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ORIGINAL ARTICLE

Effects of Malathion Acute Toxicity on Behavioral and Haematological Parameters in *Capoeta damascina* (Cypriniformes: Cyprinidae)

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KEYWORDS

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ABSTRACT: The present study investigated the effects of acute toxicity of malathion on behavioral and haematological parameters in the cyprinid *Capoeta damascina*. The specimens were collected from the Kordan River, Karaj, Iran in August, 2014 and were exposed to different concentrations of malathion at the laboratory (24, 48, 72 mg L^{-1}) based on 96h-$LC_{(10; 30; 50; 90)}$ which was 6.08 (5.22-7.18) mg L^{-1}. The animals were then exposed to 0.76, 1.00 and 1.52 mg L^{-1} of malathion for 10 days. Blood samples were collected in days 1, 5 and 10. Red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), hematocrit (PVC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were measured. Behavioral abnormalities were observed in fishes exposed to high levels of malathion. The specimens exposed to malathion had significantly lower RBC, WBC, Hb, PVC but higher MCV and MCH than those of the control group. No significant difference was detected in MCHC the exposed and control specimens. Hematological parameters (except MCHC) were significantly correlated with exposure time. In conclusion, malathion showed extensive haematological effects on *C. damascina* that might be used as bioindicator of this pesticide in flowing waters.

INTRODUCTION

Pesticides are an important group of chemical aquatic pollutants that are used widely in agricultural activities around the world [1-3]. They enter into aquatic systems through different ways [4] influencing aquatics, including fishes, and finally human health [5]. Pesticides also have ecological effects changing aquatic biota

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communities' structure [6]. Their impacts on fishes have been investigated using behavioral, physiological and biochemical methods [7-19]. Haematological parameters are a group of biomarkers, which are extensively used in order to assessing toxicity effects, health status of Immune system, and histological damages in live organisms [20-23]. Haematological parameters are sensitive to alterations in environment factors, which have made them reliable and easy-to-measure indices in biomonitoring [24]. In addition, haematological profile provides appropriate information about physiological condition of the organism [25-27].

Malathion is an organophosphate pesticide considered hazardless for mammals, and thus has been used more frequently than other pesticides [28]. It is highly toxic for aquatic biota [2, 29]. With respect to the undesirable effects of this pesticide on aquatic organisms, especially fishes as economically important organisms, studying toxic effects of such chemicals on fish species of inland water bodies is very important.

Capoeta damascina, belongs to the family *Cyprinidae*, is an important fish in Iranian waters that has widespread distribution, even in Asian countries [30]. Since habitats of this species are rivers adjacent to the agricultural lands and orchards, which receive high loads of malathion, the assessment of toxicity levels of malathion and its impacts on this species is very important. Therefore, this study was conducted to investigate the acute toxicity levels of malathion, and the effects of sublethal concentrations (or chronic exposure) on behavioral changes and haematological indices in *C. damascina* as an endemic and economically valuable species.

MATERIALS AND METHODS

Specimens (length: 12 ± 1 cm; weight: 125 ± 5 g [mean ± SD]) were collected in August, 2014 using electrocution from the Kordan River (Karaj, Iran), transferred to the laboratory, allocated randomly to plastic tanks for a week to get adapted to the experimental condition. Temperature, pH, dissolved oxygen and alkalinity of water were T: 21 ± 1 °C, pH: 7.5 ± 0.24, DO: 7.5 ± 0.5 mg L^{-1}, alkalinity: 220 ± 4 mg $CaCO_3$ L^{-1}, photoperiod: 8:16 (L: D).

Static acute toxicity bioassay was performed according to the APHA (1985) [31] to find 24, 48, 72 and 96-h lethal concentration (LC_{10-90}). Six different concentrations (1, 2, 4, 8, 16 and 32 mg L^{-1}) of malathion with three replicates were used in the test series. After finding LC_{50}96 h, three sublethal concentrations were selected in order to fish exposure, includes: 0.76 ppm ($0.125\times LC_{50}$); 1.00 ppm ($0.166\times LC_{50}$); 1.52 ppm ($0.250\times LC_{50}$). Animals were exposed to malathion for 10 days, where water was exchanged every 48 h. The specimens were allocated to three treatment and one control groups with three replicates for each treatment and 15 fish in each replicate. Most observed behavioral changes (i.e., less activity, loss of equilibrium, abnormal swimming, rapid gill movement and staying motionless on the aquarium bottom) were recorded during the experiment. Fishes were sampled in days 1, 5 and 10, when a 1-mL blood samples were collected using caudal peduncle into heparinized syringes and test tubes.

The following blood parameters were measured: number of erythrocytes (RBC, $\times10^6$ mm^{-3}), number of leukocytes (WBC, $\times10^3$ mm^{-3}), hemoglobin (Hb, g dL^{-1}), hematocrit (PCV: packed cell volume, %), the mean corpuscular volume (MCV, fL), the mean corpuscular hemoglobin (MCH, pg) and the mean corpuscular hemoglobin concentration (MCHC, %). The last three parameters were calculated according to previous studies [32, 33]:

$$\mathrm{MCV} = \left[\frac{\text{hematocrit (\%)}}{\text{RBC } (10^6)}\right] \times 10\mu^3$$

$$\mathrm{MCH} = \left[\frac{\text{hemoglobin (g)}}{\text{RBC } (10^6)}\right] \times 10\text{pg}$$

$$\mathrm{MCHC} = \left[\frac{\text{hemoglobin (g)}}{\mathrm{RBC}\ (10^6)}\right] \times 10\text{g per 100 ml}$$

LC_{50} was calculated using the probit analysis [28]. Significant differences between haematological parameters were examined using a one-way ANOVA and the Duncan's multiple range test ($\alpha = 0.05$). Relationships between exposure time/concentration and haematological parameters were examined using the Pearson's correlation coefficient. All results are presented as mean ± standard deviation. All statistical analyses were performed using SPSS version 20.00 (Chicago, IL, USA).

RESULTS

There was no mortality in the control group. The LC 10-90 calculated for *C. damascina* are presented in Table 1. LC_{50} 96 h value for malathion in fish was determined as 6.08 (5.22-7.18) mg L^{-1}. LC_{50} values decreased with increase of exposure time and for a given exposure time, lethal concentration increased from LC_{10} to LC_{90}.

Table 1. Lethal concentrations of malathion in *Capoeta damascina* during different exposure times.

Point	Concentration (ppm) (Lower Bound- Upper Bound)			
	24h	**48h**	**72h**	**96h**
LC_{10}	4.58 (2.72 - 5.66)	3.84 (2.17 – 4.90)	3.73 (2.16 – 4.72)	3.20 (1.80 – 4.12)
LC_{30}	6.45 (5.32 – 7.64)	5.86 (4.78 – 7.00)	5.53 (4.51 – 6.52)	4.90 (3.96 – 5.79)
LC_{50}	7.74 (6.69 – 9.44)	7.25 (6.21 – 8.84)	6.77 (5.83 – 8.08)	6.08 (5.22 – 7.18)
LC_{90}	10.90 (9.26 – 14.61)	10.66 (9.01 – 14.00)	9.81 (8.42 – 12.52)	8.95 (7.72 – 11.16)

In control group and 1 and 2 mg L^{-1} exposed treatments, have the same behavioral characteristics, includes active swimming, regular gill movement and stable equilibrium. Behavioral changes such as irregular movement and abnormal swimming, increase in the number of opercle movements, and swimming near the water surface were observed 4 hours after exposure in 4 and 8 mg l^{-1}, and 1 hour after exposure in 16 and 32 mg l^{-1} exposure concentrations.

Changes in RBC, Hb, PCV and WBC of the specimens exposed to different concentrations of malathion are shown in Figure 1. All parameters were significantly lower than those of the control group. RBC, Hb, PCV and WBC in specimens exposed to malathion decreased over time at a rate higher than those of the control group.

Changes of MCV, MCH, and MCHC are shown in Figure 2. MCV and MCH increased in the specimens exposed to 1.00 and 1.52 mg L^{-1} over the entire period of the experiment, but no significant change was detected in the control group and those exposed to 0.76 mg L^{-1} concentration. In the first day of exposure, MCHC values of fishes belong to the highest exposure concentration was significantly lower than other treatments and control group, but in days 5 and 10, the values of this indice at all exposure treatments were lower than controls.

The correlation coefficients and levels of significance between haematological parameters and exposure times/concentrations of malathion are presented in Table 2. There was negative correlation between RBC, Hb, PCV and WBC and exposure time, and positive correlations between MCV and MCH with exposure time. No significant correlation was found between MCHC and exposure time. There was no significant correlation between haematological parameters and concentration of malathion.

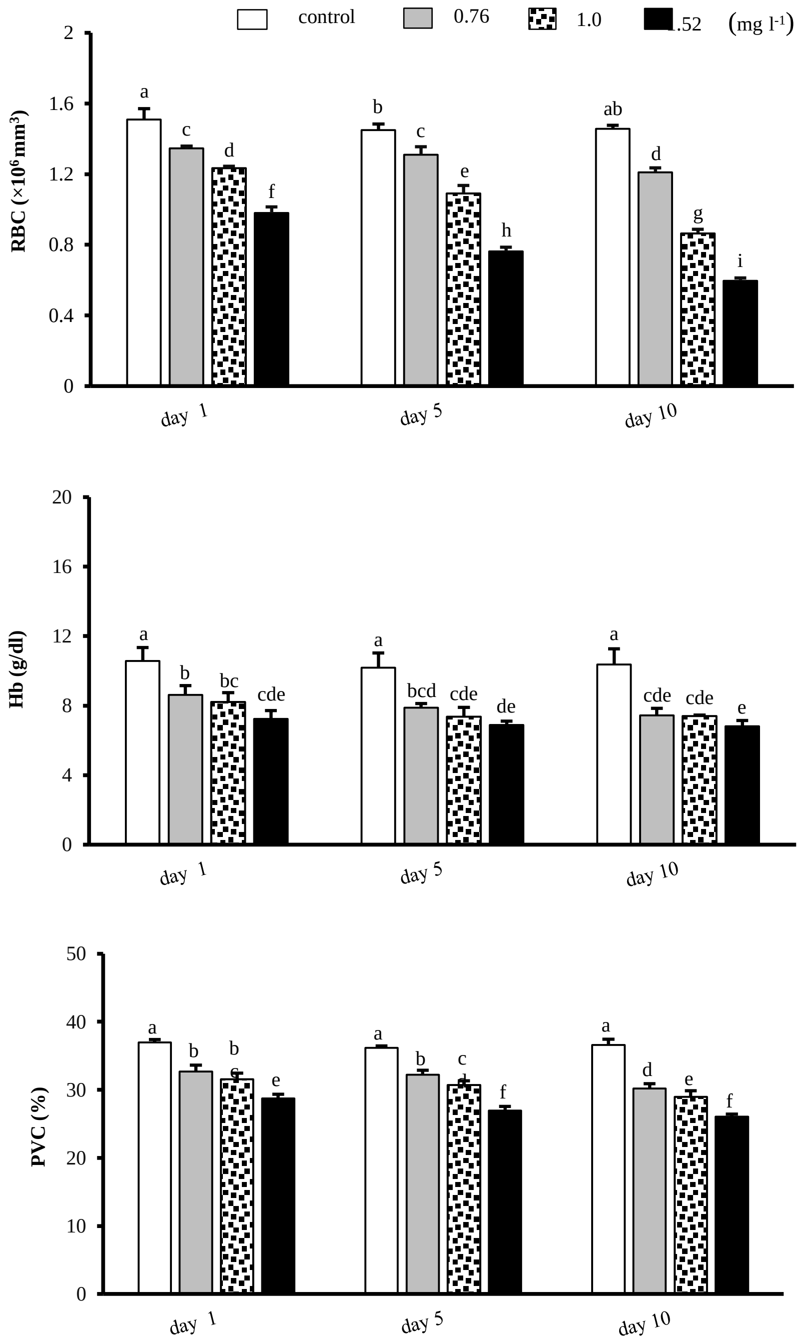

Figure 1. Main haematological parameters: red blood cells (RBC); hematoglobin (Hb), hematocrit (PVC), white blood cells (WBC) . (Error bars represents standard deviation).

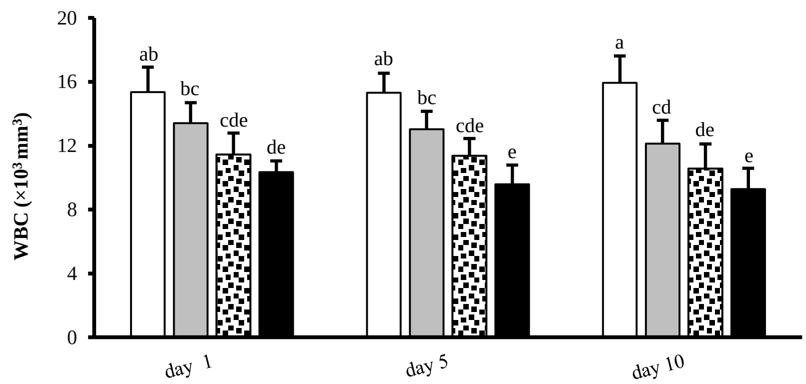

Figure 1. Continued.

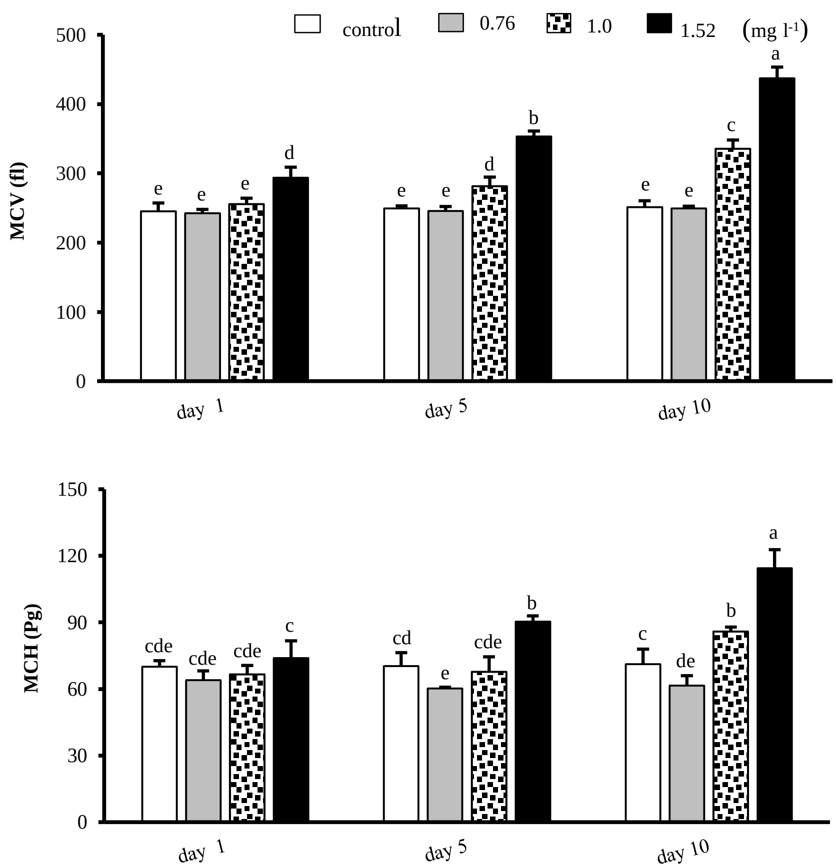

Figure 2. Secondary haematological parameters: mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC). (Error bars represents standard deviation).

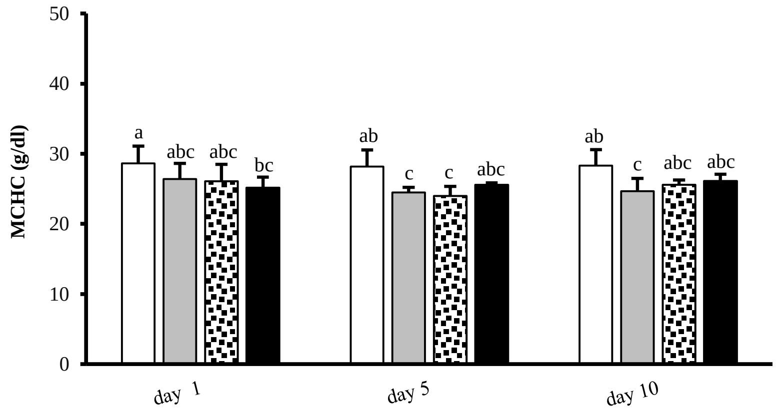

Figure 2. Continued.

Table 2. The correlation coefficients and levels of difference between levels of blood parameters and exposure times of malathion.

Blood Parameter	Exposure time		Exposure concentration	
RBC	r = - 0.839**	p < 0.01	r = - 0.492	p > 0.05
Hb	r = - 0.743*	p < 0.05	r = - 0.570	p > 0.05
PCV	r = - 0.863**	p < 0.01	r = - 0.490	p > 0.05
WBC	r = - 0.910**	p < 0.01	r = - 0.325	p > 0.05
MCV	r = 0.770*	p < 0.05	r = 0.513	p > 0.05
MCH	r = 0.761*	p < 0.05	r = 0.472	p > 0.05
MCHC	r = 0.249	p > 0.05	r = - 0.178	p > 0.05

**P < 0.01; *P < 0.05

DISCUSSION

In the present study, 96h-LC_{50} of malathion was obtained 6.08 (5.22-7.18) mg L^{-1} for *Capoeta damascina*. It has been mentioned in prior studies [36, 37, 40, 43] that malathion has moderate level of toxicity in fishes, where its 96h-LC_{50} values is ranged from 0.1 to 15 mg l^{-1} (Table 2). Our results showed that LC_{50} values is decreased with increase in exposure duration; in other words in longer periods of exposure, lower concentrations of pesticide is caused 50% mortality in studied fish population. This situation had been observed in most studies [28, 39, 41, 46], but it has been reported that the duration of exposure period has no effect on LC_{50} values [34]. Differences in lethal concentrations of pesticides in fishes are dependent on several factors includes fish size and weight, and environmental factors, such as temperature, pH, total alkalinity and dissolved oxygen [28, 35].

Table 2. Malathion LC_{50} values that have been reported for some of other fish species.

Species	LC_{50} (ppm)	Exposure time	Reference
Colisa fasciatus	2.12	96h	[36]
Ictalurus puntatus	9.65	96h	[37]
Labeo rohita	9	96h	[38]
Oreochromis niloticus	2	96h	[39]
Oryzias latipes	1.8	48h	[40]
Channa punctatus	6.61	96h	[28]
Channa punctatus	1.738	96h	[41]
Primephales prmelas	12.5	96h	[42]
Oncorhynchus mykiss	0.1	96h	[42]
Cyprinus carpio	2.1	96h	[43]

Behavior is an appropriate indice in toxicological studies [44]; because behavioral changes in fishes are good indexes of organism response toward aquatic pollutants [45]. Fishes showed behavioral abnormalities in exposure to chemical pollutants [28]. In the present study, exposed fish to malathion at high concentrations showed behavioral abnormalities, where such changes have been reported by prior studies conducted on other fish species (i.e., *Channa punctatus*; *Labeo rohita*; *Ctenopharyngodon idella*) [28, 36, 46]. Absorption of pesticides in gills, with respect to the lipophilic nature of them can cause respiratory limitations [47] that consequently can be resulted in abnormal behavior, such as fish swimming at the water surface and air swallowing by it [36].

Haematological parameters in fish are changed significantly in response to the exposure to chemical stressors in the environment, although these changes are not specific for the wide range of pollutant chemicals [48]. The results of the present study indicated that RBC, hemoglobin, hematocrit and WBC were decreased in fishes exposed to high concentrations of malathion compared to controls. This decreasing pattern has also been observed in prior studies [49-51]. Decrease in erythrocyte number, as well as, hemoglobin and hematocrit indicates anemia due to inhibitory effects of pollutants and their metabolites on erythropoiesis, disturbance in osmotic regulation performance, and increase in destruction of erythrocytes in hematopoietic organs [52-55]. This decrease can also be the result of a compensatory response that may be resulted in improving oxygen carrying capacity in order to maintaining gas exchange in gill lamellae [56]. Another factor that can cause decrease in RBC is the aggregation of them in fish gills in response to pollutants [57]. On the other hand, hematocrit decrease may be due to decrease in RBC or their contraction [58]. Generally, factors resulted in RBC decrease; subsequently reduce hematocrit and Hb content [57, 59].

Our results showed that WBC number in fish was decreased in exposure treatments compared to control group, and this decrease were higher in higher pesticide concentrations and longer exposure periods. The findings of prior studies (such as: *Cyprinus carpio* in exposure to malathion [49]; *Clarias gariepinus* in exposure to malathion [50]; and *Silurus glanis* in exposure to diazinon [53]) is in line with the present results. This decrease in WBC is may be due to the long-term destructive effects of pesticides on tissues that produces white blood cells or releases them into blood current. However, exposure of fish to cypermethrin can be resulted in increase in WBC number [47, 60]. This increase may be due to the short-term pathological responses of defensive system of the organism and releasing leukocytes from spleen into the blood circulation [39, 47, 60]. Higher MCV and MCH values in exposed fishes compared to controls, indicate the erythrocyte swelling or high density of immature red blood cells with lower hemoglobin in blood due to the hyperplasia of erythropoietic tissues [61]. Therefore, changes in these parameters as a compensatory response to the anoxic stress can be resulted in higher gas exchange in gills, because oxygen carrying capacity of blood is decreased with decrease in RBC and Hb in fish in exposure to pesticides [1]. Conflicting results had been reported about the effects of stressor factors, such as environmental pollutants on secondary haematological parameters. Exposure to iron oxide nanoparticles had been resulted to increase in MCV and MCH in *Tilapia mossambicus* [32], while exposure to cadmium had no significant effect on secondary haematological indices in *Acipenser ruthenus* [19]. It seems that response in secondary parameters depends on several factors, including fish species, living conditions, type and concentration of chemical pollutant.

Our results showed that the time of exposure period has significant effect on all of the studied parameters, as the increase or decrease levels in measured parameters were higher in longer times of exposure. Time is reported as an important factor in pollutant toxicity levels for aquatic organisms [62]. There was a significant correlation between levels of haematological parameters (except MCHC) and exposure time, but no correlation was observed between these parameters and exposure concentrations. Negative correlations have been reported between exposure times and haematological parameters [53].

CONCLUSIONS

Malathion is moderately toxic to *Capoeta damascina* and chronic exposure to this pesticide leads to significant changes in fish behavior and its haematological parameters. Besides, the ranges of change in these parameters are significantly correlated to the duration of exposure period (except MCHC). No correlation is existing between considered parameters and exposure concentrations of malathion. Totally, it can be said that very low concentrations of this pesticide in natural water can reduce populations of this species due to its harmful effects, and accordingly, releasing of this chemical to the aquatic ecosystems must be controlled.

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REFERENCES

1. Sarkar B., Chatterjee A., Mahapatra C.T., Ayyappan S., 2004. Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton). Ecotox Environ Safe. 58, 220-226.

2. Moore P.D., Patlolla A.K., Tchounwou P.B., 2011. Cytogenetic evaluation of malathion-induced toxicity in Sprague-Dawley rats. Mutat Res-Gen Tox En. 725(1), 78-82.

3. Vani T., Saharan N., Roy S.D., Ranjan R., Pal A.K., Siddaiah G.M., Kumar R., 2012. Alteration in haematological and biochemical parameters of *Catla catla* exposed to sub-lethal concentration of cypermethrin. Fish Phys Biochem. 38(6), 1577-1584

4. Pan G., Dutta H.M., 1998. The inhibition of brain acetylcholinesterase activity of juvenile largemouth Bass *Micropterus salmoidesby* exposed to sublethal concentrations of diazinon. Environ Res. 79, 133-137.

5. Das B.K., Mukherjee S.C., 2003. Toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical,

enzymatic and haematological consequences. Comp Biochem Phys C. 134, 109-121.

6. Gaafar A.Y., El-Manakhly E.M., Soliman M.K., Soufy H., Zaki S.M., Mohamed S.G., Hassan S.M., 2010. Some pathological, biochemical and haematological investigations on Nile tilapia (*Oreochromis niloticus*) following chronic exposure to edifenphos pesticide. J Am Sci. 6(10), 542-551.

7.Crane M., Maltby L., 1991. The lethal and sublethal responses of *Gammarus pulex* to stress: sensitivity and sources of variation in an in situ bioassay. Environ Toxicol Chem. 10, 1331–1339.

8. Miren P., Cajaraville M.J., Bebianno J.B., Cinta P., Carmen S., Aldo V., 2000. The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian peninsula: a practical approach. Sci Total Environ. 247, 295–311.

9. Svobodova S., 1975. Changes in the blood picture of the carp intoxicated with organophosphate pesticides. Acta Veterinaria Brno. 44, 49-52.

10. Benarji G., Rajendranath T., 1990. Hematological changes induced by an organophosphorus insecticide in a freshwater fish *Clarias batrachus* (Lnnaeus). Trop Freshwater Biol. 2, 197-202.

11.Sampath K., Velammal S., Kennedy I.J., James R., 1990. Hematological changes and their recovery in *Oreochromis mossambicus* as a function of exposure period and sublethal levels of ekalux. Acta Hydrobiolgy. 35, 73-83.

12. Singh N., Srivastava A.K., 1994. Formothion induced hematological changes in the freshwater Indian catfish, *Heteropneustes fossilis.* J Ecotox Environ Monit. 4, 137-140.

13.Nath R., Banerjee V., 1996. Effect of pesticides Methylparathion and cypermethrin on the air-breathing fish *Heteropneustes fossilis*. Environ Ecol. 14, 163-165.

14. Khattak I.U., Hafeez M.A., 1996. Effects of malathion on blood parameters of fish, *Cyprinus wabsoni*. Pak J Zool. 28, 45-49.

15. Houston H., 1997. Review: are the classical hematological variables acceptable indicators for fish health? T Am Fish Soc. 126(6), 879-894.

16. Tavares D.M., Martines M.L., Nascimento K.S., 1999. Evaluation of the hematological parameters in *Piaractus mesopotamicus* Holmberg (Osteichthyes, Characidae) with *Argulus* sp. (Crustucea, Branchiura) infestation and treatment with organophosphate. Rev Bras Zool. 16, 5553-5555.

17. Davis A.K., Maney D.L., Maerz J.C., 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct Ecol. 22(5), 760-772.

18. Adedeji O.B., Adeyemo O.K., Agbede S.A., 2009. Effects of diazinon on blood parameters in the African catfish (*Clarias gariepinus*). Afr J Biotechnol. 8(16), 3940-3946.

19. Orojali M., Paykan Heyrati F., Mahboobi Soofiani N., Dorafshan S., 2013. Cadmium sublethal concentration effects on the haematological parameters of starlet (*Acipenser ruthenus*). J Fish Sci Tech. 2(2), 11-22.

20. Larsson Å., Haux C., Sjöbeck M.L., 1985. Fish physiology and metal pollution: results and experiences from laboratory and field studies. Ecotox Environ Safe. 9(3), 250-281.

21. Thrall M.A., 2004. Veterinary hematology and clinical chemistry. Williams and Wilkins, Philadelphia, 277–289.

22. Talas Z.S., Gulhan M.F., 2009. Effects of various propolis concentrations on biochemical and hematological parameters of rainbow trout (*Oncorhynchus mykiss*). Ecotox Environ Safe. 72, 1994–1998.

23. Kavitha C., Malarvizhi A., Senthil Kumaran S., Ramesh M., 2010. Toxicological effects of arsenate exposure on hematological, biochemical and liver transaminases activity in an Indian major carp, *Catla catla*. Food Chem Tox. 48, 2848–2854.

24. Remyla, S.R., Ramesh, M., Sajwan, K.S., Kumar, K.S., 2008. Influence of zinc on cadmium induced haematological and biochemical responses in a freshwater teleost fish *Catla catla*. Fish Phys Biochem. 34(2), 169-174.

25. Verma S.R., Rani S., Dalela R.C., 1982. Indicators of stress induced by pesticides in *Mystus vittatus*: haematological parameters. Ind J Environ Health. 24, 58-64.

26. Edsall C.C., 1999. A blood chemistry profile for lake trout. J Aquat Anim Health. 11, 81-86.

27. Li Z.H., Li P., Randak T., 2010. Ecotoxicological effects of short-term exposure to a human pharmaceutical Verapamil in juvenile rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Phys C. 152(3), 385-391.

28. Pandey S., Kumar R., Sharma S., Nagpure N.S., Srivastava S.K., Verma M.S., 2005. Acute toxicity bioassays of mercuric chloride and malathion on air-breathing fish *Channa punctatus* (Bloch). Ecotox Environ Safe. 61(1), 114-120.

29. Areechon N., Plumb J.A., 1990. Sublethal effects of Malathion on channel catfish, *Ictalurus punctatus*. Bull Environ Contam Tox. 44(3), 435-442.

30. Asadollah S., Soofiani N.M., Keivany Y., Shadkhast M., 2011. Reproduction of *Capoeta damascina* (Valenciennes, 1842), a cyprinid fish, in Zayandeh-Roud River, Iran. J Appl Ichthyol. 27, 1061-1066.

31. APHA (American Public Health Association). Standard Methods for the examination of water and wastewater. Sixteenth ed., Washing-ton, DC, 1985.

32. Atcf M., Attar A., 2005. Changes in hematological parameters of the fish, *Oreochromis niloticus* treated with sublethal concentrations of cadmium. Pak J Biol Sci. 8(3), 421-424.

33. Karthikeyeni S., Vijayakumar T.S., Vasanth S., Ganesh A., Manimegalai M., Subramanian P., 2013. Biosynthesis of iron oxide nanoparticles and its haematological effects on fresh water fish *Oreochromis mossambicus*. J Acad Indust Res. 10, 645-649.

34. Lakota S., Razska A., Utracki T., Chmiel Z., 1989. Side-effect of deltamethrin and cypermethrin in the environment of water biocenoses. Organika. 71, 71-77.

35. Gupta P.K., Khangarot B.S., Durve V.S., 1981. The temperature dependence of the acute toxicity of copper to a freshwater pond snail, *Viviparus bengalensis* L. Hydrobiol. 83, 461–464.

36. Singh S. K., Tripathi P. K., Yadav R.P., Singh D., Singh A., 2004. Toxicity of malathion and carbaryl pesticides: effects on some biochemical profiles of the freshwater fish *Colisa fasciatus*. Bull Environ Contam Toxicol. 72(3), 592-599.

37. Areechon N., Plumb J. A., 1990. Sublethal effects of Malathion on channel catfish, *Ictalurus punctatus*. B Environ Contam Tox. 44(3), 435-442.

38. Patil V. K., David M., 2008. Behaviour and respiratory dysfunction as an index of malathion toxicity in the freshwater fish, *Labeo rohita* (Hamilton). Turk J Fish Aquat Sci. 8, 233-237.

39. Pathiratne A., George S.G., 1998. Toxicity of malathion to nile tilapia, *Oreochromis niloticus* and modulation by other environmental contaminants. Aquat Toxicol. 43(4), 261-271.

40. Tsuda T., Kojima M., Harada H., Nakajima A., Akoi S., 1997. Acute toxicity, accumulation and excretion of organophosphorus insecticides and there oxidation products in killifish. Chemosphere. 35, 939–949.

41. Khangarot B.S., Ray P.K., 1988. Studies on the toxicity of malathion to freshwater teleosts, *Channa punctatus* (Bloch) and *Puntius sophore* (Hamilton). Arch Hydrobiol. 113, 465–469.

42. Pickering Q.H., Henderson C., Lemke A.E., 1962. The toxicity of organic phosphorus insecticides to different species of warm water fishes. T Am Fish Soc. 91(2), 175-184.

43. Kaur K., Dhawan A., 1993. Variable sensitivity of *Cyprinus carpio* eggs, larvae, and fry to pesticides. Bull Environ Contam Tox. 50(4), 593-599.

44. Drummond R.A., Russom C.L., 1990. Behavioral toxicity syndromes: A promising tool for assessing toxicity mechanisms in juvenile fathead minnows. Environ Toxicol Chem. 9(1), 37-46.

45. Weis J.S., Candelmo A., 2012. Pollutants and fish predator/prey behavior: A review of laboratory and field approaches. Cur Zool. 58(1), 9-20.

46. El Ella S.M.A. 2008. Toxicity of malathion and its effect on the activity of acetylcholinesterase in various tissues of the grass carp, *Ctenopharyngodon idella* Val. Egyptian J Aquat Biol Fish. 12(2), 109 -117.

47. Masud S., Singh I.J., 2013. Effect of Cypermethrin on some hematological parameters and prediction of their recovery in a freshwater Teleost, *Cyprinus carpio*. Afr J Environ Sci Tech. 7, 852-856.

48. Modesto K.A., Martinez C.B., 2010. Effects of Roundup Transorb on fish: hematology, antioxidant defenses and acetylcholinesterase activity. Chemosphere. 81(6), 781-787.

49. Mişe Yonar S., Ural M.Ş., Silici S., Yonar M.E., 2014. Malathion-induced changes in the haematological profile, the immune response, and the oxidative/antioxidant status of *Cyprinus carpio*: Protective role of propolis. Ecotox Environ Safe. 102, 202-209.

50. Ahmad Z., 2012. Toxicity bioassay and effects of sub-lethal exposure of malathion on biochemical composition and haematological parameters of Clarias gariepinus. Afr J Biotechnol. 11(34), 8578-8585.

51. Al-Ghanim K.A., 2012. Acute toxicity and effects of sub-lethal malathion exposure on biochemical and haematological parameters of *Oreochromis niloticus*. Sci Res Essays. 7(16), 1674-1680.

52. Powell D.M., Perry F.S., 1997. Respiratory and acid-base pathophysiology of hydrogen peroxide in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquat Toxicol. 37, 99–l12.

53. Köprücü S.Ş., Köprücü K., Ural M. Ş., İspir Ü., Pala M., 2006. Acute toxicity of organophosphorous pesticide diazinon and its effects on behavior and some hematological parameters of fingerling European catfish (*Silurus glanis* L.). Pest Biochem Phys. 86(2), 99-105.

54. Rosety-Rodriguez M., Ordonez F.J., Rosety I., Rosety J.M., Rosety M., 2005. Erythrocyte antioxidant enzymes of gillhead as early warning bio-indicators of oxidative stress induced by malathion. Haematology. 8, 237–240.

55. Vani T., Saharan N., Mukherjee S.C., Ranjan R., Kumar R., Brahmchari R.K., 2011. Deltamethrin induced alterations of hematological and biochemical parameters in fingerlings of *Catla catla* (Ham.) and their amelioration by dietary supplement of vitamin C. Pestic Biochem Phys. 101(1), 16-20.

56. Jee J.H., Masroor F., Kang J.C., 2005. Responses of cypermethrin-induced stress in haematological parameters of Korean rockfish, *Sebastes schlegeli* (Hilgendorf). Aquac Res. 36(9), 898-905.

57. Narain A.S., Srivastava P.N., 1989. Anemia in the freshwater teleost, *Heteropneustes fossilis*, under the stress of environmental pollution. Bull Environ Contam Tox. 43, 627-634.

58. Singh N.N., Srivastava A.K., 2010. Haematological parameters as bioindicators of insecticide exposure in teleosts. Ecotox. 19, 838-854.

59. Banaee M., Mirvagefei A.R., Rafei G.R., Majazi Amiri B., 2008. Effect of sub-lethal diazinon concentrations on blood plasma biochemistry. Int J Environ Res. 2, 189-198.

60. Akinrotimi O.A., Orlu E.E., Gabriel U.U., 2013. Haematological Responses of *Tilapia guineensis* Treated with Industrial Effluents. Appl Ecol Env Sci. 1(1), 10-13.

61. Ferrando H.D., Moliner E.A., 1991. Effect of lindane on the blood of a freshwater fish. Bull Environ Contam Toxicol. 47, 465–470.

62. Richterová Z., Svobodová Z., 2012. Pyrethroids influence on fish. Slov Vet Res. 49(2), 63-72.