ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 68 52 Number 3, 2020

EFFECT OF BIO-ENZYMATIC PREPARATION ON WATER QUALITY IN PONDS AND ON QUANTITY AND QUALITY OF FISHPOND SEDIMENTS

Barbora Müllerová[1], Marija Radojičić[1], Radovan Kopp[1], Jan Grmela[1], Michal Šorf[1], Eva Poštulková[1]

[1] Department of Zoology, Fisheries, Hydrobiology and Apiculture, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Link to this article: https://doi.org/10.11118/actaun202068030551
Received: 30. 3. 2020, Accepted: 13. 5. 2020

To cite this article: MÜLLEROVÁ BARBORA, RADOJIČIĆ MARIJA, KOPP RADOVAN, GRMELA JAN, ŠORF MICHAL, POŠTULKOVÁ EVA. 2020. Effect of Bio-Enzymatic Preparation on Water Quality in Ponds and on Quantity and Quality of Fishpond Sediments. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* 68(3): 551–557.

Abstract

The aim of this study was to evaluate the ability of a bacterial-enzymatic preparation to affect water quality, particularly the composition and the amount of sediments in ponds. The PTP Plus preparation was applied during the year 2016. For evaluation of the possible effects of the preparation, the water and sediment was compared in the growing seasons 2016 and 2017. When monitoring the sediment loss, a difference ranging from 72 to 86 mm was measured. There was an increase in the level of conductivity, alongside with an increase in the amount of calcium and chlorides in the water. In the case of the sediment, there was an increase in the amount of organic substances in the aqueous leachate and a decrease in the amount of available and total calcium. Changes of other monitored parameters were not so clear. The significant changes in water and sediment quality in the individual years of monitoring were probably more influenced by climatic conditions and significant growth of macrophytes in 2017 than by the application of the product. The expected reduction of the share of organic matter in the sediments after the application of the product has not been confirmed.

Keywords: phosphorus, organic matter, bacteria

INTRODUCTION

Clogging of both the natural lakes and manmade shallow ponds is generally considered a natural phenomenon. Unlike in natural lakes, removal of sediment is an important part of pond management. Removal of mud from ponds was also understood as natural, particularly in view of the fact that sediments from ponds, following extraction, return to the location from where they ended up in the ponds, meaning back to farmland (Vrána and Beran, 2002). The legal situation of sediments, which were considered quality soil at the beginning of the 20th century, has changed dramatically. The currently valid legislation characterises sediments as waste, and their reuse in agriculture is therefore very limited.

Sediments extracted from ponds and reservoirs have a high share of organic matter, carbonates and calcium compared to arable land. The content of available magnesium and total phosphorus is also higher. The content of potassium and total magnesium is comparable. However, the share of available phosphorus is lower compared to arable land (Kopp *et al.*, 2019). Apart from the chemical composition, the physical properties of the sediment are also very important as they can significantly influence the improvement of the physical state of fertile soil and in the current climate change conditions can also increase the retention of water in soil.

As a result of increased land erosion, which has affected more than 50% of farmland, there have been significant deposits of sediments in ponds and reservoirs. It is estimated that there are approximately 200 million cubic metres of sediments in ponds in the Czech Republic (Gergel *et al.*, 2002). Another negative effect of sediment deposition is the gradual reduction, even impossibility, of pond use for the purposes of water management, ecology, recreation and above all fisheries. The high share of nutrients is leading to excessive development of primary producers and to the fluctuation of the physico-chemical parameters of the aquatic environment. Higher deposits of organic substances can also have the effect of increased oxygen consumption, which can lead to depletion of oxygen in ponds, spread of anaerobic organisms and subsequent release of toxic substances, which will have a negative effect on pond habitats (Rahman *et al.*, 2004; Muendo *et al.*, 2014).

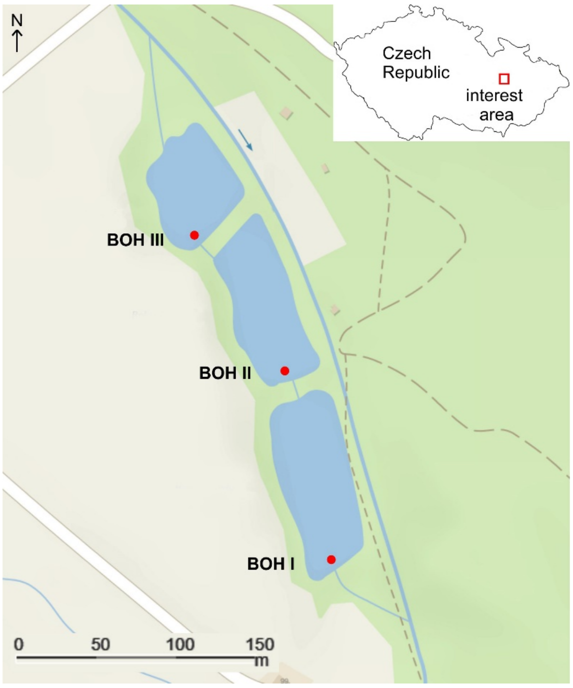
Solutions for elimination of sediments are connected with high financial costs. Sediments can be removed either in a "wet" manner using suction equipment, or in a "dry" manner using heavy machinery after drainage of water and partial drying of the pond. Mainly for economic reasons, people are looking for other methods of reducing excessive accumulation of sediments. One of these methods is the use of various bacterial-enzymatic mixtures which should reduce the share of organic matter in the sediment. The mixtures that are most frequently used in ponds are inocula with bacteria of the *Bacillus* genus, but the *Nitrobacter*, *Pseudomonas*, *Enterobacter*, *Cellulomonas*, *Rhodopseudomonas* and photosynthetic sulphur bacteria are also used (Boyd, 1990). One of these preparations is the PTP Plus produced by Baktoma s. r. o. (Czech Republic).

The product is supposed to speed up natural biodegradation processes and break down the organic part of sediments in ponds and reservoirs. It is a concentrate of spores and endospores of specially selected and custom-bred strains of primarily soil bacteria which show specific properties such as the capability of increased production of the required enzyme. According to the producer, all the strains of bacteria used are non-pathogenic and occur freely in nature. These strains of original soil bacteria have been chosen for their specific capability and have not been genetically altered or modified. After introduction of the mixture into the aquatic environment, in a short amount of time the spores and endospores should revive and should produce specific enzymes and eat the organic sediment.

Regular use of the PTP Plus should lead to reproduction of the bacteria and therefore to continuous purification of the ponds. The manufacturers of this mixture also state that after a couple of weeks of use of the preparation, a biological balance will occur in the aquatic environment and the amount of organic sediment will be reduced, as well as the turbidity of the water at the bottom and throughout the water column. There should also be a significant decrease in the level of chlorophyll a, which indicates the biomass of algae, and an increase in the amount of oxygen in the water should take place (Baktoma, 2018).

MATERIALS AND METHODS

A cascade of ponds with a total area of 2.4 ha was used to study the effect of the preparation: Bohuslavický I (BOH I, 1 ha), Bohuslavický II (BOH II, 0.8 ha) and Bohuslavický III (BOH III, 0.6 ha). The ponds are flow-through with a stream flowing through all of them. They are eutrophic, and located in the municipality of Bohuslavice u Konice in the Olomouc Region (Fig. 1). The ponds have a thick layer of organic matter on the bottom and there is a strong presence of macrophytes (*Lemna* spp., *Spirodela polyrhiza*, *Ceratophyllum demersum* and *Myriophyllum spicatum*). The ponds are used for raising juvenile fish that are later to be grown in other ponds, mainly the carp (*Cyprinus carpio*), and additionally the grass carp (*Ctenopharyngodon idella*) and the tench (*Tinca tinca*). Since fish are caught from all three ponds together in BOH I and



1: *Map of sampling localities: BOH I – Bohuslavický I, BOH II – Bohuslavický II, BOH III – Bohuslavický III, red spot – location of collection of water samples* as there is only an estimate of the weight of the caught fish, it is not possible to determine exactly the production levels in individual ponds. Based on available data, it can be estimated that production of fish in the monitored ponds does not exceed 1000 $kg.ha^{-1}$.

Monitoring of the physical and chemical parameters of the water and of the composition of sediments took place in the growing seasons of 2016 and 2017. In an interval of approximately one month, basic hydrochemical parameters were measured directly in all three ponds: the amount of dissolved oxygen, the water temperature, the pH value (measured by the Hach Hq40d multimeter manufactured by Hach-Lange, USA) and the conductivity of water (measured by the Hanna Combo conductometer manufactured by Hanna, USA). The transparency of the water was measured using a Secchi disk. The water samples for testing in the laboratory were collected in 1-litre plastic bottles, approximately 20 cm below the surface near the oulet (Fig. 1). In the chemical laboratory, the following parameters were measured: COD, BOD, total nitrogen and phosphorus, ammoniacal nitrogen, nitrites, nitrates, phosphates, chlorides, acid neutralizing capacity and calcium content. The chemical parameters were determined using the standard methods (Horáková, 2007). Cyanobacterial and algal biomass were evaluated by chlorophyll–a concentrations using heated ethanol extraction (Lorenzen, 1967).

In the same intervals as the collection of water samples, sediment samples were collected (surface layer 0–15 cm) using an Ekman-Birge sampler. The sediment samples collected from at least three different locations in the pond were homogenised and the larger particles were removed using a 2-mm mesh. In the laboratory, the dry mass of the sediment samples was determined and aqueous leachates from fresh samples of sediment were prepared in accordance with the ČSN EN 12457-4 standard. From the dried samples of the sediment, soil extracts were prepared according to the Mehlich III procedure (Zbíral, 2016). Part of the sample was burnt (550 °C, at least 6 hours) for determination of the organic share, and the burnt sample was used for the preparation of a soil extract with aqua regia in accordance with the process set out by Zbíral (2011). Using the aqueous leachates prepared according to the ČSN EN 12457-4 standard, the amount of basic available nutrients was determined (N, P) along with the content of organic substances. The amount of the available phosphorus and calcium was determined using the extracts prepared according to Mehlich III and the extracts prepared with aqua regia. The chemical parameters derived from the leachates and extracts were determined using standard methods set out by Horáková (2007), Zbíral (2011) and Zbíral (2016). The results are expressed in weight units of dry mass of the sediments used.

Application of the PTP Plus was carried out in the prescribed amount by sprinkling it onto the water surface during 2017. The supplier of the preparation (Baktoma s. r. o., Czech Republic) prepared the specific dosage based on the character of the monitored ponds. The product was applied to the BOH II pond (standard dosage 100% = 7.5 kg) and the BOH III pond (double dosage 100% = 15 kg) a total of eleven times, in accordance with the instructions from the preparation supplier. As for the BOH I pond, the preparation got there only with flowing water. The year 2017 was characterised by low precipitation, which resulted in a lower water level in the ponds and a low flow of water between the ponds. The first application took place on 9 May 2017, followed by 6 applications in weekly intervals (100, 75, 75, 50, 50, 25, 25%), and then 4 applications in monthly intervals (always 25%), the last being done on 16 October 2017. The total amount of the applied product was 75 kg (BOH III) and 38 kg (BOH II).

In 5 spots for each pond, rods for indicative measurements of the loss of sediments were installed. The measuring was done by a person from Baktoma s.r.o. after the first month, in the middle and at the end of the application of the preparation. The difference in height from the top of the rod to the surface of the sediment was monitored (mm).

Results of the two treatment groups were compared using Student's t-test. Statistical analyses were performed using Statistica for Windows® 12.0 (StatSoft, Tulsa, OK, USA). Non-metric multidimensional scaling (NMDS) based on Bray-Curtis distance measure was used to show (dis) similarity in multidimensional space. We used all the physico-chemical parameters of both the sediment and water (cf. Tab. I and Tab. II) except for samples taken during the fish catch (October). Interpretation of NMDS ordination is easy: centroids close to each other are more similar. NMDS was performed in CANOCO 5.12 (Ter Braak and Šmilauer, 2018).

RESULTS

The total loss of sediment was determined to be 74 mm in BOH III, 86 mm in BOH II and 72 mm in BOH I. This means that, based on the indicative measurements, reduction in the amount of sediment was ascertained. Monitored parameters of water or sediment quality did not show any connection with obtained loss in sediment depth. For evaluation of the possible effects of the PTP Plus preparation, the water and sediment quality in the growing season of 2016 (before the application of the preparation) and in the growing season of 2017 (after the application of the preparation) were compared. The results are shown in Tab. I and Tab. II, including the statistically significant differences. However, different climatic conditions in each year had probably a major impact on the changes of the

I: *The physico-chemical parameters of the water of the monitored ponds during growing season (average ± standard deviation). Significant differences between 2016 and 2017 parameters of the pond are indicated by asterisks (* P < 0.05; ** P < 0.01), N = 6. TN – Total nitrogen, TP – Total phosphorus, ANC – Acid neutralisation capacity, COD – Chemical oxygen demand, BOD – Biochemical oxygen demand.*

		BOH I		BOH II		BOH III	
Parameter	Unit	2016	2017	2016	2017	2016	2017
Temperature	°C	17.92 ± 3.71	18.6 ± 1.97	17.70 ± 4.19	18.83 ± 1.66	18.06 ± 4.28	18.05 ± 1.42
Dissolved oxygen	%	28.3 ± 33.1	29.6 ± 21.5	32.3 ± 47.8	18.6 ± 15.3	35.9 ± 34.8	27.4 ± 29.6
pH	pH	7.60 ± 0.37*	6.89 ± 0.31	7.67 ± 0.63	7.12 ± 0.28	7.56 ± 0.39	7.43 ± 0.40
Conductivity	$mS.m^{-1}$	33.5 ± 3.0*	41.3 ± 5.0	36.3 ± 2.4**	44.2 ± 1.4	36.8 ± 5.1	38.3 ± 2.3
Transparency	m	0.60 ± 0.30	0.75 ± 0.37	0.72 ± 0.17	0.53 ± 0.21	0.85 ± 0.32	0.79 ± 0.29
TN	$mg.l^{-1}$	1.5 ± 0.5	2.0 ± 0.6	1.3 ± 0.5*	2.0 ± 0.4	1.7 ± 0.9	1.8 ± 0.2
TP	$mg.l^{-1}$	0.24 ± 0.10	0.22 ± 0.05	0.18 ± 0.08	0.29 ± 0.14	0.20 ± 0.09	0.21 ± 0.07
COD	$mg.l^{-1}$	45 ± 21	46 ± 15	33 ± 11	47 ± 9	36 ± 12	45 ± 7
BOD	$mg.l^{-1}$	7.75 ± 4.99	6.03 ± 4.11	4.98 ± 2.33	6.11 ± 2.44	8.87 ± 4.84	4.37 ± 1.48
Chlorophyll a	$mg.l^{-1}$	66.6 ± 50.2	55.3 ± 70.7	45.6 ± 41.5	57.5 ± 42.2	125.8 ± 115.0	30.1 ± 26.2
$N\text{-}NH_4$	$mg.l^{-1}$	0.09 ± 0.14	0.26 ± 0.38	0.08 ± 0.09	0.07 ± 0.09	0.07 ± 0.11	0.09 ± 0.10
$N\text{-}NO_2$	$mg.l^{-1}$	0.011 ± 0.02	0.008 ± 0.01	0.016 ± 0.02	0.002 ± 0.00	0.020 ± 0.02	0.005 ± 0.00
$P\text{-}PO_4$	$mg.l^{-1}$	0.061 ± 0.04	0.072 ± 0.04	0.047 ± 0.03	0.055 ± 0.03	0.037 ± 0.01	0.078 ± 0.05
$N\text{-}NO_3$	$mg.l^{-1}$	0.4 ± 0.7	0.1 ± 0.1	0.4 ± 0.6	0.1 ± 0.1	0.5 ± 0.8	0.0 ± 0.1
Chloride	$mg.l^{-1}$	24.2 ± 2.1*	27.3 ± 0.9	23.7 ± 1.5**	28.7 ± 0.8	23.0 ± 1.7**	27.5 ± 0.8
ANC	$mmol.l^{-1}$	2.59 ± 0.41	2.73 ± 0.46	2.86 ± 0.75	3.50 ± 0.33	2.69 ± 0.63	2.78 ± 0.32
Calcium	$mg.l^{-1}$	39.9 ± 3.2*	51.6 ± 7.1	45.3 ± 4.1**	54.3 ± 3.1	42.3 ± 6.5	46.1 ± 3.8

II: *The physico-chemical parameters of the sediments of the monitored ponds (average ± standard deviation). Significant differences between 2016 and 2017 parameters of the pond are indicated by asterisks (* P < 0.05; ** P < 0.01), COD – Chemical oxygen demand, WE – aqueous leachates, ME – soil extracts by Mehlich III, AE – soil extract with aqua regia.*

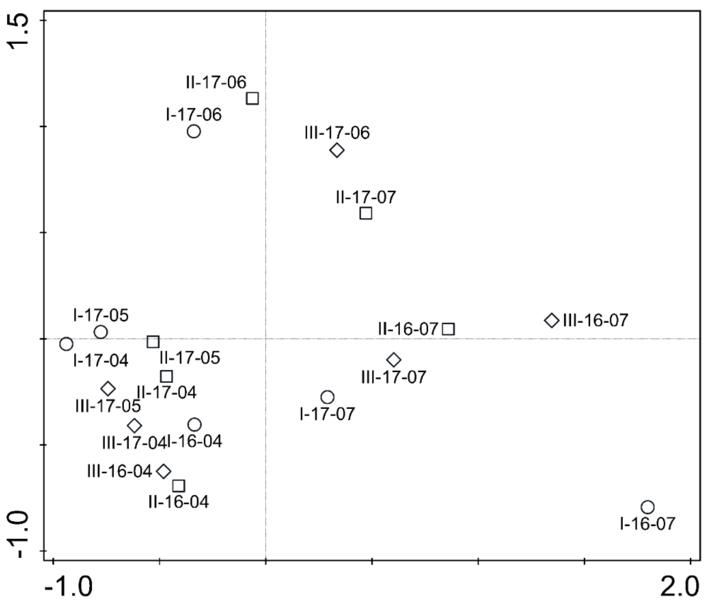
		BOH I		BOH II		BOH III	
Parameter	Unit	2016	2017	2016	2017	2016	2017
Dry matter	%	24.65 ± 7.21	29.98 ± 4.45	38.26 ± 3.32	39.27 ± 1.96	46.36 ± 2.46	44.39 ± 2.48
Organic matter	%	12.05 ± 3.10	13.21 ± 2.08	11.60 ± 2.58	9.90 ± 0.82	8.65 ± 1.43	8.56 ± 0.60
COD (WE)	$mg.l^{-1}$	30 ± 2**	41 ± 3	27 ± 8	38 ± 7	20 ± 5**	39 ± 2
Nitrogen (WE)	$mg.l^{-1}$	4.63 ± 1.60	2.42 ± 0.62	3.18 ± 0.83*	1.33 ± 0.45	2.20 ± 0.58	1.44 ± 0.36
Phosphorus (WE)	$mg.l^{-1}$	0.30 ± 0.12	0.25 ± 0.11	0.14 ± 0.02	0.23 ± 0.10	0.09 ± 0.01	0.19 ± 0.08
Phosphorus (ME)	$mg.kg^{-1}$	40.9 ± 9.2**	26.0 ± 5.4	36.2 ± 9.6	35.2 ± 7.3	24.4 ± 3.9*	48.8 ± 19.5
Phosphorus (AE)	$mg.kg^{-1}$	578 ± 174	639 ± 133	794 ± 173	716 ± 98	609 ± 152	549 ± 120
Calcium (WE)	$mg.l^{-1}$	38.6 ± 3.4	43.2 ± 18.0	58.6 ± 1.6*	37.7 ± 10.0	46.6 ± 6.3	35.0 ± 5.7
Calcium (ME)	$g.kg^{-1}$	3.11 ± 1.05	4.18 ± 0.73	5.22 ± 1.38	3.46 ± 1.52	4.13 ± 1.42*	2.51 ± 0.96
Calcium (AE)	$g.kg^{-1}$	9.74 ± 6.75	8.63 ± 1.86	12.90 ± 5.43	8.67 ± 2.34	7.38 ± 2.15	6.93 ± 1.81

monitored parameters. The ponds had lower water levels in 2017 compared to 2016 as a result of low amount of precipitation. Moreover, the water flow between the ponds was minimal and led to greater development of submerged and natant vegetation. NMDS showing water parameters did not reveal any similar groups, both studied year as well as all three ponds are randomly distributed within the ordination (Tab. I, Fig. 2). Selected parameters of sediment resulted in distinguishing among ponds but not between years (Tab. II, Fig. 3) indicating different sediment conditions of each pond. The effect of year is lower compared to pond definition.

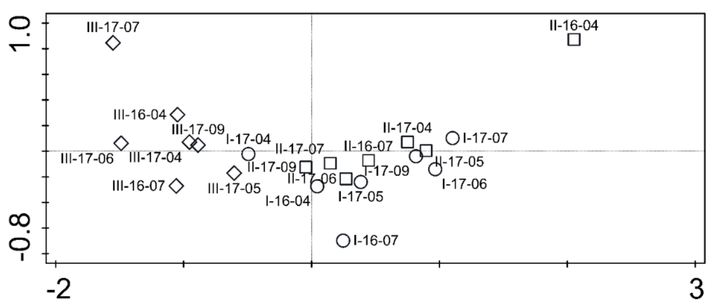
DISCUSSION

The application of the PTP Plus is supposed to reduce the share of organic substances, phosphorus and chlorophyll a. The content of dissolved oxygen should increase, along with the transparency of the water (Baktoma, 2018). None of these parameters changed significantly during our study. The sharp decrease in the values of chlorophyll a in pond BOH III in 2017 was caused mainly by the development of *Lemnion minoris*, when in 2017 a large part of the surface of the pond was covered, and the development of phytoplankton was thus significantly limited. The monitored parameters have been influenced mainly by the climatic conditions in each of the years of monitoring. As a result of low rain totals during the growing season, minimum water flow to ponds and high evaporation of water in 2017, there was a reduction in the volume of water in the monitored ponds. Reduction of water volume during the year leads to an increase in water conductivity and to increased concentration of several substances dissolved in the water, particularly chlorides and calcium (Pitter, 2015). Therefore, the significant increase in these parameters in 2017 cannot be said to be caused by the application of the PTP Plus.

Boyd *et al*. (1984), Tucker and Loyd (1985) monitored changes in water quality and in the growth rate of the fish (*Ictalurus punctatus*) after the addition of an inoculum with bacteria. They did not discover any significant differences between the testing and the control groups beyond an increase in the content of dissolved oxygen for the group with the addition of bacteria. Queiroz and Boyd (1998)



2: *Results of non-metric multidimensional scaling showing dissimilarity of ponds based on physico-chemical parameters of water (see Tab. I for the list of the parameters). Circles – BOH I, squares – BOH II, diamonds – BOH III. Labels show pond (I, II, III), year (16, 17) and month when particular sample was taken.*



3: *Results of non-metric multidimensional scaling showing dissimilarity of ponds based on physico-chemical parameters of sediment (see Tab. II for the list of the parameters). Circles – BOH I, squares – BOH II, diamonds – BOH III. Labels show pond (I, II, III), year (16, 17) and month when particular sample was taken.*

did not discover any significant changes in water or sediment quality after the addition of a bacterial inoculum called "Biostart" into ponds with channel catfish. For shrimp farming in ponds, no major differences in water quality between the ponds where commercial bacterial mixtures were added, and the control pond were recorded either. Only the value of ammoniacal nitrogen was significantly reduced after the addition of the bacteria (Shariff *et al*., 2001). During aquarium tests with the common carp, a significant decrease in the level of ammoniacal nitrogen, nitrates and phosphates in the water after the addition of bacteria of the Bacillus genus was observed (Lalloo *et al*., 2007). In general, it can be said that the effect of addition of bacteria on changes in water quality in production ponds is poor (Verschuere *et al*., 2000).

A significant increase in the amount of organic substances in the aqueous leachates of the sediment was discovered for ponds BOH III and BOH I. The amount of organic substances in the BOH III pond was also found to be higher than normal, but the results are not conclusive. This situation occurred probably as a result of a high share of dead organic matter of aquatic plants, whose biomass was higher in 2017 than in 2016. Other significant changes in the values of the monitored parameters of pond sediment are rather the result of differing physical and chemical conditions of the ponds in each of the years of monitoring than a result of the addition of the bacterial mixture. Similar results were recorded by Shariff *et al.* (2001) in the case of shrimp farming, and by Queiroz and Boyd (1998) in the case of channel catfish farming in ponds. In both cases, after the addition of a commercial bacterial mixture, no major differences in the content of carbon, nitrogen or phosphorus in sediments were recorded. The addition of the enzymatic mixture into the ponds with channel catfish farming did not cause any significant changes in the soil condition (Queiroz *et al*., 1998).

CONCLUSION

Our results confirm the findings of other authors who state that the effect of addition of bacterial mixtures into ponds on the improvement of the water and sediment quality is low. Their efficiency is better in terms of their probiotic effects in ponds where fish and other water organisms are farmed as they limit the impact of pathogenic organisms and reduce the mortality of the fish. The effects of probiotics are more pronounced if the health of the fish, crustaceans or molluscs that are being farmed is poor. The amount of knowledge about probiotic effects of various bacterial strains is not yet sufficient for their greater use in commercial farming of aquatic organisms (Boyd and Gross, 1998; Verscheure *et al*., 2000).

Acknowledgements

This study was supported by the Internal Grant Agency project No. AF-IGA-IP-2017/030 and by the project PROFISH CZ.02.1.01/0.0/0.0/16_019/0000869. The project is financed by European Regional Development Fund in the Operational Programme Research, Development and Education and the Czech Ministry of Education, Youth and Sports.

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Contact information

Barbora Müllerová: barborkamusilova@seznam.cz
Marija Radojičić: radojicic.marija88@gmail.com
Radovan Kopp: fcela@seznam.cz
Jan Grmela: JencekG@seznam.cz
Michal Šorf: michal.sorf@centrum.cz
Eva Poštulková: e.postulkova@seznam.cz