electrospray ionization (NI), both purchased from Waters Corporation. For the analysis in PI mode, the optimized separation conditions were as follows: solvent (A) methanol, solvent (B) 10 mM formic acid/ammonium formate (pH 3.2) at a flow rate of 0.5 mL/min. The gradient elution was: initial conditions 5% A; 0-4.5 min, 5-95% A; 4.5-4.6 min, 100% A; 4.6-6.0 min, 100% A; from 6.0 to 6.1 return to initial conditions; 6.1-6.7, equilibration of the

column. The analysis in NI mode was performed by using acetonitrile (A) and 5 mM ammonium acetate/ammonia (pH=8) (B) at a flow rate of 0.6 mL/min. The gradient elution was: 0-1.5 min, 0-60% A; 1.5-2.0 min, 100% A; 2.0-3.0 min, 100% A; 3.20 min return to initial conditions; 3.20-3.70 min, equilibration of the column. The sample volume injected was 5 µL, and sample analysis was repeated three times. UPLC instrument was coupled to a 5500 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer (Applied Biosystems, Foster City, CA, USA) with a turbo ion spray source. Compound-dependent MS parameters (declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP)) were optimized by direct infusion of individual standard solutions of each compound at 20 µg/L.

All data were obtained and processed using Analyst 1.5.1 software, and the end value was obtained by adding the repeated values and dividing them by three.

Saprobity Index (*SI*)

Samples intended for saprobiological analysis were taken by using a 25 µm mesh plankton net, and they were immediately analyzed without being fixated, or fixated using 96% alcohol.

The saprobity index was calculated according to Pantle-Buck method. This system involves the application of the standard sampling techniques (Grginčević and Pujin, 1986).

Phytoplankton analysis with the application of the saprobity system, as noted in [30], determination of phytoplankton in the tested water sample, along with specifying the relative frequency (u) of each type, by using the numbers 1,3,5, whereas in this study, in addition to listed numbers 1, 3 and 5, the abundance was also reported by numbers 2, 4 and 10, depending on the frequency of microalgae, which is rather common in research of this kind.

Saprobity index (*SI*) is determined by using a method explained in [30] by applying a species value index as described in [31].

Preparation for SEM:

- Freshly collected diatom material can simply be air dried onto a small coverslip (Ø 12 mm) or filtered through a 2.5 µm Millipore® filter.
- Cleaned material can be prepared in a similar manner.
- After air drying, the sample should be placed in a desiccator containing silica gel for 24 h to make sure that it is completely dehydrated before continuing with further preparation.
- When dry, the coverslip or filter should be mounted on an aluminum microscope stub with carbon tape and sputter-coated with gold-palladium.
- The samples are now ready for examination.
- 10-15 kV is usually an adequate voltage for examining diatoms.
- Image analysis is the most advanced technology for a broad range of functions such as digital image acquisition, image processing, sample analysis, database archiving, and results/report documentation [32]. A recommended software package is “analySIS” which has several expansion versions and configurations. Version 3.1 provides live overlay, 3-D surfaces, automatic scale bar, and many other functions. It is relatively user-friendly, however, expert advice is required for the most benefit.

RESULTS AND DISCUSSION

The system of the kinetic model of the reactor was operating from 05/09/2017 to 05/09/2018. Over this time, the system proved sustainable, micropollutant (NSAIDs) removal efficiency slowly stabilized, and the indicators, *i.e.*, saprobity index, auto-purification level, and the concentration of dissolved oxygen in water (DO) showed that water quality was improving. Oligotrophic microorganism phenotype monitoring showed that native samples differ from those taken 24 h after the samples passed through the kinetic reactor.

Following an empirically determined incubation period, color patterns in the 96-well matrix and intensity (O.D.) of color formation can be used to determine AMR to describe the microbial community. According to that, native sample AMR (Figure 2) ranged from 0.8 to 1.94. Initially, the values of the native sample AMR, *i.e.*, of the sample before entering into the cylinder collector, were almost identical, except for the 0.8 minimum in September 2018. In December 2018, the native sample AMR value was 1.71. The AMR values of the kinetic cylindrical reactor sample were higher and ranged from 1.3 to 2.17.

The average mean result AMR for the sample before entering into the cylinder amounted to 1.42, while the average mean result AMR after the samples passed through the kinetic reactor amounted to 1.96 (Figure 2). Standard deviation is a significant statistical element that shows us how close the values in a data set are to the mean. For the native sample AMR, the standard deviation was 0.38, and for the kinetic cylindrical reactor sample, the standard deviation was higher by 0.05. The standard deviation value being

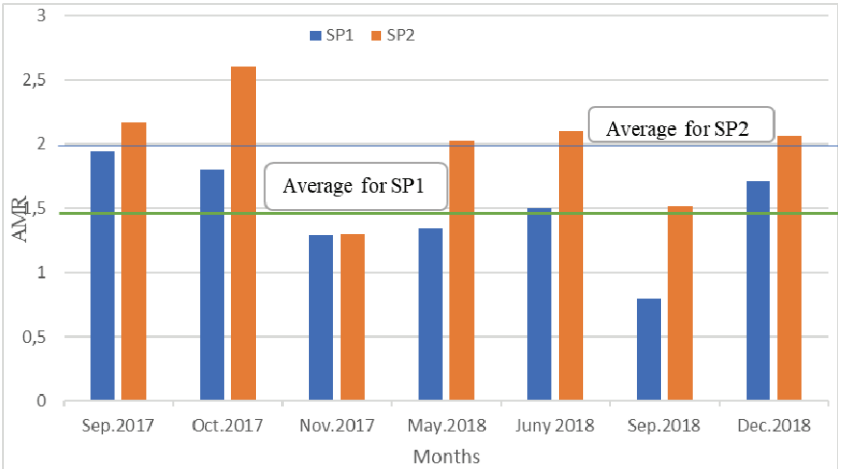

Figure 2. AMR average for research locations SP1 and SP2 for oligotrophic microorganisms.

this low only leads us to conclude that the results are mostly the closest to the average mean result.

The water contains 8.3 mg/L of dissolved oxygen at the temperature of 25 °C and it is of huge importance for many microorganisms in the water. During the operation of the model of the kinetic reactor, we established that the *DO* values in the analyzed samples were significantly higher and ranged from 9.5 to 16.9 mg/L. The average *SP*1 value was 12.64 mg/L, while the average *SP*2 value was 12.52 mg/L (Figure 3).

The average native sample value mg/L of dissolved O_2 is negligibly higher (0.12) compared to the value of dissolved O_2 in the water samples for the indigenous microorganisms of the kinetic cylindrical reactor sample. We assume that the sustainability of the system is based on the presence of the microorganisms that are capable of auto-purification, hence breaking down the micropollutants.

Based on the water self-purification categories, depending on the FO/H index, during the operating of the model (Figure 4), both *SP*1 and *SP*2 show good self-purification ability. The average FO/H mean index for *SP*1 amounted to 1.88, while the average FO/H mean index for *SP*2 amounted to 2.62 (Figure 4). FO/H range value for *SP*1 was 1.17, while the same *SP*2 value amounted to 1.98. The FO/H index values for *SP*2 were 28.05% higher compared to *SP*1 samples. Such auto-purification values are followed by the values of the NSAIDs in water samples *SP*1 and *SP*2 at the Vukovci location. During the study, we examined acetaminophen (paracetamol) which is used for reducing fever and treating pain in people of all ages [33], salicylic acid, as one of the most important components in the pharmaceutical industry [34], carbamazepine, which is used to prevent and control seizures, known as an anticonvulsant or antiepileptic drug [35], and ketoprofen class of nonsteroidal anti-inflammatory drugs (NSAID) with analgesic and antipyretic effects [36] in micropollutant concentrations at the research sites (Figure 5). With the model of the cylindric reactor for self-purification by

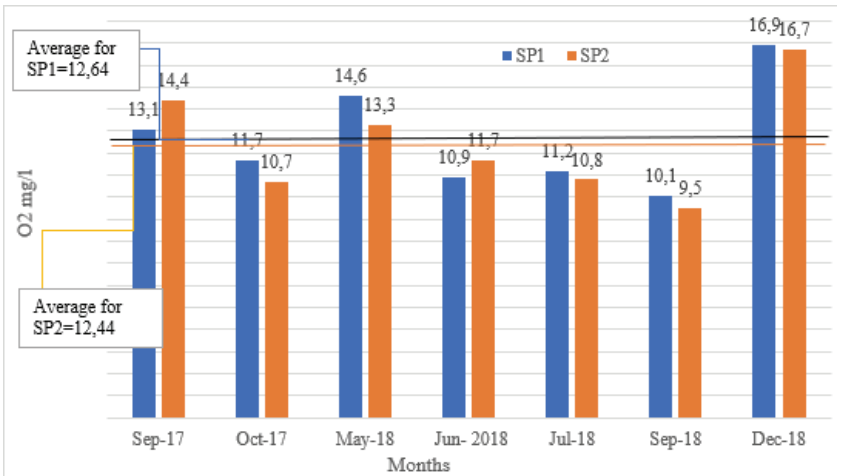

Figure 3. Dissolved oxygen concentration in the water (DO) values for locations SP1 and SP2, Vukovci locality.

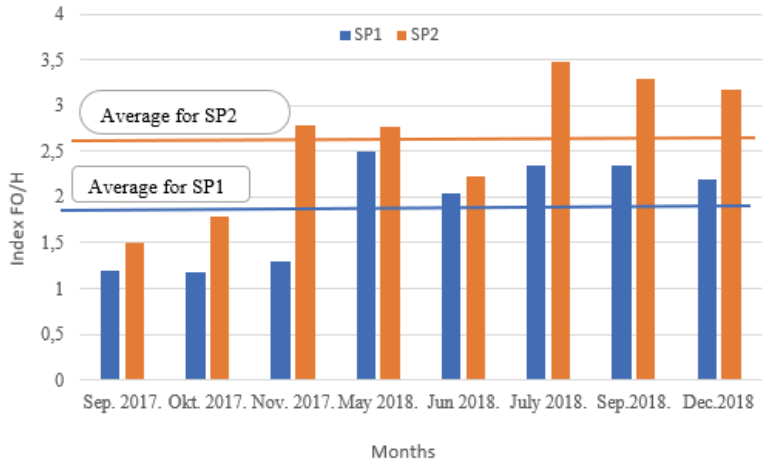

Figure 4. Index FO/H at the Vukovci locality for locations SP1 and SP2.

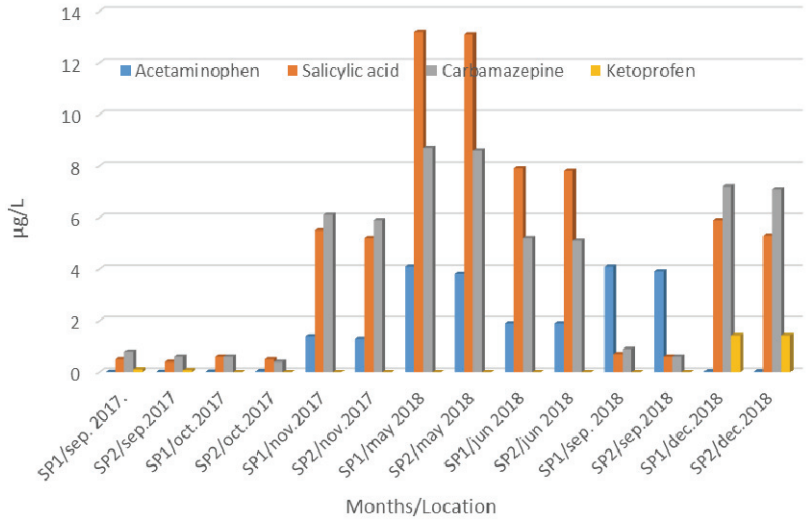

Figure 5. NSAID presence on Vukovci locality for locations SP1 and SP2.

indigenous microorganisms, we proved a 4.19% decrease in micropollutant (NSAIDs) in *SP*2 samples. When observing the pharmaceuticals individually, we noticed that the average value for the acetaminophen *SP*1 was 1.65, and 1.56 for *SP*2, with the standard deviation difference of 0.12 in favor of the *SP*1 (1.83). SP1 range value was 4.09, while that value amounted to 3.89 for *SP*2. Statistical values of the salicylic acid showed that the results for *SP*1 and *SP*2 do not differ significantly, with the averages for *SP*1 and *SP*2 being 4.9 and 4.7, respectively. Standard deviation was even for both samples, 4.73 for *SP*1, and 4.72 for *SP*2. The average mean for carbamazepine *SP*1 value was 4.21, and the average mean for *SP*2 was 4.04. The standard deviation for *SP*1 was 3.39, and 4.04 for *SP*2. *SP*1 ketoprofen range value was 1.32, and 1.35 for *SP*2, with the maximal value for *SP*1 and *SP*2 being 1.43. Minimal *SP*1 and *SP*2 values were 0.11 and 0.08, respectively. During researching, ketoprofen values for October and November of 2017, as well as May, June, and September of 2018, were under the method detection limits (*MDL*).

During the experiment, it was especially important to prove that the system does not significantly affect the composition of the microalgae/saprobity index (*SI*).

SI value for the Vukovci locality, as well as locations *SP*1 and *SP*2 (Figure 6), was increasing in *SP*1 up until May 2018. By monitoring the system operation in May 2018, we noted that the value of *SP*2 saprobity index was higher than the value in *SP*1. Then, the *SP*1 saprobity index went up during June and July 2018, until September and December of 2018, when the *SP*1 saprobity index significantly surpassed the *SP*2 value. The average saprobity index value for *SP*1 was higher than the average *SP*2 value by 0.38 (Figure 6). Standard deviation of the "sum sample" amounted to 0.39 which clearly indi-

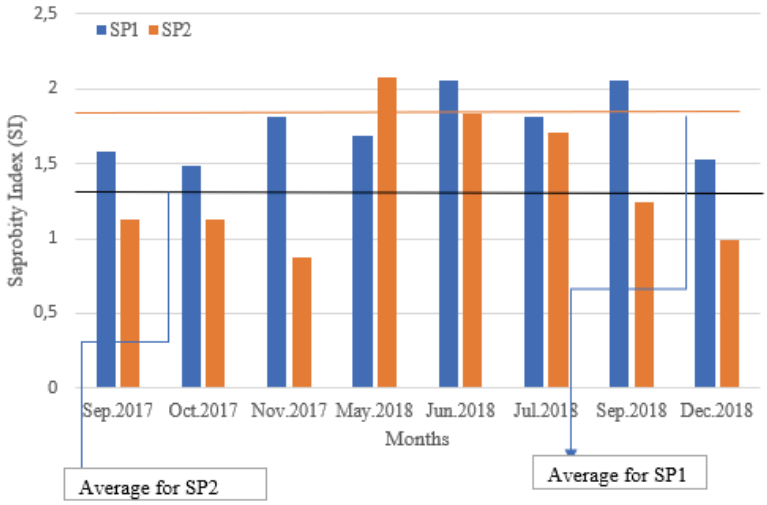

Figure 6. Saprobity index for Vukovci locality at locations SP1 and SP2.

cates that the results are grouped mostly around the average mean value, as well as low dispersion compared to the overall sample. The most prevalent division was *Bacillariophyta*, while the most prevalent species were *Cymbella lanceolata*, *Fragilaria crotonensis* and *Cymbella ehrenbergii*, captured using SEM microscope.

During the summer months, the water level at Vukovci site fell below the zero point. During that period (August-October), operating the kinetic cylindrical reactor was very difficult and saprobity index values (Figure 6) were at their highest. When the system normalized, heavy rainfall was recorded from November to June, which played a key role in the improvement of the system results.

As Figures 4 and 5 show, there is a significant connection between FO/H index, self-purification, and NSAID concentration in water samples at the Vukovci locality. By correlating these two variables using the Pearson correlation coefficient, we are able to support our results mathematically. The calculated *r*-value is 0.484, which allows us to establish a moderately positive ratio between those two variables [37]. The coefficient of determination amounts to 23%, therefore we can claim that 23% of one variable in water (FO/H index) was caused by the concentration value of the second variable in the water (NSAID concentration). Integrated effect: self-purification index, dissolved oxygen in water, removal of NSAIDs from water samples, saprobity index, complied with the

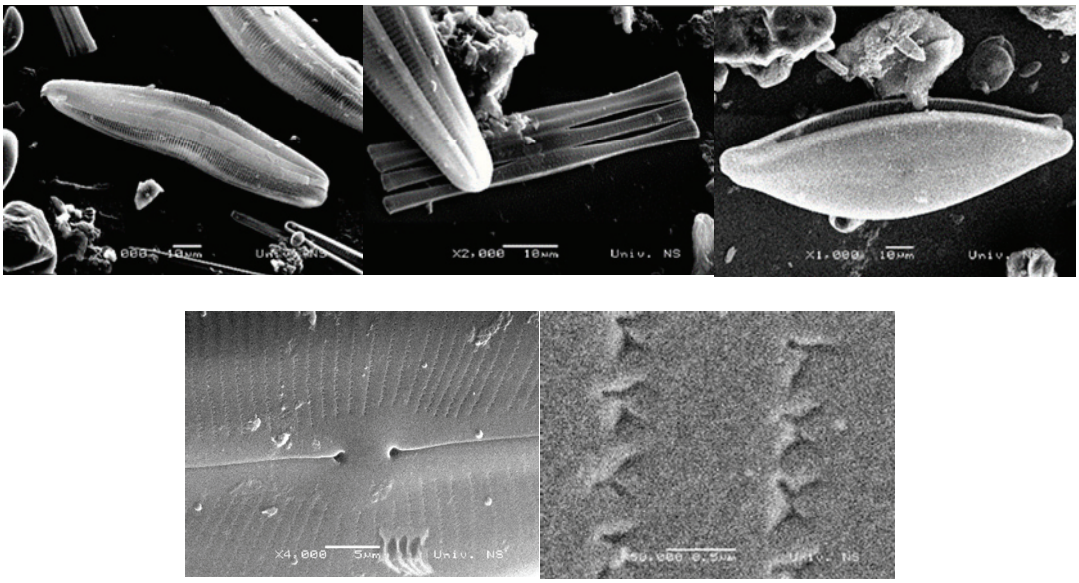

Figure 7. SEM micrographs of diatoms collected from Morača river; from left to right, first row: Cymbella lanceolata, Fragilaria crotonensis, Cymbella ehrenbergii; second row: Cymbella ehrenbergii, both.

development and change in the phenotype of the indigenous facultative oligotrophic microorganisms in the water.

During September 2017, optimal conditions regarding air and water temperature enabled our system to function optimally, and therefore the difference in the adaptation of the phenotype of indigenous oligotrophic microorganisms shown as the *AMR* value was higher by 10.6% at the *SP*2 location compared to the *SP*1, and also the value of the auto-purification index in *SP*2 was higher by 20% than the value in *SP*1.

Regarding the saprobity index, the value in location *SP*1 was 28.5% higher than the value in *SP*2. The *SP*2 values were noticeably higher until November 2017, when the difference in the phenotype of indigenous facultative oligotrophic microorganisms amounted to only 0.08%.

The CLPP approach has sufficient sensitivity to detect acute contaminant impact on the physiological processes of the indigenous microbial community while providing data for the evaluation of chronic stress-induced adaptations in microbial community structure. By examining the AMR relation for *SP*2 and *SP*1, the most significant difference in their results was noted during October 2017, when the difference amounted to 0.8, and then during the September sampling, when it amounted to 0.68. Of course, microbial communities have great potential for temporal or spatial change, and thus represent a powerful tool for understanding community dynamics in both, basic and applied ecological contexts [5]. This proves that the system has satisfactory self-purification ability. Water quality is directly correlated to its chemistry at numerous locations along the course of rivers, but also is the result of geomorphological conditions, as well as anthropological influence.

The dissolved oxygen content of water ranged from 9.5 to 16.9 mg/L during the operating of the system; therefore, the system was supersaturated with oxygen. The saprobity index was highly oligotrophic [38] during 57.15% of our analysis for *SP*1, and 85.22% of our analysis for *SP*2; for the rest of the analysis it was β-mesosaprobic. This proves that our system is sustainable.

CONCLUSION

One-year observation of the system of “cylindrical reactor for self-purification by indigenous microorganisms” showed that the system has sufficient auto-purification capacity and therefore actively improves the water quality. It was mature and stable throughout the entire year. The FO/H index values were 28.05% higher in the *SP*2 samples than the values in *SP*1. This model showed a total decrease of 4.19% of the selected pharmaceuticals and micropollutants in samples taken at location *SP*2, *i.e.*, post-reactor samples, compared to the same NSAID concentration in the *SP*1 or native sample. Indigenous microorganisms actively affected NSAID degradation.

The *AMR* for *SP*2 was 24.62% higher than for *SP*1, which proves the phenotypic adaptivity of facultative oligotrophic microorganisms. Saprobity level was highly oligotrophic for over 50% of the samples, the highest being *Bacillariophyta.*

The system integrates water purification and ecological restoration. Based on the “close to nature, multi-functional and sustainable” concept of ecological restoration, the study provides an excellent theory and practice to promote a healthy river environment and the sustainable ecological, economic, and social development of the region.

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NAUČNI RAD

PRIMJENA MODELA CILINDRIČNOG REAKTORA U CILJU SAMOPREČIŠĆAVANJU KORIŠTENJEM AUTOHTONIH MIKROORGANIZAMA

Farmaceutski aktivne komponente (PhACs), a posebno nesteroidni antiinflamatorni lekovi (NSAID) su u sve široj upotrebi i kao takvi su sve češće deo organske materije recipijentnih reka, posebno u njihovom donjem toku. Da bismo ukazali na njihov značaj kao zagađivača, kao i na značajnu ulogu koju prisustvo, autohtone mikroflore igra u rešavanju ovog pitanja, preduzeli smo da izvedemo ovaj eksperiment. Eksperiment pod nazivom „Primjena modela cilindričnog reaktora u cilju samoprečišćavanju korištenjem autohtonih mikroorganizama" sproveden je u periodu od godinu dana na lokalitetu Vukovci, u donjem toku rijeke Morače. Pod pretpostavkom da se koncentracija NSAID i PhACs u vodi može smanjiti samoprečišćavanjem, dokazano je da takvi procesi rezultiraju modifikacijom fenotipa u autohtonoj mikrobiološkoj populaciji. Imajući u vidu navedenu predpostavku, konstruisan je eksperimentalni model, koji podrazumeva da se voda kreće. Pri tome je definisana zapreminska brzina protoka po jedinici vremena i ona je iznosila 0,005 m/s; dužina cilindričnog reakora je iznosila 432 m. Za godinu dana primene modela cilindričnog reaktora za povećanje kapaciteta samoprečišćavanja od strane autohtonih mikroorganizama, autoprečišćavanje je povećano za 28,05%, fenotip autohtonih mikroorganizama se promenio za 24,62%, dok je ukupna koncentracija pojedinih PhACs, mikrozagađivača i NSAID smanjena za 4,19.

Ključne reči: farmaceutski aktivne komponente (PhACs), nesteroidni antiinflamatorni lekovi (NSAID), samoprečišćavanje, kretanje vode, fenotip autohtonih mikroorganizama.