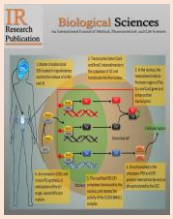
RESEARCH ARTICLE

Biological Sciences
(An International Journal of Medical, Pharmaceutical, and Life Sciences)

Journal home: https://irrespub.com/biolsciences/index.php/1/index



Assessment of haematological and histopathological effects of mixture toxicity of phenanthrene and benzo[a]pyrene in catfish

Uchechukwu O. Osuagwu*, Cosmas O. Ujowundu, Linus A. Nwaogu and Reginald N. Nwaoguikpe

Department of Biochemistry, Federal University of Technology, Owerri, Nigeria

ARTICLE HISTORY

Received: 01-04-2023
Revised: 27-04-2023
Accepted: 08-05-2023
Online: 13-05-2023

KEYWORDS

Subchronic
Phenathrene
Benzo[a]pyrene
Safe level
Hematology
Histopathology
Growth

ABSTRACT

The effect of joint mixtures of phenanthrene and benzo[a]pyrene on selected hematological and histopathological parameters of the *Clarias gariepinus* was investigated. Healthy juvenile *C. gariepinus* (n = 90) weighing 19.7 ± 1.8 g were exposed to sublethal concentrations of joint mixtures of phenanthrene and benzo[a]pyrene for a subchronic period of 35 days. Acute toxicity studies showed that phenanthrene and benzo[a]pyrene had LC50 values of 1400 and 16 µg/L respectively while the safe limits of the chemicals varied in the ranges of 0.00016 to 1.6 µg/L and 0.014 to 140 µg/L for benzo[a]pyrene and phenanthrene respectively. The joint mixture significantly ($p < 0.05$) reduced growth rate in exposed fish. The joint mixtures also led to significant ($p < 0.05$) declines in the studied hematological parameters including blood cell count (RBC), hemoglobin (Hb), and hematocrit (Hct). The erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) all showed significant ($p < 0.05$) declines in the presence of the joint toxicants. There were significant ($p < 0.05$) increases in white blood cell (WBC) count and platelet (PLT) count. Histopathological examination showed alterations in the liver and gill sections of exposed fish.

Introduction

Human activities have led to an upsurge in the release of hydrocarbons such as those found in petroleum. These hydrocarbons though of economic benefit pose significant risks to both the aquatic ecosystem and human health (1). Polycyclic aromatic hydrocarbons (PAHs) are a major component of these hydrocarbons. Although PAHs have low water-solubility and are short-lived in the water column, they persist in the sediment and can cause severe behavioural and physiological alterations in aquatic organisms (2). Phenanthrene and benzo[a]pyrene are among the sixteen PAHs that have been identified as priority contaminants by the World Health Organization (WHO) because they can bioaccumulate in the environment and result in contamination of the food chain (1). Both phenanthrene and benzo[a]pyrene occur in fossil fuels and are present in products of incomplete

*Address for correspondence
Department of Biochemistry, Federal University of Technology, Owerri, Nigeria

Email: osuagwu.uo@gmail.com
DOI: https://doi.org/10.55006/biolsciences.2023.3202
Published by IR Research Publication;

combustion. Some of the known sources of both compounds in the atmosphere are automobile emissions, coal burning, wood combustion, and coke plants (3). They are widely distributed in the aquatic environment and have been identified in surface water and tap water. They have also been identified in seafood collected from contaminated waters and in smoked fish and other foods (3).

Phenanthrene and benzo[a]pyrene have served as markers of oil pollution (4) and are reasonably anticipated as carcinogens (5). Petroleum hydrocarbons have been reported to cause structural damage to the respiratory lamellae of the gills (6). The compounds tend to adsorb to organic or inorganic matter in sediments and can be trapped in long-term reservoirs. There is substantial uptake of these compounds by aquatic organisms through diet, exposure to contaminated water, and through direct contact with sediment (7).

Clarias gariepinus was selected as the test organism, because of its hardiness, and ecological and commercial importance in several tropical countries It is also a model organism that has been used extensively in ecotoxicological research (8).

The present study aimed to study the effects of a joint mixture of phenanthrene and benzo[a]pyrene on the growth, hematology, and tissues of *C. gariepinus*.

Materials and methods

Chemicals

Phenanthrene and benzo[a]pyrene was obtained from Sigma Aldrich (Germany). Acetone was obtained from BDH Chemicals (UK).

Animals

African catfish *C. gariepinus* (19.7 ±1.8 g) were obtained from commercial aquaculture and transferred to a 100 L maintenance tank. Fish were acclimated to the laboratory condition for 2 weeks prior to the experiment and fed twice daily with commercial fish feed. Fecal matter and uneaten food were removed daily to prevent water pollution. Fish were maintained on a 12-h light/dark cycle at all times.

Acute toxicity study

A range-finding test was used to determine the appropriate concentrations of exposure. Based on the obtained pre-LC50 data, the 96-hour LC50 was determined for phenanthrene and benzo[a]pyrene according to the revised OECD guidelines for testing of chemicals (2019). Triplicate sets of 7 fish each were randomly exposed to varying concentrations of phenanthrene and benzo[a]pyrene separately. Acetone was used as a solvent carrier. The fish were placed in 30 L plastic tanks containing 20 L of clean tap water. Another set of 7 fish was also maintained with an equal amount of tap water (and solvent carrier) but without the test chemicals and considered as the solvent control. Fish were not fed throughout the experiment and lethality was the toxicity endpoint. Dead fish were removed and the mortality was recorded at intervals of 24, 48, 72, and 96 h. The 96-hour LC50 value of naphthalene for the fish was determined by probit analysis.

Sublethal toxicity study

Stock solutions of phenanthrene and benzo[a]pyrene were prepared by dissolving the chemicals in distilled water and taking acetone as a solvent carrier. Test solutions were prepared by dilution of stock solutions in tap water. During sublethal studies, fish were exposed to a mixture of 50% and 25% of the LC50 value of both chemicals (corresponding to treatment levels 1 and 2). A solvent control was included in the experimental design. Fish were kept in groups of 10 in 30 L plastic tanks containing the test solutions. Experiments were performed in triplicates. The period of exposure lasted 5 weeks.

Assays

At the end of the exposure period, hypothermia was used to anesthetize the fish. Blood was then collected from the immobilized fish by caudal vein puncture method as described by Argungu et al. (10) using a 5ml sterile disposable syringe with a 22 gauge needle. The blood was transferred to EDTA tubes and transported to the lab for analysis.

The liver and gill tissues were dissected and placed in a 10% formal saline solution prior to histopathological investigation.

Hematology

Hematological parameters were determined using an automated hematology analyzer machine (Mindray BC 2300, USA).

Weight gain (WG)

Weight gain was determined using the method of Thaller et al (2014):

$$WG = \frac{Wt\ (final\ weight) - Wi\ (initial\ weight)}{Wi\ (initial\ weight)} \times 100$$

Statistical analysis

Results were expressed as mean ± standard error. Data from the different treatment groups were compared by a one-way analysis of variance (ANOVA) followed by a Scheffes test to determine statistically different groups. All differences were considered significant at $p < 0.05$. Statistical analysis was performed using the SPSS statistical package (ver. 24.0 SPSS Company, Chicago, IL, USA).

Results

The results for the acute toxicity tests are shown in **Figures 1** and **2**, corresponding to phenanthrene (Phe) and benzo[a]pyrene (BaP) respectively. While the LC50 for Phe was 1400 µg/L, the LC50 for BaP was 16 µg/L. While there were no recorded mortalities in the control group, there was 100% mortality in the highest concentration groups corresponding to 3200 and 64 µg/L for the Phe and BaP groups respectively.

Tables 1 and **2** show the safe limit values for Phe and BaP ranged from 0.014 to 140 and 0.00016 to 1.6 µg/ L respectively.

Figure 3 shows the inhibitory effect of Phe and BaP joint compounds on the growth of *C. gariepinus*. The result shows that joint compounds of Phe and BaP inhibited growth in a concentration dependant pattern.

The impact of the joint compounds on selected blood parameters is shown in Table 3. The joint compounds lead to significant ($p < 0.05$) declines in red blood cell (RBC) count, hemoglobin (Hb) concentration, and hematocrit (Hct). Erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCV), and mean corpuscular hemoglobin concentration (MCHC) all showed significant ($p < 0.05$) declines.

Significant ($p < 0.05$) increases were observed in white blood cell (WBC) count and plate (PLT) counts.

Figures 4 to 7 show the histopathology results. **Figure 4** is the normal impression of the gills in the control group while **Figure 5** shows the gill section for the group at the second level of treatment. The gills in this group showed disorganized lamella. While **Figure 6** showed normal architecture for fish liver in the control group, **Figure 7** shows dense infiltrates for the liver tissues at the second level of treatment.

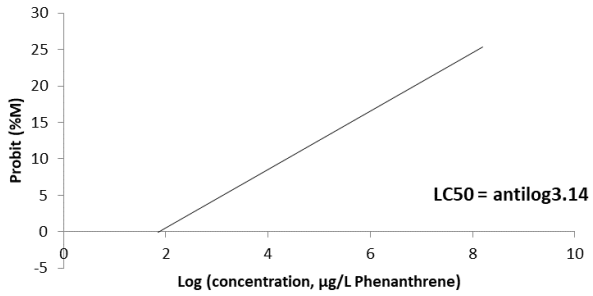


Fig 1. shows the linear regression curve of log10 concentration versus the probit of phenanthrene-induced mortality on catfish. y = 3.99x - 7.43. The LC50 was 1400 µg/L

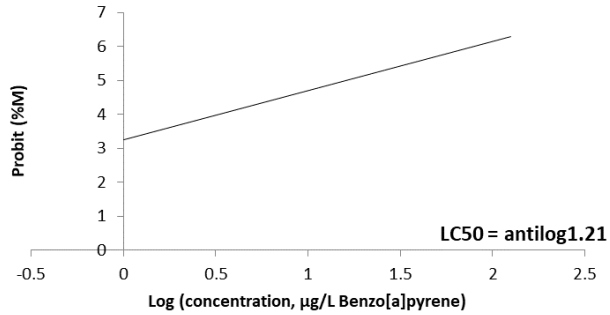


Fig 2. shows the linear regression curve of log10 concentration versus probit of benzo[a] pyrene-induced mortality on catfish. y = 1.451x + 3.24. The LC50 was 16 µg.

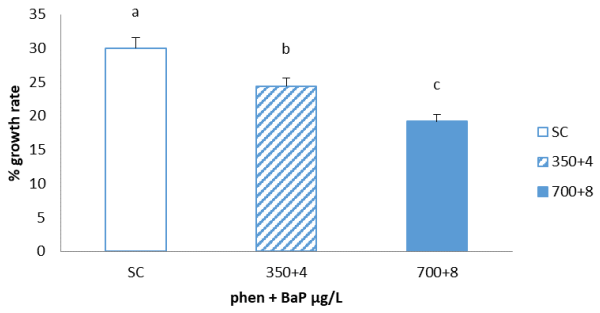


Fig 3. Growth rate of C gariepinus on exposure to joint mixtures of phenanthrene and benzo[a]pyrene. Means not sharing the same letter (a, b or c) are statistically different at $p < 0.05$.

Discussion

The anticipated toxic effect of chemical substances is usually represented as their LC50 describes the anticipated toxic effect of chemicals. The LC50 for phenanthrene and benzo[a]pyrene were to be 1400 and 16 µg/L respectively. The huge difference in acute toxicity could be as a result of benzo[a]pyrene being more strongly lipophilic compared to phenanthrene. The LC50 for phenanthrene for

Table 1. Estimated safe level limits for phenanthrene.

PAH	96h LC_{50} (μgl^{-1})	Method	AF	Safe level (μgl^{-1})
Phenanthrene	1400	Sprague (35)	0.1	140
		CWQC (35)	0.01	14
		NAS/NAE (35)	0.01-0.00001	14 – 0.014
		CCREM (35)	0.05	70
		IJC (35)	5% of 96h LC_{50}	70

Table 2. Estimated safe level limits for benzo[a]pyrene.

PAH	96h LC_{50} (μgl^{-1})	Method	AF	Safe level (μgl^{-1})
Benzo[a]pyrene	16	Sprague (35)	0.1	1.6
		CWQC (35)	0.01	0.16
		NAS/NAE (35)	0.01-0.00001	0.16 – 0.00016
		CCREM (35)	0.05	0.8
		IJC (35)	5% of 96h LC_{50}	0.8

Table 3. Haematological parameters of *C. gariepinus* exposed to joint mixtures of phenanthrene and benzo[a]pyrene.

Parameter	Control group	Phen + BaP joint 350+4 µg/L	concentration 700+8 µg/L
RBC (x10^9cell/L)	2.41±0.06^a	2.35±0.13^b	2.17±0.43^c
Hb (g/L)	102.66±3.71^a	99±1.9^b	91.28±2.8^b
Hct (%)	34.85±2.38^a	34.13±2.13^a	30.1±0.09^b
MCV (fL)	147.5±2.5^a	143.8±0.5^b	138.5±0.96^b
MCH (pg)	43.3±0.24^a	41.15±0.52^b	39.6±1.1^b
MCHC (g/L)	305.33±4.4^a	279.33±3.1^b	272±1.2^b
WBC (x10^9 cell /L)	135.56±2.98^a	146.86±3.3^b	168.74±4.1^c
PLT (x10^9 cell /L)	12.66±1.33^a	18.33±2.7^b	19±1.82^b

Means not sharing the same letter (a, b or c) are statistically different at $p < 0.05$.

different aquatic organisms as determined by other researchers are 432 µg/L for *T. obscurus* (11); 940 µg/L for *C. macropomum* (12) and 4676 µg/L for *C. fluminea* (13). The LC50 for benzo[a]pyrene in C. chanos was 1.4 µg/L (14). The observed differences in LC50 could be a result of differences in the metabolism of the different organisms. Environmental factors, size, and regional influences could also play important roles in determining acute toxicities of PAHs (14).

There were huge variations in the estimated levels of safety for both toxicants. The estimated safe levels as determined by various methods obtained in the present study showed a large variation. Variations obtained in estimated safe level measurements are controversial with regard to acceptability (15). Extrapolation of laboratory data to the field is not always consistent and may be difficult to accept based on the method employed. Furthermore, the calculation of application faction (AF) which is dependent on LC50 value is a major source of weakness (16).

Growth is a continuous increase in average mass. The present study shows that the growth rate was inhibited by sublethal concentrations of the joint mixture of phenanthrene and benzo[a]pyrene. It can be assumed that increasing the sub-lethal concentration of the joint mixture led to a decrease in food utilization which culminated in a reduced growth rate. Pollutants may induce changes at the biochemical level prior to cellular and tissue malfunction (17). Pavlov et al. (18) reported a significant decline in the growth rate of Mozambique tilapia exposed to cadmium and naphthalene. Jee et al. (19) demonstrated a significant reduction in the relative growth rate of flounder exposed to varying concentrations of phenanthrene.

Red blood cells are essential in maintaining blood pH and regulation of blood flow to tissues and organs (20). Several chemical pollutants have been shown to reduce the measured quantities of red blood cells in circulation (21). Aquatic organisms exposed to phenanthrene have been shown to have low RBC counts. Mehrnaz et al. (22) reported that phenanthrene-exposed yellowfin seabream had reduced red blood cell counts. This finding is in line with the results obtained in the present study. The observed decline in RBC count could be a result of inhibition of erythropoiesis in hematopoietic tissues, internal hemorrhage, and/or necrosis of blood cells (23). Kim et al. (24) demonstrated that subchronic

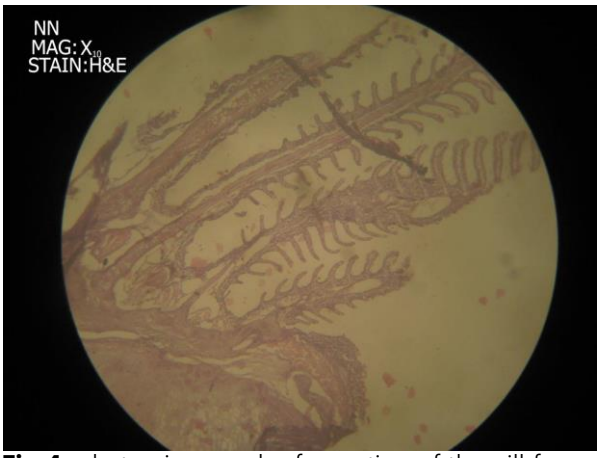


Fig 4. photomicrograph of a section of the gill from the control showing normal impression.

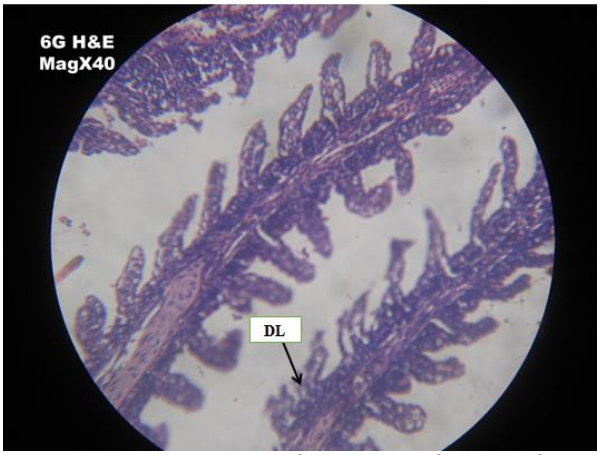


Fig 5. Photomicrograph of a section of the gill from the group exposed to Benzo[a]pyrene and phenanthrene (B+P) mixture showing disorganized lamella (DL).

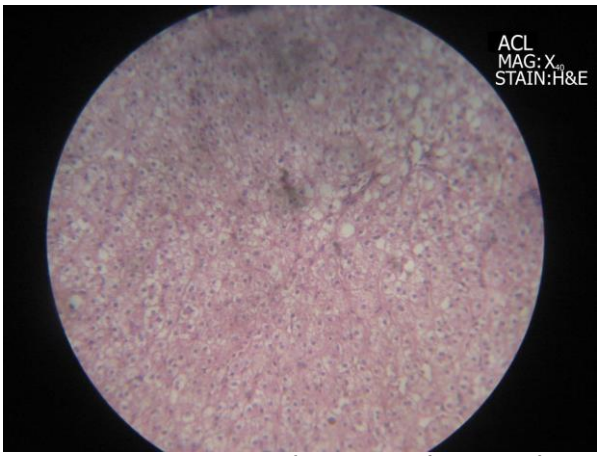


Fig 6. Photomicrograph of a section of the liver from the control group showing normal impression.

benzo[a]pyrene exposure in rockfish caused a significant reduction in erythrocytes as well as haemoglobin concentration.

Haemoglobin which is synthesized in the bone marrow is the primary intracellular polypeptide of the red blood cells (25). The reduction in

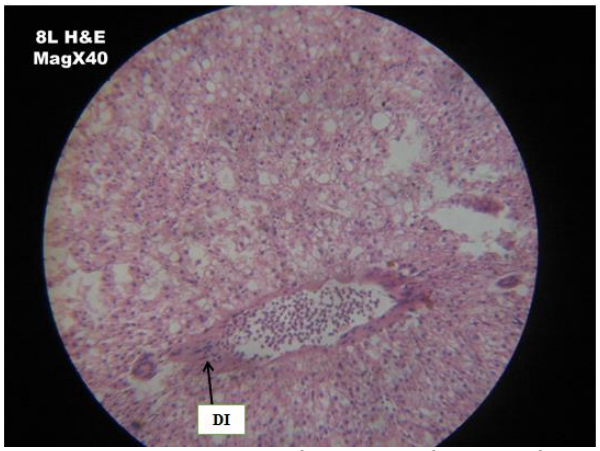


Fig. 7. Photomicrograph of a section of the liver from the group exposed to benzo[a]pyrene and phenanthrene mixture (B +P), showing dense infiltrates (DI)

hemoglobin concentration as observed in the present study may be due to the disruptive effect of the PAHs on the erythropoietic tissues. Recently, Parmar and Shar (26) demonstrated significant declines in both red blood cell count and hemoglobin concentration of C. catla exposed to RR 120 azo dye. Hemoglobin concentration can serve as a very sensitive bioindicator of alterations in ecological conditions (26).

The low HCT values observed in this work correlated with the reduction in red blood cell count as a result of PAH toxicity. A low hematocrit level is a direct consequence of a low red blood cell count (27). The observed low HCT values in this work may be attributed to the combined effects of erythropoiesis inhibition, iron metabolism alteration, and destruction of red blood cells (28). Glusczak et al. (29) reported a significant decline in HCT in L obtuseness exposed to glyophosate. Barcellos et al. (30) showed that fish species exposed to toxic chemicals had lower Hematocrit, hemoglobin concentration, and erythrocyte counts.

Erythrocyte indices are useful in understanding the etiology of anemias. Anemias are classified, according to the size of the erythrocyte, as being normocytic (normal MCV), macrocytic (increased MCV), or microcytic (decreased MCV) (31). There were significant declines ($p < 0.05$) in all studied erythrocyte indices.

There was an increase in the levels of circulating white blood cells in exposed fish pointing to the immunotoxicity of the PAH compounds. White blood cells are part of the immune system and participate in immune responses. Ramesh and Saravanan (32) reported increases in WBC count in *L. rohitato* exposed to deltamethrin at sublethal concentrations. Platelets which are not cells play important roles in the inflammatory process. Under

stress conditions such as that obtained in the present study, platelet counts are increased to ameliorate hemorrhage (21).

Histopathological alterations can be used to evaluate the health status of fish exposed to pollutants. In the present study, liver and gill sections were examined to assess the impact of the joint PAH mixtures on the tissues. While fish from the control group showed normal gill and liver architecture, exposed fish showed disorganized lamella of the gills and dense infiltrates in the liver tissues. Cell infiltrates which are seen in damaged tissues comprise plasma cells, lymphocytes, and macrophages. The presence of dense cell infiltrates indicates that PAH exposure led to structural damage in the liver (33). The observed disorganized lamella in the present work could be a protective mechanism since it leads to a reduction in the total surface area of the gills (34). A similar change has been reported in *H. fossilis* after exposure to neem extract (34).

Conclusion

The joint mixture of phenanthrene and benzo[a]pyrene had deleterious effects on the health status of exposed fish. There is a need to intensify research in the area of PAH ecotoxicology, especially using the tropical catfish as a model organism. The information obtained from this study will help to assess the effects of PAH mixtures on aquatic organisms and to establish water quality criteria in tropical countries.

Contribution of authors

Uchechukwu O. Osuagwu participated in all the experiments, performed the LC50 and subchronic study, and contributed to the writing of the manuscript. Cosmas O. Ujowundu participated in all the experiments and contributed to the writing of the manuscript. Linus A. Nwaogu participated in the experiment and coordinated the laboratory work. Reginald N. Nwaoguikpe designed and supervised the experiments and proofread the manuscript.

Acknowledgments

The authors wish to thank Adaugo, Precious and Francisca for their assistance in handling the experimental animals.

Conflict of interest

The authors declare no conflict of interest.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

References

1. Rahmanpour S, Ghorghani N. Ashtiyani S. Polycyclic aromatic hydrocarbon (PAH) in four fish species from different trophic levels in the Persian Gulf. Environmental Monitoring and Assessment. 2014; 186: 7047-7053.
2. Santos T, Gomes V, Passos M. Histopathological alterations in gills of juvenile Florida pompano Trachinotus carolinus (Perciformes, Carangidae) following sublethal acute and chronic exposure to naphthalene. Pan-American Journal of Aquatic Science. 2011; 6: 109-120.
3. U.S. EPA (U.S. Environmental Protection Agency). Drinking Water Criteria Document for Polycyclic Aromatic Hydrocarbons (PAHs). Prepared by the Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, U.S. Environmental Protection Agency, Cincinnati, OH, for the Office of Drinking Water; 1998.
4. Ogata M, Miyake Y. Disappearance of aromatic hydrocarbons and organic sulphur compounds from fish reared in crude oil suspensions. Water Resources. 1979; 13: 75– 78.
5. Andelman JB, Snodgrass JE. Incidence and significance of polynuclear aromatic hydrocarbons in the water environment. Critical Reviews in Environmental Control. 1974; 4 (1): 69– 83.
6. Prasad MS. SEM study on the effects of crude oil on the gills and airbreathing organs of climbing perch Anabas testudineus. Bulletin of Environmental Contamination and Toxicology. 1991; 47: 882– 889.
7. Johnson LL, Collier TK, Stein JE. An analysis in support of sediment quality thresholds for Polycyclic Aromatic Hydrocarbons (PAHs) to protect estuarine fish. Aquatic Conservation. 2002; 12(5): 517– 538.
8. Esenowo IK, Ugwumba OA. Growth response of catfish (Clarias gariepinus) exposed to water-soluble fraction of detergent and diesel oil. Environmental Research Journal. 2010; 4: 298–301.
9. OECD. Test No. 203: Fish acute toxicity test, OECD guidelines for the testing of chemicals, Section 2. Paris: OECD Publishing; 2019
10. Argungu LA, Siraj SS, Christanus A. A simple and rapid method for blood collection from walking catfish. Iranian journal of fisheries sciences. 2015; 16(3): 934 – 944.
11. Lee JS, Lee KT, Kim DH. Acute toxicity of dissolved inorganic metals, organotins and polycyclic aromatic hydrocarbons to puffer fish,

Takifugu obscurus. Environmental Analysis Health and Toxicology. 2004: 19(2): 141 – 151.
12. Chávez-Veintemilla C, Val A. Effects of phenanthrene on the amazonian fish Tambaqui Colossoma macropomum: LC50, growth and haematology. Environment and Ecology Research. 2019; 7(5): 293 – 302.
13. Xiao Y, Vijver MG, Chan G, Willies IG. Toxicity and accumulation of Cu and ZnO nanoparticles in Daphnia magna. Environmemntal Science and Technology. 2015; 49(7): 4657 – 4664.
14. Palanikumar N, Kumaraguru CM, Ramakritinan,M. Biochemical response of anthracene and benzo[a]pyrene in milkfish Chanos chanos L. Ecotoxicology and Environmental Safety. 2012; 75: 187–197.
15. Pandey S, Kumar R, Sharma S. Acute toxicity bioassays of mercuric chloride and malathion on airbreathing fish Channa punctatus (Bloch). Ecotoxicology and Environmental Safety. 2015; 61(1): 114- 120.
16. Kenega EF. Aquatic test organism and methods useful for assessment of chronic toxicity of chemicals. In: Dickson KL, Maki AW, Cairns J. (Eds.). Analysing the Hazard Evaluation Process. Washington DC: American Fisheries Society; 1979.
17. Sies H. Oxidative Stress. San Diego, CA: Academic Press; 2013.
18. Pavlov DF, Chuiko GM, Pavlov DD. Growth of Mozambique Tilapia (Oreochromis mossambicus Peters) Chronically Exposed to Cadmium, Naphthalene, and DDVP. Inland Water Biology. 2014; 7: 97 – 100.
19. Jee JH, Kim SG, Kang JC. Effects of phenanthrene on growth and basic physiological functions of the olive flounder Paralichthys olivaceus. Journal of Experimental Marine Biology and Ecology. 2004; 304:123 – 136.
20. Bloom JC, Brandt JT. Toxic responses of the blood. In: Klaassen, C.D. (Ed.) Casarett & Doull's Toxicology: The Basic Science of Poisons. New York: McGraw-Hill Companies; 2008.
21. Dey S, Palas S, Niladri S. Dose specific responses of Anabas testudineus (Bloch) to anthracene (PAH): Haematological and biochemical manifestation. Emerging Contaminants. 2019; 5: 232 – 239.
22. Mehrnaz S, Negin S, Mohammad TR. Effect of phenanthrene on the tissue structure of liver and aminotransferase enzymes in Yellowfin Seabream (Acanthopagrus latus) Iranian Journal of Toxicology. 2017; 11(4): 201 – 207.
23. Khaniyan M, Salamat N, Safahieh A, Movahedinia A. Detection of benzo [a] pyrene-induced immunotoxicity in orange spotted grouper (Epinephelus coioides). Environmental Toxicology. 2016; 31(3): 329 – 338.
24. Kim SG, Park DK, Jang SW, Lee JS, Kim SS, Chung MH. Effects of dietary Bezo[a]pyrene on growth and hematological parameters in juvenile rockfish, Seabastes schlegeli (Hilgendorf). Bulletin of Environmental Contamination and Toxicology 2017; 81: 470 – 474.
25. Chhabra N. Biochemistry for Medics. New Delhi: Jaypee Brothers; 2013.
26. Parmar A, Shah I. Acute toxicity, behavioural response and haematological alterations of Catla catla exposed to Reactive Red 120 textile dye. Indian Journal of Experimental Biology. 2021; 6(59): 275 – 279.
27. Mondal H. Hematocrit. 2021. Accessed 27 October, 2019. Available: http://www.statpearls.com/articlelibrary/viewarticle/36935
28. Eriegha OJ, Omitoyin BO, Jani EK. Evaluation of Haematological and Biochemical Parameters of Juvenile Oreochromis niloticus after Exposure to Water Soluble Fractions of Crude Oil. Journal of Applied Science and Environmental Management. 2017; 21(6): 1041 – 1045.
29. Glusczak L, Denis D, Crestani M. Effects of glyophosate herbicide on acetylcholinesterase activity and metabolic and haematological parameters in Piava,. Ecotoxicology and environmental safety. 2006; 65(2): 237 – 214.
30. Barcellos L, Carlos K, Rodriguez L. Hematological and biochemical characteristics of male jumdia: changes after acute stress. Aquaculture Research. 2003; 34(15): 1465 – 1469.
31. Walker HK, Hall WD, Hurst JW. Clinical Methods: The History, Physical, and Laboratory Examinations. Boston: Butterworths; 1990.
32. Ramesh M, Saravanan M. Haematological and biochemical responses in a fresh water fish Cyprinus carpio exposed to chorpyrifos. International Journal of Integrative Biology, 2008; 3(1): 80 – 84.
33. Bluemel J, Korte S, Gerhard F. The nonhuman primate in nonclinical drug development and safety assessment. New York: Elsevier; 2015.
34. Kumar M, Prasad MR, Srivastva K, Tripathi S, Srivastva AK. Branchial histopathological study of Catfish Heteropneustes fossilis following exposure to purified neem extract, Azadirachtin. World Journal of Zoology, 2010: 5(4): 239-243.
35. Crane M, Johnson A. Predictive Ecotoxicology. Boca Raton, Fl: CRC Press; 2019