The Influence of Selenium and Mercury Ratios on Body Characteristics in Common Carp

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Abstract
The aim of this study was to quantify the levels of selenium and mercury in the blood serum of common carp (Cyprinus carpio) and to investigate their interrelationship and potential influence on fish physical parameters. This study expected that the calculation of the molar ratio of these elements would reveal possible correlations with the total length (TL) and weight (W) of the fish. The quantification of selenium and mercury was quantified using the ICP-OES method and the physical parameters of the fish were measured in situ at the time of sampling by a specialized expert. Selenium, an essential bioactive element, contrasts with mercury, a non-essential toxic element, due to their antagonistic interactions in biological systems. Selenium has the ability to mitigate the toxicity of mercury by forming non-toxic complexes, such as the tetraselendimercuric compound (HgSe), also known as tiemannite. Previous studies have suggested that a Se:Hg molar ratio greater than 1 is associated with the protective effects of selenium against mercury toxicity. In this study, selenium was detected in only a subset of samples, while mercury was found in all samples analyzed. Statistical analyses, including correlation analysis and linear regression, were performed using GraphPad Prism 8.1. However, no statistically significant correlations were found between the molar ratio of selenium and mercury and the physical parameters of the fish. This result can probably be attributed to the limited sample size and the smaller number of samples in which selenium levels could be quantified.

Keywords: *Cyprinus carpio*, selenium, mercury, length, weight, Se:Hg molar ratio

1. Introduction

Mercury pollution in the aquatic ecosystem has been a concern of the general population for decades. The issue is still relevant despite multiple regulations and restrictions on using this toxic element. Mercury is frequently detected, especially in aquatic ecosystems [1, 2], and fish inhabiting these environments are exposed to mercury throughout their lives. Fish are therefore an ideal model for the study of mercury presence and toxicity in living organisms [3–5]. Mercury is particularly known for its neurotoxic effects, which may lead to oxidative stress or potential damage and subsequent failure of major organs, especially the brain and liver [1,6,7]. However, it has been found that essential trace elements such as selenium can mitigate or counteract its toxicity by forming inert complexes such as HgSe (mercury-selenide) [8,9], which is harmless in the body. Such antioxidative protection occurs when there is a sufficient quantity of selenium in the body relative to the mercury presence. It has been proven that if the molar ratio of Se:Hg is >1 [7], an organism is adequately protected from the toxic effects of mercury. Our goal was to monitor the concentrations of these two elements in blood

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serum, as we believe that with an appropriate molar ratio, additional mechanisms could also be protected, which might have a positive effect on the body metrics of the fish. As a model for our research, we selected a freshwater fish commonly consumed by humans, the common carp (*Cyprinus carpio*). This study aimed to observe body metrics - total body length (TL) and weight (W) and to seek a possible association between their variability and the molar ratio of Se:Hg (calculated based on detected concentrations of Se and Hg in blood serum).

2. Materials and methods

Sample collection

The fish were collected using nets during the summer season from a university experimental pond (Kolinany, Slovak Republic; 48° 21′ 14.6″ N 18° 13′ 03.2″ E). The pond is exposed to a variety of pollution due to the presence of a nearby sewage treatment plant and agricultural activity in the area, yet despite these factors, no previous studies have confirmed a significant risk of pollution and health hazards [5, 10, 11]. A total of 15 individuals of various sexes were collected and subjected to on-site measurement of physical parameters. Subsequently, the fish were transported within a short time to the university's institute of animal husbandry, where blood samples were extracted from the caudal vein by an experienced expert.

The collected blood samples, stored in tubes without anticoagulant, were centrifuged at 2000g for 10 minutes after 30 minutes of the clotting process. Afterward, blood serum was carefully extracted using a pipette and stored at -20 degrees until further analysis. For each fish specimen, approximately 1.5 mL of whole blood was collected to obtain at least 0.6 mL of serum, which was required for mineralization and subsequent analysis of trace elements.

Water samples were not collected in this study.

Detection of Microelements (ICP-OES)

Before analysis, samples of blood serum were mineralized using nitric acid (HNO_3). The concentrations of Se were analyzed by ICP – OES (Inductively Coupled Plasma Optical Emission Spectrometry) using the ICP OES 720 (Agilent Technologies, Santa Clara, CA, USA) [10]. Hg was measured using a selective mercury analyzer (AMA-254; Altec, Prague, Czech Republic).

Statistical analysis

The molar ratio of selenium to mercury (Se:Hg) was calculated using the following formula:

$$MR=([Se](mg/L)/78.96)/([Hg](mg/L)/200.59)$$

where, 78.96 and 200.59 represent the atomic weights of selenium and mercury, respectively.

Statistical analyses were performed using GraphPad Prism 8.1. Linear regression analysis was used to assess the relationship between the concentrations of selenium and mercury, as well as their association with body metrics.

Due to the non-parametric nature of datasets, Spearman's rank correlation coefficient (ρ) was used to evaluate the strength and direction of monotonic relationships between variables. A significance level of $\alpha = 0.05$ was applied.

3. Results and discussion

Following ICP-OES analyses, we could detect Hg concentrations in all samples, however, Se was determined in only 7 samples as shown in Table 1. Therefore, individuals whose concentration levels were below the limit of detection (LOD) were excluded from the statistical analyses. The total number of samples in which quantification of all values was determined was 7. The total length was 471.43 ± 19.09mm, the total weight was 2048 ± 335.9g and the calculated average molar ratio was 244.73.

Table 1. Detected selenium and mercury concentrations in blood serum, molar ratio of Se:Hg, body metrics of fish (TL and W)

Sample	Se (mg/L)	Hg (mg/L)	Molar ratio Se:Hg	TL (mm)	W (g)
1	0.10604	0.000798	337.57	460	1450
2	0.10052	0.001322	193.16	465	2210
3	0.07357	0.002676	69.84	465	2170
4	0.22685	0.001577	365.43	505	2316
5	0.09239	0.000775	302.85	480	2144
6	0.08397	0.001038	205.51	445	1710
7	0.12865	0.001369	238.73	480	2336

As shown in Figure 1, linear regression indicates a straight, slightly positive relationship between Se and Hg concentrations. As the concentration of Hg in the sample increases, the concentration of Se also tends to increase, and vice versa. This relationship is statistically significant but relatively weak, and although there is a mutual increase in concentration, it cannot be clearly determined from this regression whether the relationship is protective or toxic.

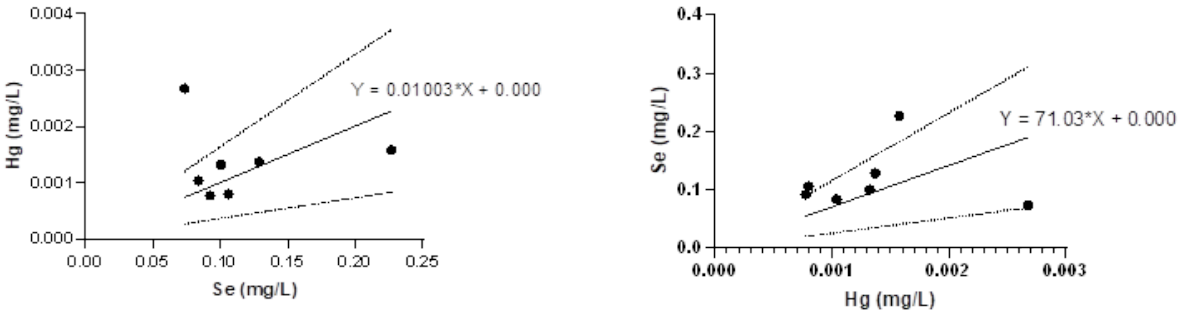

Figure 1. Linear regression of selenium and mercury

As we can see in Figure 2, the correlation of molar ratio Se:Hg showed a very weak negative relationship (r = -0.03571) with the weight of the fish, however, p-value = 0.96 indicates that there is no statistically significant difference between the results. Similarly, in the relation between total length and molar ratio, Spearman's correlation coefficient r = 0.4364 showed a slightly positive relation, but p-value = 0.34 revealed that there is no statistically significant difference. According to these results, we cannot conclude that variation in blood serum Se:Hg molar ratio has a proven influence on the physical parameters of the fish.

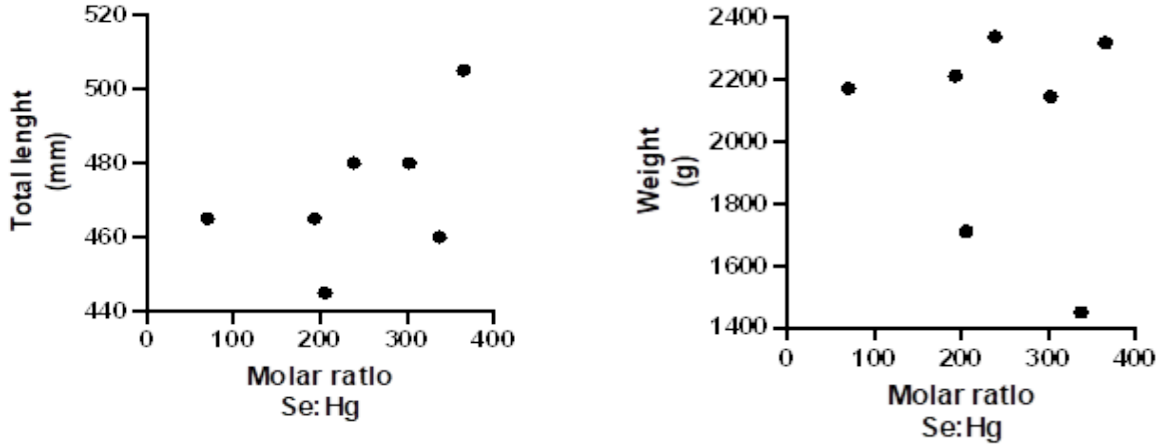

Figure 2. Correlation of molar ratio with body metrics

Discussion

Mercury is a well-known, pantoxic element, meaning that it is toxic in all its forms and to all organisms [12]. Methylmercury (MeHg) is especially harmful to fish due to its high affinity for the brain and nervous tissue, which may induce neurological damage, oxidative stress, and energy metabolism dysfunction [6]. Selenium, on the other hand, is toxic to organisms only after certain levels are exceeded, since in the appropriate levels in the body as an essential trace element it is involved in the function of various enzymes such as GPX

(glutathione peroxidase) [13]. Thereby it plays a key role in antioxidant defense and helps to maintain a balanced metabolism. One of the most important features of the interaction between these two elements is their possible antagonistic effect [9, 14].

It has been confirmed in various organisms, including fish, that selenium can bind mercury to form inert complex compounds such as HgSe (mercury-selenide), the toxicity of which to the organism is negligible [8]. In this way, selenium probably contributes to protecting the body from mercury toxicity in its various forms. The molar ratio of selenium:mercury at which protective effects of selenium have been proven has been established to be >1 [7].

Several studies have investigated the molar ratios of Se:Hg in a wide variety of species living in different areas and also investigated the variability of these ratios in different tissues. Burger et al. (2012) [15], in their comprehensive study, observed molar ratios in muscle tissue in 14 fish species, and in more than half of the samples, proved statistically significant relationships between fish size and molar ratio Se:Hg. The negative correlation that was observed reflects that with increasing body size, the molar ratio decreases, thus there is potentially less antioxidative protection in the body due to lower selenium availability. Such negative correlation was also confirmed in their other study [12], where in some cases the molar ratio dropped below 1. Another study in which they monitored molar ratios in the tissues of grebes from different areas [16] discovered that the lowest molar ratios were in the breast feathers of the birds, however, it was also influenced by the periodic dropping and regrowth of feathers. The highest values varied greatly depending on the area of occurrence, but the highest molar ratios they recorded were in the brain (23.55) and in the muscle (15.25). As part of their research, they also observed the correlation of molar ratios with body size, concluding that the largest individuals were found to have the lowest molar ratios, yet the statistical result was not significant enough to confirm this with any certainty. Compared to the results in this study, our molar ratio values are relatively higher, which is probably due to the selection of a different matrix. It is generally known that selenium accumulation in the liver is significantly higher, due to the detoxification of the organism in this organ, and on the contrary, in the brain, the concentration of mercury is high, since its toxicity primarily affects the nervous system [17]. The blood, or in our case the blood serum, unlike the internal organs, is just a transport matrix, where the concentrations of metals may vary greatly, which may result in such high molar ratios. Very few studies have investigated the levels and molar ratios of these elements in blood serum, which is one of the reasons why we decided to investigate possible interactions. In their study on dolphins [18] monitored molar ratios in whole blood, blood serum, and plasma, and discovered that the highest ratios (15-30) were in blood serum. In our research, we found even higher values. However, this may also be due to low mercury levels in the entire organism because of minor pollution in the ecosystem. Our results indicated a potentially high selenium:mercury ratios, a prerequisite for strong antioxidative protection of the organism. The absence of significant results in our work (such as the unconfirmed relationship of body metrics to molar ratios) may likely be influenced by the smaller number of samples and lower variability of measured values in fish blood serum, which may decrease the statistical strength of the analysis. These factors lead us to conclude that it would be useful to extend the study to a larger sample and use more different tissues, which may help us to further understand the dynamics of selenium and mercury interactions and their relationships with other quantifiable variables.

4. Conclusions

In our study, we were able to quantify the selenium and mercury concentrations in fish blood serum samples and calculate their molar ratios. However, we were unable to confirm the effect of the molar ratio of Se:Hg on body metrics. We consider one of the main factors for the absence of significant correlations to be the low number of samples that we could have included in the study. Yet, we plan to extend this topic with a higher number of individuals and tissues in the future.

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