Heavy metal concentrations in fish pond water after application of EM-1® effective microorganisms

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ABSTRACT

Effective microorganisms are being increasingly exploited in aquaculture. The aim of this study was to assess the effect of using effective microorganisms on heavy metal concentrations in fish pond water. This 105-day study was conducted at two fish ponds, control and experimental, used for commercial production of common carp (*Cyprinus carpio*). The EM-1® effective microorganisms were applied as a water supplement. The following elements were detected in the water of both fish ponds: aluminium, arsenic, beryllium, copper, lithium, manganese, vanadium, zinc, iron, barium, boron, uranium and strontium. Iron concentrations were highest in the fish ponds, ranging from 230.0 to 777.0 µg/L, whereas beryllium concentrations were lowest, ranging from the quantification limit to 2.5 µg/L. At the end of the study period, the levels of aluminium, beryllium, copper, manganese, vanadium, iron, barium, boron and uranium were significantly lower (P<0.05) and the levels of arsenic and strontium significantly higher (P<0.05) in the experimental fish pond as compared with the control fish pond, whereas the levels of lithium and zinc did not differ significantly (P>0.05) between the two fish ponds. Accordingly, the study results indicated the potential of EM-1® for improving fish pond water quality by reducing the concentrations of particular heavy metals in the water.

Key words: effective microorganisms; aquaculture; water quality; heavy metals; fish pond; carp farming

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Introduction

Aquaculture is the world's fastest growing sector of food production (GUILLEN et al., 2019; MAIR et al., 2023), with an average growth rate of 6.7% annually over the past three decades (FAO, 2022). EMENIKE et al. (2022) report that fish account for 20% of the average daily intake of animal protein for 3.2 billion people all over the world. Water quantity and quality are the main preconditions, but quite frequently also a restricting factor in aquaculture. Therefore, water ecosystems are a matter of interest in the production of fish and other aquatic organisms, which requires balanced physicochemical and biological environment conditions (VUČEMILO and TOFANT, 2009; POPOVIĆ, 2020).

Water pollution has become a global threat (CHAUDHRY and MALIK, 2017), mostly resulting from urbanisation, population growth, and human activities (PANDEY, 2006; LIN et al., 2022). The major sources of water pollution are industry, untreated waste water, as well as agricultural production (MATEO-SAGASTA et al., 2017). Heavy metals pose a major threat as water pollutants (SODHI et al., 2022; JAMIL EMON et al., 2023). Any metal (or metalloid) can be considered a heavy metal irrespective of its density or atomic mass if it occurs where it is unwanted, or in a form or concentration that has a detrimental effect on humans or the environment. Thus, all metals are toxic at high concentrations, and their presence in water can lead to water pollution (SINGH et al., 2011).

Effective microorganisms (EMs) are a mixed culture of microorganisms that are beneficial for nature, including humans, animals, plants, and other microbial species. EM-1® is the best known commercially available EM, which contains more than 80 types of anaerobic and aerobic microorganisms, such as photosynthetic bacteria, lactic acid bacteria, actinomycetes and yeasts (MOON et al., 2011), however, its exact composition is kept secret (SAFWAT and MATTA, 2021).

It has been demonstrated that EMs increase organic matter decomposition and phytoplankton diversity in water (DONDAJEWSKA et al., 2019), while reducing chemical oxygen demand, suspended substances, concentrations of total nitrogen and phosphorus, and water turbidity (ZHAO et al., 2013). They also have the ability to inhibit the growth of pathogenic microorganisms (SAFWAT and ROZAIK, 2017), and promote pesticide decomposition in the water (ISMAIL et al., 2015). In addition, studies have shown that the concentrations of heavy metals can be reduced in various water types with the application of EMs (ZHOU et al., 2008; SITAREK et al., 2017; YALÇIN et al., 2023).

Effective microorganisms are being increasingly used in aquaculture as either feed or water supplements. For example, ORJI and AKUKALIA (2021) found that the addition of EMs resulted in greater fish weight and length. Increased fish weight after the addition of EMs was also recorded by XU et al. (2021b) and JWHER and AL-SARHAN (2022). The results of a study carried out by OMAR et al. (2017) also revealed that the use of EMs had a favourable effect on the fish growth indices, along with lower heavy metal concentrations in the gills, liver, kidneys, muscle and skin, and thus less consequential organ damage.

However, studies dealing with the effect of EMs on fish pond water quality have reported contradictory results to date. ABDEL-SALAM et al. (2023) found the addition of EMs to have improved water quality, thus helping the fish accommodate to different temperatures, while reducing the effects of toxic pollutants. On the other hand, TANG et al. (2016) and ZHENG et al. (2017) failed to record any such effect of EMs.

This study investigated the effect of EM-1[®] on fish pond water quality by assessing heavy metal concentrations.

Materials and methods

The study was performed in commercial production conditions at Končanica d.d. fish farm, Končanica, Bjelovar-Bilogora County, Croatia. The study included two fish ponds, control and experimental ponds, rectangular in shape, area $3,800 \text{ m}^2$ and depth 1.2-2 m each, irrigated from the same Ilova river. The ponds are located near arable lands and roads. Each fish pond was populated with 7,000 common carp (*Cyprinus carpio*) juveniles, weight 7 g, length 6 cm, a week after the ponds

had been filled with water. The study lasted for 105 days in the summer-autumn period (from July to October) until the juvenile catch. The fish were fed once a day for six days a week.

In the experimental pond, EM-1® was applied in the form of 1,000 clay balls, manually distributed a week after juvenile settlement. The clay balls were made by mixing liquid EM-1® (EKO EM PLUS, Efektivni Mikroorganizmi originalna tehnologija Rijeka d.o.o., Rijeka, Croatia) and clay (ratio 1:3), and rolling the mixture in Bokashi bran (Efektivni Mikroorganizmi originalna tehnologija Rijeka d.o.o., Rijeka, Croatia). The balls were stored to dry in shade for a month.

Water samplings were performed in the morning (between 8:00 and 10:00 am) at 15-day intervals, eight times in total, including twice before using EM-1 $\mbox{\ensuremath{\mathbb{R}}}$. Each time, three composite samples were taken in 5-L glass bottles. The representative composite samples were obtained by manual sampling of particular samples of the same volume (0.5 L) using a sample grabber, at nine sites, i.e., eight sites along the edges and in the middle of the ponds, at a depth of 0.30 m below the surface. The samples were transported to the laboratory in controlled conditions (2-8°C).

The concentrations of aluminium (Al), arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lithium (Li), lead (Pb), manganese (Mn), nickel (Ni), selenium (Se), vanadium (V), zinc (Zn), iron (Fe), barium (Ba), boron (B), uranium (U), thallium (Tl), silver (Ag), strontium (Sr) and tin (Sn) in the water samples were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES), whereas mercury (Hg) concentration was determined using flow injection analysis. Sample preparation included acidification by adding 1.45 mL of nitric acid (69%, trace metal grade) to Falcon tubes (50 mL) per approximately 45 mL of the sample, and it was then made up with the sample to the tube mark. The samples used for determination of Hg concentration were acidified in the same way, but using 1.50 mL of hydrochloric acid (37%, pure acid). Hg concentration was measured on a flow injection mercury system (FIMS) 400 (Perkin Elmer, USA), whereas concentrations of all the other elements were determined on an Optima 8000 ICP-OES spectrometer (Perkin Elmer, USA). Statistical analyses were performed by using Statistica v. 14.0.1.25 (TIBCO Software Inc., USA). The Student's T-test (comparing values between the groups by study days) and one-way ANOVA (comparing values within the groups by study days) were employed for sample analysis, with Tukey HSD test for post hoc analysis, according to MORGAN (2017). These tests were used for quantitative variables (small samples) following previous checking for variance equality and normality of data distribution by using probability plots. The level of statistical significance was set at P<0.05.

Results

Heavy metal concentrations in the water of the experimental and control fish ponds throughout the study period and differences in the concentrations of particular elements according to days of observation are illustrated in Fig. 1-6, whereas differences in heavy metal concentrations between the experimental and control fish ponds according to the study days are shown in Table 1.

The presence of Al, As, Be, Cu, Li, Mn, V, Zn, Fe, Ba, B, U and Sr was demonstrated in both fish ponds, whereas Cd, Cr, Co, Pb, Ni, Se, Tl, Ag, Sn and Hg were not detected. Iron was shown to have the highest levels, ranging from 230.0 to 777.0 μ g/L, while Be had the lowest levels, ranging from the limit of quantification (LOQ) to 2.5 μ g/L.

In both fish ponds, the majority of detected elements reached the highest concentrations in the middle of the study period, followed by a decrease and then the absence of significant differences (P>0.05) until the end of the study (Fig. 1-6).

At the end of the study, the concentrations of Al, Li, Mn, Fe, Ba, B and U were significantly higher (P<0.05) and the concentrations of As, V, Zn and Sr significantly lower (P<0.05), whereas the concentrations of Cu did not differ significantly (P>0.05) compared with the beginning of the study in both fish ponds (Fig. 1-6). In the control fish pond, the concentration of Be was significantly higher (P<0.05) at the end compared with the beginning of the study. In the experimental fish pond, however, there was no significant difference (P>0.05) between Be concentrations at the end and the beginning of the study (Fig. 6).

	Day							
	1	15	30	45	60	75	90	105
	P-value							
Al	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
As	0.386	0.896	0.007	0.006	0.034	0.001	0.002	0.004
Be	1.000	1.000	0.588	0.021	0.609	0.002	0.001	0.016
Cu	0.003	0.000	0.000	0.030	0.092	0.000	0.000	0.000
Li	0.038	1.000	0.374	0.022	0.016	0.050	0.410	1.000
Mn	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000
V	0.013	0.768	0.326	0.643	0.003	0.038	0.492	0.016
Zn	0.000	0.000	0.002	0.012	0.374	0.374	0.374	0.374
Fe	0.011	0.000	0.001	0.000	0.000	0.000	0.000	0.000
Ba	0.000	0.081	0.692	0.001	0.000	0.000	0.000	0.000
В	0.001	0.011	0.003	0.007	0.009	0.008	0.058	0.001
U	1.000	0.000	0.001	0.000	0.185	0.004	0.000	0.000
Sr	0.000	0.006	0.000	0.001	0.023	0.000	0.001	0.002

Table 1. Differences (P-values) between experimental and control fish ponds according to study days

Al - aluminium, As - arsenic, Be - beryllium, Cu - copper, Li - lithium, Mn - manganese, V - vanadium, Zn - zinc, Fe - iron, Ba - barium, B - boron, U - uranium, Sr - strontium



Fig. 1. Zinc (Zn) and strontium (Sr) concentrations in experimental and control fish pond water during the 105-day study period

Values expressed as LS Mean; vertical bars denote 0.95 confidence intervals; ^{a,b,c,d,e} values recorded in the same fish pond marked with different letters differed significantly (P<0.05)



Fig. 2. Manganese (Mn) and barium (Ba) concentrations in experimental and control fish pond water during the 105-day study period

Values expressed as LS Mean; vertical bars denote 0.95 confidence intervals; $a_{a,b,c,d,e,f}$ values recorded in the same fish pond marked with different letters differed significantly (P<0.05)



Fig. 3. Arsenic (As) and boron (B) concentrations in experimental and control fish pond water during the 105-day study period

Values expressed as LS Mean; vertical bars denote 0.95 confidence intervals; a,b,c,d values recorded in the same fish pond marked with different letters differed significantly (P<0.05)





Fig. 5. Aluminium (Al) and iron (Fe) concentrations in experimental and control fish pond water during the 105-day study period

Values expressed as LS Mean; vertical bars denote 0.95 confidence intervals; $a^{b,c,d,e,f,g}$ values recorded in the same fish pond marked with different letters differed significantly (P<0.05)



Fig. 6. Beryllium (Be), copper (Cu) and uranium (U) concentrations in experimental and control fish pond water during the 105-day study period Values expressed as LS Mean; vertical bars denote 0.95 confidence intervals; ^{a,b,c,d} values recorded in the

In comparison to the control fish pond, the concentrations of B and Fe in the experimental fish pond were significantly higher (P<0.05) at the beginning, but significantly lower (P<0.05) at the end of the study (Fig. 3 and 5, Table 1). The concentrations of As, Be and U did not differ significantly (P>0.05) between the experimental and control fish ponds at the beginning of the study, but significantly lower Be and U levels (P<0.05) were measured in the experimental pond at the end of the study, along with a significantly higher As concentration (P<0.05) (Fig. 3 and 6, Table 1). The concentrations of Cu and V were significantly lower (P<0.05) in the experimental pond both at the beginning and at the end of the study, but the levels of these metals did not differ significantly (P>0.05) between the experimental and control fish ponds throughout the study period (Fig. 4 and 6, Table 1). The concentrations of Mn, Ba and Al also were significantly lower (P<0.05) in the experimental fish pond both at the beginning and the end of the study, but the differences in the final levels between the experimental and control fish ponds were greater compared with the differences recorded at the beginning of the study (Fig. 2 and 5, Table 1). In addition, the concentrations of Zn, Sr and Li were significantly lower (P<0.05) in the experimental fish pond at the beginning of the study, with no significant differences in the levels of Li and Zn (P>0.05) between the experimental and control fish ponds at the end of the study, while the level of Sr was significantly higher (P < 0.05) in the experimental fish pond (Fig. 1 and 4, Table 1).

Discussion

Pollution of water sources with heavy metals is a serious and ever-growing concern due to their toxicity, non-biodegradability and bioaccumulation, threatening both aquatic ecosystems and human health through the food chain (ALI et al., 2019; EMENIKE et al., 2022; HAMA AZIZ et al., 2023). The sources of pollution include volcanic eruptions, industrial effluents, agriculture activities, mining, electroplating, E-waste, biomedical waste, and power plants (SONONE et al., 2021). Heavy metals, such as As, Cd, Cu, Cr, Fe, Pb, Mn, Hg, Ni, Zn and Sn, are major contaminants that cause serious fish toxicity (SINGH et al., 2022). In our study, the presence of Cd, Cr, Pb, Hg, Ni and Sn was not determined in fish pond water, but As, Cu, Mn, Zn and Fe were detected, along with Al, Be, Li, V, Ba, B, U and Sr.

Heavy metal toxicity exerts unfavourable effects on fish growth, reproduction and physiology, and is associated with many fish deformities. Fish absorb metals mainly through the gills and digestive system, and to a lesser extent through the skin (SFAKIANAKIS et al., 2015; JAMIL EMON et al., 2023). Metals are mostly accumulated in the muscles, liver, kidneys and gills (SONONE et al., 2021; SINGH et al., 2022). Whereas some metals, such as Pb, Hg, Cd and As, are known to be highly toxic even at low concentrations, other metals, including Fe, Zn, Cu, Co, Cr, Mn and Ni, are essential elements in appropriate concentrations (GAUTAM et al., 2014).

the present study. Fe had highest In concentration, reaching 777.0 µg/L. The high concentration of Fe could be explained by discharge from agricultural activities in the vicinity of the fish pond, as also pointed out by ALADESANMI et al. (2014). CADMUS et al. (2018) assessed the current 1000 µg/L Fe total recoverable chronic criterion for protection of aquatic life in the United States, a value developed by use of very limited data in 1976, without any revision since. In order to develop a scientifically more reliable criterion, using literature data on chronic toxicity and their own study data, the authors calculated a final chronic value of 499 µg/L total Fe.

Heavy metal bioaccumulation in freshwater fish depends on both the characteristics of the fish and external environmental factors (ALI et al., 2019). In their study, MATAŠIN et al. (2008) demonstrated the effect of the geochemical environment on heavy metal concentrations in carp. In experimental conditions, Al concentrations as low as 50 μ g/L had unfavourable effects on common carp physiology (GARCÍA-MEDINA et al., 2010). Moreover, in the case of low pH (<5.5), exposure to Al concentrations of 12.5 μ g/L can cause severe physiological disorders in freshwater fish (ALLIN and WILSON, 2000). In our study, Al levels ranged from 49.0 to 725.0 μ g/L, suggesting a potential risk

for fish health depending on pH. In their study, DIETRICH and SCHLATTER (1989) reported that high Al concentrations (200 and 400 µg/L) at pH 5.4 and 5.6 resulted in fish gill obstruction with mucus, and thus impaired gas exchange. Al toxicity is also increased at a high pH (>9) (ANZECC and ARMCANZ, 2000). The effects of Be and Al on fish in acidic water are similar, but Be induces gill damage at lower concentrations, suggesting its role as a contaminant in acidic water. Be concentrations of \geq 10 µg/L caused increased mortality at pH 4.5 in juvenile perch, but only concentrations of \geq 50 µg/L were lethal at pH 5.5 (JAGOE et al., 1993). In our study, Be levels were lowest, reaching 2.50 µg/L.

KSZOS and STEWART (2003) report that a Li concentration as low as 600 μ g/L at long-term exposure had detrimental effects on juvenile rainbow trout survival. In the study by TKATCHEVA et al. (2007), detrimental effects in juvenile rainbow trout were demonstrated at a concentration of 528 μ g Li/L. In our study, Li concentrations ranged from 7.2 to 19.5 μ g/L. Investigating the effect of V on freshwater organisms, MEINA et al. (2020) found that V concentrations ranging between 1.8 and 6 mg/L reduced whole-body sodium and calcium in rainbow trout, while concentrations of >3.6 mg/L caused significant lipid peroxidation in fish gills and liver. In our study, V concentrations ranged from 5.0 to 29.0 μ g/L.

Copper is the main contaminant of aquatic ecosystems, which results in stressful conditions for aquatic organisms, while significantly interfering with fish growth and physiology (JAMIL EMON et al., 2023). Cu can influence the fish cardiovascular and nervous systems, sperm and egg production, glucose metabolism and cell structure, causing damage to various organs, such as the gills, liver and kidney (PADRILAH et al., 2018). Contamination of aquatic ecosystems with Zn is also well known. Zn toxicity has adverse effects on fish growth, reproduction, homeostasis, feed intake and bone formation, as well as damage to the fish liver (JAMIL EMON et al., 2023). ABDEL-TAWWAB et al. (2012) found that elevated Zn concentrations (up to 7.0 mg/L) increased ammonia excretion, which resulted in poor quality of the fish water. In their study, ALAM and MAUGHAN (1995)

determined the 96-hour 50% lethal concentration (LC_{50}) value of Cu for common carp to be 300 µg/L for small (3.5 cm) and 1,000 μ g/L for large (6.0 cm) fish, whereas the respective values varied for Zn, although they generally increased with the increase in fish size. Mn also influences fish physiology, occasionally exerting lethal effects (JAMIL EMON et al., 2023). The detrimental effects of Mn on fish mainly occur due to the increased oxidative stress induced by Mn (DOLCI et al., 2013). HARANGI et al. (2017) reported that Mn concentrations of 290 to 625 μ g/L had no effect on the survival and average body weight of common carp juveniles. In our study, the levels of Mn, Zn and Cu were 80.0-273.0 µg/L, 14.0-285.0 µg/L and 2.0-9.3 µg/L, respectively.

Arsenic is one of the most toxic heavy metals, which contaminates aquatic ecosystems by both natural and man-made actions, with multiple negative impacts on fish (JAMIL EMON et al., 2023). A study by KAR et al. (2011) demonstrated pond water as an important exposure medium for bioaccumulation of As in fish. Most of the aquaculture ponds explored by these authors had higher As concentrations than the maximum allowed (50 µg/L) in pond water in Taiwan, with the highest measured mean concentration of 75.7 μ g/L. In our study, As concentrations ranged from 1.0 to just 50.0 µg/L. Generally, LC₅₀ values of As for teleost fish species are between 7 and 29 mg/L, depending on the fish age, species, and environmental conditions (LIAN and WU, 2017).

SUZUKI et al. (1972) measured Sr concentrations in a common carp fish pond water from 28.0 to 48.5 μ g/L. In the present study, Sr levels ranged from 85.0 to 242.0 μ g/L. In a study conducted by LIU et al. (2022), Sr concentrations of \leq 60 mg/L determined in seawater had no toxic effect on *Coilia nasus* larvae development. However, toxicity may differ between freshwater and seawater fish (WHEELER et al., 2002).

Uranium poses a major threat for aquatic species such as fish, due to its toxicity and omnipresence in water sources (BARILLET et al., 2011). In a study by XU et al. (2021a), zebrafish embryos were exposed to U concentrations of 2, 20 and 100 μ g/L, and a significantly higher rate of larval malformations was recorded at a concentration of 100 μ g/L. CHEN et al. (2021) report a 96-hour LC₅₀ value of 17.785 mg/L U for zebrafish. In our study, U concentrations ranged from LOQ to 16.0 μ g/L.

Aquatic ecosystem contamination with B has recently been attracting increasing attention due to the toxic effects of high B concentrations on plants and animals (ACAR et al., 2018). These authors demonstrated the detrimental effects of B at high concentrations (50 and 100 mg/L) on the DNA integrity of blood and sperm cells, as well as on the serum biochemical parameters of *Nile tilapia*, with a 96-hour LC₅₀ value of 141.42 mg/L. ADHIKARI and MOHANTY (2012) report that the survival rate and growth of Indian major carp was reduced at 8.0 mg B/L. In our study, the levels of B ranged from 12.0 to 93.0 μ g/L.

Data on the potential unfavourable effects of Ba on fish are quite scarce. HEITMULLER et al. (1981) report on the Ba LC_{50} value for sheepshead minnow to be >500 mg/L. In our study, Ba concentrations ranged from 25.0 to 200.0 µg/L.

In a study by MANNZHI et al. (2021), the concentrations of Al, As, Cu, Mn, V, Zn and Fe in fish pond water for *Mozambique tilapia* farming were substantially lower, whereas the concentrations of Ba, B and Sr were within the range recorded in our study.

Various technologies have been used to remove heavy metals from waters, while scientists have been continuously investigating and developing new technologies (LICHT et al., 2022). Conventional technologies include physico-chemical processes, which are expensive and require large amounts of energy and specialised equipment (COLIN et al., 2012). Biological processes used to remove heavy metals from waters, such as bioremediation that involves the use of microorganisms, are an ecologically acceptable and cost-effective alternative to conventional methods (ZAKARIA et al., 2010; SAYQAL and AHMED, 2021). Bioremediation is a technique used to remove environmental contaminants from the ecosystem. Bioremediation is based on the principle of reducing the solubility of these contaminants by changing the pH, redox reactions and adsorption of contaminants from the environment. Redox

reactions involve chemical transformation of adverse contaminants into harmless or less toxic compounds that are more stable, less mobile or inert (OJUEDERIE and BABALOLA, 2017).

In our study, EM-1® was used for the removal of heavy metals from fish pond water. In comparison to the control fish pond, significantly lower concentrations of Al, Be, Cu, Mn, V, Fe, Ba, B and U were recorded in the experimental fish pond at the end of the study. ZHOU et al. (2008) found the maximum tolerant As concentration for EM bacteria to be 50 µg/L, whereas higher As concentrations could cause DNA damage, and reduce their capability for water treatment. Although the initial As concentration was almost $50 \mu g/L$ in the experimental fish pond, it decreased upon the use of EM-1[®] but was still significantly higher compared to the control fish pond at the end of the study. The final concentration of Sr was also significantly higher in the experimental fish pond. In contrast, YALCIN et al. (2023) measured lower Sr concentration in wastewater after the application of EMs. In the present study, Zn and Li concentrations did not differ significantly between the experimental and control fish ponds at the end of the study.

In both experimental and control fish ponds, the concentrations of the detected elements were generally highest in the middle of the study period, after which they decreased and remained lower until the end of the study. Nevertheless, significantly lower concentrations of most of the elements observed were recorded in the experimental fish pond at the end of the study, suggesting that the quality of fish pond water could be improved by use of EM-1[®].

Conclusions

The results of this study demonstrate that using EM-1® as a relatively inexpensive, ecologically acceptable, and easily applicable technology has the potential to reduce the levels of particular heavy metals in fish pond water. Additional EM research should be focused not only on contamination of fish pond water with heavy metals, but also on fish health and performance.

Ethics approval

The study was approved by the Committee for Ethics in Veterinary Medicine of the Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia.

Declaration of competing interest

The authors declare no conflict of interest.

References

ABDEL-SALAM, R. G., S. M. BASSEM, E. S. ABDEL-REHEIM, M. ABDEL-LATIF, G. GUERRIERO, F. K. ABDEL-GAWAD (2023): Role of effective microorganisms on hematological and biochemical indices of cultured *Oreochromis niloticus* exposed to lead, copper, and cadmium under temperature variations. J. Appl. Biol. Biotechnol. 11, 153-160.

https://doi.org/10.7324/JABB.2023.110222

- ABDEL-TAWWAB, M., G. O. EL-SAYED, S. H. SHADY (2012): Effects of dietary protein levels and environmental zinc exposure on the growth, feed utilization, and biochemical variables of Nile tilapia, *Oreochromis niloticus* (L.). Toxicol. Environ. Chem. 94, 1368-1382. https://doi.org/10.1080/02772248.2012.703202
- ACAR, Ü., B. E. İNANAN, F. ZEMHERI, O. S. KESBIÇ, S. YILMAZ (2018): Acute exposure to boron in Nile tilapia (*Oreochromis niloticus*): median-lethal concentration (LC_{50}) , blood parameters, DNA fragmentation of blood and sperm cells. Chemosphere 213, 345-350. https://doi.org/10.1016/j.chemosphere.2018.09.063
- ADHIKARI, S., M. MOHANTY (2012): Effect of waterborne boron and molybdenum on survival, growth and feed intake of Indian major carp, *Cirrhinus mrigala* (Hamilton). Chem. Ecol. 28, 113-121.

https://doi.org/10.1080/02757540.2011.627856

ALADESANMI, O. T., I. F. ADENIYI, I. M. ADESIYAN (2014): Comparative assessment and source identification of heavy metals in selected fishpond water, sediment and fish tissues/organs in Osun State, Nigeria. J. Health Pollut. 4, 42-53.

https://doi.org/10.5696/2156-9614-4-7.42

- ALAM, M. K., O. E. MAUGHAN (1995): Acute toxicity of heavy metals to common carp (*Cyprinus carpio*). J. Environ. Sci. Health – Toxic/ Hazard. 30, 1807-1816. https://doi.org/10.1080/10934529509376303
- ALI, H., E. KHAN, I. ILAHI (2019): Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. J. Chem. 2019, 6730305. https://doi.org/10.1155/2019/6730305

- ALLIN, C. J., R. W. WILSON (2000): Effects of pre-acclimation to aluminium on the physiology and swimming behaviour of juvenile rainbow trout (*Oncorhynchus mykiss*) during a pulsed exposure. Aquat. Toxicol. 51, 213-224. https://doi.org/10.1016/S0166-445X(00)00111-9
- ANZECC, ARMCANZ (2000): Australian and New Zealand guidelines for fresh and marine water quality. Volume 2. Aquatic ecosystems. Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand.

https://www.waterquality.gov.au/anz-guidelines/resources/ previous-guidelines/anzecc-armcanz-2000

BARILLET, S., C. ADAM-GUILLERMIN, O. PALLUEL, J.-M. PORCHER, A. DEVAUX (2011): Uranium bioaccumulation and biological disorders induced in zebrafish (*Danio rerio*) after a depleted uranium waterborne exposure. Environ. Pollut. 159, 495-502.

https://doi.org/10.1016/j.envpol.2010.10.013

CADMUS, P., S. F. BRINKMAN, M. K. MAY (2018): Chronic toxicity of ferric iron for North American aquatic organisms: derivation of a chronic water quality criterion using single species and mesocosm data. Arch. Environ. Contam. Toxicol. 74, 605-615.

https://doi.org/10.1007/s00244-018-0505-2

- CHAUDHRY, F. N., M. F. MALIK (2017): Factors affecting water pollution: a review. J. Ecosyst. Ecography 7, 225. https://doi.org/10.4172/2157-7625.1000225
- CHEN, L., Z. HUO, X. ZHOU, B. NIU, W. ZHANG, Z. P. TANG (2021): Acute toxic effects of uranium on two aquatic organisms, zebrafish (*Brachydanio rerio*) and ostracod (*Cypridopsis vidua*). IOP Conf. Ser.: Earth Environ. Sci. 937, 022020.

https://doi.org/10.1088/1755-1315/937/2/022020

COLIN, V. L., L. B. VILLEGAS, C. M. ABATE (2012): Indigenous microorganisms as potential bioremediators for environments contaminated with heavy metals. Int. Biodeterior. Biodegradation 69, 28-37.

https://doi.org/10.1016/j.ibiod.2011.12.001

- DIETRICH, D., C. SCHLATTER (1989): Aluminium toxicity to rainbow trout at low pH. Aquat. Toxicol. 15, 197-212. https://doi.org/10.1016/0166-445X(89)90036-2
- DOLCI, G. S., V. T. DIAS, K. ROVERSI, KR. ROVERSI, C. S. PASE, H. J. SEGAT, A. M. TEIXEIRA, D. M. BENVEGNÚ, F. TREVIZOL, R. C. S. BARCELOS, A. P. K. RIFFEL, M. A. G. NUNES, V. L. DRESSLER, E. M. M. FLORES, B. BALDISSEROTTO, M. E. BÜRGER (2013): Moderate hypoxia is able to minimize the manganese-induced toxicity in tissues of silver catfish (*Rhamdia quelen*). Ecotoxicol. Environ. Saf. 91, 103-109. https://doi.org/10.1016/j.ecoenv.2013.01.013

- DONDAJEWSKA, R., A. KOZAK, J. ROSIŃSKA, R. GOŁDYN (2019): Water quality and phytoplankton structure changes under the influence of effective microorganisms (EM) and barley straw lake restoration case study. Sci. Total Environ. 660, 1355-1366. https://doi.org/10.1016/j.scitotenv.2019.01.071
- EMENIKE, E. C., K. O. IWUOZOR, S. U. ANIDIOBI (2022): Heavy metal pollution in aquaculture: sources, impacts and mitigation techniques. Biol. Trace Elem. Res. 200, 4476-4492.

https://doi.org/10.1007/s12011-021-03037-x

FAO (2022): Food and Agriculture Organization. The state of world fisheries and aquaculture 2022. Towards Blue Transformation. The Food and Agriculture Organization of the United Nations, Rome.

https://doi.org/10.4060/cc0461en

GARCÍA-MEDINA, S., A. C. RAZO-ESTRADA, L. M. GÓMEZ-OLIVÁN, A. AMAYA-CHÁVEZ, E. MADRIGAL-BUJAIDAR, M. GALAR-MARTÍNEZ (2010): Aluminum-induced oxidative stress in lymphocytes of common carp (*Cyprinus carpio*). Fish Physiol. Biochem. 36, 875-882.

https://doi.org/10.1007/s10695-009-9363-1

- GAUTAM, R. K., S. K. SHARMA, S. MAHIYA, M. C. CHATTOPADHYAYA (2014): Contamination of heavy metals in aquatic media: transport, toxicity and technologies for remediation. In: Heavy Metals in Water: Presence, Removal and Safety. (Sharma, S. K., Ed.), The Royal Society of Chemistry, London, pp. 1-24. https://doi.org/10.1039/9781782620174-00001
- GUILLEN, J., F. ASCHE, N. CARVALHO, J. M. FERNÁNDEZ POLANCO, I. LLORENTE, R. NIELSEN, M. NIELSEN, S. VILLASANTE (2019): Aquaculture subsidies in the European Union: evolution, impact and future potential for growth. Mar. Policy 104, 19-28. https://doi.org/10.1016/j.marpol.2019.02.045
- HAMA AZIZ, K. H., F. S. MUSTAFA, K. M. OMER, S. HAMA, R. F. HAMARAWF, K. O. RAHMAN (2023): Heavy metal pollution in the aquatic environment: efficient and low-cost removal approaches to eliminate their toxicity: a review. RSC Adv. 13, 17595-17610. https://doi.org/10.1039/d3ra00723e
- HARANGI, S., E. BARANYAI, M. FEHÉR, C. N. TÓTH, P. HERMAN, L. STÜNDL, I. FÁBIÁN, B. TÓTHMÉRÉSZ, E. SIMON (2017): Accumulation of metals in juvenile carp (*Cyprinus carpio*) exposed to sublethal levels of iron and manganese: survival, body weight and tissue. Biol. Trace Elem. Res. 177, 187-195.

https://doi.org/10.1007/s12011-016-0854-5

HEITMULLER, P. T., T. A. HOLLISTER, P. R. PARRISH (1981): Acute toxicity of 54 industrial chemicals to sheepshead minnows (*Cyprinodon variegatus*). Bull. Environ. Contam. Toxicol. 27, 596-604. https://doi.org/10.1007/BF01611069 ISMAIL, A., A. DERBALAH, S. SHAHEEN (2015): Monitoring and remediation technologies of organochlorine pesticides in drainage water. Pol. J. Chem. Technol. 17, 115-122.

https://doi.org/10.1515/pjct-2015-0017

JAGOE, C. H., V. E. MATEY, T. A. HAINES, V. T. KOMOV (1993): Effect of beryllium on fish in acid water is analogous to aluminum toxicity. Aquat. Toxicol. 24, 241-256.

https://doi.org/10.1016/0166-445X(93)90074-B

JAMIL EMON, F., M. F. ROHANI, N. SUMAIYA, M. F. TUJ JANNAT, Y. AKTER, M. SHAHJAHAN, Z. ABDUL KARI, A. B. TAHILUDDIN, K. W. GOH (2023): Bioaccumulation and bioremediation of heavy metals in fishes – a review. Toxics 11, 510. https://doi.org/10.3390/toxics11060510

JWHER, D. M., M. R. AL-SARHAN (2022): Evaluation the role of effective microorganisms (EM) on the growth performance and health parameters on common carp (*Cyprinus carpio* L.). J. Appl. Vet. Sci. 7, 46-52.

https://doi.org/10.21608/JAVS.2022.122809.1128

KAR, S., J. P. MAITY, J.-S. JEAN, C.-C. LIU, C.-W. LIU, J. BUNDSCHUH, H.-Y. LU (2011): Health risks for human intake of aquacultural fish: arsenic bioaccumulation and contamination. J. Environ. Sci. Health - Toxic/Hazard. 46, 1266-1273.

https://doi.org/10.1080/10934529.2011.598814

KSZOS, L. A., A. J. STEWART (2003): Review of lithium in the aquatic environment: distribution in the United States, toxicity and case example of groundwater contamination. Ecotoxicology 12, 439-447.

https://doi.org/10.1023/a:1026112507664

LIAN, Z., X. WU (2017): Acute and chronic toxicities assessment of arsenic (III) to catfish, *Silurus lanzhouensis* in China. Cogent Biol. 3, 1334418.

https://doi.org/10.1080/23312025.2017.1334418

LICHT, K., I. HALKIJEVIĆ, H. POSAVČIĆ (2022): Overview of heavy metal removal methods from water. Hrvat. vode 30, 81-94. (in Croatian)

LIN, L., H. YANG, X. XU (2022): Effects of water pollution on human health and disease heterogeneity: a review. Front. Environ. Sci. 10, 880246.

https://doi.org/10.3389/fenvs.2022.880246

LIU, M.-Z., R.-J. JIANG, H. ZHANG, F. YANG, X.-F. LI, G.-P. FENG, R. YIN, F. CHEN (2022): Otolith marking with strontium for stock assessment in *Coilia nasus*. Front. Mar. Sci. 9, 890219.

https://doi.org/10.3389/fmars.2022.890219

MAIR, G. C., M. HALWART, Y. DERUN, B. A. COSTA-PIERCE (2023): A decadal outlook for global aquaculture. J. World Aquac. Soc. 54, 196-205. https://doi.org/10.1111/jwas.12977 MANNZHI, M. P., J. N. EDOKPAYI, O. S. DUROWOJU, J. GUMBO, J. O. ODIYO (2021): Assessment of selected trace metals in fish feeds, pond water and edible muscles of *Oreochromis mossambicus* and the evaluation of human health risk associated with its consumption in Vhembe district of Limpopo Province, South Africa. Toxicol. Rep. 8, 705-717.

https://doi.org/10.1016/j.toxrep.2021.03.018

MATAŠIN, Ž., V. OREŠČANIN, Z. PETRINEC, L. MIKELIĆ, E. GJURČEVIĆ, I. TLAK GAJGER, S. LULIĆ (2008): Heavy metals in sediment, water and cultivated common carp (*Cyprinus carpio* L.) in Croatian waters. Dtsch. Tierärztl. Wschr. 115, 71-74.

https://doi.org/10.2377/0341-6593-115-71

- MATEO-SAGASTA, J., S. M. ZADEH, H. TURRAL, J. BURKE (2017): Water pollution from agriculture: a global review. Executive summary. The Food and Agriculture Organization of the United Nations, Rome, the International Water Management Institute on behalf of the Water Land and Ecosystems research program, Colombo. https://www.fao.org/3/i7754e/i7754e.pdf.
- MEINA, E. G., S. NIYOGI, K. LIBER (2020): Investigating the mechanism of vanadium toxicity in freshwater organisms. Aquat. Toxicol. 229, 105648. https://doi.org/10.1016/j.aquatox.2020.105648
- MOON, Y. H., K. B. LEE, Y. J. KIM, Y. M. KOO (2011): Current status of EM (Effective Microorganisms) utilization. KSBB J. 26, 365-373. https://doi.org/10.7841/ksbbj.2011.26.5.365
- MORGAN, C. J. (2017): Use of proper statistical techniques for research studies with small samples. Am. J. Physiol. Lung Cell. Mol. Physiol. 313, L873-L877. https://doi.org/10.1152/ajplung.00238.2017
- OJUEDERIE, O. B., O. O. BABALOLA (2017): Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. Int. J. Environ. Res. Public Health 14, 1504.

https://doi.org/10.3390/ijerph14121504

OMAR, W. A., R. G. ABDEL-SALAM, H. M. MAHMOUD (2017): The use of effective microorganisms (EM) as a probiotic on cultured Nile Tilapia; *Oreochromis niloticus*. Egypt. J. Zool. 67, 67-90.

https://doi.org/10.12816/0037795

- ORJI, O. A., C. M. AKUKALIA (2021): Effective microorganism 'Em1' and their effects on performance of pond cat fish (*Clariasgariepinus*). IOSR J. Environ. Sci. Toxicol. Food Technol. 15, 39-43. https://doi.org/10.9790/2402-1502013943
- PADRILAH, S. N., M. K. SABULLAH, M. Y. A. SHUKOR, N. A. YASID, N. A. SHAMAAN, S. A. AHMAD (2018): Toxicity effects of fish histopathology on copper accumulation. Pertanika J. Trop. Agric. Sci. 41, 519-540.

- PANDEY, S. (2006): Water pollution and health. Kathmandu Univ. Med. J. 4, 128-134.
- POPOVIĆ, J. (2020): Management of Fishing Waters. Second supplemented edition. Croatian Sport Fishing Association, Zagreb. (in Croatian)
- SAFWAT, S. M., E. ROZAIK (2017): Growth inhibition of various pathogenic microorganisms using effective microorganisms (EM). Int. J. Res. Eng. 4, 283-286. https://doi.org/10.21276/ijre.2017.4.12.2
- SAFWAT, S. M., M. E. MATTA (2021): Environmental applications of Effective Microorganisms: a review of current knowledge and recommendations for future directions. J. Eng. Appl. Sci. 68, 48. https://doi.org/10.1186/s44147-021-00049-1
- SAYQAL, A., O. B. AHMED (2021): Advances in heavy metal bioremediation: an overview. Appl. Bionics Biomech. 2021, 1609149.

https://doi.org/10.1155/2021/1609149

- SFAKIANAKIS, D. G., E. RENIERI, M. KENTOURI, A. M. TSATSAKIS (2015): Effect of heavy metals on fish larvae deformities: a review. Environ. Res. 137, 246-255. https://doi.org/10.1016/j.envres.2014.12.014
- SINGH, A., A. SHARMA, R. K. VERMA, R. L. CHOPADE, P. P. PANDIT, V. NAGAR, V. ASERI, S. K. CHOUDHARY, G. AWASTHI, K. K. AWASTHI, M. S. SANKHLA (2022): Heavy metal contamination of water and their toxic effect on living organisms. In: The Toxicity of Environmental Pollutants. (Dorta, D. J., D. P. de Oliveira, Eds.), IntechOpen.

https://doi.org/10.5772/intechopen.105075

SINGH, R., N. GAUTAM, A. MISHRA, R. GUPTA (2011): Heavy metals and living systems: an overview. Indian J. Pharmacol. 43, 246-253.

https://doi.org/10.4103/0253-7613.81505

SITAREK, M., A. NAPIÓRKOWSKA-KRZEBIETKE, R. MAZUR, B. CZARNECKI, J. P. PYKA, K. STAWECKI, M. OLECH, S. SOŁTYSIAK, A. KAPUSTA (2017): Application of Effective Microorganisms Technology as a lake restoration tool – a case study of Muchawka Reservoir. J. Elem. 22, 529-543.

https://doi.org/10.5601/jelem.2016.21.2.1196

SODHI, K. K., L. C. MISHRA, C. K. SINGH, M. KUMAR (2022): Perspective on the heavy metal pollution and recent remediation strategies. Curr. Res. Microb. Sci. 3, 100166.

https://doi.org/10.1016/j.crmicr.2022.100166

SONONE, S. S., S. JADHAV, M. S. SANKHLA, R. KUMAR (2021): Water contamination by heavy metals and their toxic effect on aquaculture and human health through food chain. Lett. Appl. NanoBioScience 10, 2148-2166. https://doi.org/10.33263/LIANBS102.21482166

- SUZUKI, Y., R. NAKAMURA, T. UEDA (1972): Accumulation of strontium and calcium in freshwater fishes of Japan. J. Radiat. Res. 13, 199-207. https://doi.org/10.1269/jrr.13.199
- TANG, J.-Y., Y.-X. DAI, Y.-M. LI, J.-G. QIN, Y. WANG (2016): Can application of commercial microbial products improve fish growth and water quality in freshwater polyculture? N. Am. J. Aquac. 78, 154-160. https://doi.org/10.1080/15222055.2015.1116474
- TKATCHEVA, V., N. M. FRANKLIN, G. B. MCCLELLAND, R. W. SMITH, I. J. HOLOPAINEN, C. M. WOOD (2007): Physiological and biochemical effects of lithium in rainbow trout. Arch. Environ. Contam. Toxicol. 53, 632-638. https://doi.org/10.1007/s00244-006-0173-5
- VUČEMILO, M., A. TOFANT (2009): Practicum Environment and Animal Keeping Hygiene. Naklada Slap, Jastrebarsko. (in Croatian)
- WHEELER, J. R., K. M. Y. LEUNG, D. MORRITT, N. SOROKIN, H. ROGERS, R. TOY, M. HOLT, P. WHITEHOUSE, M. CRANE (2002): Freshwater to saltwater toxicity extrapolation using species sensitivity distributions. Environ. Toxicol. Chem. 21, 2459-2467. https://doi.org/10.1002/etc.5620211127
- XU, C., T. LI, C. HU, H. GUO, J. YE, L. LI, W. LIU, L. NIU (2021a): Waterborne uranium causes toxic effect and thyroid disruption in zebrafish larvae. Ecotoxicol. Environ. Saf. 208, 111585.

https://doi.org/10.1016/j.ecoenv.2020.111585

- XU, W., P. Y. MAWOLO, J. GAO, L. CHU, Y. WANG, Z. NIE, L. SONG, N. SHAO, J. GAO, P. XU, G. XU (2021b): Effects of supplemental effective microorganisms in feed on the growth, immunity, and appetite regulation in juvenile GIFT tilapia. Aquac. Rep. 19, 100577. https://doi.org/10.1016/j.aqrep.2020.100577
- YALÇIN, Z. G., M. DAĞ, E. AYDOĞMUŞ (2023): Wastewater treatment using active microorganisms and evaluation of results. IJANSER. 7, 55-61. https://doi.org/10.59287/ijanser.2023.7.4.550
- ZAKARIA, Z., S. GAIROLA, N. MOHD SHARIFF (2010): Effective microorganisms (EM) technology for water quality restoration and potential for sustainable water resources and management. Proceedings of the 5th International Congress on Environmental Modelling and Software, 5-8 July, Ottawa, Ontario, Canada, pp. 1-8.
- ZHAO, W., G. YANG, X. CHEN, J. YU, B. MA (2013): Study on integrated restoration technique for eutrophic artificial lakes. Adv. Mater. Res. 807-809, 1304-1310. https://doi.org/10.4028/www.scientific.net/AMR.807-809. 1304
- ZHENG, X., J. TANG, G. REN, Y. WANG (2017): The effect of four microbial products on production performance and water quality in integrated culture of freshwater pearl mussel and fishes. Aquac. Res. 48, 4897-4909. https://doi.org/10.1111/are.13309

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ZHOU, S., C. WEI, C. LIAO, H. WU (2008): Damage to DNA of effective microorganisms by heavy metals: impact on wastewater treatment. J. Environ. Sci. 20, 1514-1518. https://doi.org/10.1016/S1001-0742(08)62558-9

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SAŽETAK

Efektivni mikroorganizmi sve se više koriste u akvakulturi. Cilj istraživanja bio je utvrditi učinak primjene efektivnih mikroorganizama na koncentracije teških metala u vodi ribnjaka. Istraživanje je provedeno na dva ribnjaka s komercijalnom proizvodnjom običnog šarana (*Cyprinus carpio*), kontrolnom i pokusnom, u trajanju od 105 dana. U istraživanju su korišteni efektivni mikroorganizmi EM-1® primijenjeni kao dodatak vodi. Na oba ribnjaka detektirani su sljedeći elementi: aluminij, arsen, berilij, bakar, litij, mangan, vanadij, cink, željezo, barij, bor, uranij i stroncij. Koncentracije željeza u ribnjacima bile su najviše i kretale su se u rasponu od 230,0 do 777,0 μ g/L, a berilija najniže, u rasponu od granice kvantifikacije do 2,5 μ g/L. U usporedbi s kontrolnim ribnjakom koncentracije aluminija, berilija, bakra, mangana, vanadija, željeza, barija, bora i uranija na kraju istraživanog razdoblja bile su znakovito niže (P<0,05), a koncentracije arsena i stroncija znakovito više (P<0,05) u pokusnom ribnjaku, dok se koncentracije litija i cinka nisu znakovito razlikovale (P>0,05) između kontrolnog i pokusnog ribnjaka. Zaključno, rezultati istraživanja upućuju na potencijal EM-1® u poboljšanju kvalitete vode ribnjaka smanjenjem koncentracije pojedinih teških metala u vodi.

Ključne riječi: efektivni mikroorganizmi; akvakultura; kvaliteta vode; teški metali; ribnjak; uzgoj šarana