

Dietary supplements attenuate the toxicity of heavy metals and enhance their elimination in fish

Ewa Łuszczek-Trojnar, Ewa Dług-Kozak, Bartosz Bojarski

Received – 23 April 2025/Accepted – 25 March 2026. Published online: 31 March 2026;

©National Inland Fisheries Research Institute, Poland

Citation: Łuszczek-Trojnar, E., Dług-Kozak, E., Bojarski, B. (2026). Dietary supplements attenuate the toxicity of heavy metals and enhance their elimination in fish. *Fisheries & Aquatic Life* 34, 1-17.

Abstract. Heavy metals cause a threat to the aquatic environment and to its inhabitants when their concentrations exceed safe limits. This applies to both metals considered priority substances (Pb, Cd, Hg) and trace elements that are necessary for life at appropriate doses (Cu, Zn, Fe, Co, Ni, Mn). They can cause toxic effects in fish due to their non-biodegradable properties and long persistence in the environment. A decrease in heavy metal concentration in organisms observed by various researchers after the use of feed supplements is a positive sign, pointing to the possibility of inhibiting the accumulation and toxic impact of these substances on tissues and organs. In this review article, we demonstrate that certain supplements (such as vitamins, boron compounds, melatonin, lysine, and probiotics), each acting through distinct mechanisms, can reduce the accumulation of heavy metals, significantly mitigate their toxicity in fish, and accelerate their elimination from fish tissues. Consequently, dietary supplementation may positively influence the production of fish intended for human consumption by preventing avoidable losses associated with the deterioration of fish health. Indirectly, it also protects

consumers by reducing the risk of exposure to heavy metals that may be present in fish tissues.

Keywords: fish, heavy metals, health protection, supplementation

Introduction

The use of heavy metals in human activities is a common source of environmental pollution. Heavy metals enter open waters together with industrial and municipal wastewater, or with surface runoff. Heavy metals include essential metals (such as Zn or Cu), which are crucial for life and proper function of living organisms, and non-essential metals (e.g. Cd, Pb or Hg), which do not play any physiological role in organisms, but may disrupt their proper functioning (Ali et al. 2019). These non-essential heavy metals have low tolerance at very low concentrations, toxic effects at moderate concentrations, serious health damage at high concentrations, and most depressingly, lethality at excessive concentrations (Adazabra et al. 2013). Despite being essential for living organisms, microelements should be provided in amounts that do not exceed permissible thresholds; otherwise they become toxic. While our knowledge of heavy metals is rather extensive (Ali et al. 2019, Kumar et al.

E. Łuszczek-Trojnar [✉], E. Dług-Kozak
Department of Animal Nutrition and Fisheries, University of
Agriculture in Krakow, Aleja Adama Mickiewicza 24/28 30-059
Kraków, Poland
E-mail: ewa.trojnar@urk.edu.pl

B. Bojarski
Department of Animal Biology and Environment, Faculty of Animal
Breeding and Biology, Bydgoszcz University of Science and
Technology, Mazowiecka 28, 85-084 Bydgoszcz, Poland

2024), various aspects of their toxicity to aquatic animals, including fish, remain to be explored. Thus, new studies in this area continue to be undertaken. Given the negative effects of metals, their levels in fish are systematically monitored. This is especially important because fish consumption may be the main source of human exposure to heavy metals (Garcia et al. 2016). Analysis of their concentrations in fish also provides information on the contamination of the aquatic environment (López-López and Sedeño-Díaz 2015). Studies have demonstrated that metals like Cd, Pb, Cu, Fe, Cr, Zn, Hg or As can have a negative effect on fish, disrupting their reproduction and early development (Gouva et al. 2020, Łuszczek-Trojnar et al. 2014, Witeska et al. 2014, Drąg-Kozak et al. 2018, Elgaml et al. 2019, Taslima et al. 2022). These authors observed various effects, such as inhibited spermatogenesis via steroidogenesis suppression, disorders in the release of reproductive hormones, reduced reproductive output, egg production, number of spawns, average number of eggs per spawn and hatching rate, reduced embryo and larval survivability, lower larval growth rate, malformation in the yolk sac, curvature in vertebral column, body shortening, and cardiac edema. Cadmium or lead exposure may influence food intake and growth (Szczerbik et al. 2006, Łuszczek-Trojnar et al. 2013, Rahman et al. 2018, Drąg-Kozak et al. 2021a). By slowing down fish growth, heavy metals can lead to economic losses in aquaculture. Moreover, they can result in hematological (Łuszczek-Trojnar et al. 2019, Abalaka et al. 2021, 2022, Drąg-Kozak et al. 2021b, Yu et al. 2024) and hematopoietic (Kondera and Witeska 2012, 2013) changes, in addition to possibly contributing to the collapse of fish populations in their natural environment (Rahman et al. 2018). Researchers have also undertaken studies monitoring oxidative stress in fish living in metal-contaminated environments (Kirici et al. 2017, Drąg-Kozak et al. 2019) and assessing the genotoxic effects of these substances (e.g., Francisco et al. 2019, Silva et al. 2020, Drąg-Kozak et al. 2022). Therefore, the issue of heavy metals' influence on fish is complex and can be studied from many angles. In this review, we describe

the processes involved in eliminating heavy metals from fish tissues and focus on selected dietary supplements that may be used in aquaculture to reduce metal toxicity and accelerate metal elimination. These issues are of particular relevance for fish health, aquaculture production efficiency, and consumer health protection. Moreover, the present article provides a structured and integrative synthesis of current knowledge on dietary supplements that mitigate heavy metal toxicity in fish, with a particular focus on the mechanisms of detoxification and metal elimination. Unlike previous reviews, which primarily address metal accumulation or general antioxidant protection, the present article emphasizes supplements that actively enhance metal excretion and physiological recovery. By integrating mechanistic evidence with experimental outcomes reported across different fish species, this review contributes to a more comprehensive evaluation of dietary strategies to reduce heavy metal toxicity.

Heavy metal elimination

Toxicity refers to the effect displayed by a toxic agent at a target site in an organism. Thus, toxicity can be predicted based on the relationship between the concentration of a given toxicant at the target site and the ensuing toxic effect. Biologists have studied heavy metal accumulation in various fish tissues for a long time, but very few studies have focused on the process of metal elimination from the fish's body.

It is known that fish may exhibit higher or lower metabolism rates, depending on temperature, species, and age. In the process of breathing and feeding, fish take micro- and macroelements from the environment, including harmful ones, which they can then eliminate from the body in various proportions. In an unpolluted environment, where heavy metal levels do not exceed safe standards, the rate of metal uptake is equal to the rate of its elimination from the body, and therefore a constant increase in the concentration of metals in fish is not usually observed. Very often lower concentrations of heavy metals are

Table 1

Studies analyzing heavy metal elimination from the fish's body following exposure

Fish species	Metal	Dose of exposure	Exposure duration	Way of exposure	Elimination period duration	References
<i>Carassius gibelio</i>	Pb	8-49 mg kg ⁻¹	3-12 months	food	3-12 months	Łuszczek-Trojnar et al. 2013, 2014, 2015
<i>Carassius gibelio</i>	Cd	0.4-4.0 mg L ⁻¹	13 weeks and 5 months	water	3-6 weeks and 1-2 months	Drąg-Kozak et al. 2018, 2019, 2021a, 2021b
<i>Clarias gariepinus</i>	Pb, Cd	5-25 mg L ⁻¹	5 days	water	4 days	Babatunde et al. 2020
<i>Capotea fusca</i>	Cu	0.25-0.75 mg L ⁻¹	14-21 days	water	10-20 days	Mansouri et al. 2013a
<i>Capotea fusca</i>	Zn	3.6-9.75 mg L ⁻¹	21 days	water	10 days	Pourkhabbaz et al. 2015
<i>Capotea fusca</i>	Ni	4.5-12.7 mg L ⁻¹	5-15 days	water	5-15 days	Mansouri et al. 2012
<i>Capotea fusca</i>	Co	6.8 mg L ⁻¹	15 days	water	15 days	Mansouri et al. 2013b
<i>Dicentrachus labrax</i> <i>Solea senegalensis</i>	Cd	3.5-22.9 mg g ⁻¹	2 months	food	1-2 months	Le Croizier et al. 2018
<i>Tilapia niloticus</i>	Hg	10-20 µg kg ⁻¹	28 days	food	7 days	Sunardi et al. 2017
<i>Tilapia mossambicus</i>	Cd	0.5-1.5 mg L ⁻¹	30 days	water	30 days	Xue et al. 2023

observed in older fish than in younger ones, which is referred to as metal dilution in the growing organism (Canli and Atli 2003).

During exposure to increased metal concentrations, the rate of uptake is higher than the rate of elimination and an accumulation process is observed. However, after the cessation of exposure, the elimination rate increases again and the fish can effectively eliminate metals previously accumulated in the body (Łuszczek-Trojnar et al. 2013, 2015). Table 1 presents selected studies conducted over the last decade that analyze this subject. Their authors exposed fish of various marine and freshwater species to metals in water or feed during long- and short-term experiments. Łuszczek-Trojnar et al. (2013) demonstrated that long-term exposure to various doses of Pb administered to Prussian carp (*Carassius gibelio* (Bloch)) in feed caused accumulation in soft (liver, gills, kidneys, intestines, muscles) and rigid tissues (bones, scales) of this metal, always

in a dose-dependent manner. The authors observed changes in lead concentrations, ranging from a 3.5-fold increase in the intestine to a 22-fold increase in the gills of fish during the 12-month exposure period. The authors also showed that following exposure, elimination occurred in all studied fish, leading to a significant decrease in lead levels in soft tissues within 3 months. For example, when exposed to the highest Pb concentration in feed (49 mg kg⁻¹), after 3 months of elimination, the metal level decreased from 10% in the kidneys to 40% in the gills. On the other hand, rigid tissues presented higher concentrations even 12 months after the cessation of exposure, which suggests that lead accumulated in those tissues is not easily eliminated from the fish's organism.

Other studies analyzing Cd accumulation and elimination in Prussian carp revealed that the metal administered in water accumulates in tissues in a time- and dose-dependent manner, and even after

the end of the 3-month exposure period, further accumulation of Cd was observed in some tissues, such as the brain, hepatopancreas or ovaries. After two months of Cd elimination, the loss rate was 38% for the brain, 43% for the hepatopancreas, and 45% for the ovary in Prussian carp. Cd elimination from the kidneys was ineffective because two months after exposure cessation, the Cd loss rate stood at 0%, and the concentration did not change. However, in the muscles, the authors noted effective elimination, because the Cd concentration decreased by 75% (Drağ-Kozak et al. 2018, 2019, 2021a, 2021b).

Some studies assessing the effects of metal elimination in fish have been conducted on black fish (*Capoeta fusca* Nikol'skii) exposed to various heavy metals, such as copper (Cu), zinc (Zn), nickel (Ni), and cobalt (Co) (Mansouri et al. 2012, 2013a, 2013b; Pourkhabbaz et al. 2015). The authors of these studies demonstrated that the accumulation and elimination of all these metals depended on the tissue type, metal dose and exposure duration. They noted a 5- and 4.2-fold increase in Cu accumulation in the liver and kidneys, respectively, within 21 days of exposure, and a loss rate of 70% and 80% in the same tissues after 20 days following exposure cessation, reaching pre-exposure concentration (Mansouri et al. 2013a). The same authors noted an increase in the concentration of Ni, ranging from a 48-fold increase in the gills to over 92-fold in the liver during 15-day exposure to 12.7 mg dm^{-3} in water; later, during a 30-day Ni elimination period, the loss rate ranged from 71% in the muscles to 89% in the gills (Mansouri et al. 2012). Analyzing the accumulation and elimination of Zn in *C. fusca*, Pourkhabbaz et al. (2015) showed that during 21 days of exposure to 9.75 mg dm^{-3} , the fish accumulated it in their tissues at a level of 1.3 times the control level in the gills to 3.6 times in the kidneys. During the 10-day elimination period, they eliminated Zn from the tissues at a loss rate from 29% in the gills to 39% in the kidneys.

Le Croizier et al. (2018) showed that metal accumulation and elimination depended on fish species by exposing both European bass (*Dicentrarchus labrax* (L.)) and Senegalese sole (*Solea senegalensis*

Kaup) to cadmium. The researchers observed that these processes were faster in the former species as compared to the latter. Cd accumulation in the muscles of the bass reached 91 times the control concentration, in the liver 25 times and in the bile 20 times during 60 days of exposure to Cd in feed at a concentration of $22.9 \text{ mg kg}^{-1} \text{ d.m.}$ The same accumulation values for Senegalese sole were 6.3 times in the muscles, 11 times in the liver and 9.5 times in the bile, while the Cd loss rate was 53% for the muscles and bile and 10% for the liver (Le Croizier et al. 2018).

Experiments conducted at various water temperatures demonstrated faster accumulation and more effective elimination of Hg from the liver and kidney of Nile tilapia (*Oreochromis niloticus* (L.)) at a higher temperature (32°C), with no such effect observed in the muscle (Sunardi et al. 2017). Accumulation and elimination of Cd in Mozambique tilapia (*Oreochromis mossambicus* (Peters)) were dependent on water salinity (Xue et al. 2023). Among the four tissues studied, edible muscle showed the highest elimination rates, ranging from 76.7 to 81.9% at different salinities (0-25‰). The authors concluded that moderate salinity promoted the depuration of Cd in tissues.

An interesting result of fish detoxification observed by various authors is metal relocation, which involves secondary accumulation of a given metal released from other tissues (Drağ-Kozak et al. 2019, Le Croizier et al. 2018, Łuszczek-Trojnar et al. 2013). Metal relocation slows down the effective elimination of toxic substances from the fish body. Fish excrete metals mainly through the kidney (apart from the skin, gills, and liver). This organ is mainly responsible for internal osmoregulation, eliminating the excess of water and electrolytes, and excreting toxic agents. The glomerular filtrated metals are transported in the blood, whereas nephrocytes in the proximal tubule absorb and excrete them into the urine. Metals can be removed from the digestive tract in the feces or may be released by the basal lamina to hemocytes present in the visceral mass (Marigómez et al. 2002). An important way of detoxifying the body from heavy metals is their ability to bind to metallothionein, a family of proteins present in the

liver that bind excess metals and prevent their toxic effects. Metallothionein is capable of chelating metal ions via sulfhydryl groups to exert metal detoxification (Liu et al. 2022).

The above-mentioned studies demonstrate that fish can eliminate heavy metals from their bodies and the speed of this process depends on metal dose, exposure duration, tissue type, and fish species. Water temperature and salinity also seem to be important, but the significance of environmental conditions in the context of the rate of removal of these substances from the fish body requires further research.

Supplements used to protect fish from the toxic effects of heavy metals

A decrease in heavy metal concentrations in organisms is a positive sign that indicates the possibility of inhibiting the accumulation and toxic impact of metals on tissues. Studies analyzing the impact of heavy metals on fish have revealed that some supplements can reduce the accumulation of metals, significantly decrease their toxicity in fish, and accelerate their effective elimination from fish tissues (Table 2). The most commonly studied substances which act as antioxidants include melatonin, vitamins, borax, lysine, and probiotics (various *Lactobacillus* strains). Probiotics show a beneficial effect on digestive process, additionally displaying a protective effect against heavy metal toxicity.

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone secreted by the pineal gland and the retina in vertebrates and is produced from the amino acid tryptophan (Falcón 2007). It exhibits a strong daily circadian rhythm; the majority of the hormone is produced during the dark phase of the photoperiod (Reiter 1988). Melatonin is particularly effective in reducing oxidative stress under a wide range of conditions. Wongprayoon and Govitrapong (2016)

showed that melatonin could ameliorate the effects of toxic drugs on the human brain. It achieves this effect via a variety of means: direct detoxification of reactive oxygen and reactive nitrogen species and indirectly by stimulating antioxidant enzymes while suppressing the activity of pro-oxidant enzymes. In fish, the effects of melatonin are mediated through high-affinity receptors (Reiter et al. 2016). Drag-Kozak et al. (2018, 2019, 2021a, 2021b) studied Prussian carp females co-exposed to cadmium and melatonin. Melatonin had a positive influence both in terms of mitigating the effects of metal accumulation in tissues, and accelerating and enhancing effective metal elimination. The authors found that exogenous melatonin could effectively reduce Cd accumulation in the hepatopancreas, kidneys, heart, blood, brain and ovaries of Prussian carp. Observed effects could be melatonin's ability to bind heavy metals. In 1998, using absorptive voltammetry as a means of assessment, Limson et al. (1998) reported that melatonin binds aluminum, cadmium, copper, iron, lead, and zinc in a similar way to metallothionein, which plays an important role in binding of transition metals. Metallothionein plays a less important role as a binder of transition metals, particularly in the brain. Consequently, melatonin, in addition to its direct scavenging activity and indirect antioxidant effects, may replace or supplement metallothionein as a major binder of transition metals. Implanting melatonin in cadmium-treated fish resulted in marked improvements in hematological profile (red blood cell (RBC) count, hematocrit (Ht) level and hemoglobin (Hb) concentration) (Drag-Kozak et al. 2021b). The same authors observed that melatonin administered during Prussian carp exposure to Cd protected fish against toxic metal effects such as mortality, lower body weight, lowered GSI (gonadosomatic index), impaired spontaneous LH secretion, and impaired LH secretion during stimulation of fish spawning. Additionally, they found that melatonin co-treatment can effectively protect the fish against the toxic effects of cadmium on endogenous antioxidant status in hepatopancreas tissues. The authors suggest that preventive administration of melatonin may protect

Table 2

Studies analyzing the effects of dietary supplementation with protective agents against heavy metal toxicity in fish

Protective factor dose and treatment method	Metal dose, way and period of exposure	Fish species	Effects analysed	References
Melatonin in implants 18 mg	Cd 0.4 mg L ⁻¹ 4 mg L ⁻¹ in water 13 weeks and 5 months	<i>Carassius gibelio</i>	Reproductive effects (LH, GSI), oxidative stress (GR, GRx, SOD, GSH), mortality, hematological parameters (WBC, RBC, Ht, Hb, MCH, MCHC, MCV), growth, Cd accumulation and elimination in tissues	Drag-Kozak et al. 2018, 2019, 2021a, 2021b
Melatonin 50 and 200 mg kg ⁻¹ in diet	AgNPs 4000 mg L ⁻¹ in water 56 days	<i>Oreochromis niloticus</i>	The growth performance, GPx, SOD, MDA activity, plasma levels of: glucose, TP, AST, ALT Transcriptional parameters (GPx, SOD, hsp70, CYP1A), biochemical parameters (AChE, MDA, oxidative DNA damage, caspase-3 levels), immunohistochemically staining of 8-OHdG, hematological parameters (RBC, WBC, Hb, Ht, PLT, MCHC, MCH, MCV), antioxidant enzyme activities (SOD, CAT, GPx)	Veisi et al. 2021
Borax (1.25, 2.5, and 5 mg kg ⁻¹ in diet)	Cu (500 and 1000 mg kg ⁻¹) 21 days	<i>Oncorhynchus mykiss</i>	Antioxidant enzyme activities (CAT, GPx, SOD, MPO), biochemical parameters (AChE, MDA, oxidative DNA damage, caspase-3 levels, GSH), TNF- α , IL-6, Nrf-2, and BDNF levels	Alak et al 2018, 2019 a, b, c
Borax 0.75 mg L ⁻¹ in water	Al [AH,Al(OH)3] 4.41 mg L ⁻¹ 48 and 96 hours	<i>Oncorhynchus mykiss</i>		Alak et al. 2023
Bentonite – 40 g Mannan Oligosaccharide (MOS) – 4 g, organic selenium – 0.3 mg or Vit C – 50 mg	Pb 50 ppm in diet 16 weeks	<i>Oreochromis niloticus</i>	Pb concentration in fish gills, fish growth, FCR, TP, albumin, creatinine, AST, ALT, hematological parameters,	Ayyat et al. 2020
Vitamin C – 5 mg L ⁻¹ in water	Pb – 6 mg L ⁻¹ Cr – 2 mg L ⁻¹ , Cd – 8 mg L ⁻¹ 7 days	<i>Cyprinus carpio</i>	The activity of enzymes: (ALP, AST), TP in fish's blood and activity of GST and MDA level in gills	Sahiti et al. 2018
Vitamin C – 5 mg L ⁻¹ in water Vitamin E – 1000 mg kg ⁻¹ – in diet	Pb – 0.3 mg L ⁻¹ , Hg – 0.001 mg L ⁻¹ , Cd – 0.4 mg L ⁻¹ 2 weeks	<i>Cyprinus carpio</i>	Accumulation of metals in fish tissues (liver, muscle, gills and plasma)	Sahiti et al. 2020
Vitamin C 100 μ g L ⁻¹	Pb-60 μ g L ⁻¹ 48 hours	<i>Danio rerio</i>	AChE activity, lipid peroxidation (MDA), some antioxidant enzyme activities (SOD and GPx), three-dimension locomotion responses and changes of elements concentrations in the zebrafish body	Paduraru et al. 2021
Vitamin E – 20 IU kg ⁻¹ MT – metallothionein – 2.1 mg kg ⁻¹ In injections	Cd 20 μ mol kg ⁻¹ in injection 4 – 16 days	<i>Ctenopharyngodon idellus</i>	The activity of enzymes AST and ALT, oxidative stress in liver (CAT, SOD, and GSH-Px activity and related genes expression), liver histopathology	Feng et al. 2018

Protective factor dose and treatment method	Metal dose, way and period of exposure	Fish species	Effects analysed	References
Vitamin C – 500 mg kg ⁻¹	Pb – 5 mg L ⁻¹ in water 15 days	<i>Cyprinus carpio</i>	Plasma biochemical changes (Pb, MDA, creatinine, ALT, AST, LDH, GGT, albumin, glucose, triglyceride, total protein, cholesterol, urea and uric acid in plasma)	Nourian et al. 2019
Vitamin C – 50 mg L ⁻¹	Cd – 1.248 ppm 45 days	<i>Channa orientalis</i>	Hematological parameters (Hb, WBC, Ht)	Borane 2013
Vitamin C – 150 and 300 mg kg ⁻¹	Cd – 1 and 2 mg L ⁻¹ in water 8 weeks	<i>Cyprinus carpio</i>	Cd bioaccumulation, hematological parameters (Ca, cholesterol, Mg, glucose, ALP, ALT, AST, total protein, LDH, LZM), heat shock protein-related genes <i>Hsp70</i> , <i>Hsp90</i> , <i>Hsp47</i> and <i>Hsp60</i>	Wang et al. 2022
Vitamin C 500 mg kg ⁻¹	Cd 5 mg L ⁻¹ 45 days	<i>Oreochromis niloticus</i>	expression of genes (MT, GST- α 1 and GPx1), liver histopathology,	El-Sayed et al. 2016
Vitamin C – 100 mg kg ⁻¹ , Vitamin E – 50 mg kg ⁻¹ , dietary clay 3% (bentonite), probiotic 1 × 10 ¹⁰ CFU kg ⁻¹ in diet	Cd 25 and 50 mg kg ⁻¹ in diet 12 weeks	<i>Oreochromis niloticus</i>	Cd accumulation, body weight, daily gain, FCR, TP, albumin, creatinine, urea-N, AST, ALT, Hb, RBC, WBC, body composition, survival rate, Genotoxic and cytotoxic effects as micronuclei (MN), binuclei (BN), nuclear abnormalities (NAs), morphologically altered erythrocytes (MAIs)	Ayyat et al. 2017
Vitamin E 400 mg kg ⁻¹ , or A + C + E + Se (A – 300 μ g kg ⁻¹ , C – 60 mg kg ⁻¹ , E – 10 mg kg ⁻¹ , Se – 75 μ g kg ⁻¹)	Cd, Cu, Pb, Zn 1.25 mg L ⁻¹ each one in water 7 days	<i>Oreochromis niloticus</i>	Growth performance, oxidative damage in fish <i>in vivo</i> and in enterocytes <i>in vitro</i> : activity of antioxidant enzymes (SOD, GPx, GST, GR, CAT), Nrf2 in fish intestine, MTT, ALP, lactate dehydrogenase activities, PC, MDA, 8-hydroxydeoxyguanosine, and related gene expression, histopathology of stomach and intestine,	Harabawy and Mosleh 2014
Lysine 7.1-19.6 g kg ⁻¹ in diet (<i>in vivo</i>) or 0-300 mg L ⁻¹ in media (<i>in vitro</i>) 56 days	Cu 6 mg L ⁻¹ 24 hours	<i>Ctenopharyngodon idellus</i>	mortality, the growth performance parameters (WG, ADG, RGR, FCR), MN-test, Cd concentration in liver and kidney, oxidative stress (CAT, GSH-Px, SOD, MDA, ROS (reactive oxygen species), activity of LZM and ACP, C3 and C4 in the kidney, levels of TNF- α and IL-1 β)	Li et al. 2016
Lysine 0.4 mg 100 g ⁻¹ body weight per day ⁻¹ 30 days	Cr 300 ppm 30 days	<i>Clarias batrachus</i>	Concentration of Cd, Mg, Glu, TC, TP, RBC, HCT, Hb, HTC, AST, ALT, ALP, LDH, immune-related gene expression including IL-1, IL-6, IL-10, LZM, IgM, <i>Hsp70</i> and <i>Hsp90</i>	Dhami 2022
Probiotic <i>Lactobacillus acidophilus</i> 1 g kg ⁻¹	Cd 32 ppm in water or 0.1 ppm in diet 60 days	<i>Oreochromis niloticus</i>	<i>Hsp70</i> , <i>Hsp90</i> , <i>Hsp47</i> , <i>Hsp60</i> , <i>IL-1b</i> , <i>IL-6</i> , <i>IL-10</i> , <i>TNF-alfa</i> , IgM and LZM in the spleen, SIgA in the gut	Abu-Braka et al. 2017
Probiotic <i>Bacillus coagulans</i> 10 ⁸ CFU g ⁻¹	Cd 0.5 mg L ⁻¹ in water 60 days	<i>Cyprinus carpio</i>		Chang et al. 2021
Probiotic <i>Bacillus cereus</i> 10 ⁸ CFU g ⁻¹ 4 weeks	Cd 0, 1 and 2 mg Cd L ⁻¹ in water 4 weeks	<i>Carassius auratus gibelio</i>		Wang et al. 2019

Protective factor dose and treatment method	Metal dose, way and period of exposure	Fish species	Effects analysed	References
Probiotic <i>Lactobacillus bulgaricus</i> 10 ⁸ CFU g ⁻¹ 45 days	Pb 500 µg kg ⁻¹ in diet 21 days	<i>Oncorhynchus mykiss</i>	The hematological parameters	Mohammadian et al. 2020
Probiotic <i>Lactobacillus reuteri</i> 10 ⁸ CFU g ⁻¹	Pb 1 mg L ⁻¹ in water 6 weeks	<i>Cyprinus carpio</i>	Mortality, growth performances, hemato-biochemical parameters (MDA, GPx, SOD, Lysozyme, phagocytic activity, TNF-α, IL-1β, hsp70, hsp90, intestinal microbiota, and Pb accumulation in tissues (gill, spleen, liver, kidney, intestine)	Giri et al. 2018
Probiotic <i>Lactobacillus rhamnosus</i> 10 ⁶ CFU g ⁻¹ 60 days	Zn 4.56 mg L ⁻¹ and 2.28 mg L ⁻¹ 60 days	<i>Mystus montanus</i>	The hematological parameters (Hb, PCV, RBC, ESR, MCV, MCH, MCHC, HET, MON, EOS, BAS, LYM	Thuraiswami 2020
Probiotic <i>Lactobacillus buchneri</i> , <i>cesnori</i> , <i>bulgaricus</i> , <i>fermentum</i> 1:1:1:1 in diet 25 ml of solution (1x10 ⁸ CFU ml ⁻¹) kg ⁻¹ diet	Cu 0.75 mg L ⁻¹ , 1.5 mg L ⁻¹ , and 3 mg L ⁻¹ in water 15 days	<i>Oreochromis niloticus</i>	Growth performance (PWG, SGR) and total intestinal bacteria	Wanguyun et al. 2019
Probiotic <i>Lactobacillus lactis</i> In diet with 10 ⁹ and 10 ¹⁰ CFU g ⁻¹	Hg 0,13 ppm in diet 60 days	<i>Clarias gariepinus</i>	hematology, immunity, biochemical parameters and bioaccumulation. Growth performance, FCR, mortality, Hb and glucose levels; the frequencies of nuclear (notched nuclei, nuclear bridges, and karyopyknosis) and cellular (tear drop shaped, fusion, and twin) abnormalities of erythrocytes,	El-Bouhy et al. 2021
Probiotic <i>Bacillus subtilis</i> B. <i>thuringiensis</i> <i>Lactobacillus plantarum</i> <i>L. buchneri</i> 1 ml L ⁻¹	Cr 4 mg L ⁻¹ 42 days	<i>Labeo rohita</i>		Ferdous et al. 2024

GPx – glutathioneperoxidase, SOD – superoxide dismutase, GR – reductase, CAT – catalase, hsp70, hsp90 – heat shock proteins, CYP1A – cytochrome P450 1A, AChE – acetylcholinesterase, MDA – malondialdehyde, 8-OHdG – 8-hydroxydeoxyguanosine – marker of oxidative DNA damage, TNF-α – tumor necrosis factor alpha, IL-6 – interleukin 6, IL-1β – interleukin 1 Beta, Nrf-2 – nuclear factor erythroid-2, BDNF – brain-derived neurotrophic factor, ALP – alkaline phosphatase, AST – aspartate aminotransferase, ALT – alanine aminotransferase, ACP – acid phosphatase, TP – total proteins, TC – total cholesterol, RBC – red blood cells, WBC – white blood cells, Hb – hemoglobin, Ht – hematocrit, PLT – platelets, MCHC – mean corpuscular hemoglobin concentration, MCH – mean corpuscular hemoglobin, MCV – mean corpuscular volume, MPO – myeloperoxidase, PCV – packed cell volume, ESR – erythrocyte sedimentation rate, HET – heterophil, MON – monocyte, EOS – eosinophil, BAS – basophil, LYM – lymphocyte, WG – mean weight gain, ADG – average daily gain, RGR – specific growth rate, FCR – feed conversion ratio, MN – micronucleus test, PC – protein carbonyl, C3, C4 – complements, Cd – cadmium, Glu – glucose, Mg – magnesium, MT – metallothionein

fish against the adverse effects of exposure to heavy metals in the case of water contamination. Melatonin also reportedly chelates transition metals, which are involved in the Fenton/Haber-Weiss reactions; in doing so, melatonin reduces the formation of the devastatingly toxic hydroxyl radical resulting in the reduction of oxidative stress (Reiter et al. 2016). The ameliorative effect of dietary melatonin on AgNPs-induced toxicity (silver nanoparticle) in Nile tilapia was noted by Veisi et al. (2021). The authors used two doses of dietary melatonin (50 and 200

mg kg⁻¹ of dry diet) for 56 days. At the end of feeding, those fish fed with melatonin-supplemented diets were exposed to three concentrations of AgNPs for 24 h: 0.05, 0.1, and 0.5 mg L⁻¹. The authors observed various effects, depending on the doses used. The GPx (glutathioneperoxidase) and SOD (superoxide dismutase) activities were higher, and MDA (malondialdehyde) activity was lower in AgNPs-exposed Nile tilapia fed on a high melatonin-supplemented diet than the control individuals, thus indicating the ameliorative effect of

melatonin on the toxicity of AgNPs. The AST (aspartate aminotransferase) and ALT (alanine aminotransferase) activities in melatonin-treated Nile tilapia exposed to AgNPs were significantly lower than in untreated fish. Exposing Nile tilapia to AgNPs increased the plasma glucose concentration in a dose-dependent manner, while treatment with melatonin (especially at a dose of 200 mg kg⁻¹ of diet) led to a reduction in the glucose concentration, suggesting the protective effect of melatonin.

Vitamin C and E

Sahiti et al. (2018) studied the impact of vitamin C on Cr, Pb and Cd toxicity in common carp. These authors showed that exposure to a mixture of Pb, Cd and Cr resulted in significantly elevated MDA levels and increased GST (glutathione S-transferase) activity in the gills. The study also demonstrated a significant increase in AST and ALP activity, and a subsequent decrease in the total protein concentration in the blood. Vitamin C administration, however, caused a significant decrease in the level of MDA and in the activity of GST in the gills of the metal-exposed fish. Supplementation with ascorbic acid resulted in an effective decrease in the ALP and AST activity, which had been increased during the exposure. Nourian et al. (2019) recorded similar results after co-exposing common carp to Pb and vitamin C. The protective effect of vitamin C against Cd hepatotoxicity was presented by El-Sayed et al. (2016), who observed the potential of this vitamin to protect Nile tilapia by sustaining hepatic antioxidants' gene transcripts and normal histoarchitecture. These authors proved that co-treatment of Cd-exposed fish with vitamin C overexpressed antioxidant enzyme-related genes (GST- α 1 and GPx1), and maintained the expression of the MT gene close to the control, averting the toxicopathic lesions induced by Cd. In a study by Sahiti et al. (2020), Pb, Cd and Cr content decreased significantly in the tissues of common carp following supplementation with vitamins C and E (together or individually) as

compared to the exposed fish and control ones. Borane (2013) demonstrated a protective effect of vitamin C during exposure to Cd in the walking snakehead (*Channa orientalis* Bloch & Schneider). In his study, vitamin C acted as an antioxidant and effective inhibitor of cadmium chloride, mitigating toxicity symptoms observed in those fish that had not received the vitamin. It also accelerated normalization of hematological parameters after the end of fish exposure to cadmium. The administration of cadmium to grass carp (*Ctenopharyngodon idella* (Val.)) disrupted liver morphology and function. This was manifested by increased AST and ALT activity, organelle rupture, and decreased activity of SOD, CAT (catalase), and GSH-Px (glutathione peroxidase). Supplementation with metallothionein and vitamin E decreased the AST and ALT activities, repaired organelles, increased the activity of CAT, SOD, and GSH-Px, and regulated the expression of the associated mRNA transcript, thus providing protection against liver damage induced by cadmium in grass carp (Feng et al. 2018).

Wang et al. (2022) observed a significant protective effect of vitamin C on cadmium-exposed common carp after 8 weeks of exposure to the metal. This protection was related to inhibiting bioaccumulation, reducing changes in blood biochemical parameters (including the concentration of calcium, magnesium (Mg), Glu (glucose), TP (total protein), and TC (total cholesterol), and the activity of alkaline phosphatase – ALP, ALT, AST, and lactate dehydrogenase – LDH), and downregulating the transcription of the heat shock protein-related genes. Paduraru et al. (2021) observed the mitigative effects of vitamin C against behavioral and biochemical changes induced by a mixture of contaminants (with Pb) in zebrafish *Danio rerio* (Hamilton, 1822). The authors demonstrated that such a mixture can be used as an effective antioxidant. Harabawy and Mosleh (2014) observed the protective effect of vitamin E against the genotoxic and cytotoxic effects of Cd, Cu, Pb and Zn, and a more significant protective effect when vitamin E was administrated in combination with selenium and vitamins A and C. The results of the studies mentioned above indicate that supplementation with

vitamins C and E may exert an antioxidant effect against heavy metal toxicity in fish and effectively protect their health.

Borax

Boron (B) and borax (BX) are potentially detoxifying products, which affect the organism's physiological processes and metabolic reactions (Alak et al. 2018, Öz 2025). Alak et al. (2018, 2019abc) studied the properties of borax in copper-treated rainbow trout (*Oncorhynchus mykiss* (Walbaum)). Different doses of BX and/or copper (Cu) (1.25, 2.5 and 5 mg kg⁻¹ of BX; 500 and 1000 mg kg⁻¹ of Cu) were used for 21 days in pre- and co-treatment options. The oxidative response of the brain, gills, liver and kidney tissues of rainbow trout to borax applied at various doses was investigated. The authors found that copper causes a decrease in antioxidant enzyme activity and gene expression in the gill and liver tissues of *O. mykiss*, but an increase in 8-OHdG (8-hydroxydeoxyguanosine), MDA, caspase-3 levels, *hsp70* and *cyp1a* gene expressions. However, in borax-combined groups, antioxidant enzyme activity was increased and the levels of 8-OHdG, caspase-3 and MDA were decreased compared to the copper-exposed group. Similarly, *hsp70* and *cyp1a* gene expressions were decreased after treatment with BX (Alak et al. 2019c). In fish kidneys, antioxidant enzyme activity and gene expression were increased; 8-OHdG, caspase-3, and MDA levels were decreased in groups fed with borax-supplemented feeds in comparison to the copper-treated group (Alak et al. 2019b). Additionally, the authors observed that biochemical parameters (MDA, oxidative DNA damage marker 8-OHdG, and caspase-3 levels) in the brain were increased and AChE (acetylcholinesterase) activity decreased in the copper and copper-combined groups. However, in the borax group of every application, it was established that borax had a protective effect against copper toxic action by decreasing and/or increasing the relevant biochemical parameter in the brain tissue of the fish. In addition, the expression levels of the *sod*, *cat*, and *gpx*

genes in the brains of fish exposed to borax and to borax combination were significantly higher than in the groups treated with copper only; this indicates that borax supplementation provided significant protection against copper-induced neurotoxicity in rainbow trout (Alak et al. 2018). In their conclusion, the authors suggest that borax itself is not an antioxidant but it supports antioxidant defense mechanisms of fish disrupted by heavy metals. They revealed that borax displayed a protective effect, reducing oxidative stress in fish. In blood samples collected from Cu-treated fish, dose-dependent increases were observed in the following parameters: hematological indices (RBC and WBC counts, Hb concentration, Ht value, PLT (platelet) count, as well as MCHC (mean corpuscular hemoglobin concentration), MCH (mean corpuscular hemoglobin) and MCV (mean corpuscular volume), 8-OH-dG level, and rates of nuclear abnormalities. However, the treatments with BX did not alter these hematological and DNA damage endpoints. The data revealed that borax exhibited hematoprotective and genoprotective effects (Alak et al. 2019a). The potential neuroprotective effect of BX against the toxicity of aluminum hydroxide [Al(OH)₃] on the brain of rainbow trout (*O. mykiss*) was studied by the same authors (Alak et al. 2023). They observed apoptosis induced by Al in the Nrf-2/BDNF/AChE pathways (nuclear factor erythroid 2-related factor 2/brain-derived neurotrophic factor/acetylcholinesterase), as evidenced by DNA damage, enzyme inhibition and lipid peroxidations. Nevertheless, applications of BX supported antioxidant capacity without leading to apoptosis, lipid peroxidation, inflammatory response or DNA damage. BX showed a neuroprotective effect via down-regulating cytokine-related pathways, minimizing DNA damage, and apoptosis and up-regulating GSH, AChE, BDNF and antioxidant enzyme activity.

Lysine

Lysine is an essential amino acid for fish and one of the most important limiting amino acids for most

plant ingredients. The most apparent symptoms of lysine deficiency in fish are decreased feeding, stunted growth, and reduced feed efficiency. Excess lysine also exerts negative effects on the growth rate and feed utilization of fish (Dou et al. 2023). Supplementation with lysine mitigated lipid and protein oxidation in grass carp exposed to Cu by increasing both gene expression and activity of antioxidant enzymes SOD, GPx, GST, GR (reductase), and Nrf2 mRNA levels in the intestine (Li et al. 2016). The regenerative role of lysine was demonstrated by Dhimi (2022) in the alimentary tract of *Clarias batrachus* (L.) subjected to chronic chromium exposure. The author analyzed the microstructure of the stomach and intestine of fish after 30 days' exposure to the metal. The analysis revealed atrophy of the gastric epithelium, reduced mucosal folds, and degeneration of the gastric glands in fish exposed to Cr only, and nearly normal histological structure in the case of fish which were also injected with L-lysine in a dose of 0.4 mg 100 g⁻¹ body weight per day throughout the exposure period.

Unfortunately, little research has been conducted on the protective effect of lysine on heavy metal toxicity in fish, but the promising results obtained in the articles presented here provide hope that this is another supplement which may have a beneficial effect on fish health.

Probiotics

Probiotic microorganisms play a role in the formation of the largest microbial ecosystem in the intestine, namely, the gut microbiota (Wang et al. 2021). Probiotics have recently received attention as one of the potent antioxidants. The consumption of certain strains of probiotics alone or in combination with food exerts antioxidant efficacy and reduces oxidative damage. Certain probiotic strains implement their antioxidant effects by producing metabolites and antioxidant enzymes, increasing the antioxidant capacity, and reducing host oxidant metabolites (Kavyani et al. 2024). Many authors have observed

the ameliorative effect of probiotics against the toxic impact of heavy metals in fish. ThuraiSwami (2020) demonstrated a protective effect of probiotics against the toxic effects of Zn on selected blood parameters in *Mystus montanus* (Jerdon). Probiotics administered together with this heavy metal prevented an excessive decrease in the concentration of hemoglobin and MCV. Following probiotic administration to Nile tilapia, Abu-Braka et al. (2017) observed a mitigating effect on growth inhibition and genotoxicity induced by cadmium in the fish. Other authors demonstrated a positive effect of probiotic supplementation in copper-exposed Nile tilapia. Various *Lactobacillus* strains colonized fish intestines, enhancing effective digestion and thus protecting the fish against weight loss, which was observed in those fish that had not been supplemented with probiotics (Wangyun et al. 2019). The protective effects of probiotics against growth performance inhibition were also observed by Giri et al. (2018) in their study on common carp exposed to Pb. Probiotics also mitigated other toxic symptoms of lead treatment in carp, decreased mortality, reduced Pb accumulation in tissues, protected the fish against oxidative stress, and improved hemato-biochemical and immunological parameters. Furthermore, probiotic administration enhanced the activity of digestive enzymes, restored a proper balance of gut microbiota, and reversed Pb-induced changes in the expression of pro-inflammatory cytokines (TNF- α and IL-1 β), which are associated with the NF- κ B signaling pathway. Chang et al. (2021) indicated that dietary supplementation with *Bacillus coagulans* can restore growth performance and reduce Cd accumulation in the liver and kidney of common carp exposed to 0.5 mg dm⁻³ for 60 days. Wang et al. (2019) suggested that *Bacillus cereus* has the potential to countermeasure Cd-induced hematological disturbances and immunosuppression in *Carassius gibelio*. Probiotics used by the authors restored nonspecific immune and antioxidative capabilities. The beneficial impact of probiotics against the toxic effects of chromium was demonstrated by Ferdous et al. (2024) in rohu (*Labeo rohita* (Hamilton)) exposed to that metal for 42 days. Ayyat et al. (2017) studied the impact of

various supplements (vitamins C and E, probiotics, bentonite) on cadmium toxicity in Nile tilapia. Supplements added to the feed caused a decrease in Cd residue levels in the fish. The supplementation resulted in an improved growth rate and decreased serum creatinine concentration and ALT activity, irrespective of the dietary Cd concentration. El-Bouhy et al. (2021) proposed the potential use of *Lactobacillus lactis* to ameliorate Hg toxicity and as an immune stimulant in African catfish (*Clarias gariepinus* (Burchell)). Their results clarified that dietary supplementation with *L. lactis* improved the hematological picture and significantly increased lysozyme, antiprotease activity, nitric oxide, GSH, MDA, total protein, albumin and globulin, besides modulating ALT, AST, urea and creatinine levels compared with other groups. *L. lactis* mitigated the decline in the serum lysozyme, antiprotease activity, and nitric oxide of the Hg-exposed group. Additionally, *L. lactis* reversed the decline in GSH and MDA levels in the serum of *C. gariepinus* in the Hg-exposed group. This resulted in a slight accumulation of Hg in these organs (El-Bouhy et al. 2021).

Probiotics appear to exert their beneficial effects through several mechanisms: chelating metal ions, using their own antioxidant metabolites, regulating the activity of host antioxidants, increasing the level of host antioxidant metabolites, regulating host signaling pathways, reducing the activity of ROS-producing enzymes, and regulating intestinal microbiota (Kavyani et al. 2024).

Limitations

Despite the comprehensive synthesis presented in this review, several limitations should be acknowledged:

- 1) Species sensitivity – Most studies focus on a limited number of fish species (e.g., Prussian carp, Nile tilapia, rainbow trout). The effects of supplements on heavy metal toxicity may vary across species, and extrapolation to other fish or aquatic organisms is limited.
- 2) Environmental variability – Many studies were conducted under controlled laboratory conditions. Field conditions, including fluctuating temperature, salinity, and the presence of multiple pollutants may impact the effectiveness of supplements.
- 3) Tissue-specific data gaps – While some tissues (liver, kidney, gills) are studied extensively, data on other organs, such as the gonads, brain, or skin, remain scarce, thereby limiting understanding of systemic detoxification.
- 4) Dose response and long-term effects – Optimal doses, duration of supplementation, and potential long-term effects of these dietary agents are not fully established.
- 5) Interactions with other contaminants – Most studies assess metals individually, whereas real-world scenarios involve co-exposure to multiple chemicals, which may affect supplement efficacy.
- 6) Mechanistic understanding – Although antioxidant, chelation, and microbiome modulation mechanisms are proposed, the understanding of the precise molecular pathways remains incomplete across various species.
- 7) Different environmental conditions – The effects observed in the studies presented may depend on environmental factors such as temperature, water hardness (or salinity), and pH. These factors may be critically important and might significantly influence the fish's physiological response to the supplement.

Conclusion

Heavy metal exposure in fish and other aquatic organisms often results in significant bioaccumulation and toxic effects. Research confirms both the ability of fish to eliminate already accumulated heavy metals and also the effective protection provided by various feed supplements against the accumulation and toxic effects of metals. These findings are promising for the safe fish farming. Alongside probiotics, vitamins C and E or other substances with antioxidant properties exert a positive impact on fish health and

welfare. In the case of chronic or sporadic heavy metal contamination of water, these agents can protect fish against their negative effects. Therefore, supplementation may favorably impact the production of fish intended for consumption by preventing unnecessary losses. Protecting fish from contamination with heavy metals ultimately protects humans from consuming fish meat containing these substances.

Recommendations

In our opinion, ecotoxicological studies are of particular importance nowadays. In the context of heavy metals and fish, we recommend conducting studies involving different fish species simultaneously and various physicochemical parameters of water, especially with regard to eliminating these substances from tissues. However, such research is difficult to conduct for technical and logistical reasons, yet it seems that the results can fill the existing gap in knowledge. Studies that take into account the toxicity, accumulation and elimination of heavy metals in conditions of co-exposure to other toxic substances present in the aquatic environment may also be of great importance. Studies on the toxicology of heavy metals should analyze as many tissues and organs as possible and take into account different levels of exposure to these substances, as well as using different methods of treatment. We also recommend conducting application research aimed at introducing previously tested dietary supplements (melatonin, vitamins, borax, lysine, probiotics, etc.) into fish culture.

Author contributions. B.B. – conceptualization; E.Ł.T. and E.D.K. – funding acquisition; E.Ł.T. and E.D.K. – visualization; E.Ł.T. – writing (original draft); E.Ł.T., E.D.K., and B.B. – writing (review and editing).

ORCID iD

Ewa Łuszczek-Trojnar  <https://orcid.org/0000-0002-7929-267X>

Ewa Dług-Kozak  <https://orcid.org/0000-0002-2572-0034>

Bartosz Bojarski  <https://orcid.org/0000-0003-3922-0329>

References

- Abalaka, S. E., Oyelowo, F. O., Akande, M. G., Tenuche, O. Z., Sani, N. A., Adeyemo, B. E., Idoko, I. S., Ogbe, A. O., Ejeh, S. A. (2021). Effects of Moringa oleifera leaves extract, vitamin C, and taurine co-exposures on calcium and metallothionein levels, oxidative stress, and gill histopathological changes in *Clarias gariepinus* exposed to sub-lethal cadmium. *Environmental Science and Pollution Research*, 28, 52258-52271.
- Abalaka, S. E., Oyelowo, F. O., Akande, M. G., Tenuche, O. Z., Sani, N. A., Adeyemo, B. E., Idoko, I. S., Ogbe, A. O., Ejeh, S. A. (2022). Toxicopathological effects of acute cadmium chloride exposure of African Catfish, *Clarias gariepinus*. *Toxicology and Environmental Health Sciences*, 14, 25-32.
- Abu-Braka, A. Z., Zaki, M. S., Abbas, H. H., Ismail, N. E. D. A., Khalil, R., Tanekhy, M., Saad, T. (2017). Filed studies on some probiotics to minimize hazard effects of prevailing heavy metals contamination for improving immunity and growth performance of *Oreochromis niloticus*. *Electronic Physician*, 9(4), 4138-4144.
- Adazabra, A. N., Gyasi-Antwi, D., Nyaaba, R. A. (2013). Biomonitoring of non - essential heavy metals concentrations in the Tono Irrigation Dam using mussel tissues. *American Journal of Environmental Protection*, 2 (6), 121-127.
- Alak, G., Ucar, A., Yeltekin, A. Ç., Çomaklı, S., Parlak, V., Taş, I. H., Özkaraca, M., Topal, A., Kirman, E. M., Bolat, I., Atamanalp, M., Türkez, H. (2018). Neuroprotective effects of dietary borax in the brain tissue of rainbow trout (*Oncorhynchus mykiss*) exposed to copper-induced toxicity. *Fish Physiology and Biochemistry*, 44, 1409-1420.
- Alak, G., Parlak, V., Aslan, M. E., Ucar, A., Atamanalp, M., Türkez, H. (2019a). Borax Supplementation Alleviates Hematotoxicity and DNA Damage in Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Copper. *Biological Trace Element Research*, 187, 536-542.
- Alak, G., Yeltekin, A. Ç., Ucar, A., Parlak, V., Türkez, H., Atamanalp, M. (2019b). Borax Alleviates Copper-Induced Renal Injury via Inhibiting the DNA Damage and Apoptosis in Rainbow Trout. *Biological Trace Element Research*, 191, 495-501.
- Alak, G., Parlak, V., Yeltekin, A. Ç., Ucar, A., Çomaklı, S., Topal, A., Atamanalp, M., Özkaraca, M., Türkez, H. (2019c). The protective effect exerted by dietary borax on toxicity metabolism in rainbow trout (*Oncorhynchus mykiss*) tissues. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 216, 82-92.
- Alak, G., Türkez, H., Ucar, A., Yeltekin, A. C., Ozgeris, F. B., Parlak, V., Atamanalp, M. (2023). Neuroprotective properties of borax against aluminum hydroxide-induced

- neurotoxicity: Possible role of Nrf-2/BDNF/AChE pathways in fish brain. *Brain Research*, 1803, 148241 <https://doi.org/10.1016/j.brainres.2023.148241>.
- Ali, H., Khan, E., Ilahi, I. (2019). Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. *Hindawi Journal of Chemistry*, 6730305. <https://doi.org/10.1155/2019/6730305>.
- Ayyat, M. S., Mahmoud, H. K., El-Hais, A. E. M., Abd El-Latif, K. M. (2017). The role of some feed additives in fish fed on diets contaminated with cadmium. *Environmental Science and Pollution Research*, 24(30), 23636-23645. <https://doi.org/10.1007/s11356-017-9986-1>.
- Ayyat, M. S., Ayyat, A. M. N., Naiel, M. A. E., Al-Sagheer, A. A. (2020). Reversal effects of some safe dietary supplements on lead contaminated diet induced impaired growth and associated parameters in Nile tilapia. *Aquaculture*, 515, 734580. <https://doi.org/10.1016/j.aquaculture.2019.734580>.
- Babatunde B. B., Dike C. S., Ibekwe C. J. (2020). Assessing uptake kinetics and natural depuration of lead and cadmium in African catfish juvenile. *Journal of Applied Sciences and Environmental Management*, 24 (2), 207-211.
- Borane, V. R. (2013). Protective role of ascorbic acid on the cadmium induced changes in hematology of the freshwater fish, *Channa orientalis* (Schneider). *Advances in Applied Science Research*, 4(2), 305-308.
- Chang, X., Chen, Y., Fenf, J., Huang, M., Zhang, J. (2021). Amelioration of Cd-induced bioaccumulation, oxidative stress and immune damage by probiotic *Bacillus coagulans* in common carp (*Cyprinus carpio* L.). *Aquaculture Reports*, 20, 100678. <https://doi.org/10.1016/j.aqrep.2021.100678>
- Canli, M., Atli, G. (2003). The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environmental Pollution*, 121(1): 129-136.
- Dhami, B. S. (2022). Regenerative role of l-Lysine amino acid on alimentary canal of fresh water food fish due to chronic chromium toxicity- a lab study. *Acta Scientific Veterinary Sciences*, 4(2), 75-80.
- Dou, X., Liu, Y., Cao, Y., Zhang, Y., Fu, X., Deng, J., Tan, B. (2023). Effects of dietary lysine level on growth performance and protein metabolism in juvenile Leopard coral grouper (*Plectropomus leopardus*). *Aquaculture Nutrition*, 1017222. <https://doi.org/10.1155/2023/1017222>.
- Drag-Kozak, E., Socha, M., Gosiewski, G., Łuszczek-Trojnar, E., Chyb, J., Popek, W. (2018). Protective effect of melatonin on cadmium-induced changes in some maturation and reproductive parameters of female Prussian carp (*Carassius gibelio* B.). *Environmental Science and Pollution Research*, 25, 9915-9927.
- Drag-Kozak, E., Pawlica-Gosiewska, D., Gwilik, K., Socha, M., Gosiewski, G., Łuszczek-Trojnar, E., Chyb, J., Solnica, B., Popek, W. (2019). Cadmium-induced oxidative stress in Prussian carp (*Carassius gibelio* Bloch) hepatopancreas: ameliorating effect of melatonin. *Environmental Science and Pollution Research*, 26(12), 12264-12279.
- Drag-Kozak, E., Łuszczek-Trojnar, E., Socha, M. (2021a). Cadmium accumulation and depuration in the muscle of Prussian carp (*Carassius gibelio* Bloch) after sub-chronic cadmium exposure: ameliorating effect of melatonin. *Animals*, 11, 2454. <https://doi.org/10.3390/ani11082454>
- Drag-Kozak, E., Łuszczek-Trojnar, E., Socha, M., Bojarski, B. (2021b). Effects of melatonin on cadmium accumulation and haematological parameters in cadmium intoxicated Prussian carp (*Carassius gibelio* B.). *Annals of Animal Science*, 21(3), 899-923.
- Drag-Kozak, E., Kuchta-Gładysz, M., Grzesiakowska, A., Łuszczek-Trojnar, E., Socha, M. (2022). Genotoxic effect of cadmium and zinc in the peripheral erythrocytes of Prussian carp (*Carassius gibelio* B.). *Journal of Veterinary Research*, 66, 619-628.
- Elgaml, S. A., Saad, T. T., Hamed, M. F., Zaki, V. H. (2019). Effects of heavy metal pollutants on the reproduction of Nile tilapia. *International Journal of Fisheries and Aquatic Studies*, 7(5), 542-547.
- El-Bouhy, Z. M., Reda, R. M., Mahboub, H. H., Gomaa, F. N. (2021). Chelation of mercury intoxication and testing different protective aspects of *Lactococcus lactis* probiotic in African catfish. *Aquaculture Research*, 52, 3815-3828.
- El-Sayed, Y. S., El-Gazzar, A. M., El-Nahas, A. F., Ashry K. M. (2016). Vitamin C modulates cadmium-induced hepatic antioxidants' gene transcripts and toxicopathic changes in Nile tilapia, *Oreochromis niloticus*. *Environmental Science and Pollution Research*, 23, 1664-1670.
- Falcón, J. (2007). Nocturnal melatonin synthesis: How to stop it. *Endocrinology*, 148, 1473-1474.
- Feng, Y., Huang, X., Duan, Y., Fan, W., Duan, J., Wang, K., Geng, Y., Ouyang, P., Deng, Y., Chen, D., Yang, S. (2018). The effect of vitamin E and metallothionein on the antioxidant capacities of cadmium-damaged liver in Grass carp *Ctenopharyngodon idellus*. *BioMed Research International*, 7935396. <https://doi.org/10.1155/2018/7935396>.
- Ferdous, Z., Rafiquzzaman, S. M., Shahjahan, Md. (2024). Probiotics ameliorate chromium-induced growth retardation and stress in Indian major carp rohu, *Labeo rohita*. *Emerging Contaminants*. 10: 100291. <https://doi.org/10.1016/j.emcon.2023.100291>
- Francisco, C. D. M., Bertolino, S. M., De Oliveira, R. J. J., Morelli, S., Pereira, B. B. (2019). Genotoxicity assessment of polluted urban streams using a native fish *Astyanax altiparanae*. *Journal of Toxicology and Environmental Health, Part A*, 82(8), 514-523.

- García, M. Á., Núñez, R., Alonso, J., Melgar, M. J. (2016). Total mercury in fresh and processed tuna marketed in Galicia (NW Spain) in relation to dietary exposure. *Environmental Science and Pollution Research*, 23(24), 24960-24969.
- Giri, S. S., Yun, S., Jun, J. W., Kim, H. J., Kim, S. G., Kang, J. W., Kim, S. W., Han, S. J., Sukumaran, V., Park, S. C. (2018). Therapeutic effect of intestinal autochthonous *Lactobacillus reuteri* P16 against waterborne lead toxicity in *Cyprinus carpio*. *Frontiers in Immunology*, 9, 1824. <https://doi.org/10.3389/fimmu.2018.01824>.
- Gouva, E., Nathanailides, C., Skoufos, I., Paschos, I., Fotini, A., Pappas, I. S. (2020). Comparative study of the effects of heavy metals on embryonic development of zebrafish. *Aquaculture Research*, 51, 3255-3267.
- Harabawy, A. S. A., Mosleh, Y. Y. I. (2014). The role of vitamins A, C, E and selenium as antioxidants against genotoxicity and cytotoxicity of cadmium, copper, lead and zinc on erythrocytes of Nile tilapia, *Oreochromis niloticus*. *Ecotoxicology and Environmental Safety*, 104, 28-35.
- Kavyani, B., Ahmadi, S., Nabizadeh, E., Abdi, M. (2024). Anti-oxidative activity of probiotics; focused on cardiovascular disease, cancer, aging, and obesity. *Microbial Pathogenesis*, 196, 107001. <https://doi.org/10.1016/j.micpath.2024.107001>.
- Kirici, M., Turk, C., Caglayan, C., Kirici, M. (2017). Toxic effects of copper sulphate pentahydrate on antioxidant enzyme activities and lipid peroxidation of freshwater fish *Capoeta umbla* (Heckel, 1843) tissues. *Applied Ecology and Environmental Research*, 15(3), 1685-1696.
- Kondera, E., Witeska, M. (2012). Cadmium-induced alterations in head kidney hematopoietic tissue of common carp. *Fresenius Environmental Bulletin*, 21(3a), 769-773.
- Kondera, E., Witeska, M. (2013). Cadmium and copper reduce hematopoietic potential in common carp (*Cyprinus carpio* L.) head kidney. *Fish Physiology and Biochemistry*, 39, 755-764.
- Kumar, M., Singh, S., Jain, A., Yadav S., Dubey, A., Trivedi, S. P. (2024). A review on heavy metal-induced toxicity in fishes: Bioaccumulation, antioxidant defense system, histopathological manifestations, and transcriptional profiling of genes. *Journal of Trace Elements in Medicine and Biology*, 83, 127377. <https://doi.org/10.1016/j.jtemb.2023.127377>.
- Le Croizier, G., Lacroix, C., Artigaud, S., Le Floch, S., Raffray, J., Penicaud, V., Coquillé, V., Autier, J., Rouget, M. L., Le Bayon, N., Laë R., De Morais L.T. (2018). Significance of metallothioneins in differential cadmium accumulation kinetics between two marine fish species. *Environmental Pollution*, 236, 462-476.
- Li, X. Y., Liu, Y., Jiang, W. D., Jiang, J., Wu, P., Zhao, J., Kuang, S. Y., Tang, L., Tang, W. N., Zhang, Y. A., Zhou, X. Q., Feng, L. (2016). Co- and post-treatment with lysine protects primary fish enterocytes against Cu-induced oxidative damage. *PLoS One*, 26, 11(1), e0147408. <https://doi.org/10.1371/journal.pone.0147408>
- Limson, J., Nyokong, T., Daya, S. (1998). The interaction of melatonin and its precursors with aluminium, cadmium, copper, iron, lead, and zinc: An adsorptive voltammetric study. *Journal of Pineal Research*, 24, 15-21.
- Liu, Y., Wu, Z., Guo, K., Zhou, Y., Xing, K., Zheng, J., Sun, Y., Zhang, J. (2022). Metallothionein-1 gene from *Exopalaemon carinicauda* and its response to heavy metal ions challenge. *Marine Pollution Bulletin*, 175, 113324. <https://doi.org/10.1016/j.marpolbul.2022.113324>
- López-López, E., Sedeño-Díaz, J. E. (2015). Biological indicators of water quality: the role of fish and macroinvertebrates as indicators of water quality. In: *Environmental indicators*. (Ed.) R. Armon, O. Hänninen, Springer, Dordrecht, The Netherlands: 643-661.
- Łuszczek-Trojnar, E., Drąg-Kozak, E., Popek, W. (2013). Lead accumulation and elimination in tissues of Prussian carp, *Carassius gibelio* (Bloch, 1782), after long-term dietary exposure, and depuration periods. *Environmental Science and Pollution Research*, 20(5), 3122-3132.
- Łuszczek-Trojnar, E., Drąg-Kozak, E., Szczerbik, P., Socha, M., Popek, W. (2014). Effect of long-term dietary lead exposure on some maturation and reproductive parameters of a female Prussian carp (*Carassius gibelio* B.). *Environmental Science and Pollution Research*, 21, 2465-2478.
- Łuszczek-Trojnar, E., Drąg-Kozak, E., Socha, M., Szczerbik, P., Popek, W. (2015). Influence of long-term exposure to lead on its accumulation and elimination from tissues and on selected reproductive parameters in the Prussian carp (*Carassius gibelio* B.) in pond environment. *Czech Journal of Animal Science*, 60 (10), 459-472.
- Łuszczek-Trojnar, E., Grosicki, D., Drąg-Kozak, E., Guja, I., Popek, W. (2019). Changes in hematological parameters, and copper and iron concentrations in tissues of Prussian carp during depuration period after the previous exposure to copper in water. *Turkish Journal of Fisheries and Aquatic Sciences*, 19(9), 753-763.
- Mansouri, B., Ebrahimpour, M., Babaei, H. (2012). Bioaccumulation and elimination of nickel in the organs of black fish (*Capoeta fusca*). *Toxicology and Industrial Health*, 28(4), 361-368.
- Mansouri, B., Baramaki, R., Zareh, M., Pourkhabbaz, A., Hamidian, A. H. (2013a). Bioaccumulation and depuration of copper in the kidney and liver of a freshwater fish, *Capoeta fusca*. *Iranian Journal of Toxicology*, 7 (20), 816-822.
- Mansouri, B., Pourkhabbaz, A., Ebrahimpour, M., Babaei, H., Hamidian, A. H. (2013b). Bioaccumulation and elimination rate of cobalt in *Capoeta fusca* under controlled

- conditions. *Chemical Speciation & Bioavailability*, 25, 1, 52-56.
- Marigómez, I., Soto, M., Cajaraville, M. P., Angulo, E., Giamberini, L. (2002). Cellular and subcellular distribution of metals in molluscs. *Microscopy Research and Technique*, 56, 358-392.
- Mohammadian, T., Dezfuly, Z.T., Motlagh, R.G. Jangaran-Nejad, A., Hosseini, S. S., Khaj, H., Alijani, N. (2020). Effect of encapsulated *Lactobacillus bulgaricus* on innate immune system and hematological parameters in Rainbow trout (*Oncorhynchus mykiss*), post-administration of Pb. *Probiotics and Antimicrobial Proteins*, 12, 375-388.
- Nourian, K., Baghshani, H., Shahsavani, D. (2019). The effect of vitamin C on lead-induced plasma biochemical alterations in fish, *Cyprinus carpio*. *Iranian Journal of Toxicology*, (2), 25-29.
- Öz, M. (2025). Effects of boric acid on oxidative stress parameters, growth performance and blood parameters of Rainbow trout (*Oncorhynchus mykiss*). *Biological Trace Element Research*, 203, 1647-1655.
- Paduraru, E., Flocea, E. I., Lazado, C. C., Simionov, I. A., Nicoara, M., Ciobica, A., Faggio, C., Jijie, R. (2021). Vitamin C mitigates oxidative stress and behavioral impairments induced by deltamethrin and lead toxicity in zebrafish. *International Journal of Molecular Sciences*, 22(23), 12714, <https://doi.org/10.3390/ijms222312714>.
- Pourkhabbaz, A., Alipour, H., Zarei, I. (2015). Bioaccumulation and depuration rates of zinc by *Capoeta fusca* under controlled conditions. *Water Qual Expo Health*, 7, 187-191.
- Rahman, Z., Ahmad, I., Rashid, I. (2018). Effects of cadmium exposure on growth and survival and accumulation in various organs of Nile tilapia (*Oreochromis niloticus*, Linnaeus). *Journal of Agriculture and Aquaculture*, 1(1), 1-17.
- Reiter, R. J. (1988). Neuroendocrinology of Melatonin. In: *Melatonin: Clinical Perspectives* (Ed.) A. Miles, D.R.S. Philbrick, C. Thompson, Oxford University Press, New York, 1-28.
- Reiter, R. J., Mayo, J. C., Tan, D. X., Sainz, R. M., Alatorre-Jimenez, M., Qin, L. (2016). Melatonin as an antioxidant: under promises but over delivers. *Journal of Pineal Research*, 61(3), 253-278.
- Sahiti, H., Bislimi, K., Bajgora, A., Rexhepi, A., Dalo, E. (2018). Protective effect of vitamin C against oxidative stress in common carp (*Cyprinus carpio*) induced by heavy metals. *International Journal of Agriculture and Biosciences*, 7(2), 71-75.
- Sahiti, H., Bislimi, K., Rexhepi, A., Dalo, E. (2020). Metal accumulation and effect of vitamin C and E in accumulated heavy metals in different tissues in Common carp (*Cyprinus carpio*) treated with heavy metals. *Polish Journal of Environmental Studies*, 29(1), 799-805.
- Silva, E. P., Benvindo-Souza, M., Cotrim, C. F. C., Motta, A. G. C., Lucena, M. M., Filho, N. R. A., Pereira, J., Formiga, K. T. M., Silva, D. M. (2020). Genotoxic effect of heavy metals on *Astyanax lacustris* in an urban stream, *Heliyon*, 6(9), e05034, <https://doi.org/10.1016/j.heliyon.2020.e05034>.
- Sunardi, S., Astari, A. J., Pribadi, T. D. K., Rosada, K. K. (2017). Accumulation and elimination of mercury in Nile Tilapia (*Oreochromis niloticus*) under an elevated temperature and its ambient concentrations. *Nusantara Bioscience*, 9(1), 18-22.
- Szczerbik, P., Mikołajczyk, T., Sokołowska-Mikołajczyk, M., Socha M., Chyb, J., Epler P. (2006). Influence of long-term exposure to dietary cadmium on growth, maturation and reproduction of goldfish (subspecies: Prussian carp *Carassius auratus gibelio* B.). *Aquatic Toxicology*, 77, 126-135.
- Taslina, K., Al-Emran, M., Rahman, M. S., Hasan, J., Ferdous, Z., Rohani, M. F., Shahjahan, M. (2022). Impacts of heavy metals on early development, growth and reproduction of fish – A review. *Toxicology Reports*, 9, 858-868.
- Thuraiswami, S. (2020). Probiotic effect on zinc sulphate induced haematological toxicity in the fish *Mystus montanus*. *Annals of Biological Sciences*, 8(2), 34-37.
- Veisi, S., Sarkheil, M., Johari, S.A., Safari, O. (2021). Dietary supplementation with melatonin: influence on growth performance, oxidative stress status, and amelioration of silver nanoparticles-induced toxicity in Nile tilapia (*Oreochromis niloticus*). *Tropical Animal Health and Production*, 53(2), 314, <https://doi.org/10.1007/s11250-021-02760-w>.
- Wang, N., Gao, C., Zhang, P., Guan, L., Wang, Y., Quin, Y., Li, Y. (2019). Effect of *Bacillus cereus* against induced hematological disturbances and immunosuppression in *Carassius auratus gibelio*. *Fish and Shellfish Immunology*, 89, 141-148.
- Wang, X., Zhang, P., Zhang, X. (2021). Probiotics regulate gut microbiota: an effective method to improve immunity. *Molecules*, 26(19), 6076, <https://doi.org/10.3390/molecules26196076>.
- Wang, H., Feng, Y., Ming, M., Song, J., Chen, Z., Xiao, Z. (2022). Amelioration of Cd-induced bioaccumulation, hematological parameters, and heat shock protein-related genes by Vitamin C on common carp. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 258, 09362, <https://doi.org/10.1016/j.cbpc.2022.109362>.
- Wangyun, A. P., Hayati, A., Utomo, B. (2019). The effects of probiotics feed supplementation on tilapia (*Oreochromis niloticus*) in copper-tainted water. *Ecology, Environment and Conservation*, 25, 13-17.

- Witeska, M., Sarnowski, P., Ługowska, K., Kowal, E. (2014). The effects of cadmium and copper on embryonic and larval development of the *Leuciscus idus* L. *Fish Physiology and Biochemistry*, 40(1), 151-163.
- Wongprayoon, P., Govitrapong, P. (2016). Melatonin attenuates methamphetamine-induced neurotoxicity. *Current Pharmaceutical Design*, 22, 1022-1032.
- Xue, Y., Huang, J., Wang, J. Q., Li, F.Y. (2023). Tissue-specific accumulation and depuration of cadmium in tilapia: role of salinity and cadmium concentration. *Applied Ecology and Environmental Research*, 21(5), 4177-4194.
- Yu, Y. B., Lee, J. W., Jo, A. H., Choi, Y. J., Choi, C. Y., Kang, J. C., Kim, J. H. (2024). Toxic effects of cadmium exposure on hematological and plasma biochemical parameters in fish: A review. *Toxics*, 12(10), 699, <https://doi.org/10.3390/toxics12100699>.