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Histopathology and recovery of Nile tilapia after exposure to malathion insecticide used for the control of *Aedes aegypti*

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Abstract. Malathion is an organophosphate insectide that can intoxicate tilapia fish besides of causing economic losses in the tilapiculture sector due to mortaliy. Therefore, this study aimed to evaluate histological alterations and the recover of gills, liver and kidney of tilapia after acute exposure to diferent concentrations of malathion. The malathion caused in the gills hyperplasia, lamelar fusion, congestion of the venous sinus and lamelar disarrangement. In the liver was found congestion of the sinusoids, nuclear displacement, steatosis and cordonal disarrangement. In the kidney were observed vacuolization of the renal tubule cells, inflammatory infiltrate and increased Bowman's space. After 30 days, gills, liver and kidney recovered from alterations caused by malathion at concentrations of up to 5.0 mg L^{-1}. There were no significant changes ($p > 0.05$) in water in concentrations of malathion ranging from 3.0 mg L^{-1} to 9.0 mg L^{-1}. The malathion cause damage in essential organs of tilapia fish survivor at concentations tested, but these fish are capable of recover after 30 days in water free contaminants and can be maintained in aquaculture farm, without economic losses by mortality.

Key words: Pesticides, environmental toxicology, pollutants on fish, organophosphorous, aquaculture.

Resumo: Histopatologia e recupeção de tilapia-do-Nilo após exposição ao inseticida malathion utilizado no controle de *Aedes aegypti.* Malathion é um inseticida organofosforado que pode intoxicar e matar tilápias, gerando perdas econômicas no setor da tilapicultura devido a mortalidade. Neste estudo objetivou-se avaliar as alterações histológicas e a recuperação de brânquias, fígado e rim de tilápias após intoxicação aguda a diferentes concentrações de malathion. O malathion causou nas brânquias: hyperplasia, congestão do seio venoso e fusão e desarranjo das lamelas. No fígado houve congestão dos sinusoids, desarranjo cordonal e esteatose. No rim foi observado vacuolização das células dos túbulos renais, presença de infiltrado inglamatório e aumento do espaço de Bowman. Após 30 dias, as branquias, o fígado e o rim recuperaram das alterações causadas por malathion em concentrações de até 7.0 mg L^{-1}. Não houve diferença significativa ($p > 0.05$) nos parâmetros aquáticos nas concentrações avaliadas entre 3.0 mg L^{-1} e 9.0 mg L^{-1}. O malathion causa danos em órgãos essenciais para tilápias sobreviventes, porém estes peixes são capazes de recuperar após 30 dias em água livre

de contaminantes e podem ser mantidos em aquicultura, sem grandes perdas econômicas por mortalidade.

Palavras-chave: Pesticidass, toxicologia ambiental, poluentes em peixes, organofosforado, aquicultura.

Introduction

The mosquito *Aedes aegypti*, adapted to urban environments, is the main vector for dengue, Zika, Chikungunya fever and urban yellow fever, which are considered public health problems in tropical and subtropical regions (Braga & Valle 2007, Brasil 2009, Zara *et al.* 2016). Organophosphorate malathion is used to combat the mosquito in a formulation emulsible in water in disease outbreacks (Brasil 2009). With the advancement of technology, pesticides were prepared with easy dilution in aqueous medium while making them more toxic and efficient in pest control (Veiga *et al.* 2006).

The malathion is a organophosphate pesticide that acts in the peripheral and central nervous system, inhibiting the action of the enzyme acetylcholinesterase in nerve endings and causing the accumulation of acetylcholine, causes muscle spasms in animals and death for paralysis. These factors are indicative of direct influences on the sympathetic and parasympathetic central nervous system (Maddrell 1980, Varo *et al.* 2003, Pavão & Leão 2005, Pacheco-Ferreira 2008), proving the effectiveness of the product formulated for that purpose.

Pesticides such as malathion may reach and contaminate the aquatic environment by surface or percolation, after application in rainy periods (Dellamatrice & Monteiro 2014). Intercommunication among aquatic ecosystems allows any type of contaminant to reach areas far from where were initially applied, resulting in contamination (Veiga *et al.* 2006; Dellamatrice & Monteiro 2014). Due to the intercommunication among aquatic ecosystems, after nebulization of malathion for mosquito *Aedes* control, this organophosphate can reach the aquatic environment, contaminate the waters and intoxicate the species that are not targets of this insecticide such as fish (Mello *et al*. 2020). Intoxication and death of these organisms can generate economic losses for fish farming, located or not, near the initial sites of application.

The aim of this study was to evaluate histological alterations and the recover of gills, liver and kidney of tilapia *Oreochromis niloticus* after acute exposure to diferent concentrations of organophosphorous malathion and determine the recover percentage of survivors after intoxication.

Material and Methods

Legislation: The present study was according to the ethical principles for animal experimentation approved by the Brazilian College of animal experimentation (Protocol number: 011283/17). The insecticide used in the trials was malathion (Komvektor 44%®, Cheminova Brasil Ltda) with emulsion in water (EW) registered in Ministério da Agricultura, Pecuária e Abastecimento – MAPA number 014307.

Laboratory bioassay: Following a two-week acclimation period, Nile tilapia (*Oreochromis niloticus*) were submitted to sensitivity tests with potassium chloride (KCl) for 48 hours, in glass containers with a capacity of 3.0 L. The assays were performed in triplicates for concentration (control; 0.5; 0.75; 1.0; 1.25 and 1.5 g L^{-1}) in static system and heated room with photoperiod control (12h clear/dark) and temperature (26 ± 2.0ºC) (Abnt 2016). The fish were distributed completely randomized design (CRD) into 18 tanks. The LC_{50}-48h of potassium chloride for tilapia was 1.13 g L^{-1}, with the upper limit of 1.33 g L^{-1} and the lower limit of 0.98 g L^{-1}, thus attesting to sanity and susceptibility of organisms according to control letter of other batches used.

After evaluating the sanity and sensitivity of the organisms to be used in the experiments, preliminary and definitive ecotoxicological tests were carried out to determine concentrations that could cause 10%, 30%, 50% and 70% of mortality in fish. Fish were duly distributed in quadruplicates, by a CRD in cylindrical glass containers with 10 L of capacity, with five fish weighting 2 g each (Abnt 2016). The concentrations used in the tests were: control; 3.0 mg L^{-1}, 5.0 mg L^{-1}, 7.0 mg L^{-1} and 9.0 mg L^{-1} of malathion.

Mesocosm bioassay: After the laboratory bioassay, fish with mean weight of 5.0 g were aclimated during 15 days in five 1000 L plastic tanks. The tanks were supplied with constant oxygenation and water flow (0.5 L min^{-1}). After this period, 980 fish were distributed in control group and pre-determined malathion concentrations of 3.0 mg L^{-1}, 5.0 mg L^{-1},

7.0 mg L^{-1} and 9.0 mg L^{-1}, which provided a mortality after 24h exposure of 10%, 30%, 50% and 70% mortality respectively. The control group remained during the experiment period, without the addition of malathion. After 24h, 60 survivors of each concentration were distributed in CRD (quadruplicates) in the recovery mesocosms (150 L tanks). This experiment was carried out in a continuous flow of water at a flow rate of 0.5 L/ min during 30 days.

Sample collection: The fish were anesthezied by benzocaine (1:1000) and the samples of gills, kidney and liver were collected 24 hours after malathion intoxication, at 15 and 30 days after the recovery trial. The tissue samples were fixed in 10% buffered formalin (0,1 M; pH 7,3), processed routinely, embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin (H&E). Based on regression of clinical signs and tissue lesions after 30 days of recovery, it was found the concentration and percentage of surviving fish that could be kept in fish culture.

Water quality parameters: The following water quality conditions were recorded: dissolved oxygen (mg L^{-1}), pH, temperature (ºC) and electrical conductivity (µS / cm) using a multiparameter probe with system YSI 556 MPS of the Yellowspring Co.

Statistical analysis: The values were submitted to calculation of average, standard deviation and analized by the Scott-Knott. The significance level was defined as $p < 0.05$ using the statistical software GraphPad Prism 8 (GraphPad Software, San Diego, USA).

Results

Histopathology and tilapia recovery: Histopathological changes in the gills (Fig. 1a), liver (Fig. 1b) and kidney (Fig. 1c) of the control group during the 30-day period of the mesocosm experiment were not observed. In the present study, the exposure to malathion in concentrations of 3.0 and 5.0 mg L^{-1} caused gill hyperplasia. Concentrations of 7.0 mg L^{-1} and 9.0 mg L^{-1}, showed branchial hyperplasia, lamellar fusion and venous sinus congestion and secondary lamellar desarrangement (Fig. 1d).

After 15 days mesocosms recovery, the gill lesions were observed at a lower frequency and after 30 days no lesions were observed in gills of fish intoxicated with 3.0, 5.0 and 7.0 mg L^{-1} of malathion. In this way, gills surviving acute malathion intoxication at concentrations of 3.0, 5.0 and 7.0 mg L^{-1} recovered completely after 30 days. However, for the concentration of 9.0 mg L^{-1} it will take more than 30 days for the full recovery of that organ to occur.

Concentrations of 3.0 mg L^{-1}, 5.0 mg L^{-1}, 7.0 mg L^{-1} and 9.0 mg L^{-1} showed cordonal desarrangement and steatosis (Fig. 1e) in liver. However, after 15 days in water free of contaminants, the liver presented lesions at lower frecuencies in all concentrations and at 30 days the lesions completely regressed.

In the kidney, malathion caused vacuolization of renal tubule cells at concentrations of 3.0 and 5.0 mg L^{-1}, inflammatory infiltrate at 5.0 mg L^{-1} and increased Bowman space at 7.0 mg L^{-1} (Fig. 1f). After 15 days of recovery in mesocosms, vacuolization was observed at the concentrations of 9.0 mg L^{-1}, which remained until the 30 days. No histopathological changes were observed in the kidneys fragments of the fish in the other concentrations.

Water quality: No significative differences ($p > 0.05$) were observed on aquatic parameters (pH, oxygen, temperature and electric conductivity) at 24h, 15 and 30 days during the recovery period of tilapia fish (Table I).

Discussion

Possible abnormalities occurring when organisms are exposed to xenobiotics are the result of defense mechanisms (Castro *et al.* 2014). Pesticides such as malathion have the ability to cause toxic effects in exposed fish even at low concentrations (El-Nahhal 2018) as observed in the present study in all concentrations. Gill hyperplasia, for example, is a defense mechanism to increase the absorption of oxygen in order to normalize respiration, acting as compensatory mechanism (Mishra & Mohanty 2008). The lesions observed were according to Di Giulio and Newman (2012) are related because hyperplasia causes lamellar fusion due to structural disarrangement. These changes serve to increase the contact surface to allow greater gas exchange (Castro *et al.* 2014).

Similar effects were observed in fish species *Mugil cephalus* and *Chanos chanos* exposed to organophosphorus chlorpyrifos (Marigoudar *et al.* 2018) and in carp *Labeo rohita* after exposure to organophosphate malathion (Marigoudar *et al.* 2018, Karmakar *et al.* 2016) and was observed that the curima gills had lesions of hyperplasia, cell degeneration of lamellae, lamelar fusion, besides of accumulation of mucus and formation of vacuoles, and such changes are associated with the ability to

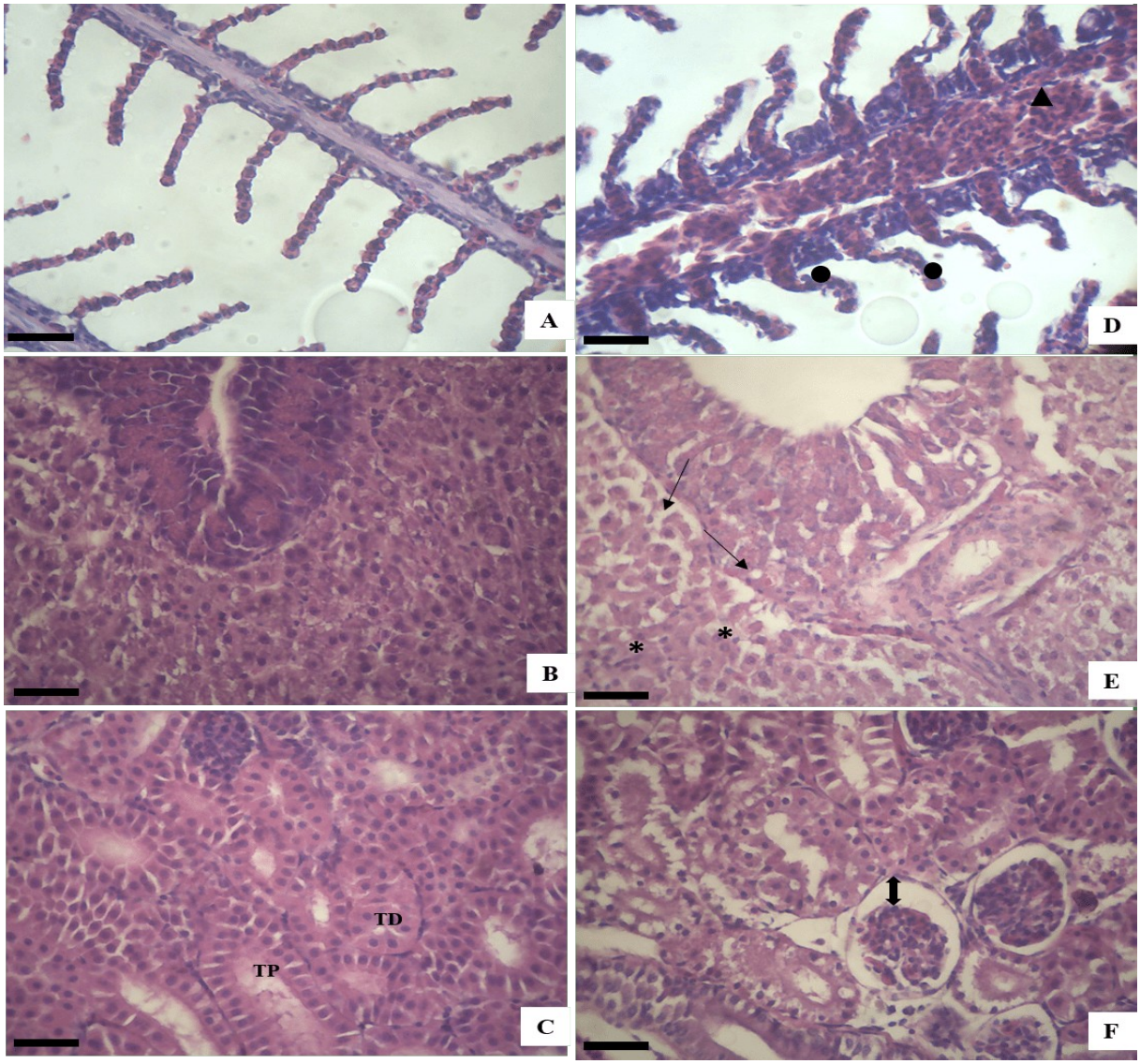

Figure 1. Histopathology of organs of Nile tilapia (*Oreochromis niloticus*) after exposure to different malathion concentrations (3.0, 5.0, 7.0 and 9.0 mg L^{-1}). **A-** Control gills. **B-** Control liver. **C-** Control kidney (TD: distal tubule; TP: proximal tubule). **D-** Gills with venous sinus congestion (▲) and derangement of the secondary lamella (●). **E-** Liver with steatosis (↑) and cordonal derangement (*). **F-** Kidney with increased Bowman's space (↔). Bar raise: 40 µm.

adapt to toxicity expressed by chlorpyrifos (Marigoudar *et al.* 2018). For *L. rohita*, fusion of primary and secondary lamellae was observed, as well as epithelial hyperplasia and lamella disarrangement (Karmakar *et al.* 2016). Similar lesions were observed in the present study, corroborating with the capacity of malathion to cause structural changes in the gills of different fish species after acute exposure.

The histopathological changes observed in gills, liver and kidney after 24 hours of exposure to malathion was a result of the stress caused by the chemical agent (Caccia *et al.* 2017). According to Anvarifar *et al.* (2018) from all existing pesticides the insecticides are generally the most toxic, with emphasis on organophosphates, organochlorines, carbamates, neonicotinoids and pyrethroids. Animals are often exposed to pesticides and other types of chemical contaminants that act as membrane disrupters, causing loss of cell and tissue function (Castro *et al.* 2014).

The effects of hepatotoxicity were also observed in the study by Karmakar *et al*. (2016) fish fingerlings of *L. rohita* showed physiological changes that increased the activity of some liver enzymes after exposure to malathion. In addition to malathion, other substances have the capacity to cause hepatic alterations, such as potassium

Table I. Means and standard deviation (SD) of the aquatic variables during the recovery period of the of Nile tilapia (*Oreochromis niloticus*) after exposure to different malathion concentrations.

Concentrations (mg L^{-1})	pH				Electric conductivity (µS/cm)			
	Mean			SD	Mean			SD
	24h	15d	30d		24h	15d	30d	
0,0	8.3aA	8.2aA	8.1aA	0.07	210.8aA	206.0aA	215.7aA	0.04
3.0	8.2aA	8.2aA	8.1aA	0.06	235.1bB	207.7aB	210.5aB	0.15
5.0	8.3aA	8.2aA	8.2aA	0.04	235.2bB	207.5aB	216.0aB	0.14
7.0	8.3aA	8.2aA	8.2aA	0.03	237.7bB	206.3aB	216.3aB	0.16
9.0	8.3aA	8.2aA	8.2aA	0.03	241.5cC	206.7aC	217.4aC	0.18
Concentrations (mg L^{-1})	**O_2 (mg L^{-1})**				**Temperature ºC**			
	Mean			SD	Mean			SD
	24h	15d	30d		24h	15d	30d	
0,0	5.6aA	5.9aA	5.7aA	0.12	26.3aA	26.3aA	26.1aA	0.14
3.0	5.6aA	5.8aA	5.7aA	0.11	26.3aA	26.2aA	26.2aA	0.05
5.0	5.6aA	5.8aA	5.7aA	0.11	26.3aA	26.1aA	26.1aA	0.11
7.0	5.6aA	5.9aA	5.6aA	0.13	26.2aA	26.1aA	26.1aA	0.06
9.0	5.6aA	5.8aA	5.7aA	0.12	26.2aA	26.3aA	26.3aA	0.06

Values (mean) of capital letters and lower case letter in the same column are significantly different within groups ($p < 0.05$).

dichromate that caused cordonal derangement, steatosis and congestion of sinusoids in *Piaractus mesopotamicus* in the study by Castro *et al.* (2014). Similar effects were observed in the present study after intoxication of *Oreochromis niloticus* to malathion at concentrations 3.0 mg L^{-1}, 5.0 mg L^{-1}, 7.0 mg L^{-1} and 9.0 mg L^{-1} corroborating hepatotoxicity effects in previous work. The changes found in the liver in response to the action of a xenobiotic happen due to the capacity of detoxification besides the regeneration after tissue or functional loss caused by environmental contaminants (Roberts & Oris 2004, Robbins & Cotran 2005, Harmut 2012, Sanchez-Valle *et al.* 2012, Deng *et al.* 2016).

The proliferation of mature cells after tissue damage allows restoration of the original tissue through the proliferation of new cells. For this, there must be a dose-response correlation (Harmut 2012) in which the recovery depends on the dose administered and the animal's exposure time. These factors explain the total recovery of liver at concentrations of up to 7.0 mg L^{-1} of malathion, unlike in the gills and kidneys that recovered, totally after 30 days in concentrations of up to 5.0 mg L^{-1}.

Studies with malathion in *Cyprinus carpio* revealed the presence of renal cell hyperplasia, changes in nucleus structure, formation of vacuoles, necrosis, and cellular degeneration (Dhanapakian & Premlata 1994). Later, in the study by Karmakar *et al.* (2016) similar lesions were observed as renal tubule narrowing and disorganization, renal epithelial degeneration and tubule epithelium necrosis in *Labeo rohita* fingerlings after exposure to sublethal concentrations of 10, 50 and 100 µg L^{-1} malathion, which correspond to 0.01, 0.05 and 1.0 mg L^{-1} respectively. In the present study, the malathion was more toxic to *L. rohita* carp than tilapias at concentrations up to 9.0 mg L^{-1} because the toxicity of a product may vary between species and stages of development, with younger species being more sensitive (Karmakar *et al*. 2016).

Histopathological changes in the kidneys are related to toxic substances that are present in the filtrate of the glomerulus (Silva & Martinez 2007). When a chemical enters the systemic circulation and is directed to the kidneys, a concentration that is nontoxic in plasma becomes toxic to the kidneys and renal tubules resulting in lesions (Schnellmann 2012). Some of these changes occur to improve the elimination of the toxic substance from the organs, such as Bowman's increased space observed at 7.0 mg L^{-1} concentration of malathion in the water. Renal tubules are the most susceptible to injury because they accumulate xenobiotics. Environmental chemical compounds cause nephrotoxicity which, depending on the intensity, may lead to recovery or permanent damage (Schnellmann 2012). Fish intoxicated with malathion recovered from kidney damage at concentrations up to 5.0 mg L^{-1}. According to Narra *et al.* (2017) the debugging process detoxifies fish exposed to insecticides that

have a deleterious action mode. The authors observed that at concentrations of 165 mg L^{-1} of chlorpyrifos and 2.14 mg L^{-1} of monocrotophos, *Clarias batrachus* recover the enzymatic functions in 30 days in free water. Therefore, Narra *et al.* (2017) points out that recovery studies have become an important tool for improving the quality of life of exposed fish.

Regarding to aquatic parameters, according to Sipaúba-Tavares (2013) the ideal pH range for breeding fish is between 6.5 and 9.5 with values above 11.0 or below 4.0 being considered lethal for these organisms. Based on this study, the pH range (8.3 - 8.5) observed during the 30 days of experimentation remained adequate for the survival of the species and did not present significant alterations (p>0,05).

El-Sherif and El-Feky (2009) reinforce that the ideal pH for tilapia production is 7.0 to 8.0 and, according to Ginneken *et al*. (1997) tilapia has the ability to maintain homeostasis at pH up to 4.0, corroborating with Lemos *et al.* (2018) who observed that this species of fish has the ability to tolerate different pH changes in water.

In relation to oxygen, studies show that *O. niloticus* supports concentrations of dissolved oxygen in the water in the interval between 0.1 – 0.5 mg L^{-1}. This species of fish supports levels of 0 mg L^{-1} of O_2, as long as they have access to the surface of the water to breathe, but in these cases, the risk of mortality was high (Sayed & Fattah, 2006). Severe conditions of hypoxia in tilapias increase the respiratory rate, which after reaching the apex, begins to decay, increasing the levels of glucose and cortisol (Ishibashi *et al.* 2002) which are released under stress conditions, resulting in fish mortality.

Values above 4.0 mg L^{-1} are considered good for fish, according to Sipaúba-Tavares (2013). Throughout the experimental period of recovery of the present study, there was no significant alteration of the dissolved oxygen (p> 0.05), which ranged from 5.6 to 5.9 mg L^{-1}, remaining adequate and corroborating Sipaúba-Tavares (2013).

For the electrical conductivity the values ranged from 210.8 to 241.5 during the 30 days of the mesocosm. There was a significant change at the 1% probability level (p <0.01) during the test between groups and time, but these small variations did not influence the cause of fish mortality. According to Delincé (1992) the values of conductivity around 10.000 µS / cm is considered an indication of pollution for the fresh water environment (Sipaúba-Tavares 2013). In this way, it can be observed that the values obtained in the present study show that the recovery waters were free of water pollution since the values were below the indicative of aquatic pollution.

Finally, among the factors that are important for the survival of aquatic organisms, we highlight the temperature that did not change significantly (p> 0.05) during the test. Ideal values are in the range of 20 to 35ºC for the *O. niloticus* (Sayed & Fattah 2006). In the present work, a temperature variation of 27.1 to 27.3ºC was observed throughout the experimental period, indicating a suitable range for homeostasis of the species, according to Sayed and Fattah (2006).

So, malathion causes hyperplasia in concentrations between 3.0 and 7.0 mg L^{-1}, in addition to lamellar fusion, venous sinus congestion and lamellar derangement of gills after acute exposure. In the liver of juvenile tilapia, it causes congestion of the hepatic sinusoids, derangement and steatosis at 3.0, 5.0, 7.0 and 9.0 mg L^{-1}. In the kidney, it causes vacuolization of renal tubule cells from 3.0 to 5.0 mg L^{-1}, inflammatory infiltrate, and increased Bowman's space at concentrations up to 7.0 mg L^{-1}.After 30 days in malathion-free water, total recovery of the kidney and gills at concentrations of up to 5.0 mg L^{-1} and total liver recovery up to 7.0 mg L^{-1} is achieved through the physiological clearance process. In mortalities up to 70% of the lot (9.0 mg L^{-1} malathion), survivor tilapia recover partially from injury and can be maintained in the culture system without the need for disposal. During the purification process of malathion there were no significant changes (p> 0.05) in water in concentrations ranging from 3.0 mg L^{-1} to 9.0 mg L^{-1}. Finally, the tilapia fish exposed during 24 hours in concentrations between 3.0 mg L^{-1} to 9.0 mg L^{-1} are capable of recovery and could be maintenance in pisciculture tank without prejudice zootechnical parameters and economical losses.

Conclusion

The malathion cause damage in essential organs of tilapia fish survivor at concentations tested, but these fish are capable of recover after 30 days in water free contaminants and can be maintained in aquaculture farm, without economic losses by mortality.

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Compliance with Ethical Standards

All applicable international, national and/or institutional guidelines for the care and use of animals were followed

Conflict of Interest

The authors declare that they have no conflict of interest.

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