

Interactions of Lead with Other Heavy Metals (Cadmium, Nickel and Zinc) in Toxic Effects on the Histological Structure of Gills of the Common Carp *Cyprinus carpio* Linnaeus, 1758

Kostadinka Todorova¹, Iliana Velcheva², Vesela Yancheva², Stela Stoyanova^{3*}, Petya Dimitrova⁴, Stoil Tomov⁵ & Elenka Georgieva³

¹ Lyuben Karavelov Branch, Plovdiv University, Kardzhali, Bulgaria

² Department of Ecology and Environmental Conservation, Faculty of Biology, Plovdiv University, Plovdiv, Bulgaria

³ Department of Developmental Biology, Faculty of Biology, Plovdiv University, Plovdiv, Bulgaria

⁴ Regional Accredited Laboratory, Executive Environment Agency, Ministry of Environment and Water, Plovdiv, Bulgaria

⁵ Department of Urology and General Medicine, Plovdiv Medical University, Plovdiv, Bulgaria

Abstract: We performed a laboratory experiment in order to study the toxic effects of five different concentrations of single lead (Pb) and its interactions with other heavy metals, cadmium (Cd), nickel (Ni) and zinc (Zn), on the gills of common carp (*Cyprinus carpio* Linnaeus, 1775). Cadmium, Ni and Pb are considered as priority polluting substances in surface waters. A short-term experiment (96 h) in laboratory conditions was performed. The fish were treated with decreasing concentrations of single Pb as well as with its combination with Cd, Ni and Zn. Then we examined the fish gill histological changes and the degree of expression of each histological alteration. Several alterations were observed, i.e. lamellar epithelium lifting, edema, proliferation of the stratified epithelium and cartilage tissue, fusion and degeneration in the gill epithelium as well as vasodilatation and aneurysms in the gill blood circulatory system. The alterations were more pronounced for the single Pb-exposed groups than for its combination with the other tested heavy metals. Overall, the study has contributed to clarify the toxicity of single Pb as well as its mixtures (Pb²⁺+Cd²⁺, Pb²⁺+Ni²⁺ and Ni²⁺+Zn²⁺) and the interactions between the metals on the commercially important freshwater common carp.

Key words: lead, heavy metals, common carp, gills, histology

Introduction

Aquatic environments are being polluted with heavy metals due to the growing urbanisation and globalisation in the last few decades (ZHOU et al. 2008, ISLAM et al. 2015, CAROLIN et al. 2017). In addition, according to DÍAZ et al. (2018), the aquatic ecosystems are more sensitive to heavy metals than the terrestrial ecosystems. Heavy metals are non-biodegradable and they can easily be accumulated in the living organisms, including fish (SIA SU et al. 2013, AKTER et al. 2014, FERRANTE et al. 2017, DALZUCHIO et al. 2017, STRUNGARU et al. 2018). They also have

extensive persistence and their ability to biomagnify is a serious threat of the food chain (CHOURPAGAR et al. 2011, EZEJLOFOR et al. 2013). Heavy metals, such as Cu, Fe and Zn, are essential for fish metabolism while others, such as Pb, Cd and Hg, have no known metabolic functions in organisms (AZAMAN et al. 2015). The main sources of Pb exposure include mining, smelting, lead battery disposal, crystal and ceramic industries. Moreover, Pb toxicity induces a wide array of physiological, biochemical, histological and behavioural dysfunctions found in

*Corresponding author: stela.st@abv.bg

laboratory animals, including fish (U.S. AGENCY FOR TOXIC SUBSTANCES DISEASE 2007). As for Pb, many preventive measures to decrease its presence in the environment have been implemented around the world, including the introduction of unleaded petrol. However, Pb toxicity is still prevalent.

Fish are well known water quality bio-indicators and, thus, they are widely used to monitor variations in the aquatic environment due to anthropogenic pollutants (ŁUCZYŃSKA et al. 2018). The possible ways of heavy metal accumulation in fish are through direct uptake by water and food and contact with polluted sediments (SHESTERIN et al. 2010). Furthermore, heavy metals entering the fish through the gills have a chance to get accumulated in different organs and excessive amounts can build up to a toxic level (KUMAR et al. 2005). Furthermore, fish have proven to be useful as sentinel organisms which display measurable biological responses (biomarkers) that vary in proportion to the extent of exposure to contaminants, i.e., changes in health status and physiological characteristics and behavioural responses of individuals or communities (GAGNON & HODSON 2012). According to SIA SU et al. (2013), the histological biomarkers are constantly used in most toxicological studies because they indicate the overall fish health and are important indicators for aquatic pollution. As fish gills are in constant contact with water, they represent an important target organ of dissolved pollutants. The morphological changes in the gills can represent adaptation strategies to keep some physiological functions or endpoints to evaluate the acute and chronic exposure to chemicals found in water and sediment (OMAR et al. 2013).

Increasing heavy metal pollution in the aquatic ecosystems, especially near industrial areas, necessitates early timely detection, prediction of consequences and possibly prevention. In such conditions, aquatic organisms are often exposed to a mixture of metals rather than a single element (MORE & RAMAMOORTHY 1984). Water chemical analyses are used to establish the nature and concentration of pollutants; however, the information is not sufficient to predict the real effects on the aquatic biota due to two main reasons: the complexity of the processes in the water and the processes taking place in the organisms. The effects of Pb on fish have been studied already for several decades. Some studies have also been carried out on the combined toxicity among heavy metals (OTITOLOJU 2003, COOPER et al. 2009). However, data from heavy metal interactions (i.e., additive, antagonistic or synergistic) between different concentrations of Pb with other heavy metals, such as Cd, Ni and Zn, is relatively limited in labora-

tory conditions. In addition, heavy metals may share common uptake routes and interact with each other, thus affecting uptake, bioaccumulation and toxicity. The results of such interactions are highly variable, ranging from antagonism to synergism depending on the metal, its external concentration and exposure scenario, length of exposure, studied species and examined organs (NORWOOD et al. 2003). According to ALONSO et al. (2004), since the toxicity of one metal or metalloid can be dramatically modulated by the interaction with other toxic or essential metals, studies addressing the chemical interactions between heavy elements are increasingly important. Cadmium, Ni and Pb are considered priority substances in surface waters according to DIRECTIVE 2013/39/EU. The main aim was to study the short-term (96 h) toxic effects of single increasing Pb concentrations and its interactions with Cd, Ni and Zn on the gills of common carp. For this purpose, histological biomarkers were applied and the degree of expression of each histological alteration was determined.

Materials and Methods

Experimental setup

For the purpose of the experiment nine 100-L water tanks were filled in advance with dechlorinated tap water (8 test and 1 control tanks). The applied metal concentrations are presented in Table 1. The metals were applied as nitrate salts because they are the most soluble: $\text{Pb}(\text{NO}_3)_2$, $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (MERCK). Lead stock solution was prepared based on the maximum allowable concentration (MAC-EQS) according to DIRECTIVE 2013/39/EU. The other three increasing Pb concentrations were prepared from the stock solution and were selected according to the obtained results in a preliminary experiment based on fish mortality (Table 1). Similarly, in the heavy metals mixtures the Pb concentration was 50 $\mu\text{g/L}$ and the concentrations of Cd, Ni and Zn were respectively 10, 200 and 3000 $\mu\text{g/L}$, the highest concentrations at which no mortality was observed. During the experiment, the physical and chemical properties of the water (pH, conductivity, temperature, dissolved oxygen and water hardness) were measured daily with a combined filled meter (WTW).

Test animals

Common carps were proposed as test organisms in toxicological assays because they can survive and accumulate contaminants even at heavily polluted sites (REYNDERS et al. 2008). The fish were purchased from a certified fish farm and transported in

Table 1. Heavy metal concentrations in the test tanks, µg/L.

Heavy metals	Pb ²⁺	Pb ²⁺	Pb ²⁺	Pb ²⁺	Pb ²⁺	Pb ²⁺ +Cd ²⁺	Pb ²⁺ +Ni ²⁺	Pb ²⁺ +Zn ²⁺
Test concentration	10	25	50	75	100	50+10	50+200	50 +3000

Table 2. Histological alterations in gills of common carp after single Pb exposure and its combination with Cd, Ni and Zn.

Histological alterations	Control	Test concentrations, µg/L							
		10 Pb ²⁺	25 Pb ²⁺	50 Pb ²⁺	75 Pb ²⁺	100 Pb ²⁺	50 Pb ²⁺ +10Cd ²⁺	50Pb ²⁺ +200Ni ²⁺	50 Pb ²⁺ + 3000 Zn ²⁺
Lamellar lifting	0	1	1	1	0	1	1	1	1
Edema	0	1	0	1	0	0	1	1	1
Proliferation of stratified epithelium	0	2	2	2	3	2	0	1	1
Fusion	0	2	2	2	2	2	0	2	1
Proliferation of cartilage tissue	0	2	2	1	2	2	0	0	1
Degeneration of gill epithelium	0	1	1	1	0	1	0	1	1
Vasodilatation of secondary lamellae:									
Along the length of blood vessel	0	1	1	2	0	1	0	1	1
At basal part of the blood vessel	0	0	1	1	0	0	0	0	1
At apical part of the blood vessel	0	0	1	1	0	0	1	1	0
Vasodilatation of central venous sinus	0	0	0	1	0	0	2	0	1
Aneurysms	0	0	0	0	0	1	1	1	1

Legend: (0) – no histological alterations which represented normal histological structure; (1) – mild histological alterations; (2) – moderate histological alterations; (3) – severe histological alterations; (4) – very severe histological alterations of the gill surface architecture. **Bold** – significant differences for the degree of expression for each histological alteration between the tested groups. *Italic* – control group

special aerated containers to the laboratory on the same day. They were of the same size-age group (12±2 cm in length and 10.5±3.5 g in weight) and without external pathological injuries. After a seven-day acclimatisation, the fish were treated with the heavy metals. Ninety-six hours acute bioassays were performed, following in general OECD guidelines for fish acute bioassays (guideline OECD 203, 92/69/EC, method C1, see OECD 1993). The experiment was performed in triplicate. During the entire duration of the experiment, the fish were maintained under a natural light/dark cycle (12:12). They were not fed. No fish mortality was recorded.

Histological analysis

Fish dissection was performed according to the procedures given in the EMERGE Protocol (ROSSELAND et al. 2003). The samples were treated following

ROMEIS (1989) and a rotary microtome (Leica RM 2125) was used. Ten slides for histological examinations from each sample were prepared. The experiment was conducted in accordance with national and international guidelines of the European Parliament and the Council on the protection of animals used for scientific purposes (DIRECTIVE 2010/63/EU). Histochemical alterations in fish gills were observed and photographed using Nikon microscope mounted digital camera with SETI.

Semi-quantitative scoring

The degree of expression of each alteration was studied, including the corresponding changes in the gill surface in relation to the normal histological structure. The histological alterations in the gill epithelium were determined semi-quantitatively, using the grading system of PIERCE et al. (1978) and ZIMMERLI

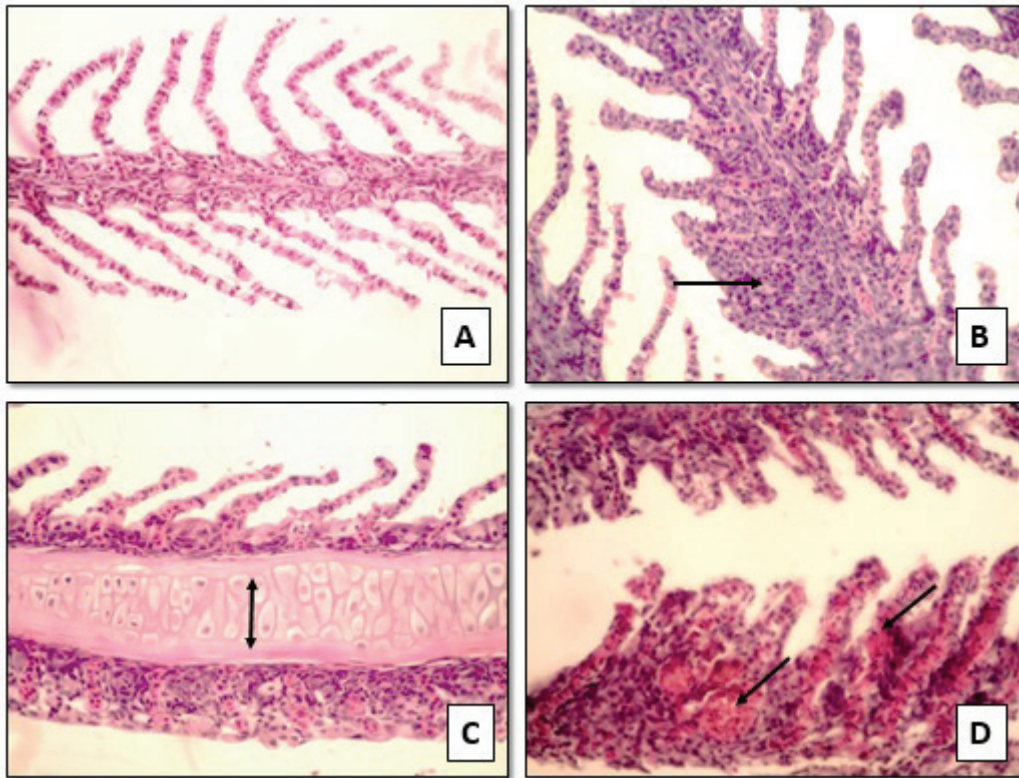


Fig. 1. Histological alterations in gills of *Cyprinus carpio* after single Pb exposure, x400, H&E: A – control group; B – epithelial proliferation in gills, exposed to 10 µg/L Pb²⁺; C – proliferation of cartilage tissue in gills, exposed to 25 µg/L Pb²⁺; D – vasodilatation in blood vessels of the secondary lamellae in gills, exposed to 50 µg/L Pb²⁺.

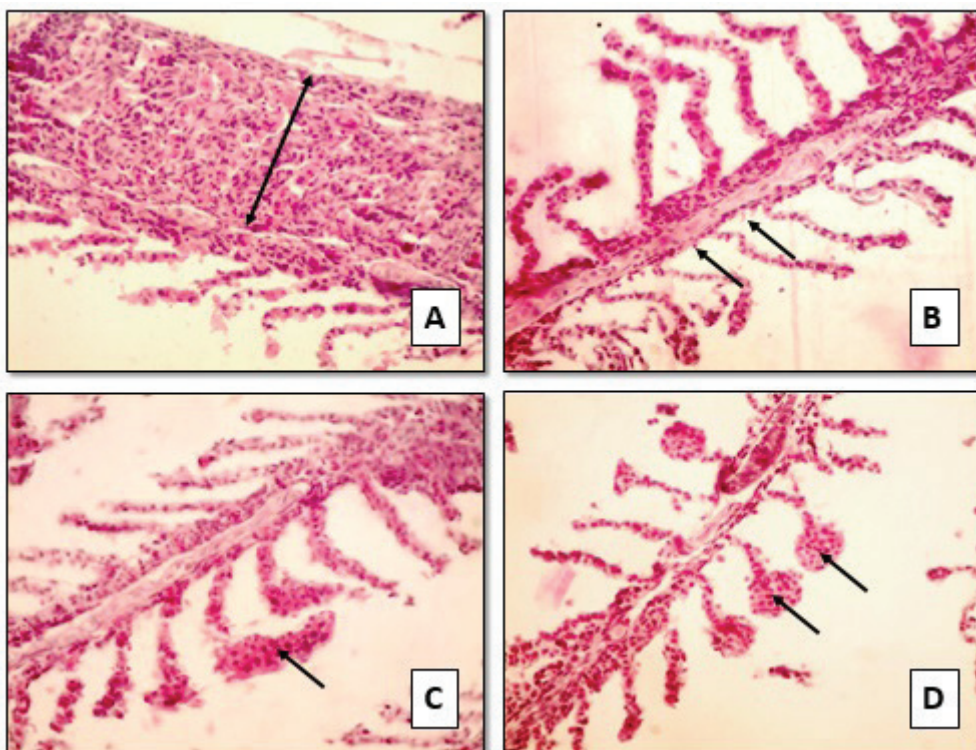


Fig. 2. Histological alterations in gills of *Cyprinus carpio* caused by combinations of heavy metals, x400, H&E: A – fusion in gills, exposed to Pb²⁺+Ni²⁺; B – degeneration in gills, exposed to Pb²⁺+Zn²⁺; C – vasodilatation in blood vessels of the secondary lamellae in gills, exposed to Pb²⁺+Ni²⁺; D – aneurysms in gills, exposed to Pb²⁺+Cd²⁺.

et al. (2007), which we combined and modified. Each grade represented specific histological characteristics and was categorised as follows: (0) – no histological alterations, which represented normal histological structure; (1) – mild histological alterations; (2) – moderate histological alterations; (3) – severe histological alterations; (4) – very severe histological alterations in the gill surface architecture.

Statistical analyses

We used Graph Pad Prism 7 for Windows for statistical analyses. In order to study the differences between the degree of expression of each histological alteration between the control and treated with different metal concentrations groups, Student t-test ($p < 0.05$) was applied. This was possible as for each degree of expression a numerical value from 0 to 4 was given. Ten fish from each test group were analysed. The results are presented as average values.

Results

The toxic effects, which were observed in the gill structure after the exposure to the different increasing Pb concentrations and its combination with Cd, Ni and Zn, are presented in Table 2. The histological structure of the control fish gills was determined to be normal as there were no changes (Fig. 1A).

Overall, in the present study the histological changes in the common carp gills were classified into three main groups: 1) proliferative changes – lamellar lifting, edema, gill epithelial proliferation covering the filaments and secondary lamellae and fusion; 2) degenerative changes in the covering gill epithelium; 3) changes in blood vessels – vasodilatation in the main blood vessel and secondary lamellae, aneurysms (Figs. 1, 2). From the observed changes in the area of the gill epithelium, we found two main mechanisms: one to activate cell division processes associated with manifestation of proliferative alterations and the other was associated with activation of necrobiotic processes and necrosis associated with degenerative changes.

Changes like epithelial lifting and edema were expressed (to a slight degree) in both types of exposure, to single Pb and to combinations of Pb with Cd, Ni and Zn. At the single Pb concentrations, the proliferative changes such as proliferation of the squamous epithelium and cartilage tissue as well as fusion were expressed to a moderate degree (Table 2, Figs. 1B, C) as compared to the effect of the combination with the other tested metals.

Regarding the degenerative changes, a general trend of less pronounced degree of manifestation

than the proliferative ones was established at both, single Pb exposure and its combination with the other metals, with a clear difference in the degree of proliferation in the gill epithelium, moderate and severe (Table 2, Fig. 2B). This confirmed more active inclusion of the compensatory-adaptive mechanisms over the necrotic ones. Overall, at single Pb concentrations the proliferative alterations prevailed over the degenerative. In addition, the predominant manifestation of cell division was particularly visual at Pb concentration of 75 $\mu\text{g/L}$, where the proliferation of the squamous epithelium was scored as severe, while degeneration was completely absent.

In terms of vasodilatation in the secondary lamellae, a variation in the localisation and extent of expression was observed for both single Pb and combined metal exposures (Table 2, Figs. 1D, 2C). The most common variation was manifested at a mild degree, with one exception at Pb 50 $\mu\text{g/L}$ (Table 2). Vasodilatation in the main sinus was expressed at a moderate degree only in the combination of Pb and Cd. Lastly, aneurysms in the blood system were found at a mild degree of expression in all metal mixtures, but only at the highest single Pb concentration (Fig. 2D).

We found significant differences between the control and fish exposed to Pb 75 $\mu\text{g/L}$ for the degree of expression of proliferation of stratified epithelium ($P < 0.05$) and between the control and fish exposed to Pb 50 $\mu\text{g/L}$ for vasodilatation of secondary lamellae (along the length of blood vessel; $P < 0.05$). No significant differences were observed for the degree of expression of each histological alteration between the different single Pb concentrations (10, 25, 50 and 75 $\mu\text{g/L}$). On the other hand, such were observed for the degree of expression of fusion and vasodilatation of central venous sinus between the fish exposed to $\text{Pb}^{2+} + \text{Cd}^{2+}$ and $\text{Pb}^{2+} + \text{Ni}^{2+}$, as well as for the degree of expression of vasodilatation of the central venous sinus between the control and fish exposed to $\text{Pb}^{2+} + \text{Cd}^{2+}$.

Discussion

According to ALDOGHACHI et al. (2016), the various histological alterations in fish gills are a sensitive tool for identifying the effects of heavy metals. In the present experiment proliferative alterations, such as lamellar lifting and edema, were observed and similarly to previous studies (KHAN et al. 2011) we consider them adaptive changes for the fish survival in a metal-polluted environment. Furthermore, we agree with KOSTIĆ et al. (2017) that epithelial lifting occurs as a response to stress conditions result-

ing in an increased diffusion distance between water and blood. As also stated by RAŠKOVIĆ et al. (2013), in conditions of reduced oxygen concentration, the blood flow increases, which in turns leads to an increase in the level of circulatory alterations, including vasodilation. In terms of the metal interactions, we observed similar degree of expression of lamellar lifting and edema for all single Pb concentrations and its combination with Cd, Ni and Zn. Thus, we consider that Pb did not increase its toxicity in the applied metal mixtures.

We think that the observed proliferative changes, such as squamous epithelial proliferation, cartilage tissue proliferation and fusion, could act as protective mechanisms in order to reduce the area of vulnerable gill surface. Thus, these mechanisms increase the diffusion barrier of the toxicant by preventing its access to the blood stream, which is in agreement with GARCIA-SANTOS et al. (2007). In addition, fusion also serves as a protective mechanism for minimising the surface area in the fish gills as demonstrated also by VASANTHI et al. (2013). The proliferative gill alterations indicated a more active cell division at the single Pb exposures. Moreover, in the present study for the combined metal exposure, these changes were generally less pronounced (Fig. 2A). We believe this could indicate that the tolerance threshold for the metal is likely to increase, resulting in the occurrence of compensatory-adaptive mechanisms but to a lesser degree. Moreover, in combination with Cd, Ni and Zn, Pb toxicity decreases. This could be the reason why in such cases fish sometimes show adaptation and survival in natural waters polluted with a mixture of toxic metals, most likely due to decreasing the toxicity of the metals mixtures.

Our results on the degenerative alterations are in agreement with EVANS (1987) and JASIM (2017), who state that the destruction of gills can disturb the respiratory function and osmoregulation, which in turn reduces fish swimming activity due to oxygen deficiency and finally, the growth rate. Furthermore, in the present study these histological changes occurred in the gills at a mild degree, both at single Pb exposures and its combination with Cd, Ni and Zn. Similarly, to the proliferative alteration, we believe the metal mixtures did not lead to increasing Pb toxicity.

In terms of the metal combinations, the proliferation and degenerative processes were equalised. Thus, our results confirm similar degree of expression for the cell division process and necrosis. On the other hand, for single Pb exposures the process of cell division prevailed over necrosis, which showed that the processes were rather compensatory-adaptive than degenerative.

In general, the toxicity of metals in the circulatory system was relatively low. Similarly to VIGARIO & SABÓIA-MORAIS (2014), we also consider vasodilatation in the main venous sinus as a compensatory mechanism for oxygen deficiency, which in our case is a result from metal exposures. The observed vasodilatation was only expressed at a moderate degree for the combination of Pb with Cd. According to HASSAN (2011), the action of Cd could strongly affect the circulatory system. In the present experiment, vasodilatation could be considered as an indicator for the increasing Pb toxicity in this particular metal mixture. Aneurysms were found for all metal mixtures at a mild degree. In addition, the degree of expression was mainly mild in the combination of Pb with all tested metals, which was probably a result of increased Pb toxicity due to metal interactions. Aneurysms were found only at the highest Pb concentration for the single Pb exposures, which was probably dose-dependent.

Conclusions

In conclusion, the degree of proliferative and degenerative changes in the gill epithelium of common carp was manifested at a more severe degree for the single Pb exposures as compared to those in its combination with Cd, Ni and Zn. Furthermore, the alterations in the circulatory system showed more often equal degree of expression between the single and combined Pb exposures. We consider that single Pb exhibited higher toxicity and, thus, in combination with the applied metals, probably entered into antagonistic interactions and showed lower toxicity. However, in terms of vasodilation and aneurysms, such clear metal interactions were not observed. Therefore, we strongly suggest that further studies in this particular field are needed, in order to better study the interactions between metals and toxic effects of metal mixtures on fish.

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References

- AGENCY FOR TOXIC SUBSTANCES DISEASE REGISTRY. 2007. Toxicological Profile for Lead. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.
- AKTER M., SIKDER T. & ULLAH A. K. M. A. 2014. Water quality assessment of an industrial zone polluted aquatic body in Dhaka, Bangladesh. *American Journal of Environmental Protection* 3: 232-237.

- ALDOGHACHI M. A., AZIRUN M. S., YUSOFF I. & ASHRAF M. A. 2016. Ultrastructural effects on gill tissues induced in red tilapia *Oreochromis* sp. by a waterborne lead exposure. *Saudi Journal of Biological Sciences* 23: 634-641.
- AZAMAN F., JUAHIR H., YUNUS K., AZID A., KAMARUDIN M. K. A. K., TORIMAN M. E., MUSTAFA A. D., AMRAN M. A., HASNAM C. N. C. & SAUDI A. S. M. 2015. Heavy metal in fish: analysis and human health – a review. *Journal Teknology* 77 (1): 61-69.
- CAROLINA C. F., KUMAR P. S., SARAVANAN A., JOSHIBA G. J. & NAUSHAD M. U. 2017. Efficient techniques for the removal of toxic heavy metals from aquatic environment: A review. *Journal of Environmental Chemical Engineering* 5: 2782-2799.
- CHOURPAGAR A. R. & KULKARNI G. K. 2011. Heavy metal toxicity to a freshwater crab, *Barytelphusa acuminata* (Westwood) from Aurangabad region. *Recent Research in Science and Technology* 3: 1-5.
- COOPER N. L., BIDWELL J. R. & KUMAR A. 2009. Toxicity of copper, lead, and zinc mixtures to *Ceriodaphnia dubia* and *Daphnia carinata*. *Ecotoxicology and Environmental Safety* 72: 1523-1528.
- DALZOCCHIO T., RESEL SIMÕES L. A., DE SOUZA M. S., RODRIGUES G. Z. P., PETRY I. E., ANDRIGUETTI N. B., SILVA G. J. H., GEHLEN G. & DA SILVA L. B. 2017. Water quality parameters, biomarkers and metal bioaccumulation in native fish captured in the Ilha River, southern Brazil. *Chemosphere* 189: 609-618.
- DÍAZ E., PÉREZ D., ACEVEDO J.D. & MASSOL-DEYÁ A. 2018. Longitudinal survey of lead, cadmium, and copper in seagrass *Syringodium filiforme* from a former bombing range (Vieques, Puerto Rico). *Toxicology Reports* 5: 6-11.
- DIRECTIVE 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. Official Journal of the European Union.
- DIRECTIVE 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending DIRECTIVES 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.
- EVANS D. H. 1987. The fish gill: site of action and model for toxic effects of environmental pollutants. *Environmental Health Perspectives* 71: 47-58.
- EZEJIOFOR T. I. N., UDEBUANI A. C., EZEJI E. U., AYALOGBU E. A., AZUWUIKE C. O., EZEJIOFOR A. N. & NGWOGU K. O. 2013. Environmental metals pollutants load of a densely populated and heavily industrialized commercial city of Aba, Nigeria. *Journal of Toxicology and Environmental Health Sciences* 5: 1-11.
- FERRANTE M., PAPPALARDO A. M., FERRITO V., PULVIRENTI V., FRUCIANO C., GRASSO A., SCIACCA S., TIGANO C. & COPAT C. 2017. Bioaccumulation of metals and biomarkers of environmental stress in *Parablennius sanguinolentus* (Pallas, 1814) sampled along the Italian coast. *Marine Pollution Bulletin* 122: 288-296.
- GAGNON M. M. & HODSON P. V. 2012. Field studies using fish biomarkers – How many fish are enough? *Marine Pollution Bulletin* 64: 2871-2876.
- GARCIA-SANTOS S., MONTEIRO S. M., CARROLA J. & FONTAINHAS-FERNANDES A. 2007. Histological alterations in gills of Nile tilapia *Oreochromis niloticus* caused by cadmium. *Brazilian Journal of Veterinary and Animal Science* 59 (2): 376-381.
- HASSAN W. 2011. The effect of copper and cadmium on oxygen consumption of juvenile carp *Cyprinus carpio* (L.). *Mesopotamian Journal of Marine Science* 26 (1): 25-34.
- ISLAM M. S., AHMED M. K., RAKNUZZAMAN M., HABIBULLAH-AL-MAMUN M. & MASUNAGA S. 2015. Metal speciation in sediment and their bioaccumulation in fish species of three urban rivers in Bangladesh. *Archives of Environmental Contamination and Toxicology* 68: 92-106.
- JASIM B. M. 2017. Impact of certain heavy metals on histology and physiology of fishes: Interpretative study. *Annual Research & Review in Biology* 19 (6): 1-21.
- KHAN H. A., SIKDAR-BAR M., KAMLESH B., ADIL AHMAD W. & AHMED P. 2011. Lead nitrate-induced histopathological changes in the gills of African catfish *Clarias batrachus*. *Journal of Applied Sciences Research* 7 (7): 1087-1092.
- KOŠTIĆ J., KOLAREVIĆ S., KRAČUN-KOLAREVIĆ M., ABORGIBA M., GAČIĆ Z., PAUNOVIĆ M., VIŠNJIĆ-JEFTIĆ Ž., RAŠKOVIĆ B., POLEKSIĆ V., LENHARDT M. & VUKOVIĆ-GAČIĆ B. 2017. The impact of multiple stressors on the biomarkers response in gills and liver of freshwater breams during different seasons. *Science of the Total Environment* 601-602: 1670-1681.
- KUMAR K. A. & ACHYUTHAN H. 2005. Heavy metal accumulation in certain marine animals along the East Coast of Chennai Tamil Nadu, India. *Journal of Environmental Biology* 28: 637-643.
- ŁUCZYŃSKA J., PASZCZYK B. & ŁUCZYŃSKI M. J. 2018. Fish as a bioindicator of heavy metals pollution in aquatic ecosystem of Pluszne Lake, Poland, and risk assessment for consumer's health. *Ecotoxicology and Environmental Safety* 153: 60-67.
- MORE J. W. & RAMAMOORTHY S. 1984. Heavy metals in natural waters. *Applied Monitoring and Impact Assessment*. New York: Springer Verlag New York Inc.
- NORWOOD W. P., BORGMANN U., DIXON D. G. & WALLACE A. 2003. Effects of metal mixtures on aquatic biota: a review of observations and methods. *Human and Ecological Risk Assessment* 9: 795-811.
- OECD 1993. OECD Guidelines for the Testing of Chemicals. Test 203: Fish, Acute Toxicity Test.
- OMAR W. A., ZAGHLOUL K. H., ABDEL-KHALEK A. A. & ABO-HEGAB S. 2013. Risk assessment and toxic effects of metal pollution in two cultured and wild fish species from highly degraded aquatic habitats. *Archives of environmental contamination and toxicology* 65 (4): 753-764.
- OTITOLOU A. A. 2003. Relevance of joint toxicity evaluations in setting realistic environmental safe limits of heavy metals. *Journal of Environmental Management* 67: 121-128.
- PIERCE K. W., MCCAIN B. B. & WELLINGS S. R. 1978. Pathology of hepatomas and other liver abnormalities in English sole (*Parophrys vetulus*) from the Duwamish river estuary, Seattle, Washington. *Journal of the National Cancer Institute* 60 (6): 1445-1543.
- RAŠKOVIĆ B., JARIĆ I., KOKO V., SPASIĆ M., DULIĆ Z., MARKOVIĆ Z. & POLEKSIĆ V. 2013. Histopathological indicators: a useful fish health monitoring tool in common carp (*Cyprinus carpio* Linnaeus, 1758) culture. *Central European Journal of Biology* 8 (10): 975-985.
- REYNDERS H., BERVOETS L., GELDERS M., DE COEN W. M. & BLUST R. 2008. Accumulation and effects of metals in caged carp and resident roach along a metal pollution gradient in Flanders, Belgium. *Science of the Total Environment* 391: 82-95.

- ROMEIS B. 1989. Mikroskopischetechnik. Munchen: Urban und Schwarzenberg. 697 p.
- ROSSELAND B. O., MASSABUAU J. C., GRIMALT J., HOFER R., LACKNER R., RADDUM G., ROGNERUD S. & VIVES I. 2003. Fish Ecotoxicology: European Mountain Lake Ecosystems Regionalisation, Diagnostic and socio-economic evaluation (EMERGE). Fish sampling manual for live fish. Norway: Norwegian Institute for Water Research (NIVA), 1-7 p.
- SHESTERIN I. S. 2010. Water pollution and its impact on fish and aquatic invertebrates. In: LYSENKO G., SQUIRES V. & VERHEYE W. H. (Eds.): Interactions: Food, Agriculture and Environment. UK: EOLSS, pp. 437.
- SIA SU G. L., RAMOS G. B. & SIA SU M. L. L. 2013. Bioaccumulation and histopathological alteration of total lead in selected fishes from Manila Bay, Philippines. Saudi Journal of Biological Sciences 20: 353-355.
- STRUNGARU S.-A., NICOARA M., TEODOSIU C., BALTAG E., CIOBANU C. & PLAVAN G. 2018. Patterns of toxic metals bioaccumulation in a cross-border freshwater reservoir. Chemosphere 207: 192-202.
- VASANTHI L. A., REVATHI P., MINI J. & MUNUSWAMY N. 2013. Integrated use of histological and ultrastructural biomarkers in *Mugil cephalus* for assessing heavy metal pollution in Ennore Estuary, Chennai. Chemosphere 91: 1156-1164.
- VIGÁRIO A. F. & SABÓIA-MORAIS S. M. T. 2014. Effects of the 2,4-D herbicide on gills epithelia and liver of the fish *Poecilia vivipara*. Pesquisa Veterinária Brasileira 34 (6): 523-528.
- ZHOU Q., ZHANG J., FU J., SHI J. & JIANG G. 2008. Biomonitoring: An appealing tool for assessment of metal pollution in the aquatic ecosystem. Analytica Chimica Acta 606: 135-150.
- ZIMMERLI S., BERNET D., BURKHARDT-HOLM P., SCHMIDT-POSTHAUS H., VONLANTHEN P., WAHLI T. & SEGNER H. 2007. Assessment of fish health status in four Swiss rivers showing a decline in brown trout catches. Aquatic Sciences 69: 11-25.

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