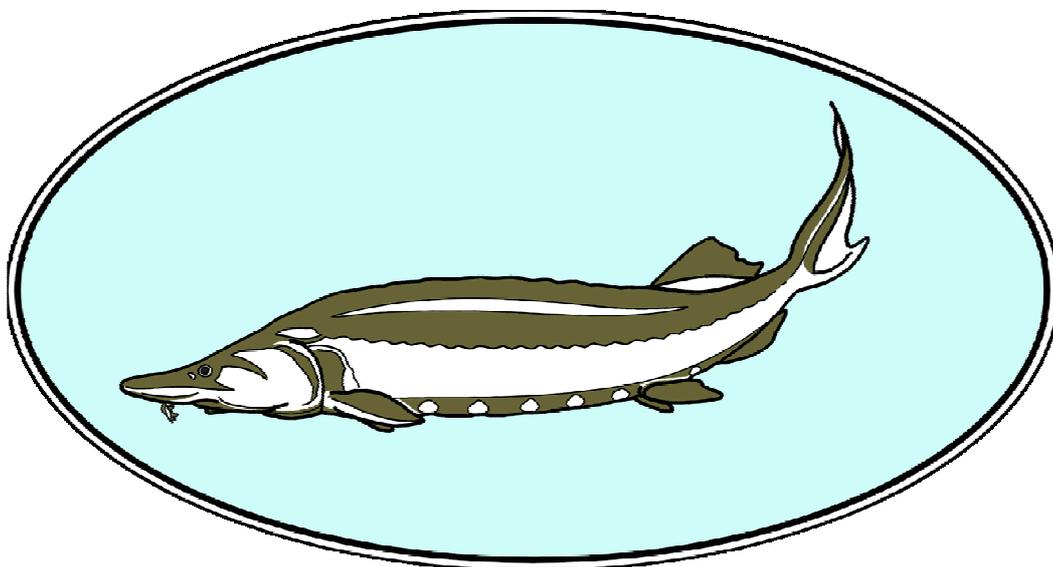


MENDEL UNIVERSITY IN BRNO
DEPARTMENT OF FISHING AND HYDROBIOLOGY



Evaluation of effects of microbial-enzymatic preparation SEKOL Lakus aqua – Čisté jezírko on the organism of common carp, composition of its muscle and sensory evaluation.

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BRNO, June 2012

Introduction

Based on a request made by **Sanbien Trade s.r.o. and ENZYMIX s.r.o.**, the producer of the preparation that limits growth of undesired blue-green and green algae, the department of fishing and hydrobiology performed in April and May 2012 a standard experiment with common carp fry to determine potential effects of the preparation on the fish organism. Apart from monitoring the quality of the environment for fish, we also conducted histological and sensory evaluation.

Material and methodology

In order to determine potential effects of the tested preparation on fish we used common carp fry (*Cyprinus carpio* L.) from a fish farm of Rybníkářství Pohořelice s.r.o. The experiment started after a necessary period of adaptation of the fish on 11 April 2011. We used three tanks with the capacity of 330 l, each of them with its own filtration system and water recirculation, with common carp fry of the average weight 0.93 kg. Physicochemical parameters were monitored on a daily basis and water in the tanks was partly changed (30-100 l), so that high levels of ammonia or nitrite nitrogen cannot put at risk the tested fish. A more detailed chemical analysis was conducted once a week.

One tank was used as a control and the other tanks were tested with different doses of the preparation. The tested concentrations corresponded to concentrations used for liquidation of blue-green and green algae in ponds (5 kg.ha⁻¹ and 15 kg.ha⁻¹, for the average pond depth of 1.5 m) and they were maintained at the same level throughout the entire time of the experiment (0.33 mg.l⁻¹, 1 mg.l⁻¹). The fish was fed with comprehensive feeding mixture for carp (Skretting F-2P B40 4mm, 41% of protein, 12% of fat). The originally planned duration of the experiment, i.e. 30 days, was extended so that the planned sensory evaluation could be performed for fresh fish muscle. The experiment was closed after 36 days on 17 May 2012.

Basic parameters of the fish, i.e. length and weight, were determined at the beginning and at the end of the experiment, as well as the content of fat, protein and ash matter in the fish tissue. Also basic indicators were calculated of the fish condition.

Concentration of oxygen, percentage of water saturation with oxygen, pH and temperature of the water were determined using HACH Hq 40d (Hach-Lange, Colorado, USA). A multipurpose instrument made by the US company Hanna Instruments - Combo HI 98129 was used to determine specific conductivity, with automatic temperature correction to 25 °C.

Values of ammonia and nitrite nitrogen, chlorides and BOD₅ were determined by means of standard methods for analysis of surface water (HORÁKOVÁ et al., 2007). To determine CHOD_{Cr}, total nitrogen and phosphorus we used commercial sets made by WTW. All photocolorimetric determinations were conducted on PhotoLab Spektral spectral photometer made by WTW.

The histological examinations of hepatopancreas and kidneys were conducted by veterinary pathologist Doc. MVDr. Ladislav Novotný, CSc. Samples of hepatopancreas and cranial kidney were examined for 18 individuals.

The sensory evaluation was performed in an accredited sensory laboratory at the Mendel University in Brno by a group of six evaluators with adequate qualifications. The individual descriptors (parameters) were evaluated by means of a non-structured scale (100 mm, 1 mm = 1 point). The evaluated parameters included intensity of smell and its agreeability, texture in mouth, juiciness, intensity of taste and its agreeability.

A statistical evaluation of the data was conducted using the t-test by means of the statistical program Statistica 8.0 (StatSoft, USA).

Results

Physicochemical parameters

Table No. 1 shows the monitored physicochemical parameters during the experiment. As it was necessary to use fish of higher weight (sufficient quantity of muscle was needed for sensory evaluation) the exposure of the breeding tanks was high and the range of some of the parameters was beyond the framework of the optimum values for carp fish farming. In order to achieve optimum living conditions in the environment up to 1/3 of water volume in the tank was replaced every day. Despite that, some different trends were found in fluctuation of certain parameters.

Tab. No. 1 Values of basic physicochemical parameters in the course of the experiment (average \pm standard deviation)

Parameter		1 mg.l ⁻¹	0.33 mg.l ⁻¹	Control
Oxygen	%	74.2 \pm 7.1	79.0 \pm 6.4	76.9 \pm 7.2
pH		7.8 \pm 0.2	7.9 \pm 0.3	7.9 \pm 0.2
Temperature	°C	22.7 \pm 0.8	22.8 \pm 0.6	22.7 \pm 0.5
Conductivity	mS.m ⁻¹	141.0 \pm 29.0	148.1 \pm 29.0	139.7 \pm 28.9
N-NO ₂	mg.l ⁻¹	1.32 \pm 2.22	2.29 \pm 3.44	1.00 \pm 1.13
N-NH ₄	mg.l ⁻¹	0.48 \pm 0.52	1.06 \pm 2.23	1.81 \pm 3.67
Cl ⁻	mg.l ⁻¹	336.4 \pm 135.5	365.9 \pm 153.1	334.9 \pm 154.7
N _{tot.}	mg.l ⁻¹	24.2 \pm 10.2	23.0 \pm 7.9	19.4 \pm 5.0
P _{tot.}	mg.l ⁻¹	0.7 \pm 0.2	0.5 \pm 0.1	0.6 \pm 0.2
CHOD _{Cr}	mg.l ⁻¹	40.1 \pm 27.3	39.7 \pm 21.6	37.2 \pm 32.7

BOD ₅	mg.l ⁻¹	10.2 ± 7.4	12.4 ± 6.0	12.6 ± 7.3
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The value of the dissolved oxygen, water temperature and pH were stable throughout the monitoring period, without significant deviations and at the optimum levels for common carp. Water conductivity values, similarly as concentration of chlorides, were high as a result of added sodium chloride that was used to eliminate negative effects of high values of nitrite nitrogen. The achieved values of water conductivity and concentrations of chlorides had no negative effect on the tested fish.

The values of nitrite nitrogen from the fourth day of the experiment started growing in all the three tanks. A clear trend was found of higher N-NO₂ values for groups with higher doses of the preparation in comparison with the control group. Values of N-NO₂ in the tanks with the added preparation were 2-3 times higher than those in the control group but, on the other hand, the concentration of nitrites decreased earlier than in the control group (approximately by one week). The results indicate that the preparation influences composition of nitrification bacteria and if the preparation is used in systems with recirculation this effect shall be taken into account. In case of application into natural water ecosystems (reservoirs, ponds etc.) the effect of the preparation on the N-NO₂ value will be negligible.

The value of ammonia nitrogen was significantly increased in the course of 10 days (8-18 day of the experiment) due to the high stock concentration (feed, products of metabolism) and low capacity of the filtration system. In this case an opposite trend was found than for the nitrite nitrogen, while an increase of N-NH₄ in the tank with a higher dose of the preparation was significantly lower than in the control group. The results make it possible to conclude that the preparation influences the composition of nitrification bacteria and speeds up degradation of ammonia nitrogen. In the case of application of the preparation into natural water ecosystems the effect of the preparation on N-NH₄ will be negligible, similarly as in the case of N-NO₂.

The content of organic matter (CHOD_{Cr}, BOD₅), total nitrogen and phosphorus did not demonstrate any significant differences and it was in the range usual for eutrophic ponds in the Czech Republic

Characterization of the fish used in the experiment

Tables No. 2 and 3 contain the basic parameters of length and weight of the fish and calculated basic somatic indexes. No significant differences were found between the individual fish groups from the viewpoint of weight gain and the changes found in

comparison with the beginning of the experiment (e.g. reduction of the body width, increase of the body height etc.) were observed in all the groups of fish, including the control group.

Significant changes were found in the values of somatic indexes (see Table No. 3), with an apparent trend of all the three indexes to decrease with the increasing concentration of the preparation. The decrease of the HSI and VSI values indicates a lower deposition of fat and glycogen in hepatopancreas and a lower content of fat in the body cavity. The duration of the experiment did not make it possible to positively determine whether this was a lasting phenomenon, with either a positive or negative effect on the fish organism. However, it is possible to conclude that the tested preparation probably influences deposition of fat in the fish body.

Table No. 2 Length and weight characteristics of the tested fish (DC - total length, DT – body length, V – height, Š – width, W – weight, Fc – Fulton coefficient of condition, Iv – height-to length ration, IŠ – weight-to-length ratio)

	Beginning of the experiment			End of the experiment		
	10 April 2012			17 May 2012		
	1 mg.l ⁻¹	0.33 mg.l ⁻¹	Control	1 mg.l ⁻¹	0.33 mg.l ⁻¹	Control
DC	391 ± 11	385 ± 21	382 ± 14	404 ± 16	388 ± 16	387 ± 15
DT	317 ± 10	314 ± 17	309 ± 14	333 ± 13	316 ± 13	316 ± 14
V	113 ± 4	113 ± 7	114 ± 5	120 ± 7	133 ± 8	122 ± 5
Š	62 ± 2	62 ± 4	62 ± 3	59 ± 2	57 ± 3	59 ± 2
W	927 ± 70	927 ± 122	913 ± 102	1025 ± 84	928 ± 129	994 ± 99
Fc	2.92 ± 0.19	2.99 ± 0.24	3.09 ± 0.26	2.79 ± 0.22	2.93 ± 0.36	3.16 ± 0.19
Iv	2.80 ± 0.09	2.78 ± 0.15	2.72 ± 0.11	2.78 ± 0.22	2.81 ± 0.19	2.58 ± 0.08
IŠ	19.51 ± 0.77	19.77 ± 0.79	20.00 ± 0.74	17.77 ± 0.79	18.14 ± 0.82	18.61 ± 0.41

Table No. 3 Average values (± standard deviation) of somatic indexes of the tested fish at the end of the experiment. (HSI – hepato-somatic index, VSI – viscerosomatic index, GSI – gonado-somatic index), * - statistical significance level (p < 0.05), ** - statistical significance level (p < 0.01) in comparison with the control group of fish

Group \ index	HSI	VSI	GSI
1 mg.l ⁻¹	2.19 ± 0.17 **	8.39 ± 0.47 *	1.42 ± 0.55
0.33 mg.l ⁻¹	2.50 ± 0.16 **	8.55 ± 0.53 *	2.10 ± 0.93
Control	3.19 ± 0.36	10.85 ± 2.14	2.96 ± 2.29

Analyses of fish tissues

At the beginning and at the end of the experiment analyses were conducted of selected fish tissues (see Table No. 4). The monitored parameters included dry matter, fat, protein and ash matter. Some differences were found between the initial values and results of analyses conducted at the end of the experiment but this was expected with regard to the significant change of fish farming conditions (the fish was moved from the natural environment into a recirculation system, higher temperature of water etc.) No significant differences were found between the tested groups and the control group at the end of the experiment. Values of some of the parameters could not be statistically evaluated (hepatopancreas, entrails), the indicated values are average values from two determinations of mixed samples of the fish. The values, however, were within the interval usual for the given type of fish. The effect of tested preparation on the composition of tissues of the tested fish was not demonstrated by the experiment.

Table No. 4 Analyses of fish tissues, the indicated values are in % of the fish body (average \pm standard deviation)

Date	Variant	Tissue	Dry matter	Fat	Proteins	Ash
10 Apr 2012	Input	Hepatopancreas*	20.16	4.74	-	-
		Whole fish	20.28 \pm 0.68	4.15 \pm 0.44	12.67 \pm 0.83	2.81 \pm 0.23
		Muscle	18.37 \pm 0.41	1.69 \pm 0.22	15.07 \pm 0.77	1.00 \pm 0.06
17 May 2012	Control	Entrails *	20.52	4.46	-	-
		Hepatopancreas*	26.71	7.48	-	-
		Muscle	21.46 \pm 0.60	3.06 \pm 0.27	17.29 \pm 0.79	1.08 \pm 0.08
	0.33 mg.l ⁻¹	Entrails *	19.04	3.64	-	-
		Hepatopancreas*	24.15	3.41	-	-
		Muscle	20.62 \pm 1.17	2.72 \pm 0.72	16.69 \pm 0.56	1.09 \pm 0.11
	1.0 mg.l ⁻¹	Entrails *	20.34	5.45	-	-
		Hepatopancreas*	25.77	5.25	-	-
		Muscle	21.60 \pm 0.58	3.59 \pm 0.72	16.35 \pm 0.25	1.13 \pm 0.03

* mixed sample from several pieces of fish

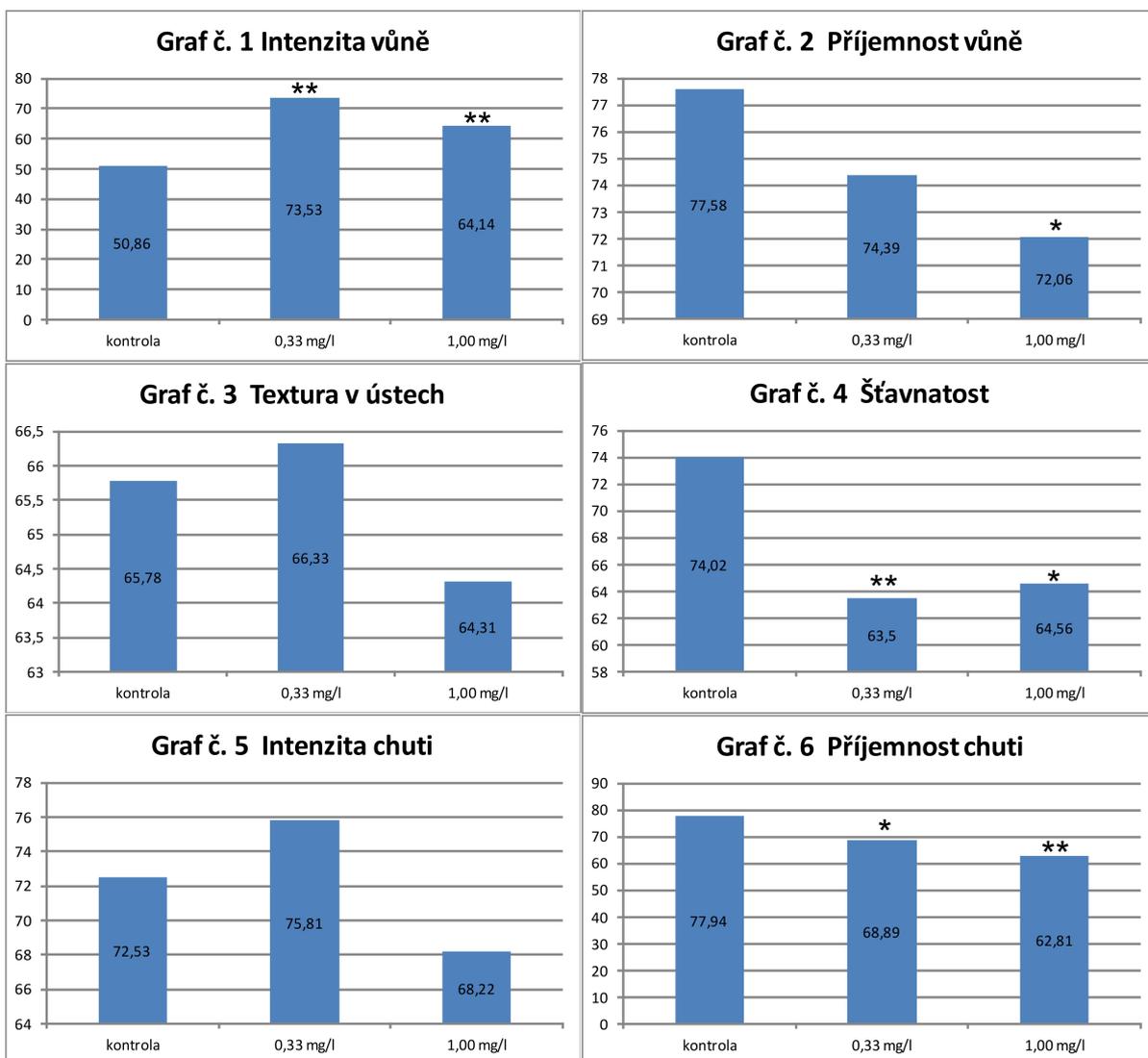
Histological examination of hepatopancreas and kidney

Samples of hepatopancreas and cranial kidney were examined for 18 individuals (6 from each tested group). Histology of hepatopancreas proved well-preserved architecture, hepatocytes and pancreatic tissues had normal morphology for all the tested groups of fish. Architecture of the cranial kidney was well-preserved, with congestions, sporadic thyroidal follicles, hemopoietic and interregional endocrinal tissue with normal morphology, sporadic melanomacrophages. Histology examination did not demonstrate any pathomorphological changes in any of the tested groups of fish.

Sensory evaluation of fish muscle

Evaluation of sensory descriptors has become a standard part of tests monitoring quality of fish muscle. Results of the sensory tests are provided in the diagrams No. 1 through 6 below. Statistically significant differences were found in the intensity of smell , where fish muscle with an addition of the preparation had a more intense smell but agreeability of the smell was higher for fish muscle from the control group of fish. Juiciness and agreeability of taste were also higher for fish muscle from the control group of fish. The parameter of texture in mouth and intensity of taste demonstrated no conclusive difference between the groups. The results indicate that the tested preparation influences some sensory descriptors of fish muscle.

Diagrams No. 1 - 6 Evaluation of the individual sensory descriptors * - level of statistical significance ($p < 0.05$), ** - level of statistical significance ($p < 0.01$) in comparison with the control group of fish



kontrola = control

Diagram No. 1 Intensity of smell

Diagram No. 2 Agreeability of smell

Diagram No. 3 Texture in mouth

Diagram No. 4 Juiciness

Diagram No. 5 Intensity of taste

Diagram No. 6 Agreeability of taste

Conclusions

The performed experiment that monitored effects of the tested preparation on common carp has demonstrated effects on some of the indicators. The preparation modulates composition of nitrification bacteria and thus, indirectly, also quantities of nitrogen compounds, it reduces values of somatic indexes of the tested fish and also influences some sensory parameters of fish muscle. No effect of the preparation was found

on length and weight parameters, composition of fish tissue or histology of hepatopancreas and kidney.

As the preparation is applied exclusively in water bodies in the natural environment the effect on chemistry of water can be considered negligible. Based on the obtained data the changes of somatic indexes of fish caused by the preparation cannot be evaluated as negative or positive. In the natural environment the effects of the preparation on sensory values of fish muscle will be manifested in a much smaller scale. Moreover, once the application of the preparation into water environment is finished a fast reduction of its effects on sensory parameters of fish muscle is expected; moreover, the target organisms (blue-green and green algae) to be suppressed by the preparation often adversely influence sensory parameters of fish meat by its metabolites.

Based evaluation of all the obtained data the preparation can be characterized as suitable for application in the water environment without any risk for fish stock and other fauna.

In Brno 23 June 2012

Doc. Ing. Radovan Kopp, Ph.D

**Czech Anglers Union
MO Šluknov**

Protocol about application of the preparation Čisté jezírko

On 26 April 2010, based on a biological survey and analysis of blue-green algae and based on a resolution adopted by the committee, workers of ENZY MIX s.r.o. and members of the committee of the local organization (MO) Šluknov of the Czech Anglers Union applied bacterial enzymatic preparation Čisté jezírko in the water body of Šluknovská nádrž, fishing district No. 441 054 with the area of 8.5 ha.

At the time of the application the water demonstrated visible contamination with green and blue-green algae, it was non-transparent and dark green and in some locations completely covered with green algae. The measured pH was 13.1.

The first application used 50 kg of the preparation Čisté jezírko.

The second application fourteen days later, i.e. on 7 May 2010, used again 50kg of the preparation while reduction of green algae was already visible and the water was slightly transparent. It was possible to observe large quantities of young zooplankton and the measured pH was 10. One month after the first application the water was completely transparent with pH 7.8. Large quantities of zooplankton were visible, as well as movement of fish.

Preventive application of 20 kg of the preparation Čisté jezírko was performed on 5 May 2011. The water was full of fallen algal bloom but otherwise it was transparent in comparison with previous years. Major movement of fish was visible and the fish stock was more agile. The current pH is in the range 7.5 – 8.1

In Šluknov, on 11 May 2011

For MO Šluknov
Pond administrator responsible for contamination
management
Rudolf Reintsch Tel:+420/606944196

Czech Anglers Union
Local organization
407 77 Šluknov, Na hrázi 837
Company Registration Number (IČO) : 00482811
Signature illegible

Český rybářský svaz
(Czech Anglers Union)
MO Krásná Lípa
Frindova 8 407 46

Evaluation of application of the preparation Čisté jezírko made by ENZYMIX s.r.o. in water bodies used for angling and fish farming and managed by MO Krásná Lípa

A resolution adopted by the committee at its meeting on 25 April 2010 approved application of the microbiological and enzymatic preparation Čisté jezírko for ponds used for angling and fish farming in the fishing district Křinice II.

The concerned bodies included the pond **Cimerák**, fishing district No. 441 018-6, 2.8 ha, **deep pool V**, 7 ha, and fish farming ponds – **deep pool IV**, **Klabeček**, **Králův**, **Březák** and others with the total area of 10.2 ha.

All the water bodies were covered with blue-green and green algae that made the water column non-transparent and the water was dark green. The values of pH were 8.2 in the **Cimerák** pond, pH 8.5 in **deep pool V**, with visible contamination with blue-green algae, pH 7.2 in **Klabeček** and on average pH 7.8-8.2 in the other water bodies while contamination with blue-green algae was less visible.

5 kg of the preparation was used in **Cimerák** and 4 kg in the deep pool V as it manifested a high level of contamination. 7.5 kg of the preparation Čisté jezírko was used in fish farming ponds with the total area of 10.2 h. The application was performed on 10 May 2010 with a follow-up inspection on 30 May 2010. The inspection of the **deep pool V** showed green algae in a strip one meter broad around the banks but otherwise the water body was clean with pH 8.2. Therefore addition 1 kg of the preparation was applied into the **deep pool V** to speed up removal of the blue-green and green algae. In the **Cimerák** pond the water was clean without any algae, pH 8 and numerous small crustaceans were visible. Significant improvement of water cleanliness was also visible in the other water bodies, they were free of green and blue-green algae and in fish farming ponds fish movement was visible and it was obvious that quantities of powder and zooplankton increased as a result of application of the preparation which was highly beneficial for the fish stock.

An inspection of the treated water bodies one year after the application was performed on **9 May 2011** and it was concluded that there were no visible blue-green and green algae in the bodies, except in the deep pool V, where some minor occurrence of blue-green algae was reported, however, very low in comparison with the previous year. Therefore we decided to use 0.5 kg of the preparation as a preventive measure in all the ponds to prevent potential occurrence of blue-green algae. We also decided to apply 1.5 kg of the preparation Čisté jezírko in the deep pool V.

In Krásná Lípa, on 12 May 2011

For ČRS MO Krásná Lípa
Tomáš Krajmer administrator
Tel. 723 839 746

Czech Anglers Union
Local organization
Krásná Lípa u Rumburka, district Děčín
Signature illegible