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Effects of in vitro exposure of mercury on sperm quality and fertility of tropical fish *Cyprinus carpio* L.

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abstract

Mercury is a widespread aquatic pollutant that adversely affects the reproductive system of male fish. Although the effects of mercuric chloride (HgCl_2) on the testicular structure, the sperm count and altered sperm morphology of fish have been investigated, the effects on sperm quality and fertilization are unknown. Therefore, the aim of this study was to investigate the effects of HgCl_2 exposure in different concentrations (0; 0.5; 1; 2.5; 5 ppm) on sperm parameters and fertility of male *Cyprinus carpio*. Sperm quality parameters, including mass and individual time of sperm motility (s), and viability (%) were measured using digital inverted microscopy. The fertilization (%) was measured by counting the number of fertilized eggs. Integrity or DNA fragmentation (%) was measured by Acridine orange test (AOT) using fluorescence microscopy. The change of sperm membrane surface was examined using scan-ning electron microscopy (SEM). The results showed that the in vitro exposure of mercury could significantly decrease some parameters of fish sperm qualities, i.e. decreased the time for mass and individual motility, viability, DNA fragmentation, and fertilization ability ($P < 0.05$) and significantly increase the malondialdehyde levels of sperm ($P < 0.05$). In addition, Hg exposure altered the morphological structure of the sperm head and interfered with the development of embryos in fish eggs.

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Introduction

River is an important ecosystem for human which provides water as a source for various activities such as agriculture, industry and domestic use (Moss, 2007). Water that comes from the spring has a good quality but in the process of water drainage, it will be affected by various pollutants. In recent years, river water quality in Indonesia has contaminated by many pollutants with different characteristics after passing through residential, industrial and agricultural areas. The river pollution in Indonesia has exceeded the quality standard and could negatively affect the life of aquatic biota including fish (Hayati et al., 2017a).

Mercury (HgCl_2) is one of the heavy metals found in highly polluting river water due to industrial activity. It is recognized as the most toxic heavy metal to organisms. High Hg concentrations in freshwater fish are from both aqueous and dietary containing mer-cury and decreased reproduction of adult fish (Pickhardt et al.,

2006). Furthermore, the existence of mercury at high concentrations in the water could inhibit metabolic processes leading to fish death (Vangronsveld and Clijsters, 1994). Mercury also impaired the testes function of the tropical fish *Gymnotus carapo* (L.), including the decrease in sperm count and the alternation in sperm morphology (Vergilio et al., 2014). An in vivo study conducted by Lahnsteiner et al. (2004) showed that mercury had significantly decreased the percentage of sperm motility and velocity of *Clarias gariepinus* and *Lota lota*.

The major mechanisms behind metal toxicity including mercury have been attributed to oxidative stress. Mercury possesses the ability to form reactive oxygen species (ROS) that cause the oxidation of DNA and lipids, DNA fragmentation, and lipid peroxidation. Moreover, malondialdehyde (MDA) will be formed after exposure to ROS which is the secondary product of lipid peroxidation (Flora et al., 2008).

Cyprinus carpio is one of the tropical fish that cultivated for human consumption. It is known as a very sensitive fish to the environmental changes. The decline in fish reproduction is associated with the decreasing of the quality and fertilization ability of sperm. Fish sperm is also used as a bioindicator of toxicity parameters (Kime et al., 2001). Previous studies showed that cadmium

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