Influence of arsenic and cyanobacteria co-exposure on plasmatic parameters of rainbow trout (*Oncorhynchus mykiss* W.)

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Abstract

OBJECTIVES: Fish can be exposed under environmental conditions to multiple stressors including natural toxins and environmental or feed contamination at the same time. This study brings new knowledge about the effects of controlled exposure to multiple stressors in fish. The aim of this study was to test the hypothesis that influence of cyanobacterial biomass and arsenic in feed can combine to enhance the effects on fish.

METHODS: Rainbow trouts were sorted into six groups, each with 25 specimens: control group (fed with commercial feed), groups exposed to toxic cyanobacterial biomass (81 $mg.kg^{-1}$ MCs of feed), two groups exposed to arsenic (concentration of 5 $mg.kg^{-1}$, and 50 $mg.kg^{-1}$ of fish feed) and two groups exposed to combination of cyanobacterial biomass and arsenic in two concentrations mentioned above. The experiment lasted 30 days. During the experiment we evaluated the influence of co-exposure on plasmatic parameters mentioned above. Samples were collected on days 10, 20 and 30 of exposure.

RESULTS: Biochemical analysis revealed a significant decrease in calcium (T20) and an increase in natrium (T10) and chlorides (T10) values in combined cyanobacterial and arsenic exposures. Our results showed a significant decrease in the values of magnesium after exposure to higher concentration of arsenic compared to control and feeding with addition of cyanobacterial biomass groups. The changes of other monitored plasmatic parameters were not significantly increased or decreased in comparison with controls.

CONCLUSIONS: Our results confirmed the hypothesis that influence of toxic cyanobacterial biomass and a chemical agent represented by arsenic can combine to enhance the effects on fish. This work originally shows that while the single agents in sub-lethal doses do not cause changes in the plasmatic parameters, their co-exposure leads to the significantly decrease or increase of the electrolytes of rainbow trout.

ORIGINAL ARTICLE

Abbreviations:

ALB	- albumin
ALT	- alanine aminotransferase
ALP	- alkaline phosphatase
AST	- aspartate aminotransferase
AMP	- adenosine monophosphate
AMS	- amylase
ANOVA	- analysis of variance
As	- arsenic
As 5; As 50	- groups fed with addition of arsenic
B	- group fed with addition of cyanobacterial biomass
B + As 5; B + As 50	- groups with combined feeding
BE	- brutto energy
C	- control group
CHOD-PAP	- enzymatic colorimetric determination of serum cholesterol
CHOL	- cholesterol
CREA	- creatinine
GLU	- glucose
LACT	- lactate
LDH	- lactate dehydrogenase
MC, MCs	- microcystin, microcystins
Na^+/K^+-ATPase	- sodium-potassium adenosine triphosphatase
NADH	- nicotinamide adenine dinucleotide
NADPH	- nicotinamide adenine dinucleotide phosphate
NFE	- nitrogen free extract
$N\text{-}NH_4$	- ammonia nitrogen
$N\text{-}NO_2$	- nitrite nitrogen
$N\text{-}NO_3$	- nitrate nitrogen
$P\text{-}PO_4$	- phosphate phosphorus
T 10	- 10 days after start of the experiment
T 20	- 20 days after start of the experiment
T 30	- 30 days after start of the experiment
TP	- total protein
TRIG	- triglycerides
UREA	- uric acid
UV/VIS	- ultraviolet-visible spectrophotometry

INTRODUCTION

Cyanobacteria and fish co-evolved in the same habitats and thus the question arises whether the cyanotoxins containing cyanobacteria given via the natural exposure route as a component of fish diet might affect the fish physiology (e.g. growth) and cause toxin accumulation in fish. The influence of cyanotoxins on fish following experimental intoxication or the impact of an environment containing cyanotoxins on fish has been studied using clinical, morphological, histological, ultrastructural, haematological and biochemical methods (Ibelings & Chorus 2007). One of the most common results of cyanobacterial blooms is the production of hepatotoxic microcystins (Microcystis) that can occasionally occur in high concentrations in shallow waters where cyanobacteria can accumulate and may induce injury of fish. A complete summary about the effects of microcystins on fish was provided by Malbrouck and Kestemont (2006).

Arsenic is graded as one of the most toxic elements to fish. In the aquatic environment fish appear to be valuable bioindicators of arsenic toxicity as they are continuously exposed to arsenic through gills respiration and intake of arsenic contaminated food (Ghosh *et al.* 2007). Acute exposure can result in immediate death because of As-induced increasing in mucus production, subsequently suffocation, or direct detrimental effects on gill epithelium (Bhattacharya *et al.* 2007). Many studies have also examined sublethal effects such as growth reduction, avoidance behavior, haematological, biochemical and ionoregulatory responses (Lima *et al.* 1984; Lavanya *et al.* 2011). Arsenic is a toxic element that is commonly found in feed mixtures for fish. Content of arsenic in complete feed for fish is limited in Czech Republic by Notice No. 356/2008 Sb. Limit is 6 $mg.kg^{-1}$.

Under environmental conditions, fish can be exposed to multiple stressors including natural toxins and chemicals simultaneously. The synergistic interaction between chemicals and natural stressors considerably complicates ecological risk assessment of chemical mixtures and interactions with cyanobacterial stressors have received very little attention so far (Cerbin et al., 2010; de Coninck *et al.* 2013).

This study brings new knowledge on the effects of controlled exposure to multiple stressors in fish. Low concentration of evaluated agens did not have any influence on the mortality of fish. Considering the fact that in the natural conditions, there are no single influences of particular factors on the organism, but conditions of the environment have cumulative effect, it is misleading to evaluate the influence of single agent on the fish organism.

The aim was to test the hypothesis that the influence of cyanobacterial biomass in feed and feed contamination represented by arsenic can combine to enhance the effects on fish. For this purpose we compared the effects of single and combined exposures and evaluated the plasmatic parameters.

MATERIALS AND METHODS

Experimental design

Fish (rainbow trout, *Oncorhynchus mykiss* W.) with average weight of 274±40 g were obtained from commercial Fishery Skalní Mlýn, Czech Republic. Fish were divided into six groups, each with 25 specimens (1 control group and 5 groups experimental) and placed to the laminated circular tanks with volume 1 m^3 and with own recirculation. Fish were exposed to a 12-h light/12-h dark photoperiod. Fish were fed twice a day in the whole amount of 0.8% of fish weight in the first 10 days and then in amount of 1% of weight of fish stock till the end of experiment. Adaptation of the feed dose was made once for 10 days on the basis of actual fish weight. Fish in the control group were fed by commercial food EFICO Enviro 920 (Biomar, Denmark, 44% proteins, 29% fat, 12.4% NFE, BE 25 $MJ.kg^{-1}$). The amount of arsenic in feed was 1.55 $mg.kg^{-1}$. Fish in the experimental groups were fed by the same food with addition of 3% of lyophilised toxic cyanobacterial biomass with microcystins or with addition of arsenic (As^{5+} in 2% nitric acid) at concentration of 5 $mg.kg^{-1}$

and 50 mg.kg^{-1} of fish feed, or with their combinations respectively. The scheme of the experiment is presented in the Table 1. In addition, cyanobacterial biomass (monospecific population of *Microcystis aeruginosa*, 43.3% proteins, 2.3% fat) has been added to the feed at specific exposure groups. The concentrations of the total microcystins in the cyanobacterial biomass were 2.70 mg.g^{-1} dry weight. Three dominant microcystin variants were present in the cyanobacteria with the following average concentrations: microcystin-RR 1.46, microcystin-YR 0.10 and microcystin-LR 1.09 mg.g^{-1} dry weight. The concentrations of microcystins in the biomass were determined by reverse phase high performance liquid chromatography with UV/VIS detection using established methods (Blaha & Marsalek 2003). The whole amount of microcystins was 81 mg microcystin.kg^{-1} of food, i.e. 0.76 mg microcystin.kg^{-1} of fish weight and day. The whole amount of arsenic in feed was 5 mg.kg^{-1} and 50 mg.kg^{-1}, i.e. 0.05 mg.kg^{-1} and 0.5 mg.kg^{-1} of fish weight and day respectively.

The exposure lasted 30 days. Samples for evaluation were taken in days 10, 20 and 30 (T10, T20, T30). Each sampling, seven specimens from each group were euthanized, necropsed and analysed. Experiment was performed in compliance with the laws for the protection of animals against cruelty as approved by the Ethical Committee of the University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic.

Tab. 1. The scheme of the experiment.

Group [marking]	Additions to feed
Control [C]	–
Single experimental [B]	3% lyophilised cyanobacterial biomass
Single experimental [As 5]	arsenic at concentration of 5 mg.kg^{-1}
Single experimental [As 50]	arsenic at concentration of 50 mg.kg^{-1}
Combined experimental [B + As 5]	3% lyophilised cyanobacterial biomass and arsenic at concentration of 5 mg.kg^{-1}
Combined experimental [B + As 50]	3% lyophilised cyanobacterial biomass and arsenic at concentration of 50 mg.kg^{-1}

Water quality analyses

A series of physicochemical parameters [temperature, dissolved oxygen, pH, conductivity, nitrite nitrogen (N-NO_2), nitrate nitrogen (N-NO_3), ammonia nitrogen (N-NH_4) and phosphate phosphorus (P-PO_4)] were determined every week. Water parameters during the experiments were as follows (given for the control and experimental groups respectively): water temperature 16.4±0.3, 15.4±0.3 °C; dissolved oxygen 91.5±4.2, 91.7±3.6%; pH 8.2±0.2, 8.3±0.1; conductivity (recalculated on 25 °C) 70.5±9.8, 70.9±9.6 mS.m^{-1}; ammonia 0.05±0.04, 0.04±0.04 mg.l^{-1} N-NH_4; nitrate 24.3±2.3, 24.2±2.1 mg.l^{-1} N-NO_3; nitrite 0.12±0.09, 0.13±0.10 mg.l^{-1} N-NO_2; and phosphate 0.66±0.04, 0.62±0.16 mg.l^{-1} P-PO_4. Water saturation by oxygen, pH and temperature were measured using the portable Oxi 340i meter (WTW, Weilheim, Germany). Conductivity measurements were taken by conductivity meter HI 98129 (HANNA Instruments, USA) and other chemical parameters were determined using standard methods (APHA 1998).

Sampling and measurement of biochemical parameters

Fish blood was collected by cardiac puncture using heparinised syringes. Heparin at a concentration of 50 I.U. per 1 ml was used for blood stabilization. The blood was centrifuged at 400 g for 15 min at 4 °C, and the resulting plasma was stored at –80 °C until the day of the analyses. Biochemical analyses were performed by the automatic clinical chemistry analyzer KONELAB T20xt (Thermo Fisher Scientific, Finland) using commercially available reagents. Serum enzymatic activities were determined at 37 °C. Alanine aminotransferase (ALT) activity determination was based on the kinetic assessment of NADPH consumption coupled with the generation of pyruvate. Aspartate aminotransferase (AST) activity was determined by the kinetic measurement of NADH consumption coupled with the formation of oxaloacetate (Expert panel on enzymes of the IFCC 1976). Lactate dehydrogenase (LDH) was determined as the formation of NADH during the conversion of L-lactate to pyruvate (Hajzer & Jagelkova 1988). Alkaline phosphatase (ALP) was determined by a modification of the enzymatic method using an AMP (adenosine monophosphate) buffer (Tietz 1980).

Total serum protein (TP) was determined by the biuret reaction (Doumas *et al.* 1981). Glucose (GLU) concentration was determined by the glucose hexokinase method at 37 °C with an endpoint reading at 340 nm (Barham & Trinder 1972). Calcium (Ca) and magnesium (Mg) concentrations were determined by the modified colorimetric methods with arzenazo III (Michaylova & Ilikova 1971; Skavrada 1999). Phosphorus (P) was determined by an endpoint method with sample blanking using an ammonium molybdenate reagent (Kratochvila & Garcic 1977). Iron (Fe) was determined by the photometric method with ferene (ferroin-type reagent) without deproteination (Higgins 1981). The concentration of lactate (LACT) in plasma was measured by the enzymatic method according to Shimojo *et al.* (1989). Albumin (ALB) was determined by the photometric method with bromocresol green (Doumas *et al.* 1971). Urea (UREA) concentrations were determined by the kinetic enzymatic method with urease (Roch-Ramel 1967). The cholesterol (CHOL) was determined by the CHOD-PAP method after enzymatic hydrolysis and oxidation (Roschlau *et al.* 1974). Electrolyte levels (Na, K, Cl) were analysed with ion selective electrodes (Eisenman 1967) by an electrolyte

analyzer EasyLyte Plus (Medica, USA). The triglycerides (TRIG) were assessed by the Fossati three-step enzymatic reaction with a Trinder endpoint (Fossati & Prencipe 1982). The creatinine (CREA) was determined by the Jaffe kinetic method without deproteination (Jaffe 1886).

Statistical analyses

Statistical analyses were performed with Statistica for Windows® 9.0 (StatSoft, Tulsa, *OK,* USA). Results from different treatment groups were compared by one-way analysis of variance (ANOVA) and post-hoc analysis of means using the Scheffe test.

RESULTS

The results of the study are presented in the Table 2. The biochemical parameters affected by cyanobacterial biomass and arsenic are presented in the Figure 1. Biochemical analysis revealed a decrease in calcium (T10) and an increase in natrium and chlorides values (T20) in combined cyanobacterial and arsenic exposures. Our results showed a significant decrease in the values of magnesium (T10) after exposure to higher concentration of arsenic compared to C and B groups. The changes of others monitored plasmatic parameters were not significantly increased or decreased in comparison with controls.

DISCUSSION

In eutrophic water bodies, fish are commonly exposed to various concentrations of cyanobacteria. During summer, this exposure can last for weeks. It was shown previously that exposure to cyanobacteria or their released toxins can cause various health problems in animals. However, the fish can be affected by multiple stressors in the environment and while mostly single

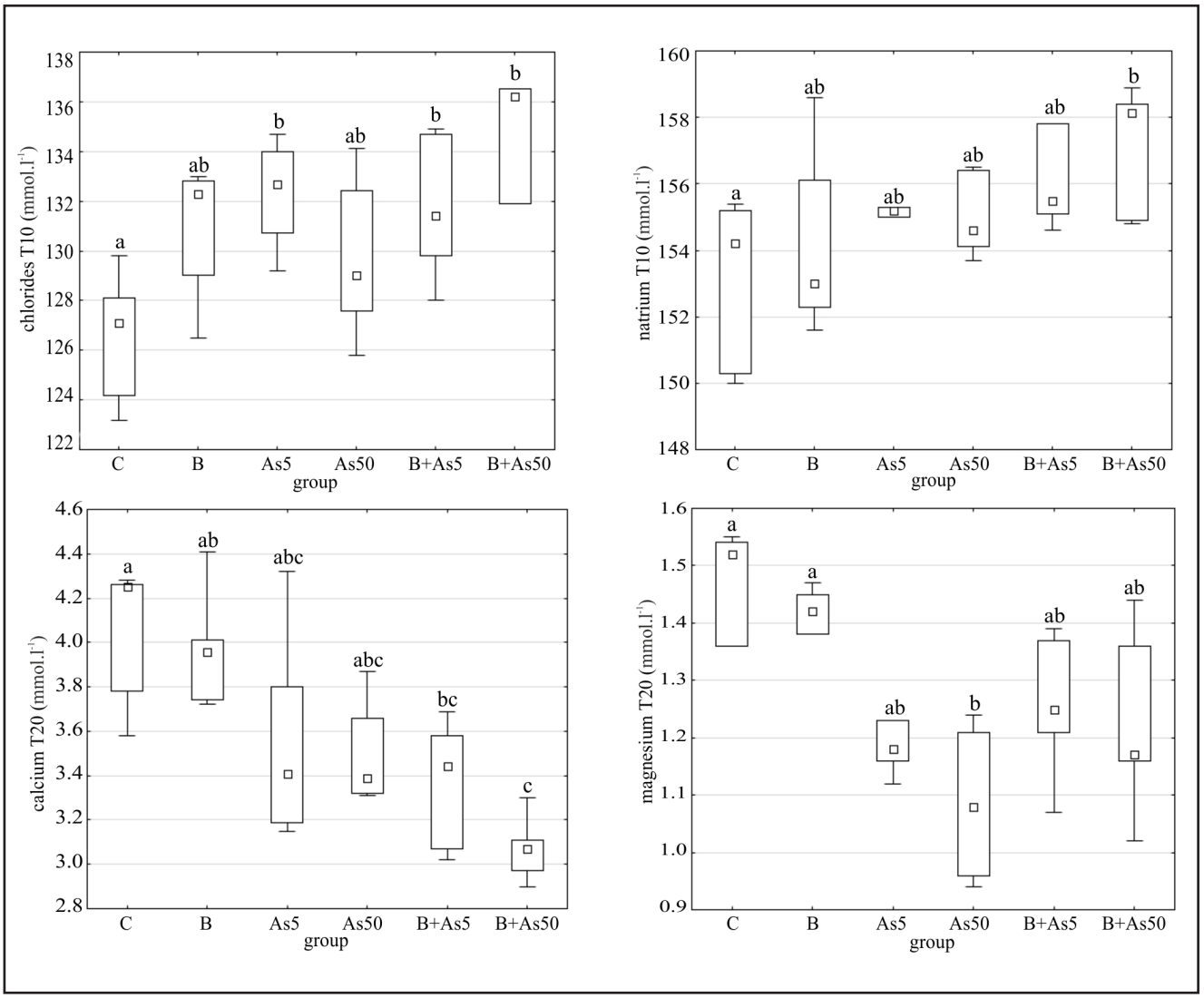

Fig. 1. The effect of cyanobacteria and arsenic on the biochemical indices of the rainbow trout. Box includes the 25th to 75th percentiles, with the middle point representing the median and the spots showing the extremes. Significantly different indices compared to the control are marked by letters ($p<0.05$).

Tab. 2. The biochemical indices in the blood plasma of rainbow trout [average±SD] during the experiment period (seven fish from the experimental group and from the control were analyzed at each sampling).

Indices	Group	C	B	As5	As50	B+As5	B+As50
ALB	T10	19.6±2.38	19.3±1.21	19.3±0.99	17.7±1.51	18.4±1.15	21.7±1.05
($g.l^{-1}$)	T20	18.7±0.97	18.9±0.95	18.2±1.14	18.9±1.29	18.1±1.14	17.4±1.49
	T30	17.8±1.98	17.4±3.22	18.4±2.29	18.7±1.99	18.8±1.24	18.6±1.28
ALP	T10	0.9±0.66	1.1±0.41	0.9±0.28	1.0±0.49	1.4±0.48	1.7±0.92
($\mu kat.l^{-1}$)	T20	1.8±0.77	1.7±0.47	1.6±0.83	1.7±0.26	1.4±0.18	1.3±0.38
	T30	1.7±0.40	2.0±0.81	1.3±0.40	1.8±0.74	1.4±0.48	1.9±0.28
ALT	T10	0.8±0.94	0.7±0.71	0.3±0.14	0.3±0.24	0.4±0.23	1.1±0.52
($\mu kat.l^{-1}$)	T20	0.6±0.32	0.2±0.06	0.4±0.26	0.3±0.24	0.2±0.07	0.2±0.07
	T30	0.2±0.08	0.2±0.08	0.4±0.22	0.3±0.34	0.2±0.07	0.2±0.08
AST	T10	2.3±2.11	1.9±3.18	3.3±3.00	3.8±3.11	3.5±3.76	1.6±2.94
($\mu kat.l^{-1}$)	T20	3.8±3.47	4.6±2.49	4.4±3.15	5.6±3.00	4.6±2.86	6.0±2.23
	T30	5.3±2.59	4.7±2.08	2.8±2.63	6.3±2.31	5.2±2.08	6.1±2.59
Ca	T10	3.2±0.27	3.7±0.31	3.5±0.24	3.3±0.13	3.7±0.28	3.9±0.00
($mmol.l^{-1}$)	T30	3.4±0.28	3.5±0.38	3.3±0.22	3.4±0.14	3.6±0.16	3.5±0.12
CHOL	T10	6.5±1.44	7.1±1.47	5.7±0.67	5.8±1.43	7.6±1.91	8.4±1.48
($mmol.l^{-1}$)	T20	7.8±1.22	7.3±0.95	7.7±1.86	7.9±0.73	8.7±1.22	7.6±1.36
	T30	8.5±2.01	8.4±2.09	7.5±1.34	7.9±1.09	9.5±2.32	8.2±1.63
CREA	T10	47.9±29.74	65.3±16.74	73.6±98.66	56.0±37.22	23.9±7.68	42.7±14.46
($\mu mol.l^{-1}$)	T20	51.3±33.96	35.7±23.28	63.7±46.04	48.2±19.88	58.4±15.58	20.0±7.76
	T30	66.9±56.31	24.5±6.47	44.2±25.96	34.2±11.89	39.6±37.63	52.8±30.78
GLU	T10	4.6±1.25	5.4±0.88	5.2±1.15	5.9±1.15	4.9±0.50	5.1±0.77
($mmol.l^{-1}$)	T20	5.3±0.74	4.9±0.71	5.8±0.87	5.7±0.74	5.7±0.93	5.6±1.33
	T30	5.6±1.00	5.5±0.67	5.1±0.54	6.2±1.64	5.1±0.40	6.0±0.64
Fe	T10	21.3±4.00	25.5±1.26	23.4±8.26	24.3±7.58	22.2±3.57	20.8±1.32
($\mu mol.l^{-1}$)	T20	22.6±0.95	23.9±3.15	20.7±4.17	21.4±6.16	23.9±1.33	15.9±2.50
	T30	25.4±2.18	20.6±4.32	19.7±2.28	23.5±2.99	18.9±5.24	19.8±1.39
LDH	T10	18.3±10.81	22.9±26.27	18.8±5.54	21.5±13.63	21.3±10.82	29.6±21.94
($\mu kat.l^{-1}$)	T20	22.1±20.43	10.8±2.57	14.4±6.02	17.8±10.13	11.0±2.57	10.2±3.21
	T30	9.2±1.57	7.8±1.36	11.6±3.22	8.3±1.68	8.2±1.17	8.5±3.84
LACT	T10	1.2±0.49	1.2±0.26	1.9±0.79	2.7±0.72	2.5±0.72	2.7±0.78
($mmol.l^{-1}$)	T20	2.6±1.14	1.7±0.75	1.5±0.34	1.6±0.36	1.4±0.57	1.5±0.43
	T30	2.1±0.84	2.1±0.86	2.0±0.83	2.1±0.69	3.0±0.98	3.0±1.02
Mg	T10	1.3±0.12	1.3±0.13	1.3±0.15	1.2±0.22	1.5±0.05	1.4±0.18
($mmol.l^{-1}$)	T30	1.3±0.06	1.3±0.13	1.3±0.10	1.3±0.11	1.4±0.06	1.2±0.15
P	T10	4.1±0.44	4.7±0.86	4.4±0.57	5.1±0.54	4.7±0.69	5.5±0.42
($mmol.l^{-1}$)	T20	5.1±0.50	4.5±0.67	4.6±0.49	4.5±0.32	4.2±0.85	3.9±0.84
	T30	4.8±0.64	4.7±0.88	4. 7±0.80	5.3±0.60	5.0±0.49	5.2±0.44
TP	T10	41.9±4.92	42.9±4.09	42.5±2.93	39.3±4.72	42.8±2.93	47.9±3.54
($g.l^{-1}$)	T20	45.0±4.14	41.5±3.11	47.0±5.49	45.95±4.23	45.8±3.54	44.2±6.17
	T30	46.5±9.97	44.7±5.74	45.3±4.38	47.2±5.63	46.7±2.70	47.1±4.65
TRIG	T10	3.7±1.49	3.6±1.39	2.3±0.72	3.8±1.91	3.5±1.70	4.1±2.00
($mmol.l^{-1}$)	T20	3.8±2.04	2.7±0.80	3.2±0.88	3.4±0.99	3.2±1.12	2.9±0.73
	T30	3.7±2.74	2.7±0.56	2.7±0.57	6.0±4.39	2.7±0.70	3.5±1.10
UREA	T10	0.5±0.46	0.5±0.11	0.3±0.18	0.5±0.29	0.4±0.16	0.6±0.40
($mmol.l^{-1}$)	T20	0.5±0.18	0.4±0.18	0.4±0.20	0.6±0.44	0.6±0.33	0.4±0.18
	T30	0.1±0.22	0.7±0.30	0.6±0.38	0.6±0.30	0.4±0.16	0.5±0.26
Na	T20	154.9±2.48	155.8±1.74	155.3±0.94	156.3±1.80	157.5±1.41	156.1±1.51
($mmol.l^{-1}$)	T30	155.6±2.78	156.3±1.86	155.2±1.19	156.8±1.79	157.5±2.63	158.7±2.61
K	T10	1.2±0.86	1.0±0.23	1.2±0.57	1.0±0.47	0.9±0.17	0.8±0.07
($mmol.l^{-1}$)	T20	0.6±0.20	0.6±0.16	0.8±0.24	0.7±0.11	0.9±0.33	1.0±0.39
	T30	0.7±0.11	0.6±0.10	0.6±0.12	0.6±0.14	0.6±0.15	0.6±0.11
Cl	T20	129.5±2.97	133.4±1.28	131.4±1.82	130.6±3.23	132.3±1.49	133.5±2.51
($mmol.l^{-1}$)	T30	131.5±3.65	135.8±1.30	130.0±2.26	136.2±3.10	134.9±3.02	135.4±2.40

agents in sub-lethal doses do not involve visible changes or mortality, their co-exposure does. The studies of co-exposure to various agents including cyanobacteria are lacking in fish.

Paskova *et al.* (2011) and Pikula *et al.* (2010) studied the combined exposure of cyanobacterial biomass, lead and the Newcastle virus in Japanese quails (*Coturnix coturnix japonica*). No mortality and no clinical signs of toxicity were found in birds exposed to cyanobacterial biomass only, but mortality occurred in combined exposures and acute effects were observed around day 10 in combined exposure. The interactive effects of chemical stressors and cyanobacteria on zooplankton have received very little attention so far (Cerbin *et al.* 2010; Bernatowicz & Pijanowska 2011). They reported synergistic interactions between chemicals and natural stressors on zooplankton.

Palíková *et al.* (2012) reported new knowledge on the effects of controlled exposure to multiple stressors including toxic cyanobacteria and infectious agents on common carp. They confirm additive action of different agents on the immune system of fish. While single agents seemed to stimulate the immune response, the combination of both caused immunosuppression.

Our results showed a significant increase in electrolyte values (Na and Cl) after 10 days of co-exposure of the fish to cyanobacteria and arsenic. Lusková *et al.* (2002) reported that levels of Na in blood plasma of carp decreased in water containing organophosphate. Common carp from environment with toxic *Microcystis aeruginosa* or dispersed microcystin, had lower values of natrium and chlorides in serum (Carbis *et al.* 1996; 1997). So, the values of electrolytes in this work do not correspond well with the results published by other authors. On the other hand, in freshwater fish the enzyme Na^+/K^+-ATPase play an important role in active transport mechanisms for ions. Gill ATPase is intimately involved in electrolyte balance and subsequent osmoregulation. Lavanya *et al.* (2011) observed inhibition activity of Na^+/K^+-ATPase of gills in carp treated with arsenic. Changes in gill Na^+/K^+-ATPase activity during experimental period could be explained as a factor for a dysfunctional regulation of chloride and sodium levels. Experimental group with high concentration of arsenic (As50) also showed significantly decreased values of magnesium (T20). Magnesium is important for normal function of the kidneys, liver and proteosynthesis.

Values of Ca significantly decreased after 20 days of co-exposure of the fish to cyanobacteria and arsenic. Kopp *et al.* (2010; 2011) reported a significant decrease in values of Ca in silver carp under the influence of a natural cyanobacteria population. Lusková *et al.* (2002) reported that levels of Ca in blood plasma of carp decreased in water with pesticide contain.

The basic function of ions in the body is to control fluid distribution, intra- and extra-cellular acidobasic balance, maintaining osmotic pressure of body fluids and normal neuro-muscular irritability. Electrolytes and other ions functionally participate in maintaining normal irritability of the heart, muscles and nerves, as well as the selective permeability of cell membranes. Decreased or increased values of ions in blood plasma indicated abnormal function of the fish organism.

Our results confirmed the hypothesis that influence of toxic cyanobacterial biomass and a chemical agent represented by arsenic can combine to enhance the effects on fish. This work originally shows that while the single agents in sub-lethal doses do not cause changes in the plasmatic parameters, their co-exposure leads to the significantly decrease or increase of the natrium, chlorides, calcium and magnesium of rainbow trout.

In our experiment, there were no significant changes in biochemical parameters in the last samples (T30). In connection with the significant differences detected in previous sampling (T10, T20), it is possible that in longer period there are occurring adaptations of fish organism to toxic stressor influence, and thus the stabilisation of biochemical parameters in fish plasma.

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REFERENCES

1 APHA (1998). Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC, USA.
2 Barham D, Trinder P (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst. **97**: 142–145.
3 Bernatowicz P, Pijanowska J (2011). *Daphnia* response to biotic stress is modified by PCBs. Ecotox Environ Saf. **74**: 711–718.
4 Bhattacharya S, Bhattacharya A, Roy S (2007). Arsenic-induced responses in freshwater teleosts. Fish Physiol Biochem. **33**: 463–473.
5 Blaha L, Marsalek B (2003). Contamination of drinking water in the Czech Republic by microcystins. Arch Hydrobiol. **158**: 21–429.
6 Carbis CR, Mitchell GF, Anderson JW, Mc Cauley I (1996). The effect of microcystins on the serum biochemistry of carp, *Cyprinus carpio,* L., when toxins are administered by gavage, immersion and intraperitoneal routes. J Fish Dis. **19**: 151–159.
7 Carbis CR, Rawlin GT, Grant P, Mitchell GF, Anderson JW, Mc Cauley I (1997). The study of feral carp, *Cyprinus carpio,* L., exposed to *Microcystis aeruginosa* at Lake Mokoan, Australia, and possible implications for fish health. J Fish Dis. **20**: 81–91.
8 Cerbin S, Kraak MHS, de Voogt P, Visser PM, Van Donk E (2010). Combined and single effects of pesticide carbaryl and toxic *Microcystis aeruginosa* on the life history of *Daphnia pulicaria*. Hydrobiologia. **643**: 129–138.
9 De Coninck DIM, Janssen CR, De Schamphelaere KAC (2013). An investigation of the inter-clonal variation of the interactive effects of cadmium and *Microcystis aeruginosa* on the reproductive performance of *Daphnia magna* Aqua Toxicol. **140–141**: 425–431.

10 Doumas BT, Bayse DD, Carter RJ, Peters T, Schaffer R (1981). Candidate reference method for determination of total protein in serum. I. Development and validation. Clin Chem. **10**: 42–50.
11 Doumas BT, Watson WA, Biggs HG (1971). Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta. **1**: 87–96.
12 Eisenman G (1967). Glass Electrodes for Hydrogen and Other Cations, Principles and Practice. G Eisenman, New York, Marcel Dekker Inc., pp. 594.
13 Expert Panel on Enzymes of The IFCC (1976). Clin Chim Acta. **70**: F19.
14 Fossati P, Prencipe L (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem. **28**: 2077–2080.
15 Ghosh D, Datta S, Bhattacharya S, Mazumder S (2007). Long-term exposure to arsenic affects head kidney and impairs humoral immune responses of Clarias batrachus. Aquat. Toxicol. **81**: 79–89.
16 Hajzer S, Jagelkova J (1988). Proposition of standard method for catalytic concentration of lactate dehydrogenase in plasma diagnosis. Biochem Clin Bohemoslovaca. **17**: 371–379.
17 Higgins T (1981). Novel chromogen for serum iron determinations. Clin Chem. **9**: 1619–1620.
18 Ibelings BW, Chorus I (2007). Accumulation of cyanobacterial toxins in freshwater „seafood" and its consequences for public health: A review. Environ Pollut **150**(1): 177–192.
19 Jaffe MZ (1886). Ueber den Niederschlag, welchen Pikrinsaure in normalem Harn erzeugt and ueber eine Reaction des Kreatinins. Zeits Fur Physiol Chem. **10**: 391–400.
20 Kopp R, Palíková M, Navrátil S, Kubíček Z, Ziková A, Mareš J (2010). Modulation of Biochemical and Haematological Indices of Silver Carp (Hypophthalmichthys molitrix Val.) Exposed to Toxic Cyanobacterial Water Bloom. Acta Vet Brno. **79**: 135–146.
21 Kopp R, Palikova M, Mares J, Navratil S, Kubicek Z, Zikova A (2011). Haematological indices are modulated in juvenile carp, Cyprinus carpio L., exposed to microcystins produced by cyanobacterial water bloom. J Fish Dis. **34**: 103–114.
22 Kratochvila J, Garcic A (1977). Assignment of organic phosphorus in biological matter. Biochem Clin Bohemoslovaca. **6**: 65–77.
23 Lavanya S, Ramesh M, Kavitha C, Malarvizhi A (2011). Hematological, biochemical and ionoregulatory responses of Indian major carp *Catla catla* during chronic sublethal exposure to inorganic arsenic. Chemosphere. **82**: 977–985.
24 Lima AR, Curtis C, Hammermeister DE, Markee TP, Northcott CE, Brooke LT (1984). Acute and chronic toxicities of arsenic (III) to fathead minnows, flagfish, daphnids, and an amphipod. Arch Environ Contam Toxicol. **13**: 595–601.
25 Lusková V, Svoboda M, Kolařová J (2002). The Effect of Diazinon on Blood Plasma Biochemistry on Carp (*Cyprinus carpio* L.). Acta Vet Brno. **71**: 117–123.
26 Malbrouck Ch, Kestemont P (2006). Effects of microcystins on fish. Environ Toxicol Chem. **25**(1): 72–86.
27 Michaylova V, Ilikova P (1971). Photometric determination of micro amounts of calcium with arsenazo III. Anal Chim Acta. **53**: 194–198.
28 Palikova M, Navratil S., Papezikova I, Ambroz P, Vesely T, Pokorova D, Mares J, Adamovsky O, Navratil L, Kopp R (2012). Combined exposure of carps (*Cyprinus carpio* L.) to cyanobacterial biomass and white spot disease. Neuroendocrinol Lett. **33**(Supp.3): 77–83.
29 Paskova V, Pikula J, Bandouchova H, Sedlackova J, Hilscherova K (2011). Combined exposure of Japanese quails to cyanotoxins, Newcastle virus and lead: Oxidative stress responses. Ecotoxicol Environ Saf. **74**: 2082–2090.
30 Pikula J, Bandouchova H, Hilscherova K, Paskova V, Sedlackova J, Adamovsky O, Knotkova Z, Lany P, Machat J, Marsalek B, Novotny L, Pohanka M, Vitula F (2010). Combined exposure to cyanobacterial biomass, lead and the Newcastle virus enhances avian toxicity. Sci Total Env. **408**: 4984–4992.
31 Roch-Ramel F (1967). An enzymic and fluorophotometric method for estimating urea concentrations in nanoliter specimens. Anal Biochem. **21**: 372–381.
32 Roschlau P, Bernt E, Gruber WJ (1974). Enzymatic determination of total cholesterol in serum. J Clin Chem Clin Biochem. **12**: 403–407.
33 Shimojo N, Naka K, Nakajima C, Yoshikawa C, Okuda K, Okada K (1989). Test-Strip Method for Measuring Lactate in Whole-Blood. Clin Chem. **35**: 1992–1994.
34 Skavrada J (1999). Diagnostic agent for magnesium determination. Utility model CZ 8152 U1.
35 Tietz NW, Burtis C, Ervin K, Petitclerc CJ, Rinker AD, Zygowicz E (1980). Progress in the Development of a Recommended Method for Alkaline-Phosphatase Activity Measurements. Clin Chem. **26**: 1023–1023.