Bioaccumulation of Zn and Cu in *Chasar bathybius* (Gobiidae) tissue and its nematode parasite *Dichelyne minutus*, southeast of the Caspian Sea, Iran

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Bioaccumulations of Zn and Cu in liver and intestinal tissue of the fish *Chasar bathybius* (Gobiidae) and its nematode parasite *Dichelyne minutus* (Rudolphi, 1819) were studied and compared. Concentrations of Zn and Cu were measured using Inductively Coupled Plasma (ICP-OES). Concentrations of Zn and Cu in *Dichelyne minutus* were markedly higher than in the liver and intestinal tissues of the specimens. Zn concentration in the fish liver increased with an increased density of the parasite. Bioconcentration Factor for intestinal tissue (BF=C parasite /C host intestine) reached to 42-194 for Cu and 25-65 for Zn. BF in the liver tissue ranged from 19 to 88 for Cu and 48 to 96 for Zn. Nematode parasites may have a beneficial influence on the health of the host fish and enable the survival of their hosts from heavy metal absorption.

**Keywords:** *Chasar bathybius, Dichelyne minutus, Caspian Sea, Heavy metals, Bioindicator*

**Introduction**

Intestinal helminth parasites (i.e. cestodes, acanthocephalans, nematodes) have attracted the interest of parasite ecologists as they have been utilized as an indicator of heavy metals in aquatic systems and demonstrated a beneficial role in accumulation of heavy metals from their host’s tissues. Recent studies support the idea that parasites should not always be deemed as an indictment of disease or pathogenicity. Sures et al., stated that there are several advantages to the selection of certain parasites as an indicator of heavy metal pollution in environmental monitoring. Study of heavy metals in parasites provides researchers with the means to determine how heavy metals transfer from a host to its predator. Recent investigations have demonstrated that the most successful parasites in accumulating heavy metals are acanthocephalans. For example, amounts of Lead and Cadmium in the acanthocephalan parasite *Pomphorhynchus laevis* were 2700 and 400 times higher respectively, than those recorded from the intestine of its host fish, Chub *Leuciscus cephalus*.

In this study, *Chasar bathybius* (Gobiidae) and its nematode parasite *Dichelyne minutus* from the Gomishan wetland, southeast of the Caspian Sea were chosen to assess the accumulation of Zn and Cu. Gobiidae fish are not themselves important in economic terms and they are not a protected species under any nature conservation scheme but they are a significant food source for sturgeon in the Caspian sea which are a commercially valuable fish.

**Material and Methods**

**Study area**

The present study was undertaken in the Gomishan wetland (3711’N, 5357’E) along the Ramsar Site with 200 km² area and an average depth of 1 m. Gomishan is a brackish shallow coastal wetland at the extreme southeast of the Caspian Sea, at the edge of the Turkmen steppe and the north of Gorgan bay which is separated from the sea by a narrow sandy barrier and is frequently over run by the sea (Fig. 1). Gomishan wetland supports various species of fish, aquatic plants and endangered birds. However pollutants from the Caspian Sea considerably effect this wetland.

A total of 41 specimens of *Chasar bathybius* (Gobiidae) were sampled in Gomishan wetland during March–April 2009 by dragging a 20 mx1.5 m seine net (5 mm mesh size) through the sandy bed. The fish specimens were identified in situ using species level Coad and were brought to the lab kept on ice.

Biometrical parameters including Total Length (TL) and Total Weight (TW) of fish were measured in the lab; then, with further dissection and examination, the intestinal nematode parasites were collected and

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identified according to levels of species Moravec. Whole liver samples and intestinal tissues, as well as nematode parasites were frozen at -18°C. Fish specimens were divided into three groups according to densities of parasites found in the fish, they were: Group 1-A, total of 15 fish lacking any parasites; Group 2-A, total of 18 fish containing 40-80 parasites; and Group 3-A, total 8 of fish containing more than 90 parasites. Groups were treated separately for analytical measurements to avoid any cross-contamination. After weighing, the parasites were placed in a Pyrex vial where 205 µl HNO₃ (65%, Merck, D-6100 Darmstadt, F.R. Germany) and finally 40 µl H₂O₂ (30%, Merck-Schuehardt, Germany) were added as a digestion solution. Digestion processes took 6.5 hours at 70°C in the oven. In order to determine the heavy metal concentrations in the intestinal and liver tissue, about 0.5g (wet weight) of each type of tissue was placed in a Pyrex vial where 2.5 mL HNO₃ (65%, Merck, D-6100 Darmstadt, F.R. Germany) and 500 µl H₂O₂ (30%, Merck-Schuehardt, Germany) were added as a digestion solution. This process took 9 hours at 70°C in the oven. The materials used in the digestion procedure were completely acid-rinsed. After completion of the digestion process, samples were diluted with doubly distilled water and then analyzed for Zn and Cu concentrations by ICP-OES simultaneous vista-pro. Some analytical blanks such as nitric acid and doubly distilled water were analyzed simultaneously by other samples in order to assess the detection limits.

**Data analyses**

Data on concentrations of Zn and Cu in intestinal tissue were normal according to Shapiro-Whilk test. Thus a one-way ANOVA was applied to compare Zn and Cu concentrations among the three groups. When a significant difference was detected, a pairwise comparison test was done using Tukey’s test. Data on concentrations of Zn and Cu in the liver tissue samples were not normal according to the Shapiro-Whilk test and other different transformation methods failed to normalize the data. Thus a Kruskal Wallis test was used to compare Zn and Cu concentrations between the three groups. When a significant difference was detected, the pairwise comparison test was done using the Mann-Whitney test. Association between concentrations of Zn and Cu elements in intestine and liver tissue with densities of parasites was explored using the Pearson and Spearman Rank Correlation coefficients, respectively. In order to determine the capability of the parasite in the accumulation of heavy metals, the Bioconcentration Factor (BF=C parasite /C host organ) was computed.

**Results**

Total length and Weight of fish ranged from 23 to 29 cm and 200 to 350 g, respectively. The dominant nematode parasite was *Dichelyne minutus*.

**Metal concentrations in fish tissue and parasites**

Mean concentrations of Zn and Cu in intestine tissue reduced with increasing numbers of nematodes (Table 1). Results of one-way ANOVA showed significant differences in concentration of Zn in the intestine tissue of fish with high density of parasites (Group 3), low density of parasites (Group 2) and those fish with no parasites (Group 1) (Table 2). Result of pairwise comparisons presented significant differences

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**Table 1**—Mean of heavy metal concentrations (µg g⁻¹ wet weight) in intestine and liver tissue of those fish with no parasite (Group 1), fish containing 40-80 parasites (Group 2) and fish containing more than 90 parasites (Group 3).

<table>
<thead>
<tr>
<th>Parasite quantity</th>
<th>Zn mean concentration in Intestine</th>
<th>Cu mean concentration in Intestine</th>
<th>Zn mean concentration in Liver</th>
<th>Cu mean concentration in Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>32</td>
<td>5.71</td>
<td>5.67</td>
<td>6.83</td>
</tr>
<tr>
<td>Group 2</td>
<td>19.27</td>
<td>3.38</td>
<td>6.01</td>
<td>5.59</td>
</tr>
<tr>
<td>Group 3</td>
<td>12.53</td>
<td>2.12</td>
<td>6.53</td>
<td>5.46</td>
</tr>
</tbody>
</table>
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Fig. 1—Location of Gomishan wetland in the southeast Caspian Sea (Curtesy of Patimar).
in Cu concentration in intestinal tissue of fish in groups 1 and 3 (Table 2). Mean concentration of Zn in liver tissue increased with numbers of nematodes in the fish (Table 1). Result of Kruskal Wallis and pairwise comparisons using Mann-Whitney test yielded significant differences of Zn concentration in liver tissue among all three groups (Chi-square = 17.67, df = 2, P<0.05). Mean concentration of Cu in liver tissue reduced when numbers of nematodes increased (Table 1); however, these reductions were not significantly different (P>0.05). Based on all collected samples, the mean concentrations of Zn and Cu present in the nematodes were markedly higher than those in the fish tissue (liver and intestinal) (Fig. 2). Bioconcentration Factor in the fish intestine (BF = C parasite /C host intestine) ranged from 42 to 194 for Cu and 25 to 65 for Zn. BF in the fish liver ranged from 19 to 88 and 48 to 96 for Cu and Zn levels.

**Association between parasite density and heavy metal concentration**

A weak significant direct correlation was found between concentration of Zn in the intestinal tissue of fish and densities of parasites (r = 0.39, N = 27, P<0.05). No statistically significant correlation was found between parasite density and Zn concentration in liver tissue.

A strong significant positive correlation was detected between densities of parasites and the Cu concentration in the liver tissue of fish. (r = 0.76, N = 27, P<0.01). There was no statistically significant association between density of parasites and Cu concentration in the intestinal tissue.

**Discussion**

The highest concentrations of Zn and Cu were found in the nematode parasites *Dichelyne minutus*, it can be suggested that nematodes have the capability to absorb and accumulate heavy metals from the body tissue of their host organisms. Several earlier studies have investigated the bioaccumulation of heavy metals by nematodes in both a terrestrial environment and aquatic systems. The result of this study paralleled those earlier studies indicating that parasitic nematodes *Dichylaeminutus* (Rudolphi, 1819) collected from *Chasar bathybius* (Gobiidae) were capable of accumulating significant levels of heavy metals. According to previous studies, only two nematodes, including *Philometracyprinirutili* (Creplin, 1825) and *Anisakis simplex* (Bullini, 1976; *Fig. 2—Mean concentrations of Zn and Cu in liver and intestine of Chasar bathybius (Gobiidae) and its nematode parasite Dichelyne minutus.*

Table 2—Summary of one-way ANOVA testing the differences in concentrations of Zn and Cu in intestine tissue (µg g⁻¹ wet wt) of fish with high density of parasites (Group 3), low density of parasites (Group2) and those fish with no parasites (Group1).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Heavy metal</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine</td>
<td>Zn</td>
<td>0.46</td>
<td>10.04 *</td>
<td>Cu 2</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>0.38</td>
<td>16.34 *</td>
<td></td>
</tr>
</tbody>
</table>
Parasites to their host fish. No similar trend in intestinal tissue, suggesting a beneficial role of heavy metals absorption from the host tissue. Thus, an increase in parasite density leads to higher absorption of Zn and Cu metals from the host tissue. This process is further exaggerated by increasing densities of nematodes in fish intestines, suggesting that the parasites can actually absorb heavy metals. In contrast, acanthocephalans are more effective than nematodes in absorbing heavy metals, as they lack mouths and guts and absorb nutrients through their thin tegument. A similar process is observed in acanthocephalans, which have a thick tegument that impedes bile absorption in high concentrations. Secondly, acanthocephalans attach very firmly to the gut-wall through their proboscis, which allows them to stay in the intestine of their host for longer periods of time presenting more opportunity to absorb the heavy metals. In contrast, nematodes may not be identical to that of acanthocephalans, nematodes however, still function as an effective parasite in heavy metals accumulation from host fish. Sures has argued that acanthocephalans are unable to synthesize their own cholesterol and fatty acid, thus they have a tendency to synthesize cholesterol and fatty acid through bile absorption. Bile-metal complex transfers across the bile-duct to the intestine and acanthocephalan then uptake the metals from the complex. A similar process may be used by nematode parasites. There might be two reasons to support the idea that acanthocephalans are more effective than nematodes in the bioaccumulation of heavy metals. Firstly, acanthocephalan worms lack mouths and guts, but they uptake nutrients through their thin tegument. Nematodes however, have a digestive system and a thick tegument that impedes bile absorption in high concentrations. Secondly, acanthocephalans attach very firmly to the gut-wall through their proboscis and this allows them to stay in the intestine of their host for longer periods of time presenting more opportunity to absorb the heavy metals. In contrast, a nematode that lacks a proboscis has a limited period of residence in the intestine of the host and therefore a limited time to absorb heavy metals.

In the context of this study, the presence of D. minutus significantly reduced concentrations of the Zn and Cu in fishes' intestines, suggesting that the parasites had a beneficial role. This process is further exaggerated by increasing densities of nematodes in host tissue. Thus, an increase in parasite density leads to higher absorption of Zn and Cu metals from the intestinal tissue, suggesting a beneficial role of parasites to their host fish. No similar trend (Zn concentration increase with increased numbers of nematodes) was demonstrated for Zn absorption in the liver tissue.

Conclusion

Dichelyne minutus is a common parasite of Gobbid fish in the Caspian Sea. Present study provides the first report of bioaccumulation capability of Dichelyne minutus in Gobbid fish in the Caspian Sea. Present study has shown that parasites may hamper environmental stress in fish from the bioaccumulation of heavy metals and that these parasites can actually assist in the survival of their host. It should be mentioned that Chasar bathybius is considered as a significant source of food for sturgeon in the Caspian Sea. C. bathybius and its nematode parasites' ability to absorb heavy metals may be beneficial to the survival of sturgeon in this area. Results of the current study also indicate that D. minutus may be an indicator species useful for assessment of heavy metals contamination.

Acknowledgment

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