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The Effect of Cadmium on Sperm Quality and Fertilization of *Cyprinus carpio* L.

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ABSTRACT

The objective of the study was to determine the effect of cadmium on sperm quality and fertilization of *C. carpio* L. Sperm and eggs were collected by abdomen striping from the mature testis and ovary of *C. carpio* L. This study used one control and four treatment groups of variation on the cadmium concentration (0, 50, 100, 150, and 200 ppm) with eight replications. Sperm motility (mass motility, mass motility duration, and individual motility duration) and viability were measured after three to four seconds of incubation in the water. The percentage of fertility success was calculated by observing embryo development after the eggs were mixed with sperm and incubated in the water for 72 hours. The success of the fertilization process was indicated by a color change of the egg that darkens after successful fertilization, and white-milk if failed. The data were analyzed using analysis of variance ($\alpha = 0.05$). The results of this study indicate that exposure of 50 ppm cadmium and control group shown success in term of sperm quality (motility and viability) and fertilization, but at 100 ppm or more decreased the sperm quality and fertilization rate. It can be concluded that cadmium exposure decreases sperm quality and fertility at 100 ppm or higher concentrations.

1. Introduction

Human activities in industry, agriculture or households have impacts on the increase of pollutants in aquatic ecosystems. The major pollutants from waste treatments are a heavy metal such as cadmium (Cd) and lead (Pb)(Mishra *et al*., 2006). Cadmium is a toxic heavy metal which is often used as the main or auxiliary material in the industry, among others nickel-cadmium battery industry (50-55%), pigment (18-20%), the coating material (8-12%), stabilizers and other synthetic materials (6-10%). Until the end of the 20th century, 45% of the total global pollution was Cd (Connell and Miller, 1995). Heavy metals exposure can disrupt physiological processes and give toxic effects to organisms (Patric, 2006).

Cell damage due to Cd exposure, among others are changes in sulfhydryl homeostasis and decreased antioxidant capacity by the inhibition of the enzyme and replacement of Zn and Se in metalloenzymes, thus reducing metabolic activity. These gave rise to the formation of reactive oxygen species (ROS) that produce oxidative stress in cells and cause damages such as lipid peroxidation, destruction of protein structure and function, and mutations in DNA (Valko *et al.*, 2006).

The toxicity of heavy metals affects water organisms such as fish and can indirectly affect the male reproductive organs that consume them. (Siu *et al*., 2009). Heavy metals were introduced to the fish body through absorption and accumulation from environment. These disrupted the structure and function of tissues and organs (Jezierska and Witeska, 2001). Heavy metal pollution has been reported to

inhibit spermatogenesis in male goldfish (Tandjung, 1992).

The common carp (*Cyprinus carpio* L) is a widely cultivated species of fish. The deadly effects and toxicities of various metals have been widely recognized in goldfish (Mason, 1981). Sperm fish become motile when released in water and sperm motility will occur in a short time (Morisawa, 1990, Cosson, 2004). The motility of sperm can be attributed to a series of cellular changes, including increased sperm respiration rates and a drastic reduction in ATP content (Dzyuba and Cosson, 2014). The objective of this study was to determine the effect of Cd on the quality of sperm and fertility of *C. carpio*, L.

2. Materials and Methods

The materials used in this experiment are $CdCl_2$, 0.9% NaCl, female and male *C. Carpio* L. from Freshwater Aquaculture Installation, Batu Malang, East Java. This study used a complete randomized design consisting of one control group and four treatments with various concentrations of Cd (50, 100, 150, and 200 ppm).

2.1. Collection of fish sperm and eggs

The sperm and eggs of the fish were collected by stripping. Semen (2-3 ml) was collected in 5 ml syringe. One ml of the semen was taken to dilute by ten times with 0.9% NaCl. As much as 20-30 ml of eggs were collected in a cup. Each treatment used 200-250 eggs and diluted using 2 to 3 ml of 0.9% NaCl.

2.2. Sperm motility

Mass motility is measured in the sperm suspension by diluting the sample in 0.9% NaCl. The suspension (40 ml) was diluted again with 10 ml of water, then incubated for 15 s. The percentage of mass motility was calculated using a score of 0 if no movement is observed, one if 25% of observed sperm move, two if 50% observed sperm move, three if 75% sperm observed move and four if more than 75% sperm can move.

Mass motility duration is measured after the first dilution. One hundred ml of suspension is placed on a glass concave then diluted again with 10 ml water or Cd solution with various concentrations. After incubated for 15 s, the sample was calculated for the duration of mass motility.

The duration of individual motility is measured after the first dilution. Forty ml of sperm suspension is placed on a concave object then dissolved with 10 ml water or Cd solution with various concentrations, incubated for 15 s, and then calculated for the duration of the individual motility. Calculations were performed on 50 sperm cells for each repetition in one treatment.

2.3. Sperm Viability

The viability is measured after the first dilution. One drop of suspension is placed into one drop of water or a Cd solution with a various concentration, then smeared and stained with 1% Eosin and 10% Nigrosin and then calculated for the percentage of living sperm. Dead sperm will absorb the color, while living sperm will not absorb the color (clear).

2.4. Fertilization

In the observation of fertilization, two ml of sperm suspension was mixed with 200 eggs. Water or Cd solution with various concentration was added and homogenized using rooster feather for five minutes. Observations were made after 24 hours of fertilization. Successful fertilization was indicated by change of egg color to clear after being fertilized, but will be cloudy like white-milk if fertilization failed.

2.5. Data analysis

The data were analyzed using Kruskal-Wallis ($p < 0,05$) for mass motility, while other parameters were analyzed by one way variance (ANOVA) ($p < 0,05$) and Duncan test.

3. Results and Discussion

3.1. Effect of Cd on motility

The results showed that the mass motility of fish sperm is active when the sperm suspension (sperm and NaCl) is diluted with water. Motility becomes hyperactive after incubation for 10-15 seconds at room temperature. The mass motility score of the control group and the Cd treatment (50 ppm) was 4 ± 0, but at concentrations of 100 ppm or more (150 and 200 ppm) there was a significant decrease in mass motility value ($p < 0.05$), 3 ± 0,53; 1.63 ± 0.52; and 1 ± 0 (Figure 1).

Mass motility duration is the time required for mass sperm motility, which is different for each group. Duration of mass motility at the control is 68±5.26 second but decreased significantly ($p<0.05$) after exposure of Cd. The higher the concentration of Cd the less duration of mass motility. The mean duration of Cd of 50, 100, 150, and 200 ppm were, respectively 64.25±2.96; 48.37±3.66; 31.12± 4.91; and 21±2.82 seconds (Figure 2A).

The duration of individual motility is the time required for the motility of each individual or sperm cell. The results showed the duration required for each sperm cell varies

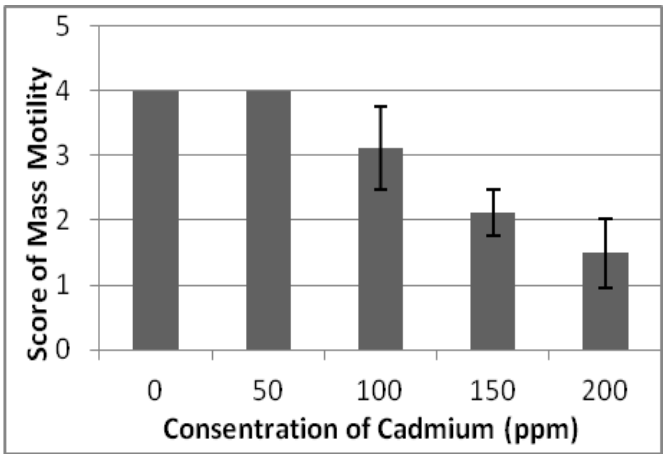

Figure 1. Score of the mass motility of *C. carpio* L. sperm after exposure Cd

between groups. The highest duration was found in control, whereas the Cd treatment decreased the motility duration of each sperm cell significantly ($p<0.05$). Individual duration at control 93.81±7.69 second, but on the treatment of various concentration of Cd respectively 89.29 ± 6.81; 67.68±7.74; 50.51±5.34; and 35.72±3.91 seconds (Figure 2B).

Cadmium toxicity in aquatic organisms decreases cell and tissue function when the concentrations are low, but at high concentrations leads to the death of the organism. Cd can be introduced into the body can be through the accumulation or biomagnification. The impact of Cd toxicity is a physiological disorder of enzyme function in cells and its metabolism (Darmono, 2001). Many heavy metal effects on the structure and physiology of sperm, including inhibiting activation and sperm motion. The structure of the sperm membrane is highly permeable to the toxic material, making it easier for heavy metals to enter sperm cells (Lahnsteiner *et al*., 2004).

Free radicals produced by toxic compounds (Cd) can reduce the quality and function of sperm through inhibition of enzyme activity. The formation of new compounds from heavy metal reactions with the sulfhydryl (SH) group is easy to occur since SH is readily bonded to the Cd ions that enter the sperm membrane. The bonding of these two compounds results in a decrease of enzyme synthesis or does not work because the enzyme undergoes denaturation (Palar, 2002). Free radicals from Cd can enter the sperm cells through lipid peroxidation reactions so that the reaction can decompose the unsaturated fats into many aldehydes. The formation of this aldehyde causes sperm cells susceptible to free radicals (Li *et al*., 2010; Cabrita *et al*., 2014).

Motility depends on energy supply in the form of ATP metabolism (Rizal and Herdis, 2005). Motility can be affected by stress due to heavy metals causing increased lipid

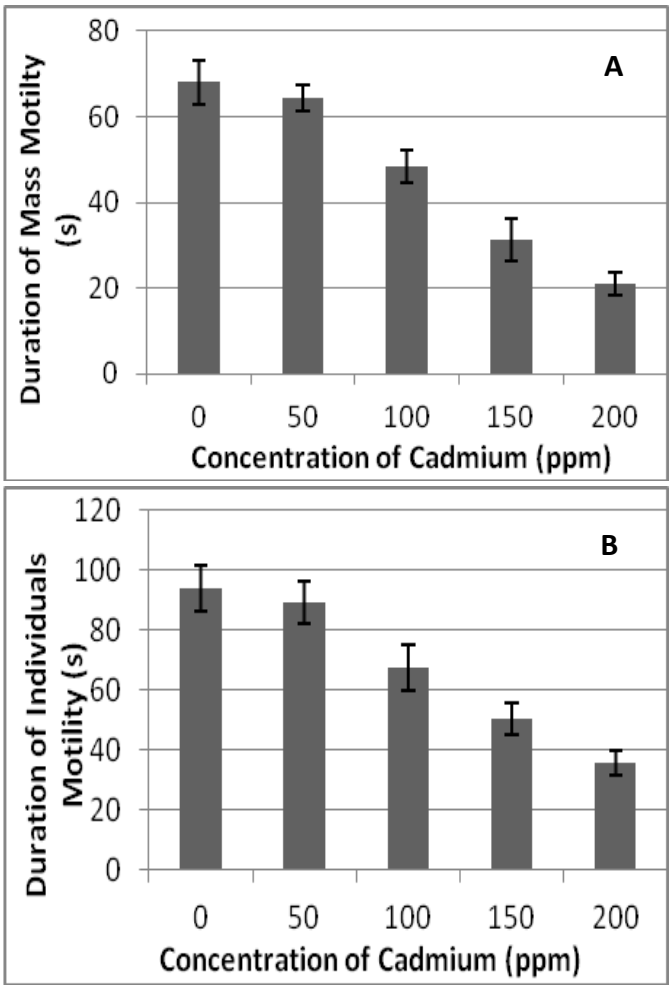

Figure 2. Duration of the mass and individual motility of *C. carpio* L. sperm after exposure Cd

peroxidation thus disrupting the nutrient transport required for sperm movement (Nichi *et al*., 2006). Morita *et al.* (2003) state that the rate of sperm motility and long motion is influenced by the availability of Ca ions. Reddy et al. (1988) suggest that Cd is known to interfere with the activity of Ca ions, so that Ca activity decreases with high Cd concentrations.

According to research that has been done by Warnecke (2005), Cd causes a 50x decrease in the sperm motility at 10 mg/L. Cd ions can bind proteins that affect sperm movements and beat-cross enzyme frequencies, or bindings that affect sperm cell metabolism (Lahnsteiner *et al*. 1999), leading to a decrease in sperm motility rates.

3.2. Effects of Cd on sperm viability

Sperm viability is the percentage of living and healthy sperm in semen. It's important to move and its lifespan for fertility. The sperm viability was observed using 1% Eosin and 10% Nigrosin staining, where living sperm was transparent in color while the dead sperm was red (Figure 3). In control, the fish sperm viability is 78 ± 0.065%. Like other sperm quality

parameters, exposure of Cd in water with various concentration decreases the percentage of fish sperm viability. The percentage decrease after the exposure of Cd at 50, 100, 150, and 200 ppm respectively was 71±0.02% (not significant p> 0.05); but significant (p<0.05) at 62± 0.06%; 42±0.05%; and 27±0.07% (Figure 4).

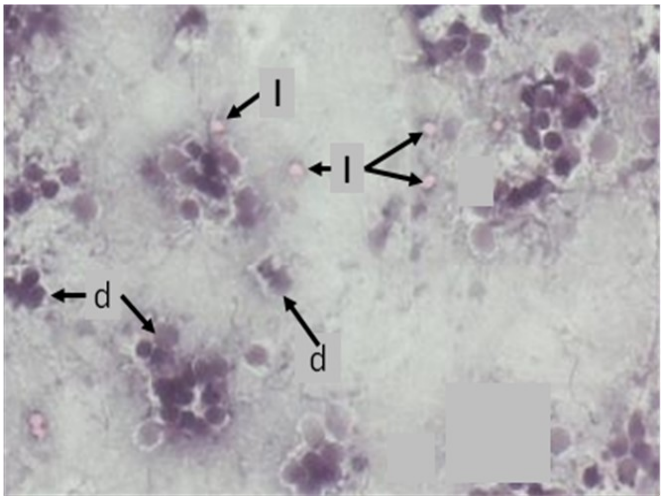

Figure 3. Sperm viability of *C. carpio* L. l: live sperm; d: dead sperm

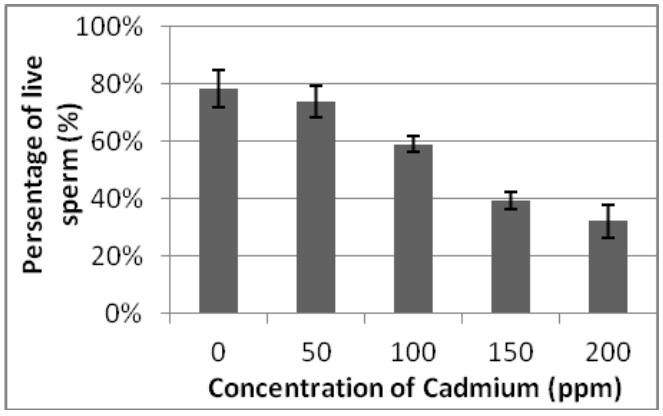

Figure 4. Sperm viability of *C. carpio* L. sperm after exposure Cd

The viability of sperm is the survival ability of the sperm. The percentage of sperm viability decreased at high levels of Cd in the media. This could be caused by oxidative stress and increases ROS levels (Agarwal *et al*., 2014). The high level of ROS caused the formation of aldehydes, resulting in changes in the permeability of membranes, consequently enables many molecules to penetrate the sperm membrane. The presence of these molecules inside the cells interferes with cell metabolism, resulting in cell death. According to Ginzburg (1972), the presence of heavy metals in waters causes the formation of many vacuoles in the cell and changes the permeability of the tail membrane of the sperm. Fraser *et al.* (2011) added that hypertonic solutions due to the high concentration of heavy metals in waters lead to an increase in osmosis, resulting in cell death.

The presence of plasma membrane damage can be proven by using sperm staining. The damaged membrane causes the dye to enter cells. Fraser *et al.* (2011) also state that the exposure to metals causes the solution to be hypertonic. Hypertonic solutions can cause the membrane of the sperm to contract. These caused the sperm to loss its integrity of the membrane, leading to cell damaged or cell death. Dandan *et al*. (2013) stated that the accumulation of Cd could reach the concentrations of 116 mg/L in sperm cells, which showed that all treatment groups experienced significant increases in cell death compared with controls.

3.3. Effect of Cd on fish fertilization

In fish, fertilization of eggs can be done either externally or internally, but in *C. carpio* L is externally in the water. A total of 200 eggs were taken it into two ml sperm suspension, and not all the fish eggs were fertilized by the sperm. The result showed that fertilized eggs in control have fertilization rate of 78 ± 0.06% and the 50 ppm Cd has fertilization rate of 74 ± 0.055% (not significant p> 0.05). Higher concentrations of Cd (100, 150, and 200 ppm) showed a significant decrease in fertility (p<0.05). The percentage of fertility for a higher concentration of Cd were 59±0.03%; 39±0.03%; and 32± 0.058% (Figure 5).

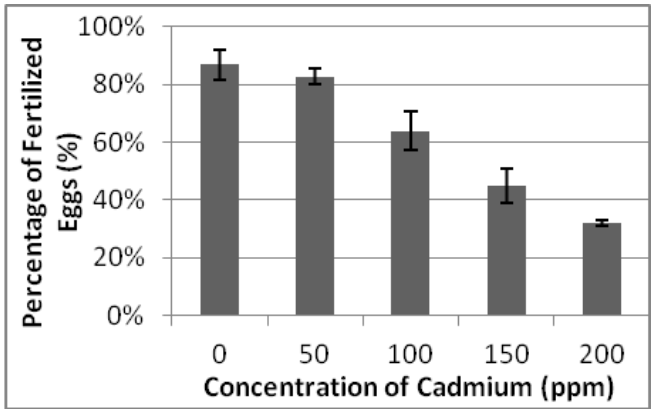

Figure 5. Fertilized of *C. carpio* L. eggs after exposure Cd

Each sperm has an equal chance to fertilize an egg. According to Fraser *et al.* (2011), the complete chromatin structure and DNA is a prerequisite of sperm in the fertilization rate. Woynarovich and Horvath (1980) stated that the entry of sperm into the egg through microphilia only lasted between 45-50 seconds, after that, the microphilia is shut. In addition to the limited time of the sperm to enter the

egg, the sperm survival time is also very short. Mass active movement of fish sperm in fresh water is only 30-60 seconds. According to Hermawaty (2008), successful fertilization can be seen by the color of the egg that turns into clear after being fertilized, but will be cloudy like white-milk if it fails to be fertilized (Figure 6).

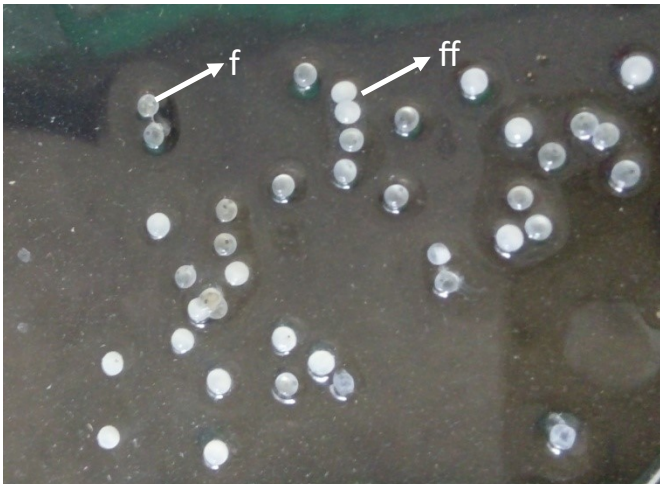

Figure 6. Fertilization of *C. carpio* L eggs. f: the success of fertilization, but ff: cloudy like white milk if it fails to be fertilized.

Not all fertilized fish eggs will hatch into larvae, due to the low quality of eggs caused by heavy metal contamination (Setyono, 2009). The toxicity of Cd is related to the transport of calcium (Ca) ions into the cell membrane. Cd and Ca ions have the same characteristics so that Cd ions can enter cells through Ca channels, inhibiting the removal of Ca ions. This causes adverse effects, because Ca ions are very important in many cell signalling pathways, especially steroidogenesis which may decrease testosterone levels (Monsefi *et al.,* 2009). This can also reduce sperm concentration and motility (Coward *et al*., 2002), the increase of lipid peroxidation, and influences sperm maturation (Abascal *et al*., 2007).

This study indicates that the motility and viability of sperm were affected by the large concentrations of Cd, followed by a decline in the percentage of successful fertilization. This is consistent with the statement of Herdis *et al.* (2005), that sperm has low of motility and viability will have a lower fertilization anyway. Dietrich *et al*. (2010) that Cd exposure in the process of fertilizing the fish causes swelling of sperm to reduce the motility and fertilization of fish.

4. Conclusions

Cd is toxic to sperm and fertilization of C. *carpio* Linn. Exposure to Cd at concentrations of 100 ppm or more can decrease sperm quality both in motility and viability, and affect the fertilization of fish.

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