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The Effect Of Heavy Metal Cd On The Histology Of Male Nilem Fish (*Osteochilus hasselti*)

Norman Arie Prayogo[1]*, Purnama Sukardi[2], Asrul Sahri Siregar[1], Yasumasa Bessho[3], Ren Fitriadi[2], and Waheti Aulia Sarasih[1]

[1]Program Study of Aquatic Resources Management and Magister SDA Program, Faculty of Fisheries and Marine Sciences, Universitas Jenderal Soedirman. Jl. Dr Soeparno, Banyumas 53122, Central Java, Indonesia

[2]Departmen of Aquaculture, Faculty of Fisheries and Marine Science, Jenderal Soedirman University, Karangwangkal, Purwokerto 53122, Indonesia

[3]Nara Institute of Science and Technology, Gene Regulation Research Laboratory, Division of Biological Science, Graduate School of Science and Technology, Takayama, Ikoma, Nara, Japan

***Corresponding Author: -** Norman Arie Prayogo*

**Email: norman_s2biologi@yahoo.com*

Abstract

Cd is a heavy metal that is found in many aquatic environments and has toxic effects on aquatic organisms. Cadmium has a negative impact on organisms reproductive systems, especially during the process of spermatogenesis. The test biota used in this study was male nilem fish (*Osteochilus hasselti*). The purpose of this study was to determine the effect of heavy metal Cd on the level of damage to the testicular tissue of male nilem fish and to determine the ratio of the level of damage to the testicular tissue of male nilem fish in four treatments of Cd heavy metal for 4 weeks. The method used was an experimental method with a completely randomized design research design (CRD). The research was conducted in three stages, namely taking the testes, making histological preparations, and laboratory analysis using a microscope. The test biota was given four treatments of Cd 0 ppm, 2 ppm, 4 ppm, and 6 ppm with an exposure time of 28 days and sampling was carried out every two weeks. Quantitative data in the form of the proportions of the spermatogenesis stages were analyzed by One Way ANOVA. The results showed that there was no change in shape at the spermatogenesis stage and showed fluctuating results. The results showed that giving 4 Cd treatments to fish for 4 weeks did not affect spermatogenesis

Keywords: Cd heavy metal, nilem fish, testes, histology, spermatogenes

INTRODUCTION

Heavy metals are one of the pollutants that are often found in waters (Mohiuddin et al., 2011; Tantri et al., 2022). Heavy metals can cause certain effects and can become toxic substances that can poison the bodies of

living creatures. Heavy metal pollution into the environment can pose a health hazard, both to humans, animals, plants and the environment itself (Nisak et al., 2013; Isroni & Maulida., 2022). Some heavy metals that are often found in the environment include Hg (Hydrargyrum), Cu (Cuprum), Pb (lead), Zn (Zink), and Cd (Cadmium). There are many uses for these metals, such as rubber manufacture, batteries, TV tubes, paint, soap, and textile printing. If the concentration in the waters exceeds the threshold, it can cause pollution and be dangerous to the aquatic environment including the organisms in it (Darmono, 1995; Soegianto et al., 2012).

In animals, Cadmium metal (Cd) may cause problems with organs, such as gills and kidneys, if it is absorbed by their bodies (Erlangga, 2007; Zulkarnain et al., 2013). Cd metal can come from industrial waste, if it enters the body of an organism it can accumulate in the body as a poison and as a barrier to the work of enzymes in metabolic processes (World Bank, et al., 1983). Cd metal can enter organisms that live in water through oral, inhalation or dermal (Palar, 1994).

Cd metal can enter the organism's body through the body of the organism through the food chain, gills or diffusion through the surface of the skin, as a result the metal can be absorbed into the tissue, accumulate in the tissue (bioaccumulation) and at certain concentrations can damage organs in the body's tissue. Acute conditions describe cells that are damaged in a short time but are lethal, where the cell membrane ruptures so that the cell contents are completely dissolved, which occurs in a number of cell groups. In chronic conditions, cells experience damage over a long period of time and are sub-lethal (Banks, 1986; Anggitasari et al., 2019; Prayogo et al., 2023). Fish are very susceptible to exposure to heavy metals in waters, which can reduce fish populations (Palar, 1994; Ardiyansyah et al., 2019).

The Nilem fish (*Osteochilus hasselti*) is used because it is a native fish in Banyumas Regency and a food fish there. Nilem fish is a freshwater fish that belongs to the Cyprinidae family. Male nilem fish have a smaller distribution than female nilem fish in waters (Effendi, 2006; Fira et al., 2020). This reason is the background for research on the histology of the reproductive organs of male nilem fish. There are many environmental factors that affect fish reproduction, including food availability, lighting duration, temperature, rainfall, currents, and water pressure (Taylor & Francis, 2009). The organs that play an important role in the fish reproduction process are the testes in male nilem fish (Sumantadinata, 1983).

Testicles are the reproductive organs of fish. Testicles contaminated with heavy metals will disrupt the reproductive process and can even reduce the number of Nilem fish. If the level of toxicity in the water is higher and the exposure time is longer, the higher the level of damage that occurs in fish ovarian tissue (Atli, 2015). Cadmium has an effect on the spermatogenesis process (Purwoko, 2010). Cd compounds that accumulate in fish testes are very dangerous because they reduce the quality of gametes and affect sperm metabolism, including spermatozoa motility (Gage et al., 2004). Cd can inhibit spermatogenesis in male goldfish, and in female fish it causes follicles to fail to reach the stage of maturation and ovarian atrophy (Tandjung, 1992). Histological analysis can be a very sensitive parameter and is very important in determining changes in cell structure that occur in internal organs such as the kidneys, liver and gonads (Dutta, 1996). Histology is the science of anatomical biology which studies the structural arrangement of cells. A change in cell structure may indicate that there has been a change in the environment in which the fish lives, such as due to disease, bacteria, or heavy metal exposure (Banks, 1986; Insivitawati et al., 2015; Hayati et al., 2023). Therefore, research is needed on the effect of exposure to cadmium metal on the gonad histology of nilem fish. The research will be carried out for 4 weeks.

MATERIALS AND METHODS

2.1 Time and Location

This research was conducted from November 2019 – November 2020 at the Research Laboratory of the Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University, and the BBVet Laboratory (Veterinary Center) Yogyakarta.

2.2 Experimental Design

This research applies the experimental method Completely Randomized Design (CRD) with 4 treatments based on those used for the sublethal test, namely 0%, 30%, 60%, 90% of the LC-50 test value for 96 hours 6.5 ppm, namely a cadmium concentration of 0 ppm, 2 ppm, 4 ppm, and 6 ppm with three individual replications of male nilem fish in each treatment for each sampling (Shah & Altindag, 2005). Treatment was given for 4 weeks with sampling intervals every 14 days.

2.3 Research Procedure
2.3.1 Research Preparation

The experimental tubs used were 4 round fiber tubs, 1 for control (without heavy metal treatment) and 3 for Cd heavy metal treatment, each tub was filled with 300 L of water and equipped with a closed recirculation system to ensure oxygen supply. The fish used as test animals were 4 month old nilem fish from 1 parent. First, prepare 50 nilem fish per tank. The 4 month old nilem fish came from the same parent and were then acclimated in the aquarium for three days. Fish were divided into three groups treated with different heavy metal Cd and one control group (Prayogo & Siregar, 2017).

2.3.2 Testicular Sample Collection and Storage Procedures

Fish testicle samples were taken at the 2nd and 4th weeks of exposure to the heavy metal Cd, 3 individuals at each concentration. The fish was dissected with surgical scissors, starting from the anus towards the top of the stomach to the back of the operculum then descending ventrally to the bottom of the stomach. Separate the gonads from other internal organs with tweezers then put them in a sample bottle to be fixed with NBF solution and then used as a histology preparation.

2.3.3 Procedure for Making Histology Preparations

Histology preparations were made using the paraffin method with Hematoxylin Eosin staining. The stages of making histological preparations according to Mescheq, (2009) are as follows:

1. Fixation
The organ sample (testis) was put into a sample bottle then added with NBF solution and left for 2x24 hours.

2. Dehydration
Organ samples (testicles) were dehydrated gradually with 70%, 80% and 90% alcohol once for 45 minutes each, and with absolute alcohol twice for 45 minutes.

3. Clearing
Organ samples (testicles) were immersed in a mixture of xylol-alcohol (3:1; 1:1; 1;3) for 30 minutes each. Then with a solution of pure xylol 1 and pure xylol 2 for 30 minutes each.

4. Infiltration
Organ samples (testicles) that look clear are placed in a mixture of xylol-paraffin (1:3; 1:1; 3:1) for 30 minutes each, paraffin I and II for 45 minutes each in an oven at a temperature of 60°C.

5. Embedding
The organ sample (testis) was placed in a mold filled with liquid paraffin, positioned in the direction of the cut and left to solidify to form a paraffin block.

6. Cutting
Paraffin blocks containing organs (testicles) was placed in a holder and then cut transversely using a microtome with a thickness of 4 μm to produce ribbon-shaped pieces.

7. Pasting
A ribbon-shaped piece of paraffin was placed on a glass object that had been dripped with water containing egg white, then heated with a water heater at 40oC until the ribbon expanded. The slices that have been attached to the glass object are dried for 24 hours.

8. Coloring
Dried tissue slices were deparaffinized in pure xylol 2 times, first for 10 minutes and second for 5 minutes. Then proceed to immersion in 100%, 90%, 80%, 70% alcohol and distilled water. After that, put it in Hematoxylin dye for 15 minutes, then wash it in running water for 2 minutes, then put it in Eosin dye for 1-2 minutes, then wash it in running water for 2 minutes. In the next stage, the samples were successively immersed in 70%, 80%, 90% and 100% alcohol for 5 minutes each. After that, the sample was put back in

pure xylol for 2-5 minutes, removed and dripped with Canada balsam before drying, then covered with a cover glass. The results of the histology preparations from each treatment that had been stained were observed under a light microscope with a magnification of 1000 times.

2.3.4 Proportions of Spermatogenesis Stages

Ten samples were examined histologically and then sliced into pieces of 4µm ribbon pieces. Sections of cells from each stage in spermatogenesis. Observations were made using a light microscope. Observe and calculate the percentage of each stage of spermatogenesis with the total number of cells in spermatogenesis. The formula for calculating cell proportions at the spermatogenesis stage refers to the research of Almeida-Santos et al. (2014) with the formula:

Stage Proportions X = $\frac{\sum spermatogenesis\ cells\ stage\ x}{\sum total\ spermatogenesis\ cells} \times 100\%$

Where: x is the stage of development of certain sperm cells in spermatogenesis.

2.3.5 Gonadosomatic Index (IGS)

The percentage of gonad weight that has been weighed is then divided by the total body weight of the fish. The gonadosomatic index in Effendie (1979) is calculated using the following formula:

$$IGS = \frac{Bg}{Bt} \times 100\%$$

Where:

IGS = Gonadosomatic Index
Bg = Gonad Weight
Bt = Body Weight

2.4 Data Analyst

In this study, quantitative data was collected on male nilem fish testicular histology in each treatment in order to determine the damage caused by cadmium on their testicular histology. Meanwhile, the comparison of the concentration of the heavy metal Cd which can inhibit spermatogenesis in the testes of male nilem fish was analyzed using the ANOVA test and continued with the BNT test on significantly different data.

RESULTS AND DISCUSSION

4.1 The Effect of Cd Metal on Testicular Histological Damage in Male Nilem Fish

The testes have two main functions, namely producing spermatogonia and secreting androgen hormones. The process of forming sperm cells in the testicles is called spermatogenesis. The spermatogenesis process consists of 5 stages, namely the formation of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa (Nalbandov, 1990).

The heavy metal Cd is a dangerous metal and one of its effects can cause damage to the testicles (Harsanto, 1997). Damage to the testicular organs is closely related to the spermatogenesis process. The spermatogenesis process occurs in the seminiferous tubules of the testicles. Where there are spermatogonia stem cells. Sertoli cells which function to feed spermatozoa are also Leydig cells found in interstitial tissue which function to produce testosterone. The presence of disturbances in the reproductive organs due to chemicals will indirectly affect the spermatogenesis process (Guyton and Hall, 2001).

The treatment was conducted by administering the heavy metal Cd at concentrations of 2 ppm, 4 ppm and 6 ppm. The 0 ppm treatment was not exposed to Cd metal and was the control for each treatment. The sampling times carried out were week 0, week 2, and week 4 with three repetitions of each. Histological images of spermatogonia stages can be seen in **Figure 1**.

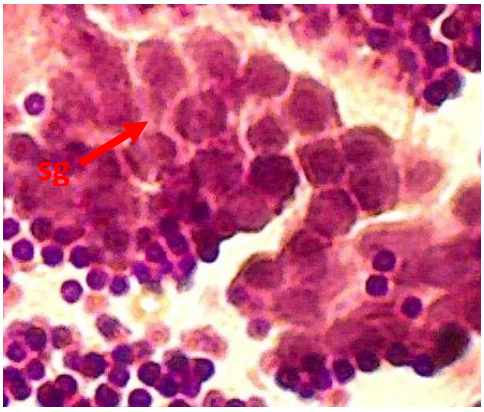

Figure 1. Histology of Spermatogonia in Nilem Fish After Exposure to Cd Metal for 4 Weeks

The shape of the spermatogonia (sg) cells in Figure 1 shows a large round shape with dark and light colors. Spermatogonia cells observed in nilem fish when given 2 ppm, 4 ppm and 6 ppm of Cd metal did not change shape. Immature spermatogenic cells or spermatogonia cells are cells that are located near the basement membrane of the seminiferous tubules. Spermatogonia divide by mitosis to produce several cells (Eroschenko, 2013). In general, exposure to high levels of the heavy metal Cd can inhibit the production of the hormone adrenaline, thus affecting the spermatogenesis process in fish testes (Soemirat, 2005). However, the treatment carried out in this study did not provide a difference in the shape of nilem fish spermatogonia before and after exposure to Cd metal. The stage after spermatogonia proliferate is spermatocyte cells. Histological images of spermatocyte cells can be seen in **Figure 2.**

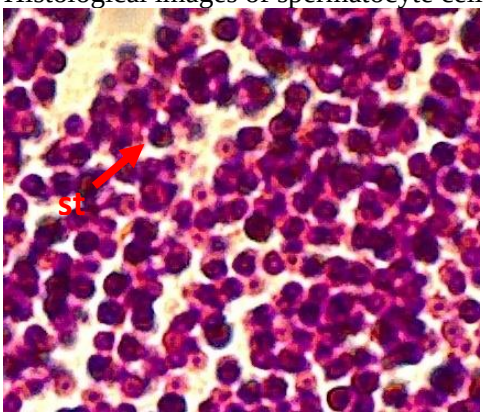

Figure 2. Histology of Spermatocytes in Nilem Fish After Exposure to Cd Metal for 4 Weeks

Based on histological observations, there was no change in the shape of spermatocyte cells between week 0 and week 4 with different treatments. **Figure 2** shows the shape of spermatocytes (st) where the nucleus is still visible inside and there is a light colored layer covering the outside of the spermatocyte. At this stage two divisions occur. The first meiotic division produces smaller secondary spermatocytes with less dense nuclear chromatin (Eroschenko, 2013). Histology results showed that it was difficult to distinguish between primary and secondary spermatocytes. This is because secondary spermatocyte cells undergo a second meiotic division within a short time after their formation, so these cells are rarely seen in the seminiferous tubules (Mescheq, 2009). Next, the spermatocyte cells divide to form spermatids. Histological images of spermatid cells can be seen in **Figure 3.**

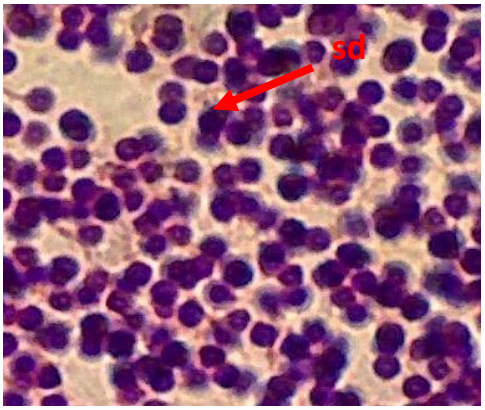

Figure 3. Histology of Nilem Fish Spermatids After Exposure to Cd Metal for 4 Weeks

Based on **Figure 3**, the shape of the spermatids (sd) are completely round. Spermatids are formed from the second division process in spermatocytes. There was no change in shape from week 0 to week 4 with four different treatments. The discovery of spermatids in the spermatogenesis process supports the results of the discovery of spermatocytes where in the treatment given, the meiotic division process can still proceed to form spermatids. The spermatids then metamorphose to form sperm through the process of spermiogenesis (Mescheq, 2009). In the histological analysis, no spermatozoa stages were found. This could be caused by the observed fish not having mature enough gonads.

4.2. Comparison of Spermatogenesis Stages in Testes of Male Nilem Fish Exposed to Cd Metal

In the comparison of each treatment described, the spermatogonia stages in the 0 ppm treatment showed results that were not significantly different. This shows that the conditions in the 0 ppm treatment were still stable because they were not exposed to Cd metal. In the 2 ppm treatment, the results showed that spermatogonia cells increased in number. This can happen because in the 2nd week and 4th week there begins to be an accumulation of the heavy metal Cd, so that some spermatogonia do not proliferate properly to form spermatocytes (Eroschnko, 2013). This was also shown in the 4 ppm and 6 ppm treatments. According to Gosling (1992), the accumulation of heavy metals will affect the gametogenesis process, where lysosomes are unstable and will experience degeneration so that the cells will die.
The results of calculating the average proportion of spermatogonium stages in the testes of nilem fish exposed to the heavy metal Cd for 4 weeks can be seen in **Table 1.**

Table 1. The Proportion of Nilem Fish Spermatogonia Stages Calculated

	Week 0			Average	Week 2			Average	Week 4			Average
	1	2	3		1	2	3		1	2	3	
0 ppm	3,79	1,66	1,53	2,32	0,25	7,05	3,24	3,51	0,93	10,15	0,45	3,85
2 ppm	3,79	1,66	1,53	2,32	13,88	0,25	6,76	6,96	12,80	11,11	0,29	8,07
4 ppm	3,79	1,66	1,53	2,32	4,89	1,59	1,40	2,63	6,27	52,87	8,85	22,66
6 ppm	3,79	1,66	1,53	2,32	24,42	18,07	8,52	17,01	0,98	68,18	0,57	23,25

*1: 1st repetition *2: 2nd repetition *3: 3rd repetition

The data results in **Table 1** show that in week 0 the resulting value has the same average value, namely 2.32%. The nilem fish were not exposed to the heavy metal cadmium at week 0, so their spermatogenesis stage values were the same in each treatment. In the 2nd week of the spermatogonia stage there was an increase and decrease in the average value with results between 2.63% -17.01%. In the 4th week there was an increase in the proportion value of the spermatogonia stage with a value between 3.85% -23.25%. The decrease in the number of spermatogonia cells is caused by inhibited proliferation of germ cell development. The decrease in the number of spermatogonia cells occurs due to disturbances in the spermatocytogenesis process (Heryani, 2011). The histogram of the proportion of spermatogonia stages can be seen in **Figure 4.**

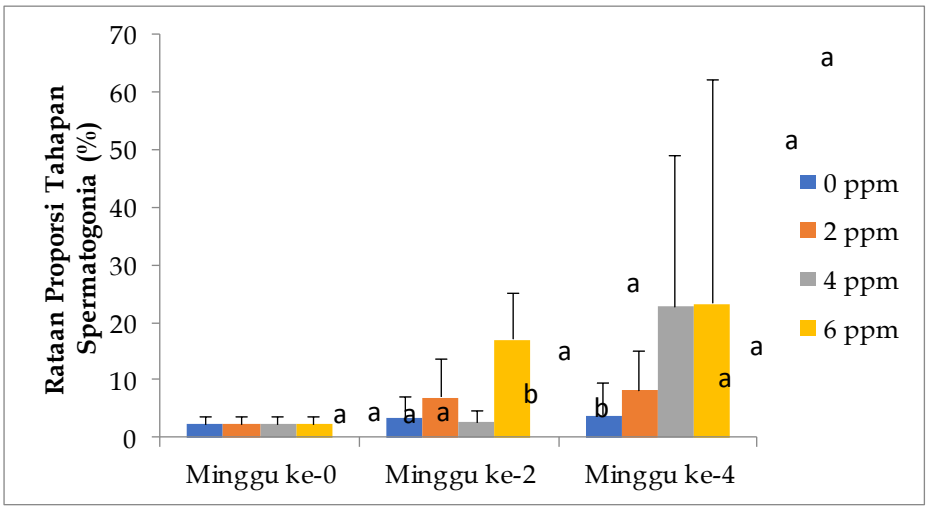

Figure 4. Proportion of Nilem Fish Spermatogonia Stages

Figure 4 shows that the results at week 0 are not significantly different with a significance level of >0.05 when the treatment is the control.In the 2nd week, the results showed fluctuations where the results of 0ppm and 6ppm were significantly different, whereas the results of 4ppm and 6ppm were significantly different. The results of the 4th week histogram showed an increase in the number of spermatogonia produced in each treatment. The results of the analysis carried out showed that the sig value in the 4th week was >0.05. When compared with the results of the proportion of spermatocyte stages, the results of the proportion of spermatogonia stages are lower. This can happen because the spermatogonia cells produced have undergone many divisions to form spermatocyte cells. The pretesticular mechanism inhibits spermatogenesis through the hypothalamus, pituitary and testicular axis. Decreased LH in the serum will reduce intratesticular testosterone which is followed by a decrease in FSH so that sperm production is hampered (Sukmaningsih, 2012).

Spermatocyte stages in the 0 ppm treatment showed no significant changes. Due to the fact that the fish were not exposed to Cd metal during the 0 ppm treatment. In the 2 ppm treatment there was an increase in spermatocytes every week. However, at concentrations of 4 ppm and 6 ppm there were fluctuations in the spermatocyte cells produced. According to Eroschenko (2013), fluctuations in sperm formation are caused by changes in the two hormones that make testosterone, namely follicle stimulating hormone (FSH) and luteinizing hormone (LH).

Table 2. Calculation of the Proportion of Nilem Fish Spermatocyte Stages

	Week 0			Average	Week 2			Average	Week 4			Average
	1	**2**	**3**		**1**	**2**	**3**		**1**	**2**	**3**	
0 ppm	31,00	55,12	46,78	44,30	53,81	29,13	47,12	43,35	42,59	56,46	48,82	49,29
2 ppm	31,00	55,12	46,78	44,30	55,64	51,56	30,18	45,80	55,51	51,19	68,26	58,32
4 ppm	31,00	55,12	46,78	44,30	35,34	32,80	37,05	35,06	45,06	44,16	41,65	43,63
6 ppm	31,00	55,12	46,78	44,30	39,00	40,26	51,78	43,68	37,20	37,30	37,64	37,38

*1: 1st repetition *2: 2nd repetition *3: 3rd repetition

The results in Table 2 show that at week 0 there was no fluctuation in the average value of the proportion of spermatocyte stages. In the 2nd week there was a decrease and increase in the average value between 35.06%-45.80%. In the 4th week there was another fluctuation in the average value between 37.38%-58.32%. According to Purbonegoro et al., (2017), exposure to fish in small amounts of the heavy metal cadmium does not affect physiological function in fish. Low exposure time to the heavy metal cadmium can also cause effects that are not significantly different, and in this study a relatively short period of time showed results that were not significantly different. Next, a comparison graph of the proportion of spermatocyte stages is presented in Figure 5**.**

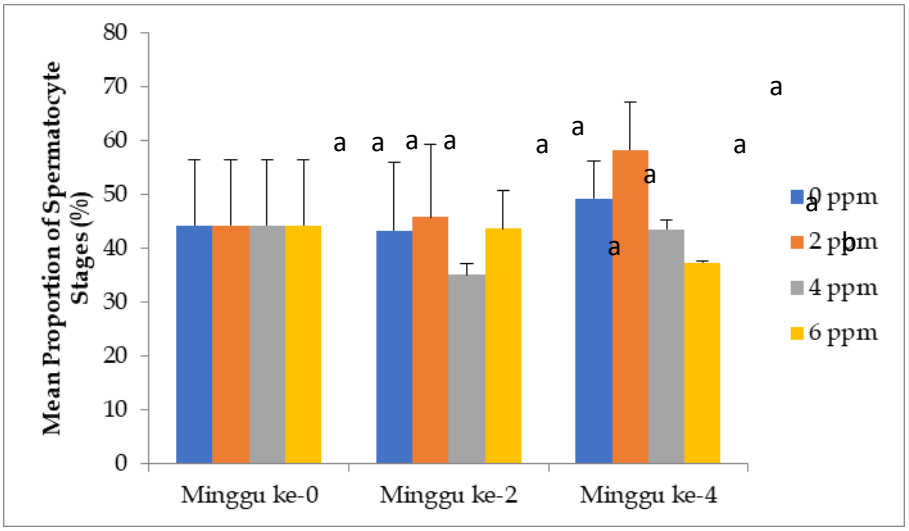

Figure 5. Proportions of Nilem Fish Spermatocyte Stages

According to Figure 5, the results of the analysis in the second week of sampling showed a sign >0.05, which indicated that the proportion of spermatocyte stages did not differ significantly from the proportion in the first week based on exposure time. Meanwhile, the 4th week of sampling showed that the results of treatment at concentrations of 2 ppm and 6 ppm were significantly different. The discovery of spermatocyte cells in the 2nd week and 4th week shows that the spermatogonia cells in each treatment were able to proliferate well and divide optimally into spermatocytes (Eroschenko, 2013).

The results of the proportion of spermatocyte stages showed that at week 2 and week 4, the values obtained fluctuated. Fluctuating results can be caused by the exposure time given. According to Soeprijanto (2003), the rate of bioaccumulation of Cd metal is higher at the beginning of exposure, namely in the period of 0-10 days. In the middle of exposure, the increase in bioaccumulation rate tends to decrease and will be relatively stable at exposure times above 20 days. Meanwhile, in week 0, the results obtained were average and did not experience an increase or decrease in the average value. At week 0 the fish can still grow well because there are no toxic substances that inhibit their metabolism (Rahayu et al., 2017).

Table 3. Calculation of the Proportion of Nilem Fish Spermatid Stages

	Week 0			Average	Week 2			Average	Week 4			Average
	1	**2**	**3**		**1**	**2**	**3**		**1**	**2**	**3**	
0 ppm	65,22	43,21	51,69	53,38	45,94	63,82	49,64	53,13	56,48	33,38	50,73	46,86
2 ppm	65,22	43,21	51,69	53,38	30,48	48,44	63,06	47,33	31,70	37,70	31,74	33,71
4 ppm	65,22	43,21	51,69	53,38	59,78	65,61	61,55	62,31	48,66	37,46	49,49	45,21
6 ppm	65,22	43,21	51,69	53,38	36,57	41,67	39,69	39,31	61,81	59,21	62,36	61,12

*1: 1st repetition *2: 2nd repetition *3: 3rd repetition

The results in **Table 3** show that at week 0 there was no fluctuation in the average value of the proportion of spermatid stages. in week 2, the average value ranged between 39.31%-62.31%. In the 4th week, the resulting average value ranged between 33.71%-61.12%. There were fluctuations in the 2nd and 4th weeks with results experiencing increases or decreases in value. This could be due to the influence of heavy metal concentrations that can still be tolerated by nilem fish and exposure times that are not long enough. Based on research by Siregar and Prayogo (2017), the heavy metal mercury does not have a significant effect on sGnRH gene expression with a length of exposure of around 2 - 4 weeks..

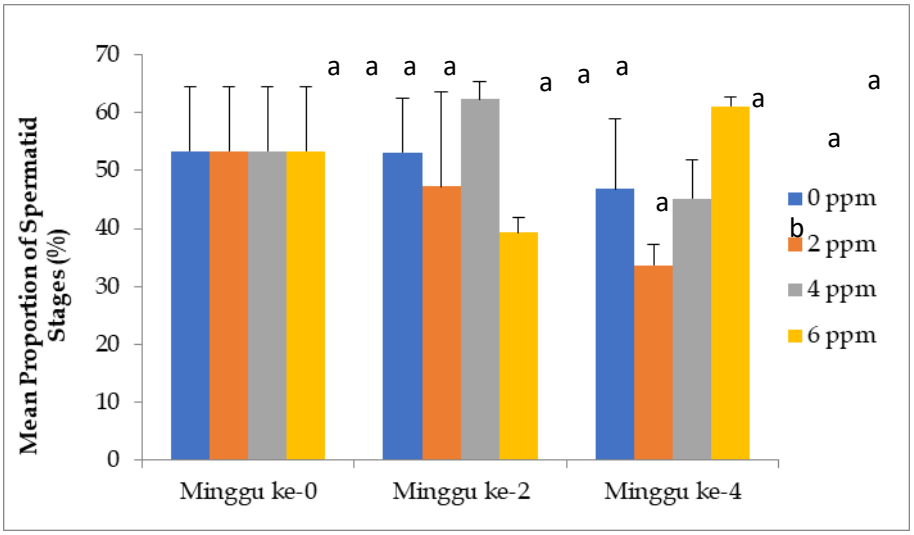

Figure 6. Proportion of Nilem Fish Spermatid Stages

Based on **Figure 6,** it can be seen that both spermatid counts at week 2 and week 4 were lower than those at week 0. The results of the analysis in the 2nd week showed sig >0.05 so the results were not significantly different. In the 4th week, the results of the comparison of the 2nd and 6th weeks showed significantly different results. Fluctuations and differences in significance values can occur due to short exposure times. Based on research conducted by Prabowo (2005), the accumulation of heavy metals in the body of milkfish did not show a major influence on metabolism and protein synthesis after an exposure time of 2 weeks (Prabowo, 2005).

The spermatid stage with 0 ppm treatment showed results that were still in stable condition. Meanwhile, there were fluctuations in the 2 ppm, 4 ppm and 6 ppm treatments. The spermatids produced tend to be unstable and fluctuate. Fluctuating results can occur because the concentration of Cd metal given has not significantly influenced the spermatogenesis process in the testes of nilem fish. Based on research conducted by Hayati et al. (2017), exposure to the heavy metal cadmium at 50 ppm, 100 ppm, 150 ppm and 200 ppm can have an effect on reducing sperm quality and fertility in goldfish. The decrease in spermatid cells could be caused by the absorption of the Cd metal given into the treatment pool. According to Soemirat (2005), the fish absorbs Cd metal and binds it to the protein thionein, synthesizes it by the liver, and distributes it throughout the fish, including the testes, through blood circulation, causing damage to the fish's testicles and inhibiting sperm production.

The reproduction process in fish can be affected by high concentrations of Cd metal. Based on research conducted by Prabowo (2005), cadmium significantly had a toxic effect on milkfish with a concentration of 46.55 ppm, in research by Almeida (2009), cadmium significantly had a toxic effect on *Oreochromis niloticus* with a concentration of 40.96 and 81.92 ppm, and in research by Zikic et.al., (2001), cadmium with a concentration of 20 ppm after exposure for 15 days had a significant effect on the *Carassius auratus* gibelio fish. The concentrations used in this research were 2 ppm, 4 ppm, and 6 ppm, lower than the concentrations used in the research of Prabowo (2005), Almeida (2009) and Zikic et.al., (2001) so that good results could occur fluctuates.

Fish that have immature gonads are supported by the gonadosomatic index data in **Figure 7.**

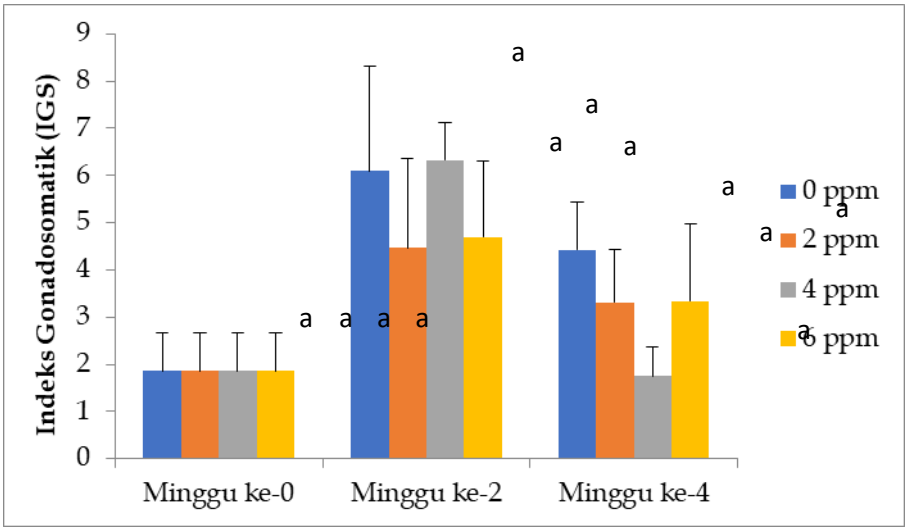

Figure 7. Gonadosomatic Index (IGS) of Nilem Fish

A Gonadosomatic Index determines the type of spawning and the level of maturity of fish gonads. According to Effendie (2002), the increase in IGS numbers is in line with the increase in TKG. The results regarding the proportion of spermatogenesis stages showed significantly different results, supported by IGS data which showed a significance figure of >0.05, which means it was not significantly different. The absence of spermatids that differentiate into spermatozoa in the fish studied can also be supported based on IGS data, which indicates that the fish may not yet have mature gonads (Hendri & Baihaqi, 2015). Based on research conducted by Nugraha (2017), the gonad maturity level of male nilem fish in Rawa Pening is at 19.67%. Meanwhile, the observed IGS results on nilem fish ranged from 0.63% -6.33%.

The results of treatment on nilem fish for 4 weeks did not provide significant results on the proportion of spermatogenesis stages of nilem fish. This is possible because nilem fish have adapted to the heavy metal cadmium given during the exposure time (Prayogo et al., 2016). The time of exposure to heavy metals greatly influences aquatic organisms (Mehmood et al., 2019). According to Drąg-Kozak et al. (2019), that exposure to heavy metals for a short time still has little effect on the fish's body. Longer exposure times to heavy metals will have a greater influence and have worse chronic impacts on fish and other aquatic organisms (Purbonegoro, 2017).

Based on the results of this study, Cd at concentrations of 0 ppm, 2 ppm, 4 ppm, and 6 ppm has no effect on inhibiting spermatogenesis in nilem fish, as the concentrations used are still tolerable. The presence of the heavy metal cadmium in Banyumas waters such as in the Serayu River has a range of cadmium heavy metal levels of 0.045-0.079 ppm (Suwarsito and Sarjanti, 2014). The levels of the heavy metal Cd in the river have exceeded the water quality standards determined according to PP No. 82 of 2001, namely 0.01 ppm. The water used by the community for fish cultivation in Banyumas comes from the surrounding rivers. This suggests that the water used for fish cultivation may also be contaminated with heavy metal Cd, and that the nilem fish may be able to adapt to it.

CONCLUSION

Based on the heavy metal Cd with treatment of 0 ppm, 2 ppm, 4 ppm, and 6 ppm exposed for 4 weeks, it had no effect on testicular damage to male nilem fish. It was found that differences in Cd metal concentrations of 0 ppm, 2 ppm, 4 ppm, and 6 ppm that were exposed did not affect spermatogenesis in male nilem fish testes.

CONFLICT OF INTEREST:

The authors declare that there are no conflicts of interest regarding the publication of this manuscript

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