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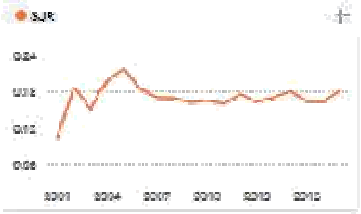

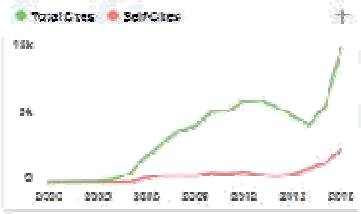

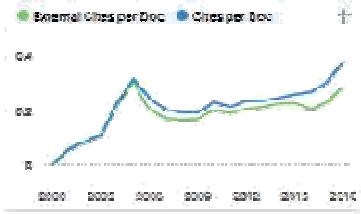

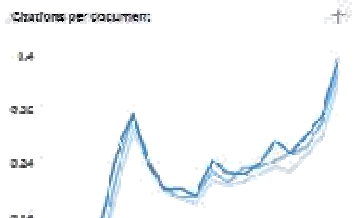

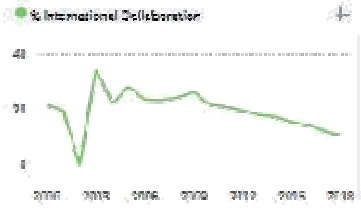

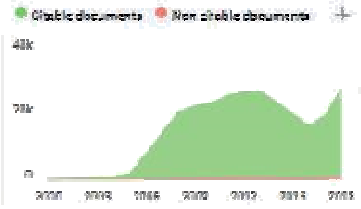

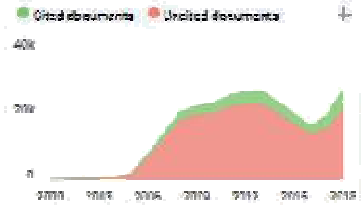

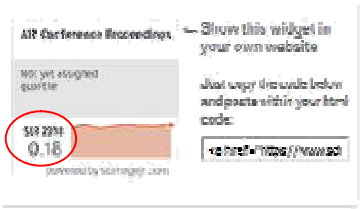

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Histopathological Assessment of Cadmium Effect on Testicles and Kidney of *Oreochromis niloticus* in Different Salinity

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Abstract. This study was aimed to determine the effect of cadmium on testicles and kidney structure of *Oreochromis niloticus* in different salinity. Twenty-seven *Oreochromis niloticus* at age of 5±0.5 months with average size 11±1 cm and average weight 250±50 g were used and divided into nine treatment groups with variations in salinity (0, 5 and 10 $^{0}/_{00}$) and cadmium levels (0, 2.5, and 5 ppm). After two weeks of treatment periods, testicles and kidney was collected and then processed into histological slide. Result showed that cadmium and salinity variations caused change in diameter of seminiferous tubules in the testicles. Kidney structure also showing various damage such as necrosis and inflammation from groups treated with various concentration of salinity and cadmium. Smallest diameter of seminiferous tubules of the testicles and the highest percentage necrosis and inflammation of kidney was found from salinity:cadmium = $0^{0}/_{00}$: 5 ppm treatment.

Key words : *Oreochromis niloticus*, cadmium, salinity, seminiferous tubules, testicles, kidney

INTRODUCTION

Environmental pollution, especially on water is public problem, because water is essential for the life. Some pollutant such as microbiology materials (bacteria and parasites); organic substances (pesticides, detergents, insecticides, and household waste); and inorganic materials (salts, acids, and metals), as well as other chemicals have been found in the river (Qiao et al., 2007). In the increasingly industrialized society, heavy metal waste concentration in the water is also found to be increased that it was possible to achieve toxicity level for aquatic life. One of the heavy metals having toxic effect was cadmium (Cd). The Cd was found in the river and marine environment (Almeida et al., 2009). Changes of heavy metal in aquatic systems was depended on specific factor caused by chemical or physical effect of the surrounding environment. The different of the salinity would affect Cd absorption rate in fish tissue. Variation of salinity level could affected on the toxicity of Cd, the higher the salinity Cd toxicity on fish would lower. Water in the river and reservoir was commonly used as source of water supply of fish, but increasing level of heavy metal pollution in the water threatened aquaculture activities. Development and deployment of tilapia in the river and reservoir was very rapid, because this fish had rapid growth, large body size, tasteful flesh, and less thorn, also high breeding and survival rate. Tilapia species was generally characterized by large tolerance to salinity, however, its capacity to adapt to brackish or seawater might be modulated by environmental factors. Major osmoregulation mechanisms involved in salinity adaptation was presented.

Cadmium was known to possess long half-life inside the body of living organism (Patrick, 2003). Such contaminant was health-endangering towards organisms which admitted through food chain and accumulated in tissues such as kidney and reproductive organs (Kostnett, 2007). Accumulation of Cd in the reproductive organ of organism could affect reproductive processes thus affecting survival of the species. Cadmium had carcinogenic and

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endocrine-damaging properties (Bobocea et al., 2008). In fish, contaminant was able to enter across biological barrier that separated internal medium of the organism from the environment by means of absorption through the gills, especially from epithelium branchiale. The chemicals would then enter respiratory system until eventually penetrated capillary endothelial cells to enter the blood circulation. As it followed bloodstream, the contaminant would eventually participated in the various metabolic processes (Connell, 2006). Incoming contaminant was also indirectly contacted the surface of intestinal microvilli (Miller 2007, Kostnett 2007). Blood was filtered in the kidneys, thus Cd contained in the blood was filtered. So, kidneys was exposed to Cd through blood filtration, while gill was exposed through respiratory water circulation which then would absorbed it. Cadmium traveled to hypothalamus was able to lower LH release, thereby lowering testosterone level. Cadmium might inhibit gametogenesis in both male and female fish. In the female fish, Cd inhibited follicle maturation and follicle would fail to reach the stage of ovarian atrophy, otherwise it caused estrogen could not initiated vitelogenin synthesis in the liver. As vitellogenin level was reduced, oocytes would be lacking materials for yolk (vitelogenin) constitution, and consequently oocyte growth would be hampered.

MATERIALS AND METHODS

This study used *Oreochromis niloticus* with age of 5±0.5 months, size 11±1 cm, and weight 250±50 g; Cadmium ($Cd(NO_3)_2$, $4H_2O$), 10% of neutral buffered formalin, xylol, alcohol (70, 80, and 96%, absolute), paraffin, hematoxyllin – eosin, Mayer's albumin, entellan, Olympus light microscope, and camera.

The cadmium levels used were 0, 0.25, and 5 ppm in 0, 5, and 10% salinity. Paraffin method was used for testicles dan kidney histological slide preparation. Thirty-six samples of male fish (n=4) were collected and acclimated for a week. After that, fishes were transferred into aquarium filled with water at different salinity and cadmium level appropriated for each treatment for 2 weeks duration.

The diameter of seminiferous tubules, testicular weight, and structure of spermatogenic cells and kidneys were evaluated. Proximal convolutus tubule of the kidney was evaluated using graticule. Data analysis was performed statistically using analysis of variance (ANOVA) with 5% significance ($P > 0.05$).

RESULT

The morphometric of *Oreochromis niloticus* testicles was presented on Figure 1.

FIGURE 1. The morphometric of *Oreochromis niloticus* (A); Testicles (yellow arrow) (B) Weight and size of *Oreochromis niloticus* testicles that has been excised from each treatment variation of Cd and salinity level was measured (Figure 2).

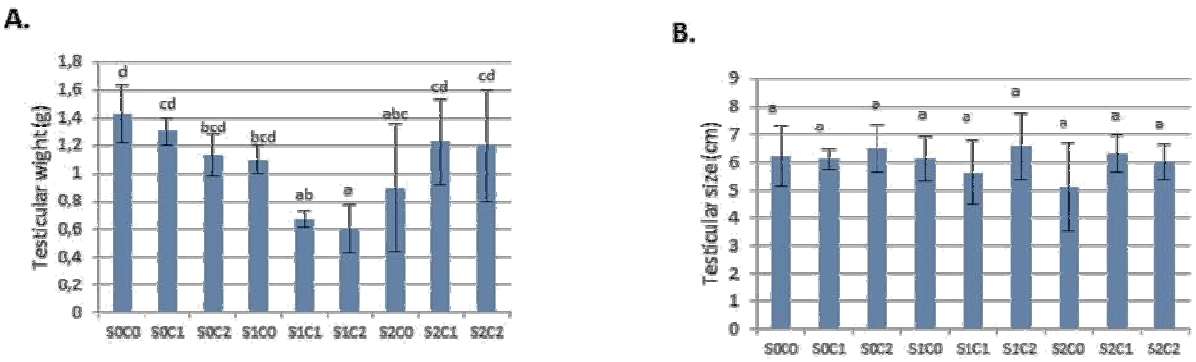

FIGURE 2. Effect of salinity and Cd level on testicular weight (A) and size (B). S_0C_0 ($0^0/_{00}$: 0 ppm), S_0C_1 ($0^0/_{00}$: 2,5 ppm), S_0C_2 ($0^0/_{00}$: 5 ppm), S_1C_0 ($5^0/_{00}$: 0 ppm), S_1C_1 ($5^0/_{00}$: 2,5 ppm), S_1C_2 ($5^0/_{00}$: 5 ppm), S_2C_0 ($10^0/_{00}$: 0 ppm), S_2C_1 ($10^0/_{00}$: 2,5 ppm), S_2C_2 ($10^0/_{00}$: 5 ppm)

Cadmium was one of chemicals able to cause endocrine disruption of metabolic and reproductive neuroendocrine system. Salinity was also able to affect toxicity level of heavy metal on water towards fish. Decrease of salinity due to desalination process would increase toxicity of heavy metal, therefore bioaccumulation level of heavy metal was also elevated.

Testicle collected from the fish was then processed into histological slides. Testicle histological structure of *Oreochromis niloticus* was presented in Figure 3.

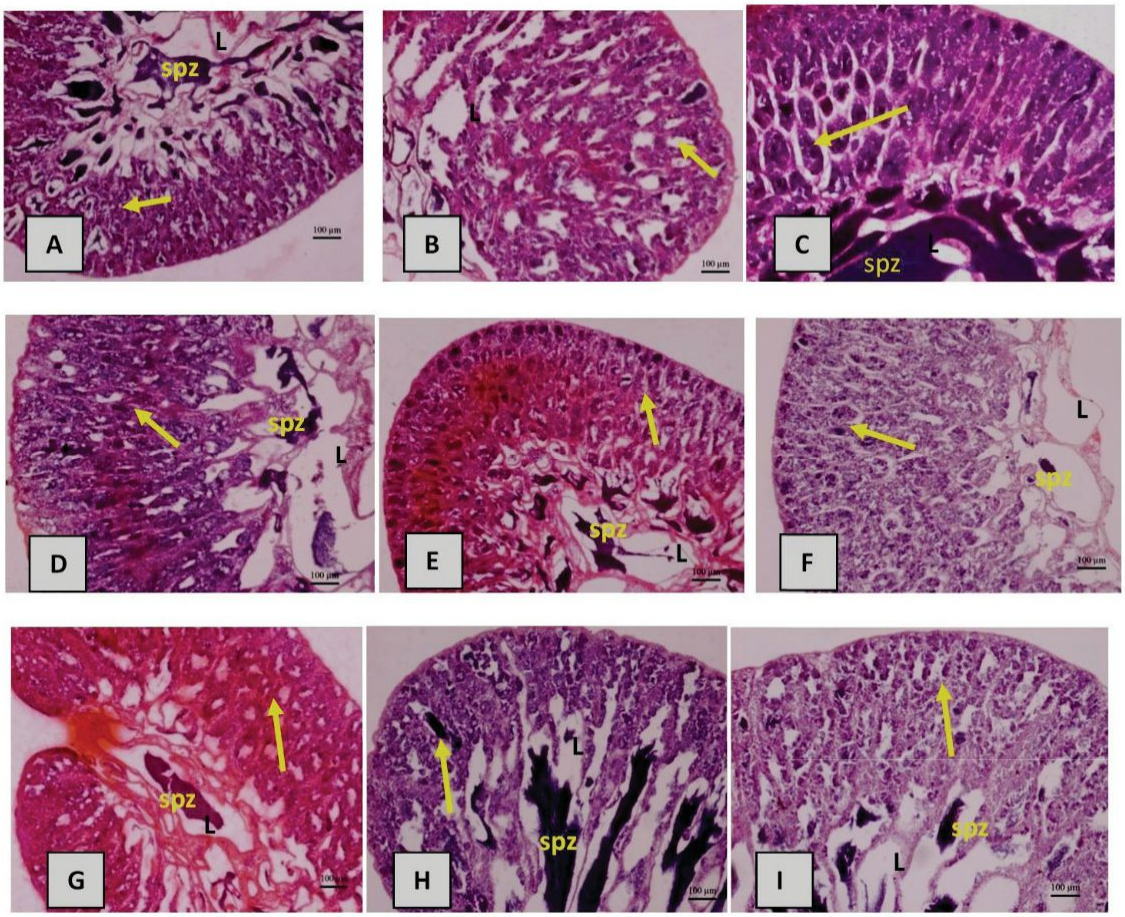

FIGURE 3. Testicular structure of *Oreochromis niloticus* treated with various salinity and Cd level. $0^0/_{00}$, 0 ppm (A); $0^0/_{00}$, 2,5 ppm (B); $0^0/_{00}$, 5 ppm (C); $5^0/_{00}$, 0 ppm (D); $5^0/_{00}$, 2,5 ppm (E); $5^0/_{00}$, 5 ppm (F); $10^0/_{00}$, 0 ppm (G); $10^0/_{00}$, 2,5 ppm (H); $10^0/_{00}$,

5 ppm (I); spz: spermatozoa; L: lumen; seminiferous tubules (yellow arrow)

Spermatogenic cell structure and diameter of seminiferous tubules testicular was found to be altered when treated with various salinity and Cd level. Figure 3 and 4 showed that the size of seminiferous tubule's diameter was found to be rising along with increasing salinity and Cd level.

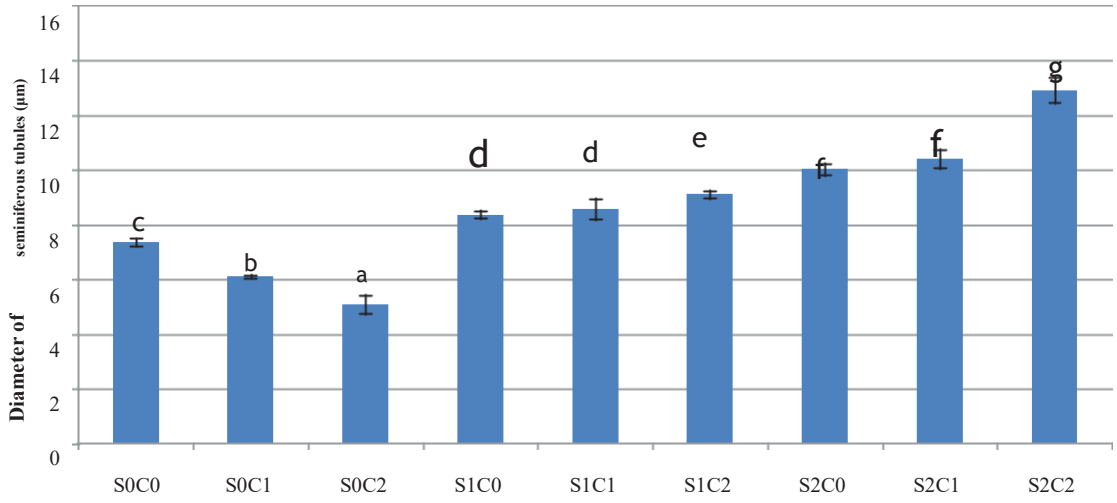

FIGURE 4. Diameter of seminiferous tubules *Oreochromis niloticus* treated with various salinity and Cd level. S_0C_0 ($0^0/_{00}$: 0 ppm); S_0C_1 ($0^0/_{00}$: 2,5 ppm); S_0C_2 ($0^0/_{00}$: 5 ppm); S_1C_0 ($5^0/_{00}$: 0 ppm); S_1C_1 ($5^0/_{00}$: 2,5 ppm); S_1C_2 ($5^0/_{00}$: 5 ppm); S_2C_0 ($10^0/_{00}$: 0 ppm); S_2C_1 ($10^0/_{00}$: 2,5 ppm); S_2C_2 ($10^0/_{00}$: 5 ppm)

Increasing diameter of seminiferous tubule was shown in Figure 4. Fish from S_2C_2 had the biggest diameter compared to control (S_0C_0) and other group. It was shown that on S $_2C_2$, there was significant increase on spermatogenesis process. Increasing number of spermatogenesis process in the seminiferous tubules could increase tubule diameter. On the other hand, when testicular spermatogenesis was inhibited, seminiferous tubules diameter was reduced. Tubules diameter from S_0C_0 (control), S_0C_1 and S_0C_2 group had respectively found to be reduced, because Cd accumulated in testicle would reduce the number of gamete cell and affect metabolism of cell undergoing spermatogenesis (Gage *et al.*, 2004). Effect caused by Cd toxicity including physical damage (degeneration, necrosis) and physiological disorder (disruption of enzyme function and cell metabolism). Furthermore, as found from S_0C_2 group, Cd toxicity level was higher on 0 $^0/_{00}$ salinity. Low salinity means the number of Cd bonded to ion was also low, thus the level of toxic Cd^{2+} was higher.

Tubules from fish given S_1C_0, S_1C_1 and S_1C_2 treatments had larger diameter. Although the diameter appeared to be larger, there was no significant different found from fish given S_1C_0 and S_1C_1 treatments. It was likely that $5^0/_{00}$ salinity combined with 2.5 ppm Cd caused no damage towards testicle, in which result was found to be similar as those treated with various salinity without Cd addition. This tendency could also observed from S_2C_0 and S_2C_1. Meanwhile, in groups treated with different salinity level without additional Cd (S_0C_0, S_1C_0, and S_2C_0), it resulted on significantly different and larger tubular diameter. Treatment with 10 $^0/_{00}$ salinity had smallest osmotic gradient, inidicated that on 10 $^0/_{00}$ salinity level, energy required for osmoregulation process was less than on 0 $^0/_{00}$ salinity level. Thus, more energy was saved for growth and immunity. This energy was then later used for immunity so fish testicular health was improved.

Fish treated with 2.5 ppm Cd at various salinity level (S_0C_1, S_1C_1, and S_2C_1) showed larger tubular diameter. Fish from S_2C_1 treatment had highest tubular diameter because the number of salinity ions was higher compared to other group. Salinity ions (Cl^-) in water was able to decrease Cd toxicity level because Cd $^{2+}$ bonded with salinity ions, causing formation of more stable bond and lower its toxicity level. Result also showed that fish treated with 5 ppm Cd at different salinity levels (S_0C_2, S_1C_2, and S_2C_2 respectively) also had larger seminiferous tubular diameter.

The structure of kidney cell constituent of *Oreochromis niloticus*

The effect of Cd on the constituent cells structure of *Oreochromis niloticus* kidney at different salinity level was presented at Figure 5.

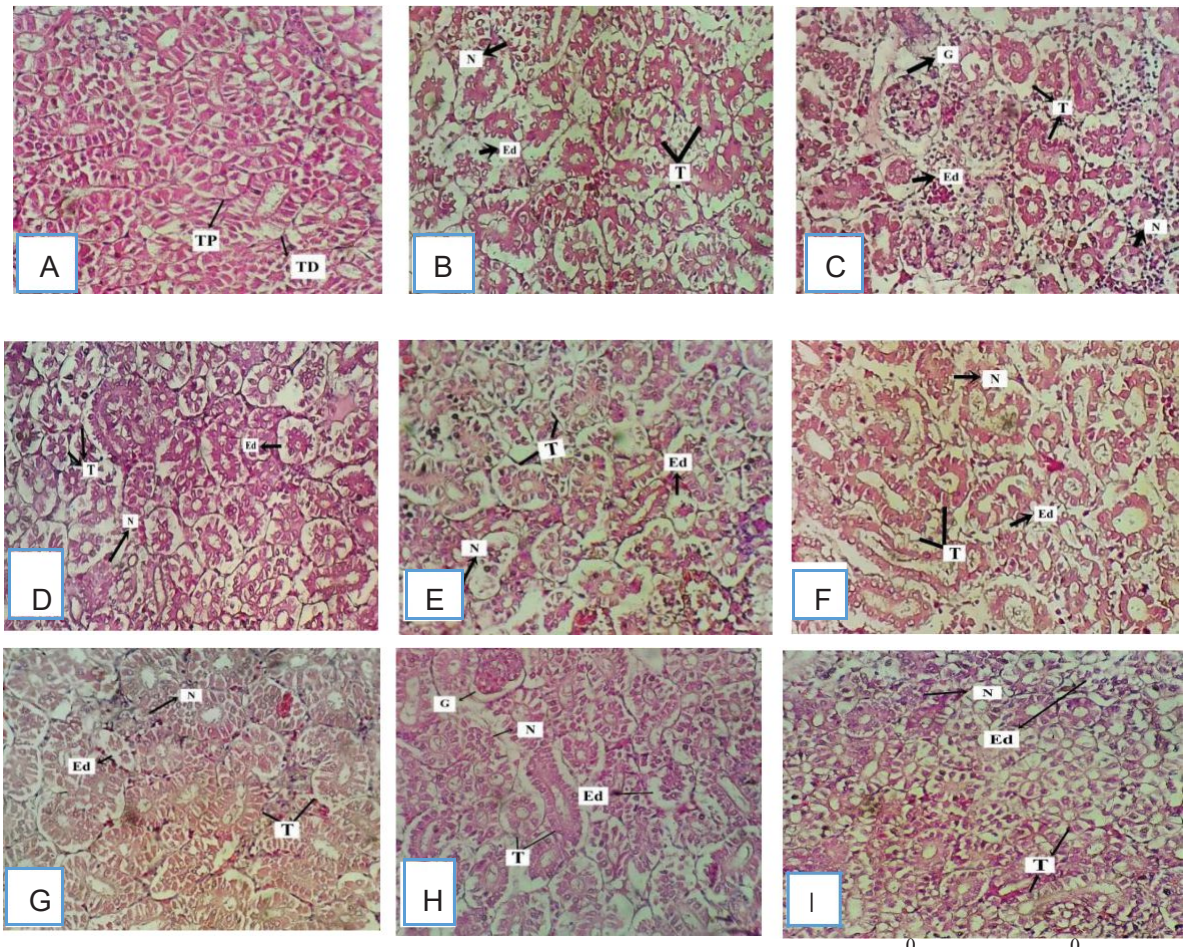

Figure 5. The structure of kidney *Oreochromis niloticus* treated with various level of salinity and Cd. $0\,^{0}/_{00}$, 0 ppm (A); $0\,^{0}/_{00}$, 2,5 ppm (B); $0\,^{0}/_{00}$, 5 ppm (C); $5\,^{0}/_{00}$, 0 ppm (D); $5\,^{0}/_{00}$, 2,5 ppm (E); $5\,^{0}/_{00}$, 5 ppm (F); $10\,^{0}/_{00}$, 0 ppm (G); $10\,^{0}/_{00}$, 2,5 ppm (H); $10\,^{0}/_{00}$, 5 ppm (I); Tubules proximal (TP), Tubules distal (TD), Necrosis (N), Edema (Ed), glomelures (G). HE, 40x100

Kidney cell was shown to be swollen and undergone necrosis (Figure 5) as indicated by changes in cell nucleus and cytoplasm, in addition of several lysogenic tubules. Intertubular space was also appeared wider due to the loosening of tubule basement membrane.

On salinity $0\,^{0}/_{00}$ and 0 ppm of Cd, the damage on kidney tubule was found not as significant as kidney given 2,5 and 5 ppm of Cd, which clearly showed damage on tubules constituent cells. Increasing salinity level (5 and $10\,^{0}/_{00}$) was also found to cause cell damage. Great damage was shown on fish treated with 5 ppm Cd. Changes of salinity level in aquatic condition was found to affect heavy metal's toxicity (Cd). Abdel dan Wafeek (2008) stated that salinity level was inversely proportional to heavy metal's toxicity, if salinity level was reduced, it would consequently elevate toxicity level, as heavy metal became increasingly difficult to be decomposed and more toxic to aquatic organism.

The damage observed on kidney tubule cells was presented in the following table.

Table 1 Kidney cell damage evaluation on *Oreochromis niloticus* after exposure to various salinity level (S) and Cadmium (C)

Treatment	Kidney Cell	
	Necrosis (%)	Edema (%)
S_0C_0	$7,82 \pm 3,47^{a}$	$4,87 \pm 0,25^{a}$
S_0C_1	$39,94 \pm 8,75^{g}$	$26,65 \pm 7,20^{h}$
S_0C_2	$41,63 \pm 4,33^{h}$	$30,47 \pm 3,62^{i}$
S_1C_0	$17,54 \pm 5,37^{b}$	$10,40 \pm 2,87^{b}$
S_1C_1	$28,81 \pm 6,31^{f}$	$17,57 \pm 4,23^{f}$
S_1C_2	$29,08 \pm 5,09^{f}$	$19,25 \pm 6,13^{g}$
S_2C_0	$18,60 \pm 4,47^{c}$	$11,59 \pm 4,77^{c}$
S_2C_1	$21,18 \pm 3,72^{d}$	$13,32 \pm 4,50^{d}$
S_2C_2	$25,18 \pm 6,96^{e}$	$15,43 \pm 7,12^{e}$

Note :
S_0C_0 ($0\ ^{0}/_{00}$, 0 ppm), S_0C_1 ($0\ ^{0}/_{00}$, 2,5 ppm), S_0C_2 ($0\ ^{0}/_{00}$, 5 ppm), S_1C_0 ($5\ ^{0}/_{00}$, 0 ppm), S_1C_1 ($5\ ^{0}/_{00}$, 2,5 ppm), S_1C_2 ($5\ ^{0}/_{00}$, 5 ppm), S_2C_0 ($10\ ^{0}/_{00}$, 0 ppm), S_2C_1 ($10\ ^{0}/_{00}$, 2,5 ppm), dan S_2C_2 ($10\ ^{0}/_{00}$, 5 ppm). Note: diferent letters indicated significant difference

On similar level of salinity, damage on the fish kidney cells was found to be elevated along with the increase of Cd concentration. Meanwhile, given similar Cd Concentration, impairment of kidney cells was also found to be increased as water salinity also rising. However, Cd concentration was inversely proportional to salinity level, because higher salinity was able to affect toxicity caused by Cd. Thus, higher salinity level in the water would caused Cd toxicity to lower (Table 1).

DISCUSSION

Salinity described total ion concentration contained in the organic or inorganic water. Sea water salinity was found to be caused by 7 main ions i.e. Sodium (Na^{+}), Potassium (K^{+}), Calcium (Ca^{2+}), Chlorida (Cl^{-}), Sulphate (SO_4^{2-}), and Bicarbonate (HCO_3^{-}) (Effendi, 2003). Naturally, heavy metals dissolved in water was in the form of free ions, pairs of organic ions, organic and inorganic complexes (Connell & Miller, 2006). Cationin Cd dissolved in sea water normally would interact with previously existing anions (Cl^{-}, $S04^{2-}$, HCO_3^{-}) to form inorganic or organic complexes which would reduce free Cd ions. In low salinity, concentration of free Cd^{2+} ions would increase, as the water contained less anion to form complexes (Yudiati, 2009). It was likely that this was the main cause of hugely increasing Cd toxicity level in low salinity water. According to report from Mance (1990), salinity level determined the toxicity level of heavy metals. The decrease in salinity level was found to increase heavy metal toxicity in the water (Sullivan, 2000).

Reproductive system of fish was mainly controlled by *Hypothalamic – pituitary – Gonad – Liver* (HPGL) axis (Hachfi *et al*., 2012). Cadmium was endocrine-disrupting metal able to affect homeostasis, reproduction, and also impair the function of HPGL axis. Moreover, it was also able to disrupt hormone synthesis and damage plasma protein binding (Hachfi *et al*., 2012). Cadmium entering the hypothalamus could possibly increase serotonin release in posterior part and lower neurotransmitter level in both anterior and mediobasal part. Cadmium was found to compete with acetylcholine to bind acetylcholinesterase enzyme. Cadmium was also found to inhibit acetylcholine and cause vasoconstriction. Long term vasoconstriction would give rise to poor blood circulation and keep oxygen and nutrition from supplying to cells, thus it would impede fish metabolism. Less supply of oxygen and nutrition in reproduction organs would eventually hamper spermatogenesis process (Abdelhamid, 2013).

The damage in kidney's constituent cells caused by Cd and salinity was found on various level according to Cd concentration and water salinity of each treatment. From treatment given, necrosis and edema was found on the kidney tissue evaluated. Necrosis was indicated by condition in where tissue activity was ceasing and several parts of the cell from a tissue was missing, leading to cell death. Death of cells and tissues following cell degeneration was the end of irreversible degeneration (Almeida, *et al* 2009).

Damaged kidney occurred because cell constituent of kidney tissue was unable to divide completely or division process was delayed due to radiation exposure. Based histological evaluation on fish kidney, the effect of Cd on tilapia fish kidney could be toxic. Fish from S0C2 treatent given 0 concentration of salinity and 5 ppm of Cd showed serious damage such as necrosis (cell death) and edema mainly found in epithelial of proximal convoluted tubules. Edema was observed from narrowing tubules lumen resuted from epithelials outgrow as space between each tubules grew larger. This condition occurred because of increasing cell permeability in the kidney enabled potassium to diffuse into the cells following water osmosis. Less damage was observed from fish treated with 10 salinity and 5 ppm Cd, possibly because low salinity in water leading to elevated Cd toxicity. Except for both groups, necrosis and edema was observed from other groups treated with various Cd and salinity level. Cadmium as toxic compound entered fish body into kidney through blood flow and body surface. Toxic compounds mainly entered the body by forming metal ions which could be dissolved in fat. Those metal ions was able to penetrate cell membrane and accumulated inside the cells of various organ. Unlike Cd^{2+} ion, saline ion such as N+ and Cl- was non toxic. Non-toxic saline ions in the water, Cl- would bind to toxic Cd^{2+}, form CdCl2, and cause toxicity level of metal ion to increase (Portier, 2012).

Toxicant in the kidney could affect activity of various biological enzymes. Toxicant was found to be able to bind enzymes, due to heavy metals tended to be able to exchange its metal group with other metals functioning as enzyme co-factor. The bonds between enzyme substrates and heavy metals would cause various imbalance on the physiological system. These imbalances would initiate disease manifestation of toxicant poisoning. The cell tended to maintain its various environment and intercelular factor within narrow range of physiological parameters in order to maintain its normal homeostatis. When cells undergone physiological stress, cell was able to adapt for reaching different homeostasis suitable for its current condition and maintain its viability. But if the cell adapted excessively, lesion would happen to the cell. Within certain period of time, lesion was reversible and cell would be able to return to its normal condition, such as the case with swollen cell. If stress endured by the cell was too severe, irreversible lesion would then happened and cell would eventually undergone necrosis.

CONCLUSION

Various concentration of Cd at different salinity levels affected testicle weight and structure, and also kidney cells constituent of *Oreochromis niloticus,* however those concentrations did not affected testicular length. Lowest weight and smallest diameter of the testicular tubule and highest kidney cells impairment was found from fishes treated with $0^0/_{00}$ salinity and 5 ppm Cd.

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