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Full Length Research Paper

Effect of lead on gill and liver of blue spotted ray (*Dasyatis kuhlii)*

Joeharnani Tresnati* and Iqbal Djawad

Fisheries Department, Faculty of Marine Science and Fisheries, Hasanuddin University.
Jalan Perintis Kemerdekaan Km 10, Makassar 90245, Indonesia.

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The aim of study was to analyze the effect of lead nitrate $Pb(NO_3)_2$ on gill and liver of blue spotted ray *Dasyatis kuhlii* using a histological observation. The rays were placed in five tanks filled by seawater with three fishes for each tank. Blue spotted rays, *Dasyatis kuhlii* were treated for 12 days by using different concentrations of lead nitrate ($Pb(NO_3)_2$), that is, 0 ppm as control (treatment A), 0.2 ppm (treatment B), 0.5 (treatment C), 0.9 ppm (treatment D) and 1.8 ppm (treatment E). The rays were placed in the tanks until all fishes exposed to Pb (treatments B to E) were dead. Results of the study show that $Pb(NO_3)_2$ exposure altered the gill and liver microscopic structure. Gill lamella alterations were found on hypertrophy, hyperplasia, club shaped, necrosis and formed bunch texture. Liver alterations included cloudy swelling, atrophy, necrosis, vacuolar degeneration and fatty degeneration. Death time of blue spotted ray became shorter when lead nitrate $Pb(NO_3)_2$ concentration increased.

Key words: Histological observation, seawater, lead nitrate.

INTRODUCTION

Intensive progresses of human activities in marine and coastal areas increase waste volume in waters. It could cause many pollution problems. Pollutants could enter into fish through various routes, that is, skin, gills, oral consumption of water, food and non-food particles. Blood stream transports pollutant absorbed to either a storage point, that is, bone or liver. In the liver, pollutants could be stored there or excreted in bile or passed back to blood for possible excretion via gills or kidneys or stored in fat (Heath, 1991). Aquatic organisms could accumulate pollutants such as metals and organic compounds from water (WHO, 1985; Obasohan, 2008). The metal accumulation in organism body could take place, if rate of uptake by organism exceeds rate of elimination (Oronsaye, 1987; Oguzie, 2003). As fish constitute an important link in food chain, its contamination by toxic metals could cause a direct threat to humans that consumed it as food; some metal accumulation levels exceeded WHO and PEPA recommended limits in food fish, might not be fit for human consumption (Obasohan, 2008).

Some heavy metal substances that found in the waste are highly poisonous toxicants, that is, lead (Pb). Pb is a toxic and dangerous pollutant for marine organism. Some micro algae, that is, *Chlorella* and *Dunaliella*, were significant factor responsible to bioaccumulation of Pb. *Chlorella* and *Dunaliella* are great absorption and have a high tolerance level to Pb (Muhaemin, 2004). The Pb that have been bioaccumulation by micro algae could transfer to next food chain level, such as fish and shellfish. The Pb was found in muscular of commercial fish in marine systems. The certain values of muscular Cd, Hg, Pb and Zn were however above thresholds for human consumption (Kojadinovic et al., 2007).

Pb in the marine waters could come from fuel residue, industry, and mine. The accumulation of Pb in organism tissue could alterate some fish essential organ, that is, gill and liver. Vinodhini and Narayanan (2008) revealed that bioaccumulation of Pb in the gill and liver tissues of *Cyprinus carpio* were statistically significant. Pb bioaccumulation in the gill and liver was more important than Cd, Ni and Cr, but it is very poisonous for the fish. Javid et al. (2007) revealed that bioaccumulation in the

*Corresponding author. E-mail: jtresnati@yahoo.com. Tel: +62-8124250631. Fax: +62-411-852825.

Figure 1. Morphological and anatomical structures of Blue Spotted Ray *Dasyatis kuhlii*. 1) Gill; 2) Liver.

bodies of three fish species *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* was performed during 96 h LC_{50} exposure.

Blue spotted ray, *Dasyatis kuhlii*, is an economic fish and abundance in the marine and coastal area. It is a demersal fish that has a biological adaptation form to live in the bottom side of sea. The ray's mouth and gill fissure are located at underside of the body (Hoeve, 1992). Its mouth and gill position could increase the contamination risk because pollutant that highly concentrated in seabed could come easily to ray's respiratory and digestion organ, and then go through to excretion organ.

Pb could disturb physiological function of the gill. The ray's gill is a very important respiration and osmoregulation organ. Gill is a sensitive organ concerning metal toxicity. Pb could cause biochemistry interference. It could cause failure of gill anatomical structure, that is, epithelium thickened, secondary lamella thickened, hypertrophy, hyperplasia and necrosis (Darmono, 2001). Pb could also intrude enzyme system in fish liver (Darmono, 1995). The fish liver synthesizes some digestion substance, that is, bile salt. The enzyme system interference could decrease the bile salt production that may have consequences, such as decrease of fat absorption and vitamins that dissolved in fat. The serious physiological malfunction could be appearing when Pb concentration increase in water. Therefore, the objective of study was to analyze the effect of various Pb concentrations on two important organ, gill and liver, of blue spotted ray, *D. kuhlii* using a histological observation.

MATERIALS AND METHODS

The total length and weight of blue spotted ray used were from 39 to 65 cm and from 230 to 700 g, respectively. Blue spotted rays, *D. kuhlii*, were treated for 12 days by using different concentrations of Plumb Nitrate ($Pb(NO_3)_2$), that is, 0 ppm as control (treatment A), 0.2 ppm (treatment B), 0.5 (treatment C), 0.9 ppm (treatment D), 1.8 ppm (treatment E). The rays were placed in the tanks until all fishes exposed by Pb (treatment B-E) have been dead. Gill and liver specimens (Figure 1) for histological preparate were conserved and fixed by using Bouins' Solution, haematoxylin and eosin according to Carson (1990).

The study was a descriptive analysis by using a histological observation to determine the gill and liver alterations. The Alterations were determined by using histological features of Takashima and Hibiya (1995). The Gill tissue alterations could be hypertrophy, distal hyperplasia, club shaped, and necrosis. Whereas, the tissue liver alterations could be cloudy swelling, atrophy, necrosis, vacuolar degeneration, and fatty degeneration (Takashima and Hibiya, 1995).

RESULTS AND DISCUSSION

Gill

The study reveals that $Pb(NO_3)_2$ is an important alterations on gill of blue spotted ray. The Gill tissue at normal condition, without $Pb(NO_3)_2$ treatment, presented some normal organelles, that is, primary lamellae (PL), secondary lamellae (SL), blood cell in capillary lumen, pillar cell (PC) and cell inters lamellae (CIL) (Figure 2A). The normal gill tissue did not present epithelium thickening. Treatment of 0.2 ppm $Pb(NO_3)_2$ caused gill hypertrophy (HT) at epithelium of secondary lamellae (Figure 2B). The gill hypertrophy signed by the increase of cell or tissue size due to thickened of lamellae epithelium. The Epithelium hypertrophy of gill tissue could obstruct the binding of gazes and ions during respiration process that could decrease gazes and ions diffusion into the lamellae. $Pb(NO_3)_2$ could also increase mucus. The mucus secreted more by the gill as effort to protect the gill from strange substance as Pb. As mucus is not soluble enough in water, it could block ions and oxygen diffusion in gill surface (Sorensen, 1991).

Treatment 0.5 ppm of $Pb(NO_3)_2$ caused the gill alteration at secondary lamellae. Lamelle tissue presented hyperplasia at periphery (distal hyperplasia, DH) (Figure 2C). Hyperplasia was indicated by swelling at secondary lamellae caused by increasing the number of abnormal cells. Hyperplasia could plug capillary lumen that has functioned as blood tract that contain erythrocyte. Hyperplasia could disturb the ions and gazes

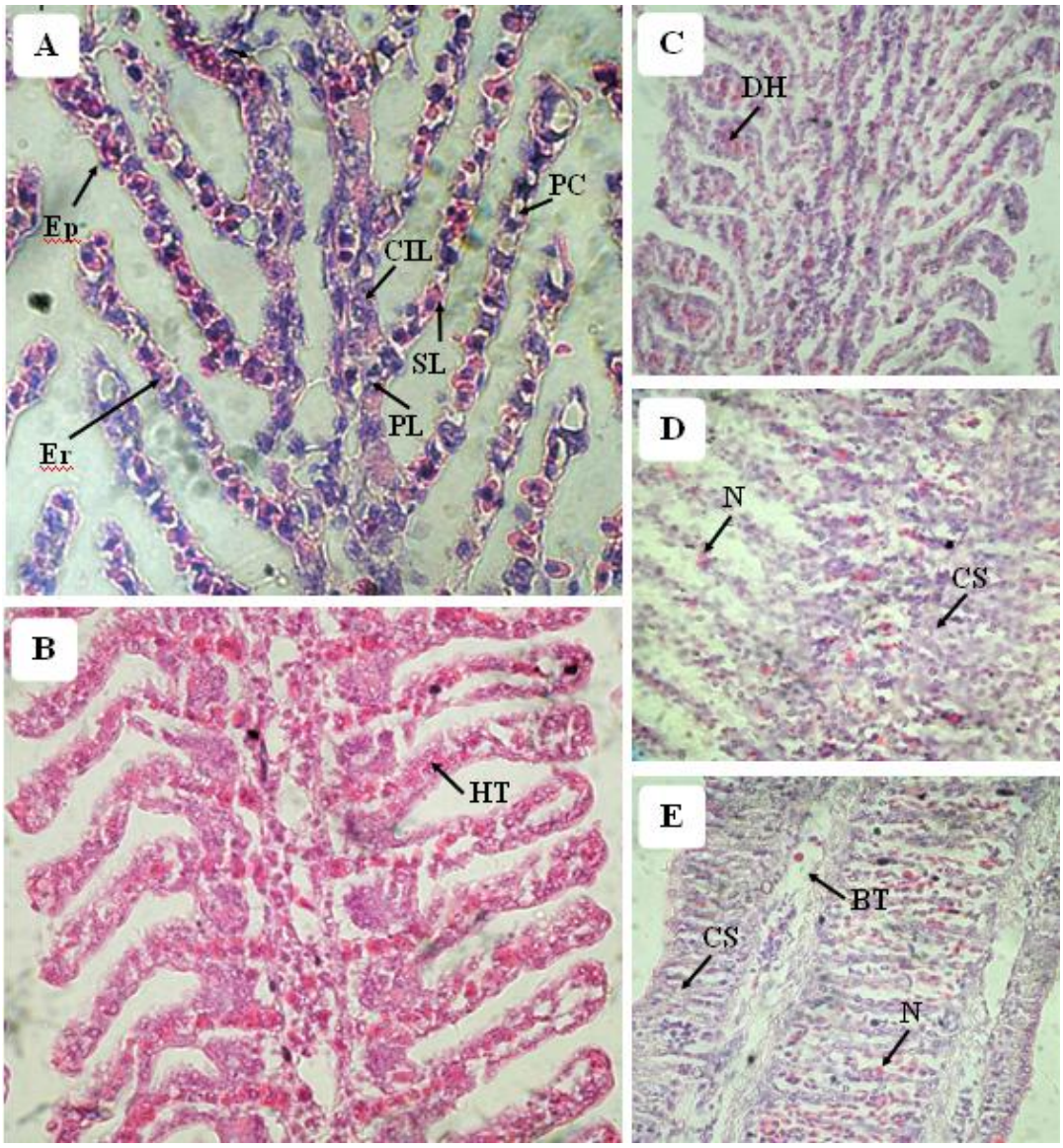

Figure 2. Gill histological structure of blue spotted ray *Dasyatis kuhlii* after exposure at different concentrations of $Pb(NO_3)_2$. A, Control; B, 0.2 ppm; C, 0.5 ppm; D, 0.9 ppm; E, 1.8 ppm. CIL, Cell inters lamellae; CS, cloudy swelling/club-shaped; DH, distal hyperplasia; Ep, epithellium; Er, erythrocyte; HT, hypertrophy; BT, bunch tissue N, necrocis; PC, pillar cell; PL, primary lamellae; SL, secondary lamellae.

absorption by gill due to capillary lumen size become tight, and cell in centre of lamellae move to periphery at secondary lamellae (Heath, 1987).

Treatment 0.9 ppm of $Pb(NO_3)_2$ caused serious gill alteration indicated by fusion of several secondary lamellae (club-shaped, CS) (Figure 2D). It was probably caused by excessive mucus production. Fissure inter-secondary lamellae are closed due to lamellae stuck each other that could block water passage into the gill. This condition could extremely disturbed osmoregulation. Djawad (1998) revealed that excessive mucus production causes respiration difficulties due to increase the distance of oxygen diffusion around lamella surface. Treatment 0.9 ppm of $Pb(NO_3)_2$ caused pillar cell necrosis (CN), disappear of capillary lumen at secondary lamellae, and autolysis of some tissue. Takashima and Hibiya (1995) revealed that necrosis was signed by disappear of some parts in lamellae, as pillar cell. It could indicate that gill function have already decreased due to the death of lamellae tissue.

Treatment 1.8 ppm of $Pb(NO_3)_2$ caused more serious gill alterations indicated by presence of club-shaped (CS) and bunch tissue (BT) at primary lamellae (Figure 2E). The club-shaped formed due to excessive mucus production and swelling and increase of cell hyperplasia at all secondary lamellae. It could indicate that gill function is already failed. This study reveals that treatment 0.2 ppm of $Pb(NO_3)_2$ caused hypertrophy, 0.5 ppm caused

Table 1. Gill alteration condition of blue spotted Ray (*Dasyatis kuhlii*) after treatment of different $Pb(NO_3)_2$ concentrations.

Tissue alteration	$Pb(NO_3)_2$ concentration				
	Control	0.2 ppm	0.5 ppm	0.9 ppm	1.8 ppm
Hypertrophy	-	+	-	-	-
Distal hyperplasia	-	-	++	-	-
Club shaped	-	-	-	+++	++++
Necrosis	-	-	-	+++	++++

-, No alteration; +, light alteration; ++, moderate alteration; +++, serious alteration; ++++, very serious alteration.

distal hypertrophy, and 0.9 and 1.8 ppm caused club shape and necrosis, respectively at gill tissue of blue spotted ray *D. kuhlii* (Table 1).

Liver

This study reveals that $Pb(NO_3)_2$ have important influence on liver of blue spotted ray. The liver tissue at normal condition, without $Pb(NO_3)_2$ treatment, presented some normal organelles, that is, hepatocyte (Hp), sinusoid (Sn) and nucleus (Nc) (Figure 3A). Treatment of 0.2 ppm $Pb(NO_3)_2$ caused alteration of liver cell. The nucleus swells or become bigger, cloudy or misty cytoplasm, and formation of smooth granules (Figure 3B) that absorb eosin color at its cytoplasm (cloudy swelling, CS). It due to effect of $Pb(NO_3)_2$ exposure on liver enzymatic process. $Pb(NO_3)_2$ in liver could change hepatocyte form, and then cause liver alteration, even in the low concentration of $Pb(NO_3)_2$. Pb is very poisonous at cell metabolism because it could reach with protein, sulfur and nitrogen. Contamination of cytoplasm by Pb could disturb bimolecular process and decrease ability of detoxification process. Pb could also obstruct enzymatic function (Darmono, 1995).

Treatment of 0.5 ppm $Pb(NO_3)_2$ caused atrophy (At) and necrosis (N) of hepatocyte (Figure 3C). At atrophy hepatocyte, cell border was indistinguishable, and eosin did not quietly absorbed by cytoplasm. Liver tissue atrophy due to Pb could alterate erythrocyte, and block hemoglobin synthesis, by consequence, nutrition supplies to liver cell could not function normally. By the time, liver size decreased, sinusoid disappears slowly due to cell constriction. Takashima and Hibiya (1995) revealed that atrophy is an abnormal condition where cell volume lost is normal condition, outline cell is indistinguishable, nucleus become smaller and totally disappear due to cell death. Cell death (necrosis) is an advanced effect of cell alterate due to atrophy cell. Necrosis signed by the color change and decreased of cell size. Cytoplasm was dominated by pink, and nucleus was bluish. Necrosis in liver cell could be due to obstruction of sinusoid that normally transports blood to hepatocyte. Blockage of blood current to hepatocyte could decrease cell nutrition supply. Hudson et al. (1984) revealed that liver metabolism is strongly related to blood supply; and hepatocyte is the first organ that receives blood.

Treatment of 0.9 ppm $Pb(NO_3)_2$ caused alteration at liver tissue, that is, vacuolar degeneration (FD) (Figure 3D) that could have negative effect on enzyme activity in liver tissue. Pb is a toxic inhibitor and could block metabolism enzyme function, and alterate protein synthesis. Takashima and Hibiya (1995) revealed that vacuolar degeneration is due to protein deficiencies at nucleus protein. Treatment of 1.8 ppm $Pb(NO_3)_2$ caused alteration of lever tissue, that is, fatty degeneration (FD) which histological appearance is in the form of empty vacuoles at most part of tissue (Figure 3E). That alteration could cause liver disfunction. Fat is fish energy principal source to maintain the structure and function of cell membrane. The fat also functioned as saving place of soluble vitamins in fat, that is, vitamins A, D, E, and K. These vitamins are useful as antioxidant, growth, and tissue regeneration. Pb could disturb fat metabolism process and could cause deficit of soluble vitamins in fat. Steven et al. (2002) revealed that fat degeneration in liver tissue could disturb metabolism due to energy deficiency. This study revealed that treatment 0.2 ppm of $Pb(NO_3)_2$ caused cloudy swelling, 0.5 ppm of $Pb(NO_3)_2$ caused atrophy and necrosis, 0.9 ppm of $Pb(NO_3)_2$ caused vacuolar degeneration and 1.8 ppm of $Pb(NO_3)_2$ caused fatty degeneration at liver of blue spotted ray *D. kuhlii* (Table 2).

Death time

Death time of blue spotted ray *D. kuhlii* during $Pb(NO_3)_2$ exposures was dependent on concentration. Fish died after exposure 208 h at concentration 0.2 ppm of $Pb(NO_3)_2$; 179 h at 0.5 ppm; 84 h at 0.9 ppm; and 43 h at 1.8 ppm (Figure 4). These results indicate that death time blue spotted ray *D. kuhlii* become shorter when $Pb(NO_3)_2$ concentration increase. Gravity of the high concentration of Pb could be related to dissolve oxygen in water, and increase of oxygen consumption. Javid et al. (2007) revealed that increasing lead concentrations in water decreased dissolved oxygen in water; and oxygen requirements of fish increased with concomitant increase in metal concentrations.

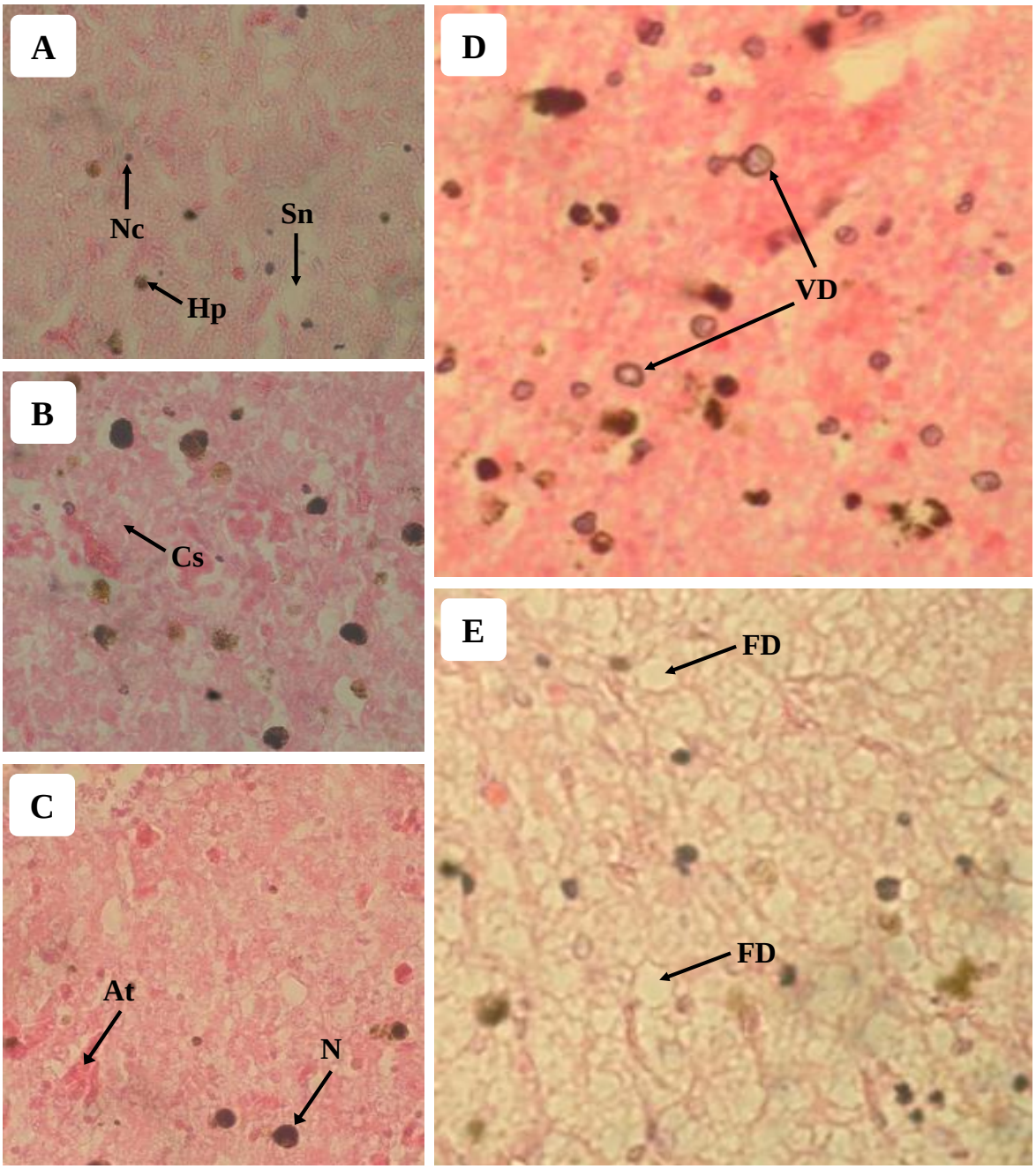

Figure 3. Liver histological structure of blue spotted ray *Dasyatis kuhlii* after exposure at different concentrations of $Pb(NO_3)_2$. A, control; B, 0.2 ppm; C, 0.5 ppm; D, 0.9 ppm; E, 1.8 ppm. Nc, Nucleus; Hp, hepatocyte; Sn, sinusoid; Cs, cloudy swelling; At, atrophy; N, necrosis; VD, vacuolar degeneration; FD, fatty degeneration.

In summary, gill alterations due to $Pb(NO_3)_2$ exposures were hypertrophy, hyperplasia, club-shaped, necrosis and bunch tissue. While liver alterations were cloudy swelling, atrophy, necrosis, vacuolar degeneration and fatty degeneration. Death time became shorter when $Pb(NO_3)_2$ concentrations increased. Blue spotted ray *D. kuhlii* died after exposure 208 h at concentration 0.2 ppm of $Pb(NO_3)_2$; 179 h at 0.5 ppm; 84 h at 0.9 ppm; and 43 h at 1.8 ppm.

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Table 2. Liver alteration condition of Blue Spotted Ray (*Dasyatis kuhlii*) after treatment of different concentrations of $Pb(NO_3)_2$.

Tissue alteration	$Pb(NO_3)_2$ concentration				
	Control	0.2 ppm	0.5 ppm	0.9 ppm	1.8 ppm
Cloudy swelling	-	+	-	-	-
Atrophy	-	-	++	-	-
Necrosis	-	-	++	-	-
Vacuolar degeneration	-	-	-	+++	
Fatty degeneration	-	-	-	-	++++

-, No alteration; +, light alteration; ++, moderate alteration; +++, serious alteration; ++++, very serious alteration.

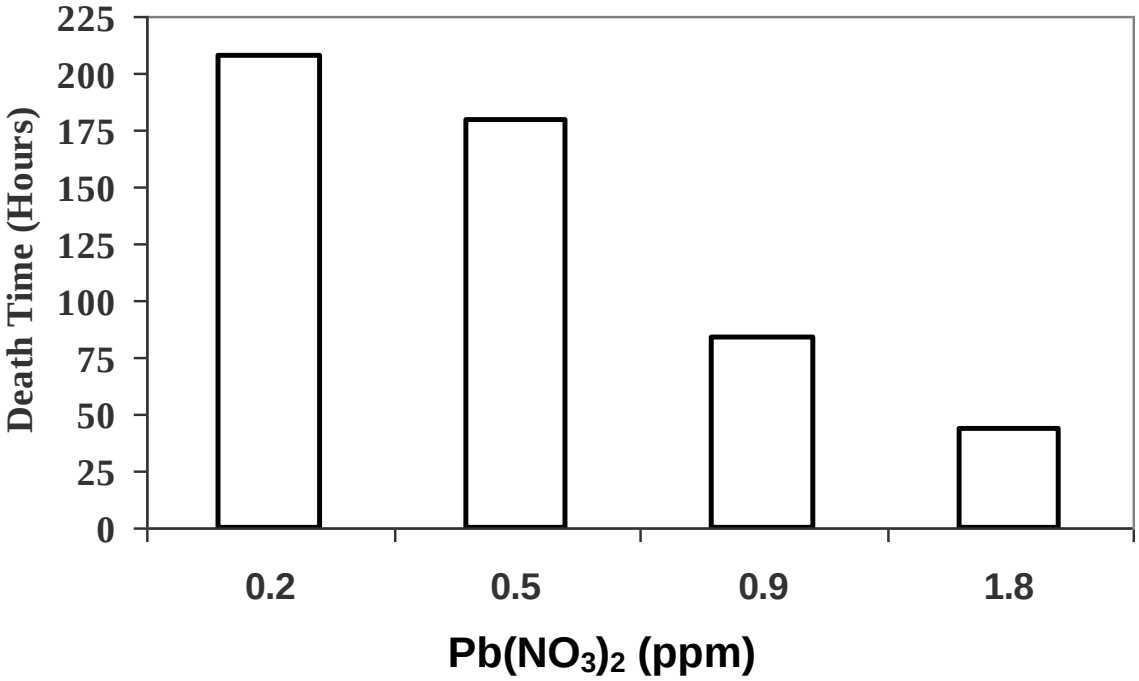

Figure 4. Death time of blue spotted ray *Dasyatis kuhlii* during $Pb(NO_3)_2$ exposure.

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