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MERCURY AND SELENIUM ACCUMULATION ASSESSMENT IN FISH MOST CONSUMED BY CUBATÃO COMMUNITY, SÃO PAULO, BRAZIL

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ABSTRACT

High correlation between mercury (Hg) and selenium (Se) concentrations in fish organs is well known. In the present study, 58 fish samples of five fish species most commonly consumed by the Cubatão city population, São Paulo State, Brazil were analyzed. The Cubatão Estuary, located in southeastern São Paulo State, is an economically important area suffering severe environmental water quality problems due to industrial wastes, domestic sewage and solid residues. The waters of the bay suffer the impact of the immense industrial complex of Cubatão. In this study, Hg concentration in muscle, liver and kidney tissues and Se concentration in liver tissue from three predatory fish species: *Macrodon ancylodon (*Pescada*), Menticirrhus americanus (*Perna de Moça*) and Micropogonias furnieri (*Corvina*)* and two planctivorous species: *Mugil liza (*Tainha*) and Sardella braziliensis (*Sardinha*)* were determined. Mercury determination was performed using Cold Vapour Atomic Absorption Spectrometry (CV AAS) and selenium by Instrumental Neutron Activation Analysis (INAA). The muscle-Hg concentration variation (wet weight) was: (8 to 40 µg kg^{-1}) - Sardinha; (12 to 62 µg kg^{-1}) – Pescada; (3 to 23 µg kg^{-1}) – Tainha; (43 to 184 µg kg^{-1}) – Perna de Moça and (41 to 348 µg kg^{-1}) – Corvina. The general concentration ranges of the analyzed elements in all species studied were (dry weight): muscle-Hg (13 to 1512 µg kg^{-1}); liver-Hg (21 to 1804 µg kg^{-1}); kidney-Hg 47 to 9912 µg kg^{-1}) and liver-Se (2.10 to 43.00 mg kg^{-1}). Se concentrations were higher than those of Hg in the liver.

1. INTRODUCTION

Mercury (Hg) is a persistent element found in oceans, lakes and rivers that can be converted to methylmercury (MeHg) by aquatic biota and bioaccumulate in aquatic food chains including fish and shellfish [1, 2]. Once Hg is released in aquatic environments, bacteria transform this Hg into the highly toxic MeHg organic form. This toxic MeHg can move up the food chain to fish and then eventually reach humans. Mercury is also bioconcentrated, mainly as MeHg, in the trofic fish chain and can be especially dangerous since this methylated form represents 73-98% of the total Hg [3, 4]. Brazilian legislation determines 0.5 mg kg^{-1} (wet weight) as the maximum allowable limit for total Hg in fish, crustaceans and mollusks [5].
Coastal areas, in general, tend to suffer intense human occupation, provoking severe pressure on fragile ecosystems. The Cubatão Estuary, located in southeastern São Paulo State, is an economically important area. However, it is an area with severe environmental water quality

problems due to industrial effluents and emissions, domestic sewage and solid residues. Some studies have already detected the environmental pollution for mercury in this area. The main sources of Hg pollution of those waters are metals processing industries, chloro-alkali and battery production, fluorescent lamps, increased crop harvests and fungicides containing Hg. Sewage and other residues can also be sources of contamination [6, 7, 8].

Fish are very sensitive to environmental changes and have been considered good bioindicators of environmental and contamination status. Fish have high potential for accumulating some trace elements, such as Hg, Se and the analysis of tissues from different species of fish can be used as a tool for the assessment of environmental pollution [9]. The accumulation of Hg in its organic form in piscivorus species can reach up to 9 times more than in other species [10, 11]. It has been suggested that several factors may influence a population's vulnerability to the effects of MeHg. Among those are age, gender, health and nutritional status, as well as, the intake of other foods or nutrients that might influence the absorption, uptake, distribution and metabolism of both Hg and MeHg. One of the most discussed possibilities is related to the effect of selenium (Se) compounds counteracting the toxic effects of Hg.

Although we have extensive knowledge on toxic and protective effects of Se in mammals [12, [13, 14], there is very little information on the protective role of Se in fish species. Some experiments have investigated the reduction in bioavailability of some trace elements, such as Hg by Se [15]. Selenium seems to present protection in regards to fetus toxicity of Hg. Studies with mice showed that the lethal fetus toxicity of the MeHg is exacerbated by a Se deficiency in the maternal diet [16, 17]. Selenium alters the toxic-kinetics of Hg, modifying its distribution among organs (an increase of their amounts in liver, spleen, pancreas, etc and a decrease in the kidneys and muscle) [18, 19]. It has also been reported that the liver of aquatic organisms may act as an organ for demethylation and/or the sequestration of both organic and inorganic forms of Hg [20, 21]. The exact mechanisms of interaction between Hg and Se in fish are not yet fully understood but, data obtained from fish studies indicate that Se, like Hg in aquatic organisms, is mostly found in concentrations that increase proportionally with the trophic species level [22].

The purpose of this study is to contribute to a better understanding in the accumulation of Hg and Se in different tissues and fish species. For that, Hg and Se levels, in different fish body organs, acquired in the street markets of Cubatão, São Paulo, Brazil, were evaluated. Hg distribution in the muscle, liver and kidney and the relationship between Hg and Se concentrations in the liver of different fish species were also investigated

2. MATERIAL AND METHODS

2.1. Description of the studied area

The vegetation of the Atlantic Forest on Serra do Mar near the industrial complex of Cubatão (state of São Paulo, Brazil) has been subject of severe damage caused by massive emissions of pollutants, as a result of the progressive industrialization in the area (Fig. 1).

Coastal regions, generally, present an intense human occupation and high pressure on the ecosystem. The estuarine systems of Santos and Sao Vicente are the most important examples of environmental degradation of coastal regions in Brazil. In this region there is the largest harbor of Latin America, the Santos Harbor and the most important industrial complex in Brazil, situated in Cubatao

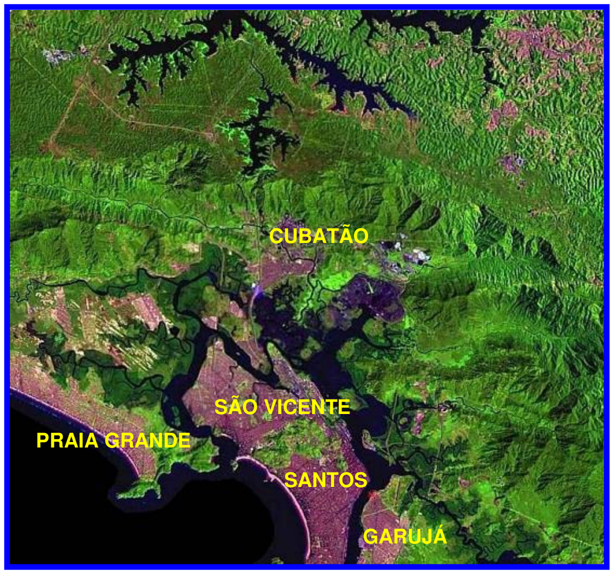

Figure 1. Cubatão city location [23].

2.2. Fish species selection

A questioner was distributed to families that took part in another study in order to ascertain which fish species most consumed in the daily diet for the Cubatão community. This questioner was distributed in three public schools where children's hair was also collected for Hg bioaccumulation studies. From this questioner the results were tabulated and the most consumed fish species were identified. The five fish species selected were: three carnivorous, *Macrodon ancylodon (*Pescada*), Menticirrhus americanus (*Perna de Moça*) and Micropogonias furnieri (*Corvina*)* and two planktivorous species, *Mugil liza (*Tainha*) and Sardella braziliensis (*Sardinha*)*

2.3. Sampling

The fish samples were acquired directly from local street markets in Cubatão during March of 2008. All samples were assessed for quality indicators and external organoleptic characteristics (eyes, gills and scales). Fish were conditioned in isothermic boxes in crushed ice and identified [24]. In the laboratory fish were evaluated according to total length, total weight and body weight. After this muscle tissues were separated from 58 individuals and dried at 50^0 C until constant weight. Dried samples were ground, homogenized and prepared for chemical analysis. Samples for INAA technique were dried. Also kidney and liver tissues were separated from these individuals. They were analyzed "*in natura*" for total Hg, because these organs are too small. These analyses were performed at the Neutron Activation Analysis Laboratory – LAN/IPEN.

2.4. Total Hg by CV AAS and Se determination by INAA

Total mercury determination was performed by Cold Vapor Atomic Absorption Spectrometry (CV AAS) using FIMS (Flow Injection Mercury System) from Perkin Elmer. About 200 to 500 mg of fish muscle, liver and kidney were separately digested with a mixture of concentrated HNO_3 and H_2SO_4 in Teflon vials. The vials were closed and left overnight at room temperature. The following day, the vials were put into an aluminum block at 90 ^{0}C and left there for 3 hours. The samples were allowed to cool at room temperature and the final volume was completed to 50 mL with Milli-Q water. The analytical procedure used (wet digestion) was that of HORVAT (1996) with some modifications [25].

Selenium determination was done using Instrumental Neutron Activation Analysis (INAA). For analysis, approximately 100 mg of fish liver and about 150 mg of reference materials were accurately weighed and sealed in pre-cleaned double polyethylene bags, for irradiation. Fish liver samples, reference materials and Se synthetic standard were irradiated for 8 hours, under a thermal neutron flux of 10^{12} cm^{-2} s^{-1} in the IEA-R1 nuclear research reactor at IPEN. Details for the INAA experimental procedure were already described by Favaro *et al.* (2000) [26], with some modifications.

Both analytical methods were validated for precision and accuracy by means of reference material analyses with certified values for the elements determined: Dogfish liver (DORM-1, NRCC) and Dogfish liver (DOLT-1, NRCC).

3. RESULTS AND DISCUSSION

Table 1 shows the results obtained for the reference material analyzes by INAA and Hg by CV AAS. Relative standard deviation ranged from 3.7 to 7.7% and relative error from 0.9 to 9.3%, showing good precision and accuracy for both analytical methodologies.

Table 1. Results (mg kg^{-1}) for the reference material analysis: total Hg by CV AAS and Se by INAA

Reference Materials	Total Hg (mean ± s.d.)				Se (mean ± s.d.)			
	Certified Value	Found Value	RSD (%)	RE (%)	Certified Value	Found Value	RSD (%)	RE (%)
DOLT-1	0.225 ± 0.037	0.246 ± 0.019	7.7	9.3	7.340 ± 0.420	7.460 ± 0.284	3.7	1.6
DORM-1	0.798 ± 0.074	0.754 ± 0.035	4.6	5.5	1.620 ± 0.120	1.605 ± 0.091	7.4	0.9

RSD = relative standard deviation; RE = relative error

3.1. Total Mercury Bioacumulation and Selenium Determination

Due to the differences in the ways of metabolizing xenobiotic components, there is a need to detect and to evaluate the impact of pollutants in the internal organs of exposed organisms and not only in the environment have they lived in. This study evaluated the bioaccumulation for planktivorous, detritivorous and carnivorous species that play an important role in the trofic balance estuarine ecosystem [27, 28].

Table 2 presents feeding habits, biometric measurements, total Hg in muscles, liver and kidneys and total Se in liver in the fish species. Seawater fish from unpolluted areas usually show Hg levels lower than 0.15 mg kg^{-1}. In contaminated areas, levels can reach up to 2 mg kg^{-1} or more and in benthic feeder species may go as high as 10 or 20 mg kg^{-1}, values already considered lethal for fish [29]. This study found higher levels of total Hg (dry weight) in the liver (21 to 1804 µg kg^{-1}) and lower levels in the muscle (13 to 1512 µg kg^{-1}), as expected. The results obtained for Hg in the kidneys (17 to 9912 µg kg^{-1}) and liver Se (2.10 to 43.00 mg kg^{-1}) values were also high.

From the data presented in Table 2 it can be seen that Corvina (178 – 1512 µg kg^{-1}) (dry weight) presented higher Hg levels in muscle than the other fish species studied: Perna-de-Moça (206 - 878 µg kg^{-1}), Pescada (51 – 257 µg kg^{-1}), Sardinha (26 - 135 µg kg^{-1}) and Tainha (13 –90 µg kg^{-1}).

Corvina and Perna de Moça fish species presented higher total Hg levels in liver and kidneys when compared to Pescada, Tainha and Sardinha species. The lower levels of total Hg in muscle than liver or kidneys can possibly reflect an exposure process, which can be the case of fish species offered in the street market of Cubatão.

Table 2. Food habits, biometric measurements, total Hg and total Se concentrations (µg kg^{-1}) in different tissues in fish species (median and range)

Fish species (N)[a]	Food Habits	TL. (mm)	Hg Total (µg kg^{-1}) Muscle (wet weight)	Hg Total (µg kg^{-1}) (dry weight)			Se Liver (dry weight) (mg kg^{-1})
				Muscle	Liver	kidneys	Liver
Corvina (11)	Detritivorous	426 360 - 453	181 41 - 348	789 178 - 1512	799 462 - 1804	2828 314 - 9912	20.00 19.00 – 43.00
Perna de Moça (12)	Carnivorous	324 301 - 385	105 43 - 184	499 206 - 878	266 21 - 619	641 358 - 1600	3.50 2.10 – 6.20
Pescada (16)	Carnivorous	355 200 - 368	46 12 - 62	193 51 - 257	187 111 - 609	193 51 - 257	4.20 3.00 – 6.10
Sardinha (16)	Planktivorous	223 210 - 235	27 8 – 40	91 26 - 135	164 102 - 503	774 120 - 1621	7.55 5.70 – 10.00
Tainha (5)	Planktivorous	547 520 – 555)	8 3 - 23	29 13 - 90	85 35 - 146	250 47 - 417	10.90 8.80 – 17.00

[a] – number of samples analyzed.

On the other hand Tainha presented lower total Hg levels in muscle when compared to liver and kidneys levels. This can be attributed to either more efficient detoxification or elimination process.

Higher total Hg in kidneys could indicate that fish species acquired in Cubatão were exposed to higher Hg levels and/or external factors that can affect kidney function.

When total Hg levels were compared to limits set by Brazilian legislation we could observe that no species surpassed the limits, 0.5 mg kg^{-1} for carnivorous species and 1.0 mg kg^{-1}, for non-predatory species (wet weight) [30].

Se concentrations in liver were significantly differents between fish species studied and Corvina presented the highest Se levels in liver (19 – 43 mg kg^{-1}), followed by Tainha (8.80 – 17.00 mg kg^{-1}), Sardinha (5.70 – 10.00 mg kg^{-1}), Pescada (3.00 – 6.10 mg kg^{-1}) and Perna-de-Moça (2.10 – 6.20 mg kg^{-1}), respectively.

As was expected, Se concentrations in liver were higher than Hg. Se intake can protect the fish organism from exposition to Hg and its harmful effects in predatory fish, but on the other hand, high Se levels can be toxic to the animals.

Previous studies verified the influence of feeding habits and tropic position in the Se accumulation (Seixas e col., 2007). Watanabe *et al* (1997) monitoring Se and Hg levels in fish, refers to Se intake values in fish diets ranging from 0.15 to 0.5 mg kg^{-1}(dry weight) as necessary to adequate metabolic activity in cells and tissues.
In the present study the carnivorous species Corvina presented higher Se contents, suggesting that the higher Hg levels seem to have a considerable impact on Se status. Azevedo (2003)

also describes that selenium alters the toxic-kinetic of Hg, modifying its distribution among the organs (an increase of its amounts in the liver, spleen, pancreas, etc. and a decrease in the kidneys and muscles), its elimination rates and its biological half-life in the organism [22].

Figure 2 presents the triangular diagram for the five fish species studied from Cubatão city regards to total Hg distribution in different fish organs. In diagram A, ***Sardella brasiliensis*** (Sardinha), showed a high displacement of total Hg to the kidney suggesting an attempt of Hg elimination by this organ. In the present study, this fish species presented low total Hg in muscles (<10 to 40 $\mu g\ kg^{-1}$).

Diagram B presents results for ***Macrodon ancylodon*** (Pescada). As can be seen total Hg had a tendency to accumulate in muscle and liver.

Diagram C presents results for ***Mugil platanus*** (Tainha). It can be observed that most of the total Hg accumulated in the kidneys.

In diagram D for ***Menticirrhus americanus (***Perna de Moça) there was a tendency to accumulate total Hg in the muscle and kidney. This organ is much more sensitive to deleterious effects of Hg.

In diagram E results for ***Micropogonias furnieri*** (Corvina) are shown. It can be observed that this fish species present a tendency of accumulating total Hg in liver and kidney. This species also presented the highest total Hg levels in muscle.

In general, it can be concluded that fish species acquired in the street markets in Cubatão presented a tendency of Hg kidney elimination. As already mentioned, higher total Hg in kidneys could indicate that fish species acquired in Cubatão were exposed to higher Hg levels and/or external factors that can affect kidney function.

Finally we could verify that none of the fish species studied in the present work surpassed the total Hg limits recommended by Brazilian legislation for non-predatory species (0.5 $mg\ kg^{-1}$) and predatory species (1.0 $mg\ kg^{-1}$) [30].

3.2. Statistical analysis

Since the results obtained for Hg and Se in fish tissues did not present a normal distribution verified by Shapiro-Wilks test ($p<0.05$), the Krushal-Wallis non parametric test was applied to the results. Due to the high variability of Hg and Se concentration the median of these two elements differ significantly ($p<0.05$).The concentrations of total Hg and Se in the liver presented a significant correlation ($R=0.7264$ and $p<0.001$).

As expected, Se concentration in liver was higher than that of Hg. High Se intake can reduce Hg toxicity in predatory fish but on the other land, it can also be toxic to the organism.

Se concentrations in the liver of the studied fish species were quite different being Corvina the fish species which presented the highest concentration.

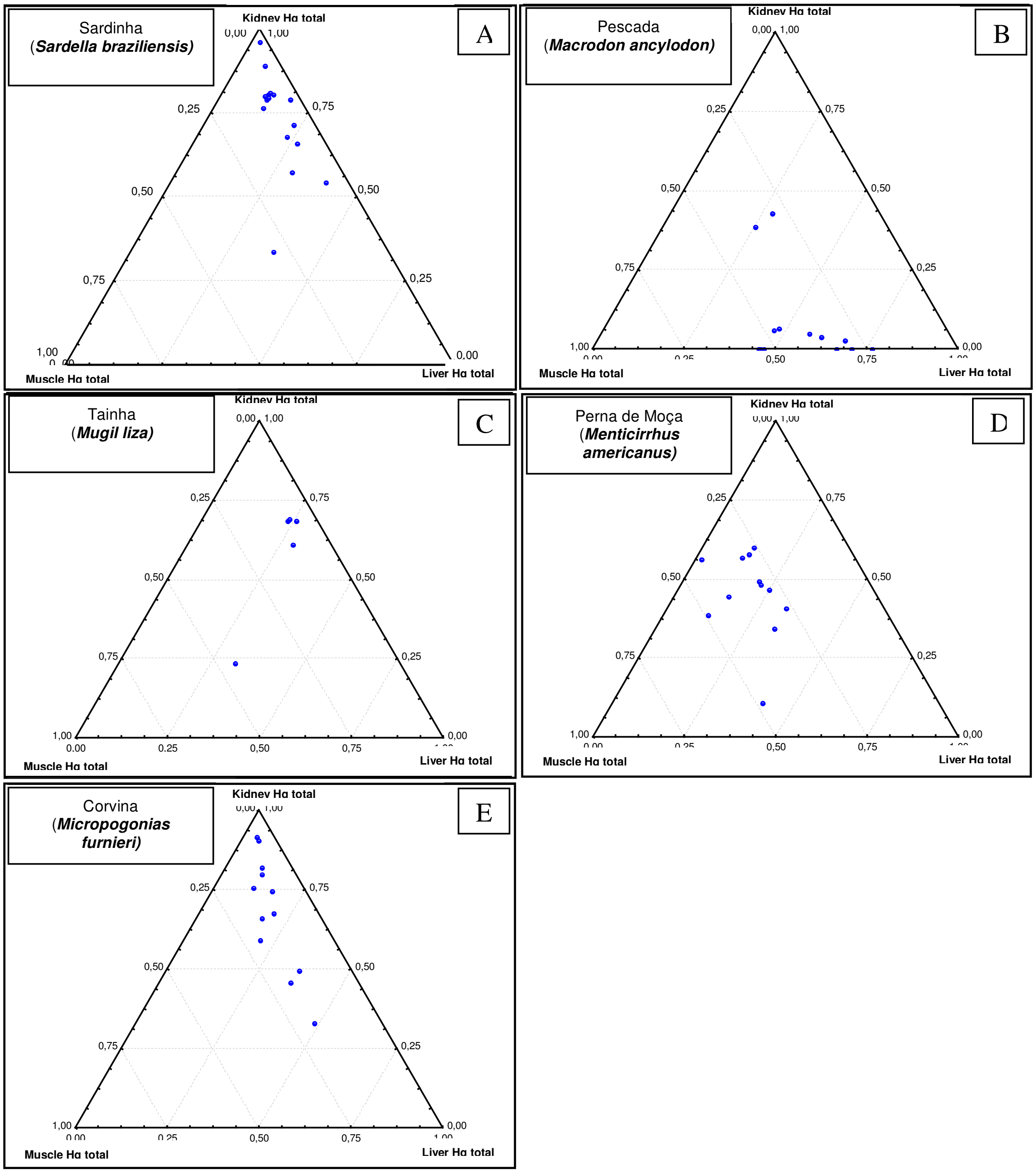

Figure 2. Triangular diagram for total Hg concentration in different fish tissues (muscle, liver, kidney)

3.3. Hg and Se relationship

In the last decades, the possibility of selenium (Se) to act as an inhibitor of toxicity caused by mercurial contamination has been considered. It is known that the levels of Hg and Se in the aquatic biota vary among the species of a single location and for the same species in different locations. Several factors influence this relationship, such as, the variation among species in

metabolism, feeding habits, the capacity of migration, etc [3, 31]. Koemam et al (1975), apud Azevedo (2003), evaluated Hg and Se levels in the liver of sea lions and dolphins and the results varied from 0.37 to 326 mg kg^{-1} for both elements [22]. A perfect relationship of 1:1 was observed among Se and Hg contents by the cited author. Lima *et al.* (2005), studying the correlation between Hg and Se concentrations in fish from Cachoeira do Piriá Municipality, Pará State, Brazil, reported a Hg range from 1.15 to 13.44 nmol g^{-1} and Se from 2.44 to 14.56 nmol g^{-1}, for carnivorous species [32]. It is important to note that Vasconcellos *et al*. (2000) and Campos *et al.* (2002) reported similar significant correlations between these elements in hair samples from different sites of the Amazon region. According to these authors, this correlation may originate from the high fish consumption, since it constitutes the most important food source of Hg and Se in the diet of the Amazon population [33], [34].
Although the interaction between Hg and Se has been studied, the interaction mechanism is still not entirely clear. However, the importance of this interaction can be seen by the many studies published on the matter [35, 36].

As previously mentioned, the correlation between these metals can suffer the interference of several factors. Thus, the high contents of Hg in the liver and kidneys found in this study and the lack of official reports of mercury poisoning in the literature and its interaction with Se, suggest that Se can be acting as a detoxification agent in the fish species analyzed. However, a more comprehensive study concerning the interaction of specific Hg and Se chemical forms along with hydrodynamic process should be undertaken.

3. CONCLUSIONS

This study verified that predatory species do in fact present higher Hg levels in muscle tissue as also described in other studies. On the whole, the Hg levels in the kidneys of the fish acquired in Cubatão street markets used in this study presented higher levels than those found in liver and muscle tissues.

This study also showed that the fish species analyzed presented higher Se contents in livers. These high hepatic Hg and Se concentrations are probably related to the role played by the liver in terms of pollutant bio-transformation and this can be indicative that high Hg levels seem to have a considerable impact on Se status.

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