

Health Condition of *Tridacna* sp. In The Waters of Obi Island, Indonesia

Tamrin, Muhammad Aris*

Department of Aquaculture, Fisheries, and Marine of Faculty, University of Khairun, Ternate, Indonesia

* Corresponding Author: amboasse100676@gmail.com

Abstract

Tridacna sp. is one of the protected heritage in Indonesia, because its population has declined dramatically. *Tridacna* sp. is the largest type of shellfish in water. Like a clam, *Tridacna* sp. can be used as a bioindicator of the aquatic environment because it can accumulate more heavy metals than other aquatic organisms due to its nature as a filter feeder. This study aims to determine the health condition of *Tridacna* sp. in the waters of Obi Island with a histopathological analysis approach. The histopathological analysis aims to see the level of tissue damage due to the accumulation of heavy metals. Observation of water quality was also observed in this study. Water quality parameters observed in-situ are temperature, brightness, salinity, pH, and dissolved oxygen. While the water quality parameters observed ex-situ are nitrate, orthophosphate, ammonia, iron (Fe), and nickel (Ni). Observations show that the water temperature is at 27.99 °C. Water brightness is at 13 m. Salinity is at 32.13 mg / l. The acidity of the waters is 8.64. Ammonia is at 0.4 mg / L. Nitrate is at 0.009 mg / L. Orthopedics are at 0.016 mg / L. Dissolved oxygen waters are at 3.77 mg / L. The iron (Fe) level of water is at 0.6 mg / L. The level of nickel (Ni) waters is at 0.06 mg / L. This study shows the health condition of *Tridacna* sp. the histological approach shows that the condition of the network has changed. Symptoms of this change indicate the condition of the *Tridacna* sp. degeneration and cell necrosis. This change is thought to be influenced by heavy metals. Heavy metal content in liquids exceeds the quality standard threshold.

Keywords: *Tridacna* sp.; Heavy metal; Water quality; histopathological.

INTRODUCTION

Tridacna sp. is one of the protected aquatic organisms in Indonesia, because its population has declined dramatically (PERMENLHK, 2018). *Tridacna* sp. is the largest type of shellfish in water. Like a clam, *Tridacna* sp. can be used as a bioindicator of aquatic environments (Azizi et al. 2018; Jia et al. 2018; Saidov and Kosevich, 2019; Nour 2020).

Shells can accumulate more heavy metals than other aquatic organisms due to their nature as filter feeders (Kodama et al. 2012; Murphy et al. 2019; Feng et al. 2020). Heavy metals can be toxic to aquatic organisms because heavy metals are difficult to degrade, so they are easily accumulated in aquatic environments and their presence is naturally difficult to remove (Baramaki et al. 2012; Mansouri et al. 2012; Hao et al. 2019).

Industrial activities around the waters can contribute to heavy metals entering the waters around the river flow (Karbassi

et al. 2008; Kim et al. 2009; Abdolvand et al. 2014; Khan et al. 2019; Paschoalinia et al. 2019). One of the industrial activities on Obi Island is the mining industry. This study aims to determine the health condition of *Tridacna* sp. in the waters of Obi Island with a histopathological analysis approach. The histopathological analysis aims to see the level of tissue damage due to the accumulation of heavy metals (Poleksic et al. 2010; Dane and Şişman, 2020).

MATERIALS AND METHODS

Study Area

This research was conducted in the waters of Obi Island, South Halmahera Regency, North Maluku, Indonesia (01022,517'S, and 127033,934'E).

Water Quality Data Collection

Observation of water quality data is done in-situ and ex-situ. Water quality parameters observed in-situ are temperature, brightness, salinity, pH, and

dissolved oxygen. While the water quality parameters observed ex-situ are nitrate, orthophosphate, ammonia, iron (Fe) and nickel (Ni). For ex-situ observations, water samples are taken based on the Indonesian National Standard (SNI).

Histopathological examination

The aquatic organisms that were the target of sampling were (*Tridacna* sp.). The sample handling and histopathological analysis followed the Korun and Timur procedure (2008). Samples of fish organs were fixed with NBF 10%, dehydrated using ethanol solution in stages, then clearing using xylene and embedded using paraffin. Next, the sample was cut to a thickness of 5 µm with a microtome and stained using hematoxylin and eosin (H&E).

RESULTS AND DISCUSSION

Water quality parameters

Temperature is one of the most important factors in regulating life processes and the spread of organisms in waters. Water temperature controls the condition of aquatic ecosystems (Burt et al. 2011). Observations show that the water temperature is at 27.99 °C. These results indicate that the water temperature

is below the optimal range of quality standards or low temperatures (KepMenLH, 2004). Seawater brightness is very influential in the growth of marine organisms. Brightness is very high (Boyd and Pine, 2010). The observations showed the brightness of the waters was at 13 m. The results of this observation are following the quality standards for seawater organisms (KepMenLH, 2004).

Physiologically, salinity is closely related to osmotic pressure adjustment (Varsamos et al. 2005). Observations show salinity at 32.13 mg / l. This result shows salinity below the quality standard range (KepMenLH, 2004). The degree of acidity or pH is one of the important chemical parameters in monitoring water stability. The observations showed that the pH of the waters was at 8.64. These results indicate that the pH is in high or alkaline conditions (KepMenLH, 2004). The condition of waters that are acidic or basic will endanger the survival of the organism because it will cause metabolic and respiratory disorders. Besides that, a very low pH will cause the mobility of various toxic heavy metal compounds to be higher, while a high pH will increase the concentration of ammonia (Kale. 2016).

Table 1. Water Quality Observation Results

Parameters	Observation result	Quality standards	Unit
Temperature	27.99	28-30*	°C
Brightness	13	> 5*	Meters
Salinity	32.13	33 – 34*	Mg/L
pH	8.64	7-8.5*	
Dissolved oxygen	3,7	> 5*	mg/L
Nitrate	0.009	0.008*	mg/L
Ortophospat	0.016	0.015*	mg/L
Ammonia	0.400	.,3*	mg/L
Iron (Fe)	0.600	0.5**	mg/L
Nickel (Ni)	0.060	0.05*	mg/L

Information = (*): Kep-51 / MenKLH / 2004; (**): USEPA.

The analysis showed that ammonia was at 0.4 mg / L. These results indicate that ammonia exceeds the range of quality standards (KepMenLH, 2004). Ammonia can be toxic to organisms if the levels exceed the maximum threshold. High

ammonia levels can be indicated by the presence of organic material pollution from domestic waste, industrial waste, and agricultural fertilizer runoff (Franklin and Edward, 2019). Also, ammonia compounds in waters can also be derived

from the results of animal metabolism and the results of the process of decomposition of organic matter by bacteria (Kuypers, 2015).

Besides ammonia, the decomposition process by decomposing organisms also produces nitrates. The process is a nitrification process, namely the oxidation process of ammonia to nitrite and nitrate. This process is important in the nitrogen cycle and takes place in aerobic conditions. The oxidation of ammonia to nitrite is carried out by *Nitrosomonas* bacteria while the oxidation of nitrite to nitrate is carried out by *Nitrobacter* (Cáceres et al. 2017).

The analysis showed that nitrate was at 0.009 mg / L. These results indicate that nitrate exceeds the threshold range in the range of quality standards (KepMenLH, 2004). The high concentration of nitrate is thought to be influenced by temperature because high temperatures will cause a higher metabolic rate (He et al. 2019). The higher metabolic rate of phytoplankton can cause nitrates to be absorbed by phytoplankton more and so the nitrate measured is getting smaller (Akomeah et al. 2019). Observations (Table 1) show that the temperature is below the optimal range of quality standards or low temperatures (KepMenLH, 2004).

Besides temperature, nitrate concentration is also influenced by pH. If the pH in water is getting closer to base it will affect the concentration of nitrate, because the nitrate will tend to be higher when in an alkaline state (Jaramillo et al. 2018). The observations (Table 1) show that the pH is in a high or base condition. Besides, pH also affects the concentration of orthophosphate in water, if the pH approaches base, orthophosphate will tend to be higher in concentration (Guo et al. 2011). The analysis showed that orthophosphate was at 0.016 mg / L. This result shows that orthophosphate exceeds the range of quality standard threshold (KepMenLH, 2004).

Dissolved oxygen (DO) is needed by all living bodies for breathing, metabolic processes, or exchange of substances which then produce energy for growth and

culture (Keene et al. 2017). Besides, oxygen is needed for the oxidation of organic and inorganic materials in the aerobic process (Roots et al. 2019). Observations show DO waters are at 3.77 mg / L. This result shows DO is lower than the range of quality standards (KepMenLH, 2004).

The analysis shows that iron (Fe) waters are at 0.6 mg / L. These results indicate that Fe exceeds the threshold range of quality standards (USEPA, 1986). The analysis shows that nickel (Ni) waters are at 0.06 mg / L. These results indicate that nickel also exceeds the threshold of the quality standard range (KepMenLH, 2004).

High concentrations of heavy metals (Fe and Ni) that exceed the threshold are thought to originate from the activity of the mining industry. Also, the concentration of heavy metals is also influenced by temperature and dissolved oxygen. Low water temperature will make it easier for heavy metals to soak into the sediment. While temperatures are high, heavy metal compounds will dissolve in water (Huang et al. 2018). Low oxygen content causes lower solubility of heavy metals (Jitar et al. 2014). Observations show that temperature and dissolved oxygen are lower than the optimal range of quality standards (KepMenLH, 2004).

Histopathological analysis

Heavy metal pollution in waters is a very serious problem because it disrupts the health of aquatic organisms. The presence of heavy metals in waters can lead to the process of accumulation in the body of aquatic organisms naturally. Heavy metals that enter the body cannot be removed from the body anymore, because heavy metals tend to accumulate in the body. Besides, accumulation can also occur through direct absorption of heavy metals contained in water. Exposure to heavy metals results in physiological disorders that make the body of aquatic organisms have to adapt and can even cause tissue damage (Maleki et al. 2015; Benjamin et al. 2019; Thabet et al. 2019).

The results showed the Kima tissue (Figure 1) experienced an infiltration of lymphocytes, degeneration, and cell necrosis. Heavy metals can endanger the health of aquatic organisms. Heavy metal accumulation influences the immune response resulting in the infiltration of lymphocytes. Excessive accumulation leads to degeneration and necrosis or death of tissue making cells. Necrosis can threaten the sustainability of populations of aquatic organisms because of its low survival rate due to death (Athikesavan et al. 2006; Dohaish et al. 2018; Haredi et al. 2020).

Heavy metal pollution can also threaten human health through the food chain. Materials containing heavy metals that are wasted in the waters are eaten by microorganisms. Microorganisms are eaten by fish, shrimp, and shellfish so that the heavy metals accumulate in the body tissues of fish, shrimp, and shellfish and are finally consumed by humans (Turkmen et al. 2005; Triebkorn et al. 2008).

Heavy metal Fe is an essential metal whose existence in a certain amount is needed by living organisms, but in excessive amounts can affect living organisms, such as ferrous metals (Fe) and nickel (Ni) (Ibrahim and Tayel, 2005). The high content of Fe metal will have an impact on human health including poisoning (vomiting), intestinal damage, premature aging to sudden death, arthritis, birth defects, bleeding gums, cancer, kidney cirrhosis, constipation, diabetes, diarrhea, dizziness, fatigue, hepatitis, hypertension, insomnia (Youdim, 2001; Abbaspour et al. 2014; Wessling-Resnick, 2017). Nickel absorption can be through inhalation, oral, and dermal. Health problems that arise can be in the form of systemic disorders, immunological disorders, neurological disorders, reproductive disorders, developmental disorders, carcinogenic effects, and death (Das et al. 2019; Buxton et al. 2019).

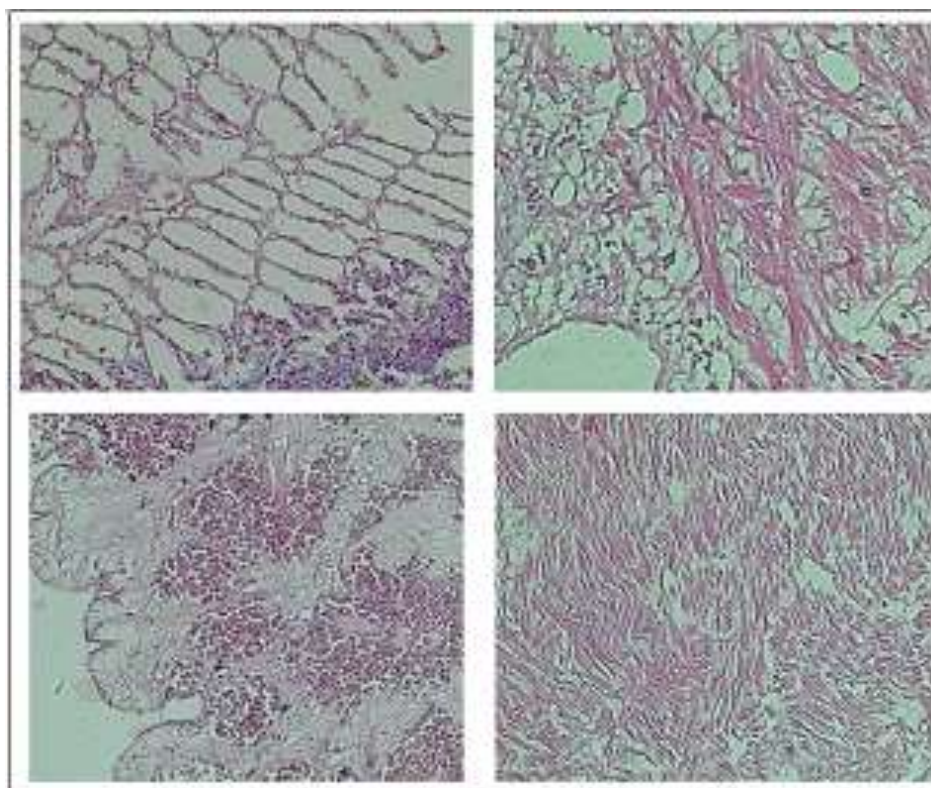


Fig. 1. Histopathological description of the Kima shell

CONCLUSION

The histological approach shows that the condition of the network has changed. Symptoms of this change indicate the condition of the *Tridacna* sp. degeneration and cell necrosis. This change is thought to be influenced by heavy metals. Heavy metal content in liquids exceeds the quality standard threshold.

REFERENCES

- Abdolvand S, Esfahani SK, Dmirchi S. 2014. Mercury (Hg) and Methyl Mercury (MMHg) Bioaccumulation in Three Fish Species (Sea Food) from Persian Gulf. *Toxicol. Environ. Health. Sci.* 6(3), 192-198. DOI: 10.1007/s13530-014-0204-y
- Akomeah E, Davies J, Lindenschmidt K. 2019. Water Quality Modeling of Phytoplankton and Nutrient Cycles of a Complex Cold-Region River-Lake System. *Environmental Modeling & Assessment*, DOI: 10.1007/s10666-019-09681-x
- Athikesavan S, Vincent S, Ambrose T, Velmurugan B. 2006. Nickel induced histopathological changes in the different tissues of freshwater fish, *Hypophthalmichthys molitrix* (Valenciennes). *Journal of Environmental Biology*, 27(2): 391-395
- Azizi G, Akodad M, Baghour M, Layachi M, Moumen A. 2018. The use of *Mytilus* spp. mussels as bioindicators of heavy metal pollution in the coastal environment. A review. *J. Mater. Environ. Sci.* 9, 1170–1181.
- Baramaki YR, Ebrahimpour M, Mansouri B, Razaeei MR, Babaei H. 2012. Contamination of Metals in Tissues of *Ctenopharyngodon idella* and *Perca fluviatilis*, from Anzali Wetland, Iran. *Bull. Environ. Contamin. Toxicol.* 89, 831-835. DOI: 10.1007/s00128-012-0795-4
- Benjamin KB, Co EL, Competente JL, de Guzman DGH. 2019. Histopathological Effects of Bisphenol A on Soft Tissues of *Corbicula fluminea* Mull. *Toxicol. Environ. Health. Sci.*, 11(1): 36-44. DOI: 10.1007/s13530-019-0386-4
- Boyd CE, Pine H. 2010. Application of agrometeorology to aquaculture and fisheries. In: *Guide to Agricultural Meteorological Practices (GAMP)*, Chapter 13. (PP. 1–25). Geneva, Switzerland: World Meteorological Organization
- Burt JM, Hinch SG, Patterson DA. 2011. The importance of parentage in assessing temperature effects on fish early life history: a review of the experimental literature. *Rev Fish Biol Fisheries*, 21: 377–406. DOI: 10.1007/s11160-010-9179-1
- Buxton S, Garman E, Heim KE, Lyons-Darden T, Schlekot CE, Taylor MD, Oller AR. 2019. Concise Review of Nickel Human Health Toxicology and Ecotoxicology. *Inorganics*, 7(89): 1-38. DOI: 10.3390/inorganics7070089
- Cáceres R, Malińska K, Marfà O. 2017. Nitrification within composting: A review. *Waste Management*, <https://doi.org/10.1016/j.wasman.2017.10.049>
- Dane H, Şişman T. 2020. A morpho-histopathological study in the digestive tract of three fish species influenced with heavy metal pollution. *Chemosphere*, 242: 125212. DOI: 10.1016/j.chemosphere.2019.125212
- Das KK, Reddy RC, Bagoji IB, Das S, Bagali S, Mullur L, Khodnapur JP, Biradar MS. 2019. Primary concept of nickel toxicity – an overview. *J Basic Clin Physiol Pharmacol*, 30(2): 141–152. DOI: 10.1515/jbcpp-2017-0171
- Dohaish EJAB. 2018. Impact of some heavy metals present in the coastal area of Jeddah, Saudi Arabia on the gills, intestine and liver tissues of *Lutjanus monostigma*. *Journal of Environmental Biology*, 39: 253-260. DOI: 10.22438/jeb/39/2/PRN-121
- Feng W, Wang z, Xu H, Chen L, Zheng F. 2020. Trace metal concentrations in commercial fish, crabs, and bivalves

- from three lagoons in the South China Sea and implications for human health. *Environmental Science and Pollution Research*, <https://doi.org/10.1007/s11356-019-06712-8>
- Franklin DA, Edward LL. 2019. Ammonia toxicity and adaptive response in marine fishes - A review. *Indian Journal of Geo Marine Sciences* 48(03): 273-279
- Guo H, Zhou J, Zhang S, Guo Z. 2011. Characteristics of nitrogen and phosphorus removal in a sequencing batch reactor. *J. Environ. Sci.* 23, S110-S113. [https://doi.org/10.1016/S1001-0742\(11\)61089-9](https://doi.org/10.1016/S1001-0742(11)61089-9)
- Hao Z, Chen L, Wang C, Zou X, Zheng F, Feng W, Zhang D, Peng L. 2019. Heavy metal distribution and bioaccumulation ability in marine organisms from coastal regions of Hainan and Zhoushan, China. *Chemosphere* 226: 340-350. DOI : [10.1016/j.chemosphere.2019.03.132](https://doi.org/10.1016/j.chemosphere.2019.03.132)
- Haredi AMM, Mourad M, Tanekhy M, Wassif E, Abdel-Tawab HS. 2020. Lake Edku pollutants induced biochemical and histopathological alterations in muscle tissues of Nile Tilapia (*Oreochromis niloticus*). *Toxicol. Environ. Health. Sci.* DOI: 10.1007/s13530-020-00042-w
- He J, Strezov V, Kan T, Weldekidan H, Asumadu-Sarkodie S, Kumar R. 2019. Effect of temperature on heavy metal(loid) deportment during pyrolysis of *Avicennia marina* biomass obtained from phytoremediation. *Bioresource Technology* 278: 214–222. <https://doi.org/10.1016/j.biortech.2019.01.101>
- Huang H, Yao W, Li R, Ali A, Du J, Guo D, Xiao R, Guo Z, Zhang Z, Awasthi MK. 2018. Effect of pyrolysis temperature on chemical form, behavior and environmental risk of Zn, Pb and Cd in biochar produced from phytoremediation residue. *Bioresour. Technol.* 249, 487–493. <https://doi.org/10.1016/j.biortech.2017.10.020>
- Ibrahim SA, Tayel SI. 2005. Effect of heavy metals on gills of *Tilapia zillii* inhabiting the River Nile water (Damietta branch) and El-Rahawy drain. Egypt. *J. Aquat. Biol. and Fish*, 9: 111-128.
- Jaramillo F, Orchard M, Muñoz C, Zamorano M, Antileo C. 2018. Advanced strategies to improve nitrification process in sequencing batch reactors - A review. *J. of Env. Man*, 218: 154-164. <https://doi.org/10.1016/j.jenvman.2018.04.019>
- Jia YY, Wang L, Qu ZP, Yang ZG. 2018. Distribution, contamination and accumulation of heavy metals in water, sediments, and freshwater shellfish from Liuyang River, Southern China. *Environ. Sci. Pollut. Res.* 25, 7012–7020.
- Jitar O, Teodosiu C, Oros A, Plavan G, Nicoara M. 2014. Bioaccumulation of heavy metals in marine organisms from the Romanian sector of the Black Sea. *N. Biotechnol.* DOI: 10.1016/j.nbt.2014.11.004
- Kale VS. 2016. Consequence of temperature, Ph, turbidity and dissolved oxygen water quality parameters. *Int J Adv Res Sci Eng Technol* 3:186–190
- Karbassi AR, Monavari SM, Nabi Bidhendi GR, Nouri J, Nematpour K. 2008. Metal pollution assessment of sediment and water in the Shur River. *Environ Monit Assess*, 147: 107–116. DOI: 10.1007/s10661-007-0102-8
- Keene NA, Reusser SR, Scarborough MJ, Grooms AL, Seib M, Santo Domingo J, Noguera DR. 2017. Pilot plant demonstration of stable and efficient high rate biological nutrient removal with low dissolved oxygen conditions. *Water Res.* 121: 72-85. <https://doi.org/10.1016/j.watres.2017.05.029>
- Khan UA, Kujala K, Nieminen SP, Räsänen ML, Ronkanen A. 2019. Arsenic, antimony, and nickel

- leaching from northern peatlands treating mining influenced water in cold climate. *Science of the Total Environment* 657 : 1161–1172. DOI: [10.1016/j.scitotenv.2018.11.455](https://doi.org/10.1016/j.scitotenv.2018.11.455)
- Kim Y, Kim BK, Kim K. 2009. Distribution and speciation of heavy metals and their sources in Kumho River sediment, Korea. *Environmental Earth Sciences*, 60 : 943-952. DOI: 10.1007/s12665-009-0230-2
- Kodama K, Lee JH, Oyama M, Shiraishi H, Horiguchi T. 2012. Disturbance of benthic macrofauna in relation to hypoxia and organic enrichment in a eutrophic coastal bay. *Mar. Environ. Res.* 76, 80–89
- Korun J, Timur G. 2008. Marine Vibrios Associated With Diseased Sea Bass (*Dicentrarchus labrax*) In Turkey. *Journal of Fisheries Sciences.* 2(1): 66-76. DOI: 10.3153/jfscm.2008
- Kuypers MMM. 2015. Microbiology: a division of labour combined. *Nature* 528: 487–488
- Mansouri B, Ebrahimpour M, Babaei H. 2012. Bioaccumulation and elimination of nickel in the organs of black fish (*Capoeta fusca*). *Toxicol. Ind. Health.* 28, 361-368
- Maleki A, Azadi NA, Mansouri B, Majnoni F, Rezaei Z, Gharibi F. 2015. Health Risk Assessment of Trace Elements in Two Fish Species of Sanandaj Gheshlagh Reservoir, Iran. *Toxicol. Environ. Health. Sci.* 7(1), 43-49. DOI: 10.1007/s13530-015-0219-z
- Menteri Negara Lingkungan Hidup, Indonesia. 2004. Keputusan Menteri Negara Lingkungan Hidup Nomor 51 Tahun 2004 tentang Baku Mutu Air Laut Untuk Biota Laut. Jakarta
- Menteri Lingkungan Hidup Dan Kehutanan Republik Indonesia. 2018. Peraturan Menteri Lingkungan Hidup Dan Kehutanan Republik Indonesia Nomor P.92/MENLHK/SETJEN/KUM.1/8/2018 Tentang Perubahan Atas Peraturan Menteri Lingkungan Hidup Dan Kehutanan Nomor P.20/MENLHK/SETJEN/KUM.1/6/2018 Tentang Jenis Tumbuhan Dan Satwa Yang Dilindungi
- Murphy AE, Kolkmeier R, Song B, Anderson IC, Bowen J. 2019. Bioreactivity and Microbiome of Biodeposits from Filter-Feeding Bivalves. *Microbial Ecology*, <https://doi.org/10.1007/s00248-018-01312-4>
- Nour HES. 2020. Distribution and accumulation ability of heavy metals in bivalve shells and associated sediment from Red Sea coast, Egypt. *Environ Monit Assess*, 192: 353. <https://doi.org/10.1007/s10661-020-08285-3>
- Paschoalinia AL, Savassia LA, Arantesb FP, Rizzoa E, Bazzolib N. 2019. Heavy metals accumulation and endocrine disruption in *Prochilodus argenteus* from a polluted neotropical river. *Ecotoxicology and Environmental Safety* 169: 539–550. DOI : 10.1016/j.ecoenv.2018.11.047
- Poleksic V, Lenhardt M, Jaric I, Djordjevic D, Gacic Z, Cvijanovic G, Raskovic B. 2010. Liver, Gills, And Skin Histopathology And Heavy Metal Content Of The Danube Sterlet (*Acipenser ruthenus* Linnaeus, 1758). *Environmental Toxicology and Chemistry*, 29(3): 515–521
- Roots P, Wang Y, Rosenthal AF, Griffin JS, Sabba F, Petrovich M, Yang F, Kozak JA, Zhang H, Wells GF. 2019. Comammox *Nitrospira* are the dominant ammonia oxidizers in a mainstream low dissolved oxygen nitrification reactor. *Water Research* 157, 396-405. <https://doi.org/10.1016/j.watres.2019.03.060>
- Saidov DM, Kosevich IA. 2019. Effect of Heavy Metals (Cu, Co, Cd) on the Early Development of *Mytilus edulis* (Mollusca; Bivalvia). *Russian Journal of Ecology*, (50)1: 58–64. DOI: 10.1134/S1067413619010077
- Thabet IA, Tawadrous W, Samy AM. 2019. Pollution induced change of liver of *Oreochromis niloticus*: metals accumulation and histopathological response. *World Journal of*

- Advanced Research and Reviews* 02(02), 025–035. DOI : [10.30574/wjarr.2019.2.2.0020](https://doi.org/10.30574/wjarr.2019.2.2.0020)
- Turkmen A, Turkmen M, Tepe YI, Akyurt I. 2005. Heavy metals in three commercially valuable fish species from Iskenderun Bay, Northern East Mediterranean Sea, Turkey. *Food Chemistry*, 91, 167–172
- Triebskorn R, Telcean I, Casper H, Farkas A, Sandu C, Stan G, Colărescu O, Dori T, Köhler H. 2008. Monitoring pollution in River Mureş, Romania, part II: Metal accumulation and histopathology in fish. *Environ Monit Assess* 141:177–188. DOI : [10.1007/s10661-007-9886-9](https://doi.org/10.1007/s10661-007-9886-9)
- USEPA. 1986. Quality criteria for water EPA 440/5-86-001. United States Environmental Protection Agency, Office of Water Regulations and Standards, Washington DC
- Varsamos S, Nebel C, Charmantier G. 2005. Ontogeny of osmoregulation in postembryonic fish: A review. *Comparative Biochemistry and Physiology, Part A* 141: 401–429. DOI: [10.1016/j.cbpb.2005.01.013](https://doi.org/10.1016/j.cbpb.2005.01.013)