Potential of probiotics and vitamin C on metallothionein and hematological parameters in tilapia (*Oreochromis niloticus*) affected by cadmium exposure

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Abstract. Water pollution is a problem for the health of aquatic organisms. In this study, blood parameters of tilapia (*Oreochromis niloticus*), which received probiotic and vitamin C were investigated after heavy metal cadmium (Cd) exposure in different concentration (0 ppm, 0.3 ppm, and 0.6 ppm). The study was prepared by incorporating probiotics from lactic acid bacteria (LAB), vitamin C, and a combination of probiotics and vitamin C. Thirty-six healthy adult fish were used in this study. The experiment was carried out for 15 days. At the end of experiment, metallothionein (Mt) levels and following blood parameters such as white blood cell (WBC), procalcitonin (PCT), hemoglobin (Hb), red blood cell (RBC), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were measured from fish blood. The result showed that probiotics, vitamin C, and a combination of them could decrease Mt levels and improve blood parameters significantly ($p < 0.05$). Overall, the combination of probiotics and vitamin C produced the best result for the parameters improvement. This result indicated positiveness for the health status of fish against toxic environment.
Key Words: cadmium, fish, hematological, probiotics, vitamin C.

Introduction. Industrialization, housing, and agricultural development, especially those located around rivers and reservoirs are important sources of water pollution, if the waste is not managed properly. Rivers that have been polluted by heavy metals, such as lead (Pb) and chromium (Cr), will contain high levels of heavy metals that exceed the quality standard. High levels of heavy metals in water can cause damage to tissues and organ structures in the liver, gills, and gonads through the formation of free radicals, DNA damage, and lipid peroxidation when consumed by organisms such as freshwater fish (Hayati et al 2017). This condition also can increase the risk of cancer (Chen et al 2014). Fish farmers use rivers or reservoirs as water sources to raise their fish. This condition can make fish easily contaminated with waste including heavy metals. The consumption of this fish has raised concerns about the potential for the transfer of toxic heavy metals to the human body.

Heavy metals pollution occurs when many industries dump their waste obtained from various industrial processes to the environment especially in rivers and water reservoirs (Kovarova et al 2009; Hayati et al 2019). Cadmium (Cd) is one of the metals that has been included in the group of non-essential heavy metals that are harmful to the physiological functions of the body (Milad et al 2016). It can enter the body of fish through various channels including gills and digestive systems. After that, Cd will move through the bloodstream to the organs, including testicles and liver where cause damages to cells and their constituent tissues. In the kidney, it causes inflammation and cell necrosis (Hayati et al 2016).

Probiotics are a consortium of bacteria, such as LAB, which can be used as an agent to reduce exposure to heavy metals (Mohapatra et al 2018). Moreover, a characteristic possessed by probiotics is to eliminate and reduce toxicity from heavy metal. Bacteria can easily bind heavy metals from inside or outside the cell, thus preventing hazardous interaction in the host cell. Also besides, the bacteria can actively transport metal out from the cytosolic cell. Furthermore, this bacteria also can neutralize heavy metals by absorbing and storing them in their cell vacuoles (Monachese et al 2012).

Metallothionein (Mt) is a protein that contains cysteine in about 25%-35% which has potential to bind metal ions (Kovarova et al 2009). Mt concentration can be used as an indicator of heavy metal accumulation in the body. It is a single-chain protein with a low molecular mass of 6000-7000 Da with scavenging metal radical ability (Chen et al 2014). The primary function of Mt is to maintain homeostasis between Zn and Cu and protect the body against reactive oxygen species (ROS). Furthermore, the sublethal concentration of Ag, Cu, Hg, and Cd can stimulate the Mt gene to produce Mt (Kovarova et al 2009; Chen et al 2014). Abundant amounts of Mt are found in the liver, kidneys, gills and digestive tract. In hepatopancreas, Mt is divided into two classes namely Mt-I and Mt-II in a small amount. Many factors such as environmental stress, hunger, bacterial infection, interleukin-1, glucocorticoids, interferon, and heavy metal can elevate the molecular levels of Mt (Kovarova et al 2009).

The hematopoietic system is more delicate to dangerous toxins, drugs, and infections. It can be used as an indicator to determine the physiological and health status of fish (Khalesi et al 2017). The effects and changes of hematological parameters in fish and other organisms depend on factors such as age, gender or genetic background, sexual maturity of spawners, and diseases (Tête et al 2015; Khalesi et al 2017). Cd can establish a formation of ROS which cause oxidative damage and then increase the impaired function of the blood cell membrane, organs, and blood parameters including red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), platelets, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (Ashour 2014). In the blood, Cd can affect hypocoagulation by decreasing blood platelets and fibrinogen. Otherwise, the liver being susceptible to Cd it can affect the formation of procoagulant factors such as vitamin K, protein C, protein S, protein Z, fibrinogen, antithrombin, α2-PI (plasminogen inhibitor), and plasminogen (Strachan et al 2018; Ashour 2014). In erythrocytes, Cd can cause anemia from suppression of hematopoietic tissues which increase penetrability of the membrane, elevated rate of RBCs mechanical breakability in cells, and distorted iron metabolism (Ashour 2014).

Bioremediation action using a biological agent to centralize or remove toxins from heavy metal exposure has been increased. Several microorganisms have been used as bioremediation agent, among them is one type of gram-positive bacteria known as lactic acidic bacteria (LAB). Various types of *Lactobacillus* have been successfully used as a neutralizer of heavy metals. They can also improve cell and tissue performance but another supplement is still necessary. Vitamins can play a role as the supplement, it can maintain and prevent cell damage caused by heavy metals. Vitamins play an important role as a nutrient in carrying out physiological functions of fish (Okhionkpamwonyi & Edema 2017). Moreover, vitamin C can also act as an antioxidant against free radicals and also facilitate the inflammatory response (Chen et al 2004; Petric et al 2003). Previous studies show that vitamin C could provide improvements in fish growth performance and blood parameters (Fazio et al 2019). The combination of fish feed containing probiotic supplement and vitamin C is expected to improve the hematology of fish contaminated with Cd. The aim of this study was to provide information on fish farmers using water from heavy metal polluted rivers by analyzing the potential of additional fish feed (probiotics and vitamin C) for the recovery of damaged blood cells and metallothionein levels after exposure to Cd.

Material and Method

Animal research ethics. This research was followed by internationally recognized guidelines for the ethical use of animals (Håstein 2004; Grigorakis 2010). Some freshwater fish are tolerant of hypoxia, thus, oxygen levels in the holding unit must be optimal. Before sacrificed, the fish were anesthetized with 0.1 mL/L of clove oil containing eugenol (Fernandesa et al 2017). This immersion is made to avoid the occurrence of stress and pain before death. By using a clove oil solution, it is possible to soothe the fish for several minutes, so as not to cause a rejection reaction in fish (Håstein 2004).

Fish feed supplement preparation. This study used a group of heavy metal bioremediation bacteria consisting of several strains namely *Lactobacillus buchneri* (DSM 20057), *Lactobacillus casei* (DSM 20011), *Lactobacillus bulgaricus* (NBRC13953), and *Lactobacillus fermentum* (ME3), with a ratio of 1:1:1:1. The bacterial consortium (108 CFU/ml) was then mixed with fish feed. Synthetic fish feed was made from soy flour, fish meal, flour, vitamin, and fish oil. Mixing probiotics and vitamin C in synthetic fish feed was done by spraying. Dilution of the bacterial consortium was done by using water, then it was sprayed on the fish feed with concentration of 50 mL kg^{-1} bw. The dose of vitamin C used was 50 mg kg^{-1} bw of feed.

Experimental animals. Thirty-six male tilapia (*Oreochromis niloticus*) weighing 250-300 g were obtained from freshwater fish farms in Pandaan, Pasuruan, East Java, Indonesia. Each fish is reared in a glass tank measuring 90×60×50 cm, in fresh water with aeration at a temperature of 25°C±1.5 °C, in the fish laboratory of Airlangga University. Experiments were carried out from June to September 2019. The fish were kept in light and dark cycles of 12 h and a pH of 7.5 ± 0.03. Fish were randomly divided into 12 equal groups each comprising of three fish. Each group was kept in a separate glass tank. The first group was kept in a tank with normal water without any treatment, this group was used as negative control. The treatment groups were exposed to a variation of Cd concentrations (0, 0.3, and 0.6 ppm) with some type of fish feeds (feed, feed + vitamin C, feed + probiotic, and feed + combination of vitamin C and probiotics), and the experiment was conducted for 15 days. The fish were acclimatized at the 15th day. In this study, the fish condition was always monitored.

Mt determination. Gill samples were washed in phosphate-buffered saline (PBS) solution, then resected and homogenized with 3 ml homogenizing buffer (0.5 M sucrose; 20 mM Tris-HCl buffer; pH 8; 0.01 β-mercaptoethanol) to produce gill homogenate then centrifuged at 3000 RPM for 20 min at 4°C. The levels of Mt in the supernatant was then measured using ELISA kits, according to the manufacturer's instructions. The absorbance was measured using an ELISA reader at a wavelength of 450 nm (Santa Cruz Biotechnology, Cat # J0410).

Hematological examination. The fish were anesthetized using clove essential oil (0.1mL/L). The blood sample was obtained directly via the severance of the caudal peduncle. The caudal peduncle was severed with a sharp knife, a few millimeters posterior to the dorsal fin. Then, the blood was collected using a disposable syringe and placed in Eppendorf tubes containing EDTA anticoagulant (Mgbenka et al 2003). After that, the blood was analyzed using a hematology analyzer. The blood parameters such as WBC, PCT, RBC, Hb, MCH, and MCHC were analyzed by following standard method (Dacie & Lewis 2011).

Statistical analysis. Data was analyzed by using the Kolmogorov-Smirnov normality test and Levene homogeneity test, followed by the ANOVA in SPSS 21 software. Post hoc tests were assessed by Duncan test at $p<0.05$.

Results and Discussion. Metallothionein increased significantly after Cd exposure (Nursanti et al 2017). In the bloodstream, Cd bind red blood cells and albumin for transport to the liver where it combines with Mt to form Cd-Mt complex which then will be released back to the blood circulation and accumulates to other organs (Ashour 2014). They were known that one molecule of Mt can bind six to seven Cd molecules and become very important in the detoxification system of Cd and other heavy metal (Kovarova et al 2009). Vitamin C, probiotics, and the combination of vitamin C and probiotics which were mixed in the feed did not decrease significantly Mt levels in 0 and 0.3 ppm concentrations ($p > 0.05$). However, Mt levels decreased significantly on the combination of vitamin C and probiotics in 0.6 ppm concentration Cd exposure ($p<0.05$) (Figure 1). In this study, decreasing Mt levels could indicate the lack of free radicals in the fish body due to a decrease in heavy metal content. The combination of vitamin C and probiotic provide a protective effect from binding heavy metals with bacteria and also antioxidants against free radicals.

All of the results of blood parameters showed different result in every concentration (Table 1, Table 2, and Table 3). Probiotics, vitamin C, and both of them could decrease and increase WBC significantly on 0 ppm and 0.6 ppm after Cd exposure compared to control respectively ($p<0.05$). However, probiotics and vitamin C produced a different results in 0.3 ppm which only vitamin C could decrease WBC significantly and the others were giving a significant increase. Oxidation stress from Cd exposure could reduce fish's immune potential. This was seen from the significant decrease in WBC after exposure with different concentrations. Decreasing in WBC is triggered by the Cd toxicity which causes high damage and death of WBC. Furthermore, lymphocyte cells are more susceptible to heavy metal than phagocytic activity that they were significantly reduced following Cd exposure (Witeska & Wakulska 2007).

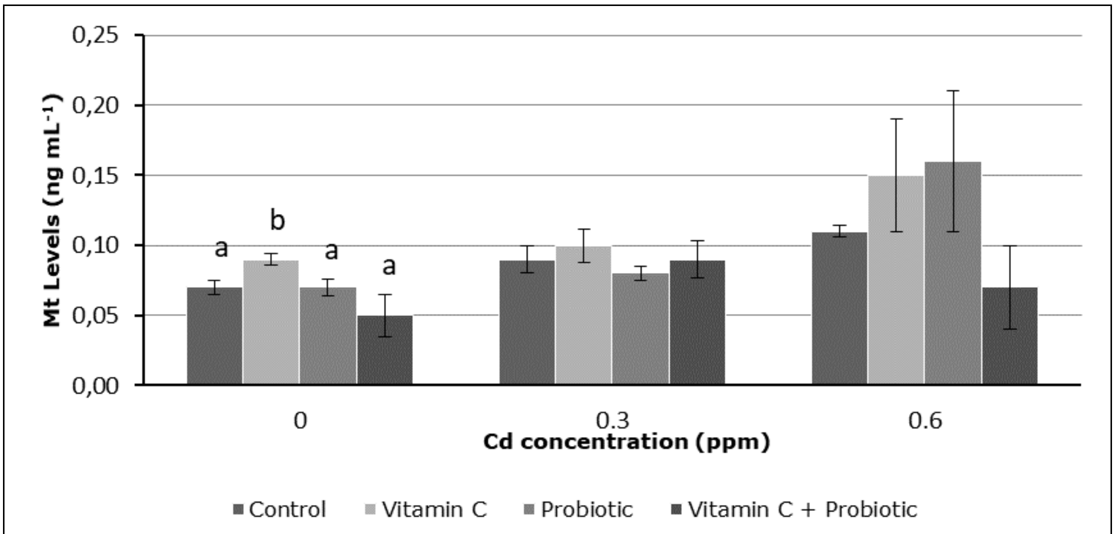

Figure 1. Mt levels of tilapia (*Oreochromis niloticus*) after 15 days Cd exposure in 0 ppm, 0.3 ppm, and 0.6 ppm. The different letter indicates significant differences between control group and treatment groups in the same fish species ($p<0.05$).

Table 1

Mean values ± SD of blood parameters of tilapia (*Oreochromis niloticus*) in 0 ppm Cd exposure after 15 days treatment. The different letter indicates significant differences control group and treatment groups in the same fish species ($p<0.05$)

Blood parameters	*Cd concentration (ppm)*			
	Control	*Vitamin C*	*Probiotic*	*Vitamin C + Probiotic*
WBC ($\times 10^3$ μL^{-1})	137 ± 1.5^a	129 ± 0.4^b	129 ± 0.8^b	130 ± 0.2^b

PCT (%)	0.09±0.01^{a}	0.13±0.01^{b}	0.14±0.01^{b}	0.02±0.01^{c}
Hb (g dL^{-1})	8.8±0.1^{a}	8.6±0.1^{a}	9.95±0.49 ^{b}	8.5±0.1^{a}
RBC (× 10^{6} μL^{-1})	1.72±0.12^{a}	1.56±0.02^{b}	1.91±0.15^{a}	1.71±0.14^{a}
MCH (pg)	44.55±2.04^{a}	48.5±1.9^{b}	45.8±3.6^{b}	48.28±2.33 ^{b}
MCHC (%)	32±0.28^{a}	36.7±0.7^{b}	31.1±0.7^{a}	35.8±2.4^{b}

There was a significant increase in PCT levels after probiotic and vitamin C induction. Otherwise, giving a combination of them could decrease significantly in PCT levels ($p<0.05$). Probiotics, vitamin C, and a combination of them also increased Hb levels significantly in 0.6 ppm Cd exposure ($p<0.05$). However, there was no significant diffrence in 0.3 ppm concentration after probiotic induction ($p > 0.05$).

Both probiotics and the combination with vitamin C could increase significantly in RBC levels compared to the control group ($p<0.05$). However, there was no significant difference after inducted with vitamin C ($p > 0.05$). Hb levels also decreased, possibly due to hypochromic microcytic anemia which contributes to iron deficiency or decreased erythrogenesis process. The addition of vitamin C and supplementation of probiotics in fish feed makes Hb levels increased.

Table 2

Mean values ± SD of blood parameters of tilapia (*Oreochromis niloticus*) in 0.3 ppm Cd exposure after 15 days treatment. The different letter indicates significant differences control group and treatment groups in the same fish species ($p<0.05$)

Blood parameters	*Cd concentration (ppm)*			
	Control	*Vitamin C*	*Probiotic*	*Vitamin C + Probiotic*
WBC (× 10^{3} μL^{-1})	134±1.7^{c}	127±1.6^{b}	137±1.4^{a}	138±0.3^{a}
PCT (%)	0.09±0^{a}	0.14±0.02^{b}	0.16±0.03^{b}	0.04±0.03^{c}
Hb (g dL^{-1})	8.2±0.37^{c}	7.9±0.3^{c}	8.4±0.3^{a,c}	8.9±0.2^{b}
RBC (× 10^{6} μL^{-1})	1.93±0.04^{a}	1.85±0.01^{a}	2.05±0.07^{c}	2.15±0.21^{c}
MCH (pg)	41±0.1^{c}	42.5±2.1^{a}	41.9±0.7^{a}	45.4±3.3^{b}
MCHC (%)	29.9 ±0.1^{c}	31.2±0.2^{a}	32.6±0.1^{b}	34±3.3^{b}

Table 3

Mean values ± SD of blood parameters of tilapia (*Oreochromis niloticus*) in 0.6 ppm Cd exposure after 15 days treatment. The different letter indicates significant differences control group and treatment groups in the same fish species ($p<0.05$)

Blood parameters	*Cd concentration (ppm)*			
	Control	*Vitamin C*	*Probiotic*	*Vitamin C + Probiotic*
WBC (× 10^{3} μL^{-1})	134±0.7^{c}	137±0.7^{a}	141±0.7^{a}	154±0.1^{e}
PCT (%)	0.09±0.01^{a}	0.12±0.01^{b}	0.13±0.01^{b}	0.05±0.02^{c}
Hb (g dL^{-1})	8.57±0.2^{c}	9.1±0.6^{b}	10.2±0.85^{b}	9.3±0.1^{b}
RBC (× 10^{6} μL^{-1})	1.92±0.02^{a}	1.9±0.01^{a}	2.29±0.08^{c}	2.15±0.06^{c}
MCH (pg)	40.2±1.1^{c}	42.1±0.1^{a}	41.9±0.7^{a}	38.2±0.1^{d}
MCHC (%)	30.65±0.5^{c}	27.4±0.3^{d}	31.3±0.2^{a}	31.8±0.2^{a}

Many studies showed heavy metal exposure including Cd can inhibit heme biosynthesis through copro-porphyrinogen enzymes, δ-ALAD, and inhibition of ferrochelatase (Hunter et al 2008; Ajioka et al 2006). Inhibition of ALAD can induce heme oxygenase which inhibits the degradation of heme into biliverdin, carbon monoxide, and free divalent iron. Decreasing this enzyme causes low Hb levels in the blood (Wang et al 2008). The addition of supplementations prevented the risk caused by Cd toxicity. Furthermore, Cd can also inhibit the induction of cytochrome P450 enzyme in fish.

This enzyme is involved in detoxifying xenobiotic organic chemical and steroid hormone homeostasis and also it can affect the toxicity from organic chemical and fish reproduction (Wang et al 2008). Heavy metals also causes blood cells and Hb synthesis disruption. After that, this condition will become anemia. A significant change in the number of RBC can reduce oxygen consumption in fish which results in organ damage due to heavy metal toxicity (Singh et al 2008).

MCH levels increased significantly in 0.3 ppm Cd exposure for probiotic, vitamin C, and both of them ($p<0.05$). Therefore, the combination of probiotics and vitamin C showed different result which could decreased significantly in MCH levels on 0.6 ppm Cd exposure ($p<0.05$). Probiotic and the combination with vitamin C showed significant increases in MCHC levels on all of the Cd exposure, but vitamin C on 0.6 ppm Cd exposure decreased significantly instead ($p<0.05$). In this study, the value of MCH and MCHC of fish blood decreased after exposure to Cd. This situation indicates a deficiency from anemia after decreasing the number of Hb and inhibition of erythropoiesis.

Conclusions. Probiotics (50 mL kg^{-1} bw of feed), vitamin C (50 mg kg^{-1} bw of feed), and combination of probiotics and vitamin C could restore Mt levels and blood parameters of fish after Cd exposure and are indicated to improve the health status of fish while in a toxic environment. However, the combination of them showed the best result than probiotics and vitamin C without combination.

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References

Ajioka R. S., Phillips J. D., Kushner J. P., 2006 Biosynthesis of heme in mammals. Biochimica et Biophysica Acta 1763:723-736.

Ashour T. H., 2014 Preventative effects of caffeic acid phenyl ester on cadmium intoxication induced hematological and blood coagulation disturbances and hepatorenal damage in rats. ISRN Hematology 2014:1-7.

Chen R., Lochmann R., Goodwin A., Praveen K., Dabrowski K., Lee K-J., 2004 Effects of dietary vitamins C and E on alternative complement activity, hematology, tissue composition, vitamin concentrations and response to heat stress in juvenile golden shiner (*Notemigonus crysoleucas*). Aquaculture 242(1–4):553–569.

Chen L., Ma L., Bai Q., Zhu X., Zhang J., Wei Q., Chen W., 2014 Heavy metal-induced metallothionein expression is regulated by specific protein phosphatase 2a complexes. Journal of Biological Chemistry 289(32):22413-22426.

Dacie, Lewis, 2011 Practical Haematology. 11th Edition, Churchill Livingstone:Elsevier, London, UK, 668 pp.

Fazio F., Iaria C., Saoca C., Costa A., Piccione G., Spano N., 2019 Effect of dietary vitamin C supplementation on the blood parameters of striped bass *Morone saxatilis* (Walbaum, 1752). Turkish Journal of Fisheries and Aquatic Science 20(6):491-497.

Fernandesa I. M., Bastosb Y. F., Barretob D. S., Lourencoc L. S., Penha J. M., 2017 The efficacy of clove oil as an anaesthetic and in euthanasia procedure for small-sized tropical fishes. Brazilian Journal of Biology 77(3):444-450.

Grigorakis K., 2010 Ethical Issues in Aquaculture Production. Journal of Agricultural and Environmental Ethics 23(4):345-370.

Hayati A., Rasyad M. F. M., Putra I. D. S., Nurhariyati T., Amin M. H. F., Putranto T. W. C., Wanguyun A. P., Sugiharto., Affandi M., 2019 Morphometric variations of fish

from Brantas river, East Java, Indonesia. Ecology, Environment and Conservation 25(April Suppl. Issue):57-61.
Hayati A., Abdizen M. M., Antien R. S., Solikha B. M., Maulidyah N., Tiantono N., Widyana H., Sugiharto., Winarni D., 2017 Bioaccumulation of heavy metals in fish (*Barbodes sp*.) tissues in the Brantas River, Indonesia. Journal of Applied Environmental and Biological Sciences 7(3):139-143.
Hayati A., Pratiwi H., Khoiriyah I., Winarni D., Sugiharto., 2016 Histopathological assessment of cadmium effect on testicles and kidney of *Oreochromis niloticus* in different salinity. In AIP Conference Proceedings of International Biology Conference 1854:1-8.
Håstein T., 2004 Animal welfare issue related to aquaculture. Proceeding Global Conference on Animal Welfare, Paris, pp. 219-231.
Hunter G. A., Sampson M. P., Ferreira G. C. 2008 Metal ion substrate inhibition of ferrochelatase. Journal of Biological Chemistry 283(35):23685-23691.
Khalesi M. K., Abedi Z., Behrouzi S., Eskandari S. K., Sciences S. A., Sea C., 2017 Haematological, blood biochemical and histopathological effects of sublethal cadmium and lead concentrations in common carp. Bulgarian Journal of Veterinary Medicine 20(2):141–150.
Kovarova J., Kizek R., Adam V., Harustiakova D., Celechovska O., Svobodova Z., Republic C., 2009 Effect of cadmium chloride on metallothionein levels in carp. Sensors (Basel) 9(6):4789–4803.
Mgbenka B. O., Oluah N. S., Umelke I., 2003 Effect of Gammalin 20 (Lindane) on the differential white blod cell counts of the African catfish, *Clarias albopunctatus*. Bull of Enviromental Contamination Toxicology 71:248-254.
Milad A., Dadar M., Fakhri Y., Conti G. O., Ferrante M., 2016 Heavy metal concentration in muscle of pike (*Esox lucius* Linnaeus, 1758) from Anzali international wetland, southwest of the Caspian Sea and their consumption risk assessment. Toxin Reviews 35(3-4):217-223.
Mohapatra R. K., Parhi P. K., Patra J. K., Panda C. R., 2018 Biodetoxification of toxic heavy metals by marine metal resistant bacteria a novel approach for bioremediation of the polluted saline environment. Microbial Biotechnology, pp. 343-376.
Monachese M., Burton J. P., Reid G., 2012 Bioremediation and tolerance of humans to heavy metals through microbial processes: a potential role for probiotics? Applied and Environmental Microbiology 78(18):6397–6404.
Nursanti L., Nofitasari E., Hayati A., Hariyanto S., Irawan B., Soegianto A., 2017. Effects of cadmium on metallothionein and histology in gills of tilapia (*Oreochromis niloticus*) at different salinities. Toxicological & Environmental Chemistry 99(5-6): 926-937
Okhionkpamwonyi O. N., Edema C. U., 2017 Effects of supplemental vitamin C (ascorbic acid) on the growth and health of African catfish *Clarias gariepinus*. Journal of Applied Sciences and Environmental Management 21(1):177–183.
Petric M., Martins M., Onaka E., Moraes J., Moraes F., Malheiros E., 2003 [Food supplementation with vitamin C potentiates the formation of polycariont macrophages in *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes: Characidae)]. Boletim Do Instituto de Pesca 29(1):69–76 [in Spanish].
Singh D., Nath K., Trivedi S. P., Sharma Y. K., 2008 Impact of copper on haematological profile of freshwater fish, *Channa punctatus*. Journal of Environmental Biology 29(2):253-257.
Strachan A., Abeysundara L., Mallett S. V., 2018 A concise summary of coagulation in liver disease. Anesthesia and Intensive Care Medicine 6(1):001-004.
Tête N., Afonso E., Bouguerra G., Scheifler R., 2015 Blood parameters as biomarkers of cadmium and lead exposure and effects in wild wood mice (*Apodemus sylvaticus*) living along a pollution gradient. Chemosphere 138:940-946.
Wang D., Zhong X. P., Qiao Z. X., Gui J. F., 2008 Inductive transcription and protective role of fish heme oxygenase-1 under hypoxic stress. The Journal of Experimental Biology 211:2700-2706.

Witeska M., Wakulska M., 2007 The effects of heavy metals on common carp white blood cells in vitro. Alternative to laboratory animals 35(1):87-92.

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