REPRODUCTIVE BIOLOGY

SHORT COMMUNICATION

The effect of copper, zinc, mercury and cadmium on some sperm enzyme activities in the common carp (*Cyprinus carpio L.*)

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SUMMARY

The objective of the study was to determine the effect of copper, zinc, cadmium and mercury ions (100, 10 and 1 mg/l) on the activity of some enzymes of carp spermatozoa. Acid phosphatase activity was proved to be relatively insensitive to zinc ions, while copper, mercury and cadmium ions effectively inhibited the activity of this enzyme. β-N-acetylglucosaminidase activity was sensitive only to mercury ions. Lactic dehydrogenase activity remained unaffected by heavy metals. Our results showed that, among the examined metals, mercury had the strongest inhibitory effect on enzymatic activities. *Reproductive Biology 2009* ***9*** *3: 295-301.*

Key words: acid phosphatase, β-N-acetylglucosaminidase, lactic dehydrogenase, spermatozoa, common carp

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INTRODUCTION

The toxicity of accumulated metals is determined not only by the type of metal, but also by the physical and chemical properties of water and the protective mechanisms of fish [12]. Owing to their bioaccumulation and non-degradability, heavy metals pose a serious pollution hazard to the aqueous environment. Fish exposed to metals are usually characterized by increased metal levels in the gonads. Gonadal levels of copper and zinc of *Catostomus commersoni* living in polluted lakes were higher than those in fish inhabiting unpolluted waters [8]. Cadmium caused deformation of the testicles and induced seasonal changes in the androgen level in brook trout [10]. In our preliminary study we observed a dose-dependent reduction of common carp sperm motility related to the concentration of heavy metal in the activation solution. This may be due to the sperm membrane disruption or inhibition of spermatozoa enzymes. In the present study we examined the effect of zinc, copper, cadmium and mercury on the activity of three sperm enzymes in order to evaluate the sensitivity of these proteins to heavy metal ion contamination. Enzyme activity may be an indicator of the toxicity of pesticides, heavy metals and other pollutants [9].

MATERIALS AND METHODS

Carps (body weight, bw, 5-7 kg, 5-6 year old) were maintained under ambient temperature in ponds of the Institute of Ichthyobiology and Aquaculture in Golysz, Poland. Before hormonal stimulation (May 2001), the fish were transferred from ponds to tanks with a water temperature of 20^{o}C reached after seven days. Sperm production was stimulated with Ovopel (one pellet containing of 18-20 μg of GnRH analog and 8- 10 mg of metoclopramide per one kg of fish bw; Interfish Ltd, Hungary; [4]). Sperm was collected 24 h after hormonal stimulation. The abdomen of the fishes was wiped dry and semen was collected by stripping into beakers. Samples contaminated with excrement or urine were excluded

from further work. Milt was centrifuged (10 min, 8000×g) to obtain seminal plasma and spermatozoa. Sperm pellets (from 10 ml of milt; n= 5 males) were stored at -79°C. Spermatozoa from each male were thawed separately at room temperature, suspended in 5 ml 0.5% NaCl and frozen at -79°C to evaluate the extent of enzyme leakage after sperm damage related to freezing/thawing cycles. Then, supernatants obtained after centrifugation of thawed material were used for *in vitro* studies and biochemical analyses.

Stock solutions of $CuSO_4$, $ZnCl_2$, $HgCl_2$ and $CdCl_2$ were dissolved in distilled water and diluted to 1, 10 and 100 mg/l final ion concentrations in the sperm supernatants. The enzymatic activities of the sperm supernatants were determined after 0, 4 or 24 hours incubation with heavy metals (+4°C). Metal concentrations were selected according to results of our preliminary experiments. First, we tested the metal influence on the parameters of sperm motility. Then, we selected the minimal doses which did not affect the sperm motility parameters and the maximal doses which caused total inhibition of sperm motility.

Acid phosphatase activity was measured using 5 mM p-nitrophenylphosphate (disodium salt) in 20 mM citrate buffer, pH 5.0 [3]. The activity of β-N-acetylglucosaminidase (β-NAGase) was measured using 0.5 mM p-nitrophenyl β-N-glucosaminide as a substrate in 0.1 M citrate buffer, pH 5.0 [5]. Lactic dehydrogenase (LDH) activity was measured with the UV-method with pyruvate and NADH [13]. Enzymatic activities were expressed as U/l (mM of adequate substrate hydrolyzed per min). Protein concentration was measured by the Lowry method [7]. Enzymatic activities and protein concentration were measured in triplicates.

Acid phosphatase affinity to the substrate was tested at 0.312; 0.625; 1.25; 2.5; 5.0 and 10 mM p-nitrophenyl phosphate with or without 10 mg/l Cu^{2+}, 100 mg/l Cd^{2+} or 10 mg/l Hg^{2+} ions. The Michaelis constant (K_m) was calculated from linear plots of reciprocal of the reaction velocity versus the reciprocal of the substrate concentration (Woolf's rule). All values were expressed as mean±SD. Statistical significance was assessed by one way analysis of variance. Tukey's multiple comparison test was used for post-hoc comparisons. Differences were significant at $p<0.05$.

RESULTS AND DISCUSSION

Contrary to zinc ions, copper ions (10 and 100 mg/l) decreased the activity of acid phosphatase by about 70% in time "0" (fig. 1A). Mercury and cadmium ions also inhibited acid phosphatase activity in this study (fig. 1B). In sturgeon semen [11], a significant drop in acid phosphatase activity was induced only by the highest doses of mercury and cadmium (100 mg/l). The effect of heavy metals on phosphatases is determined by the type of tissue from which the enzyme is extracted [2]. Our result showed that the activity of acid phosphatase from common carp spermatozoa are sensitive to all tested heavy metal ions.

The activity of β-N-acetylglucosaminidase proved to be insensitive to zinc, copper (data not shown) and cadmium ions (fig. 2). The activity of β-NAGase was significantly inhibited only by mercury at a dose 10 mg/l after 24 hours and 100 mg/l at time "0" (fig. 2). Other authors [1] also suggest that the β-NAGase is sensitive to mercury ions, while the presence of other metals did not decrease its activity. The introduction of zinc, copper, cadmium and mercury ions into sperm supernatants did not inhibit the activity of LDH (data not shown). This indicates that carp semen LDH is much more resistant to heavy metals than LDH of sturgeon semen whose activity was entirely inhibited at mercury concentrations of 10 mg/l [11].

The effect of copper ions on the affinity of acid phosphatase for substrate was determined with the use of a sample containing copper ions at 10 mg/l concentration. Copper ions decreased the affinity of acid phosphatase for p-nitrophenyl phosphate. The value of constant K_m in the presence of copper ions was 11×10^{-4} M, while in the control sample 4.51×10^{-4} M. The Michaelis constant in the sample containing cadmium at a concentration level of 10 mg/l reached 4.4×10^{-4} M, while in the sample containing mercury ions (100 mg/l), the constant K_m was more than twofold higher than in the control sample reaching 9.3×10^{-4} M.

The results of the study indicate that heavy metal ions not only distort the motility parameters of spermatozoa [2] but also inhibit the activity of sperm enzymes, thus, consequently, may inhibit their fertilizing capacity. In view of the common carp's sensitivity to pollution [6] and the accumula-

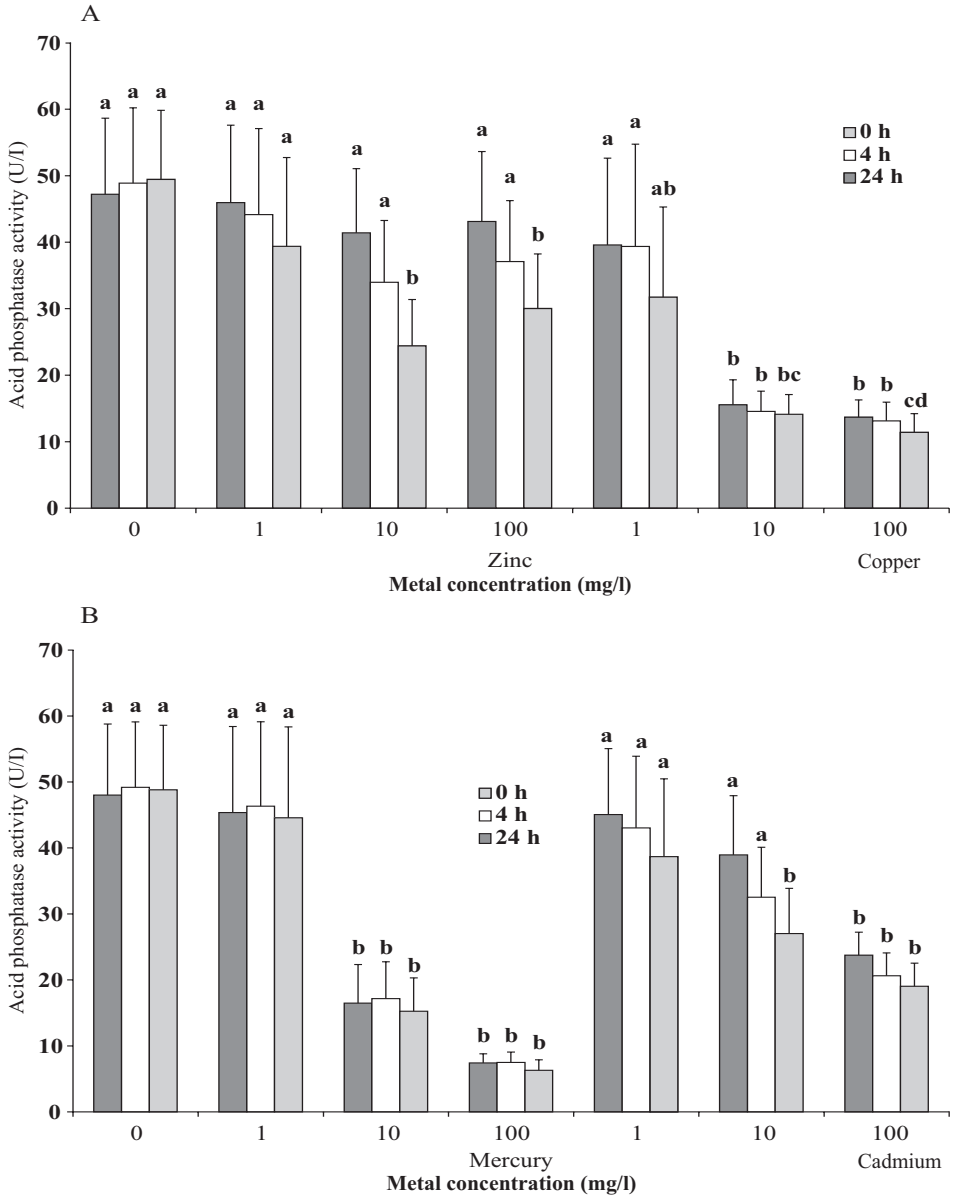

Figure 1. Acid phosphatase activity (means±SD) in the common carp sperm incubated for 0, 4 or 24 hours with zinc, copper (A), mercury and cadmium (B) ions. Different letters designate significant differences ($p<0.05$).

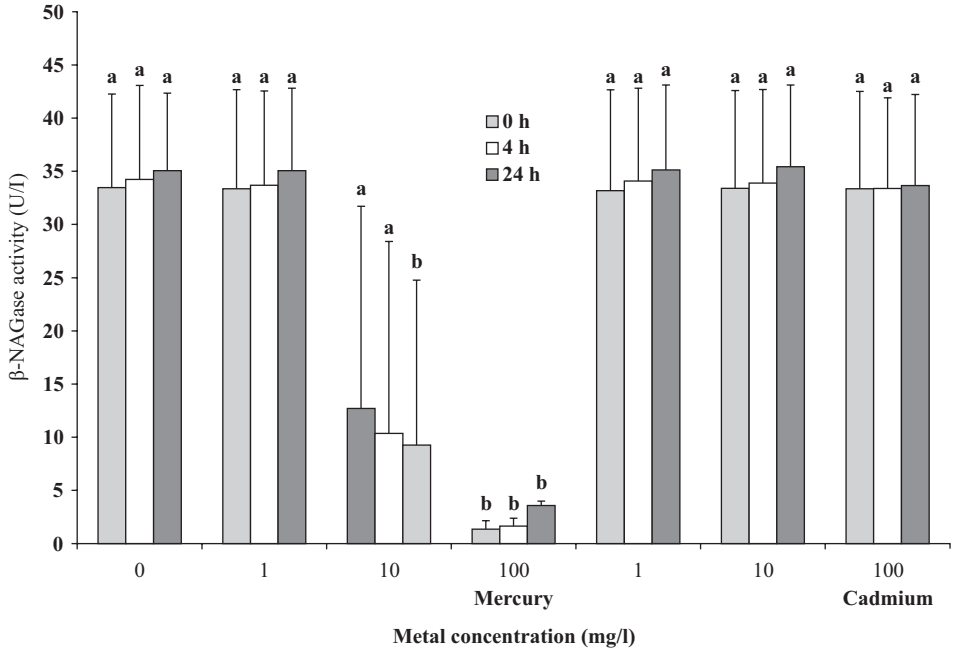

Figure 2. β-N-acetylglucosaminidase (β-NAGase) activity (means±SD) in the common carp sperm incubated for 0, 4 or 24 hours with mercury and cadmium ions. Different letters designate significant differences ($p<0.05$).

tion of high quantities of heavy metals in fish ponds [12], our results indicate that the activity of sperm enzymes in common carp may serve as a marker of pollution in water bodies.

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