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THE INFLUENCE OF MERCURY ON COMPUTER ANALYZED SPERM MOTILITY OF COMMON CARP, *CYPRINUS CARPIO* L., *IN VITRO*

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ABSTRACT. The aim of this work was to evaluate the impact of different mercury concentrations (ranging from 0.05 to 1 ppm) on common carp sperm motility. Computer assisted sperm analysis (CASA) and microscopic observations of the time of sperm motility were applied in the experiment. According to analysis using the CASA method, it was found that all the tested concentrations of mercury decreased sperm motility, and that lethal effects were observed at concentrations of 0.5 and 1 ppm. On the other hand, microscopic observations showed that mercury decreased the time of sperm movement at doses as low as 0.2 ppm, and that lethal effects were evident at 1ppm. These results suggest the negative impact of mercury on sperm motility. The reasons for the differences in the results obtained using these two techniques are also discussed.

Keywords: COMMON CARP, SPERM MOTILITY, MERCURY, TOXICITY, CASA

INTRODUCTION

One of the most dangerous pollutants that can be found in waters is mercury. This metal does not play any physiological role in living creatures. The mercury concentration in inland waters rarely exceeds 1 μg dm^{-3} (Kabata-Pendias and Pendias 1979), but it frequently exists in higher concentrations as a result of industrial activity. Mercury content in rivers is higher than in sea-water; in the lower Vistula River the concentration of mercury ranged from 0.9 to 2.1 μg dm^{-3} (Kabata-Pendias and Pendias 1979). Data concerning mercury content in fish is not abundant; in roach, *Rutilus rutilus* (L.) and pike-perch, *Stizostedion lucioperca* (L.) caught in the Odra River mouth between 1984 and 1988 concentrations of mercury ranged from 0.034 to 0.115 μg g^{-3} wet weight (Protasowicki 1991).

Extremely high mercury concentrations in fish tissues obviously leads to the death of the specimen. However, careful attention should be paid to the effects of sub-lethal tissue concentrations of this metal, as their effects are difficult to evaluate in short-term observations.

The most common and most toxic mercurial chemical compound found in fish tissues is methylmercury (Nielsen 1992). In tilapia, *Oreochromis aureus* (Steindechner), Allen et al. (1994) found that mercury accumulates mostly in the kidneys, and that the lowest concentrations were in the muscle tissue.

Mercury reaches fish tissues and organs mainly through the gills, and it initially accumulates in the liver.

Mercury causes multiple negative physiological effects in fish, e.g. the destruction of immunological mechanisms (Voccia et al. 1994) and the destruction and hemolysis of erythrocytes (Gwoździński 1994). Mercury also affects many reproductive processes in fish (see Kime 1998 for a review). It decreases GSI (Dey and Bhattacharya 1989, Kirubagaran and Joy 1988, 1992), influences vitellogenesis (Kirubagaran and Joy 1988), affects gonadotrophs (Ram and Sathyanesan 1984, Joy and Kirubagaran 1989), and decreases the levels of some hormones and enzymes involved in reproductive processes (Kirubagaran and Joy 1988).

Mercury also affects the final phases of reproduction; it decreases fertilization (Khan and Weis 1987a, b) and hatching rates (Heisinger and Green 1975), and sperm viability and movement (Khan and Weis 1987a, b)

The ability of sperm to move is one of the necessary factors of successful fertilization. Thus, determining the influence of mercury on spermatozoa motility would provide us with important information regarding the toxic impact of mercury on the final phases of reproduction.

The aim of this work was to evaluate the impact of mercury on the different parameters of sperm motility (time of motile activity, velocity) using manual microscopic observations as well as a new tool for the determination of spermatozoa motility, namely, computer-assisted sperm analysis (CASA). This method, initially used in medicine, has recently been applied in the analysis of fish sperm (Toth et al. 1995, Ebrahimi et al. 1996, Kime et al. 1996).

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS

The experiment was performed at the Department of Ichthyobiology and Fisheries, Agricultural University of Cracow, Poland. Six spermiating male carp, *Cyprinus carpio* L. were used in the investigation; they were netted in conventional earth ponds,

transferred to concrete flow-through basins and acclimated to 20°C under a simulated natural photoperiod three days before the experiment.

SPERM SAMPLING

The fish were anesthetized for 5 min using 2-phenoxyethanol (3 ml for 10 dm^{-3} of water), wiped with a wet cloth and then stripped of milt using a 1 ml sterile syringe. The sperm collected from each fish was then immediately subjected to dilutions with different pollutant concentrations.

EXPERIMENTAL DESIGN

The two-step method of sperm dilution according to Billard and Cosson (1992) was used in the experiment. In the first step, the sperm was diluted 100-fold in 10 ml polypropylene tubes in a basal solution (control group) consisting of KCl 200 mM - Tris 30 mM pH = 8.0, or in a basal solution containing the following concentrations of pollutant ($HgCl_2$ - P.O.Ch., Poland): 0.05; 0.1; 0.2; 0.5; 1 ppm (expressed as concentrations of pure metal). After gentle mixing, the tubes were incubated for 2 h at 4°C. In the second step, just before registration, 1 μl of each mixture was placed on a polyvinyl alcohol (PVA) coated microscope slide (Chance Propper Ltd., UK) and quickly mixed with 20 μl of distilled water giving a final dilution of 2100 times.

COMPUTER ASSISTED SPERM ANALYSIS (CASA)

Sperm movement was recorded using a video-camera connected to a microscope and video-recorder as described in Chyb et al. (2000). Videotapes were computer-analysed using a Hobson sperm tracker (Hobson Tracking Systems Ltd., Sheffield, UK) from 20 s after the mixing point (to allow for focusing and stabilization of water solution movement) for four consecutive 15 s periods. The following parameters were analyzed: VCL - curvilinear velocity (μm s^{-1}) is the sum of the incremental distances moved in each frame along a sampled path divided by the total time of the track; VSL - straight line velocity (μm s^{-1}) is the straight line distance between the start and end points of the track divided by the time of the track; VAP - angular path velocity (μm s^{-1}) is a derived path based on the average number of points and divided by the time of the track. For more details regarding the analysis, see Kime et al. (1996).

TIME OF SPERM MOTILITY

The sperm motility time was measured using a timer from the moment of sperm activation in distilled water until approximately 80% of the observed spermatozoa had ceased exhibiting motile activity.

STATISTICS

The results of VCL, VAP, VSL and time of sperm motility (MOT) were subjected to variance analysis followed by Duncan's multiple range test. The differences between the control and experimental groups were considered significant at $P < 0.05$.

RESULTS

VCL

The 2-hour incubation of carp sperm with mercury showed that all the concentrations of this metal significantly decreased sperm curvilinear velocity in all the analyzed periods. VCL1 velocity decreased from 79.04 μm s^{-1} in the control group to 6.12 μm s^{-1} in the group incubated with 0.2 ppm of mercury. There was no sperm movement in the 0.5 and 1 ppm mercury concentrations in any of the analyzed periods. Moreover, the action of mercury was more potent in later measurement periods resulting in the complete inhibition of sperm movement at a concentration of 0.2 ppm for VCL2, VCL3 and VCL4 (Fig. 1).

VAP

The average path velocity in the control group was 45.5 μm s^{-1}. Incubation of the sperm with mercury decreased VSL1 to 27.5, 12.43 and 4.14 μm s^{-1} at mercury concentrations of 0.05, 0.1 and 0.2 ppm, respectively. The two highest mercury concentrations (0.5 and 1 ppm) blocked sperm movement completely. Similarly to the results of VCL, the 0.2 ppm concentration blocked velocity in the VAP2, VAP3, and VAP4 measurement periods (Fig. 2).

VSL

The average VSL1 motility in the control group reached 21.75 μm s^{-1}, whereas the VSL1 of the mercury-treated sperm decreased to 11.68, 5.02 and 2.4 μm s^{-1} at concentrations of 0.05, 0.1 and 0.2 ppm, respectively. No motility was observed at mercury

concentrations of 0.5 and 1 ppm (for VSL1, VSL2, VSL3 and VSL4), or in the case of 0.2 ppm of mercury (for VSL2, VSL3 and VSL4) (Fig. 3).

TIME OF SPERM MOTILITY (MOT)

Microscopic observations of the control group showed that the average sperm motility time was 86.25 s. Mercury concentrations of 0.05 and 0.1 ppm did not significantly influence this parameter, whereas higher concentrations significantly reduced motility time or completely blocked sperm movement at a concentration of 1 ppm (Fig. 4).

DISCUSSION

Despite improvement of the quality of inland waters, industrial and agricultural activities emit excessive amounts of pollutants into the atmosphere, which, in turn, contaminate water. Heavy metals, such as mercury, are especially dangerous due to their toxic effects and strong accumulative properties. Water contamination leads to the increased accumulation of these compounds in living organisms, including fish. A consequence of accumulation is physiological abnormality including the malfunction of the reproductive system, which affects the number and health of the progeny. Thus, the analysis of the impact of these compounds on different aspects of reproduction is necessary in order to assess the toxicity of the pollutants.

In this work, we have evaluated the influence of mercury on sperm motility, which, together with the quality of the genetic material in the spermatozoa, is the key factor responsible for successful reproduction. Using computer assisted sperm analysis, it was found that all the mercury concentrations used in the experiment significantly decreased VCL, VAP and VSL velocities of sperm, and that doses of 0.5 and 1 ppm were lethal (Figs. 1, 2 and 3).

In spite of the attention scientists pay to the problems of heavy metal toxicity in fish, literature concerning the impact of mercury on sperm motility is very scarce. The incubation of *Fundulus heteroclitus* L. sperm in the presence of mercury concentrations ranging from 0.01 to 0.05 ppm for 4.5 min led to a significant decrease in sperm motility and fertilization rate (Khan and Weis 1987b). The toxic influence of mercury was more robust than in the case of our experiment. Such a rapid toxic effect could be attributed to the type of mercury compound used in the experiment; methylmercury chloride was used, whereas mercury chloride was used in the current experiment.

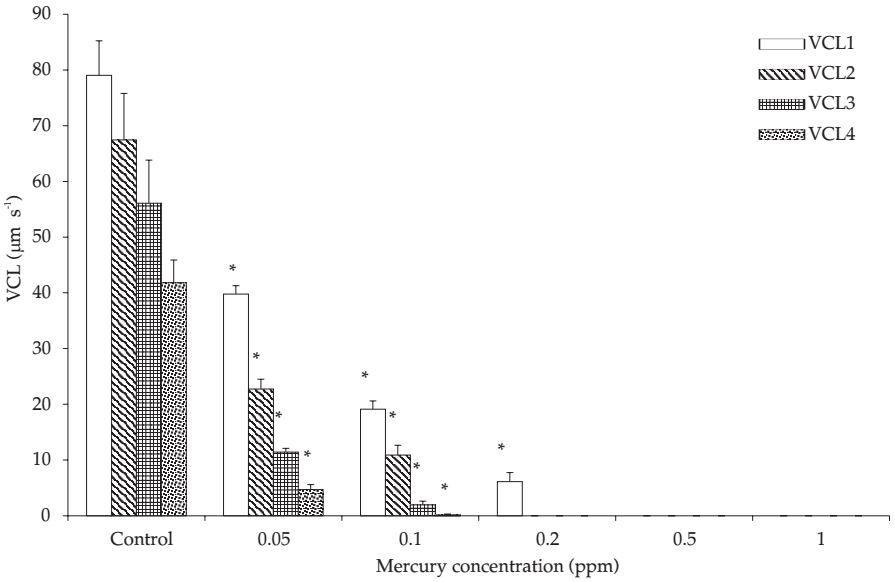

Fig. 1. Effects of different concentrations of mercury on curvilinear velocity (VCL) of common carp sperm. Subsequent periods of motility measurement are presented as VCL1, VCL2, VCL3 and VCL4. The values are expressed as mean (main bars) ± SEM (error bars). * - statistically different vs. appropriate control group ($P < 0.05$).

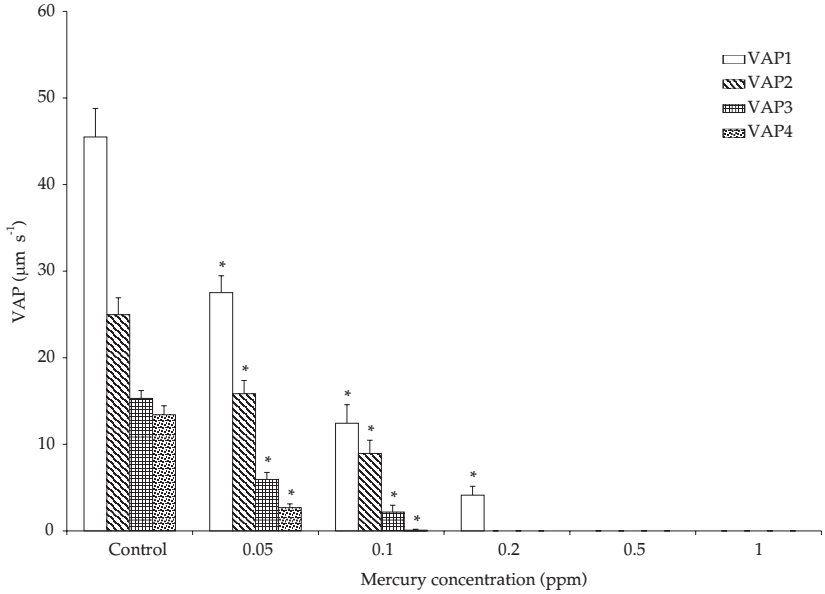

Fig. 2. Effects of different concentrations of mercury on average path velocity (VAP) of common carp sperm. The subsequent periods of motility measurement are presented as VAP1, VAP2, VAP3 and VAP4. The values are expressed as mean (main bars) ± SEM (error bars). * - statistically different vs. appropriate control group ($P < 0.05$).

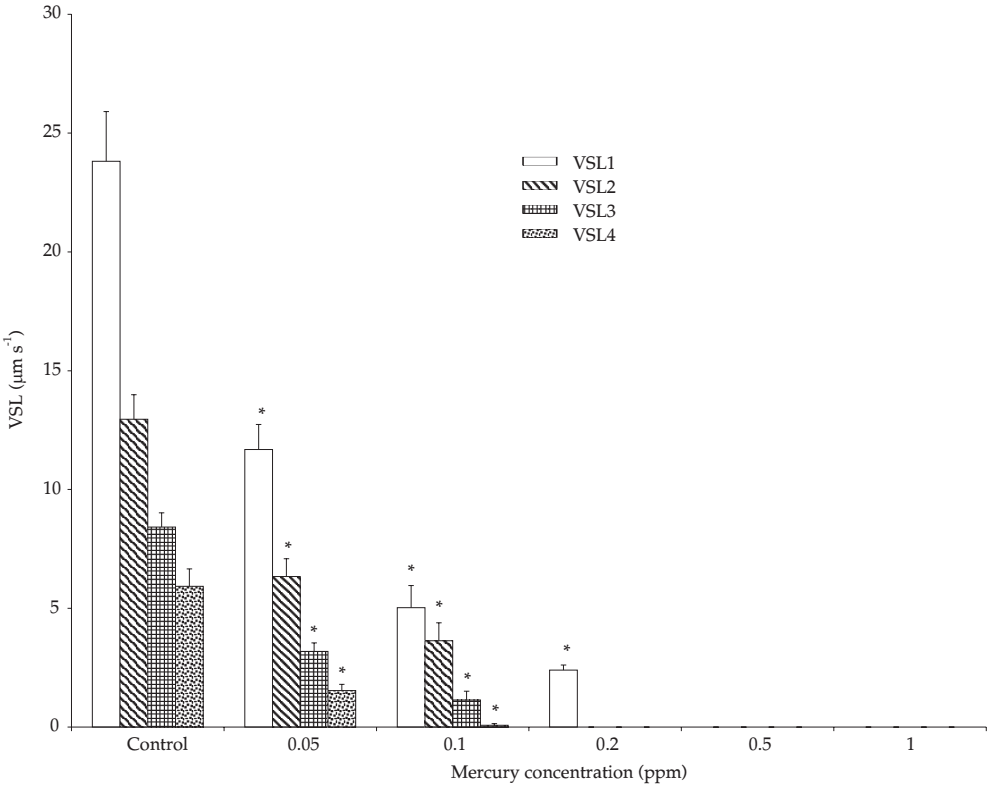

Fig. 3. Effects of different concentrations of mercury on straight line velocity (VSL) of common carp sperm. The subsequent periods of motility measurement are presented as VSL1, VSL2, VSL3 and VSL4. The values are expressed as mean (main bars) ± SEM (error bars). * - statistically different vs. appropriate control group ($P < 0.05$).

Methylmercury was found to be much more toxic, and its half-life is two to five times longer than other inorganic mercury compounds (Tollefson and Cordle 1986). The same compound also decreased the motility of rainbow trout, *Oncorhynchus mykiss* (Walbaum) sperm (McIntyre 1973), however, the incubation time was longer (30 min) and the mercury concentrations were higher (ranging from 1 to 10 ppm).

Billard and Roubaud (1985) found that rainbow trout sperm incubated for 41 min in mercury chloride at doses ranging from 0.001 to 1 ppm showed a lower fertilizing ability than intact sperm did.

The rapid and very toxic effects of mercury on sperm movement obtained in this work, as well as data from the literature, suggest that the absorption of mercury by spermatozoa is very high or that mercury directly influences the factors responsible

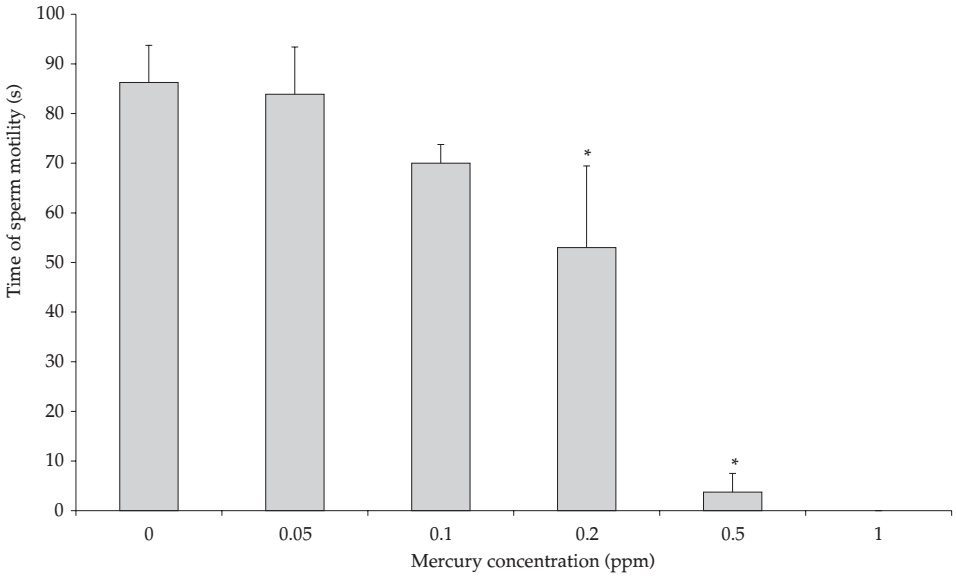

Fig. 4. Effects of different concentrations of mercury on average time of common carp sperm motility. Values are expressed as mean (main bars) ± SEM (error bars). *statistically different vs. appropriate control group ($P < 0.05$).

for sperm movement. At present, there is no data which can explain the mechanisms of the toxic effect of mercury in fish, but it is highly possible that mercury could interfere with the microtubule sliding assembly as is suggested by Mohamed et al. (1986).

The results of sperm motility time (MOT) assessments by microscopic observations (Fig. 4) differ from those obtained by means of CASA. Firstly, mercury did not have significant effects on the MOT parameter at doses of 0.05 and 0.1 ppm. Moreover, sperm movement was detected even at a concentration of 0.5 ppm; this concentration was found to be lethal when computer assisted analysis was performed. Since the results of CASA are much more consistent and repetitive, we suggest that these differences are the result of the higher level of errors made during subjective microscopic analysis. This suggestion concurs with the results of Centola (1996), who found that the CASA technique provides much more precise results than microscopic observation of sperm motility.

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STRESZCZENIE

WPŁYW RTĘCI NA ANALIZOWANĄ KOMPUTEROWO RUCHLIWOŚĆ PLEMNIKÓW KARPIA, *CYPRINUS CARPIO* L., *IN VITRO*

Celem niniejszej pracy było zbadanie wpływu różnych stężeń rtęci (od 0,05 do 1 ppm) na ruchliwość plemników karpia. W badaniach wykorzystano zarówno komputerową analizę ruchliwości plemników (CASA), jak i subiektywne mikroskopowe obserwacje czasu trwania ruchu plemników. Stwierdzono, że rtęć we wszystkich użytych koncentracjach istotnie obniżyła ruchliwość plemników wyrażoną wszystkimi komputerowo analizowanymi parametrami (VCL, VAP, VSL), mając letalny wpływ w koncentracjach 0,5 oraz 1 ppm. Subiektywne pomiary czasu trwania ruchu plemników wykazały, iż rtęć istotnie obniża czas trwania ruchu plemników, począwszy od koncentracji 0,2 ppm, całkowicie hamując ich ruch w stężeniu 1 ppm. Powyższe wyniki dowodzą negatywnego wpływu rtęci na ruchliwość plemników oraz rozbieżności w dokładności pomiarów obydwu zastosowanych metod analizy ruchliwości plemników.

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