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Research Article

Expression of metallothionein gene in kidney tissue of Persian sturgeon (*Acipenser persicus*) after exposure to cadmium chloride

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Abstract

This 14-day trial was conducted on 24 Persian sturgeons with a mean weight of 300 g. The treatments consisted of the control group (no cadmium), treatment 1: 200 µg/L of dissolved cadmium, treatment 2: 400 µg/L of dissolved cadmium, and treatment 3: 800 µg/L of dissolved cadmium in triplicates. Results showed that the expression level of metallothionein (MT) at 200 µg/L on the first day was 1.06±0.02, which increased to 2.51±0.13 in the control with prolonged exposure until the fourteenth day. The rate rose from 1.43±0.19 on the first day of exposure to 2.60±0.36 folds the control by the seventh day at a concentration of 400µg/L. Moreover, the expression level at the concentration of 800 µg/L rose from 1.1±0.13 on the first day to 2.15±0.09 folds in the control on the fourteenth day. The results also indicated that the highest level of MT expression (2.60±0.36) was observed in Treatment 2. Accordingly, all three concentrations of dissolved cadmium (200, 400, and 800 µg/L) significantly increased the MT expression on the last day of the trial (p<0.05 and p<0.01). Therefore, MT can be considered a biomarker of cadmium exposure in Persian sturgeons. This study also demonstrated that the MT expression was increased in the kidney tissue of the samples with longer times of exposure to cadmium, but it did not increase by increasing cadmium concentration. Hence, the length of cadmium exposure can affect the kidney MT expression level of Persian sturgeon.

Introduction

Pollutants pose a continuous threat to aquatic ecosystems, as studies have shown that these ecosystems contain pollutants, particularly heavy metals (Garai *et al*., 2021; Liu *et al*., 2022; Mitra *et al*., 2022). Since heavy metals can threaten ecosystems as well as human health, it is necessary to investigate their concentrations in aquatic ecosystems (Hertika *et al*., 2023). As a toxic and unwanted foreign substance, cadmium is a heavy metal that negatively affects the life of fish species through bioaccumulation. Studies have proven the harmful effects of cadmium, even at low concentrations, on aquatic organisms (Philippe *et al*., 2018; Qiu *et al*., 2021). For water monitoring purposes, biomarkers have been employed as a tool to identify environments contaminated with heavy metals (Milinkovitch *et al*., 2019). Biomarkers can describe the toxicity and effects of heavy metals at a particular level absorbed by organisms. Biomarkers also allow to monitor and control of pollution even at very low levels. Biomarkers are biological reactions that are measured at the molecular, biochemical, or cellular level within an organism, or in the organism's products, such as urine, feces, feathers, and other items, to indicate a deviation from the normal state as a result of exposure to a potentially toxic chemical (M'kandawire *et al*., 2017). Cadmium is classified as a non-essential element that exhibits significant toxicity to fish. Exposure to even low concentrations of this metal can result in various toxic effects, such as tissue damage and alterations in respiratory function, ultimately leading to mortality in fish (Rose *et al*., 2014). Aquatic organisms utilize MT to mitigate the toxic impacts of heavy metals. The severity of heavy metal toxicity differs among species, influenced by the specific metal and the species' capacity to naturally synthesize MT (El-Khayat *et al*., 2020). MT reflects a high concentration of metals and thiolate sulfate, along with the presence of 20 types of cysteine amino acids in its internal structure, resulting in the unique properties of this protein when combined with metals (Dabrio *et al*., 2002). MT serves as a crucial binding protein within the cell, characterized by its high affinity for metal ions, which can be attributed to its unique structural properties, particularly its elevated thiol content. This function mitigates the toxicity of metals to surrounding cells (Sigel *et al*., 2009). The primary characteristic of metallothionein is its facile transcriptional activation by heavy metals in the environment. This induction is associated with heavy metal concentration, which serves as an indicator of heavy metal levels in the environment and the stress response and detoxification capacity of organisms to these metals (Mourgaud *et al*., 2002). The MT genes are expressed in various tissues of organisms, which can be a potential biomarker. The MT genes are expressed in non-polluted conditions; however, their expression increases in polluted environments, aiding in structural maintenance through binding to target proteins. Consequently, variations in their expression levels may serve as a biomarker for alterations, including those induced by pollution. Previous studies have reported that the MT genes can serve as a biomarker for heavy metal contamination in fish species (Iman *et al*., 2013).

MT is a perfect biomarker for heavy metal pollution that can be used as a warning indicator to detect cases of heavy metal exposure as well as to monitor contaminated water (Fabrin *et al*., 2018; Hertika *et al*., 2018). MT has been utilized in numerous monitoring studies as a recognized biomarker for heavy metals such as cadmium in the bodies of invertebrates and vertebrates (Hamza Chafai, 2014; Wang *et al*., 2014). Previous studies have demonstrated that MT is a conclusive biomarker for heavy metal contamination in fish species (Iman *et al*., 2013). MT has been reported in various tissues of the body of aquatic vertebrates, such as the liver (hepatopancreas), kidney, testis, gills, and nervous tissues (Wang *et al*., 2014).

The Persian sturgeon, *Acipenser persicus*, is one of the most important species of the sturgeon. It is abundantly found in the southern Caspian Sea, especially on Iranian coasts. The Persian sturgeon is an economically valuable fish species endemic to the Iranian coasts of the Caspian Sea and has a special place in Iran's fisheries sector. Moreover, it has the lion's share of sturgeons caught from the Iranian coasts of the Caspian Sea (Keyvan, 2003). The International Union for Conservation of Nature (IUCN) has updated the status of the Persian sturgeon from endangered (EN) to critically endangered (CR) due to the drastic reduction in the reserves of this species. This issue necessitates the development of more stringent laws to protect the reserves of this species (CITES, 2020). Many studies about the effects of cadmium on fish have shown the extensive damage this heavy metal causes to different organs of fish. Studies have demonstrated that different concentrations and times of exposure to cadmium can influence the physiological, biochemical, enzymatic, and molecular expression of genes, depending on the target sites and tissues (Liu *et al*., 2022). Cadmium can cause extensive damage to fish species, such as disturbing respiratory metabolism (McRae *et al*., 2018) and the immune system functions (Zhang *et al*., 2017), reducing endocrine and ion regulatory capacity (Da Silva and Martinez, 2014; Liu *et al*., 2017), inhibiting growth and reproductive capacity (Tan *et al*., 2010; Wang *et al*., 2014), and threatening fish survival (Renieri *et al*., 2017). Furthermore, cadmium bioaccumulation can affect the structure of tissues, energy metabolism system, and nervous system and also change the blood parameters of fish. It also causes cytotoxicity and oxidative damage, affects the expression of stress genes, and inhibits multi-enzyme activities (Liu *et al*., 2022). Similar to other heavy metals, cadmium enters the body of fish through gills, skin, or food and then travels to different organs through blood circulation. Because of the high level of metabolism in organs such as kidneys and liver, heavy metals, including cadmium, usually accumulate in these organs (Emadi *et al*., 2019). Numerous reports state that the water, sediments, and aquatic organisms on the Caspian Sea contain substantial concentrations of heavy metals, including lead and cadmium, above the recommended limits (Nasrollahzadeh Saravi *et al*., 2014; Sinka Karimi and Sadeghi Bajgiran, 2015). Additionally, industrialization, urbanization, and oil

extraction activities will probably exacerbate the contamination of the Caspian Sea with heavy metals in the future (CEP, 2002; Pourang *et al*., 2005). As a result, it is necessary to address the effects of cadmium on the expression of marker genes including MT. The meat and caviar of the Persian sturgeon are highly valued for human consumption, making it one of the most commercially valuable fish species (Zahedi *et al*., 2013). This study aims to investigate the MT expression in the Persian sturgeon in exposure to cadmium chloride to achieve a better understanding of the relationship of fish physiology with environmental stress, adaptation, and cellular changes of this element. Since the Persian sturgeon is a carnivorous species that obtains most of its food from the seabed, especially in the early stages of life, this fish species is more likely to absorb the pollutants accumulated in sediments. The accumulation of pollutants in the body of fish, along with direct exposure to them, will lead to behavioral, physiological, and molecular changes (Safari *et al*., 2014). Therefore, it is necessary to examine the detrimental effects of this metal on this important fish species to ensure both biodiversity preservation and health and food safety. The significance of this study lies in the paucity of studies investigating the MT expression in the Persian sturgeon exposed to cadmium.

Materials and methods

Fish

In this study, 24 Persian sturgeons, *Acipenser persicus*, with an approximate weight of 300 g, were provided from Dr. Yousefpour Center for Restoration and Protection of Living Marine Fish Genetic Resources, Siyahkal, Iran. The fish were introduced to four 500-liter ponds (6 subjects apiece) to adapt to the experimental conditions at the same center. All fishes were kept under the same feeding, aeration, and water exchange regimen.

Treatments and sampling

Experimental treatments were established based on the method proposed by Roy and Battacharrya (2006). Cadmium solution was prepared by dissolving an appropriate amount of Cdcl2 (Merck, Germany) in deionized distilled water. The different concentrations of cadmium were calculated based on the LC_{50} of this element to the Persian sturgeon (4000 µg/L) reported by Mirzaee *et al*. (2003). Accordingly, three sub-lethal concentrations of cadmium chloride, *i.e.*, 200, 400, and 800 µg/L, were utilized as the main treatments, and one treatment served as the control with zero cadmium chloride (Safari *et al*., 2014). To minimize the reduction of the metal concentration after feeding and to prevent water pollution due to excess food, the experiment was conducted statically by renewing the desired concentration of the solution and changing the water once every two days (Shariati *et al*., 2011). After 14 days of exposure to different concentrations of cadmium, a few subjects from each treatment were randomly selected for sampling. The selected subjects were anesthetized with 0.5 g/L of clove powder and then their kidney tissue was removed. After being washed with deionized water, the samples were put inside numbered

microtubes wrapped with foil paper and immediately transferred to a molecular biotechnology laboratory in a tank containing liquid nitrogen. The samples were stored at -80°C until molecular experiments (Fig. 1).

Figure 1: Kidney tissue of Persian sturgeon.

RNA extraction and quantitative and qualitative assessment

A column kit (QIAGen, Germany) was used to extract RNA from 100 mg of kidney tissue frozen at -80°C. To this end, the tissue was defrosted, crushed in a mortar, and covered with the lysis buffer. The remaining steps were completed as directed by the manufacturer. RNA was then removed from the gel using the elution buffer. The quantity of extracted RNA was measured by examining the concentration of the lysing buffer at 260 nm in a Nanodrop device with an absorption ratio at a wavelength of 260 to 280 (280/260 OD) nm. Its quality was also assessed by examining the RNA-containing solution on a 1% gel using ethidium bromide staining and a UV trans laminator based on the formation of RNA-related 18 S and 28 S bands. Additionally, the absorbance ratio of 260 to 280 was measured for each sample using a Nanodrop device (1000 Nanodrop, Fisher Thermo, US), in order to verify the purity of the extracted RNA. One µl of RNA was directly loaded onto the probe of the loading device. The sample concentration was calculated by measuring the absorbance at a wavelength of 260 nm, with the concentration displayed automatically on the monitor. DNA was removed using the DNase enzyme provided by the kit's manufacturer to prevent its interference with the evaluation reaction of the gene expression.

Preparation of cDNA (Complementary DNA)

To synthesize cDNA, 1 µg of RNA was isolated using a kit that included the Reverse Transcriptase enzyme. The process involved the addition of a dt oligo universal primer and followed the protocol provided by the manufacturer (Roche, Germany). The reaction was carried out at 55°C, and the enzyme was deactivated at the end of the procedure at 85°C for 5 minutes.

Primer design for the initial identification and evaluation of MT

The primers used for the amplification of the β-actin gene in the Persian sturgeon were designed based on the method proposed by Gilannejad *et al*. (2019). The primers used for the identification of MT in the Persian sturgeon were also designed using mRNA regions of this gene in the white sturgeon, *Acipenser transmontanus*, (Recovery No. KP164836), and the lake sturgeon, *Acipenser fulvescens*, (Recovery No. KP164837) in Primer3 software (Table 1). The designed primers were checked in Blast-Primer to ensure their specificity for the Persian sturgeon. The initial identification of genes was conducted using conventional PCR in a 10-µL reaction mixture. The reaction mixture consisted of

5 µL of Ampliqon red PCR master mix buffer from Denmark, 0.3 µL of left and right primers (10 pmol/µL each), 4.4 µL of deionized distilled water, and 1 µL of the product obtained from the cDNA synthesis reaction. The PCR was performed in an Eppendorf thermocycler from Germany.

Table 1: The studied gene, designed primers, and size amplified fragments.

Studied gene	The code of the gene bank of the fragment used for primer design	Primer and its sequence	Size of amplified fragment	Primer efficiency
β-actin	KF766533.1	ß-actin F: CCATCCTTCTTGGGTATGGA ß-actin R: GCCAGGGTACATGGTGGTAC	143	99.98
MT	KP164836 KP164837	metal F: ATGGATCCGCAATCTTGCAC metal R:GGTGGCTCCCCCTTTGCA	164	99.87

The thermal regimen used for gene amplification was as follows: 94˚C for 5 min, 35 thermal cycles including denaturation at 94˚C for 30 s, annealing at 54-62˚C for 30 s, and denaturation at 72˚C for 20 s. The final extension was also done at 72˚C for 5 min.

Quantitative assessment of MT expression

A quantitative real-time PCR technique was employed to measure and compare the MT expression (96CFX, Rad Bio, US). To this end, samples were initially prepared in 96-well plates, and then a quantitative method based on Green SYBR was applied. A final volume of 20 µL was prepared for each sample. One µL of template cDNA from 1 µg of RNA at a concentration of 500 ng (RNA produced by Reverse Transcriptase), 0.25 µM of forward and reverse primers (each in a volume of 0.3 µL), 10 µL of Premix EX Syber Green, 0.5 µL of Rox Reference Day, and 7.9 µL of injectable distilled water were the materials used in each reaction. Two sets of negative controls were established for each series of reactions to trace the possible genomic DNA contamination: one contained all the above-mentioned items other than the template, while the other contained only extracted RNA. The final output of the Real-time PCR reaction indicates the number of genes amplified during each cycle. The quantitative real-time PCR analysis involved determining the threshold cycles (Ct) of the tested samples (treatments exposed to different concentrations of cadmium), compared to the control group (negative control). The ratio of the target gene to the reference (R) was then calculated using Equation 1 (Livak and Schmittgen, 2001):

$R=2^{-\Delta\Delta Ct}$ (Eq. 1)

The data obtained from Real-time PCR were analyzed based on the threshold cycle for the target and reference genes. The difference between the mean Ct of the reference gene and the mean Ct of the target gene was calculated as the ΔCt index for both test and control groups (Eqs 2 and 3).

The difference between the test and control groups in Ct values was also utilized to calculate the ΔΔCt index (Eq. 4):

Control ΔCt = (mean Ct of target gene) – (mean Ct of reference gene) (Eq. 2)
Test ΔCt = (mean Ct of target gene) – (mean Ct of reference gene) (Eq. 3)
ΔΔCt = Control ΔCt - Test ΔCt (Eq. 4)

The ratio of expression changes between the test and control samples was calculated using the following equation.
Expression change ratio = $2^{-\Delta\Delta Ct}$

Statistical analysis

The normal distribution of data was examined by the Kolmogorov-Smirnov test. Moreover, one-way analysis of variance (ANOVA) and Tukey's test were employed to compare treatments and the mean values between them at the 95% confidence level. All statistical analyses were performed in SPSS-18 and GraphPad Prism-6.

Results

Quantitative and qualitative assessment of extracted RNA

The ratio of absorption intensity in RNA samples taken from the kidney of Persian sturgeons treated with varying concentrations of cadmium ranged between 1.8 and 2 at two wavelengths of 260 and 280 nm, indicating the acceptable quality of extracted RNA. The appropriate quality of RNA extracted on a 1% agarose gel is shown in Figure 2, where the high-quality gel presents the 28S and 18S bands.

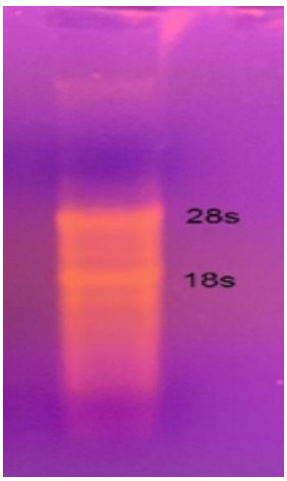

Figure 2: An RNA sample extracted from the kidneys of Persian sturgeons on 1% agarose gel.

The RNA sample is extended, exhibiting numerous weaker bands between the s18 and s28 bands. This indicates the presence of tissue RNA in aquatic organisms.

Figures 3 and 4 show the results of RT-PCR of β-actin and MT in the kidney samples of Persian sturgeons under different cadmium treatments. β-actin, as an internal control, and MT, as the target gene were amplified.

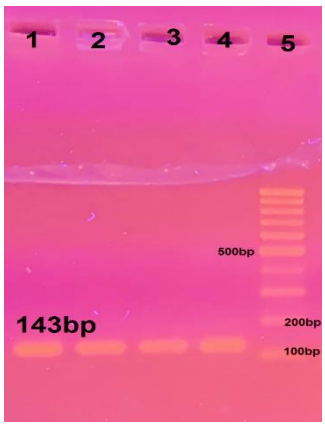

Figure 3: Amplified 143-bp fragment of β-actin (internal control); Rows 1: control treatment; 2: Treatment 1 (200 µg/L); 3: Treatment 2 (400 µg/L); 4: Treatment 3 (800 µg/L); 5. 100-kb ladder marker.

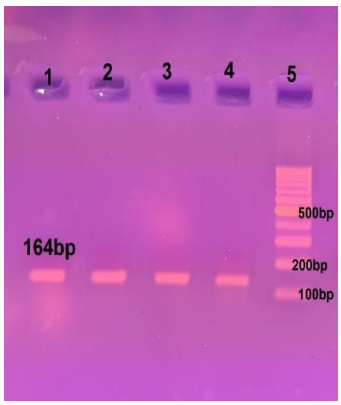

Figure 4: Amplified 164-bp fragment of MT (RT-PCR); Rows 1: control treatment; 2 Treatment 1 (200 µg/L); 3: Treatment 2 (400 µg/L); 4: Treatment 3 (800 µg/L); 5: 100-kb ladder marker.

MT expression changes in the kidney tissue

Figure 5 presents the MT expression changes in the kidney tissue of Persian sturgeons exposed to different concentrations of cadmium. The highest level of MT expression (2.60±0.36) was observed in Treatment 2. The values for treatments 1 and 3 were 2.51±0.13 and 2.15±0.09, respectively. The columns in the figure indicate significant differences between the experimental treatments and the control group at 95% and 99% levels of confidence (p<0.05, p<0.01). The double star signs indicate the significance of MT expressions in these treatments compared to the control group (p<0.01). The single star sign indicates the significance of MT expression in this treatment compared to the control group (p<0.05). The results were compared at a confidence level exceeding 99% due to the small numerical difference.

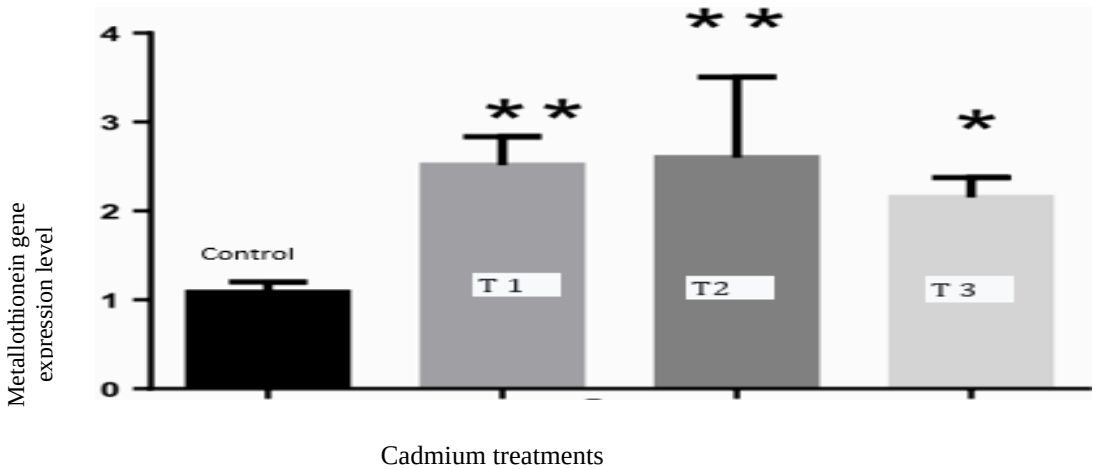

Figure 5: MT expression level in Persian sturgeons exposed to different concentrations of cadmium (mean±standard error); Treatment 1: 200 µg/L, Treatment 2: 400 µg/L, and Treatment 3: 800 µg/L; The double star sign indicates the significance of MT expression in these treatments compared to the control group (p<0.01). The single star sign indicates the significance of MT expression in this treatment compared to the control group (p<0.05).

Discussion

This study investigated the expression level of MT genes in Persian sturgeon (*Acipenser percicus*) after exposure to cadmium, a water-soluble heavy metal. The study findings enhance our understanding of the relationship between physiology and environmental stress, adaptation, and cellular changes in this fish species.

This study identified cadmium as the most significant heavy metal found in the water and sediments of the Caspian Sea, characterized by the lowest LC_{50} and highest toxicity. This is consistent with the findings of Mirzaee *et al*. (2003). This study examined Persian sturgeons during 14 days of exposure to 200, 400, and 800 µg of water-soluble cadmium, which are considered sublethal concentrations for this fish species, and found no mortality. This reflects the precision and validity of the process for selecting the intended concentration.

A review of the research literature reveals that this was the first study that examined the effects of exposure to water-soluble cadmium on the expression of MT genes in the Persian sturgeon. Cadmium and its compound solutions exhibit toxicity even at low concentrations, leading to environmental accumulation and organ retention, which results in quick liver and kidney toxicity (Shahrtash *et al*., 2010). The findings showed that cadmium, as a toxic heavy metal, significantly increased the MT expression on the 14th day of the trial at 200, 400, and 800 µg/L, with the highest increase (2.60) observed in the 400 µg/L treatment. The increase in MT concentration was entirely dependent on time in this tissue. The MT level in all three kidney tissue treatments depended on cadmium concentration, revealing a positive correlation between the expression of MT genes in kidney tissue and cadmium concentration in the body. Therefore, changes in the MT expression in the kidneys of the Persian sturgeon can be attributed to defensive mechanisms against metal pollution. Considering the importance of MT detoxification, this gene can be considered a bioindicator of metal pollution in the studied Persian sturgeons. The indicator oran used in biological and environmental monitoring is crucial, and kidney tissue is regarded as one of the key organs in the removal of heavy metals. Heavy metals usually accumulate in the body tissues of aquatic organisms, such as the Persian sturgeon, that feed mainly on mollusks, invertebrates, small crustaceans, and small fish. Therefore, this eating habit is associated with an increased risk of exposure to higher levels of heavy metals. The accumulation of high levels of hazardous metals in the body of aquatic organisms can negatively affect their physiological functions as well as the health of their consumers. Finally, they will be vulnerable to excessive metal concentrations in the environment (EL-Agri *et al*., 2022). Human activities are posing a growing threat to the population of sturgeon, particularly the Persian sturgeon, in the southern Caspian Sea. As a result, this valuable fish has been listed as a critically endangered species. The disturbance of reproductive function caused by water pollution is one of the possible causes of this population decline (Zahedi *et al*., 2013). Recent reports regarding the presence of cadmium in the rivers in the

north of Iran and the southern coasts of the Caspian Sea raise serious concerns (Shariati *et al*., 2011; Edalat Sarvestani, 2018; Panahandeh and Morovati, 2018). Since the Persian sturgeon is a benthic and carnivorous fish species, various organic and inorganic pollutants may enter its body and bioaccumulate in the food chain of this fish. Considering the bioaccumulation of heavy metals in the Persian sturgeon, this valuable fish species can be used as an indicator in biological studies.

There are uncertainties about the relative sensitivity of sturgeons to metals such as cadmium and zinc. Vardy *et al*. (2014) showed that the white sturgeon is the most sensitive species of this family to cadmium and zinc. Doering *et al*. (2015) demonstrated a difference between sturgeons in this regard, as the lake sturgeon is more sensitive than the white sturgeon to cadmium. Studies have also shown that sturgeons, especially in the early stages of life, are generally more sensitive to metals compared to other fish species. This can be attributed to the less developed compensatory response mechanisms of the sturgeons, as ancient fish species, that may reduce their ability to tolerate metals. Shekh *et al*. (2021) showed that the white sturgeon, *Acipenser transmontanus*, is more resistant and less sensitive to cadmium and copper, respectively, compared to the rainbow trout, *Oncorhynchus mykiss*. Studies on controlled acute toxicity revealed that the white sturgeon was up to 70 times more sensitive than the rainbow trout to cadmium. In contrast, sturgeons exhibited 4.7–7.9 times greater sensitivity to copper in their early life stages compared to the rainbow trout (based on a 96-hour mortality rate) (Calfee *et al*., 2014). The difference between fish species in the intracellular distribution of metals is a factor responsible for the difference between them in sensitivity to metals (Doering *et al*., 2015). Various mechanisms have been studied to further understand differences between fish species in metal sensitivity, some of which are ion dysregulation, oxidative damage, and selective detoxification strategies such as metal sequestration by MT (De Boeck *et al*., 2003; Doering *et al*., 2015; Shekh *et al*., 2021). Physiological differences between sturgeons and bony fishes, such as the rate of acid-base regulation and plasma calcium levels, are other factors causing such differences (Doroshov and Cech, 2011).

Studies on the distribution pattern of cadmium in the body tissues of different freshwater fishes have shown that the highest concentration of cadmium is usually observed in kidneys (Li and Xie, 2018; Qiu *et al*., 2021). Cadmium enters the body of freshwater fishes mainly through their gills and then accumulates in other organs (Wu *et al*., 2007). Cadmium eventually accumulates in the kidneys for excretion, where it triggers MT synthesis (Zahedi *et al*., 2013). The MT-rich tubules of kidneys reabsorb cadmium through the active transport process (Nordberg, 2009). The liver and kidneys are the body tissues that have been studied the most in relation to the accumulation of heavy metals and MT because these are the primary sites of metal accumulation in laboratory experiments, particularly during the acute phase (Cho *et al*., 2005). Cadmium enters the kidney directly from the blood. The synthesis of renal MT to store cadmium and

the reabsorption of the cadmium-MT complex are the next steps that lead to the higher expression of MT in the kidney. Consequently, the kidney reabsorbs a sizable portion of the cadmium-MT complex that was released from external areas and increases the synthesis of MT (Chowdhury *et al*., 2005). In acute cases of cadmium poisoning, cadmium enters the kidney directly from the blood, which increases the MT expression in the kidney (Wangsongsak *et al*., 2007; Shariati *et al*., 2011). Most studies indicate that MT expression is dependent on the concentration and length of exposure to heavy metals; however, MT expression typically falls when the concentration and length of exposure to heavy metals surpass the resistance thresholds of fish (Hadian *et al*., 2017). Bhardwaj *et al*. (2021) showed that long-term exposure to cadmium causes several morphological changes in the kidneys. Cadmium accumulates in the kidney because it is preferentially absorbed by receptor-mediated endocytosis, then freely filtered and bound to MT in the proximal tubule. This MT-cadmium binding is broken down in endosomes and lysosomes, releasing cadmium into the cytosol, where it can produce reactive oxygen species (ROS) and activate cell-death pathways (Liu *et al*. 2022). According to Roggeman *et al*. (2014), cadmium is the primary metal that triggers MT expression in the kidneys, and MT plays a major role in cadmium detoxification due to the high-affinity cadmium-MT binding. MT is highly concentrated in the kidney and liver, and cadmium excretion is generally slower than its absorption. As a result, cadmium must be detoxified and stored using a lower elimination mechanism, resulting in a slower removal rate.

A few studies have addressed the effects of heavy metals on the expression of MT genes in fish species. Studies on *Acipenser oxyrinchus* (*Atlantic sturgeon*), *Acipenser brevirostrum*, and *Scaphirhynchus platorynchus* have shown that sturgeons are among the most sensitive fish species to increased levels of copper and cadmium (Dwyer *et al*., 2005; Vardy *et al*., 2014). Doering *et al*. (2015) conducted a study on the white sturgeon (*Acipenser transmontano*) that demonstrated an increase in the expression of MT genes with rising cadmium levels from 0.1 µg/L to 30 µg/L. However, at a higher concentration, *i.e.*, 100 µg/L, the expression of MT genes not only increased but also showed a reduction. This is attributed to the insufficient presence of MT for transporting the toxic metal, the protein's inability to bind to the metal, or a diminished response ratio in animals exposed to elevated metal levels. The same study on the lake sturgeon (*Acipenser fulvescens*) also showed that changes in cadmium concentration had no significant effect on the expression of MT genes. Sturgeons are among the most sensitive fish species to metals. Studies conducted by Vardy *et al*. (2011, 2012, and 2014) demonstrated the greater sensitivity of the white sturgeon (*Acipenser transmontano*) to the increased level of copper, cadmium, and zinc in the water during the early stages of its life. Other studies have also reported the different effects of metals in different life stages of other fish species. For example, Dwyer *et al*. (2005) showed

different effects on members of the Cypriniformes order, such as the fathead minnow from the Cyprinidae family, when compared to other families such as Acipenseridae. Wahid *et al*. (2017) investigated the expression of MT genes in swamp eels in Thailand exposed to different levels of cadmium in upstream and downstream river water. The findings indicated that cadmium concentrations, both upstream and downstream, did not correlate with the expression of MT genes. Therefore, they concluded that this is not a suitable indicator for cadmium exposure in the swamp eel. The expression and synthesis of MT are correlated with the increased concentration of heavy metals such as cadmium, copper, and zinc. Moreover, the overexpression of MT genes can be considered a response to a variety of adverse environmental conditions, including exposure to heavy metals (Osman *et al*., 2019).

Some researchers believe that there is a typical inverted U-shaped relationship between the concentration of heavy metals and the expression of MT genes in the organism under stress conditions (Chan *et al*., 2004; Ge *et al*., 2020). In fact, when the concentration of heavy metals reaches a specific threshold, the expression of MT genes increases in response to rising heavy metal levels. However, surpassing this threshold results in irreversible toxicity to the organism due to elevated heavy metal concentrations. Therefore, the expression of MT genes decreases with the increase in the concentration of heavy metals. For example, when Scophthalmus maximus was exposed to 100 g/kg of water-soluble cadmium, there was a significant increase in the expression of MT genes in the liver. However, when the concentration exceeded 200 g/kg, the expression level gradually declined despite the rising cadmium levels (Ge *et al*., 2020). Hadian *et al*. (2017) discovered that the expression of MT genes in the liver and kidneys of sterlets diminished with elevated copper concentrations after 14 days of exposure. Their findings indicated a greater expression of MT genes in the liver compared to the kidneys. Studies on other sturgeons indicate that the lack of correlation between the expression of MT genes and cadmium concentration may stem from the protein's inability to bind to the metal, inadequate levels of MT for metal transport, and a diminished response to elevated metal concentrations in the environment.

Consistent with the findings of this study, Shariati *et al*. (2011) reported that exposure to cadmium led to the concentration- and time-dependent increase in the MT expression in the liver, kidneys, and gills of the Persian sturgeon. This suggests the ability of the Persian sturgeon can induce this metal-binding protein as a compromise to detoxify cadmium. By contrast, the study findings are not consistent with the results of Hadian *et al*. (2017). These findings suggest that concentration-dependent changes in the MT expression occur only when a fish is exposed to levels of heavy metals that do not cause harmful physiological effects (Hadian *et al*., 2017). The toxic effects of heavy metals differ from species to species, influenced by the specific metal involved and the species' capacity for natural synthesis of MT (El-Khayat *et al*., 2020).

The findings of Kuthethur *et al*. (2022) confirmed that there is a positive relationship between the expression of MT genes and the concentration of heavy metals. Because of the significance of MT detoxification, many studies have employed it as a cadmium bioindicator in fish species (Iman *et al*., 2013; Hamza-Chafai, 2014; Wang *et al*., 2014; Fabrin *et al*., 2018). Consistent with the findings of this study, Shariati *et al*. (2011) reported the sensitivity of MT to cadmium in the Persian sturgeon, *Acipenser percicus*, and, therefore, introduced MT as a biomarker of heavy metals in this fish.

Conclusions

The results showed that cadmium increased the expression of the metallothionein gene in Persian sturgeon on the seventh and fourteenth days, but there was no increase on the first and second days. Based on similar results obtained in some other fish, it seems that the mechanism of risk reduction due to toxic metal modulation, in addition to metallothionein production, follows another process that has not yet been identified in Sturgeon fishes, either the metallothionein protein is unable to bind to the metal or is insufficient for transport. It was also found that gene expression changes increased with increasing duration of exposure to cadmium.

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Conflicts of interest

Authors declare no conflict of interest.

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