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Chronic effect after acute exposure to commercial petroleum fuels on physiological status of Nile tilapia, *Oreochromis niloticus* (L.)

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Abstract

This study was conducted to explore the effect of kerosene, gasoline, or diesel, on the health status of Nile tilapia, *Oreochromis niloticus* (L.). Healthy fish (49.5 ± 1.3 g) were exposed to 0.01% (*v/v*) CPFs in glass aquaria for 5 min and then returned to clean freshwater for 4 weeks. Signs of poisoning in intoxicated fish were air gulping, increased opercular movement, loss of balance, and dyspnea. At the end of this experiment, fish were collected, counted, and weighed. Fish in the control group grew gradually up to the end of the experiment; their growth was better than those exposed to any of CPFs. Moreover, weight gain, specific growth rate, feed intake, feed conversion ratio, and survival rate of the exposed fish were poor when compared to the control fish group. The physiological variables including red blood cells (RBCs) and hemoglobin content in fish exposed to CPFs changed by time. The maximum count of RBCs was obtained at the 1st week, and decreased gradually to the 4th week. The glucose and cortisol levels were maximized after the exposure to kerosene, diesel or gasoline and decreased gradually to the end of the experiment. Plasma lipid, protein, aspartate aminotransferase, and alanine aminotransferase in the exposed fish were significantly altered by CPF exposure. They were gradually close to the control values after 3 to 4 weeks of recovery. This study concluded that the acute exposure to CPFs significantly reduced the growth performance of Nile tilapia and significantly affected its physiological status, which may be recovered after awhile.

Keywords: Nile tilapia, Commercial fuels, Petroleum hydrocarbons toxicity, Physiological profiles

Background

Pollution, which is caused by the spilling of commercial petroleum fuels (CPFs), is a major environmental constraint that causes toxic effects in aquatic ecosystems. The main CPF sources in aquatic ecosystems are the leakage of oil transport pipelines, storage tanks, and accidents involving petroleum transport vehicles. The mining of oil shale reserves may also pose a risk to freshwater ecosystems. A variety of pollutants including crude oil and its products are known to induce stress conditions, which impair the health of aquatic life (Kori-Siakpere 2000; Agbogidi et al. 2005). Earlier reports have also shown that oil pollution impacts negatively on fishery resources (Kilnhold 1980). Ekweozor (1989) reported that frequent spillage of crude oil and its products in

creeks and rivers may have resulted in a marked reduction in the number of both freshwater and marine organisms. Azad (2005) observed that eggs and young stages (fingerlings) of fishes are especially vulnerable to the toxic effects of crude oil and its refined products. The ecophysiological effects of crude oil on *Machaerium lunatus* had also been reported by Bamidele and Agbogidi (2006).

Nile tilapia, *Oreochromis niloticus* (L.), is native to Egypt and widely distributed all over the world (El-Sayed 2006). This species has been used previously in laboratory studies and has been shown to be a suitable organism for monitoring the effects of xenobiotics. This study used Nile tilapia as a model to measure the potential toxic effects of CPFs on fish performance and to test the ability of this fish species to recover from the exposure effect. Therefore, the present study has been undertaken to evaluate the physiological alterations of Nile tilapia following the acute exposure to kerosene, gasoline, and diesel.

Materials and methods

Fish culture regime

The study was done in accordance with the code of ethics of the World Medical Association for animal experiments (Declaration of Helsinki).

Healthy Nile tilapia, *O. niloticus* (L.), were obtained from the nursery ponds, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. All 120 fish (49.5 ± 1.3 g) were acclimated in indoor tanks for 2 weeks where they fed on a commercial diet containing 20% crude protein (CP). After the acclimation period, the fish were distributed into eight 120-L glass aquaria at a ratio of 15 fish per aquarium.

Kerosene, gasoline, and diesel were brought from a commercial gas station, and their specific gravities were 740, 700, and 820 g/L, respectively. Twelve milliliters of kerosene, gasoline, or diesel were separately added to six aquaria, and they were vigorously mixed with the aquaria water. Fish were then stocked into the aquaria containing kerosene, diesel, or gasoline for 5 min. Fish were then transferred into other 120-L aquaria containing dechlorinated tap water and left to recover for 4 weeks. Each treatment was represented by two aquaria. In the control group, fish were placed in dechlorinated tap water and were not exposed to any CPF. All aquaria were supplied with compressed air from air pumps via airstones. During the recovery trial, fish were fed on 25% CP up to satiation twice daily at 9:00 and 14:00 h, 5 days a week for 4 weeks. The total feed intake for each aquarium was calculated as a summation of the given feed amounts during the feeding days. Fish in each aquarium were weekly group-weighed, and dead fish were removed and recorded daily. Three-quarters of the water in each aquarium were siphoned every day to remove fish excreta and replaced by well-aerated water provided from a storage fiberglass tank.

The blood samples were taken, after transfer from the treated water to the dechlorinated water within 1 h of the end of the 5-min exposure, to represent zero time for the sample. At 1, 2, 3, and 4 weeks of the recovery period, blood samples were taken to measure the different physiological variables.

Fish performance

At the end of the experiment, the fish were collected, counted, and weighed. Growth performance was determined, and feed utilization was calculated as following:

(a) specific growth rate (SGR; percentage per day) = 100 (Ln W_2– Ln W_1)/T, where W_1 and W_2 are the initial and final weights, respectively, and T is the number of days in the experimental period; and (b) feed conversion ratio (FCR) = feed intake (g)/weight gain (g).

Physiological measurements

Fish were not fed during the 24 h immediately prior to blood sampling for all sampling dates. Two fish from each aquarium were anesthetized with buffered tricaine methanesulfonate (30 mg/L), and blood was collected from the caudal vasculature in Eppendorf tubes containing 500 U of sodium heparinate/mL, used as an anticoagulant. Blood was used for red blood cell (RBC) count (Dacie and Lewis 1984) and hemoglobin (Hb) content (van Kampen and Zijlstra 1961). The plasma was obtained by centrifugation at 3,000 rpm for 15 min, and the non-hemolyzed plasma was stored at −20°C for further assays. Total lipid and total protein contents in plasma were determined colorimetrically according to Joseph et al. (1972) and Henry (1964), respectively. Concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma were determined colorimetrically according to Reitman and Frankel (1957). Glucose was determined colorimetrically according to Trinder (1969). Plasma cortisol levels were measured by radioimmunoassay as previously validated by Chiu et al. (2003).

Statistical analysis

The obtained data were subjected to Kolmogorov-Smirnov and Cochran's tests for normality and homogeneity of variance, respectively. The data were homogenous and showed normal distribution. Data were analyzed using a two-way ANOVA with fuel sources and time intervals as factors. Statistical significance was set at the 5% probability level, and means were separated using Duncan's new multiple range test. The software SPSS version 10 (SPSS, Richmond, USA) was used as described by Dytham (1999).

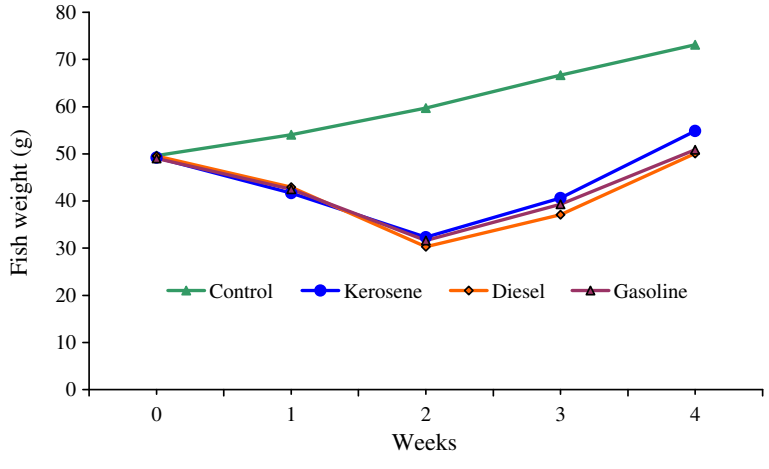

Figure 1 The live body weight (g) of Nile tilapia after short-term exposure to commercial petroleum fuels.

Table 1 Growth performance and feed utilization of Nile tilapia after short-term exposure to commercial petroleum fuels

Variable	Control	Kerosene	Diesel	Gasoline
Initial weight (g)	49.6 ± 1.39	49.2 ± 1.44	49.5 ± 0.59	49.0 ± 1.30
Final weight (g)	73.1^{a} ± 2.14	54.8^{b} ± 1.56	50.1^{b} ± 0.73	50.9^{b} ± 1.47
Weight gain (g)	23.5^{a} ± 0.75	5.6^{b} ± 0.12	0.6^{c} ± 0.23	1.9^{d} ± 0.19
SGR (%/day)	1.62^{a} ± 0.005	0.45^{b} ± 0.003	0.05^{c} ± 0.016	0.16^{d} ± 0.010
Feed intake (g/fish)	39.2^{a} ± 0.58	17.8^{b} ± 0.55	17.6^{b} ± 0.55	17.7^{b} ± 0.52
FCR	1.67^{c} ± 0.07	3.2^{d} ± 0.03	29.3^{a} ± 2.04	9.4^{b} ± 0.81
Survival (%)	97.6^{a} ± 2.22	84.4^{b} ± 2.22	82.2^{b} ± 5.87	82.2^{b} ± 2.22

Fish were exposed to commercial fuels and recovered for 4 weeks. Means having the same letter in the same row are not significantly differed at $P < 0.05$.

Results and discussion

Fish subjected to CPF pollution were removed from the tanks after 5-min exposure. During this period, symptoms of poisoning in exposed fish were air gulping, increased opercular movement, loss of balance, and dyspnea. Meanwhile, no symptoms of poisoning were observed in control fish. The increased respiration of fish suggested that

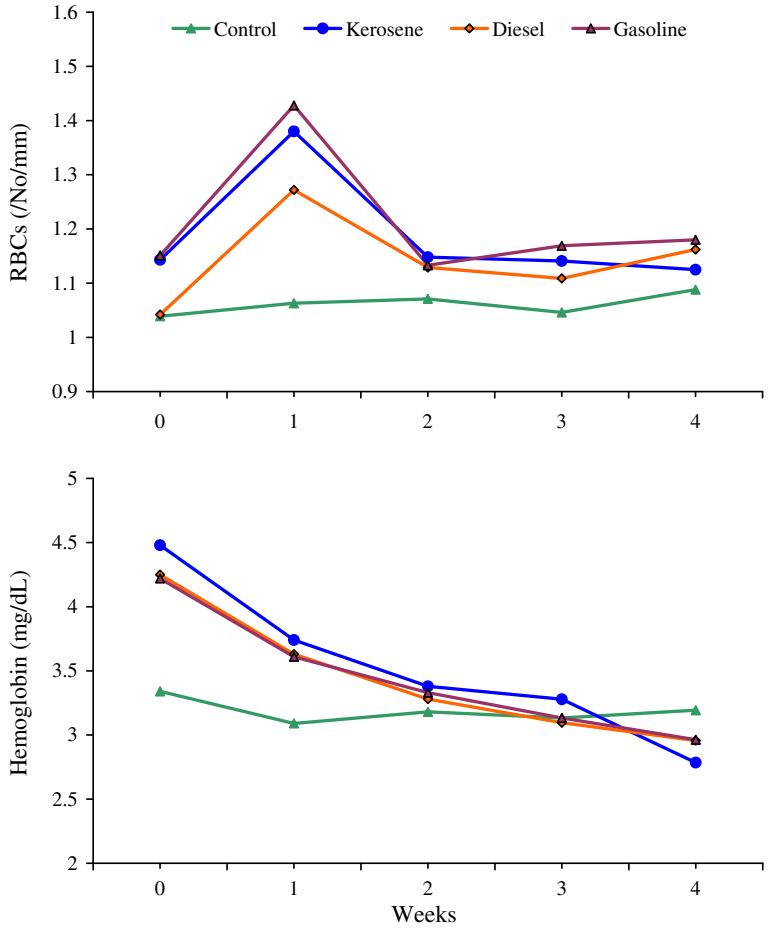

Figure 2 The mean values of RBC count (number/mm) and hemoglobin (mg/dL) in Nile tilapia. After short-term exposure to commercial petroleum fuels and recovered for 4 weeks.

fish had respiratory problems. Furthermore, these pollutants have been reported to cause structural damage to the respiratory lamellae of the gills (Poirier et al. 1986; Correa and Garcia 1990; Prasad 1991), to impede gas exchange, and result in hypoxemia (Perry et al. 1989; Ristori and Laurent 1989; Randall and Perry 1992). In addition, the fusion of secondary lamellae, gill hyperplasia, and edema have been reported in fish exposed to petroleum hydrocarbons (Correa and Garcia 1990; Prasad 1991; Dede and Kaglo 2001).

The growth of fish in the control group was gradual and continued until the end of the experiment. Meanwhile, the growth of fish exposed to the three CPFs was ceased for 2 weeks; then, they started to grow again (Figure 1). The maximum fish growth was observed in the control fish group ($P < 0.05$, Table 1). Moreover, weight gain and SGR of fish exposed to diesel or gasoline were less than those exposed to kerosene. Feed intake, FCR, and survival rate of the fish exposed to treatments were significantly low when compared to the control fish group.

The obtained results, herein, indicated that CPFs had negative impacts on fish performance and survival. However, CPF-exposed fish consumed low feed amounts and ceased to grow, suggesting that CPF exposure may lead to a reduction in fish appetite or complete fish fasting causing growth retardation. The findings of this study were in

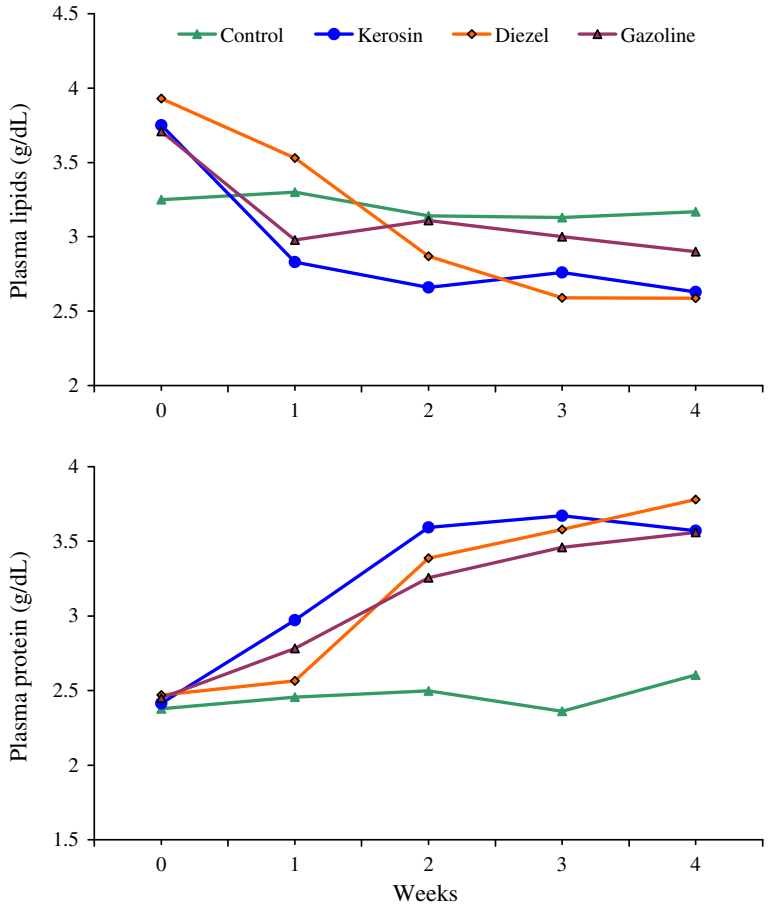

Figure 3 The mean values of plasma lipids (g/L) and protein (g/L) in Nile tilapia. After short-term exposure to commercial petroleum fuels.

concomitant with Kicheniuk and Khan (1981) and Kori-Siakpere (2000) who reported that fish exposed to water soluble fractions (WSFs) of crude oil could result in reduced feed intake and low body weight. Dede and Kaglo (2001) reported that the survival of Nile tilapia decreased by increasing concentration of diesel fuel. Additionally, delayed growth and reduced survival of pink salmon (*Onchorhynchys gorbuscha*) embryos had been observed following their exposure to crude oil (Heintz et al. 2000). In a juvenile turbot study, fish exposed to higher concentrations of the petroleum fuel exhibited reduced growth and feed consumption (Saborido-Rey et al. 2007).

Red blood cell counts in CPF-exposed fish increased with time. The maximum RBC count was observed during the 1st week. Subsequently, RBC counts decreased gradually up to the 4th week (Figure 2). Hemoglobin content in the blood of Nile tilapia increased suddenly after their exposure to CPFs and decreased with time, and reached control levels at the end of the experiment (Figure 2). These results are in concomitant with Alkindi et al. (1996) who found significant increases in RBC and Hb contents after 3-h exposure of flounder to 50% WSF of crude oil. These increases may be due to the release of catecholamines, which stimulate splenic release of erythrocytes to aid O_2 carrying capacity, to avoid difficulties in respiration due to stimulation of Na^+/H^+ exchange

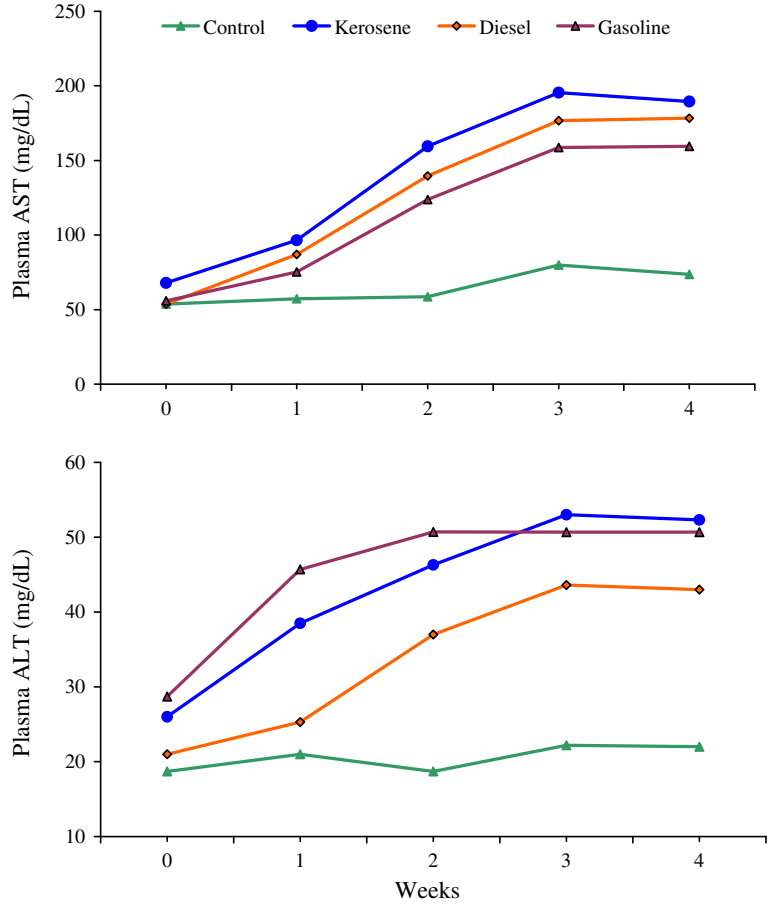

Figure 4 The mean values of plasma AST and ALT activities (mg/dL) in Nile tilapia. After short-term exposure to commercial petroleum fuels.

in erythrocytes, and increase the hemoglobin-oxygen affinity (Kita and Itazawa 1990; Pearson et al. 1992; Alkindi et al. 1996).

Plasma lipids in CPF-exposed fish decreased, while plasma protein increased significantly with time increase (Figure 3). At the end of the experiment, in CPF-exposed fish, plasma lipid contents were lower; meanwhile, plasma protein contents were higher than that of the control group fish. Moreover, activities of plasma AST and ALT in CPF-exposed fish increased gradually, reaching their maxima after 3 to 4 weeks of recovery (Figure 4). The control fish exhibited the lowest AST and ALT activities, which did not significantly differ throughout the experimental period.

The fluctuation in plasma lipids, protein, AST, and ALT may be due to the disturbance in metabolic pathways due to CPF exposure. In addition, the increase in AST and ALT activities are indicative of liver damage, which might have occurred due to the exposure to CPF and, hence, lead to the leakage of these enzymes into the blood. In this regard, Martin-Skilton et al. (2008) demonstrated that acute exposure of juvenile turbot, *Scophthalmus maximus*, to the Prestige fuel oil elicits alterations in some hepatic biotransformation enzymes with different sensitivities. Pollino and Holdway (2003) reported that short-term exposure of petroleum hydrocarbons to rainbow fish potentially alter metabolic and detoxification enzymes, with metabolic enzymes recovering after depuration (17 days).

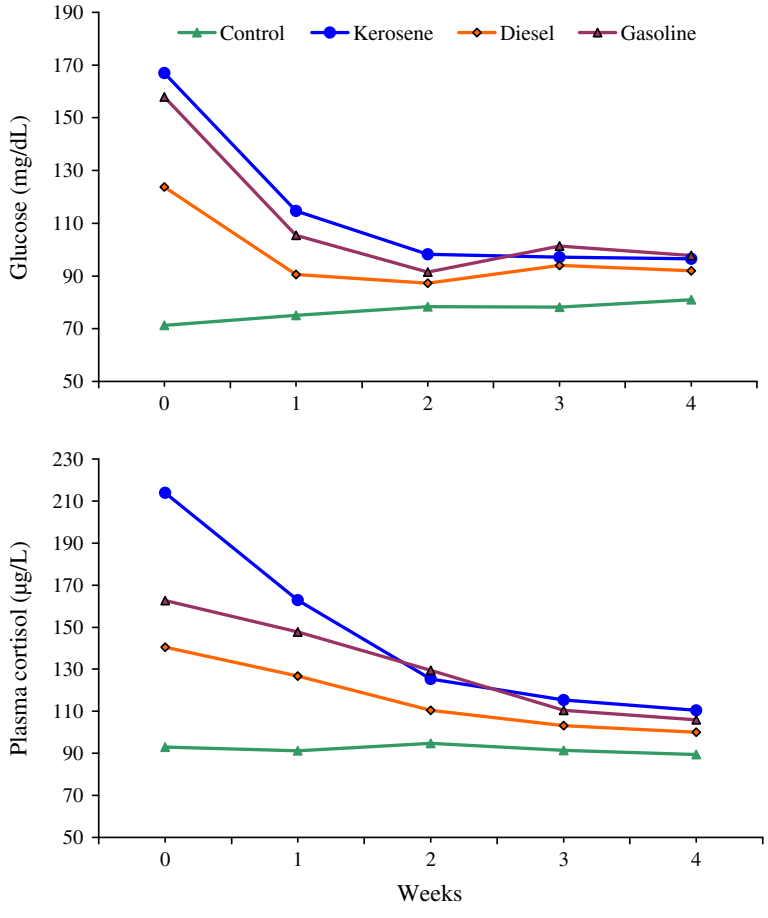

Figure 5 The mean values of plasma glucose (mg/L) and cortisol (µg/L) in Nile tilapia. After short-term exposure to commercial petroleum fuels.

Glucose level was maximized after CPF exposure and decreased gradually up to the end of the experiment (Figure 5). Glucose levels in CPF-exposed fish were higher than those of control fish. Moreover, plasma cortisol in CPF-exposed fish increased suddenly and decreased gradually up to the end of the experiment (Figure 5). Moreover, the cortisol value was significantly affected by the CPF source, and the CPF order was kerosene > gasoline > diesel. Plasma cortisol was lower in the control fish compared to the CPF-exposed fish.

The rise in glucose and cortisol concentrations indicates a stress-induced mobilization of energy reserves. These results are similar to those reported by Alkindi et al. (1996) who found that the exposure of flounders to a 50% dilution of the WSF of Omani crude oil, containing a mix of aromatic hydrocarbons (benzenes, toluene, and xylenes, and lower amounts of naphthalenes), resulted in a progressive increase in plasma cortisol concentrations continuing over the 48-h exposure period. Moreover, cortisol has a direct effect on carbohydrate metabolism by stimulating glycogenolysis and gluconeogenesis. However, cortisol also interacts with catecholamines which may exert pronounced effects in the immediate stages of stress (Wright et al. 1989; Vijayan and Moon 1994; Vijayan et al. 1994).

Conclusion

This study has conclusively demonstrated that the acute exposure to CPF significantly reduced the growth performance of Nile tilapia and affected their physiological status. The study also showed that Nile tilapia can serve as a bio-indicator of CPF toxicity. Particular attention should also be given to CPF process aimed at minimizing their toxicity to the aquatic ecosystem.

Competing interests
The author declares that he has no competing interests.

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