**Gene expression of metallothionein in gills tissues of *Cyprinus carpio* L. and *Oreochromis niloticus* L. fish after exposure to some heavy metals in Thi Qar province, Iraq.**

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# **Abstract**

The main aim of the current study is to access the metal pollution on gill gene expression of metallothionein as defence response against this pollution. Two fish species: *Cyprinus carpio* and *Oreochromis niloticus* in this study were exposed to two heavy metals( Cu and Pb). Three different concentrations are used to metal. The results showed a significant increased (P< 0.01) in the gene expression of the gill MT in all fish groups treated with heavy metals.

**Key words:** *Cyprinus carpio. Oreochromis niloticus*, gene expression , metallothionein.

# **Introduction**

Human activities are the main sources of many pollutants including plastics, metals, pesticides etc. Minerals are a major group of pollutants that have been shown to disrupt the biochemical and physiological integrity of aquatic species, especially fish. There is special attention to some elements for their effect on aquatic organisms, including copper, zinc, cadmium and lead (Vieira *et al.,* 2009).

Some heavy metals like copper (Cu) and zinc (Zn) are essential elements with fundamental biological roles, but other metals like cadmium (Cd) and lead (Pb) are more toxic elements in the aquatic systems without unknown biological roles. All of their effects are toxic. WHO has classified Cd and Pb as two of ten pollutants substances that negatively affect the public health (Osredkar and Sustar, 2011; Satarug *et al*., 2020).

Metallothioneins (MT) are a group of low molecular weight (500 to 1400 D.), water-soluble and cysteine-rich proteins or metal binding polypeptides, that have been found mostly in all living cells. The first isolation of MT was reported by Kagi and Vallee in (1957) from the horse kidney as specific cadmium binding proteins (Dallinger *et al*., 1993; Park *et al*., 2001).

MTs have many functions, including detoxification of heavy metals like Cd or Hg, homeostatic regulation of absorption and hepatic metabolisms of Zn and Cu ions, also the donation of essential metals to apo metalloproteins (Isani and Carpenè, 2014).

MTs take part in many cellular functions. Because of their capacity to bind metal ions, they .play a role of chelators. They also help in reducing of cellular events of reactive oxygen species (Liu *et al*., 2020).

According to the previous studies of Atli and Canli (2008); Kovarova *et al*., (2009); Abumourad *et al*., (2013), they noted that the MT gene expression and MT level in fish tissues have been increased after exposure to heavy metals.

The present study was designed to evaluate the role of exposure to copper (Cu) and lead (Pb) on MT gene expression in the gill tissue of two freshwater fish species: *Cyprinus carpio* and *Oreochromis niloticus*.

Common carp (*Cyprinus carpio*) and Nile tilapia (*Oreochromis niloticus*) are two species of globally farmed fishes. They are among top ten species in global aquaculture (Abdul-Hassan, 2010; ALKhafaji *et al*., 2012; Cai *et al*., 2019).

# **Materials and methods**

**Fish collection**

Male and female fingerlings of *C. carpio* fish with sizes ranging from (8.5 to 9.2 gm, 9.5-10 cm length) and *O. niloticus* fish (7.5-8.3 gm and 8.5-9.0 cm length) were collected from fish farms in Al- Refaei city in Thi-Qar province, southern Iraq. Fish were transferred and kept to acclimatize in 50 L. ventilated plastic aquariums with temperature-controlled heaters for 15 days before starting the experiments under fasting conditions for the first 3 days of acclimation. Fish samples were fed once daily with commercial pellets containing 38% proteins. In addition, the water in the aquariums is replaced once every two days.

**Exposure to heavy metals**

Before the official experiment, a preliminary study was conducted to determine the minimum lethal concentration of the experimental fish and the maximum tolerable concentration. Afterthen,140 healthy fish fingerlings from both species were used in this study. The fingerlings were randomly divided in to 7 groups for each species. The experiment was carried out at fish density of 20 fish/group. Six groups for each species were exposure to heavy metals ( Table 1). Two groups were served as control groups, one for each species that not treated with any heavy metals. Fishes were exposed to the selected concentrations of heavy metals for 30 days. In 31th behavioural study was carried out.

**Table 1: The studied fish groups and the used concentration of heavy metals.**

|  |  |
| --- | --- |
| Group | Concentration of heavy metals (ppm) |
| C1 | 2.78 |
| C2 | 4.17 |
| C3 | 8.35 |
| P1 | 2.35 |
| P2 | 3.53 |
| P3 | 7.07 |

## **Gene expression of gill MT**

Total RNA was isolated from tissue samples collected from fish gills using AccuZolTM kit, BIONEER according to the instructions of the manufacturer, and the quantity and purification of the total RNA were analyzed using Nano-drop spectrophotometers. All DNA and RNA were measured at 260 nm. Purified RNA was subjected to reverse transcription to cDNA by cDNA synthesis kit (EasyScriptTM kit) according to the reagent's instructions. For real-time quantitative PCR analysis of MT gene expression, the real-time qPCR primer of MT gene Table 2. Real-time PCR assay were carried out in a quantitative thermal cycler (LightCycler s 480 II, Roche Diagnostics Ltd., Rotkreuz, Switzerland) in a final volume of 20 ml containing 10 ml 2 x Master Mix (LightCyclers 480 SYBR Green I Master, Roche Diagnostics Ltd., Rotkreuz, Switzerland), 1 ml of cDNA mix. MT gene-specific primers were applied to evaluate the mRNA levels of MT in gill. The real-time qPCR amplification was done as what To analyze the mRNA expression level, the comparative CT methods (ΔΔCT method) was used.

**Table 2: primer design and PCR conditions for qRT-PCR for MT and Beta-actin**

|  |  |  |
| --- | --- | --- |
| **Gene and primer sequence** | **PCR conditions**  | **PCR product (bp)** |
| Metallothionein gene |
| Fw- Metallothionein5’- GCCAAGACTGGAACCCGCAACTGC-3’Rv- Metallothionein 5’- TTCCTTTGCACACGCAGCCAGAGG -3’ | **1 cycle:****Initial denaturation**: 95oC, 5 min**45 cycles: each cycle**Denaturation: 94 °C, 1min Annealing : 60 °C, 1 minExtension : 72 °C, 25 sec**Hold :** 55-95oC, 5 min | 136 |
| Beta-actin gene |
| Fw-Beta-actin5’-CAGGATGCAGAAGGAGATCACAG-3’Rv- Beta-actin5’- GTACTCCTGCTTGCTGATCCACAT -3’ | **1 cycle:****Initial denaturation:** 95 °C, 5 min**45 cycles: each cycle**Denaturation: 94 °C, 1min Annealing : 60 °C, 1 minExtension : 72 °C, 25 sec.**Hold :** 55-95°C, 5 min | 155 |

## **Statistical analysis**

Current study results were analysed by using SPSS (Statistical Package for the Social Sciences) based on one way ANOVA for variation,also it used the least significant difference (rLSD) and independent t test at p < 0.01 and p <0.05.

# **Results**

Fig (1) revealed a significant increased (P< 0.01) in the gene expression of the gill MT in all fish groups treated with heavy metals. After heavy metal exposure, MT gene expression was significantly (P< 0.01) in *O. niloticus* higher than that of *C. carpio*.



Figure 1: Relative MT mRNA after 30 days of exposure. Values with different superscript are significantly different at (P< 0.01).

There is no significant differences in RNA concentrations in all fish groups except *O. niloticus* treated with copper ( Table 3).

**Table 3: RNA concentration in the gills of the studied species.**

|  |  |  |
| --- | --- | --- |
| **Groups** | **RNA concentration (ng/µl)** | **T test*****p. value*** |
| ***O.niloticus*** | ***C. carpio*** |
| **Control**  | 1074.3 ± 260.8a | 1613.0 ± 394.4 | > 0.05 |
| **C1** | 1145.3 ± 242.9a | 2010.6 ± 68.8 | < 0.01 |
| **C2** | 1263.0 ± 327.4a | 1208.6 ± 290.0 | > 0.01 |
| **C3** | 1979.0 ± 162.2b | 1732.0 ± 381.3 | > 0.01 |
| ***p. value***  | **< 0.01** | **> 0.05** |  |
| **LSD** | **480.7** | **Non-Sig** |
| **LSD** | **Non-Sig** | **Non-Sig** | > 0.05 |
| **Control**  | 1074.3 ± 260.8 | 1613.0 ± 394.4 | > 0.05 |
| **P1** | 1283.3 ± 339.2 | 1623.0 ± 357.1 | > 0.05 |
| **P2** | 1282.6 ± 328.9 | 2064.0 ± 84.8 | < 0.05 |
| **P3** | 1701.0 ± 120.0 | 1341.6 ± 393.9 | > 0.05 |
| ***p. value***  | **> 0.05** | **> 0.05** |
| **LSD** | **Non-Sig** | **Non-Sig** | > 0.05 |

# **Discussion**

MT mRNA levels in the gills of *Gobiomorphus cotidianus*, *Crassostrea glomerata* and *Crassostrea iredalei* was considered as a sensitive biomarker to various metal ion exposure since the gills are the main source of uptake of dissolved metals (Cheung *et al*., 2004; Laurie, 2004; Hertika *et al*., 2018). So, The current results showed a significant increased (P< 0.01) in the gene expression of the gill metallothionein in all fish groups treated with heavy metals except *C. carpio* treated with zinc and cadmium.

 Wahid *et al*., (2017) suggested that hepatic MT gene expression biomarkers might be not suitable for prolonged cadmium exposure, so the results of the current study indicated that no significant differences between the control group and C. carpio exposed to Zn and Cd may be result from downregulation of metallothionein gene expression. According to Huang *et al* (2014), downregulation of metallothionein gene expression after longer exposure may have resulted from saturation or adaptive process of the metal burden in the fish organs. It may also be possible that after a longer exposure metal accumulation might have reached a toxic threshold limit and thus MT biosynthesis was compromised. Metallothionein mRNA expression in the liver of adult female mosquitofish (*Gambusia affinis*) increased at early exposure to three heavy metals (Zn, Cd and Pb). Also, there was a significant joint effect between heavy metal type and exposure time on the level of the gene expression. Zn and Cd significantly inhibited hepatic metallothionein mRNA expression after 1 and 8 days of exposure respectively.

 Min *et al.,* (2016) they observed that metallothionein levels in the gills of mullet (*Mugil cephalus*) exposed to hexavalent chromium (Cr+6) didn't show any significance from the control.

Significant increase of metallothionein gene expression in the current study after metal exposure is similar to noted of Choi *et al*., 2008). Cd treatment significantly increased metallothionein mRNA expression in the gill and digestive gland *Crassostrea gigas* fish in a dose- and time-dependent manner.

Metallothionein mRNA levels appeared a 4.5-fold induction (over control) of MT mRNA after the first and second week, and a 6.5-fold induction at the third week of exposure to 100 ppb zinc (Lam *et al*., 1998). Differences in metallothionein gene expression for different heavy metals may be partly due to differences in the interactions between the metals and metal-binding transcription factors (MTFs) (Boldrin *et al*., 2002; Mireji *et al*., 2010).

 Faverney *et al* .,(2000) concluded that a CYP1A1 inducer (3-MC) can modulate the induction of metallothionein and this must be taken into consideration in biomarker monitoring.

**Conclusion**

It can be concluded that Cu and Pb increased the gene expression of metallothionein as a defence mechanism, but further studies are necessary to study the effect of heavy metals on the other defence mechanisms.

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