Available online at sjuoz.uoz.edu.krd

journals.uoz.edu.krd

Science Journal of University of Zakho

Vol. 12, No.3, pp.382–387 July-September, 2024

p-ISSN: 2663-628X
e-ISSN: 2663-6298

COPPER AND MICROPLASTIC EXPOSURE AFFECTS THE GILL GENE EXPRESSION OF COMMON CARP DURING SALTWATER CHALLENGE

Seyyed M. Hoseini[1*], Basim S.A. Al Sulivany[2]

[1] Inland Waters Aquatics Resources Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization, Gorgan, Iran

[2] Department of Biology, College of Science, University of Zakho, Zakho, Duhok, Republic of Iraq

* Corresponding author: seyyedmorteza.hoseini@gmail.com

Received: 14 Jul., 2024 / Accepted: 10 Aug., 2024 / Published: 25 Aug., 2024. https://doi.org/10.25271/sjuoz.2024.12.3.1335

ABSTRACT:

The aim of the present study was to assess if pre-exposure to water copper and/or polyvinyl chloride microparticles affects the transcriptional responses of common carp, *Cyprinus carpio*, to saltwater exposure. Fish were exposed to 0.25 mg/L copper alone (Cu) or in the presence of 0.5 mg/L polyvinyl chloride microparticles (Cu-MPVC) for 14 days, followed by 72 hours of salt water exposure (0 to 13 ppt NaCl). The copper content in the gills and the expression of heat shock protein (*hsp70*) and cytochrome P450 family 1 (*cyp1a*) transcripts were examined. The results showed that gill copper levels increased significantly (P = 0.008) in the Cu and Cu-MPVC treatments after 14 days of exposure, compared to the control fish; the Cu and Cu-MPVC treatments had similar gill copper levels. After 14 days of exposure, branchial expression of the hsp70 and cyp1a genes was significantly up-regulated in the Cu and Cu-MPVC treatments. Exposure to salt water led to a significant down-regulation of the gene transcripts in all treatments after 24 h of exposure. At this point, the Cu and Cu-MPVC treatments showed transcripts similar to those of the control fish prior to saltwater exposure. The fish treated with Cu-MPVC showed significantly higher *hsp70* expression 72 h after saltwater exposure than the other treatments. At this time point, the control and Cu fish had significantly lower *cyp1a* expression than before saltwater exposure. In conclusion, the present data suggest that copper exposure induces stress in the fish gills, and the presence of MPCV in the water hampers normal transcriptomic responses of the fish gills to saltwater exposure.

KEYWORDS: gene expression, water contamination, carp rearing, osmotic challenge, co-exposure

1. INTRODUCTION

Water pollution is one of the major threats to the aquaculture industry as it affects fish health and fillet quality (Sonone et al., 2020). Microplastic (MP) and copper are two main pollutants in surface water and seawater that have negative effects on fish health and welfare (Malhotra et al., 2020; Kim et al., 2021). It has been demonstrated that these pollutants induce oxidative conditions, histopathological damage, and stress in fish (Gopi et al., 2019; Naz et al. 2021). Moreover, MPs have a great affinity to adsorb other water pollutants and are known as carriers of metals, facilitating the metals' transfer to fish bodies via the oral route (Banaee et al., 2019). Therefore, the presence of MPs in water resources may intensify metal accumulation in fish bodies and increase the toxic effects of metal on the fish. As a result, the combined effects of MPs and metals on fish health have been focused on in recent studies (Lu et al., 2018; Wen et al., 2018; Jinhui et al., 2019; Roda et al., 2020).

The common carp, *Cyprinus carpio*, is one of the popular species in the aquaculture industry throughout the world. It is because of people's cultural orientations as well as the hardy nature of this species that it is a suitable candidate for rearing in waters with a wide range of physicochemical properties. Despite being a freshwater species, common carp have a good ability to live in natural resources of brackish water (Barus et al., 2001). As a result, fish culturists consider it as a candidate for brackish water ponds.

Transfer of common carp from freshwater to brackish water induces stress and other physiological pathways that enable the fish to regain homeostasis under hyperosmotic conditions (Ghelichpour et al., 2018). The fish gill has pivotal roles under such conditions, as it is the main organ of osmoregulation and ionoregulation in the fish. The gill Na/K-ATPase is an important enzyme that participates in osmoregulation (Ghelichpour et al., 2020). The enzymes have several subunits with different roles; among them, subunits α1a and α1b have higher activities, respectively, in freshwater and saltwater (Bystriansky et al., 2006; McCormick et al., 2009). The enzyme activity is controlled by cortisol through glucocorticoid receptors (gr) (McCormick et al., 2008). Along with this role in controlling Na/K-ATPase activity, cortisol has a major role in fish metabolism under stressful conditions such as osmotic challenges (Aluru & Vijayan, 2009); as a consequence, or is involved in energy supply during stress (Brun et al., 2019). Osmotic stress induces heat shock protein elevation in fish (Smith et al., 1999; Choi, 2010); these proteins help cells under stressful conditions by maintaining the protein structure and normal folding (Roberts et al., 2010).

Cytochrome P450 proteins have different roles in the fish, including detoxification of the xenobiotics (Bengtson Nash et al., 2014). However, it has been reported that osmotic shock up-regulates the expression of Cytochrome P450 genes in fish (Leguen et al., 2010; Nguyen et al., 2016). This might be due to stress-induced oxidative stress and the formation of toxic substances in the fish body. Despite the involvement in osmotic shock responses, exposure of fish to copper and MPs also modulate the aforementioned proteins (Chao Dang et al., 2000; Eyckmans et al. 2010; Eyckmans et al., 2011; Wang et al., 2015; Brunet al., 2019; Espinosa et al. 2019; Yu et al. 2020; Kim et al. 2021). Therefore, it is interesting to determine the relationships between copper/MPs and saltwater exposure to these proteins in fish.

In the present study, the effects of water pollution with copper alone and in combination with MPs on common carp during saltwater challenges have been investigated. Such data are

* Corresponding author

This is an open access under a CC BY-NC-SA 4.0 license (https://creativecommons.org/licenses/by-nc-sa/4.0/)

important as MP pollution is a universal problem, and copper is used for carp pond management (as a therapeutic agent), although it is a common water pollutant as well as MPs. Thus, the desire to rear common carp in brackish waters requires further evaluation of the toxic effects of copper and MPs on saltwater challenges in this species.

2. MATERIALS AND METHODS

MP and copper sources

Polyvinyl chloride MPs (MPVC) were purchased from Arvand Petrochemical Co. (S65 type, Khuzestan province, Bandar-e-Mahshahr, Iran) and characterized by scanning electron microscope (SEM- MIRA3-XMU-TESCAN, Brno, Czech Republic). Morphological characteristics and size of the particles are presented in Fig. 1. Copper sulfate pentahydrate was purchased from Merck KGaA (Darmstadt, Germany). Working concentrations of 10 g/L MPVC and five g/L copper were prepared for further use.

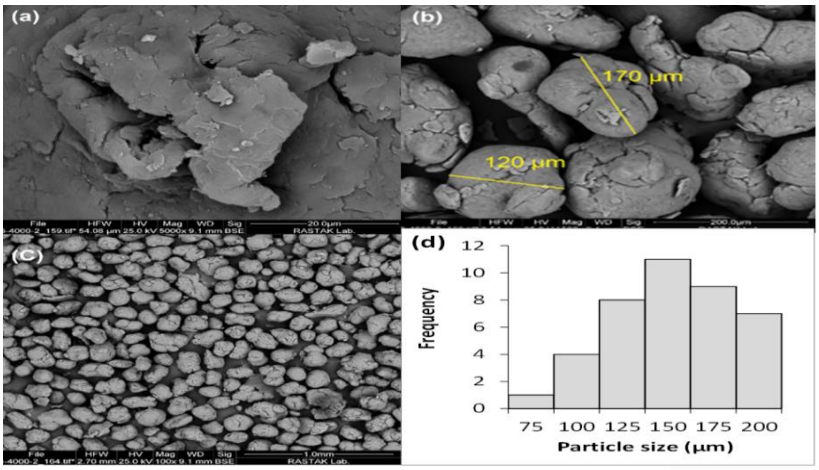

Figure1: SEM micrograph of PVC-MP at different magnifications (a, b and c) and particle size distribution histogram (d).

Ethical statement

All experimental procedures, including ethics of laboratory animal treatments, were in accordance with the regulations adjusted by the Inland Waters Aquatic Resources Research Center, Gorgan, Iran.

Experimental protocol

Juvenile common carp with individual weights of 37.5 ± 3.24 g were randomly distributed in 9 aquaria (40 L), seven fish per aquarium. The fish were allowed to acclimatize to the new conditions for ten days, during which they were fed a commercial diet (Beyza et al.) at a daily rate of 2% of biomass and the aquaria were supplied with continuous aeration. Every day, half of the aquaria water was exchanged with clean water to maintain the water quality. After the acclimation, the aquaria were assigned to three treatments: control, Cu, and Cu-MPVC. The control fish were kept in clean water for 14 days, followed by 72-h exposure to saltwater (13 g/L); the Cu fish were kept in water with 0.25 mg/L copper for 14 days, followed by 72-h exposure to saltwater; and the Cu-MPVC fish were kept in water with 0.5 mg/L MPVC for 14 days, followed by 72-h exposure to saltwater. The aquaria water was renewed by 30% to avoid fish waste pollution. The water concentrations of copper and MPVC were maintained by adding the required amounts of each material daily. The fish were kept under these conditions for 14 days, during which they were fed, similar to the acclimation period. After that, gill samples were taken for copper assay and gene expression analyses and the fish were directly exposed to salt water (13 g/L, without copper/MPVC) and gill-sampled again at 24 and 72 h exposure. Two fish were caught from each aquarium and killed by a sharp blow on the head; then, the gills were cut and washed with distilled water. Six-gill samples were dried at 70°C (24 h) and used for copper assay (n = 6), whereas three-gill samples were immediately frozen in liquid nitrogen and used for gene expression analysis.

Water temperature, pH, dissolved oxygen, hardness, and unionized ammonia levels were determined by digital apparatuses (Hach portable probe; Model HQ40D, Loveland, Colorado, USA; Palintest photometer; Model 7100, Gateshead, Tyne & Wear, NE11 0NS, United Kingdom) were 23.5 ± 1°C, 7.79 ±0.65, 6.39 ± 0.45 mg/L, 195 ± 8.99 mg $CaCO_3$/L, and 0.01 ± 0.005 mg N/L, respectively.

Copper assays

The water and gill copper concentrations were measured by graphite atomic absorption spectrophotometry (Agilent 240z, Santa Clara, California, USA). The sample digestion was performed by adding concentrated nitric acid [ratio of 1: l (v: v) for the water and 1: 20 (w: v) for the gill samples].

Gene expression analysis

Specific primers for common carp were designed using the species data in the Gene Bank and Geneious IR9 and Oligoanalyzer software (Table 1). A commercial kit was used for RNA extraction (Dena et al.). RNA was treated with DNase I (Thermo et al., USA) to remove DNA contamination. A commercial kit (SMOBIO Technology, Hsinchu City 30075, Taiwan) was used for cDNA synthesis, and genes' expressions were determined using a real-time PCR (Applied Biosciences, Step One, Foster City, California, USA). Details of the reaction mixture constituents are described before (Hoseini et al., 2022). Each sample was analyzed twice, and normalization was conducted using a reference gene (*beta-actin*). After calculating ΔΔCt, the data were log2-transformed and expressed as fold-change relative to control fish in freshwater.

Table 1: Forward and reverse sequences, length, amplicon size, and accession number of the selected genes' primers

Primer	Sequence (5-3)	length	Tm	Amplicon (bp)	Accession no.
hsp70-F	ATGTTGCCTTCACAGACACTG	21	60	120	AY120894.1
hsp70-R	GGTCATCAAACTTTCTGCCGA	21	60		
cyp1a-F	GAAGAAGTTCGTGGCCATCAA	21	60	101	AB048939.1
cyp1a-R	TGATGTCTCGGATGTTGTCCT	20	60		
beta-actin-F	TCTGCTATGTGGCTCTTGACT	21	60	118	XM_019106214.1
beta-actin-R	AACCTCTCATTGCCAATGGTG	20	60		

Statistical analysis

Data on the gill copper concentrations were log_{10}-transformed before a one-way ANOVA analysis, as they failed to meet ANOVA assumptions (Shapiro-Wilk and Levene tests). The gene expression data were log_2-transformed and analyzed by two-way ANOVA (toxicant exposure × saltwater challenge). Significant differences among the treatments were delineated by the Tukey test. All data were analyzed by the statistical software SPSS v.22 at a significance level of $P < 0.05$ and presented as mean ± SE.

3. RESULTS

There was no mortality in the treatments during the experiment. The water copper contents in control, Cu, and Cu-MPVC treatments were 0.0035 ± 0.001, 0.242 ± 0.022, and 0.250 ± 0.020 mg/L, respectively. Cu and Cu-MPVC treatments exhibited similar gill copper contents and significantly ($P = 0.008$) higher gill copper contents than that of the control treatment after 14-d exposure (Fig. 2).

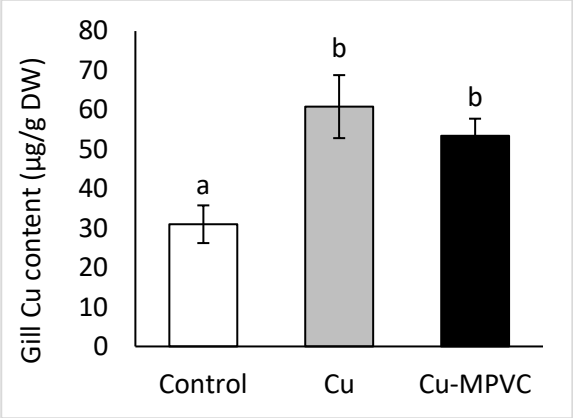

Figure. 2: Gill copper content following 14 days of exposure to 0.25 mg/L copper alone or in combination with 0.5 mg/L MPVC. Different letters above the bars indicate significant differences among the treatments (Tukey; n = 6).

Exposure to copper/MPVC and saltwater challenge induced a significant interaction effect on gill *hsp70* gene expression ($P < 0.001$). Cu and Cu-MPVC treatments exhibited similar gill *hsp70* gene expression after 14-d exposure, which was significantly higher than that of the control. The gill *hsp70* expression decreased after a 24-hour saltwater challenge in all treatments; Cu and Cu-MPVC treatments exhibited similar gene expressions and were significantly higher than that of the control treatment at this time. The gill *hsp70* gene expression in Cu and Cu-MPVC was statistically similar to the control fish after a 72-h saltwater challenge (Fig. 3A).

Exposure to copper/MPVC and saltwater challenge induced a significant interaction effect on gill *cyp1a* gene expression ($P = 0.048$). Cu and Cu-MPVC treatments exhibited similar gill *cyp1a* gene expression after 14-d exposure, which was significantly higher than that of the control. The control fish exhibited no significant changes in the gill *cyp1a* gene expression during 24 and 72 h saltwater exposure. The gill *cyp1a* gene expression decreased in Cu and Cu-MPVC treatments after 24 h saltwater exposure and remained unchanged until 72 h (Fig. 3B).

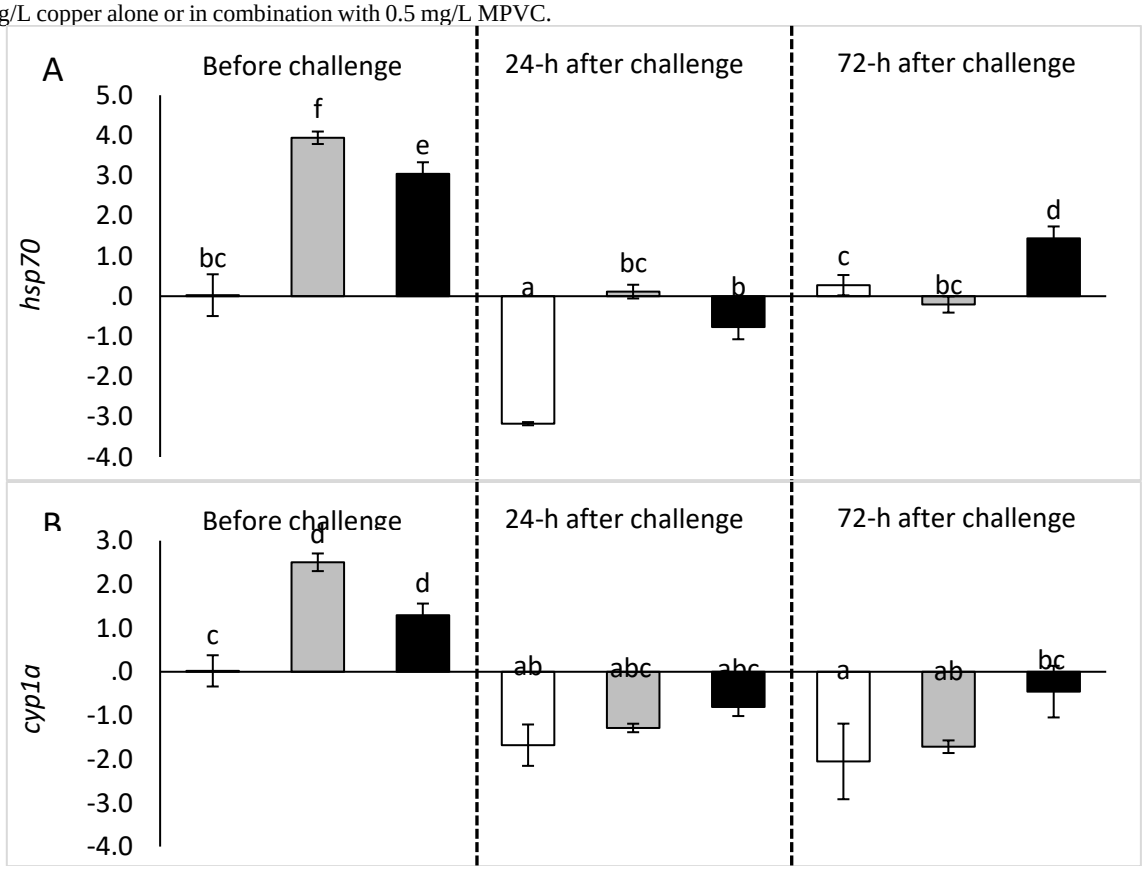

Figure 3: Expression of *hsp70* (A) and *cyp1a* (B) in the fish gill after 14 days of exposure to 0.25 mg/L copper alone or in combination with 0.5 mg/L MPVC, followed by 24 and 72 h saltwater exposure. White bars: control fish, grey bars: Cu fish, and black bars: Cu-MPVC fish. Different letters indicate significant differences among the treatments (Tukey; n = 3).

4. DISCUSSION

MPs act as vectors of metals in aquatic media, which facilitate metal accumulation in the fish body. The present results are in line with Roda et al. (2020), who have reported polyethene MPs did not increase copper accumulation in the gill of *Prochilodus lineatusa*. On the contrary, Lu et al. (2018) have reported that polystyrene MPs facilitate cadmium accumulation in the liver, gut, and gill of zebrafish *Danio rerio* after 3-week exposure. Moreover, Wen et al. (2018) have demonstrated that polystyrene MPs suppress whole-body cadmium accumulation in the discus *Symphysodon aequifasciatus*, which seems to be due to lower metallothionein content in the fish co-exposed to MPs and cadmium. Thus, it is worth determining if no change in the gill copper accumulation between the Cu and MPVC-Cu is a result of metallothionein content in the tissue.

hsp70 is a protein that helps fish during stress by maintaining normal protein' folding and functions (Sung et al., 2012).
Induction of *hsp70* in the fish tissue has been reported after exposure to different xenobiotics (Ghelichpour et al., 2019; Ghelichpour et al., 2020). The present results are in agreement with previous studies that show copper (Wang et al., 2015; Yu et al., 2020) or MPs (Espinosa et al., 2019) exposure up-regulate *hsp70* gene expression. The saltwater challenge down-regulated the gill *hsp70* in all fish in the present study. This is an unexpected result, as previous studies suggest that osmotic shock up-regulates *hsp70* expression in fish (Smith et al., 1999; Choi, 2010). Moreover, common carp exhibited *hsp70* up-regulation when transferred from 2.6 to 12 g/L salinity (Ghelichpour et al., 2020). Such an inconsistency may be due to a difference in water temperature between the present and Ghelichpour et al. (2020) study (23.5 vs. 27.5°C). Supporting this hypothesis, Eissa et al. (2017) have shown that *hsp70* is down-regulated when yellow perch, *Perca flavescens*, is transferred from freshwater to brackish water at 26°C, but not 14 and 20°C. Similar results have been reported in rainbow trout, *Oncorhynchus mykiss*, thermally-stressed before saltwater exposure (Niu et al., 2008). According to the present results, the gill *hsp70* responses to saltwater exposure in Cu and MPVC-Cu fish were similar to the control after 24 h. However, a significant up-regulation in the gene expression of MPVC-Cu fish after 72-h may be a further stress response to saltwater exposure.

cyp1a is involved in detoxification processes and increases in the fish exposed to water pollutants (Altun et al., 2017; Romano et al., 2020). Both copper (Eyckmans et al., 2011) and MPs (Kim et al., 2021) are known to induce oxidative stress and the formation of free radicals and reactive oxygen species. These toxic substances should be neutralized in the detoxification process. The present results show that detoxification processes have been initiated to protect the gill cells against copper- and MPVC-derived formation of toxic substances. Similar to the present results, copper and MPs have been found to induce *cyp1a* gene expression in fish (Kumari et al., 2018; Romano et al., 2020). Unexpectedly, the fish gill *cyp1a* was down-regulated in the fish exposed to saltwater, which is in contrast to the results obtained in rainbow trout (Leguen et al., 2010) and striped catfish, *Pangasianodon hypophthalmus* (Nguyen et al., 2016) transferred from freshwater to saltwater. However, there are inconsistencies in *cyp1a* responses to osmotic stress in fish, as saltwater exposure significantly up-regulated Cytochrome P450 family 2 and 3, but down-regulated *cyp1a* genes' expressions in coho salmon, *Oncorhynchus kisutch* (Lavado et al., 2014). Therefore, further investigations are needed to illustrate the *cyp1a* role in saltwater adaptation in fish. For example, water salinity, degree of oxidative stress and content of antioxidants in the fish tissue are determinants in *cyp1a* responses to stressors (Leguen et al., 2010; Rahman & Thomas, 2012). According to the results, MPVC-Cu may interfere with *cyp1a*'s roles in the saltwater adaptation of common carp, as the gene expression of these fish differed from that of the control fish after 72-h saltwater exposure.
The present results show that simultaneous exposure to copper and MPVC leads to lower expression of the *hsp70* and *cyp1a* genes compared to exposure to copper alone, indicating the role of MPVC on the gill transcript of fish. Such effects can be interpreted as additive toxic effects of copper and MPVC that suppress the normal responses of fish gills to copper exposure. Such additive effects seem to lead to higher stress in fish after saltwater exposure, which is characterized by high expression of *hsp70* and *cyp1a* genes.

CONCLUSION

The present study offers empirical evidence to support the assertion that prior exposure to copper in water, either alone or in combination with polyvinyl chloride microparticles (Cu-MPVC), has a significant effect on the transcriptional responses of common carp (Cyprinus carpio) when subsequently exposed to saltwater. Specifically, copper exposure led to increased copper levels in the gills and upregulation in the expression of heat shock protein (hsp70) and cytochrome P450 family 1 (cyp1a) genes. However, the introduction of saltwater resulted in a noticeable downregulation of these gene transcripts within a 24-hour period across all treatment groups. Interestingly, fish exposed to Cu-MPVC exhibited consistently higher levels of hsp70 expression over time compared to the other treatments, indicating a prolonged stress response. These findings indicate that copper exposure induces stress in fish gills, and the presence of microparticles in the water disrupts the normal transcriptomic responses of fish gills to saltwater exposure. This interaction between copper and microparticles highlights the complex nature of environmental stressors on aquatic organisms.

Funding

No fund was received for this study

REFERENCES

Altun, S., Özdemir, S. & Arslan, H. (2017). Histopathological effects, responses of oxidative stress, inflammation, apoptosis biomarkers and alteration of gene expressions related to apoptosis, oxidative stress, and reproductive system in chlorpyrifos-exposed common carp (*Cyprinus* et al.). *Environmental Pollution*, **230**, 432-443.

Aluru, N. & Vijayan, M. M. (2009). Stress transcriptomics in fish: A role for genomic cortisol signalling. *General and Comparative Endocrinology*, **164**, 142-150.

Banaee, M., Soltanian, S., Sureda, A., Gholamhosseini, A., Haghi, B. N., Akhlaghi, M. & Derikvandy, A. (2019). Evaluation of single and combined effects of cadmium and micro-plastic particles on biochemical and immunological parameters of common carp (*Cyprinus carpio*). *Chemosphere*, **236**, 124335.

Barus, V., Peaz, M. & Kohlmann, K. (2001) *Cyprinus carpio* (Linnaeus, 1758). In: *The freshwater fishes of Europe* (ed. by Banarescu P, Paepke H). Aula Verlag GmbH, Wiebelsheim, Germany, pp. 85-179.

Bengtson Nash, S., Dawson, A., Burkhard, M., Waugh, C. & Huston, W. (2014). Detoxification enzyme activities (CYP1A1 and GST) in the skin of humpback whales as a function of organochlorine burdens and migration status. *Aquatic Toxicology, pp.* **155**, 207–212.

Brun, N. R., van Hage, P., Hunting, E. R., Haramis, A.-P. G., Vink, S. C., Vijver, M. G., Schaaf, M. J. M. & Tudorache, C. (2019) Polystyrene nanoplastics disrupt glucose metabolism and cortisol levels with a possible link to behavioural changes in larval zebrafish. *Communications Biology*, **2**, 382.

Bystriansky, J., Richards, J., Schulte, P. & Ballantyne, J. (2006). Reciprocal expression of gill Na+/K+-ATPaseα-subunit

isoforms α1a and α1b during seawater acclimation of three salmonid fishes that vary in their salinity tolerance. *Journal of Experimental Biology*, **p. 209**, 1848-1858.

Chao Dang, Z., Flik, G., Ducouret, B., Hogstrand, C., E. Wendelaar Bonga, S. & A.C. Lock, R. (2000). Effects of copper on cortisol receptor and metallothionein expression in gills of *Oncorhynchus mykiss*. *Aquatic Toxicology*, **51**, 45–54.

Choi, C. Y. (2010). Environmental stress-related gene expression and blood physiological responses in olive flounder (*Paralichthys olivaceus*) exposed to osmotic and thermal stress. *Animal Cells and Systems*, **pp. 14**, 17–23.

Eissa, N., Wang, H.-P., Yao, H., Shen, Z.-G., Shaheen, A. A. & Abou-ElGheit, E. N. (2017). Expression of Hsp70, Igf1, and three oxidative stress biomarkers in response to handling and salt treatment at different water temperatures in yellow perch, *Perca flavescens*. *Frontiers in Physiology*, **8**, 1-15.

Espinosa, C., Esteban, M. Á. & Cuesta, A. (2019). Dietary administration of PVC and PE microplastics produces histological damage, oxidative stress and immunoregulation in European sea bass (*Dicentrarchus labrax* L.). *Fish & Shellfish Immunology*, **95**, 574-583.

Eyckmans, M., Tudorache, C., Darras, V. M., Blust, R. & De Boeck, G. (2010). Hormonal and ion regulatory response in three freshwater fish species following waterborne copper exposure. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, **pp. 152**, 270–278.

Eyckmans, M., Celis, N., Horemans, N., Blust, R. & De Boeck, G. (2011). Exposure to waterborne copper reveals differences in oxidative stress response in three freshwater fish species. *Aquatic Toxicology*, **103**, 112-120.

Ghelichpour, M., Taheri Mirghaed, A. & Zargar, A. (2020). The response of lufenuron- and flonicamid-exposed *Cyprinus carpio* to saltwater challenge: Study on ion-regulation and stress genes expression and plasma antioxidant characteristics. *Aquaculture Research*, **51**, 4829-4837.

Ghelichpour, M., Mirghaed, A. T., Mirzargar, S. S., Joshaghani, H. & Mousavi, H. E. (2018). Modification of saltwater stress response in *Cyprinus carpio* (Linnaeus, 1758) pre-exposed to pesticide indoxacarb. *Ecotoxicology and Environmental Safety*, **147**, 139-143.

Ghelichpour, M., Mirghaed, A. T., Hoseinifar, S. H., Khalili, M., Yousefi, M., Van Doan, H. & Jimenez, A. P. (2019). Expression of immune, antioxidant and stress-related genes in different organs of common carp exposed to indoxacarb. *Aquatic Toxicology*, **208**, 208-216.

Gopi, N., Vijayakumar, S., Thaya, R., Govindarajan, M., Alharbi, N. S., Kadaikunnan, S., Khaled, J. M., Al-Anbr, M. N. & Vaseeharan, B. (2019). Chronic exposure of *Oreochromis niloticus* to sub-lethal copper concentrations: Effects on growth, antioxidant, non-enzymatic antioxidant, oxidative stress and non-specific immune responses. *Journal of Trace Elements in Medicine and Biology*, **55**, 170-179.

Hoseini, S. M., Khosraviani, K., Hosseinpour Delavar, F., Arghideh, M., Zavvar, F., Hoseinifar, S. H., Van Doan, H., Zabihi, E. & Reverter, M. (2022). Hepatic transcriptomic and histopathological responses of common carp, *Cyprinus carpio*, to copper and microplastic exposure. *Marine Pollution Bulletin*, **175**, 113401.

Jinhui, S., Sudong, X., Yan, N., Xia, P., Jiahao, Q. & Yongjian, X. (2019). Effects of microplastics and attached heavy metals on growth, immunity, and heavy metal accumulation in the yellow seahorse, *Hippocampus kuda* Bleeker. *Marine Pollution Bulletin*, **149**, 110510.

Kim, J.-H., Yu, Y.-B. & Choi, J.-H. (2021) Toxic effects on bioaccumulation, haematological parameters, oxidative stress, immune responses and neurotoxicity in fish exposed to microplastics: A review. *Journal of Hazardous Materials*, **413**, 125423.

Kumari, K., Pathakota, G.-B., Kumar, S. & Krishna, G. (2018). Gene structure and comparative and phylogenetic analyses of *Catla catla* CYP1A full-length cDNA and its responsiveness to benzo(a)pyrene and copper sulphate at early developmental stages. *Fish Physiology and Biochemistry*, **pp. 44**, 95–108.

Lavado, R., Aparicio-Fabre, R. & Schlenk, D. (2014). Effects of salinity acclimation on the expression and activity of Phase I enzymes (CYP450 and FMOs) in coho salmon (*Oncorhynchus kisutch*). *Fish Physiology and Biochemistry*, **40**, 267-278.

Leguen, I., Odjo, N., Le Bras, Y., Luthringer, B., Baron, D., Monod, G. & Prunet, P. (2010) Effect of seawater transfer on CYP1A gene expression in rainbow trout gills. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **156**, 211-217.

Lu, K., Qiao, R., An, H. & Zhang, Y. (2018). Influence of microplastics on the accumulation and chronic toxic effects of cadmium in zebrafish (*Danio rerio*). *Chemosphere*, **pp. 202**, 514-520.

Malhotra, N., Ger, T.-R., Uapipatanakul, B., Huang, J.-C., Chen, K. H. C. & Hsiao, C.-D. (2020). Review of copper and copper nanoparticle toxicity in fish. *Nanomaterials*, **10**.

McCormick, S. D., Regish, A. & Christensen, A. (2009). Distinct freshwater and seawater isoforms of Na+/K+-ATPase in gill chloride cells of Atlantic salmon. *Journal of Experimental Biology*, **pp. 212**, 3994–4001.

McCormick, S. D., Regish, A., O'Dea, M. F. & Shrimpton, J. M. (2008). Are we missing a mineralocorticoid in teleost fish? Effects of cortisol, deoxycorticosterone and aldosterone on osmoregulation, gill Na+, K+-ATPase activity and isoform mRNA levels in Atlantic salmon. *General and Comparative Endocrinology*, **157**, 35–40.

Naz, S., Hussain, R., Ullah, Q., Chatha, A. M. M., Shaheen, A. & Khan, R. U. (2021). Toxic effect of some heavy metals on haematology and histopathology of major carp (*Catla catla*). *Environmental Science and Pollution Research*, **pp. 28**, 6533–6539.

Nguyen, T. V., Jung, H., Nguyen, T. M., Hurwood, D. & Mather, P. (2016). Evaluation of potential candidate genes involved in salinity tolerance in striped catfish (*Pangasianodon hypophthalmus*) using an RNA-Seq approach. *Marine Genomics, pp.* **25**, 75-88.

Niu, C. J., Rummer, J. L., Brauner, C. J. & Schulte, P. M. (2008). Heat shock protein (Hsp70) induced by a mild heat shock slightly moderates plasma osmolarity increases upon salinity transfer in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, **148**, 437-444.

Rahman, M. S. & Thomas, P. (2012). Effects of hypoxia exposure on hepatic cytochrome P450 1A (CYP1A) expression in Atlantic croaker: molecular mechanisms of CYP1A down-regulation. *PloS One*, **7**, e40825.

Roberts, R. J., Agius, C., Saliba, C., Bossier, P. & Sung, Y. Y. (2010). Heat shock proteins (chaperones) in fish and shellfish and their potential role in relation to fish health: a review. *Journal of Fish Diseases*, **33**, 789–801.

Roda, J. F. B., Lauer, M. M., Risso, W. E. & Bueno dos Reis Martinez, C. (2020). Microplastics and copper effects on the neotropical teleost *Prochilodus lineatus*: Is there any interaction? *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **242**, 110659.

Romano, N., Renukdas, N., Fischer, H., Shrivastava, J., Baruah, K., Egnew, N. & Sinha, A. K. (2020). Differential modulation of oxidative stress, antioxidant defence, histomorphology, ion-regulation and growth marker gene expression in goldfish (*Carassius auratus*) following exposure to different doses of virgin microplastics. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, **238**, 108862.

Smith, T. R., Tremblay, G. C. & Bradley, T. M. (1999). Hsp70 and a 54 kDa protein (Osp54) are induced in salmon (*Salmo salar*) in response to hyperosmotic stress. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, **pp. 284**, 286–298.

Sonone, S. S., Jadhav, S., Sankhla, M. S. & Kumar, R. (2020). Water contamination by heavy metals and their toxic effect on aquaculture and human health through the food Chain. *Letters in applied NanoBioScience*, **pp. 10**, 2148–2166.

Sung, Y., Roberts, R. & Bossier, P. (2012). Enhancement of Hsp70 synthesis protects common carp, *Cyprinus carpio* L., against lethal ammonia toxicity. *Journal of Fish Diseases*, **35**, 563-568.

Wang, T., Long, X., Liu, Z., Cheng, Y. & Yan, S. (2015). Effect of copper nanoparticles and copper sulphate on oxidation stress, cell apoptosis and immune responses in the intestines of juvenile *Epinephelus coioides*. *Fish & Shellfish Immunology*, **44**, 674-682.

Wen, B., Jin, S.-R., Chen, Z.-Z., Gao, J.-Z., Liu, Y.-N., Liu, J.-H. & Feng, X.-S. (2018) Single and combined effects of microplastics and cadmium on the cadmium accumulation, antioxidant defence and innate immunity of the discus fish (*Symphysodon aequifasciatus*). *Environmental Pollution*, **243**, 462-471.

Yu, Z., Dai, Z.-Y., Qin, G.-X., Li, M.-Y. & Wu, L.-F. (2020) Alleviative effects of dietary microbial floc on copper-induced inflammation, oxidative stress, intestinal apoptosis and barrier dysfunction in *Rhynchocypris lagowski* Dybowski. *Fish & Shellfish Immunology*, **106**, 120–132.