# Assessment of Anodonta cygnea as a Biomonitor Agent for Copper and Zinc in Anzali Wetland, Iran

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## ABSTRACT

**Background:** Anzali wetland has been subjected to high levels of pollution due to contamination from several industrial sites in addition to agricultural chemicals. Mussels have been widely used for monitoring pollution in aquatic ecosystems, because they, as filter feeders, bioaccumulate pollutants. Therefore we decided to evaluate Anodonta cygnea for its application as a bio-monitor for copper (Cu) and zinc (Zn).

**Methods:** A. cygnea specimens and their surface sediments were gathered from three locations in Anzali wetland. Afterwards, the collected samples (the soft tissues and shells of A. cygnea as well as surface sediments) were analyzed for Cu and Zn by a flame atomic absorption spectrophotometer (Model 670G).

**Results:** The Cu and Zn concentrations in the sediments obtained from Anzali wetland were in the range of 21.05 to 25.53 for copper and 37.84 to 82.26 µg g-1 dw for zinc. The Cu and Zn levels in the soft tissue were 1.09-1.5 times and 5-7.3 times, respectively, higher than those of the shells. The CV values (%) in the soft tissues and shells were 36 and 53 for Cu, and 53.5 and 150.3 for Zn respectively.

**Conclusions:** The lower Zn variability (CV) in the soft tissues of A. cygnea and significant correlation between Zn levels in the soft tissues of A. cygnea and the surface sediments indicated that the soft tissues of A. cygnea are more appropriate for bio-monitoring of Zn. Cu concentration in the sediment and Zn levels in the soft tissues were found to be comparatively higher than some of the international standards of reference.

**Keywords:** Anodonta, Chemical Water Pollutants, Copper, Iran, Wetlands, Zinc.

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## INTRODUCTION

Anzali wetland is one of the most important water bodies in northern Iran. The wetland represents an internationally important wildlife reserve, which was listed under Ramsar Convention [1]. The pollution in marine environments has been progressively increasing over the years due to anthropogenic activities [2]. Metals are categorized as the most critical pollutants, which can cause environmental degradation in coastal areas [3]. High level of metals in aquatic ecosystems is regarded as serious pollution, because they can get incorporated into the food chain [4]. Copper (Cu) and zinc (Zn) are essential elements for all living organisms [5]. However, they can become serious threats to aquatic organisms if their levels increase [4]. In addition, Parkinson disease has been proved to be associated with chronic exposure to copper and zinc [6] and these elements may act alone or together to propagate the disease over time [7].

Recently, new directions for monitoring marine pollution and several implications for estimation of metal bioavailability in mussel watch programs have been recommended [8]. Many studies have been carried out concerning the biomonitoring of metals in different parts of mussels [8-11]. One of the largest freshwater mussels in the family Unionidae in the Anzali wetland is the swan mussel (Anodonta cygnea).

The objective of this study was to determine the contamination status of Cu and Zn in the swan mussels' soft tissues and sediments from four sites of Anzali wetland.

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We also examined the relationship between copper and zinc concentrations in mussels' soft tissues and corresponding levels in the sediments. Other major objectives were to evaluate this species for its application in serving as a bio-monitor for Cu and Zn. Finally, a comparison between the metal concentrations and international standards was made.

## MATERIALS AND METHODS

A total of 36 Anodonta cygnea with relatively similar sizes were collected from three sampling sites in Anzali wetland (Fig. 1). Afterwards, all the total soft tissues were dissected and separated from the shells. These mussel samples were brought back to the laboratory and were stored at minus 10°C until the analysis. The sampling dates, number of analyzed mussels, the shell lengths, and site descriptions are given in Table 1. The surface sediments were collected from near the mussel habitats (three replicates of sediments were sampled from each site). The soft tissues and shells were dried in a freeze drier to reach a constant weight and homogenized in an agate mortar. The sediments samples were dried in an oven at 105 0C for 48 hours until reaching constant dry weights (dw).

The soft tissues and shells of A.cygnea were digested in concentrated nitric acid (69%). They were placed in a hot block digester at low temperature for 1 hour and were then fully digested at high temperature (140 °C) for at least 3 hours. For the analysis of sediment samples, approximately 1 gram of each dried sample was digested in a combination of concentrated nitric acid (69%) and perchloric acid (60%) in a ratio of 4:1, first at low temperature (40 °C) for 1 hour and then the temperature was increased to 140 °C for 3 hours [12]. Similarly, the digested samples were then diluted with double distilled water (DDW). The sample was then filtered through Whatman No. 1 filter paper and the filtrate was stored for metal determination. The prepared samples were then analyzed for Cu and Zn by a flame atomic absorption spectrophotometer (Model 670G).

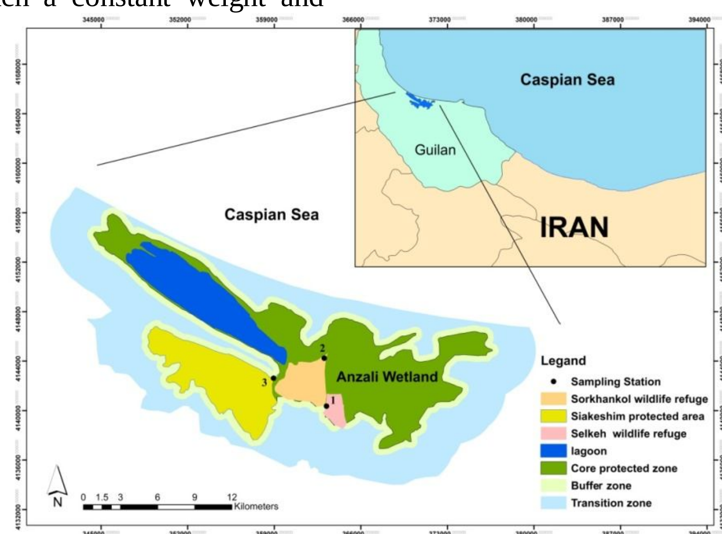


**Figure 1.** Sampling sites in the Anzali wetland.

**Table 1.** Location of sampling sites and number of the analyzed A.cygnea samples (N), collected from the Anzali wetland.

| Station | Location | Latitude (N) | Longitude (E) | N | Site description |
|---|---|---|---|---|---|
| 1 | Selke | 36° 32′ 25″ | 41° 40′ 367″ | 16 | Wildlife Refuge |
| 2 | Sorkhankol | 36° 30′ 47″ | 41° 44′ 245″ | 10 | Center of wetland |
| 3 | Mahroze | 35° 89′ 43″ | 41° 42′ 628″ | 10 | West of wetland |

The whole data were tested for normal distribution in the first place. One way analysis of variance (ANOVA) was employed to find any major difference between metal concentrations in different sites. In cases with significant differences, LSD test (least significant difference) was utilized. The homogeneity of variance for data was performed with Levene test. The CV value was calculated to determine the degrees of variability of metal concentrations in the shells and soft tissues of A. cygnea as follows:

**CV% = (standard deviation/mean) × 100**

## RESULTS

Table 2 shows the concentrations (μg/g dry weight) of metals in the surface sediments and the soft tissues and shells of A. cygnea. Cu and Zn concentrations in the surface sediments ranged from 21.05 to 25.53 and 37.84 to 82.26 μg g-1 dw, respectively. The highest Cu and Zn concentration was recorded in station two. The results disclosed that there was no significant differences in Cu concentrations among different stations (one-way ANOVA, $p > 0.05$) but there was considerable difference in Zn concentrations between stations ($p<0.05$).

The concentration of Cu and Zn in the soft tissues and shells of the A. cygnea are demonstrated in Table 2. The Cu and Zn concentrations in the soft tissue were 1.09-1.5 times and 5-7.3 times, higher than those of the shells (Table 2). The CV values in the soft tissues and shells were 36 and 53 for Cu and 53.5 and 150.3 for Zn respectively. The Cu and Zn variability in the shells were higher than those of the soft tissues.

Comparison of Zn concentrations in the soft tissues of samples demonstrated significant differences between various locations ($p<0.05$). There was no significant correlation between Cu concentrations in soft tissues of A. cygnea and the sediments ($p>0.05$, $r= -0.42$, Fig. 2) but the correlation between the Zn levels in the soft tissues and Zn levels in sediments was significant ($p<0.05$, $r= 0.8$, Fig. 3).

Table 2. Mean ± SD concentrations of Cu and Zn (μg g-1 dw) in the sediment samples and soft tissue; and shell; of A.cygnea, collected from the Anzali wetland.

| Station | Location | Sediment | | Soft tissues (ST) | | Shell (SH) | | Ratio (ST/ SH ) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Cu | Zn | Cu | Zn | Cu | Zn | Cu | Zn |
| 1 | Selke | 22.66 ± 0.23 | 37.84±0.61 | 8.2±8.8 | 59.17±10.06 | 7.47±4.46 | 11.82±11.4 | 1.09 | 5 |
| 2 | Sorkhankol | 25.53 ± 13.69 | 82.26±1.83 | 5.16±2.2 | 158.27±22.89 | 4.17±1.6 | 28.96±41.82 | 1.23 | 5.4 |
| 3 | Mahroze | 21.05 ± 6.09 | 47.15±2.1 | 8.58±5.37 | 65.23±16.82 | 5.77±2.2 | 8.97±12.82 | 1.48 | 7.27 |

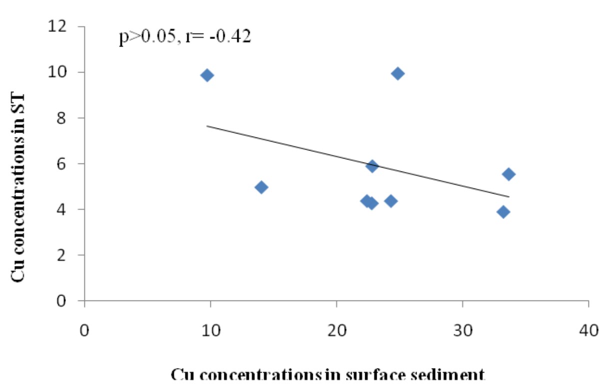


**Figure 2.** The correlation between log10 (mean) Cu in soft tissue and log10 (mean) Cu in sediment of Anodonta cygnea (n = 9).

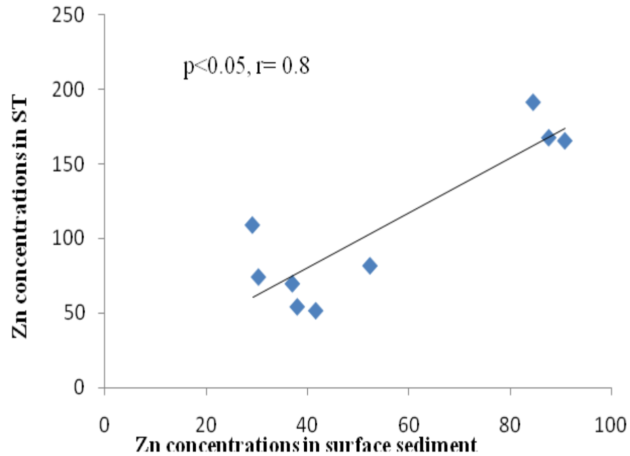


**Figure 3.** The correlation between log10 (mean) Zn in soft tissue and log10 (mean) Zn in sediment of Anodonta cygnea (n = 9).

## DISCUSSION

The results indicated a significant correlation between Zn concentrations in the soft tissues of A.cygnea and Zn concentrations in the sediments. The obtained results are in line with the results reported by Jamil et al. which confirmed the significant correlation between Zn in soft tissues of three clam species (Anodonta anatina, Unio pictorum, U. tumidus) and surface sediments in the Danube Delta, Romania [9]. In addition, our results are in agreement with those reported by Noorhaidah et al. [11], who found significant correlations between Zn levels in soft tissues of T. telescopium and some geochemical fractions of Zn in the sediments. There was no significant correlation in Cu concentrations between soft tissues of A. cygnea and sediments as Hummel et al. [13] who reported that the Cu concentration of M. edulis was not significantly related to the total Cu concentrations in the sediment.

In the present study, Cu and Zn in the soft tissues of A .cygnea were higher than the shells. It has been stated that the mollusks soft tissues accumulate higher concentrations of Cu and Zn than those in the shells [14]. Higher levels of Zn and Cu in the soft tissue could be due to the role of these metals as essential elements for the aquatic organisms [4]. We found lower Zn variability in the soft tissues of A. cygnea. According to the results gained by Yap et al. [12], the Zn variability in the shells was higher than in the soft tissues.

A comparison between the concentrations of Cu and Zn in soft tissues of A. cygnea with other studies is presented in Table 3. Cu and Zn concentrations in the soft tissue of A. cygnea obtained by the present study are in the same range compared to the data reported by Barsyte Lovejoy et al. [15] and Ravera et al. [16]. The significant correlation between Zn concentrations and Zn levels in sediments and its lower variability (CV) in the soft tissues of A. cygnea verified that this species' soft tissues can be used as precise biomonitor for Zn.

Cu levels in surface sediments was comparatively higher than the ISQG standards set by CCME, (18.7 µg g-1 dw). A comparison of Cu and Zn concentrations in soft tissues with international standards (the guidelines on seafood safety set by different countries) showed that the Cu contents of the app sampling sites were lower than the guidelines values. The Zn concentrations were lower than the standards set by ABIA, MFR, and NHMRC, but higher than those provided by MAFF and TPHR (Table. 4).

**Table 3.** Comparison of mean (±SD) or range of heavy metal concentrations in the soft tissues of different bivalve mollusks from all around the world (µg g-1 dw).

| Location | Species | Zn | Cu | Reference |
|---|---|---|---|---|
| Malku Bay, Lithuania, Channel near Dreverna | *Anodonta cygnea* | 146 | 5.4 | [14] |
| Malku Bay, Lithuania, Channel near the waterworks | *Anodonta cygnea* | 118.5 | 5.5 | [14] |
| Danube Delta, Romania | *Anodonta anatina* | 83.6 | 4.2 | [9] |
| Italy, Ranco Bay | *Anodonta cygnea* | 201 | 7 | [16] |
| SKEZ, Vietnam | *Anadara granosa* | 80.2 | 7.25 | [18] |
| Estuaries in Sabah, North Borneo | *M .meretrix* | 108- 190 | 6.64- 8.34 | [8] |
| Italy, Ranco Bay | *Unio pictorum mancus* | 150 | 5 | [16] |
| Anzali wetland | *Anodonta cygnea* | 85.59 | 5.59 | Present study |

**Table 4.** Guidelines for Zn and Cu for seafood safety, set by different countries.

| Location | Species | Zn | Cu | Reference |
|---|---|---|---|---|
| Malku Bay, Lithuania, Channel near Dreverna | *Anodonta cygnea* | 146 | 5.4 | [14] |
| Malku Bay, Lithuania, Channel near the waterworks | *Anodonta cygnea* | 118.5 | 5.5 | [14] |
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NHMRC: National Health Medical Research Council; TPHR: Tasmania Public Health Regulation; ABIA: Brazilian Ministry of Health; MAFF: Ministry of Agriculture, Fisheries and Food.

## CONCLUSION

According to our findings, the CV values in the shell of A. cygnea are higher than the soft tissue. Moreover, the significant correlation between Zn concentrations in soft tissues of A. cygnea and the sediments suggests that its soft tissues could be used as biomonitor agent of Zn pollution in the Anzali wetland. It is proposed that due to the comparatively higher concentrations of Cu and Zn than some guidelines, the concentrations of these metals should be continuously monitored. Although Cu and Zn are essential metals for living organisms, they can be harmful and may cause damage at the elevated concentrations [17]. Future studies should assess the level of risk to humans for Cu, Zn, and other toxic metals such as Pb and Cd in Anzali wetland.

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