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Contaminant metals in black scabbard fish (*Aphanopus carbo*) caught off Madeira and the Azores

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Abstract

Total mercury, cadmium and lead concentrations were measured in black scabbard fish (*Aphanopus carbo*), caught off Madeira and the Azores archipelagos (Portugal). The metal contents were expressed in mg kg^{-1} wet weight. The mean mercury levels in liver were 4.50 and 2.37 whereas, in skin, such values were 0.59 and 0.36 (Madeira and Azores, respectively). On average, the muscle content was around 0.9 in fish from both areas. Cadmium maximum levels in muscle and skin were found in Azores samples (0.09 and 0.11, respectively). Mean lead contents in all samples were always below than 0.10. In general, the results obtained did not allow the establishment of any relationship between metal accumulation and fish weight or length. According to the permissible WHO and FAO levels, these results, suggest that this species does not represent a risk for human consumption if the liver is excluded and the edible part consumed with moderation.

Keywords: *Aphanopus carbo*; Black scabbard fish; Bioaccumulation; Mercury; Lead; Cadmium; Madeira and Azores (Portugal)

1. Introduction

Marine pollution by organic and inorganic chemicals has been identified as one of the most important factors in the poisoning of marine organisms, including fish (Al-Ghais, 1995). The accumulation of such compounds in fish tissues depends on several endogenous factors, namely physiological condition, geographic habitat, fat content, adaptation capacity and the biotope characteristics (Driscoll et al., 1995; Reicherbach-Klinke, 1980).

Non-essential metals, such as mercury, cadmium and lead, are not known to play any metabolic function and can be toxic for humans, even at very low concentrations (Belitz & Grosch, 1999). They may occur naturally in the environment or be released by human activities (Chen, Shih, Chou, & Chou, 2002; Garcia-Montelongo, Díaz, Galindo, Larrechi, & Rius, 1994; Renzoni, Zino, & Franchi, 1998). Although toxic metal contents in the oceans are very low, higher contents are found in some areas, such as waters polluted by chemical and geochemical processes. Natural releases of mercury, and probably other trace metals, into coastal waters are possible in the Atlantic islands of Madeira and the Azores due to their volcanic nature (Depledge, Weeks, Frias Martins, Tristao da Cunha, & Costa, 1992; Renzoni et al., 1998). Biochemical phenomena regulate mercury transmission into trophic levels, leading to bioaccumulation in aquatic organisms. Such bioaccumulation occurs when the rate of uptake of a pollutant exceeds its rate of elimination (Khan & Weis, 1993; Soto & Marigómez, 1995). This mercury concentration along the food chain represents a toxic hazard, especially at the end of chain, for human populations, who consume fish (Richard, Arnoux, Cerdan, Reynouard, & Horeau, 2000).

Mercury, cadmium and lead are toxic metals that can be assimilated, stored and concentrated by organisms, through the food chain, resulting in physiological damage (Pigott & Tucker, 1990; Ruiter, 1995). Regarding these toxic metal ions, most of the chemical reactions that explain their toxicity at cellular level, involve electronic

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transference, formation of free radicals and their influence on the DNA chain, with possible consequences in mutagenicity, genotoxicity and carcinogenicity phenomena (Halliwell & Gutteridge, 1996), as well as competition reactions with essential elements.

The distribution of metals in certain fish tissues is extremely important. The removal of those tissues can reduce their levels in a fish dish (Allen, 1994), decreasing consumer risk.

Actually, there are European limits for mercury, cadmium and lead, defined by European Community Regulation 78/2005 (EU, 2005).

Black scabbard fish (*Aphanopus carbo*) is an oceanic benthopelagic species living at depths of 200–1600 m (Muus, Nielsen, Dahlstrom, & Nyström, 1998). This fish is an important species for Madeira and the Azores Islands, especially for Madeira in terms of its economic value and local consumption. Like other top predators it can accumulate some toxic metals due to the aquatic environment and through its diet (Andersen & Depledge, 1997; Mormede & Davies, 2001). The purpose of this work was to characterise the levels of mercury, cadmium and lead in some tissues of black scabbard fish caught off Madeira and the Azores Islands and, to establish suitable relationships between the animal length and weight and accumulation of these metals.

2. Materials and methods

2.1. Raw material and sample preparation

Black scabbard fish (*Aphanopus carbo*) used in these experiments were caught off Madeira and the Azores archipelagos located in the Atlantic-Northeast (Fig. 1) by commercial longline fishery over one year and were supplied by a fish processing factory located in Funchal (Madeira Island).

Fifty fish from Madeira and twenty from Azores waters were used for contaminant metal analysis. Total length in cm and total weight in kg were measured. Skin, muscle and liver samples were removed from all fish. Samples were immediately frozen and transported by aeroplane from the Island to the laboratory (in mainland) and they were kept at −30 °C prior to further analysis.

2.2. Sample analysis

Total mercury (Hg) was determined in duplicate by cold vapour atomic absorption spectrometry (CVAAS) using a Bacharach Coleman MAS-50D Mercury Analyser System. Of each specimen, 1 g of homogenized tissue (muscle, skin or liver) was digested with concentrated sulphuric acid. Then the mercury in the sample (Hg^0 and Hg_2^{2+}) was oxidized, with potassium permanganate (5% w/v), to Hg^{2+}. After reducing the Hg^{2+} to Hg^0 with 20% (w/v) stannous chloride, the volatile Hg^0 was bubbled into the closed system of the MAS 50D analyser ($\lambda = 253.7$ nm). The method used was developed by Hatch and Ott (1968) and described in detail by Joiris, Holsbeek, Bouquegneau, and Bossicart (1991). The accuracy was tested using the Certified Reference Material CRM-463 (tuna fish muscle: 2.85 ± 0.16 mg kg^{-1} Hg dry weight) of the Community Bureau of Reference. The CRM-463, analysed in duplicate ($n = 5$), was in the range of the certified material (2.87 ± 0.09 mg kg^{-1} Hg dry weight). The analytical

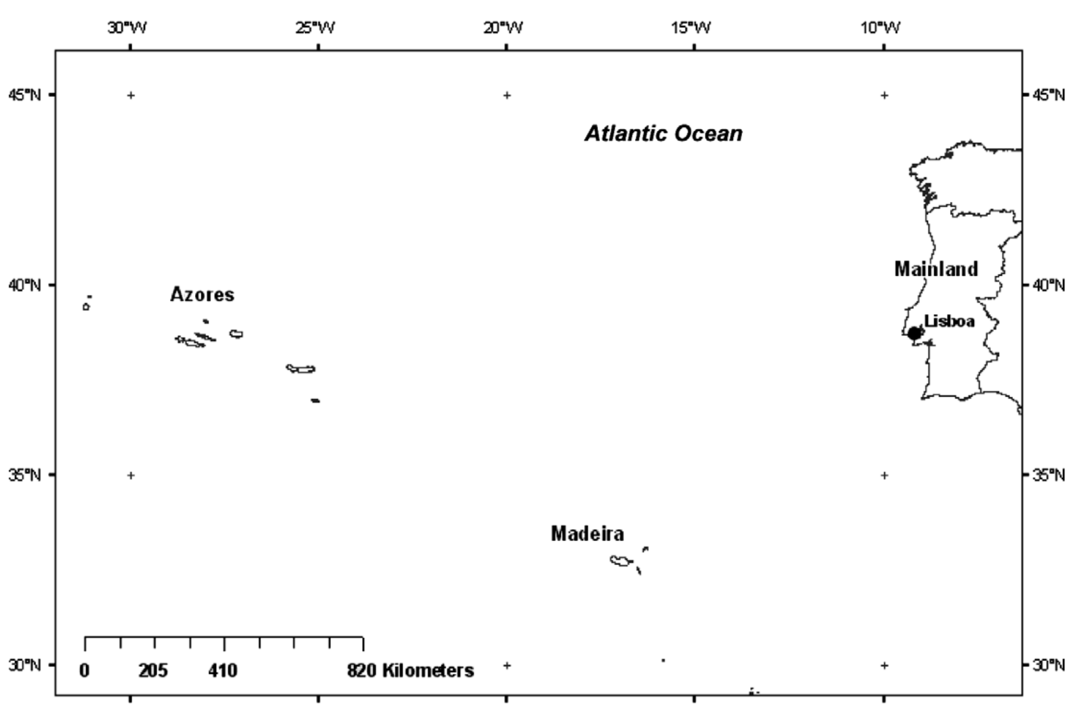

Fig. 1. Location of Madeira and Azores area (*source:* ESRIdata).

precision, evaluated in 15 homogeneous replicates of the same sample, was 11.4% as a coefficients of variation.

Cadmium (Cd) and lead (Pb) analyses were based on the methods described by Jorhem (2000). Muscle and skin samples (10 g wet weight) were dried and then washed at 500 °C with gradual increase in temperature. Nitric acid (65% w/w) was added, and the solution obtained was evaporated to dryness. The final residue was dissolved in 15% (v/v) nitric acid. Cd and Pb contents were determined by an atomic absorption method by flame atomisation in the Varian apparatus Spectr AA-20 with a background deuterium correction ($\lambda = 228.8$ and 217.0 nm, respectively). The accuracy was tested by Certified Reference Material BCR 422 (cod muscle: 0.017 ± 0.002 mg kg^{-1} Cd dry weight and 0.085 ± 0.015 mg kg^{-1} Pb dry weight) analysed in duplicate ($n = 2$). It was in the range of the certified material (0.019 ± 0.0006 mg kg^{-1} Cd dry weight and 0.081 ± 0.002 mg kg^{-1} Pb dry weight) and checked by participating in an intercalibration exercise (z-score: −1.1 for Pb and −0.3 for Cd) conducted by the Central Science Laboratory (Metallic Contaminants – FAPAS Series 7 Round 40). The analytical precisions, evaluated in 12 homogeneous replicates of the same sample, were 5.0% for Cd and 13.0% for Pb, as coefficient of variation. The detection limits were calculated through linear regression, according to ISO 5725-1 (1994) and ISO 5725-2 (1994). Obtained values were 0.01 mg kg^{-1}. Analytical data for Hg, Cd and Pb are reported as mg kg^{-1} of metal on a wet weight basis.

2.3. Statistical analysis

To test the normality and the homogeneity of variance of data, the Kolmogrov–Smirnov's test and Levene's F-test, respectively, were used. Data, which corroborate these assumptions, were analysed by the Student's t distribution to determine the difference between the metal levels in the same tissue sampled from Madeira and the Azores archipelago. Data, which do not assume the normality or homogeneity of variance, were log-transformed. When the conditions of the Student's t distribution were not verified, data were tested nonparametrically with Mann–Whitney's U-test. The Pearson coefficient was calculated to determine the correlation between the values from the same area of sampling. Statistical significance was considered when $p < 0.05$. All data analysis was performed using STATISTICA (Statsof, Inc. USA, 2000).

3. Results and discussion

The length and weight of black scabbard fish ranged from 98.1 to 125.6 cm and 1.4–3.0 kg in Madeira, and the corresponding values in fish caught off the Azores were, respectively, 97.5–120.6 cm and 1.5–3.0 kg (Table 1). All fish sampled were adults and their size was within the commercial range (Martins, Martins, & Cardador, 1989; Morales-Nin & Sena-Carvalho, 1996). Length and weight were not significantly different in fish from the two areas (Mann–Whitney's U-test, $p > 0.05$).

Table 1
Length and weight of black scabbard fish caught off Madeira and the Azores archipelagos

	Madeira archipelago	Azores archipelago
Length (cm)		
Mean ± SD	110.9 ± 5.9^a	108.9 ± 6.3^a
Median	110.8	109.3
Range	98.1–125.6	97.5–120.6
Weight (kg)		
Mean ± SD	2.1 ± 0.4^a	2.1 ± 0.4^a
Median	2.2	2.1
Range	1.4–3.0	1.5–3.0
Number of fish	50	20

Different letters in the same line denote significant differences ($p < 0.05$).

Metals results obtained for each archipelago and different tissues are summarised in Table 2. The concentrations of mercury in muscle, for the two areas, were similar, with a mean near 0.9 mg kg^{-1} wet weight (maximum was around 1.4 mg kg^{-1} wet weight). Renzoni et al. (1998) reported similar values for the same species caught off Madeira; however, in that study, the maximum concentration determined was near 1.8 mg kg^{-1} wet weight. Forty-six percent of samples caught off Madeira and 35% from Azores exhibited mercury levels in muscle equal to or above the limit proposed by the EU (2005), 1 mg kg^{-1}. Total mercury mean level in liver was higher ($p < 0.05$) in fish caught off Madeira, 4.50 ± 2.47 mg kg^{-1} wet weight, the maximum content being 11.3 mg kg^{-1} wet weight. For Azores fish, these values were 2.37 ± 1.70 and 7.63 mg kg^{-1} wet weight, respectively. These levels indicate that mercury accumulation is greater in liver than in muscle in fish from both areas. However, the opposite was also verified by Polak-Juszczak (1997) in cod species. Black scabbard fish is a deep-water species with a long life, living up to 8 years (Morales-Nin & Sena-Carvalho, 1996) and, besides, is considered a large carnivorous predator (Muus et al., 1998). Consequently, such a species is a bioaccumulator and biomagnifier, thus justifying the results found in this study. About 98% (Madeira) and 85% (Azores) of fish livers analysed showed mercury levels equal to or above 1 mg kg^{-1}. The high level of mercury in liver, when compared with the muscle (liver:muscle ratio > 1), may be due to uncontaminated black scabbard fish that recently migrated into a mercury-polluted area (Riisgård & Hansen, 1990). In fact, the Azores has a volcanic nature, like Madeira (Renzoni et al., 1998), which probably influences the natural release of mercury and other trace metals into biota (Depledge et al., 1992).

On average, the content of mercury in skin was lower than that in the other analysed tissues; however, the maximum values reached were 1.4 mg kg^{-1} wet weight (Madeira) and 1.1 mg kg^{-1} wet weight (Azores). For Madeira fish, only 10% of the values obtained were equal to or above to the EU limit. In the Azores this percentage was around 5%.

Table 2
Total mercury, cadmium and lead (mg kg^{-1} wet weight) in tissues of black scabbard fish caught off Madeira and the Azores archipelagos

	Madeira archipelago	Azores archipelago
Mercury		
Muscle		
Mean ± SD[a]	0.90 ± 0.27[a]	0.89 ± 0.26[a]
Median	0.92	0.84
Range	0.19–1.44	0.45–1.43
Number of fish	50	20
Liver		
Mean ± SD	4.50 ± 2.47[a]	2.37 ± 1.70[b]
Median	4.26	2.02
Range	0.30–11.29	0.28–7.63
Number of fish	47	20
Skin		
Mean ± SD	0.59 ± 0.30[a]	0.36 ± 0.27[b]
Median	0.58	0.31
Range	0.07–1.44	0.04–1.07
Number of fish	50	20
Cadmium		
Muscle		
Mean ± SD	0.01 ± 0.01[a]	0.03 ± 0.03[b]
Median	0.01	0.02
Range	ND[2]–0.05	0.01–0.09
Number of fish	29	8
Skin		
Mean ± SD	0.04 ± 0.01[a]	0.08 ± 0.02[b]
Median	0.04	0.07
Range	0.02–0.08	0.05–0.11
Number of fish	29	8
Lead		
Muscle		
Mean ± SD	0.02 ± 0.03[a]	0.04 ± 0.02[b]
Median	ND[b]	0.03
Range	ND[b]–0.10	ND[b]–0.07
Number of fish	29	8
Skin		
Mean ± SD	0.01 ± 0.03[a]	0.06 ± 0.04[b]
Median	ND[b]	0.07
Range	ND[b]–0.10	ND[b]–0.10
Number of fish	29	8

Different letters in the same line denote significant differences ($p < 0.05$).
[a] Mean ± standard deviation.
[b] Below detection limit.

In contrast to that observed for liver and skin, mercury concentrations in the fish muscle of both studied areas were not statistically different (Student's t test, $p > 0.05$).

For cadmium, the levels found were higher in the fish caught off the Azores. Thus, the mean values registered in the muscle were 0.01 ± 0.01 mg kg^{-1} wet weight for Madeira and 0.03 ± 0.03 mg kg^{-1} wet weight for Azores fish. Such values are above those reported by Mormede and Davies (2001) for the same species (maximum level was 0.017 mg kg^{-1} wet weight). On the other hand, this contaminant's levels were higher in skin than in muscle. This was also verified by Vas (1991) in some shark species. On the other hand, Handy (1996) considered that cadmium accumulated in fish internal organs and in muscle was minimal.

Around 3% of Madeira and 13% of Azores samples, revealed cadmium values in muscle equal to or higher than the proposed EU limit (2005) (0.05 mg kg^{-1} wet weight). Such percentages in skin were 24% and 100%, respectively.

The maximum lead concentration in all tissues was 0.10 mg kg^{-1} wet weight for fish from both studied areas. Mean values were lower than 0.05 mg kg^{-1} wet weight, except skin of the fish caught off the Azores (0.06 ± 0.04 mg kg^{-1} wet weight). Like cadmium, the values of lead found in the present study were higher than those reported by Mormede and Davies (2001).

All samples analysed showed lead levels below the EU limit (0.2 mg kg^{-1} wet weight).

These two contaminants, in Madeira and the Azores, showed significant differences either in muscle or skin (Mann–Whitney's U-test, $p < 0.05$).

Pearson correlation coefficients were calculated for specimens from Madeira and the Azores archipelagos for each tissue, length and weight (Table 3). Length and weight were highly positively correlated ($p < 0.0001$) in both areas. The obtained values were higher than those reported by Martins et al. (1989) for the same species caught off the mainland (Sesimbra), but lower than those reported by Morales-Nin and Sena-Carvalho (1996) in fish from Madeira.

The rate of physiological processes that influence uptake, distribution and elimination of chemical pollutants is recognized to be influenced by the animal size. This observation is particularly true for mercury because the levels of this element usually increase in fish with body size, so larger and/or older fish generally have higher concentrations than smaller or younger fish (Storelli, Giacominelli-Stuffler, & Marcotrigiano, 2002). However, in general, no significant correlation was obtained between the level of total mercury in the tissues and fish length or weight. Nevertheless, there is a significant correlation between the level of mercury in liver and length of the fish caught off the Azores ($p < 0.05$). As in the present study, Renzoni et al. (1998) verified that there was no clear correlation between the values of mercury and the weight of black scabbard fish from Madeira. All mercury levels in the different tissues were positively correlated, either for Madeira or the Azores (at least $p < 0.05$).

Relatively to cadmium and lead levels, only one high correlation between cadmium in the muscle and in the skin ($p < 0.05$) in fish from Madeira was found. No other correlation was evident, probably due to the number of fish sampled ($n = 29$ for Madeira; $n = 8$ for the Azores). In muscle of black scabbard fish from the Rockall Trough area, cadmium contents were very highly negatively correlated with length (Mormede & Davies, 2001).

The mentioned results allow some preliminary conclusions. The obtained levels of contaminant metals were similar to those reported for the same species by other authors, except for cadmium, which is sometimes, present in higher concentration. There is a selective accumulation of mercury

Table 3
Pearson correlation coefficients for relationships between weight, length, and levels of total mercury, cadmium and lead in tissues of black scabbard fish caught off Madeira and the Azores archipelagos

	Weight	Length	Hg muscle	Hg skin	Cd muscle	Pb muscle
Madeira						
Length	0.870***	–	–	–	–	–
Hg – muscle	−0.130	−0.110	–	–	–	–
Hg – skin	−0.203	−0.182	0.600***	–	–	–
Hg – liver	0.039	0.085	0.563***	0.296*	–	–
Cd – muscle	0.153	0.139	–	–	–	–
Cd – skin	0.165	0.086	–	–	0.494*	–
Pb – muscle	0.161	0.243	–	–	–	–
Pb – skin	−0.095	−0.027	–	–	–	0.124
Azores						
Length	0.761***	–	–	–	–	–
Hg – muscle	0.382	0.342	–	–	–	–
Hg – skin	0.042	0.328	0.707**	–	–	–
Hg – liver	0.438	0.489*	0.704**	0.681**	–	–
Cd – muscle	−0.207	−0.179	–	–	–	–
Cd – skin	−0.325	−0.675	–	–	0.263	–
Pb – muscle	−0.000	−0.017	–	–	–	–
Pb – skin	−0.113	−0.069	–	–	–	0.025

Positive correlation; −, negative correlation.
Different letters in the same line denote significant differences ($p < 0.05$).
* $p < 0.05$.
** $p < 0.001$.
*** $p < 0.0001$.

in liver and of cadmium in skin. On the other hand, the mercury muscle levels found in fish from both areas are similar.

The results obtained are important because black scabbard fish is well appreciated in Portugal, particularly in the Madeira archipelago. The World Health Organization (WHO) established a provisional tolerable weekly intake (PTWI) of 5 μg kg^{-1} body weight (bw)/week for total mercury (WHO, 1972), of which no more than two thirds (3.3 μg kg^{-1} bw/week) should be from methylmercury (WHO, 1989, 2000). In Madeira, especially in the fishing village of Câmara de Lobos, there is a large daily consumption of fish products, mainly black scabbard fish (Gaggi, Zino, Duccini, & Renzoni, 1996; Renzoni et al., 1998). Being this in mind and the WHO recommendation, it is easy to understand that this population is exposed to high levels of mercury. This has also been noted by other authors (Gaggi et al., 1996; Renzoni et al., 1998; Murata et al., 1999). This situation provokes some concerns, especially because the liver is extensively consumed, locally, as a delicatessen.

The Food and Drug Administration (FDA) also has the responsibility to safeguard human health through regulation of food and drugs. This agency suggests maximum tolerable daily intakes for cadmium (55 μg/person/day) (FDA, 1993a) and lead (75 μg/person/day) (FDA, 1993b). Despite cadmium values in the skin of Azores fish being above 0.05 mg kg^{-1} wet weight, the FDA maximum tolerable daily intake will hardly be reached. For these two metals, considering all the results observed in this work, it can be concluded that the black scabbard fish do not represent any risk for human consumption.

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