Universidade de Aveiro
2021

Hugo Miguel Coelho da Silva Vieira

Distribuição, bioacumulação e avaliação de risco do mercúrio em diferentes áreas climáticas no Oceano Atlântico Norte

Mercury distribution, bioaccumulation and risk assessment in different climate areas in the North Atlantic Ocean

Universidade de Aveiro
2021

Hugo Miguel Coelho da Silva Vieira

Distribuição, bioacumulação e avaliação de risco do mercúrio em diferentes áreas climáticas no Oceano Atlântico Norte

Mercury distribution, bioaccumulation and risk assessment in different climate areas in the North Atlantic Ocean

Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia e Ecologia das Alteração Globais, realizada sob a orientação científica do Prof. Doutor Fernando Manuel Raposo Morgado, Professor Associado com Agregação do Departamento de Biologia da Universidade de Aveiro e co-orientação do Doutor Sizenando Nogueira de Abreu do Departamento de Biologia e Centro de Estudos do Ambiente e do Mar (CESAM), Universidade de Aveiro e do Doutor Jaime Rendón-von Osten, Professor do Instituto de Ecología, Pesquerías y Oceanografía del Golfo de México (EPOMEX), da Universidade Autónoma de Campeche.

Apoio financeiro da FCT e do FSE no âmbito do III Quadro Comunitário de Apoio através de uma Bolsa de Doutoramento atribuída a Hugo Miguel Coelho da Silva Vieira (PD/BD/127808/2016)

o júri

presidente

Doutor José Carlos Esteves Duarte Pedro
Professor Catedrático, Universidade de Aveiro

Doutor Amadeu Mortágua Velho da Maia Soares
Professor Catedrático, Universidade de Aveiro

Doutor Fernando Manuel Raposo Morgado (Orientador)
Professor Associado Com Agregação, Universidade de Aveiro

Doutor Nuno Gonçalo de Carvalho Ferreira
Investigador Associado, Cardiff University

Doutor Henrique Miguel Veiga Simão de Azevedo Pereira
Investigador, Universidade de Coimbra

Doutor Tiago Fernandes Grilo
Investigador, Universidade de Lisboa

agradecimentos

A realização deste trabalho de investigação nunca poderia ter sido concretizada sem a ajuda de todos aqueles que de certa forma me apoiaram e incentivaram. Deste modo, gostaria de agradecer:

Aos meus orientadores, o Prof. Morgado, ao Siz e ao Jaime, por me darem a oportunidade de realizar este trabalho, assim como pelo apoio concedido ao longo do mesmo;

Ao Abel Ferreira, pela paciência e pelas várias maneiras que me ajudou a superar os constantes problemas do analisador de mercúrio;

Um agradecimento especial à Masha, pela ajuda prestada nos momentos chave do trabalho e sem dúvida essencial à realização do mesmo;

Ao Gonçalo e à “malta da ilha”, que apesar de longe sempre se mostraram disponíveis para ajudar;

À Malta do “Call center”, especialmente ao Monteiro, à Sónia, à Luísa, à Patrícia, à Diana e ao Diogo, pelos momentos de descontração e boa disposição;

Como não podia deixar de ser, agradecer novamente à Masha, à Luisa, ao Monteiro, à Sónia, assim como à Andreia pelos “cartões de 10” que naqueles dias mais duros funcionaram como “anti-depressivos”;

Por fim, mas nunca com menos importância, fica o agradecimento à família, especialmente aos meus pais e à minha avó, pois sem eles seria difícil ou mesmo impossível chegar até aqui, e à pequena “I” que tornou estes quatro anos ainda mais desafiantes.

Obrigado por tudo,

Hugo C. Vieira

palavras-chave

Bioacumulação de mercúrio; zonas costeiras; espécies com interesse comercial; consumo de peixe; exposição ao mercúrio.

resumo

Devido à da sua frequência, toxicidade e potenciais efeitos para a saúde humana e da vida selvagem, o mercúrio (Hg) está classificado como uma substância perigosa prioritária pela Convenção para a Proteção do Meio Ambiente Marinho do Atlântico Nordeste (Convenção OSPAR) e pela Diretiva-Quadro da Água da União Europeia (UE-WFD). Uma vez presente no meio ambiente, o Hg pode ser convertido em diferentes formas de Hg (por exemplo, metilmercúrio – MeHg). O MeHg é uma potente neurotoxina que devido à sua alta taxa de absorção pelo trato gastrointestinal e afinidade pelos grupos tiol das proteínas, tem a capacidade de acumular nos organismos. Nos humanos, a exposição ao Hg ocorre principalmente através do consumo de peixe. Apesar ser indispensável para uma dieta equilibrada e saudável, devido ao elevado teor em proteína e ácidos gordos, os peixes podem apresentar elevados níveis de Hg nos seus tecidos.

Assim, este estudo teve como questões centrais avaliar a distribuição, bioacumulação e exposição ao Hg em diferentes áreas geográficas localizadas no Atlântico Norte (costa noroeste Portuguesa, arquipélago dos Açores e costa sudeste Mexicana), tendo sido delineados os seguintes objetivos específicos de modo a avaliar: (**i**) a presença de Hg no sedimento em zonas costeiras das diferentes áreas geográficas, (**ii**) uma possível transferência do Hg presente no sedimento para a cadeia trófica marinha, (**iii**) o efeito da temperatura da água do mar dos diferentes locais na bioacumulação e toxicidade do Hg, (**iv**) a concentração de Hg presente em espécies marinhas de interesse comercial nas diferentes áreas costeiras (peixe e lula) e (**v**) a contribuição de cada espécie para a exposição humana ao Hg.

Os resultados revelaram que as concentrações de Hg presente nos sedimentos das diferentes áreas refletem a proximidade a possíveis focos de contaminação por Hg. A fracção fina é aquela que apresenta maiores níveis de Hg em comparação à fracção de sedimentos mais grosseiros (areia). Contudo, apesar de em alguns locais estes níveis de Hg ultrapassarem os valores estabelecidos pelas agências internacionais através dos critérios para a qualidade dos sedimentos, a fracção fina representa menos de 1% da fracção total do sedimento, o que faz com que a concentração de Hg presente na fracção total do sedimento se situe abaixo dos limites estabelecidos pelas diversas agências.

Relativamente à transferência do Hg para a cadeia trófica marinha, os resultados demostraram que os níveis de Hg presente nas espécies estudadas não parecem estar relacionados com a transferência do Hg presente nos sedimentos, mas sim com ingestão de Hg através da alimentação.

resumo (cont.)

Este estudo também conclui que mesmo numa exposição de curta duração a baixas concentrações de Hg, o aumento da temperatura da água pode promover a acumulação de Hg em peixes, sendo que é no fígado que essa acumulação é mais evidente. Este aumento da concentração de Hg nos tecidos, assim como o aumento da temperatura da água, podem induzir a produção de espécies reactivas de oxigénio (ROS) por parte do organismo. Contudo, as defesas antioxidantes do fígado parecem actuar de forma eficiente no combate a esses ROS, evitando danos nas células por stress oxidativo. Por outro lado, no músculo, o combate contra os ROS não foi tão eficiente resultando em danos ao nível das proteínas e diminuição do consumo de energia.

No que diz respeito à concentração de Hg nas diferentes espécies de peixe com valor comercial, observou-se que características como hábitos alimentares e estilo de vida influenciam a acumulação de Hg, sendo que as espécies de peixes carnívoras são aquelas que apresentam maiores concentrações de Hg, reforçando a ideia de que o Hg tem a capacidade de biomagnificar ao longo da cadeia trófica. Finalmente, considerando a acumulação de Hg das diferentes espécies com interesse comercial e o limite máximo de 0,5 µg g^{-1} de Hg permitido para consumo humano, apenas duas espécies de peixes (*Mora moro* e *Zeus faber*) apresentaram uma concentração de Hg acima deste limite. Apesar disso, o consumo de 14 das espécies de peixes estudadas podem levar a uma ingestão semanal de Hg maior do que 1,3 µg kg de peso corporal^{-1} semana^{-1} sugerido pelo JECFA (Comité Conjunto de Especialistas FAO/OMS sobre Aditivos Alimentares). Destas 14 espécies, 9 dizem respeito ao arquipélago dos Açores e 4 à costa noroeste de Portugal, áreas que segundo a FAO, tem um consumo de peixe per capita três vezes superior ao da costa sudeste do México. De facto, dados relativos ao arquipélago dos Açores demonstraram que as espécies que mais contribuem para a exposição humana ao Hg nesta área são as que apresentam baixo [Hg] mas que são capturadas em maiores quantidades.

Embora o Hg seja um contaminante amplamente estudado, os resultados obtidos no âmbito desta tese permitiram obter conhecimento sobre a presença de Hg em áreas costeiras do Atlântico Norte, que além de fornecerem múltiplos serviços de ecossistema são áreas até agora pouco estudadas. Ao mesmo tempo contribuem com mais informações sobre a exposição humana ao Hg por meio do consumo de peixes e derivados na análise de risco, uma vez que a concentração de Hg costuma receber maior importância independente do consumo per capita.

keywords

Mercury bioaccumulation; coastal zones; commercial fish species; fish consumption; mercury exposure.

abstract

Mercury (Hg) is classified as a priority hazardous substance by the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR Convention), the European Union Water Framework Directive (EU-WFD) due to the combination of its frequency, toxicity and potential effects on human health and wildlife. In the environment, Hg can be converted into other forms of Hg (e.g. methylmercury – MeHg). MeHg is a potent neurotoxin, which can accumulate in the organism's tissues due to its high rate of absorption by the gastrointestinal tract and its affinity for thiol groups of proteins. The human exposure to Hg occurs mainly through the consumption of fish. Despite being essential for a healthy balanced diet owing to a high content of protein and fatty acids, fish ingestion can result in the intake of large amounts of Hg since some species have high Hg levels in their tissues.

Thus, this study presents as central question the evaluation of the distribution, bioaccumulation and exposure to Hg in different geographic areas located in the North Atlantic (Portuguese northwest coast, Azores archipelago and Mexican southwest coast). In this sense, the following specific objectives were outlined: (**i**) to determine the presence of Hg in the sediment of coastal areas from different geographic areas; (**ii**) to evaluate a potential transfer of the Hg present in the sediment to the marine food chain; (**iii**) to assess the effect of different sea water temperature scenarios on the bioaccumulation and toxicity of the Hg; (**iv**) to evaluate the [Hg] present in marine species of commercial interest in the different coastal areas (fish and squid) and (**v**) to understand the contribution of each species to human exposure to Hg.

The results revealed that the Hg concentration in the sediments in the different areas reflect the proximity to possible sources of contamination by Hg. The fine fraction is the one with the higher levels of Hg when compared to the fraction of coarser sediments (sand). However, although in some places these Hg levels exceed the values established by international agencies through the criteria for the quality of sediments, the fine fraction represents less than 1% of the total sediment fraction. Therefore, the Hg concentration present in the total sediment fraction was found to be below the limits established by the different agencies.

Regarding the transfer of Hg from sediments to the marine trophic chain, the results showed that levels of Hg present in the studied species do not seem to be related to the transfer of Hg present in sediments, but rather to the ingestion of Hg through food.

abstract (cont.)

This study also concludes that even in a short-term exposure to low Hg concentrations, the increase of water temperature can promote the Hg accumulation in fish. The accumulation was is most evident in the liver. This increase in the Hg concentration in tissues, as well as the increase of water temperature, can induce the production of reactive oxygen species (ROS) by the organisms. However, the antioxidant defenses of the liver seem to act efficiently in combating these ROS, preventing cells damage by oxidative stress. On the other hand, in the muscle, the fight against ROS was not so efficient resulting in damage at the proteins' level and decreased energy consumption.

Regarding the Hg concentration in different fish species with commercial value, it was observed that characteristics such as feeding habits and lifestyle, have an influence on Hg accumulation. Carnivorous fish species are those that have higher concentrations of Hg, which reinforces the notion that Hg can biomagnify along the food chain. Finally, considering the Hg accumulation in the different species of commercial interest and the maximum limit of Hg allowed for human consumption (of 0.5 µg g^{-1}), only two fish species (*Mora moro* and *Zeus faber*) showed a Hg concentration above this limit. Despite this, consumption of 14 of the studied fish species may lead to a weekly intake of Hg greater than 1.3 µg kg body $weight^{-1}$ $week^{-1}$ suggested by JECFA (Joint FAO/WHO Expert Committee on Food Additives). From these 14 species, 9 concern the Azores archipelago and 4 the Northwest Portuguese coast, which are areas with a fish consumption per capita estimated by the FAO three times higher than the southeast coast of Mexico. In fact, data from the Azores archipelago demonstrated that the species that contributed the most to human exposure in this area were those that presented low Hg concentration but were captured in larger quantities.

Even though Hg is a widely studied contaminant, the results obtained in the framework of this thesis allowed us to obtain knowledge regarding the presence of Hg in the North Atlantic coastal areas that provide multiple ecosystem services and have until now been poorly studied. It also contributes to the state of the art regarding human exposure to Hg through the consumption of fish and seafood in risk analysis, since Hg concentration usually receives greater importance regardless of the fish consumption per capita.

Table of contents

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
ADI	Acceptable Daily Intake
AzEEZ	Azorean Exclusive Economic Zone
BCs	Background concentrations
BHT	Butylated hydroxytoluene
BMF	Biomagnification factor
BSAF	Biota-sediment accumulation factor
Bw	Body weight
CAT	Catalase
CEA	Cellular Energy Allocation
DHA	Docosahexaenoic acid
dw	Dry weight
Ea	Energy available
EACs	Ecotoxicological Assessment Criteria
EF	Enrichment factor
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid
EQS	Environmental quality standard
ETS	Electron transport system
EU	European Union
EU-WFD	European Union Water Framework Directive
FAO	Food and Agriculture Organization
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione

GSH/GSSG	Glutathione system
GST	Glutathione S-transferase
Hg	Mercury
Hg^+	Mercurous salts
Hg^{++}	Mercuric salts
Hg^0	Elemental mercury
HSP	Heat shock proteins
Igeo	Geo-accumulation index
IHg	Inorganic mercury
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LDH	Lactate dehydrogenase
LPO	Lipid peroxidation
MAR	Mid-Atlantic Ridge
MeHg	Methylmercury
MT	Metallothionein
NOAA	National Oceanic and Atmospheric Administration
NW	Northwest
OHg	Organic mercury
OSPAR	Marine Environment of the North-East Atlantic convention
PC	Protein carbonylation
PEL	Probable effect level
PTWI	Provisional Tolerable Weekly Intake
RfD	Reference dose
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SPM	Suspended particulate matter

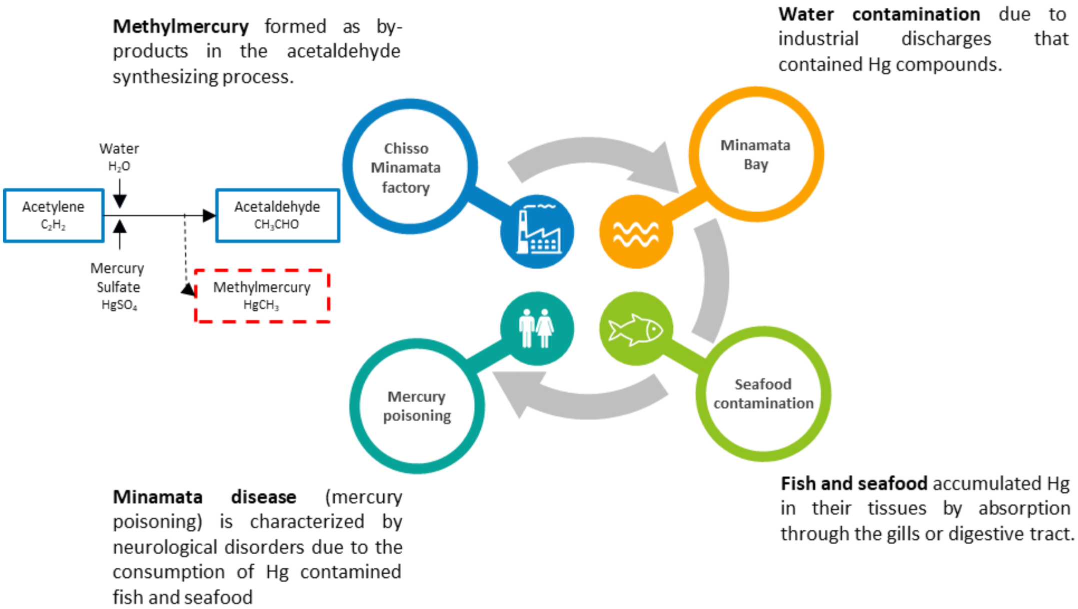
SQG	Sediment quality guidelines
SQuiRTs	Screening Quick Reference Tables
TEL	Threshold effect level
tGSH	Total glutathione
THg	Total mercury
THQ	Target hazard quotient
THQ	Target hazard quotient
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
ww	Wet weight

Chapter I:

General Introduction and research objectives

1. General Introduction

Environmental and health issues are the main concerns for the modern society. Indeed, half or more of European Union citizens (EU-28) claim they are worried about air (56%) and water pollution (50% – Eurobarometer 2014). Over four out of ten people are worried about the impact on health derived from the chemicals used in everyday products (43%) and about the growing amount of waste (43%), while more than a third are worried about the depletion of natural resources (36% – Eurobarometer 2014). One of the most catastrophic events in modern history, related to water quality, happened in Minamata Bay, Japan, where more than hundred people lost their lives and several thousand people have suffered permanently since the 1950s due to the consumption of mercury (Hg) contaminated seafood (Kudo et al. 1998). From 1932 until 1968 (date of the end of production), the Chisso Minamata factory used Hg as a catalyst in the process of manufacturing acetaldehyde and vinyl chloride (Fig. 1.1). During this period, it is said that approximately 70-150 tons or more of Hg, mixed in with effluent from the factory, was discharged into Minamata Bay (Minamata Disease Municipal Museum 2007).



Methylmercury formed as by-products in the acetaldehyde synthesizing process.

Water contamination due to industrial discharges that contained Hg compounds.

Fish and seafood accumulated Hg in their tissues by absorption through the gills or digestive tract.

Minamata disease (mercury poisoning) is characterized by neurological disorders due to the consumption of Hg contamined fish and seafood

Fig. 1.1 The route of methylmercury from generation in acetaldehyde process to the human body. Adapted from Minamata Disease Municipal Museum (2007).

Naturally present in the Earth's crust, Hg is released into the environment through natural sources (e.g. forest fires, volcanic activity, and another biomass burning – Pacyna and Keeler 1995). However, throughout the centuries, Hg was intensely used in human activities (anthropogenic sources) such as large-scale gold mining, non-ferrous metal production and combustion of fossil fuels have been mobilizing increasing amounts of the element in the atmosphere, ocean, and terrestrial systems (Selin 2009, Sun et al. 2016). The contribution of natural sources to the release of Hg to the environment is relatively small (around 76-300 tons per year) compared to anthropogenic activities that are equivalent to about 2000 tons per year (Streets et al. 2019). The predominant source sector is artisanal and small-scale gold mining (about 38%) followed by stationary combustion of coal (about 21%). These are followed by emissions from non-ferrous metal (about 15%) and cement production (about 11%). Emissions associated with the disposal of Hg-added product waste (7%), stationary combustion of other fuels including biomass (3%), ferrous-metal production (2%), and other sources (2%) make up the rest (Fig. 1.2 – UN Environment 2019).

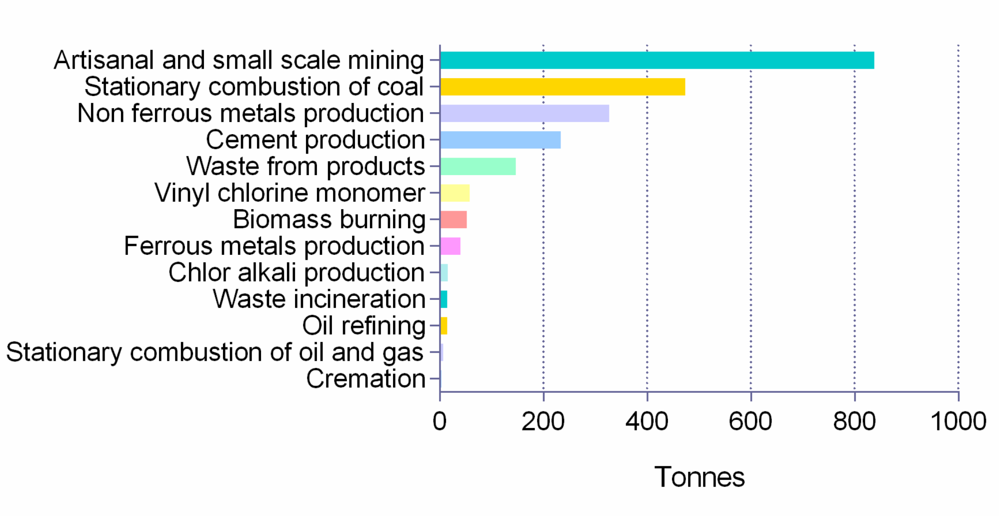


Fig. 1.2 Hg emissions estimates by sector in 2018. Adapted from UN Environment (2019).

In order to protect human health and the environment from anthropogenic emissions and releases of Hg, the multilateral environmental agreement called Minamata Convention on Mercury was adopted in 2013 (Evers et al. 2016, Selin et al. 2018). The Minamata Convention on Mercury became the first international agreement designed to specifically address contamination from a heavy metal (Buck et al. 2019) and the 128 countries that have ratified the Minamata Convention on Mercury, pledged to control, reduce or eliminate the main sources of Hg (Streets et al. 2019). Despite this, Hg emissions increased at a rate of 1.8% per year between 2010 and 2015. During this time, some countries managed to reduce their Hg emissions by 10% (United States), 5.8% (OECD Europe) and 3.2% (Canada). On the other hand, other countries from Central America (5.4%), South Asia (4.6%) and Eastern Africa (4.0%) increased their emissions (Streets et al. 2019). Hg emissions to the atmosphere must be seen as a global problem, considering that the Hg released can travel long distances through the atmosphere (Sundseth et al. 2017). For example, about 50% of anthropogenic Hg deposited annually in Europe originates outside Europe, with 30% originating only in Asia (European Environmental Agency 2018).

In the environment, Hg is found in three different forms: elemental Hg (Hg^0), inorganic Hg (IHg) and organic Hg (OHg – Bjørklund et al. 2017). Hg^0 also known as metallic Hg (Guzzi and La Porta 2008) is liquid at room temperature, and is used to produce thermometers, electrical parts, dental fillings (Budnik and Casteleyn 2019). Due to its high vapor pressure it is easily released into the atmosphere as Hg vapor (Park and Zheng 2012). The high volatility and an atmospheric useful life of approximately 6 – 24 months, makes it the most abundant form of Hg in the atmosphere (Budnik and Casteleyn 2019). IHg can be found in two oxidative states: mercurous (Hg^+) and mercuric salts (Hg^{++}) (Park and Zheng 2012); however, mercurous forms can be easily converted into Hg^0 and Hg^{++} (Ajsuvakova et al. 2020). IHg has been found in laxatives, cosmetic products, teething powders, diuretics, and antiseptics (Bjørklund et al. 2017). Finally, OHg is considered the most hazardous and most frequent form of Hg exposure, being frequently detected as methylmercury (MeHg – Crowe et al. 2017). The different Hg forms can be converted from one form to another (Fig. 1.3) by natural processes (European Union 2017).

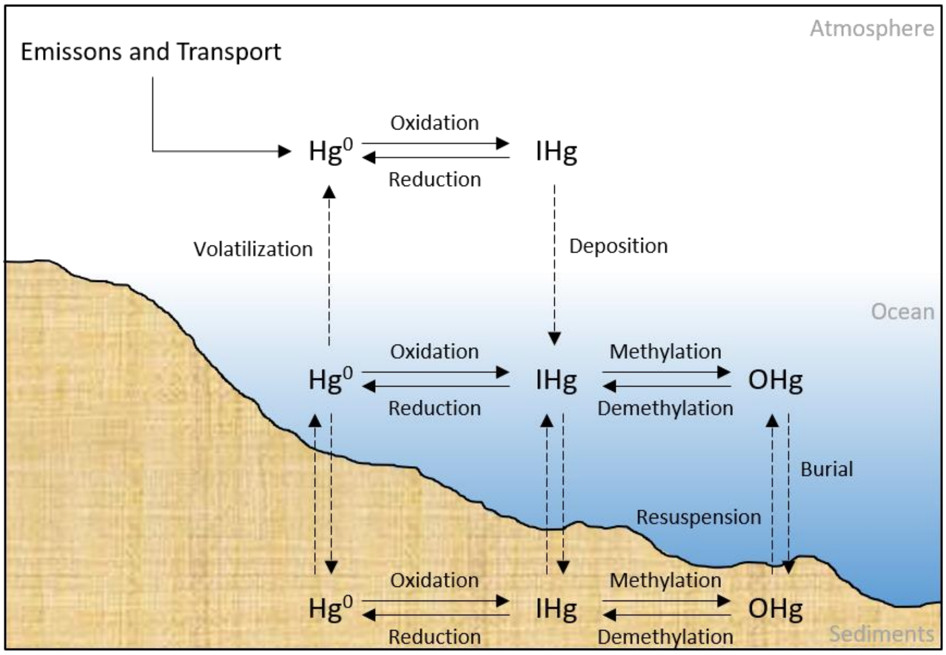


Fig. 1.3 Conceptual diagram of Hg biogeochemical transformation in the different environmental compartments. Adapted from Selin et al. (2009).

For example, Hg^0 can be easily transformed to IHg through an oxidation process when exposed to oxygen (Ozuah 2000) and IHg can be converted to OHg in aquatic systems by the action of anaerobic organisms (Cossa et al. 2009). These conversions allow Hg to be transported and recycled between the various environmental compartments (atmosphere, hydrosphere and lithosphere); however, it can be removed from the environmental compartments by burying in the lithosphere compartment (AMAP/UN Environment 2019).

All forms of Hg are toxic (Broussard et al. 2002); however, this toxicity depends on the form of Hg and exposure route (Ajsuvakova et al. 2020). Less than 0.01% of Hg^0 and approximately 7% to 15% of IHg ingested are absorbed by the gastrointestinal tract (Park and Zheng 2012). Unlike other forms of Hg, the ingested OHg is mostly absorbed by the gastrointestinal tract (about 95%) (Guzzi and La Porta 2008). Hg has a strong affinity for sulfhydryl groups (thiol; -SH) (Ajsuvakova et al. 2020) and when it is absorbed by the body, it binds to small molecules that contain thiol groups (e.g. cysteine and glutathione). The formation of these complexes is important in the transport process and in the distribution of Hg through the different tissues since they facilitate transport across cell membranes (Clarkson and Magos 2006, Kershaw and Hall 2019). The Hg-thiol interaction can lead to the inhibition of enzymes and proteins that contain free cysteine residues, resulting in health problems to animals or humans (Li et al. 2017, Ajsuvakova et al. 2020).

Due to the associated negative health effects, Hg and its compounds are considered by the World Health Organization (WHO) as pollutants of particular concern for public health (WHO 2010). The negative health effects from exposure to Hg includes tingling and numbness in fingers and toes, loss of coordination, difficulty in walking, generalized weakness, impairment of hearing and vision, tremors, loss of consciousness, developmental neurological problems such as delayed onset of walking and talking, cerebral palsy (Díez 2009, Matta and Gjyli 2016, Jyothi and Farook 2020). Although in humans, exposure to Hg can occur in three ways (inhalation, ingestion and dermal contact – Jyothi and Farook 2020), it is the ingestion of contaminated fish and seafood that most contributes to Hg exposure (especially in the form of MeHg – Rice et al. 2014). Apart from being appointed as the main route of MeHg to humans, fish consumption is also recognized as an important component of a healthy diet (Díez 2009). Considering MeHg intake and associated risk, some definitions such as Acceptable Daily Intake (ADI), Reference Dose (RfD) and Provisional Tolerable Weekly Intake (PTWI) have been implemented by international agencies. Joint FAO/WHO Expert Committee on Food Additives (JECFA) refers ADI as “an estimated maximum amount of an agent, expressed on a body mass basis, to which individuals in a (sub)population may be exposed daily over their lifetimes without appreciable health risk” and RfD as “an estimate of the daily exposure dose that is likely to be without deleterious effect even if continued exposure occurs over a lifetime” (IPCS 2004). PTWI is also defined by JECFA, as “the value that represents permissible human weekly exposure for food contaminants such as heavy metals with cumulative properties” (WHO 1987).

The transformation of IHg into OHg occurs essentially in the aquatic environment, leading to aquatic animals having higher concentrations of Hg in their tissues than terrestrial animals (UN Environment 2019). Furthermore, the affinity of Hg for thiol groups of proteins results in a low rate of excretion by organisms (Li et al. 2017). The difficulty in excreting Hg can compromise the balance between intake and excretion, and when Hg uptake exceeds Hg elimination, occurs an increase of Hg in tissues (bioaccumulation process – Chojnacka and Mikulewicz 2014). In the aquatic systems, Hg bioaccumulation process be a result of direct water absorption where Hg crosses the cell membrane by passive or active transport (phytoplankton and algae) and of absorption by food intake (invertebrates and fish – Chojnacka and Mikulewicz 2014). The bioaccumulation process also depends on several biotic factors including fish length, age, and trophic position (Mills et al. 2019) and on abiotic factors such as water chemistry (pH, organic matter richness, oxygen saturation, salinity – de Almeida Rodrigues et al. 2019), being also strongly dependent of the temperature (Chojnacka and Mikulewicz 2014).

Over the years, several attempts have been made in order to project a global map based on the different types of climates, e.g. Köppen–Geiger climate classification system (Fig. 1.4 – Rueda et al. 2017).

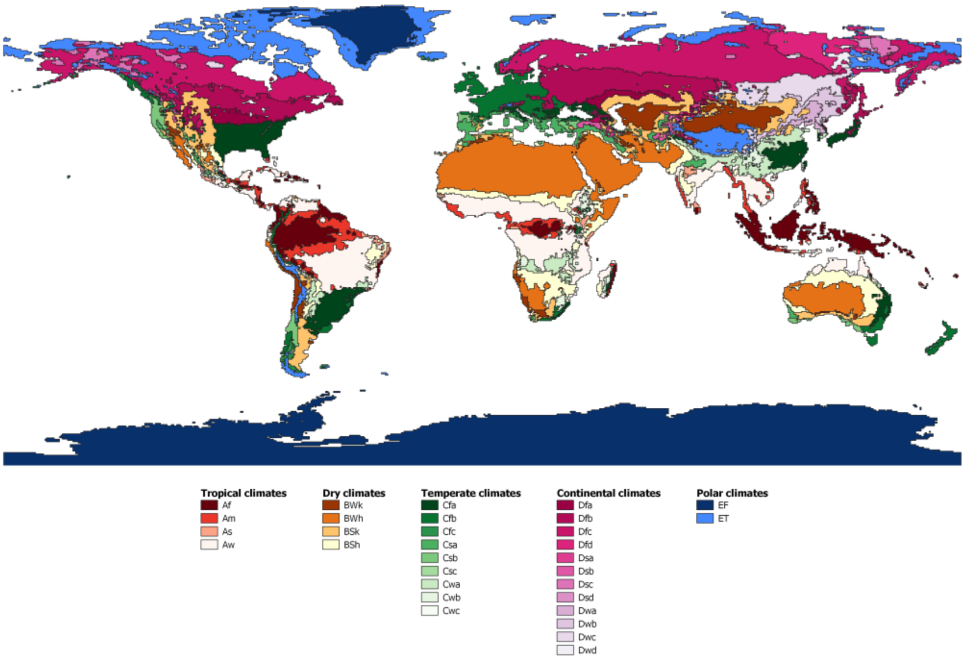


Fig. 1.4 World Map of Köppen-Geiger climate classification calculated from observed temperature and precipitation data for the period 1976-2000, adapted from Rubel and Kottek (2010).

The Köppen-Geiger climate classification is widely used and categorizes world climates into five main types: A (tropical climates), B (dry climates), C (temperate climates), D (continental climates), and E (polar climates – Hodgkins et al. 2017, Wang et al. 2017, MacLeod and Korycinska 2019). These five main climate types can be further subdivided using one or two more letters. The first division of a main climate type (second letter) refers to seasonal precipitation (e.g. Dw continental and dry winter) and the next division (third letter) concerns air temperature (e.g. Dwb for continental, dry winter with warm summer – MacLeod and Korycinska 2019). For example, according to the Köppen-Geiger climate classification it is possible find various types of climates in the North Atlantic Ocean, such as tropical AW (Tropical savanna climate) in south of Mexico, BWh (Hot desert climate) in north of Africa, different types of temperate climates in Ocidental Europe countries (e.g. Csa, Csb, Cfb).

The accumulation of heavy metals in the aquatic environment can be influenced by the climate and it is generally possible to find a positive correlation between warmer water temperatures and accumulation of Hg (Dijkstra et al. 2013). This correlation between the rise in water temperature and the accumulation of Hg may be due to the fact that increased water temperature stimulates the methylation of Hg, which can lead to higher levels of bioavailable MeHg (Hammerschmidt and Fitzgerald 2004, Fitzgerald et al. 2007).

In general, the long-term exposure of living organisms to toxicants results primarily in their accumulation in organs and tissues and secondly in irreversible molecular alterations due to their continuous deleterious action (Lopes et al. 2001). In addition to having the ability to bioaccumulate in aquatic organisms, Hg can also induce the formation of reactive oxygen species (ROS), responsible for cellular and tissue damage (Elia et al. 2003). Defenses against ROS include enzymes with antioxidant activities such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-*S*-transferases (GST – Tomás-Zapico and Coto-Montes 2005) and scavenger compounds, such as glutathione system (GSH/GSSG) and metallothionein (MT). These antioxidant defenses are essential to combat oxidative stress and maintain the redox state of cells (Monteiro et al. 2009); however, when the increase in ROS production exceeds the ability of antioxidant defenses to act, it can lead to the appearance of cellular damage at the level of lipids (lipid peroxidation – LPO – Jancic and Stosic 2014) and proteins (protein carbonylation – PC – Catalán et al. 2018). Over the years, there has been an increasing use of biomarkers in monitoring the environmental quality and health of organisms since these biochemical mechanisms involved in cell detoxification are particularly relevant in understanding the effects of environmental pollutants on organisms (Lopes et al. 2001).

2. Research objectives and thesis outline

The main objectives of this thesis were to assess the presence of Hg in coastal areas of different geographical areas located in the North Atlantic (northwest Portuguese coast, Azores archipelago and southeast Mexican coast) and whether Hg can be transferred from the sediment to the marine trophic chain. Afterwards, it was assessed whether the Hg found in sediments influences the concentration of Hg present in the biota and if the average temperature of seawater in the different coastal zones may have an influence on the Hg bioaccumulation and toxicity. Finally, the concentration of Hg present in marine species of commercial interest in the different coastal areas was evaluated considering the characteristics of each species and the contribution of each species to human exposure to Hg was assessed, considering the concentration of Hg present in the muscle and international guidelines.

To achieve the main study goals, several approaches were carried out and are expressed in the present thesis organized into nine chapters (Fig. 5), as described below:

Chapter I – General Introduction and research objectives provides an overview of the problem of Hg contamination, briefly describing how Hg appears in the environment, how and where it is transformed, how it enters the food chain and the potential negative health effects. This chapter also describes the main objectives of this study.

Chapter II – Hg distribution and enrichment in coastal sediments from different geographical areas in the North Atlantic Ocean assesses the [Hg] in sediments from different coastal areas considering the granulometry and proximity to possible contamination hotspots. It also assesses whether the [Hg] present in these sediments comes from natural or anthropogenic sources.

This chapter is based on the submitted manuscript: *Vieira, H.C., Bordalo, M.D., Figueroa, A.G., Soares, A.M.V.M., Morgado, F., Abreu, S. N., Rendón-von Osten, J. (submitted) Mercury distribution and enrichment in coastal sediments from different geographical areas in the North Atlantic Ocean. Marine Pollution Bulletin.*

Chapter III – Mercury contamination in coastal sediments and biota in the Azores archipelago: the influence of a 16th century shipwreck assesses the [Hg] in sediments, macroalgae and crustaceans from five coastal areas in the Azores archipelago and relates [Hg] to the distance of the sinking of a Spanish ship that in colonial times transported Hg to the new world to be used in gold mining. It also classifies the contamination of sediments considering the Portuguese law's guidelines for sediment quality and compares the current [Hg] with the background levels.

This chapter is based on the submitted manuscript: *Vieira, H.C., Rendón-von Osten, J., Soares, A.M.V.M., Abreu, S. N., Morgado, F. (submitted) Mercury contamination in coastal sediments and biota in the Azores archipelago: the influence of a 16th century shipwreck. Environmental Science and Pollution Research.*

Chapter IV – Mercury in superficial sediments and trophic transfer assessment in the NW coast of Portugal evaluates the concentration of Hg in sediments and intertidal species (macroalgae and mollusks) from eight sampling sites on the northwest coast of Portugal. Compares the concentrations of Hg present in the sediment with the reference value of the European Union Water Framework Directive (EU-WFD), with maximum established by OSPAR Convention through the Ecotoxicological Assessment Criteria (EACs) and with the Portuguese law's guidelines for sediment quality. In addition, it assesses the transfer of Hg from the sediment to the biota, as well as between prey and predator.

This chapter is based on the submitted manuscript: *Vieira, H.C., Maia, M.E., Bordalo, M.D., Soares, A.M.V.M., Rendón-von Osten, J., Abreu, S. N., Morgado, F. (submitted) Mercury in superficial sediments and trophic transfer assessment in the NW coast of Portugal. Biological Trace Element Research.*

Chapter V – Water temperature modulates mercury bioaccumulation and oxidative stress status of Common Goby (*Pomatoschistus microps*) investigates the effect of the average seawater temperature of the different areas from chapter II on the bioaccumulation of Hg in different tissues (liver and muscle) of the fish species *Pomatoschistus microps* (Krøyer, 1838) when exposed to an environmentally relevant [Hg] during a short period (7 days). In addition, it assesses the role of Hg and temperature in inducing oxidative stress in marine organisms.

This chapter is based on the published paper: *Vieira, H.C., Bordalo, M.D., Rodrigues, A.C.M., Pires, S.F.S., Rocha, R.J.M., Soares, A.M.V.M., Rendón-von Osten, J., Abreu, S. N., Morgado, F. (2020) Water temperature modulates mercury bioaccumulation and oxidative stress status of Common Goby (Pomatoschistus microps). Environmental Research. DOI: 10.1016/j.envres.2020.110585.*

Chapter VI – Total and organic mercury in fish from different geographical areas in the North Atlantic Ocean and health risk assessment assesses the [Hg] in 38 species of fish caught in different FAO fishing areas in the North Atlantic (areas 27 and 31) and relates this [Hg] with food habits, food preference and lifestyle of each species. Moreover, it evaluates the human exposure to Hg through the consumption of these species considering [Hg], the intake limits established by JEFCA, as well as the consumption of fish per capita in each area. In addition, it establishes recommendations for the weekly consumption of these species.

This chapter is based on the submitted manuscript: *Vieira, H.C., Figueroa, A.G., Bordalo, M.D., Rodrigues, A.C.M., Soares, A.M.V.M., Abreu, S. N., Morgado, F., Rendón-von Osten, J. (submitted) Occurrence and health risk assessment of mercury in fish species from different FAO fishing areas. Food and Chemical Toxicology.*

Chapter VII – Contribution of commercial fish species to human mercury exposure: an evaluation near the Mid-Atlantic Ridge evaluates the contribution of 28 commercial fish species to the human Hg exposure in the Azores archipelago (Portuguese region with highest fish consumption per capita), considering the [Hg] present in the muscle and the amount of fish landing (from 1994 to 2018) of each species. It also evaluates through the non-carcinogenic target hazard quotient (THQ), the implications for human health that may result from the consumption of these fish species.

This chapter is based on the published paper: *Vieira, H.C., Rendón-von Osten, J., Soares, A.M.V.M., Morgado, F., Abreu, S. N. (2020) Contribution of commercial fish species to human mercury exposure: an evaluation near the Mid-Atlantic Ridge. Journal of Food Composition and Analysis, 103688. DOI: 10.1016/j.jfca.2020.103688.*

Chapter VIII – Mercury bioaccumulation in the long-fin squid *Loligo forbesi* near the Mid-Atlantic Ridge: implications to human exposure analyzes the accumulation of Hg in different tissues (mantle and stomach), as well as in the stomach contents of squid of the long-fin squid species (*Loligo forbesi*) captured in the Azores Archipelago. It also assesses the transfer of Hg from prey to predator, as well as estimating the amount of squid that can be consumed weekly without exceeding the weekly tolerable Hg intake established by international agencies.

This chapter is based on the published paper: *Vieira, H.C., Rendón-von Osten, J., Soares, A.M.V.M., Morgado, F., Abreu, S. N. (2020) Mercury bioaccumulation in the long-fin squid Loligo forbesi near the Mid-Atlantic Ridge: implications to human exposure. Ecotoxicology and Environmental Safety, 203, 110957. DOI: 10.1016/j.ecoenv.2020.110957.*

Chapter XI – Final considerations combines the specific conclusions of each chapter (2 - 8) in an overall conclusion.

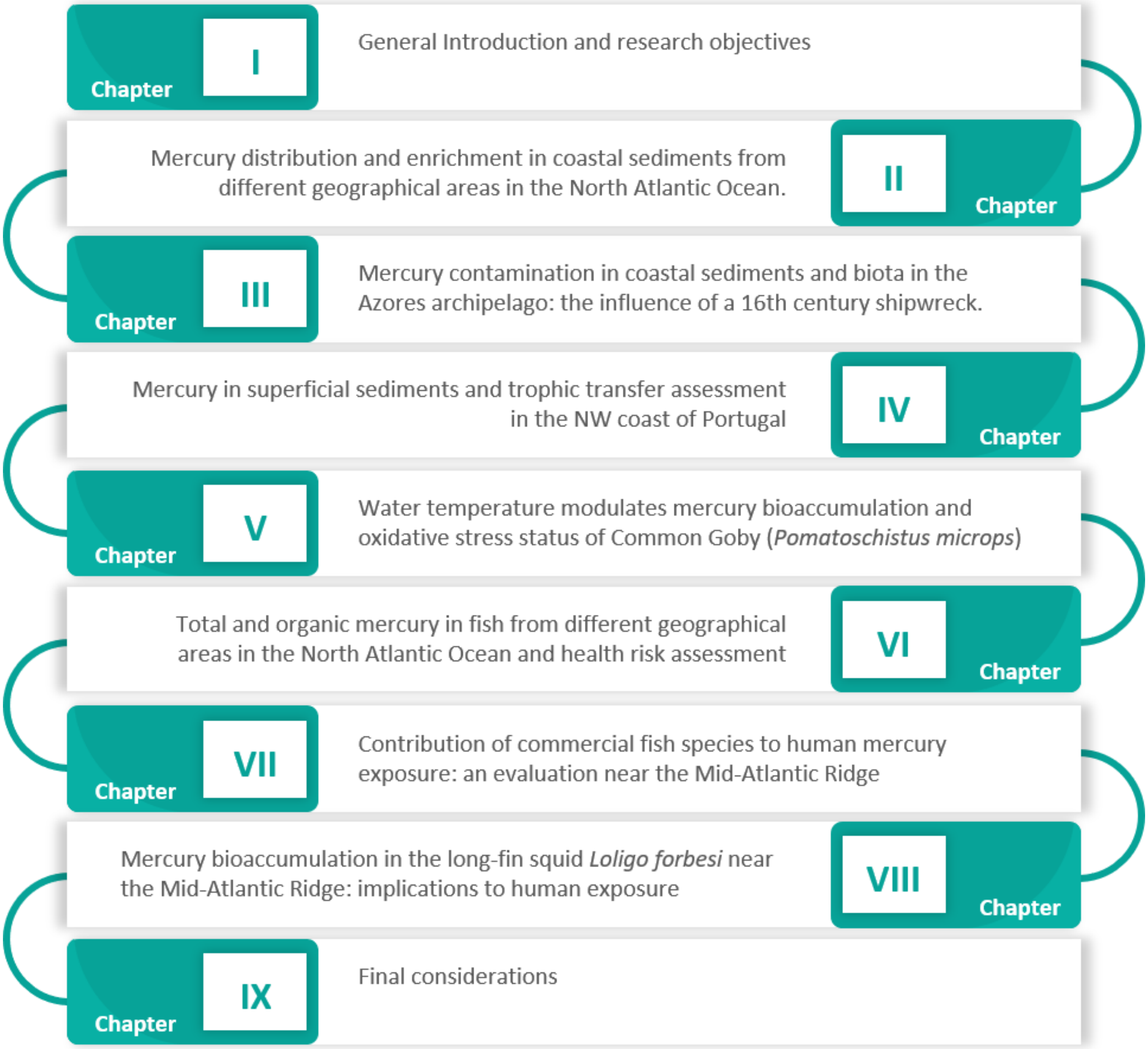


Fig. 1.5 Illustrative scheme of the thesis organization.

Reference

Ajsuvakova, O. P., A. A. Tinkov, M. Aschner, J. B. T. Rocha, B. Michalke, M. G. Skalnaya, A. V. Skalny, M. Butnariu, M. Dadar, I. Sarac, J. Aaseth, and G. Bjørklund. 2020. Sulfhydryl groups as targets of mercury toxicity. Coordination Chemistry Reviews 417:213343.

AMAP/UN Environment. 2019. Technical Background Report for the Global Mercury Assessment 2018. Arctic Monitoring and Assessment Programme, Oslo, Norway/UN Environment Programme, Chemicals and Health Branch, Geneva, Switzerland. viii + 426 pp including E-Annexes.

Bjørklund, G., M. Dadar, J. Mutter, and J. Aaseth. 2017. The toxicology of mercury: Current research and emerging trends. Environmental Research 159:545-554.

Broussard, L. A., C. A. Hammett-Stabler, R. E. Winecker, and J. D. Ropero-Miller. 2002. The toxicology of mercury. Laboratory medicine 33:614-625.

Buck, D. G., D. C. Evers, E. Adams, J. DiGangi, B. Beeler, J. Samánek, J. Petrlik, M. A. Turnquist, O. Speranskaya, K. Regan, and S. Johnson. 2019. A global-scale assessment of fish mercury concentrations and the identification of biological hotspots. Science of The Total Environment 687:956-966.

Budnik, L. T., and L. Casteleyn. 2019. Mercury pollution in modern times and its socio-medical consequences. Science of The Total Environment 654:720-734.

Catalán, V., G. Frühbeck, and J. Gómez-Ambrosi. 2018. Chapter 8 - Inflammatory and Oxidative Stress Markers in Skeletal Muscle of Obese Subjects. Pages 163-189 in A. M. del Moral and C. M. Aguilera García, editors. Obesity. Academic Press.

Chojnacka, K., and M. Mikulewicz. 2014. Bioaccumulation. Pages 456-460 in P. Wexler, editor. Encyclopedia of Toxicology (Third Edition). Academic Press, Oxford.

Clarkson, T. W., and L. Magos. 2006. The Toxicology of Mercury and Its Chemical Compounds. Critical Reviews in Toxicology 36:609-662.

Cossa, D., B. Averty, and N. Pirrone. 2009. The origin of methylmercury in open Mediterranean waters. Limnology and oceanography 54:837-844.

Crowe, W., P. J. Allsopp, G. E. Watson, P. J. Magee, J. J. Strain, D. J. Armstrong, E. Ball, and E. M. McSorley. 2017. Mercury as an environmental stimulus in the development of autoimmunity – A systematic review. Autoimmunity Reviews 16:72-80.

de Almeida Rodrigues, P., R. G. Ferrari, L. N. dos Santos, and C. A. Conte Junior. 2019. Mercury in aquatic fauna contamination: A systematic review on its dynamics and potential health risks. Journal of Environmental Sciences 84:205-218.

Díez, S. 2009. Human Health Effects of Methylmercury Exposure. Pages 111-132 in D. M. Whitacre, editor. Reviews of Environmental Contamination and Toxicology. Springer New York, New York, NY.

Dijkstra, J. A., K. L. Buckman, D. Ward, D. W. Evans, M. Dionne, and C. Y. Chen. 2013. Experimental and natural warming elevates mercury concentrations in estuarine fish. PLoS ONE 8:e58401.

Eurobarometer. 2014. Special Eurobarometer 416, Attitudes of European Citizens Towards the Environment. Available from http://ec.europa.eu/public_opinion/archives/ebs/ebs_416_en.pdf Accessed on 21 May 2020.

European Environmental Agency. 2018. Mercury in Europe's environment: A priority for European and global action. 1977-8449. EEA Report No 11/2018.

European Union. 2017. Science for environment policy. In-depth report 15: tackling mercury pollution in the EU and worldwide. Available from: http://ec.europa.eu/environment/integration/research/newsalert/pdf/tackling_mercury_pollution_EU_and_worldwide_IR15_en.pdf Accessed on 31 October 2020.

Evers, D. C., S. E. Keane, N. Basu, and D. Buck. 2016. Evaluating the effectiveness of the Minamata Convention on Mercury: Principles and recommendations for next steps. Science of The Total Environment 569-570:888-903.

Fitzgerald, W. F., C. H. Lamborg, and C. R. Hammerschmidt. 2007. Marine Biogeochemical Cycling of Mercury. Chemical Reviews 107:641-662.

Guzzi, G., and C. A. M. La Porta. 2008. Molecular mechanisms triggered by mercury. Toxicology 244:1-12.

Hammerschmidt, C. R., and W. F. Fitzgerald. 2004. Geochemical Controls on the Production and Distribution of Methylmercury in Near-Shore Marine Sediments. Environ Sci Technol 38:1487-1495.

Hodgkins, G. A., P. H. Whitfield, D. H. Burn, J. Hannaford, B. Renard, K. Stahl, A. K. Fleig, H. Madsen, L. Mediero, J. Korhonen, C. Murphy, and D. Wilson. 2017. Climate-driven variability in the occurrence of major floods across North America and Europe. Journal of Hydrology 552:704-717.

IPCS. 2004. Harmonization Project Document No.1 - IPCS risk assessment terminology. WHO, Geneva.

Jancic, S. A., and B. Z. Stosic. 2014. Chapter Fourteen - Cadmium Effects on the Thyroid Gland. Pages 391-425 in G. Litwack, editor. Vitamins & Hormones. Academic Press.

Jyothi, N. R., and N. A. M. Farook. 2020. Heavy Metal Toxicity in Public Health. Heavy Metal Toxicity in Public Health. IntechOpen.

Kershaw, J. L., and A. J. Hall. 2019. Mercury in cetaceans: Exposure, bioaccumulation and toxicity. Science of The Total Environment 694:133683.

Kudo, A., Y. Fujikawa, S. Miyahara, J. Zheng, H. Takigami, M. Sugahara, and T. Muramatsu. 1998. Lessons from Minamata mercury pollution, Japan-after a continuous 22 years of observation. Water Science and Technology 38:187.

Li, R., H. Wu, J. Ding, W. Fu, L. Gan, and Y. Li. 2017. Mercury pollution in vegetables, grains and soils from areas surrounding coal-fired power plants. Scientific reports 7:46545.

Lopes, P. A., T. Pinheiro, M. C. Santos, M. da Luz Mathias, M. J. Collares-Pereira, and A. M. Viegas-Crespo. 2001. Response of antioxidant enzymes in freshwater fish populations (*Leuciscus alburnoides* complex) to inorganic pollutants exposure. Science of The Total Environment 280:153-163.

MacLeod, A., and A. Korycinska. 2019. Detailing Köppen–Geiger climate zones at sub-national to continental scale: a resource for pest risk analysis. EPPO Bulletin 49:73-82.

Matta, G., and L. Gjyli. 2016. Mercury, lead and arsenic: impact on environment and human health. J. Chem. Pharm. Sci 9:718-725.

Mills, N., M. J. Weber, C. L. Pierce, and D. Cashatt. 2019. Factors influencing fish mercury concentrations in Iowa rivers. Ecotoxicology 28:229-241.

Minamata Disease Municipal Museum. 2007. Minamata Disease: Its History and Lessons. . Minamata City Planning Division Minamata City, Japan. Available from https://minamata195651.jp/pdf/kyoukun_en/kyoukun_eng_all.pdf Accessed on 21 May 2020.

Monteiro, D. A., F. T. Rantin, and A. L. Kalinin. 2009. Inorganic mercury exposure: toxicological effects, oxidative stress biomarkers and bioaccumulation in the tropical freshwater fish matrinxã, Brycon amazonicus (Spix and Agassiz, 1829). Ecotoxicology 19:105.

Ozuah, P. O. 2000. Mercury poisoning. Current Problems in Pediatrics 30:91-99.

Pacyna, J., and G. J. Keeler. 1995. Sources of mercury in the Arctic. Pages 621-632 Mercury as a Global Pollutant. Springer.

Park, J.-D., and W. Zheng. 2012. Human exposure and health effects of inorganic and elemental mercury. Journal of preventive medicine and public health = Yebang Uihakhoe chi 45:344-352.

Rice, K. M., E. M. Walker, Jr., M. Wu, C. Gillette, and E. R. Blough. 2014. Environmental mercury and its toxic effects. Journal of preventive medicine and public health = Yebang Uihakhoe chi 47:74-83.

Rubel, F., and M. Kottek. 2010. Observed and projected climate shifts 1901–2100 depicted by world maps of the Köppen-Geiger climate classification. Meteorologische Zeitschrift 19:135-141.

Rueda, A., S. Vitousek, P. Camus, A. Tomás, A. Espejo, I. J. Losada, P. L. Barnard, L. H. Erikson, P. Ruggiero, B. G. Reguero, and F. J. Mendez. 2017. A global classification of coastal flood hazard climates associated with large-scale oceanographic forcing. Scientific reports 7:5038.

Selin, H., S. E. Keane, S. Wang, N. E. Selin, K. Davis, and D. Bally. 2018. Linking science and policy to support the implementation of the Minamata Convention on Mercury. Ambio 47:198-215.

Selin, N. E. 2009. Global Biogeochemical Cycling of Mercury: A Review. Annual Review of Environment and Resources 34:43-63.

Streets, D. G., H. M. Horowitz, Z. Lu, L. Levin, C. P. Thackray, and E. M. Sunderland. 2019. Global and regional trends in mercury emissions and concentrations, 2010–2015. Atmospheric Environment 201:417-427.

Sun, R., D. G. Streets, H. M. Horowitz, H. M. Amos, G. Liu, V. Perrot, J.-P. Toutain, H. Hintelmann, E. M. Sunderland, and J. E. Sonke. 2016. Historical (1850–2010) mercury stable isotope inventory from anthropogenic sources to the atmosphere. Elem Sci Anth 4.

Sundseth, K., J. M. Pacyna, E. G. Pacyna, N. Pirrone, and R. J. Thorne. 2017. Global Sources and Pathways of Mercury in the Context of Human Health. International journal of environmental research and public health 14:105.

Tomás-Zapico, C., and A. Coto-Montes. 2005. A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes. Journal of Pineal Research 39:99-104.

UN Environment. 2019. Global Mercury Assessment 2018. UN Environment Programme, Chemicals and Health Branch Geneva, Switzerland.

Wang, L., R. V. Rohli, X. Yan, and Y. Li. 2017. A new method of multi-model ensemble to improve the simulation of the geographic distribution of the Köppen–Geiger climatic types. International Journal of Climatology 37:5129-5138.

WHO. 1987. Principles for the Safety Assessment of Food Additives and Contaminants in Food, Environmental Health Criteria, No. 70. World Health Organization, Geneva.

WHO. 2010. Preventing diases through healthy environments: actions is needed on chemicals of major public health concern.

Chapter II:

Mercury distribution and enrichment in coastal sediments from different geographical areas in the North Atlantic Ocean

Mercury distribution and enrichment in coastal sediments from different geographical areas in the North Atlantic Ocean

Vieira, H.C.[1], Bordalo, M.D.[1], Figueroa, A.G.[2], Soares, A.M.V.M.[1], Morgado, F.[1], Abreu, S. N.[1], Rendón-von Osten, J.[2]

[1] CESAM - Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

[2] EPOMEX Institute, Autonomous University of Campeche, Campeche, Mexico

Abstract

This study evaluated mercury (Hg) in three coastal areas in the North Atlantic Ocean (Azores archipelago, Northwest Portugal and Southeast Mexico) and calculated their enrichment factor and geoaccumulation index. Hg presented greater affinity with fine sediment fraction. In all sampling areas the sites with proximity to possible Hg hotspots presented increased [Hg] in fine sediments. However, this fraction only represents less than 1% of the whole sediment, resulting in a significantly lower [Hg] in the total sediment fraction.

After comparing [Hg] in the fine fraction and the values established by the Sediment Quality Guidelines, two sites in the Azores exceeded these values. Nevertheless, when considering the total sediment fraction, adverse biological effects are not expected to occur. Finally, both enrichment factor and geoaccumulation index indicated that the Hg present in 86% comes from natural sources and only sampling sites close to potential hotspots showed some degree of enrichment and contamination.

Keywords: Mercury; sediment fractions; sediment quality guidelines; enrichment factor; geoaccumulation index.

1. Introduction

Mercury (Hg) is considered one of the most hazardous contaminants in the aquatic environment (Hassan et al. 2019). It is classified a high-priority environmental pollutant by the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR Convention – Nyström 2018), the European Union Water Framework Directive (EU-WFD – EU 2008) and the United States Environmental Protection Agency (U.S. EPA – USEPA

2014). Hg is an element that occurs naturally in the environment (Hassan et al. 2019); however, its presence may increase due to human activity (anthropogenic sources – Bonsignore et al. 2020). Once released, Hg can travel long distances through the atmosphere and waterways (Mason et al. 1994), potentially resulting in a widespread pollution in the marine environment by accumulating in coastal sediments and bioaccumulating in marine biota (Walker 2016).

Hg is not degradable in the environment (Sajed et al. 2019) and due to its potential harmful effects on the ecosystem and human health, Hg assessment in marine ecosystems continues to attract the attention of environmental researchers (Shulkin et al. 2003). The ecological risk of exposure of aquatic organisms to Hg has led many countries and international entities to define sediment quality criteria (Esen et al. 2010). Since then sediment quality guidelines (SQG) have been published by Canada (CCME 1999), the European Union (Bignert et al. 2004) and the United States (Buchman 2008). These SQG establish the [Hg] in sediments which represents the concentration below which toxic effects are rarely or never observed (threshold effect level – TEL). On the other hand, the probable effect level (PEL) is assumed to represent the concentration above which toxic effects on living organisms are usually or frequently observed (Szalinska 2020).

In the aquatic environment, Hg tends to bind to fine sediment particles by different processes (Scanu et al. 2016), which means that coastal sediments can act both as a sink (Chakraborty et al. 2014) and as a source of Hg to offshore pelagic food webs (Hammerschmidt and Fitzgerald 2006, Fitzgerald et al. 2007). For this reason, the degree of contamination of a region can only be assessed if natural levels are known. In this sense, it is important to evaluate the background concentrations (BCs) of Hg in marine ecosystems to distinguish natural concentrations from concentrations introduced by human action (Palma et al. 2009). The OSPAR Commission defines BC as "*the concentration of a contaminant at a "pristine" or "remote" site based on contemporary or historical data*" and in order to distinguish between natural concentrations and anthropogenically-induced concentrations, [Hg] of 0.05 μg g^{-1} was established as BC for all regions of the OSPAR area (OSPAR Commission 2008).

The main objective of this manuscript is to evaluate the [Hg] present in the surface layer of coastal sediments from three distinct geographical areas of the North Atlantic, aligned in the track of the Gulf Stream (Mexican south-eastern coast, Azores archipelago, and Portuguese north-western coast), and to compare these levels to TEL and PEL values established in the different SQG. Moreover, this paper also proposes to assess the level of Hg enrichment using the enrichment factor (EF) and the level of contamination through the Geo-accumulation index (Igeo) considering the BCs established by OSPAR.

2. Materials and Methods

2.1. Sampling and sample preparation

The sampling was performed in three different areas in the North Atlantic Ocean aligned in the track of the Gulf Stream (Fig. 2.1). Surface sediment samples were collected, in 0 – 2 cm depth during low tide, in 50 sampling sites (ten in the Southeast Mexican coast, 23 in the Azores archipelago, and 17 in the Northwest Portuguese coast); sediment samples were placed in clean zip bags properly identified and stored in cooler boxes.

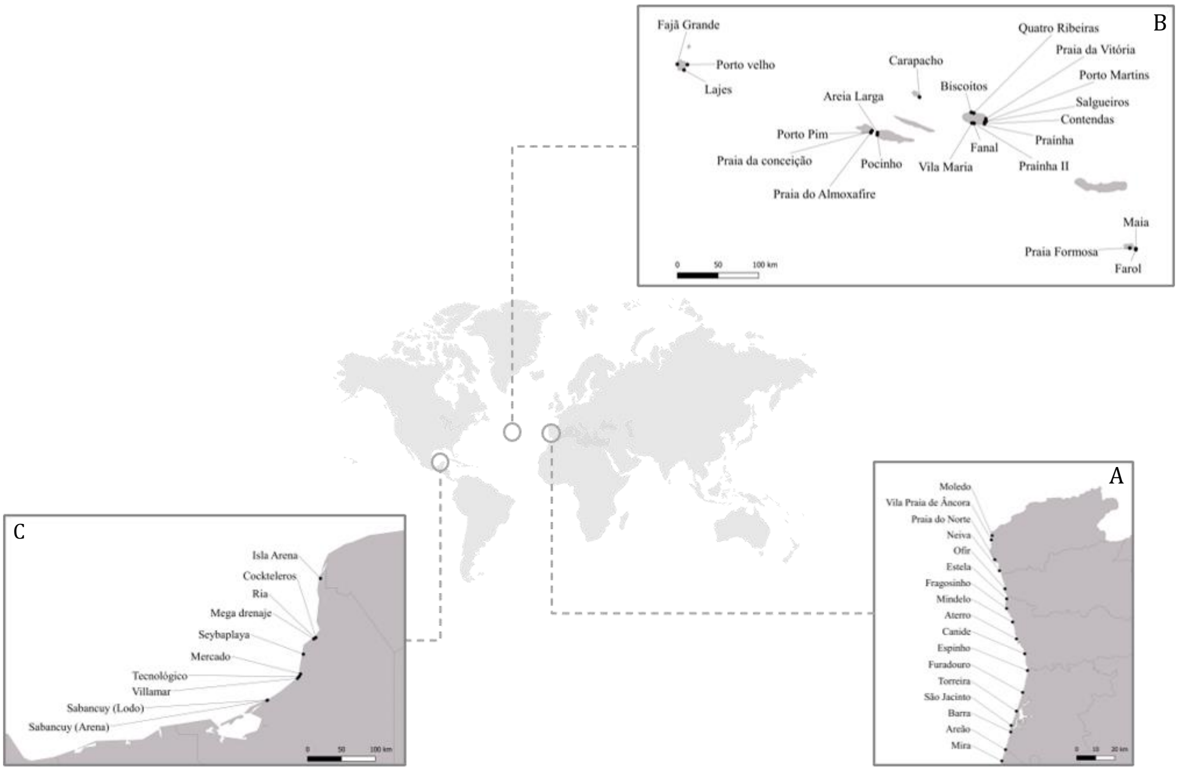


Fig. 2.1 Sampling sites in the Northwest Portuguese coast (A), Azores archipelago (B) and Southeast Mexican coast (C).

Once in the laboratory, all sediment samples were oven dried at 35 ºC for 3 days and subsequently homogenized and sieved (shaken continuously at amplitude 8) with stainless steel sieves with meshes sizes of 2 mm, 1 mm and 63 µm, and classified according to the Wentworth scale. The Wentworth scale groups in the sand class, all particles which diameter varies between 2 mm and 63 µm (Wentworth 1922). However, the sediment fraction with diameter between 2mm and 1mm represents less than 10% of the sediment particles in most of the sampling sites study. Based on that evaluation, the authors considered a <1 mm sediment particle standardization for Hg quantification in the sediments, having the sand sediment fraction with particles between 1 mm and 63 μm and the fine sediments fraction with <63 μm diameter sediment particles.

2.2. Hg quantification

Hg quantification of the sediment samples was performed with the Advanced Mercury Analyzer (AMA-254, made by ALTEC and distributed by LECO). The procedure is based in a pyrolysis process of the sample using a combustion tube heated at 750 ºC under an oxygen atmosphere and the released Hg is trapped in a gold amalgamator and subsequently detected and quantified by atomic absorption spectrometry (Costley et al. 2000).

Sample analysis were made in triplicate to check the reproducibility of the results and three blank analyses (analysis without sample) were performed intercalated among samples to verify that Hg was not being accumulated over the samples. Blank readings typically correspond to values < 0.02 ng of Hg. Based on three times the standard deviation of the blank readings, the limit of detection was calculated to be 0.01 ng g^{-1} of Hg.

Analytical quality of the procedure was validated using the inorganic reference material PACS-3 (Marine Sediment Reference Material for Trace Metals and other Constituents, National Research Council of Canada). Obtained data (2.90±0.06 μg g^{-1} of Hg for PACS-3) and reference values of Hg (3.00±0.5 μg g^{-1} of Hg for PACS-3) were not statistically different ($p>0.05$).

The organic matter content in sediments was determined according to Chen et al (2015), where the sediment sample was re-weighted immediately after the Hg analysis. The sample weight loss after Hg analysis pyrolysis corresponds to the amount of organic matter present in the sediment.

2.3. Assessment of Hg pollution

2.3.1. Enrichment factor (EF)

The enrichment factor is widely used by several authors to study the degree of contamination of the sediment (Molamohyeddin et al. 2017, Ruiz-Fernández et al. 2019, Williams and Antoine 2020). This factor compares the [Hg] present in the sediment sample with a background [Hg] (Molamohyeddin et al. 2017). The enrichment factor can be determined using the formula:

$$EF = \frac{[Hg]_{sample}/[Al]_{sample}}{[Hg]_{background}/[Al]_{background}}$$

where $[Hg]_{sample}$ and $[Hg]_{background}$ are metal concentrations in sample and background reference, respectively and the [Al]sample and [Al]background are the aluminum contents in sample and background reference, respectively. Due to the lack of data on background [Hg] in the North Atlantic, this study used the background [Hg] of 0.05 µg g^{-1} (obtained with a normalization to 5% of aluminum) established by the International Council for the Exploration of the Sea (ICES 2004). The Al values (5.5%) observed in intertidal sediments from southern Gulf of Mexico by Ruiz-Fernández et al. (2019) were used as [Al] in sediment samples. Values of EF were interpreted as suggest by Williams and Antoine (2020); where: EF less than 1 are not considered enriched, EF from 1 to 3 are classified as minor enrichment, 3 – 5 as moderate enrichment, 5 – 10 moderate-severe enrichment, 10 – 25 as severe enrichment and 25 – 50 as very severe enrichment, finally EF above 50 are classified and extremely severe enrichment.

2.3.2. Geoaccumulation index (Igeo)

Geo-accumulation index (Igeo) assesses the Hg concentration of sediments by comparing present elemental concentrations with background levels. The present calculation enables the assessment of contamination by comparing the present and crustal average values of elemental concentrations (Müller 1986). The Igeo can be calculated by using the following formula:

$$I_{geo} = \log_2\left(\frac{[\mathrm{Hg}]_{\mathrm{sample}}}{1.5\ [\mathrm{Hg}]_{\mathrm{baseline}}}\right)$$

Here, $[Hg]_{sample}$ is the measured concentration of the metal in the sediment, $[Hg]_{baseline}$ is the geochemical background value for the metal, and the factor 1.5 is introduced to include possible differences in the background values due to lithological variations. The Igeo index was calculated using the same background value (0.05 µg g^{-1}) used for the enrichment factor, and the results are interpreted in accordance with the seven levels (from uncontaminated to extremely contaminated) of metal pollution, suggested by Bolaños-Álvarez et al. (2016); where, Igeo <0 practically unpolluted, 0 – 1 unpolluted to moderately polluted, 1 – 2 moderately polluted, 2 – 3 moderately to strongly polluted, 3 – 4 strongly polluted, 4 – 5 strongly to very strongly polluted, and >5 very strongly polluted.

2.4. Statistical analysis

Data normality was examined through Shapiro-Wilk test. Since data were not normally distributed, non-parametric statistical methods, Wilcoxon on rank sum test, and Kruskal–Wallis test were used to compare the granulometric composition of the surface sediments and [Hg] present in sediment fraction of the different sampling sites. Spearman correlation was used to test the correlation between [Hg] and the organic matter (%) and [Hg] and the fine sediment fraction (%).

Statistical analyses were performed using GraphPad Prism (version 6.01). Statistical significance was defined as $p < 0.05$. [Hg] data are presented as mean value ± standard error (mean ± SE).

3. Results and Discussion

The granulometric composition of the surface sediments collected in all sampling sites (Fig. 2.2), using the Wentworth scale (Wentworth 1922), show significant differences ($p<0.05$) between the sampling sites of Azores sea and Mexico coast. Significant differences ($p<0.05$) were also found between the sampling sites from the North-western coast of Portugal and the sampling sites of Mexico.

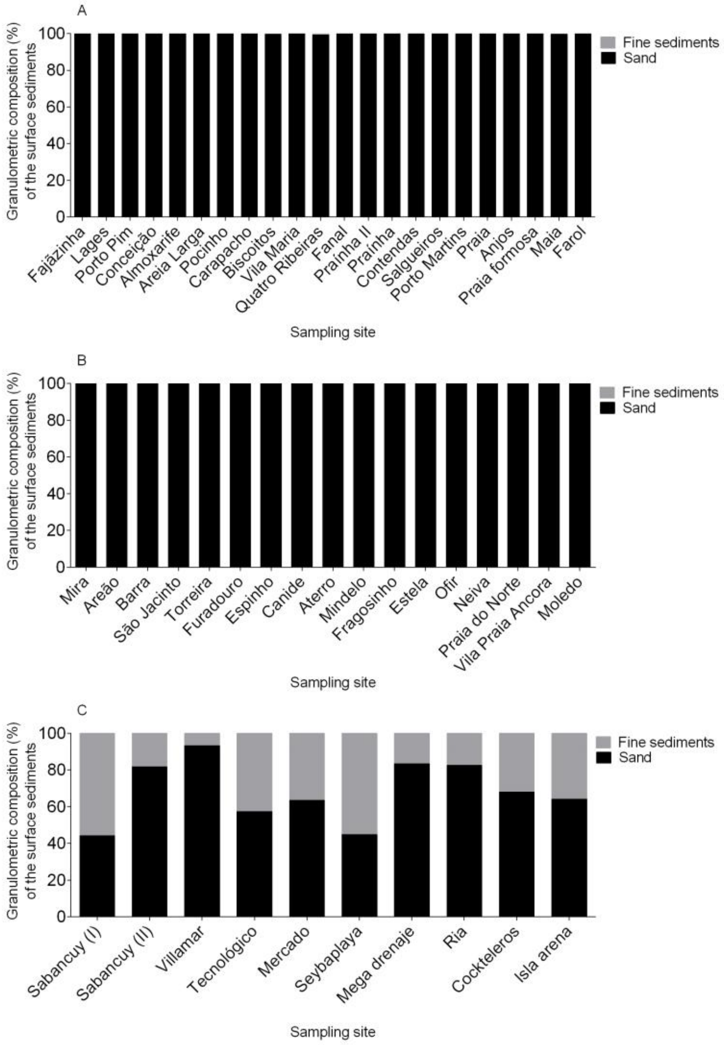


Fig. 2.2 Granulometric composition (%) of the surface sediment in the different sampling sites (A – Azores archipelago B – Northwest Portuguese coast and C – Southeast Mexican coast).

The contribution of the grain-size fraction 1 mm – 63 μm (sand) is more than 99% in all sites from Azores sea (Fig. 2.2A) and from North-western coast of Portugal (Fig. 2.2B). On the other hand, the sand fraction in the sampling sites from Mexico (Fig. 2.2C) ranged between 44% and 93%. Sediment deposition can be influenced by meteorological events and oceanographic characteristics such as waves, tides and wind currents (Chowdhury et al. 2020). For instance, the lack of wave exposure frequently leads to settling of fine sediments (Mangor 2007). The western littoral of Portugal has a NW dominant wave climate with 2 – 2.5 m mean wave height (Ramos Pereira et al. 2005) and in the Azores sea the average winter time wave height is about 3.8 m and the average for the total year is of about 2.7 m (Rusu and Guedes Soares 2012). On the other hand, the wave heights in Gulf coastal environments are less than 0.6 m high (Mendelssohn et al. 2017). Thus, the differences observed in the granulometric composition between the Azores and Mexico and the Portuguese mainland and Mexico, suggests that the size of the sediment grains are strongly controlled by the waves. Deposition and re-suspension of suspended particulate matter (SPM), including SPM transported along the Gulf stream could also be considered but is the influence of the wave energy on the dispersion and remobilization of sediments that has already been observed by other authors. Calliari et al. (2009) states that a reduction in the energy of local waves can lead to a greater deposition of sediments in Cassino beach, Brazil. Manighetti et al. (1999) found that the large waves formed during Cyclone Drena in Hauraki Gulf, New Zealand, stirred the bottom sediments to more than 100 m in depth; however, in shallow waters the effect of waves on the remobilization of sediments is more pronounced.

Regarding [Hg] obtained in the two fractions of the surface sediments (Fig. 2.3), the average [Hg] in the sampling sites of the Azores (Fig. 2.3A) varied between 0.01 μg g^{-1} and 1.31 μg g^{-1} in the fine sediments, while in the sand sediments the [Hg] ranged between 0.01 μg g^{-1} and 0.2 μg g^{-1}. At the same time, the maximum and minimum [Hg] found in the Portuguese NW coast (Fig. 3B) was 0.17±0.01 μg g^{-1} and 0.01±0.001 μg g^{-1} in the fine sediments whereas the sand fraction [Hg] varied between 0.0001 μg g^{-1} and 0.003±0.001 μg g^{-1}. Concerning the Mexican sampling sites, [Hg] ranged between 0.02±0.00 μg g^{-1} and 0.13±0.01 μg g^{-1} in the fine sediments and between 0.002±0.000 μg g^{-1} and 0.025±0.002 μg g^{-1} in the sand sediments. The increase in [Hg] in the fine sediment fraction was significantly different ($p<0.05$) in all sampling sites compared to [Hg] present in the sand fraction from the same sampling site. The Hg content in sediments is often associated to the grain size and the concentration of organic matter (Bravo et al. 2011). The differences between the sediment fractions in this study can be explained by the fact that the small particles of sediment (<63 μm) present higher capacity to adsorb Hg, given their higher surface area per unit of mass ratio (Pereira et al. 1998, Green-Ruiz et al. 2005) and its higher organic matter content comparatively to the other sediment fractions (Bengtsson and Picado 2008).

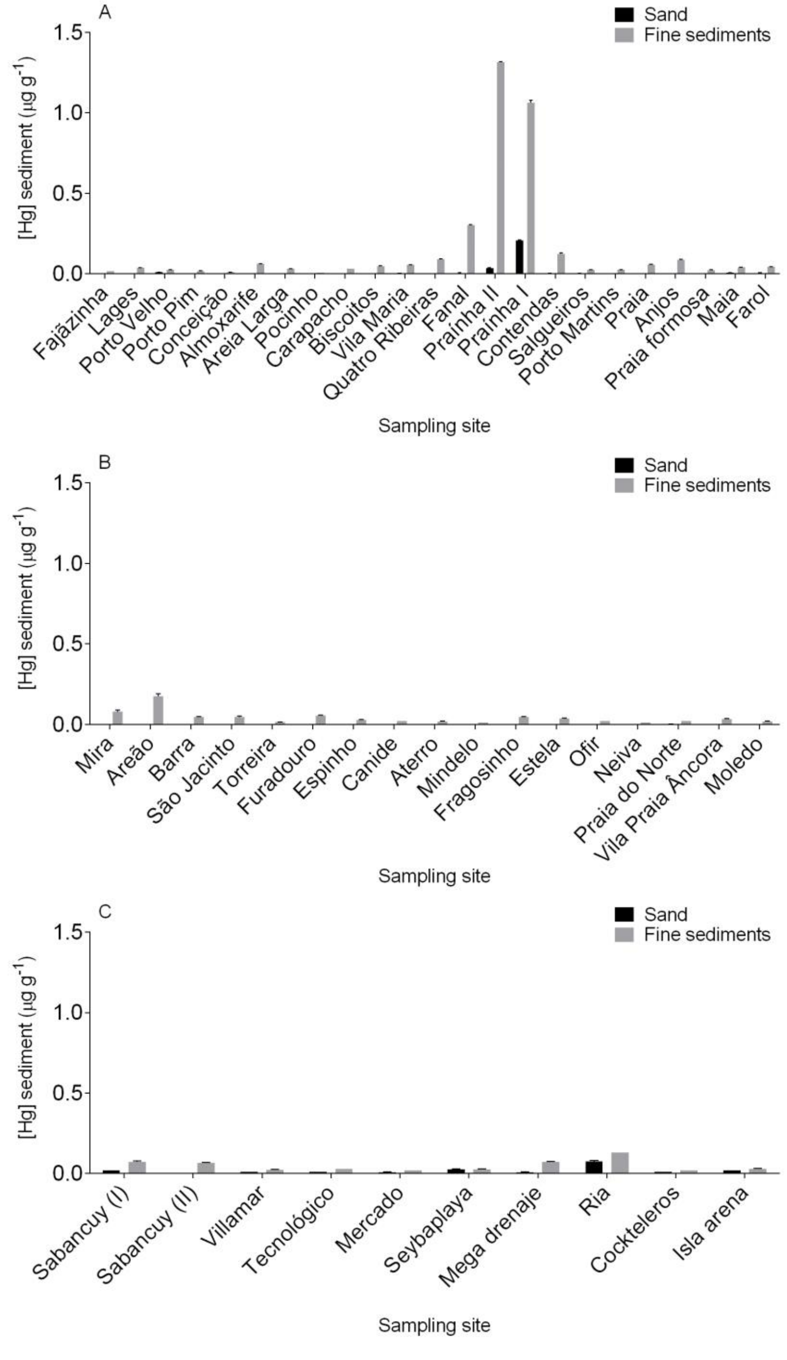


Fig. 2.3 Hg concentration (μg g^{-1}) in the sediment fraction (sand and fine sediments) from the different sampling sites (A – Azores archipelago B – Northwest Portuguese coast and C – Southeast Mexican coast).

Considering the fine sediments in the Azores sea (Fig. 2.3A), the sampling sites with higher [Hg] (Praínha I and II) are located close to the shipwreck dated from the last quarter of the fifteenth century or the first quarter of the sixteenth century. During the archaeological survey, more than 150ml of elemental Hg was recovered from the ship's hull (Garcia and Monteiro 2001). The presence of Hg in the ship bilge suggests that the vessel was carrying or had been carrying in the past a cargo with great quantities of that metal, which was used mainly as a component for the extraction of silver and gold in Central and South America (Garcia and Monteiro 2001). Shipwrecks can bring benefits to the economy of the places where they are found (e.g. through the cultural tourism industry). However, they may likewise represent local sources of pollution due to the potential release of contaminants into the surrounding environment (Ndungu et al. 2017). The German submarine U-864, which was sunk in 1945 by a British submarine on the Norwegian island of Fedje in the North Sea, carried 65 t (estimated load) of elementary Hg. After its discovery in 2003, the analysis of [Hg] present in the surrounding sediments revealed concentrations higher than 200 μg g^{-1} in the vicinity of the wreck and 0.01 μg g^{-1} in sampling sites further from the wreck (Ndungu et al. 2017). Thus, a possible explanation for the high [Hg] found in Fanal (Fig. 3A) lower than the sampling sites near the wreck, but higher than [Hg] in other sampling sites is the existence of a contamination gradient pointing the wreck as the hotspot.

Surprisingly, in the north-western Portuguese coast, Areão and Mira were sampling sites with the highest [Hg] (0.17±0.01 μg g^{-1} and 0.08±0.01 μg g^{-1}, respectively; (Fig. 2.3B). Barra was expected to be the sampling site with the highest [Hg] in sediments due to the proximity to the only opening between the Atlantic Ocean and the Aveiro lagoon. For almost five decades (from the 1950s to 1994), this lagoon received continuous discharges of Hg from a chlor-alkali plant (Pereira et al. 2009, Matos et al. 2016), leading to a deposition of discharged Hg over the time. Laranjo Bay is currently the most contaminated area in the Aveiro lagoon, from where Hg can be dispersed to the rest of the lagoon towards the mouth of the estuary, mainly in periods of increased water currents, (Pato et al. 2008). Areão is the sampling site located close to Barra on the south side, and Mira is located south of Areão. Statistically significant differences ($p<0.05$) were found in both locations when compared to Barra. These values may be explained by the fact that the Portuguese coast current flows predominantly from North to South (Álvarez-Salgado et al. 2003), which may potentially transport Hg from Aveiro lagoon to the Atlantic Ocean, and over time leading to higher [Hg] in the adjacent coastal areas. The lack of studies in these areas makes it difficult to compare [Hg] at present with levels of Hg from previous studies. Despite that, [Hg] found in the superficial sediments of sampling sites on the north-western coast of Portugal are in line with [Hg] found in two recent studies carried out along the Portuguese coast. Coelho et al. (2006) quantified [Hg] in sediments from eight sampling sites spread throughout the Portuguese coast and found that [Hg] in these sampling sites did not exceed 0.1 μg g^{-1} and Cairrão et al. (2007) found [Hg] between 0.0010±0.0001 and 0.112±0.007 µg g^{-1} in 11 sampling sites along the NW Portuguese coast.

Regarding the sampling sites located in Mexico, Ría was the one with the highest [Hg] in both fractions, being 0.13±0.01 µg g^{-1} in the fine sediment fraction, and 0.08±0.01 µg g^{-1} in the sand fraction. These [Hg] are significantly higher ($p<0.05$) than [Hg] found in all other sampling sites. Ría is located at the mouth of the natural channel "la Ría de San Francisco" (Ramirez et al. 2019). Between 1540 and the middle of the 20th century, there was no plumbing system in Campeche city that would allow the collection of domestic wastewater and rainwater (Arriaga et al. 2020). The absence of such system charged "la Ría" as the drainage channel for the wastewater from Campeche city and its periphery (Ruiz Martínez 2014). Therefore, [Hg] present in this sampling site may be related to runoff over time from “la Ría”. As in the sampling sites of the Azores and the Portuguese coast, it is also difficult to compare the present results due to the lack of studies in these areas. Notwithstanding, [Hg] of approximately 0.03 µg g^{-1} was reported in sediments from an area very close to the southernmost sampling site of the present study (Isla arena) by Ruiz-Fernández et al. (2019), which is similar to the Hg levels obtained in six of the ten sampling sites.

Some authors such as Sunderland et al. (2006) and Chakraborty et al. (2014) reported a strong relationship between [Hg] and organic matter, as well between [Hg] and fine sediment particles from coastal sediments. [Hg] found in the fine sediment fraction from the different areas do not show any correlation with the organic matter or with the amount of fine fraction in each location, probably due to the heterogeneity of the sampling sites. Some of the sites from this study are in urban areas (e.g. Praínha, Barra, Ria) while others are in more remote areas. The proximity of sampling sites to urban areas can result in greater enrichment of organic matter due to human activity (Soana et al. 2012), on the other hand, the organic matter present in the sediments of the sampling sites located in areas less impacted by human activity can be influenced by rivers (Lee et al. 2019) or continental runoff (González-Macías et al. 2006). Despite the lack of correlation between [Hg] and organic matter and the amount of sludge fraction (Fig. 2.4), Hg seems to demonstrate a greater affinity to organic matter, especially in the sampling sites located on the Portuguese coast (Fig. 2.4B) and Mexican coast (Fig. 2.4C).

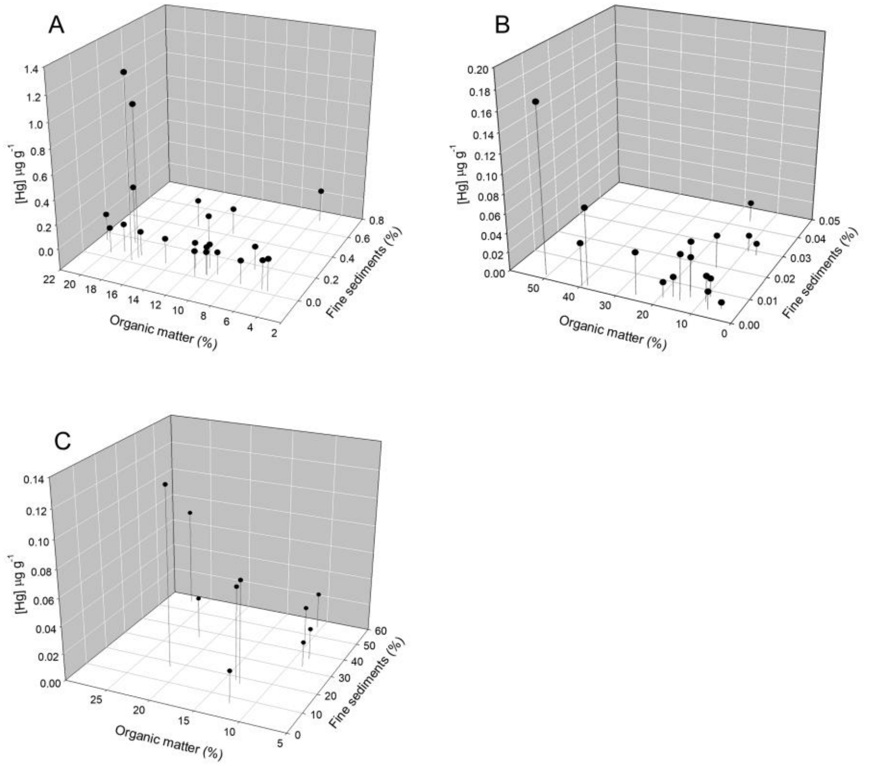


Fig. 2.4 Relationship between Hg concentration, organic matter (%) and mud fraction (%) from the different sampling sites (A – Azores archipelago B – Northwest Portuguese coast and C – Southeast Mexican coast).

Considering the TEL values of the various guidelines (NOAA SQuiRTs, EU-WFD, the Canadian SQG – Table 2.1) and the [Hg] obtained in the 23 sampling sites in the Azores, it is possible to verify that only the 3 sampling sites influenced by the shipwreck (Fanal, Prainha and Praínha II) presented higher values than the TEL established by the NOAA SQuiRTs. However, this number drops to 2 (Praínha and Praínha II) when compared to the maximum value of TEL established by EU-WFD.

Table 2.1 TEL and PEL values (µg g⁻¹) established by the different guidelines.

Guideline	Criteria	TEL	PEL	Reference
NOAA SQuiRTs	Establish associations between concentrations of chemicals in sediments and adverse biological effects	0.13	0.7	(Buchman 2008)
EU-WFD	Establish associations between concentrations of chemicals in sediments and adverse biological effects	0.05 - 0.5	▬	(Bignert et al. 2004)
Canadian SQG	Establish associations between concentrations of chemicals in sediments and adverse biological effects	▬	0.7	(CCME 1999)

In the sampling sites of Portuguese coast, Areão has a concentration higher than the values established by the TEL of NOOA squirts but lower than the EU-WFD and none of Mexico's sampling sites have [Hg] higher than those established for TEL. At the same time and considering the values established for the PEL by NOOA SQuiRTs and Canadian SQG, only Praínha and Praínha II (located in the Azores) exceed the established values.

Although the [Hg] present in the fine sediment fraction of some sampling sites in the Azores and the Portuguese coast exceeds the values established for the PEL in the different guidelines, this fraction represents less than 1% of the total standardized sediment in these places. Thus, since the total [Hg] of the sediment can be understood as the sum of the [Hg] present in the different fractions (fine sediment and sand) and taking into account their percentage, it is possible to verify a decrease in the [Hg] between the fraction of fine sediment and the assumed total (<1 mm) fraction (Table 2.2). Thus, considering the [Hg] obtained for the total sediment fractions, or even for the total sediments considering overall <2 mm diameter sediment particles, none of the 50 sampling sites in this study have [Hg] above levels where toxic effects to organisms could be observed (PEL).

Table 2.2 Hg concentration percentage of each sediment fraction and Hg concentration ($\mu g\ g^{-1}$) obtained for the total standardized sediment (<1.0 mm particle diameter), taking into account Hg concentration ($\mu g\ g^{-1}$) and mass (weight) percentage of every fraction.

	Sampling sites	**[Hg] ($\mu g\ g^{-1}$)**		**Fraction (%)**		**Obained [Hg]$_{\text{total fraction}}$**
		Sand	**Fine sediments**	**Sand**	**Fine sediments**	
Azores archipelago	Fajãzinha	0.001	0.017	99.975	0.025	0.001
	Lages	0.003	0.039	99.936	0.064	0.003
	Porto Pim	0.003	0.018	99.842	0.158	0.003
	Conceição	0.002	0.012	99.964	0.036	0.002
	Almoxarife	0.001	0.062	99.979	0.021	0.001
	Areia Larga	0.001	0.031	99.995	0.005	0.001
	Pocinho	0.000	0.008	99.943	0.057	0.000
	Carapacho	0.001	0.031	99.958	0.042	0.001
	Biscoitos	0.002	0.049	99.579	0.421	0.002
	Vila Maria	0.005	0.057	99.967	0.033	0.005
	Quatro Ribeiras	0.005	0.090	99.302	0.698	0.006
	Fanal	0.006	0.302	99.841	0.159	0.007
	Praínha II	0.037	1.314	99.983	0.017	0.037
	Praínha	0.209	1.064	99.953	0.047	0.209
	Contendas	0.006	0.126	99.777	0.223	0.006
	Salgueiros	0.003	0.027	99.931	0.069	0.003
	Porto Martins	0.002	0.027	99.944	0.056	0.002
	Praia	0.006	0.058	99.929	0.071	0.006
	Anjos	0.002	0.088	99.983	0.017	0.002

	Praia formosa	0.003	0.024	99.909	0.091	0.003
	Maia	0.006	0.040	99.581	0.419	0.007
	Farol	0.006	0.044	99.864	0.136	0.006
Northwest Portuguese coast	Mira	0.000	0.080	99.999	0.001	0.000
	Areão	0.000	0.172	99.998	0.002	0.000
	Barra	0.000	0.044	99.998	0.002	0.000
	São Jacinto	0.000	0.045	99.999	0.000	0.000
	Torreira	0.000	0.016	99.997	0.003	0.000
	Furadouro	0.000	0.057	99.994	0.006	0.000
	Espinho	0.001	0.027	99.995	0.005	0.001
	Canide	0.001	0.019	99.992	0.008	0.001
	Aterro	0.001	0.019	99.998	0.003	0.001
	Mindelo	0.001	0.007	99.996	0.004	0.001
	Fragosinho	0.000	0.047	99.996	0.004	0.000
	Estela	0.000	0.035	99.992	0.008	0.000
	Ofir	0.001	0.021	99.995	0.005	0.001
	Neiva	0.001	0.013	99.972	0.028	0.001
	Praia do Norte	0.003	0.021	99.957	0.043	0.003
	Vila Praia Ancora	0.000	0.034	99.981	0.019	0.000
	Moledo	0.002	0.017	99.971	0.029	0.002
Southeast Mexican coast	Sabancuy (I)	0.018	0.072	44.213	55.787	0.048
	Sabancuy (II)	0.002	0.068	81.775	18.225	0.014
	Villamar	0.010	0.024	93.267	6.733	0.011
	Tecnológico	0.010	0.030	57.354	42.646	0.019
	Mercado	0.009	0.023	63.572	36.428	0.014
	Seybaplaya	0.026	0.027	44.813	55.187	0.026
	Mega drenaje	0.010	0.075	83.259	16.741	0.021
	Ria	0.078	0.129	82.499	17.501	0.087
	Cockteleros	0.013	0.018	68.009	31.991	0.015
	Isla arena	0.021	0.031	64.161	35.839	0.025

The EF analysis (Table 2.3) suggests that [Hg] present in the coastal sediments of 86% of the sampling sites originates from natural processes (not enriched). The other 14% are sampling sites close to Hg hotspots (shipwreck, Aveiro lagoon and an Urban channel in Campeche city) and their degree of enrichment varies between minor enrichment and moderate-severe enrichment, depending on the proximity and type of hotspot. On the other hand, the Igeo values of the sediments show that the contamination near the Hg hotspots varied from moderately polluted to heavily polluted in the sampling sites close to the shipwreck in the Azores. In sampling sites near the Mainland Portuguese Coast hotspot (Ria de Aveiro lagoon), the degree of contamination ranged from unpolluted to moderately polluted and moderately polluted. The most contaminated site in Southern Mexico is considered unpolluted to moderately polluted and it is found close the potential main source of contamination of coastal sediments in that area.

Table 2.3 Enrichment factor (value and class) and Igeo (value and class) for each sampling site

	Sampling sites	EF		Igeo	
		Value	Class	Value	Class
Azores archipelago	Fajãzinha	0	Not enriched	-2	Practically unpolluted
	Lages	0	Not enriched	-1	Practically unpolluted
	Porto Pim	0	Not enriched	-2	Practically unpolluted
	Conceição	0	Not enriched	-3	Practically unpolluted
	Almoxarife	0	Not enriched	0	Practically unpolluted
	Areia Larga	0	Not enriched	-1	Practically unpolluted
	Pocinho	0	Not enriched	-3	Practically unpolluted
	Carapacho	0	Not enriched	-1	Practically unpolluted
	Biscoitos	0	Not enriched	-1	Practically unpolluted
	Vila Maria	0	Not enriched	0	Practically unpolluted
	Quatro Ribeiras	0	Not enriched	0	Unpolluted to moderately polluted
	Fanal	1	Minor enrichement	2	Moderately polluted
	Praínha II	5	Moderate-severe enrichement	4	Strongly polluted
	Praínha	4	Moderate enrichement	4	Strongly polluted
	Contendas	1	Minor enrichement	1	Unpolluted to moderately polluted
	Salgueiros	0	Not enriched	-1	Practically unpolluted
	Porto Martins	0	Not enriched	-1	Practically unpolluted
	Praia	0	Not enriched	0	Practically unpolluted
	Anjos	0	Not enriched	0	Unpolluted to moderately polluted
	Praia formosa	0	Not enriched	-2	Practically unpolluted
	Maia	0	Not enriched	-1	Practically unpolluted
	Farol	0	Not enriched	-1	Practically unpolluted
Northwest Portuguese coast	Mira	0	Not enriched	0	Unpolluted to moderately polluted
	Areão	1	Minor enrichement	1	Moderately polluted
	Barra	0	Not enriched	-1	Practically unpolluted
	São Jacinto	0	Not enriched	-1	Practically unpolluted
	Torreira	0	Not enriched	-2	Practically unpolluted
	Furadouro	0	Not enriched	0	Practically unpolluted
	Espinho	0	Not enriched	-1	Practically unpolluted
	Canide	0	Not enriched	-2	Practically unpolluted
	Aterro	0	Not enriched	-2	Practically unpolluted
	Mindelo	0	Not enriched	-4	Practically unpolluted
	Fragosinho	0	Not enriched	-1	Practically unpolluted
	Estela	0	Not enriched	-1	Practically unpolluted

	Ofir	0	Not enriched	-2	Practically unpolluted
	Neiva	0	Not enriched	-3	Practically unpolluted
	Praia do Norte	0	Not enriched	-2	Practically unpolluted
	Vila Praia Ancora	0	Not enriched	-1	Practically unpolluted
	Moledo	0	Not enriched	-2	Practically unpolluted
Southeast Mexican coast	Sabancuy (I)	0	Not enriched	0	Practically unpolluted
	Sabancuy (II)	0	Not enriched	0	Practically unpolluted
	Villamar	0	Not enriched	-2	Practically unpolluted
	Tecnológico	0	Not enriched	-1	Practically unpolluted
	Mercado	0	Not enriched	-2	Practically unpolluted
	Seybaplaya	0	Not enriched	-2	Practically unpolluted
	Mega drenaje	0	Not enriched	0	Practically unpolluted
	Ria	1	Minor enrichement	1	Unpolluted to moderately polluted
	Cockteleros	0	Not enriched	-2	Practically unpolluted
	Isla arena	0	Not enriched	-1	Practically unpolluted

4. Conclusion

Intertidal sediments are an important matrix for assessing and identifying signs of anthropogenic Hg pollution. [Hg] analysis in the different sediment fractions from 50 sampling sites divided into three distinct areas of the North Atlantic (Portuguese mainland, Azores and Mexico), indicated that the fine sediments fraction is the one with the highest [Hg]. The [Hg] found in the fine sediments reflect the proximity to Hg contamination hotspots or locations with a history of Hg contamination. Nevertheless, this fraction represents less than 1% of the total sediment fraction which means that the [Hg] present in the sediment (as a whole) is significantly lower than those of the fine sediments.

Regarding the ecotoxicological aspects and considering the [Hg] present in the fine sediments, the PEL values set by international guidelines are exceeded in only two of fifty sampling sites present in this study. However, considering the [Hg] present in the total sediment fraction, adverse biological effects are not expected to occur due to the Hg present in the sediments.

Finally, the enrichment factor and geoaccumulation index indicated that the coastal sediments from most of the sampling sites (86%) are enriched with Hg from natural inputs and only sampling sites with some proximity to Hg hotspots show some degree of pollution that can vary from unpolluted to moderately polluted to strongly polluted.

Conflict of interest

The authors declare that they have no conflict of interests (financial or non-financial).

Acknowledgements

Thanks are due for the financial support to CESAM (UIDB/50017/2020+UIDP/50017/2020), to FCT/MEC through national funds. The authors also thank FCT (Fundação para a Ciência e Tecnologia) for the PhD grant of Hugo C. Vieira (PD/BD/127808/2016). Maria D. Bordalo is funded by national funds (OE), through FCT – Fundação para a Ciência e a Tecnologia, I.P., in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19.

References

Álvarez-Salgado, X. A., F. G. Figueiras, F. F. Pérez, S. Groom, E. Nogueira, A. V. Borges, L. Chou, C. G. Castro, G. Moncoiffé, A. F. Ríos, A. E. J. Miller, M. Frankignoulle, G. Savidge, and R. Wollast. 2003. The Portugal coastal counter current off NW Spain: new insights on its biogeochemical variability. Progress in Oceanography 56:281-321.

Arriaga, E. R., B. E. V. Serratos, G. P. Vanegas, and E. A. M. Che. 2020. Building adaptation to extreme rain effects in San Francisco de Campeche, Mexico. Atmósfera 33:159-174.

Bengtsson, G., and F. Picado. 2008. Mercury sorption to sediments: Dependence on grain size, dissolved organic carbon, and suspended bacteria. Chemosphere 73:526-531.

Bignert, A., D. Cossa, R. Emmerson, R. Fryer, C. Füll, J. Fumega, R. Laane, H. Calls, B. McHugh, and B. Miller. 2004. OSPAR/ICES workshop on the evaluation and update of background reference concentrations (B/RCs) and ecotoxicological assessment criteria (EACs) and how these assessment tools should be used in assessing contaminants in water, sediment, and biota: Workshop The Hague, 9-13 February 2004. Final Report.

Bolaños-Álvarez, Y., C. M. Alonso-Hernández, R. Morabito, M. Díaz-Asencio, V. Pinto, and M. Gómez-Batista. 2016. Mercury contamination of riverine sediments in the vicinity of a mercury cell chlor-alkali plant in Sagua River, Cuba. Chemosphere 152:376-382.

Bonsignore, M., D. S. Manta, M. Barsanti, F. Conte, I. Delbono, M. Horvat, E. M. Quinci, A. Schirone, Y. Shlyapnikov, and M. Sprovieri. 2020. Mercury isotope signatures in sediments and marine organisms as tracers of historical industrial pollution. Chemosphere 258:127435.

Bravo, A. G., S. Bouchet, D. Amouroux, J. Poté, and J. Dominik. 2011. Distribution of mercury and organic matter in particle-size classes in sediments contaminated by a waste water treatment plant: Vidy Bay, Lake Geneva, Switzerland. Journal of Environmental Monitoring 13:974-982.

Buchman, M. F. 2008. Screening Quick Reference Tables (SQuiRTs).

Cairrão, E., M. J. Pereira, M. R. Pastorinho, F. Morgado, A. M. V. M. Soares, and L. Guilhermino. 2007. *Fucus* spp. as a Mercury Contamination Bioindicator in Costal Areas (Northwestern Portugal). Bull Environ Contam Toxicol 79:388-395.

Calliari, L. J., J. C. Winterwerp, E. Fernandes, D. Cuchiara, S. B. Vinzon, M. Sperle, and K. T. Holland. 2009. Fine grain sediment transport and deposition in the Patos Lagoon–Cassino beach sedimentary system. Continental Shelf Research 29:515-529.

CCME. 1999. Canadian sediment quality guidelines for the protection of aquatic life: Mercury. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg.

Chakraborty, P., P. V. Raghunadh Babu, K. Vudamala, D. Ramteke, and K. Chennuri. 2014. Mercury speciation in coastal sediments from the central east coast of India by modified BCR method. Marine Pollution Bulletin 81:282-288.

Chen, J., P. Chakravarty, G. R. Davidson, D. G. Wren, M. A. Locke, Y. Zhou, G. Brown Jr, and J. V. Cizdziel. 2015. Simultaneous determination of mercury and organic carbon in sediment and soils using a direct mercury analyzer based on thermal decomposition–atomic absorption spectrophotometry. Analytica Chimica Acta 871:9-17.

Chowdhury, S. R., A. Talukder, S. M. Sharifuzzaman, and M. S. Hossain. 2020. Onshore barrier affects sediment deposition in a subtropical mudflat. Regional Studies in Marine Science 37:101344.

Coelho, J. P., J. Pimenta, R. Gomes, C. M. Barroso, M. E. Pereira, M. A. Pardal, and A. Duarte. 2006. Can *Nassarius reticulatus* be used as a bioindicator for Hg contamination? Results from a longitudinal study of the Portuguese coastline. Marine Pollution Bulletin 52:674-680.

Costley, C. T., K. F. Mossop, J. R. Dean, L. M. Garden, J. Marshall, and J. Carroll. 2000. Determination of mercury in environmental and biological samples using pyrolysis atomic absorption spectrometry with gold amalgamation. Analytica Chimica Acta 405:179-183.

Esen, E., F. Kucuksezgin, and E. Uluturhan. 2010. Assessment of trace metal pollution in surface sediments of Nemrut Bay, Aegean Sea. Environ Monit Assess 160:257.

EU. 2008. Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. Official Journal of the European Union 348:84-97.

Fitzgerald, W. F., C. H. Lamborg, and C. R. Hammerschmidt. 2007. Marine biogeochemical cycling of mercury. Chemical Reviews 107:641-662.

Garcia, C., and P. Monteiro. 2001. The excavation and dismantling of Angra D, a probable Iberian seagoing ship, Angra bay, Terceira Island, Azores, Portugal. Preliminary assessment. Pages 431-447 in International Symposium on Archaeology of Medieval and Modern Ships of Iberian-Atlantic Tradition. Trabalhos de Arqueologia.

González-Macías, C., I. Schifter, D. B. Lluch-Cota, L. Méndez-Rodríguez, and S. Hernández-Vázquez. 2006. Distribution, Enrichment and Accumulation of Heavy Metals in Coastal Sediments of Salina Cruz Bay, México. Environ Monit Assess 118:211-230.

Green-Ruiz, C., J. Ruelas-Inzunza, and F. Paez-Osuna. 2005. Mercury in surface sediments and benthic organisms from Guaymas Bay, east coast of the Gulf of California. Environ Geochem Health 27:321-329.

Hammerschmidt, C. R., and W. F. Fitzgerald. 2006. Bioaccumulation and trophic transfer of methylmercury in Long Island Sound. Archives of Environmental Contamination and Toxicology 51:416-424.

Hassan, H., A. A. Elezz, M. Abuasali, and H. AlSaadi. 2019. Baseline concentrations of mercury species within sediments from Qatar's coastal marine zone. Marine Pollution Bulletin 142:595-602.

ICES. 2004. Report of the ICES Advisory Committee on the Marine Environment, 2004. ICES Advice. Volume 1, Number 1. 283 pp.

Lee, J., B.-O. Kwon, B. Kim, J. Noh, K. Hwang, J. Ryu, J. Park, S. Hong, and J. S. Khim. 2019. Natural and anthropogenic signatures on sedimentary organic matters across varying intertidal habitats in the Korean waters. Environment International 133:105166.

Mangor, K. 2007. General guidelines for good quality artificial beaches and lagoons, and case stories. DHI Water and Environment.

Manighetti, B., and L. Carter. 1999. Across-shelf sediment dispersal, Hauraki Gulf, New Zealand. Marine Geology 160:271-300.

Mason, R. P., W. F. Fitzgerald, and F. M. M. Morel. 1994. The biogeochemical cycling of elemental mercury: Anthropogenic influences. Geochimica et Cosmochimica Acta 58:3191-3198.

Matos, P., E. Sousa, M. A. Pardal, E. Pereira, and P. G. Cardoso. 2016. Structural and Functional Responses of Macrobenthic Communities to Mercury Contamination. Water, Air, & Soil Pollution 227:41.

Mendelssohn, I. A., M. R. Byrnes, R. T. Kneib, and B. A. Vittor. 2017. Coastal Habitats of the Gulf of Mexico. Pages 359-640 in C. H. Ward, editor. Habitats and Biota of the Gulf of Mexico: Before the Deepwater Horizon Oil Spill: Volume 1: Water Quality, Sediments, Sediment Contaminants, Oil and Gas Seeps, Coastal Habitats, Offshore Plankton and Benthos, and Shellfish. Springer New York, New York, NY.

Molamohyeddin, N., H. Ghafourian, and S. M. Sadatipour. 2017. Contamination assessment of mercury, lead, cadmium and arsenic in surface sediments of Chabahar Bay. Marine Pollution Bulletin 124:521-525.

Müller, G. 1986. Schadstoffe in sedimenten-sedimente als schadstoffe. Mitt, Österreichische Geologische Gesellschaft 79:107-126.

Ndungu, K., B. A. Beylich, A. Staalstrøm, S. Øxnevad, J. A. Berge, H. F. V. Braaten, M. Schaanning, and R. Bergstrøm. 2017. Petroleum oil and mercury pollution from shipwrecks in Norwegian coastal waters. Science of The Total Environment 593-594:624-633.

Nyström, J. 2018. The OSPAR list of chemicals for priority action: Suggestions for future actions. Uppsala universitet.

OSPAR Commission. 2008. Co-ordinated Environmental Monitoring Programme (CEMP) Assessment Manual for contaminants in sediment and biota. OSPAR Commission.

Palma, C., A. I. Lillebo, M. Valenca, E. Pereira, M. P. Abreu, and A. C. Duarte. 2009. Mercury in sediments of the Azores deep sea platform and on sea mounts south of the archipelago--assessment of background concentrations. Mar Pollut Bull 58:1583-1587.

Pato, P., M. Válega, E. Pereira, C. Vale, and A. C. Duarte. 2008. Inputs from a Mercury-Contaminated Lagoon: Impact on the Nearshore Waters of the Atlantic Ocean. Journal of Coastal Research:28-38.

Pereira, M. E., A. C. Duarte, G. E. Millward, S. N. Abreu, and C. Vale. 1998. An estimation of industrial mercury stored in sediments of a confined area of the Lagoon of Aveiro (Portugal). Water Science and Technology 37:125-130.

Pereira, M. E., A. I. Lillebø, P. Pato, M. Válega, J. P. Coelho, C. B. Lopes, S. Rodrigues, A. Cachada, M. Otero, M. A. Pardal, and A. C. Duarte. 2009. Mercury pollution in Ria de Aveiro (Portugal): a review of the system assessment. Environ Monit Assess 155:39-49.

Ramirez, M. M. B., R. D. Caamal, and J. R. von Osten. 2019. Occurrence and seasonal distribution of microplastics and phthalates in sediments from the urban channel of the Ria and coast of Campeche, Mexico. Science of The Total Environment 672:97-105.

Ramos Pereira, A., J. Trindade, and M. Neves. 2005. Portugal: coastal dynamics: Field Trip Guide-A1.in Sixth International Conference on geomorphology. International Association of Geomorphologists.

Ruiz-Fernández, A. C., M. Rangel-García, L. H. Pérez-Bernal, P. G. López-Mendoza, A. Gracia, P. Schwing, D. Hollander, F. Páez-Osuna, J. G. Cardoso-Mohedano, T. Cuellar-Martinez, and J. A. Sanchez-Cabeza. 2019. Mercury in sediment cores from the southern Gulf of Mexico: Preindustrial levels and temporal enrichment trends. Marine Pollution Bulletin 149:110498.

Ruiz Martínez, G., S. Maldonado Villanueva, G. Posada Vanegas, R. Silva Casarín y B. E. Vega Serratos. 2014. Clasificación de playas campechanas para su manejo integral y desarrollo sostenible. Modelo hidrodinámico. Universidad Autónoma de Campeche. 134 p.

Rusu, L., and C. Guedes Soares. 2012. Wave energy assessments in the Azores islands. Renewable Energy 45:183-196.

Sajed, S., F. Arefi, M. Kolahdouz, and M. A. Sadeghi. 2019. Improving sensitivity of mercury detection using learning based smartphone colorimetry. Sensors and Actuators B: Chemical 298:126942.

Scanu, S., D. Piazzolla, F. M. Frattarelli, E. Mancini, F. Tiralongo, M. V. Brundo, D. Tibullo, R. Pecoraro, C. Copat, M. Ferrante, and M. Marcelli. 2016. Mercury Enrichment in Sediments of the Coastal Area of Northern Latium, Italy. Bull Environ Contam Toxicol 96:630-637.

Shulkin, V., B. Presley, and V. I. Kavun. 2003. Metal concentrations in mussel *Crenomytilus grayanus* and oyster *Crassostrea gigas* in relation to contamination of ambient sediments. Environment International 29:493-502.

Soana, E., M. Naldi, and M. Bartoli. 2012. Effects of increasing organic matter loads on pore water features of vegetated (*Vallisneria spiralis L.*) and plant-free sediments. Ecological Engineering 47:141-145.

Sunderland, E. M., F. A. P. C. Gobas, B. A. Branfireun, and A. Heyes. 2006. Environmental controls on the speciation and distribution of mercury in coastal sediments. Marine Chemistry 102:111-123.

Szalinska, E. 2020. A review of heavy metals contamination within the Laurentian Great Lakes.

USEPA, U. 2014. Priority Pollutant List (p. 2).

Walker, T. R. 2016. Mercury concentrations in marine sediments near a former mercury cell chlor-alkali plant in eastern Canada. Marine Pollution Bulletin 107:398-401.

Wentworth, C. K. 1922. A scale of grade and class terms for clastic sediments. The journal of geology 30:377-392.

Williams, J. A., and J. Antoine. 2020. Evaluation of the elemental pollution status of Jamaican surface sediments using enrichment factor, geoaccumulation index, ecological risk and potential ecological risk index. Marine Pollution Bulletin 157:111288.

Chapter III:

Mercury contamination in coastal sediments and biota in the Azores archipelago: the influence of a 16th century shipwreck

Mercury contamination in coastal sediments and biota in the Azores archipelago: the influence of a 16th century shipwreck

Vieira, H.C.[1], Rendón-von Osten, J.[2], Soares, A.M.V.M.[1], Abreu, S. N.[1], Morgado, F.[1]

[1] CESAM - Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

[2] EPOMEX Institute, Autonomous University of Campeche, Campeche, Mexico

Abstract

During Spanish colonial times ships frequently transported Hg across the Atlantic ocean to the New World, to be used in gold mining. Many of those ships eventually sank, thus being a local source of Hg contamination in the marine environment, that tends to biomagnify through the food webs.

This study evaluated Hg concentration in coastal sediments and biota from distinct trophic levels (macroalgae: *Asparagopsis armata*, *Ulva lactuca*; gastropods: *Littorina striata*, *Patella candei gomesii*; crustaceans: *Paleamon elegans*, *Pachygrapsus marmoratus*). Samples were collected in five sampling sites in the Azores Archipelago, Portugal (1 sampling site near a 16th century shipwreck and the other 4 further away).

[Hg] from both sediment fractions (sand and fine sediments) of the shipwreck-area was significantly higher than [Hg] from the more distant areas. Moreover, according to the Portuguese law's guidelines for sediment quality, the shipwreck-area was classified as dredged material with trace contamination and the other four sampling sites as Clean dredged material. When the current [Hg] was compared with background levels (igeo), the sampling sites were classified from heavily to extremely contaminated to uncontaminated depending on the proximity to the wreck. As observed in the sediment samples, all species collected in the shipwreck-area showed higher [Hg] than the ones from the more distant areas. An increase in [Hg] was also observed from primary producers to consumers, reinforcing the biomagnification potential of this metal. However, these values do not pose a risk to either marine food webs or humans.

Keywords: Aquatic toxicology; heavy metals; mercury bioaccumulation; intertidal species; biomagnification; geoaccumulation index

1. Introduction

Over the centuries, mercury (Hg) has been used for a variety of purposes, being employed in the manufacture of thermometers, barometers, explosives, with a significant role in dentistry, pharmaceutical industry and agriculture (Tack et al. 2005, Klys 2010). Hg is also used in gold and silver mining processes (Engstrom et al. 2014) to separate the metal particles from the sediments (aquatic and terrestrial) through a process called amalgamation (Lacerda 1997, Gibb and O'Leary 2014). Amalgamation process was brought to South America (mainly Mexico, Peru, and Bolivia) by Spanish colonizers in the 16th century (Malm 1998, Veiga et al. 2006), becoming increasingly popular after depleting gold and silver resources in the West Indies (Leshikar-Denton 2002). It is estimated that between the years 1550 and 1880, nearly 200,000 metric tonnes Hg were released to the environment (Nriagu 1994). During the amalgamation process, Hg is usually heated in the open air and lost to rivers and soils through handling as metallic Hg (Lacerda 1997). Hg is then emitted to the atmosphere as Hg vapor by volatilization of the Hg present in the amalgam. Shipwrecks are another source of Hg contamination in the marine environment (Dimitrakakis et al. 2014). In Spanish colonial times the Hg necessary to the mining process was carried in galleons along the Atlantic, from Spain to the new world (Clarkson and Magos 2006). However, some of these vessels occasionally sank due to storms or collisions with reefs (Leshikar-Denton 2002). Over the years some archaeological evidences of the transport of Hg on Spanish ships have been found. In 1992, at Pensacola, Florida, Emanuel Point Ship was identified as a galleon of the fleet of Tristan de Luna, whose fleet was wrecked during a hurricane in 1559 and where more than 250ml of Hg have been collected from the ship's bilge (Smith 1998). Other wrecks have been discovered, such as the galleons of Nuestra Senora de Guadalupe and Conde de Tolosa, caught in a hurricane and sunk in 1724 in Sarnana Bay, Dominican Republic with a large cargo of Hg used in the process of precious metals extraction (Leshikar-Denton 2002). According to Monteiro (2013), at least 96 shipwrecks crossed the Azorean waters between 1522 and 1998, the majority being Portuguese and Spanish ships coming back from India or the New World. In 1996, during an archaeological survey of the Angra bay in Terceira Island, Azores archipelago, two wrecks were found. Wood samples taken from both wrecks provided evidences that they were dated from the last quarter of the fifteenth century or the first quarter of the sixteenth century. In one of these shipwrecks (Fig. 1), more than 150ml of elemental Hg was recovered from the ship's hull. The presence of Hg in the ship bilge suggests that the vessel was carrying or had carried in the past a cargo with great quantities of that metal, which was used mainly as a component for the extraction of silver and gold in Central and South America (Garcia and Monteiro 2001).

Fig. 3.1 Images of the archaeological work survey of the Angra bay in Terceira Island, Azores archipelago. Traces of elemental Hg found in the ship's hull are indicated with white circles.

Hg is found in several inorganic and organic forms in the environment (Rice et al. 2014). Inorganic Hg includes forms such as elemental (metallic) Hg (Hg^0) or mercuric (Hg^{++}) salts (e.g. mercuric chloride – $HgCl_2$). Organic Hg includes compounds in which Hg is bonded to a structure containing carbon atoms (e.g. methylmercury – MeHg) (Bernhoft 2012). When compared to other Hg species, Hg^0 is the form that has the lowest bioavailability (Rua-Ibarz et al. 2016); however, in the aquatic environments Hg^0 can be converted through an oxidation process, resulting in Hg species with higher toxicity and bioavailability (Batrakova et al. 2014).

Hg has been considered a pollutant of primary importance since the occurrence of the organic Hg poisoning incident of Minamata, Japan, in the 1950s resulting in adverse effects for human health (Vieira et al. 2015). Once in the environment, Hg has the particularity to bioaccumulate and biomagnify in aquatic food webs (Tsui et al. 2014), which means that [Hg]s found in predator species can be millions of times higher than those observed in surface waters. On the other hand, biomagnification of Hg can lead to toxic concentrations in fish and fish-eating wildlife (Lavoie et al. 2013, Horowitz et al. 2014).

Many marine organisms may be considered as accumulation bioindicators, i.e. they are used to monitor the pollutant impact on the marine environment, through analysis of pollutant content in these species' tissues (Amoozadeh et al. 2014). Different types of organisms such as macroalgae (Costa et al. 2011), plankton, molluscs (Arcagni et al. 2018) and fish (Sinkus et al. 2017) are used to evaluate Hg contamination in the marine environment.

The objective of this study was to assess the influence of the presence of a shipwreck on the Hg contamination in the surrounding environment, due to the potential Hg release over the years. Therefore, we evaluated the Hg present in sediments and in three different groups of marine organisms (algae, gastropods and crustacea) in intertidal rocky shores of two Azorean islands (Terceira and Graciosa), more specifically in Angra bay where a 16th century shipwreck can be found and in other bays without the presence of shipwrecks.

2. Materials and Methods

2.1. Sampling and sample preparation

This study was performed in the Azores archipelago, located in the North Atlantic Ocean (Fig. 3.2). Surface sediment samples were randomly collected, in 0 – 2 cm depth, in five different sampling sites, during low tide: Shipwreck bay (S1 – 38°39'13.1"N 27°13'13.1"W) and other bays without evidence of shipwrecks in the Terceira Island (S2 – 38°40'48.5"N 27°03'19.0"W, S3 – 38°48'04.4"N 27°14'49.5"W, S4 – 38°39'25.0"N 27°14'56.2"W) and Graciosa Island (S5 – 39°00'38.6"N 27°57'48.3"W).

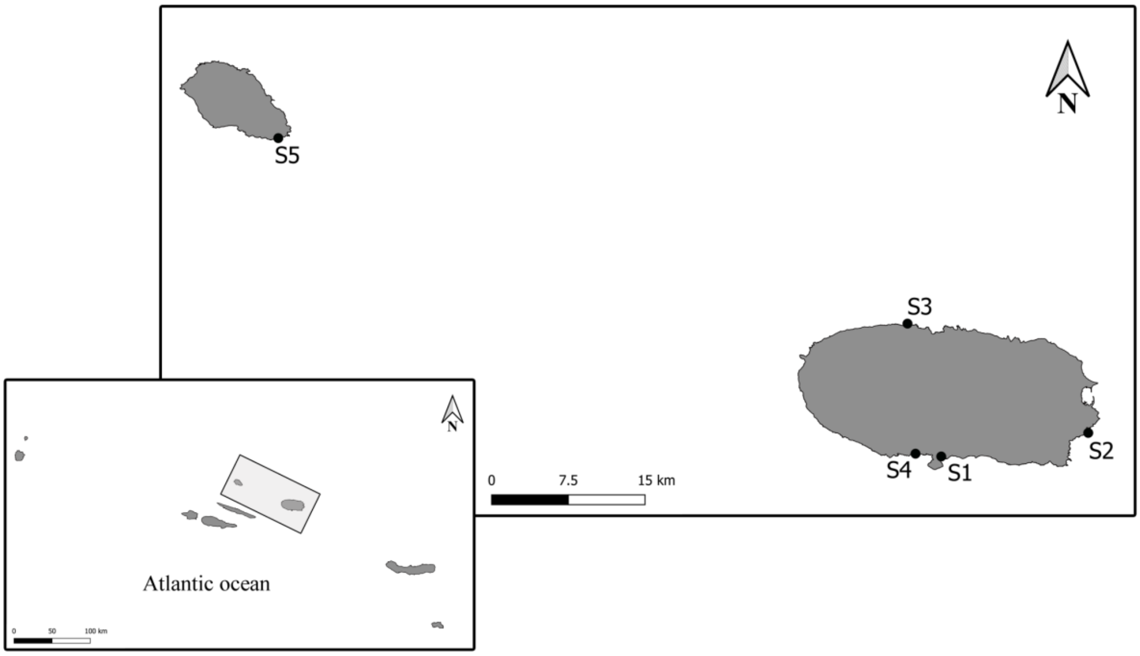


Fig. 3.2 Location of the sampling sites: S1, S2, S3, S4 (Terceira Island); S5 (Graciosa Island).

At the same time, biotic samples were also collected: the red alga *Asparagopsis armata*, the green alga *Ulva lactuca*, the gastropods *Littorina striata* and *Patella candei gomesii* and the crustaceans *Palaemon elegans* and *Pachygrapsus marmoratus*. All samples were placed in clean zip bags properly identified and stored at -20ºC for later Hg determination. Once in the laboratory, all samples were defrosted and prepared for Hg quantification. Algae were carefully cleaned of some remaining particulate matter; *L. striata* shells' were cracked in a bench vice and animals carefully removed using plastic tweezers; *P. candei gomesii* were separated from the shell with the help of a stainless steel scalpel; *P. elegans* and *P. marmoratus* were dissected in order to remove the muscle tissue. Gastropods and crustaceans were also measured for their length before removal of the soft tissues.

Sediment samples were oven dried at 35 ºC for three days and subsequently homogenized and sieved (shaken continuously at amplitude 8) with stainless steel sieves with meshes sizes of 2 mm, 1 mm and 63 µm. Sediment fractions between 1 mm and 63 µm (sand fraction) and the fraction <63 µm (fine sediment fraction) were used for Hg quantification.

2.2. Hg quantification

Hg quantification of the biotic and sediment samples was performed with the Advanced Mercury Analyzer (AMA-254, made by ALTEC and distributed by LECO). The procedure is based in a pyrolysis process of the sample using a combustion tube heated at 750 ºC under an oxygen atmosphere and the released Hg is trapped in a gold amalgamator and subsequently detected and quantified by atomic absorption spectrometry (Costley et al. 2000).

Sample analysis were quintupled to check the reproducibility of the results and three blank analyses (analysis without sample) were performed between samples to verify that Hg was not being accumulated over the samples. In this study, blank readings typically correspond to values <0.02 ng of Hg. Based on three times the standard deviation of the blank readings, the limit of detection was calculated to be 0.012 ng g^{-1} of Hg.

Analytical quality of the procedure was validated using the biological reference material TORT-2 (Lobster Hepatopancreas Reference Material for Trace Metals, National Research Council of Canada) and the inorganic reference material PACS-3 (Marine Sediment Reference Material for Trace Metals and other Constituents, National Research Council of Canada). Obtained data (0.28±0.004 µg g^{-1} of Hg for TORT-3 and 2.88±0.06 µg g^{-1} of Hg for PACS-3) and reference values of Hg) (0.29±0.02 µg g^{-1} of Hg for TORT-3 and 2.98±0.36 µg g^{-1} of Hg for PACS-3) were not statistically different ($p>0.05$).

The organic matter content in sediments was determined according to Chen et al (2015), where the sediment sample was re-weighted immediately after the Hg analysis. The sample weight loss after Hg analysis pyrolysis corresponds to the amount of organic matter present in the sediment.

2.3. Assessment of Hg pollution

2.3.1. Geoaccumulation index (Igeo)

Geo-accumulation index (Igeo) assesses the [Hg] of sediments by comparing present elemental concentrations with background levels. The present calculation enables the assessment of contamination by comparing the present and crustal average values of elemental concentrations (Müller 1986). The Igeo can be calculated by using the following formula:

$$I_{geo} = \log_2\left(\frac{[\mathrm{Hg}]_{\mathrm{sample}}}{1.5\ [\mathrm{Hg}]_{\mathrm{baseline}}}\right)$$

Here, [Hg]sample is the measured concentration of the metal in the sediment, [Hg]baseline is the geochemical background value for the metal, and the factor 1.5 is introduced to include possible differences in the background values due to lithological variations. The Igeo index was calculated using a background value of 0.03 μg g^{-1} (obtained through the analysis of [Hg] of the Island soil), and the results are interpreted in accordance with the six levels (from uncontaminated to extremely contaminated) of metal pollution, suggested by Müller (1986).

2.4. Statistical analysis

All data were assessed for normality by using Kolmogorov-Smirnov test. Data were Log-transformed to meet the normal distribution and the parametric statistical one-way ANOVA was used to compare [Hg] in sediments fractions and intertidal species of each sampling site. Tukey's test was used to determine significant differences in [Hg] between the bay with evidences of shipwreck and the other bays without evidence of shipwrecks. On the other hand, t-test was used to compare the [Hg] between producers (macroalgae) and consumers (crustaceans and gastropods).

Statistical analyses were performed using Sigmaplot (version 11.0). Statistical significant differences were considered when $p<0.05$. [Hg] data is presented as mean value ± standard error value (mean ± SE).

3. Results and Discussion

The granulometric composition of the surface sediments collected in all sampling sites was classified according to the Wentworth scale (Wentworth 1922) as sand and fine sediment (Table 3.1).

Table 3.1 Granulometric composition (%) of the surface sediments in sampling sites according to the Wentworth scale.

	Gravel		Sand		Fine sediments	
Site	Weight (g)	%	Weight (g)	%	Weight (g)	%
S_1	0	0	320.38	99.95	0.15	0.05
S_2	0	0	426.89	99.94	0.24	0.06
S_3	0	0	297.75	99.58	1.26	0.42
S_4	0	0	499.85	99.97	0.166	0.03
S_5	0	0	313.78	99.96	0.13	0.04

Sediment samples were composed mostly by sand (more than 99%) in all sites. These results are in line with the ones obtained by Quartau (2007) in a study carried out on another island in the Azores archipelago (Faial Island), where the author found a very insignificant percentage of finer material (normally less than 1% of silt and clay) in coastal sediments. On the other hand, S3 presented a higher percentage of fine sediment than the remaining sites. This may be explained by the fact that S3 is a protected bay, while the other sampling sites are under a higher exposure to the wave action. Thus, the lack of wave exposure frequently leads to settling of fine sediments (Mangor 2007).

Regarding the Hg content determined in the surface sediments, the values in S1 were very high in the sand and in the fine sediments fractions when compared with the Hg content in the sediment fractions from the other sampling sites (Fig. 3.3).

On average, the [Hg] found in the sand fraction was 0.21±0.01 µg g^{-1} in S1, 0.002±0.000 µg g^{-1} in S2, 0.002±0.000 µg g^{-1} in S3, 0.005±0.003 µg g^{-1} in S4 and 0.001±0.000 µg g^{-1} in S5. Higher concentrations were found in the fine sediments fraction, corresponding to 1.04±0.004 µg g^{-1}, 0.03±0.001 µg g^{-1}, 0.04±0.002 µg g^{-1}, 0.06±0.001 µg g^{-1} and 0.03±0.001 µg g^{-1} at S1, S2, S3, S4 and S5, respectively. The increase in [Hg] in the fine sediments fraction was statistically significant ($p<0.05$) in all sampling sites when compared to the [Hg] present in the sand fraction of the same sampling site.

A significant difference ($p<0.05$) was found in [Hg] between S1 and the sites farther from the wreck area (S2, S3, S4 and S5) in both sediment fractions. These results are in agreement with the ones reported by Ndungu et al. (2017) who found higher [Hg]s in the sediments adjacent to different wrecks in relation to the sediments of more distant areas. Leino et al. (2011) also states that metals such as Hg found in shipwrecks may influence the surrounding environment.

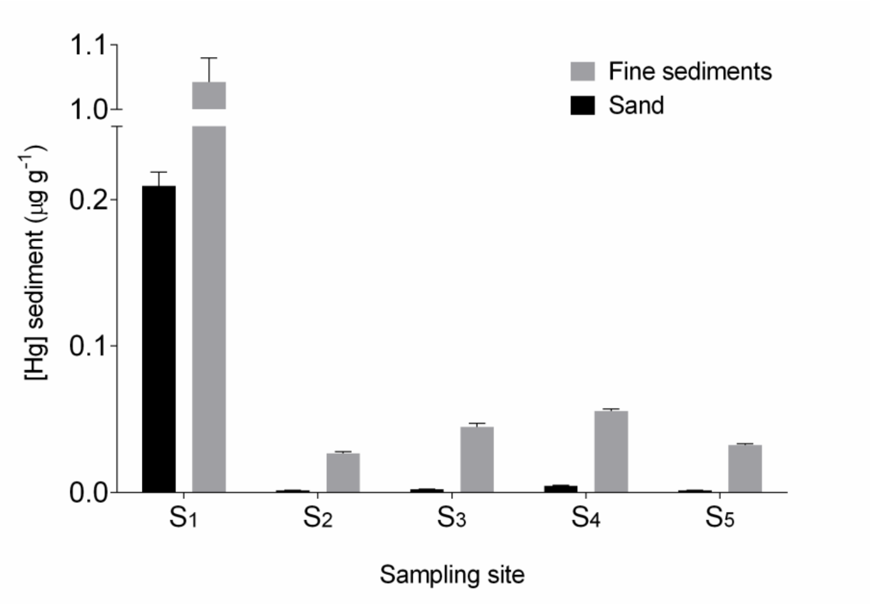


Fig. 3.3 Hg concentration (µg g^{-1}) in the sediment fraction (sand and fine sediments).

Hg particles have a strong affinity with organic matter, which plays a fundamental role in the Hg accumulation dynamics in marine sediments (Chakraborty et al. 2015, Oliveri et al. 2016). This relationship may explain the differences ($p<0.05$) in the [Hg] found in the sediment fraction sand and fine sediments (Fig. 3.4) since smaller sediment particles often trap more organic matter than larger particles, as they have more surface area per unit weight (Bengtsson and Picado 2008).

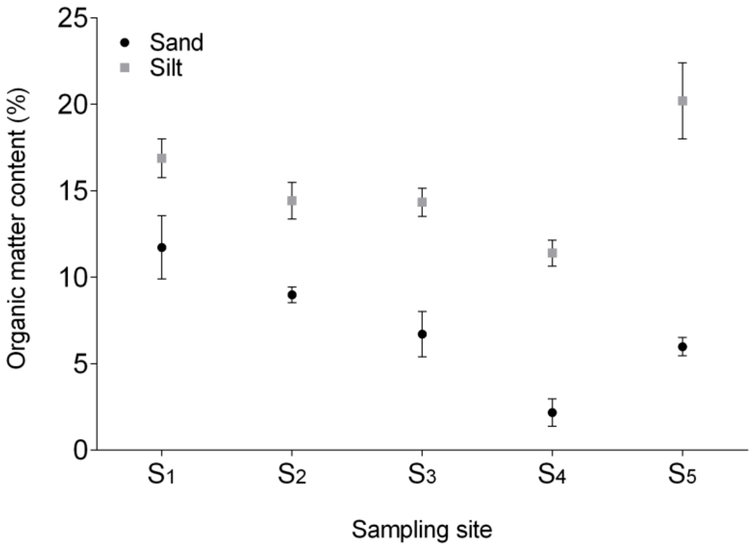


Fig. 3.4 Percentage of organic matter present in the sediment fractions in the different sampling sites.

The Portuguese law's guidelines for sediment quality (Portaria n.º 1450/2007 de 12 de Novembro) classifies marine sediment quality into 5 classes. Marine sediments with [Hg] <0.5 µg g^{-1} are classified as class 1 (Clean dredged material) and concentrations in the range 0.5 – 1.5 µg g^{-1} are classified as class 2 (Dredged material with trace contamination). At the same time, [Hg]s between 1.5 – 3 µg g^{-1} are classified as Class 3 (Lightly contaminated dredged material), [Hg]s in the range 3 – 10 µg g^{-1} are classified as class 4 (Contaminated dredged material) and marine sediments having a [Hg] >10 µg g^{-1} are classified as class 5 (highly contaminated material). Therefore, considering the Hg content of the sediment fraction <63µm, S1 is classified as class 2 and the sites farther from the sink (S2, S3, S4 and S5) are classified as class 1.

To assess the contamination class of Hg in the surface sediments, Igeo indexes were calculated for each sampling site (Fig. 3.5), using the [Hg] of the sediment fraction <63µm and a background value of 0.03 µg g^{-1} of Hg, which was obtained through the analysis of [Hg] of the Island soil. Kim (2018) tested three different methods for calculating the Igeo Index taking into account the concentration of a metal in: a) the whole sediment sample, b) only the fine fraction of a sample and c) by a standard equation, their data indicated use of the concentration found in the fine sediment fraction is the one that presents the best results. Similar [Hg]s (0.047 µg g^{-1}) were found in the topsoil of others volcanic islands in the Mediterranean, more precisely on the Canaries archipelago (Martín et al. 2013).

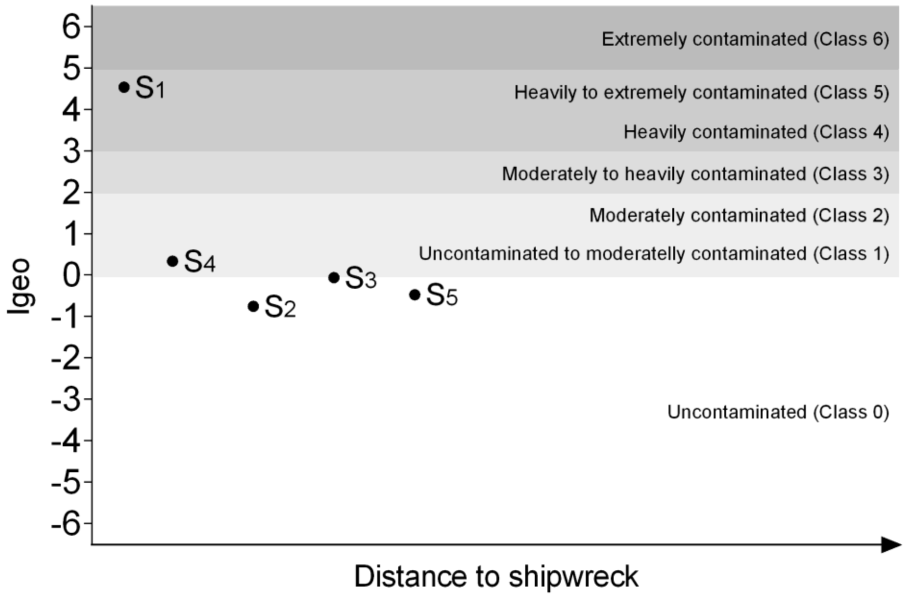


Fig. 3.5 The Igeo values and classes of metal pollution suggested by Müller of each sampling site.

Igeo values of the sediments showed a contamination degree ranging from uncontaminated (class 0) to heavily to extremely contaminated (class 5) in the different sampling sites. These values were less than 0 in the sampling sites S2, S3 and S5 classifying these areas as uncontaminated. On the other hand, the Igeo value of the sampling site S4 was slightly higher ($0<\text{Igeo}\leq 1$) when compared with the Igeo value of S2, S3 and S5, possibly due to a higher proximity to S1, indicating that this area is relatively uncontaminated. The sampling site S1 (shipwreck bay) showed a heavily to extremely high contamination degree ($4<\text{Igeo}\leq 5$). The results show that [Hg]s in sediments in S2, S3 and S5, can be considered near the background levels found in the Azores Archipelago and the Igeo values of S1 and S4 support the theory that the levels of Hg contamination in sediment decrease with distance from the wreck.

Concerning the intertidal species, no significant differences ($p>0.05$) were found in the length of the individuals from the same species between sampling sites. [Hg] ranged between 0.001 – 0.016 µg g^{-1} for *A. armata*, 0.001 – 0.022 µg g^{-1} for *U. lactuca*, 0.015 – 0.054 µg g^{-1} for *L. striata*, 0.004 – 0.045 µg g^{-1} for *P. candei*, 0.005 – 0.045 µg g^{-1} for *P. elegans* and 0.002 – 0.027 µg g^{-1} for *P. marmoratus*. All species collected at S1 presented higher levels of Hg ($p<0.05$) than the ones collected at the other sampling sites (Fig. 3.6).

None of these concentrations exceed the [Hg] (0.05 µg g^{-1} and 0.5 µg g^{-1}) established through the ecotoxicological assessment criteria (EACs - concentration of contaminants in the environment below which it is not expected to occur chronic effects on marine species), created by the OSPAR Convention (Bignert et al. 2004). Despite this, the Hg content in the biotic samples followed the same trend found in the sediments from the sites where they were collected, indicating that along with the influence of the [Hg] in the sediments, the shipwreck has also the ability to influence the Hg bioaccumulation in marine trophic chains. This assumption is corroborated by Rua-Ibarz (2016), who suggests that [Hg] found in the brown tissue of the crab *Cancer pagurus* was affected by the Hg released from the German submarine U-864 sunk at the end of World War II. Rogowska (2015) also states that the presence of shipwrecks can be considered an important source of pollution for marine ecosystems, thereby influencing the surrounding environment (e.g. metal contamination).

Some authors such as Álvaro (2016), Cabral-Oliveira (2016), Ho (1990) and Kamala-Kannan (2008) indicated some of the species present in this study as good bioindicators of metal contamination, since they have the capacity to reflect the environmental bioavailability of elements where the organisms live.

Macroalgae (producers) were the group with the lowest [Hg] in all sites and no significant differences ($p>0.05$) were found between *A. armata* and *U. lactuca* within sampling sites. On the other hand, a significant increase of [Hg] ($p<0.05$) was observed when comparing the Hg levels of producers with the Hg levels of consumers (crustaceans and gastropods). These results suggest Hg biomagnification in the trophic chain reinforcing the importance of the role that macroalgae may play in the metals' transfer cycle throughout the food chain (Cairrão et al. 2007).

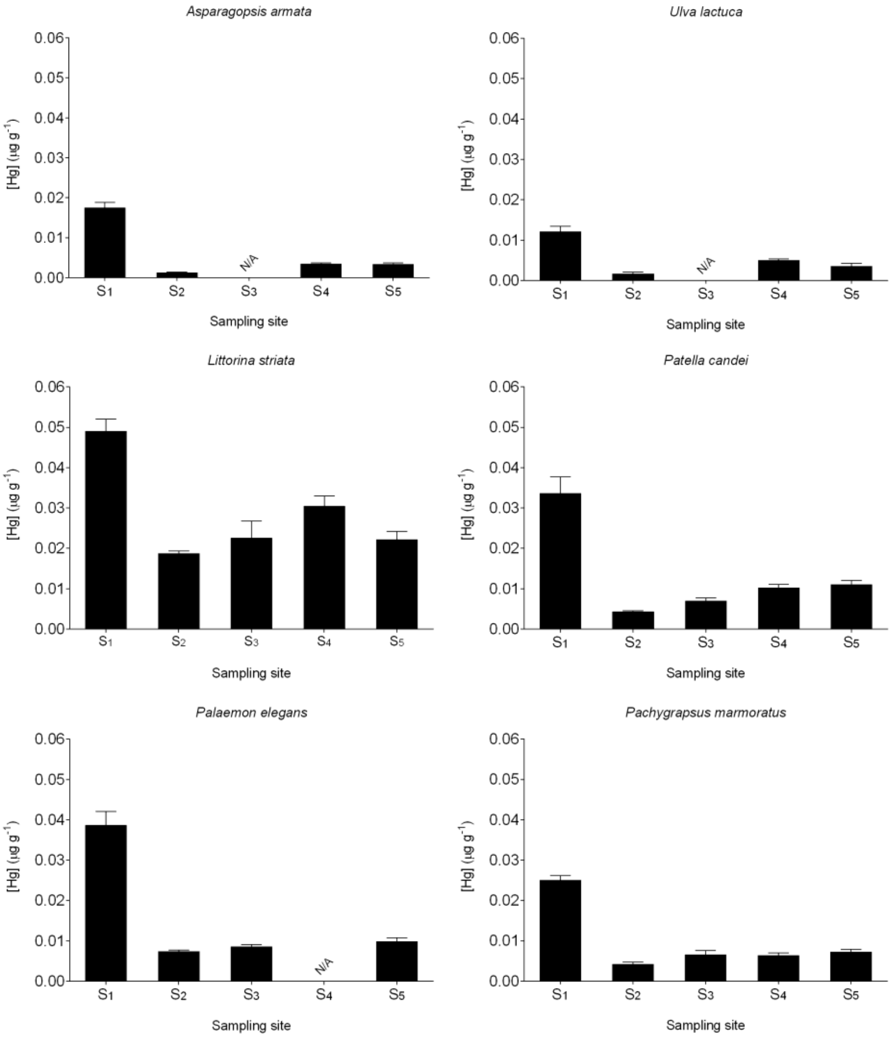


Fig. 3.6 Hg concentration (μg g^{-1}) in different intertidal species. N/A (not analyzed) means that species were not analyzed in these sampling sites.

Hg data in the available literature for these organisms are scarce and the few existing studies show only the [Hg] in dry weight and do not provide the percentage of moisture after drying, being difficult to compare the Hg values obtained in this study with those already published. Nevertheless, a study by Shiber and Washburn (1978) reported [Hg]s in *U. lactuca* between

0.00 and 8 µg g^{-1} (dw) collected in nine locations along the rocky coast of Ras Beirut, Lebanon and Green-Ruiz (2005) found [Hg] of 0.058±0.029 µg g^{-1} (dw) in *U. lactuca* from Guaymas Bay, Gulf of California. The Hg levels found by Cunha (2008) in different populations of *P. candei* collected in São Miguel Island, Azores, ranged between 0.01 – 0.03 µg g^{-1} (dw) and in Azores also, Álvaro (2016) reported [Hg] in *P. marmoratus* muscle between 0.07 and 0.09 µg g^{-1} (dw).

Despite the increasing [Hg] in both sediment and intertidal species with proximity to the shipwreck, the measured values are far from being considered dangerous, and therefore, do not pose a significant impact on both marine food webs and human welfare.

4. Conclusion

This study suggests that the presence of a Hg-containing shipwreck increases the levels of this metal in the surrounding environment, in both biotic and abiotic compartments. [Hg]s found in all the intertidal species and the Igeo values reflected the proximity of the sampling site to the shipwreck bay. Hg levels were higher in this sampling site, remaining relatively similar, stable and lower in the other areas. Consequently, the impact of Hg released from the shipwreck is localized. Lastly, differences on [Hg]s found between producers (*A. armata* and *U. lactuca*) and consumers (*L. striata*, *P. candei*, *P. elegans* and *P. marmoratus*) suggest a potential biomagnification through the food web. Nevertheless, none of the [Hg]s found in both sediments and intertidal species pose a threat to marine life or human consumption.

Competing interests

The authors declare that they have no conflict of interests (financial or non-financial).

Acknowledgements

The study was financially supported by FCT/MCTES to CESAM (UIDP/50017/2020+UIDB/50017/2020), through national funds. FCT also financed a doctoral grant to Vieira, H.C. (PD/BD/127808/2016). We are thankful for the financial support by the project INSIDER (PTDC/CTA-AMB/30495/2017 and POCI-01-0145-FEDER-030495) funded by FEDER, through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES. The authors would also like to thank Direção Regional de Cultura do Governo Regional dos Açores for providing and authorizing the use of photographs related with the shipwreck.

References

Álvaro, N. V., A. I. Neto, R. P. Couto, J. M. N. Azevedo, and A. S. Rodrigues. 2016. Crabs tell the difference – Relating trace metal content with land use and landscape attributes. Chemosphere 144:1377-1383.

Amoozadeh, E., M. Malek, R. Rashidinejad, S. Nabavi, M. Karbassi, R. Ghayoumi, G. Ghorbanzadeh-Zafarani, H. Salehi, and B. Sures. 2014. Marine organisms as heavy metal bioindicators in the Persian Gulf and the Gulf of Oman. Environmental Science and Pollution Research 21:2386-2395.

Arcagni, M., R. Juncos, A. Rizzo, M. Pavlin, V. Fajon, M. A. Arribére, M. Horvat, and S. Ribeiro Guevara. 2018. Species- and habitat-specific bioaccumulation of total mercury and methylmercury in the food web of a deep oligotrophic lake. Science of The Total Environment 612:1311-1319.

Batrakova, N., O. Travnikov, and O. Rozovskaya. 2014. Chemical and physical transformations of mercury in the ocean: a review. Ocean Sci 10:1047-1063.

Bengtsson, G., and F. Picado. 2008. Mercury sorption to sediments: Dependence on grain size, dissolved organic carbon, and suspended bacteria. Chemosphere 73:526-531.

Bernhoft, R. A. 2012. Mercury Toxicity and Treatment: A Review of the Literature. Journal of Environmental and Public Health 2012:460508.

Bignert, A., D. Cossa, R. Emmerson, R. Fryer, C. Füll, J. Fumega, R. Laane, H. Calls, B. McHugh, and B. Miller. 2004. OSPAR/ICES workshop on the evaluation and update of background reference concentrations (B/RCs) and ecotoxicological assessment criteria (EACs) and how these assessment tools should be used in assessing contaminants in water, sediment, and biota: Workshop The Hague, 9-13 February 2004. Final Report.

Cabral-Oliveira, J., H. Coelho, J. Pratas, S. Mendes, and M. A. Pardal. 2016. Arsenic accumulation in intertidal macroalgae exposed to sewage discharges. Journal of Applied Phycology 28:3697-3703.

Cairrão, E., M. J. Pereira, M. R. Pastorinho, F. Morgado, A. M. V. M. Soares, and L. Guilhermino. 2007. *Fucus* spp. as a Mercury Contamination Bioindicator in Costal Areas (Northwestern Portugal). Bull Environ Contam Toxicol 79:388-395.

Chakraborty, P., A. Sarkar, K. Vudamala, R. Naik, and B. N. Nath. 2015. Organic matter — A key factor in controlling mercury distribution in estuarine sediment. Marine Chemistry 173:302-309.

Chen, J., P. Chakravarty, G. R. Davidson, D. G. Wren, M. A. Locke, Y. Zhou, G. Brown Jr, and J. V. Cizdziel. 2015. Simultaneous determination of mercury and organic carbon in sediment and soils using a direct mercury analyzer based on thermal decomposition–atomic absorption spectrophotometry. Analytica Chimica Acta 871:9-17.

Clarkson, T. W., and L. Magos. 2006. The Toxicology of Mercury and Its Chemical Compounds. Critical Reviews in Toxicology 36:609-662.

Costa, S., D. Crespo, B. M. G. Henriques, E. Pereira, A. C. Duarte, and M. A. Pardal. 2011. Kinetics of Mercury Accumulation and Its Effects on *Ulva lactuca* Growth Rate at Two Salinities and Exposure Conditions. Water, Air, & Soil Pollution 217:689-699.

Costley, C. T., K. F. Mossop, J. R. Dean, L. M. Garden, J. Marshall, and J. Carroll. 2000. Determination of mercury in environmental and biological samples using pyrolysis atomic absorption spectrometry with gold amalgamation. Analytica Chimica Acta 405:179-183.

Cunha, L., A. Amaral, V. Medeiros, G. M. Martins, F. F. M. M. Wallenstein, R. P. Couto, A. I. Neto, and A. Rodrigues. 2008. Bioavailable metals and cellular effects in the digestive gland of marine limpets living close to shallow water hydrothermal vents. Chemosphere 71:1356-1362.

Dimitrakakis, E., J. Hahladakis, and E. Gidarakos. 2014. The “Sea Diamond” shipwreck: environmental impact assessment in the water column and sediments of the wreck area. International Journal of Environmental Science and Technology 11:1421-1432.

Engstrom, D. R., W. F. Fitzgerald, C. A. Cooke, C. H. Lamborg, P. E. Drevnick, E. B. Swain, S. J. Balogh, and P. H. Balcom. 2014. Atmospheric Hg Emissions from Preindustrial Gold and Silver Extraction in the Americas: A Reevaluation from Lake-Sediment Archives. Environ Sci Technol 48:6533-6543.

Garcia, C., and P. Monteiro. 2001. The excavation and dismantling of Angra D, a probable Iberian seagoing ship, Angra bay, Terceira Island, Azores, Portugal. Preliminary assessment. Pages 431-447 in International Symposium on Archaeology of Medieval and Modern Ships of Iberian-Atlantic Tradition. Trabalhos de Arqueologia.

Gibb, H., and K. G. O'Leary. 2014. Mercury Exposure and Health Impacts among Individuals in the Artisanal and Small-Scale Gold Mining Community: A Comprehensive Review. Environmental health perspectives 122:667-672.

Green-Ruiz, C., J. Ruelas-Inzunza, and F. Páez-Osuna. 2005. Mercury in surface sediments and benthic organisms from Guaymas Bay, east coast of the Gulf of California. Environ Geochem Health 27:321.

Ho, Y. B. 1990. *Ulva lactuca* as bioindicator of metal contamination in intertidal waters in Hong Kong. Hydrobiologia 203:73-81.

Horowitz, H. M., D. J. Jacob, H. M. Amos, D. G. Streets, and E. M. Sunderland. 2014. Historical Mercury Releases from Commercial Products: Global Environmental Implications. Environ Sci Technol 48:10242-10250.

Kamala-Kannan, S., B. Prabhu Dass Batvari, K. J. Lee, N. Kannan, R. Krishnamoorthy, K. Shanthi, and M. Jayaprakash. 2008. Assessment of heavy metals (Cd, Cr and Pb) in water, sediment and seaweed (*Ulva lactuca*) in the Pulicat Lake, South East India. Chemosphere 71:1233-1240.

Kim, B. S. M., J. L. F. Angeli, P. A. L. Ferreira, M. M. de Mahiques, and R. C. L. Figueira. 2018. Critical evaluation of different methods to calculate the Geoaccumulation Index for environmental studies: A new approach for Baixada Santista – Southeastern Brazil. Marine Pollution Bulletin 127:548-552.

Klys, M. 2010. [Mercury (and...) through the centuries]. Arch Med Sadowej Kryminol 60:298-307.

Lacerda, L. D. 1997. Global Mercury Emissions from Gold and Silver Mining. Water, Air, and Soil Pollution 97:209-221.

Lavoie, R. A., T. D. Jardine, M. M. Chumchal, K. A. Kidd, and L. M. Campbell. 2013. Biomagnification of Mercury in Aquatic Food Webs: A Worldwide Meta-Analysis. Environ Sci Technol 47:13385-13394.

Leino, M., A. T. Ruuskanen, J. Flinkman, J. Kaasinen, U. E. Klemelä, R. Hietala, and N. Nappu. 2011. The Natural Environment of the Shipwreck Vrouw Maria (1771) in the Northern Baltic Sea: an assessment of her state of preservation. International Journal of Nautical Archaeology 40:133-150.

Leshikar-Denton, M. E. 2002. Problems and Progress in the Caribbean. Pages 279-298 in C. V. Ruppé and J. F. Barstad, editors. International Handbook of Underwater Archaeology. Springer US, Boston, MA.

Malm, O. 1998. Gold Mining as a Source of Mercury Exposure in the Brazilian Amazon. Environmental Research 77:73-78.

Mangor, K. 2007. General guidelines for good quality artificial beaches and lagoons, and case stories. DHI Water and Environment.

Martín, J. A. R., G. Carbonell, N. Nanos, and C. Gutiérrez. 2013. Source identification of soil mercury in the Spanish Islands. Archives of Environmental Contamination and Toxicology 64:171-179.

Monteiro, A. 2013. Underwater Archaeological Trails and Preserves in Portugal. Lisbon: Instituto de Arqueologia e Paleociências, Faculdade de Ciências Socias e Humanas, Universidade Nova de Lisboa.

Müller, G. 1986. Schadstoffe in sedimenten-sedimente als schadstoffe. Mitt, Österreichische Geologische Gesellschaft 79:107-126.

Ndungu, K., B. A. Beylich, A. Staalstrøm, S. Øxnevad, J. A. Berge, H. F. V. Braaten, M. Schaanning, and R. Bergstrøm. 2017. Petroleum oil and mercury pollution from shipwrecks in Norwegian coastal waters. Science of The Total Environment 593-594:624-633.

Nriagu, J. O. 1994. Mercury pollution from the past mining of gold and silver in the Americas. Science of The Total Environment 149:167-181.

Oliveri, E., D. Salvagio Manta, M. Bonsignore, S. Cappello, G. Tranchida, E. Bagnato, N. Sabatino, S. Santisi, and M. Sprovieri. 2016. Mobility of mercury in contaminated marine sediments: Biogeochemical pathways. Marine Chemistry 186:1-10.

Quartau, R. 2007. The insular shelf of Faial: Morphological and sedimentary evolution. Universidade de Aveiro, Aveiro.

Rice, K. M., E. M. Walker, Jr., M. Wu, C. Gillette, and E. R. Blough. 2014. Environmental mercury and its toxic effects. Journal of preventive medicine and public health = Yebang Uihakhoe chi 47:74-83.

Rogowska, J., B. Kudłak, S. Tsakovski, A. Gałuszka, G. Bajger-Nowak, V. Simeonov, P. Konieczka, L. Wolska, and J. Namieśnik. 2015. Surface sediments pollution due to shipwreck s/s “Stuttgart”: a multidisciplinary approach. Stochastic Environmental Research and Risk Assessment 29:1797-1807.

Rua-Ibarz, A., E. Bolea-Fernandez, A. Maage, S. Frantzen, S. Valdersnes, and F. Vanhaecke. 2016. Assessment of Hg Pollution Released from a WWII Submarine Wreck (U-864) by Hg Isotopic Analysis of Sediments and Cancer pagurus Tissues. Environ Sci Technol 50:10361-10369.

Shiber, J., and E. Washburn. 1978. Lead, mercury, and certain nutrient elements in *ulva lactuca* (linnaeus) from Ras Beirut, Lebanon. Hydrobiologia 61:187-192.

Sinkus, W., V. Shervette, J. Ballenger, L. A. Reed, C. Plante, and B. White. 2017. Mercury bioaccumulation in offshore reef fishes from waters of the Southeastern USA. Environmental Pollution 228:222-233.

Smith, R. C. 1998. Discovery, Development, and Interpretation of Florida’s Earliest Shipwreck: A Partnership in Research and Historic Preservation. Underwater Archaeology:115-121.

Tack, F. M. G., T. Vanhaesebroeck, M. G. Verloo, K. Van Rompaey, and E. Van Ranst. 2005. Mercury baseline levels in Flemish soils (Belgium). Environmental Pollution 134:173-179.

Tsui, M. T.-K., J. D. Blum, J. C. Finlay, S. J. Balogh, Y. H. Nollet, W. J. Palen, and M. E. Power. 2014. Variation in Terrestrial and Aquatic Sources of Methylmercury in Stream Predators as Revealed by Stable Mercury Isotopes. Environ Sci Technol 48:10128-10135.

Veiga, M. M., P. A. Maxson, and L. D. Hylander. 2006. Origin and consumption of mercury in small-scale gold mining. Journal of Cleaner Production 14:436-447.

Vieira, H. C., F. Morgado, A. M. V. M. Soares, and S. N. Abreu. 2015. Fish consumption recommendations to conform to current advice in regard to mercury intake. Environmental Science and Pollution Research:1-8.

Wentworth, C. K. 1922. A scale of grade and class terms for clastic sediments. The journal of geology 30:377-392.

Chapter IV:

Mercury in superficial sediments and trophic transfer assessment in the NW coast of Portugal

Mercury in superficial sediments and trophic transfer assessment in the NW coast of Portugal

Vieira, H.C.[1], Maia, M.E.[2], Bordalo, M.D.[1], Soares, A.M.V.M.[1], Rendón-von Osten, J.[3], Abreu, S. N.[1], Morgado, F.[1]

[1] CESAM - Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

[2] Department of Geoscience, University of Aveiro, 3810-193 Aveiro, Portugal

[3] EPOMEX Institute, Autonomous University of Campeche, Campeche, Mexico

Abstract

Mercury (Hg) contamination is a serious problem for wildlife due to its toxic effect on the biota. All environments are subject to Hg contamination; however, coastal areas are considered one of the most vulnerable as they are located on the border between the terrestrial and the oceanic environments.

This study evaluated [Hg] in coastal sediments and intertidal species representing three different groups (Chlorophyta, Rhodophyta and Mollusca) from eight sampling sites from the northwest (NW) coast of Portugal.

Sediment samples from sites located further south presented higher [Hg], possibly due to the transport of sediments with a higher Hg load by the sea current from the Hg contamination focus in the Aveiro lagoon. Notwithstanding, all sampled sediments showed Hg concentrations below the European Union Water Framework Directive (EU-WFD) reference value and below the maximum established by OSPAR Convention through the Ecotoxicological Assessment Criteria (EACs). Moreover, according to the Portuguese law's guidelines for sediment quality, the sediment of all sampling sites is classified as uncontaminated.

In addition, Hg levels found in the species from the different sampling sites indicate a significant increase in the Hg concentration from lower trophic levels (producers) to higher trophic levels (consumers) as well as the existence of Hg transfer from prey for the predator. Furthermore, biota-sediment accumulation factor (BSAF) was less than 1 for all species, suggesting that their Hg levels do not depend on the concentration found in the sediments but rather on the Hg present in the prey/food.

Keywords: Mercury bioaccumulation; marine sediments; Intertidal species; BSAF; BAF

1. Introduction

Mercury (Hg) is one of the most hazardous toxicants in the environment because of its toxicity, ability to accumulate in the biota, high biomagnification rate along the trophic chains (Tchounwou et al. 2003, Wiener et al. 2003, Scheuhammer et al. 2007, Shore et al. 2011) and the adverse effects to wildlife and human health (Chakraborty et al. 2014, Vieira et al. 2015). Hg occurs naturally in the environment (e.g. volcanic eruptions, geothermal sources, deep oceanic water upwelling, biomass burning), but it may be also introduced through human activities, such as industrial wastes, coal-fired power plants, mining processing (Pirrone et al. 2010, Belabed et al. 2013).

Hg is identified as a priority substance in the field of water policy by Directive 2013/39/EU of the Parliament of the European Union and the European Council on environmental quality standards in the field of water policy (EU 2013) as well as by the Convention for the Protection of the Marine environment of the North-East Atlantic (OSPAR Convention – Commission 2013). At the same time, the European Union has defined maximum concentrations of contaminants in the aquatic environment below which chronic effects are not expected to occur (EQS – McHugh et al. 2016). To protect the piscivorous wildlife (birds and mammals) against secondary poisoning (Vignati et al. 2013), the Directive 2013/39/EU establishes an EQS in biota of 0.02 µg g^{-1} ww, expressed as total Hg (EU 2013). On the other hand, considering the list published by the Commission Regulation (EC) No 1881/2006 of 19 December 2006, setting maximum levels for certain contaminants in foodstuffs, the maximum limit of Hg in fish and seafood for human consumption is 0.5 µg g^{-1} and above these levels, the fish is not recommended for consumption (EU 2006). Furthermore, the European Union Water Framework Directive (EU-WFD) has established a reference value for the [Hg] in sediments (0.36 µg g^{-1}) and the OSPAR Convention has created the Ecotoxicological Assessment Criteria (EACs) values for Hg between 0.05 µg g^{-1} and 0.5 µg g^{-1} (Bignert et al. 2004). The EACs represent the contaminant concentration in the environment below which no chronic effects are expected to occur in marine species, including the most sensitive ones (McHugh et al. 2016).

Hg has a great affinity for the fine particles of the sediment in the aquatic environment, binding to them by different processes (Scanu et al. 2016). This way, sediments play an important role in the retention of Hg (Chakraborty et al. 2014) and at the same time they act as a source of Hg for aquatic food webs (Hammerschmidt and Fitzgerald 2006, Fitzgerald et al. 2007). For this reason, the coastal contamination by this metal continues to attract the attention of environmental researchers (Shulkin et al. 2003), who use bioaccumulation factors as useful tools to assess the ecological risk of Hg exposure for aquatic organisms (Burkhard 2003). For example, biota–sediment accumulation factors (BSAFs) seek to establish a correlation between the concentration of a contaminant present in the sediments and the concentration of that contaminant in fish and/or wild animals (Knightes 2008). However, a successful risk assessment using these factors must take into account factors such as spatial and temporal gradients in chemical concentrations as well as patterns of the organism's life history (e.g. migration, diet, and structure and composition of the food chain) (Burkhard 2009). For an organism to be considered a good bioindicator, it must have reduced mobility or be sessile, be abundant in the sampling site, be available all year round, be easy to sample, be able to accumulate contaminants from the surrounding environment (Barbaro et al. 1978). Different types of organisms have been routinely used to evaluate Hg contamination in marine ecosystems: macroalgae (Costa et al. 2011, Fostier et al. 2016), plankton, molluscs (Arcagni et al. 2018) and fish (Sinkus et al. 2017, Bonsignore et al. 2018).

Portugal, located in southwestern Europe, has an extended coastal area in the Atlantic Ocean, where most of the Portuguese population is settled and most industrial, agricultural, and port activities take place. As a result, estuaries, rivers, and all the Norwest Portuguese coastal areas are subjected to intensive anthropogenic pressure from a wide range of pollutants (Ribeiro et al., 2015). Given the high sensitivity of South European marine coastal environment to climate variability (Rubal et al. 2013, Intergovernmental Panel on Climate Change 2014) and recognizing the geographical importance of the Northwest (NW) coast of Portugal and of its resources, a deeper analysis of the Hg bioaccumulation and biomagnification in superficial sediments and biota can be useful to understand major coastal Hg processes and answer questions regarding coastal pollution management. The aims of the current study were to analyze Hg in superficial sediments and trophic ecotoxicity biota assessment in the NW coast of Portugal, namely: i) assess the Hg concentrations in the superficial sediments from the Northwest (NW) coast of Portugal; ii) determine the Hg content in the tissues of intertidal species (flora and fauna) and iii) investigate the correlation between the Hg concentrations present in the intertidal species and Hg concentration found in the sediments.

2. Materials and Methods

2.1. Study area

The Northwest (NW) coast of Portugal is located on the Iberian Peninsula in southwest Europe, facing the Atlantic coast. The northwest Portuguese coast, at latitudes 40 – 42°N, is a highly energetic region, as may be inferred from the average wave power obtained with data from the wave analysis model archive of the European Centre for Medium-Range Weather Forecasts (Cruz 2007). The Portuguese northwest coast may be divided into two stretches based on the geomorphological characteristics: one, from Caminha to Espinho consists of low rocky formations; the other, from Ovar to Marinha Grande is mostly a low-lying open sandy shore, vulnerable to wave action, and backed by dunes that have already been destroyed in some places. Eight sampling sites were selected along the Northwest Atlantic coast of Portugal (Fig. 4.1): Mira (40°26'53.99"N; 8°48'27.27"W), Areão (40°31'7.69"N; 8°47'3.10"W), Barra (40°37'41.25"N; 8°45'6.13"W), Furadouro (40°52'24.52"N; 8°40'42.58"W), Canide (41°06'48.1"N; 8°39'53.1"W), Aterro (41°12'21.3"N; 8°42'59.2"W), Mindelo (41°18'37.8"N; 8°44'27.2"W), Viana do Castelo (41°41'50.4"N; 8°51'05.5"W). The geographical coordinates were determined using a mobile global positioning system (GPS12 Personal Navigator®, Garmin®) in each sampled site.

Fig. 4.1 Sampling sites in the Northwestern Portuguese coast.

2.2. Sampling and sample preparation

Fifteen species (Table 4.1) belonging to three different Phyla (Chlorophyta, Rhodophyta and Mollusca) were randomly collected in the intertidal zone of each sampling site at low tide, placed in cleaned zip bags properly identified and stored at -20ºC for later Hg determination. Chlorophyta includes three Ulva species, Rhodophyta includes the species *Chondrus crispus*, *Gracilaria multipartita*, *Procamium cartilagineum*, *Corallina officinalis*, *Ceramium virgatum*, *Asparagopsis armata*, *Pterocladiella capilacea*, *Gelidium pulchellum* and *Chondracanthus teedei* and Mollusca includes the species *Mytilus galloprovincialis*, *Nucella napillus* and *Gibbula umbilicalis*.

Table 4.1 List of intertidal species collected in the different sampling sites

	Mira	**Areão**	**Barra**	**Furadouro**	**Canide**	**Aterro**	**Mindelo**	**Viana do Castelo**
Chondrus crispus	✖	✖		✖				
Gracilaria multipartita		✖					✖	
Ulva rigida	✖		✖		✖			✖
Ulva intestinalis	✖					✖	✖	
Ulva lactuca				✖	✖			
Procamium cartilagineum								✖
Corallina officinalis								✖
Ceramium virgatum								✖
Asparagopsis armata								✖
Pterocladiella capillacea								✖
Gelidium pulchellum								✖
Chondracanthus teedei								✖
Mytilus galloprovincialis	✖	✖	✖	✖	✖			✖
Nucella lapillus	✖	✖	✖	✖	✖			
Gibbula umbilicalis							✖	✖

At the laboratory, before Hg quantification, the algae species (Chlorophyta and Rhodophyta) were defrosted and carefully cleaned of particulate matter. *N. lapillus* and *G. umbilicalis* were defrosted before their shells were cracked in a bench vice and animals carefully removed using plastic tweezers. *M. galloprovincialis* was thawed and separated from the shell by using a stainless steel scalpel.

On the other hand, surface sediment samples (0 – 2 cm depth) were collected from all sites and transported to the laboratory in carefully labelled plastic bags where they were oven dried at 35 ºC for 3 days and later homogenized and sieved (shaken continuously at amplitude 8) with stainless steel sieves with meshes sizes of 2 mm, 1 mm and 63 µm. Sediment fraction <63 µm were used for Hg quantification. In parallel, water samples were also collected in the same stations using 5 L polyethylene bottles and filtered in the laboratory using polyethylene filter holders (previously acid cleaned) and weighted Millipore filters of 0.45 µm in order to separate the suspended particulate matter (SPM) and the dissolved matter fraction. After the filtration process, filters were oven dried at 35 ºC overnight and stored until Hg analysis.

2.3. Hg quantification

Hg quantification was performed with the Advanced Mercury Analyzer (AMA-254, made by ALTEC and distributed by LECO). According to Costley et al. (2000), this kind of Hg quantification does not requires a previous digestion of the sample; the procedure is based in a pyrolysis process of the sample using a combustion tube heated at 750 °C under an oxygen atmosphere and the released Hg is trapped in a gold amalgamator and subsequently detected and quantified by atomic absorption spectrometry.

Analytical quality of the procedure was validated using the biological reference material TORT-3 (Lobster Hepatopancreas Reference Material for Trace Metals, National Research Council of Canada) and the inorganic reference material PACS-3 (Marine Sediment Reference Material for Trace Metals and other Constituents, National Research Council of Canada). Obtained data (0.28±0.01 µg g^{-1} of Hg for TORT-3 and 2.88±0.08 µg g^{-1} of Hg for PACS-3) and reference values of Hg (0.29±0.02 µg g^{-1} of Hg for TORT-3 and 2.98±0.36 µg g^{-1} of Hg for PACS-3) were not statistically different ($p>0.05$).

The organic matter content in sediments was determined according to Chen et al (2015), where the sediment sample was re-weighted immediately after the Hg analysis. The sample weight loss after Hg analysis pyrolysis corresponds to the amount of organic matter present in the sediment.

2.4. Biomagnification factors (BMF)

The biomagnification factor (BMF) provides information about the Hg biomagnification process from prey to predators (Liu et al. 2019a). The BMF was calculated according to Moreno-Sierra et al. (2016) using the equation:

$$\mathrm{BMF} = \frac{[\mathrm{Hg}]_{\mathrm{predator}}}{[\mathrm{Hg}]_{\mathrm{prey}}}$$

A BMF value >1 indicates that the Hg is biomagnifying, whereas a BMF <1 implies that a Hg is not accumulated by the organism being metabolized and eliminated (Liu et al. 2019b).

2.5.Biota sediment accumulation factor

BSAF is normally calculated for studies related to the biota-sediment interaction. In this study, BSAF was calculated to determine the ratio between the concentration of Hg found in the different species and the concentration present in the surface sediment of the respective sampling site and was calculated following the formula suggested by Szefer et al. (1999):

$$\text{BSAF} = \frac{[\text{Hg}]\text{ in organism}}{[\text{Hg}]\text{ in associated sediment}}$$

BSAF was used to classify the aquatic species as macroconcentrator (BSAF>2), microconcentrator (1 < BSAF<2) or deconcentrator (BSAF<1 – Mohsen et al. 2019).

2.6.Statistical analysis

Data were checked for normality using Shapiro-Wilk test. Since data were normally distributed, parametric statistical methods, Pearson correlations were performed between Hg concentrations in sediments and organic matter, sediments and SPM, *N. lapillus* and *M. galloprovincialis*, finally between sediments and biota. A one-way ANOVA was used to compare [Hg] of the different sampling sites and the different species.

Statistical analyses were performed using GraphPad Prism (version 6.01). Statistical significance was defined as $p<0.05$. Hg concentration data are presented as mean value ± standard error (mean ± SE).

3. Results and Discussion

The obtained results for Hg content in surface sediments of the NW Portuguese coast (Fig. 4.2A) showed levels of 0.080±0.010 µg g^{-1} in Mira, 0.175±0.015 µg g^{-1} in Areão, 0.045±0.015 µg g^{-1} in Barra, 0.056±0.001 µg g^{-1} in Furadouro, 0.020±0.001 µg g^{-1} in Canide, 0.023±0.003 µg g^{-1} in Aterro, 0.010±0.001 µg g^{-1} in Mindelo and 0.030±0.001 µg g^{-1} in Viana do Castelo.

These concentrations are in line with other studies that assessed Hg levels along the Portuguese coast. Coelho et al. (2006) quantified the Hg concentration in sediments from 8 sampling sites spread throughout the Portuguese coast and found that the Hg concentrations in these sampling sites did not exceed 0.1 µg g^{-1}. Cairrão et al. (2007) found [Hg] between 0.001±0.000 and 0.112 ± 0.007 µg g^{-1} in 11 sampling sites along the NW Portuguese coast.

Contrary to what was expected, Barra was not the sampling site with the highest Hg levels, with the highest values found in adjacent sampling sites, located to the South (Areão and Mira). The sampling site "Barra" is located very close to the only connection between the Atlantic Ocean and the Aveiro lagoon. The Aveiro lagoon has received continuous Hg discharges from a chlor-alkali plant for almost five decades (from the 1950s until 1994 – Pereira et al. 2009, Matos et al. 2016). The discharged Hg accumulated over time mostly at Laranjo bay (currently the most contaminated area), from where it can be dispersed, in periods of increased water currents, to the rest of the lagoon towards the mouth of the estuary (Pato et al. (2008). The highest [Hg] found in surface sediments was observed in Areão, which is the sampling site located closest to Barra on the south side and in Mira, which is located south of Areão. Statistically significant differences ($p<0.05$) were found in both locations when compared to Barra. This may be explained by the fact that on the Portuguese coast the current flows predominantly to the south (Álvarez-Salgado et al. 2003), which may potentially transport Hg from the Aveiro lagoon to the Atlantic Ocean over time leading to higher concentrations in the adjacent areas. Despite these differences, the highest [Hg] found in the sediments (Areão) was 2 folds lower than the reference value established by EU-WFD and 3 times less than the maximum established by the OSPAR Convention through the EACs. Furthermore, all sampling sites are classified as class 1 (Clean dredged material) by the Portuguese law's guidelines for sediment quality (Portaria n.º 1450/2007 de 12 de Novembro).

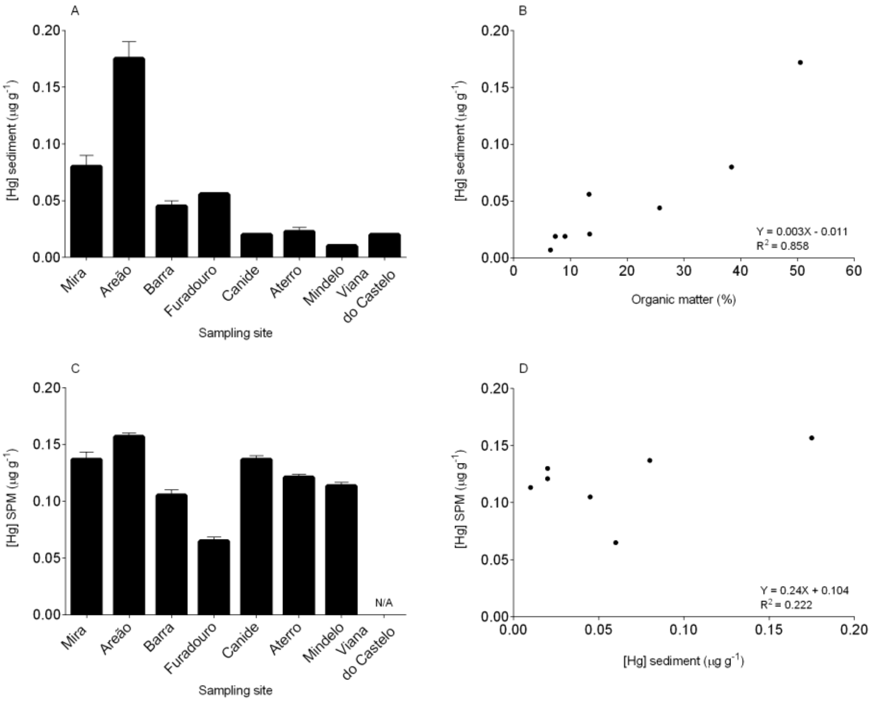


Fig. 4.2 Average Hg concentration (μg g^{-1}) in sediments (A); relationship between the [Hg] and the organic matter (B); Average Hg concentration (μg g^{-1}) in SPM (C) and relationship between the Hg concentration present in sediments and SPM (D). N/A (not analized) means that SPM was not analyzed in this sampling site.

The Hg association with particulate organic matter is well documented. Some authors such as Kainz et al. (2003), Sanei and Goodarzi (2006) and Hassan et al. (2019) have reported a strong correlation between the distribution of organic matter and Hg in numerous types of sediments (e.g. coastal and lake sediments). The results of the present study confirm this association (Fig. 4.2B), as demonstrated by the percentage of organic matter positively correlated with the [Hg] in sediments ($p<0.05$), which reflects the affinity of Hg to the organic matter. [Hg] in SPM (Fig. 4.2C) presented 0.138±0.007 $\mu g\ g^{-1}$ in Mira, 0.157±0.003 $\mu g\ g^{-1}$ in Areão, 0.105±0.005 $\mu g\ g^{-1}$ in Barra, 0.064±0.004 $\mu g\ g^{-1}$ in Furadouro, 0.138±0.003 $\mu g\ g^{-1}$ in Canide, 0.121±0.003 $\mu g\ g^{-1}$ in Aterro and 0.113±0.003 $\mu g\ g^{-1}$ in Mindelo. As observed for sediments, Areão remains the sampling site with the highest Hg levels; however, although differences continue to be significant ($p<0.05$) when compared to the Hg levels from other sampling sites, they are not as evident as those found in the sediments. At the same time, it is possible to verify that the Hg content on the SPM is higher than in the sediment in most sampling sites. This decrease in [Hg] of the sediment when compared to the Hg present in the SPM was also verified by Cranston and Buckley (1972) in a study conducted on the southeast coast of Nova Scotia, Canada. According to Ortiz et al. (2015) and Gagnon et al. (1997), the small particles which remain suspended in the water column have a higher surface to volume ratio than the sedimentary material, providing more binding sites between Hg and the sediment particle. Finally, [Hg] found in the SPM did not show any correlation with the [Hg] in the sediments (Fig. 4.2D).

As observed in Table 1, it was not possible to collect the same species in all sampling sites. Viana do Castelo was the sampling site with the highest number of collected species (10), which may be explained by the fact that this was the only sampling site with rock pools. Rock pools are extremely productive microhabitats that contain a diverse range of plant, invertebrate and fish life (White et al. 2015), presenting increased species richness and diversity when compared to sandy beaches (Santos et al. 1994).

Considering the average [Hg] found in the three groups of species (Phyla – Fig. 4.3A), Mollusca (consumers) presented significantly higher levels of Hg than the other two groups (producers). These results suggest a transfer of Hg from the lower level to higher levels of the food chain. This Hg transfer to higher levels has already been demonstrated by Bu-Olayan and Thomas (2017) who verified an increase of Hg from the primary producer (phytoplankton) to the secondary (zooplankton) and tertiary consumers (mollusc and fish) collected from Kuwait Bay. Bargagli et al (1998) have also found a progressive rise in Hg concentrations in organisms at different levels of the marine food web (phytoplankton < zooplankton and benthic primary consumers < detritivores and opportunistic benthic invertebrates < epipelagic fish < demersal fish and plankton-feeding seabirds < fish-eating penguins < predatory birds and Weddell seal).

If we look at the average [Hg] per species (Fig. 4.3B), *N. lapillus* has the highest Hg levels (0.021±0.003 $\mu g\ g^{-1}$) and *C. teedei* is the species with the lowest [Hg] (0.002±0.001 $\mu g\ g^{-1}$), while the remaining species showed [Hg] below 0.01 µg g^{-1}, except for *M. galloprovincialis*, which presented a slightly higher [Hg] (0.011±0.003 µg g^{-1}). The higher significant [Hg] found ($p<0.05$) in *N. lapillus* in relation to other species may be related to the trophic level that this gastropod occupies in the food web, as observed by Giltrap et al. (2013). These authors analysed stable isotope (δ15N) data which suggested that metal accumulation in *N. lapillus* may potentially result as a consequence of its predatory nature. In this sense, since *N. lapillus* (predator) feeds on *Mytilus sp.* (prey) (Torossian et al. 2020) a significant and positive correlation ($p <0.05$) was found between the Hg levels present in the predator and the Hg levels found in the prey considering the different sampling site s (Fig. 4.3C).

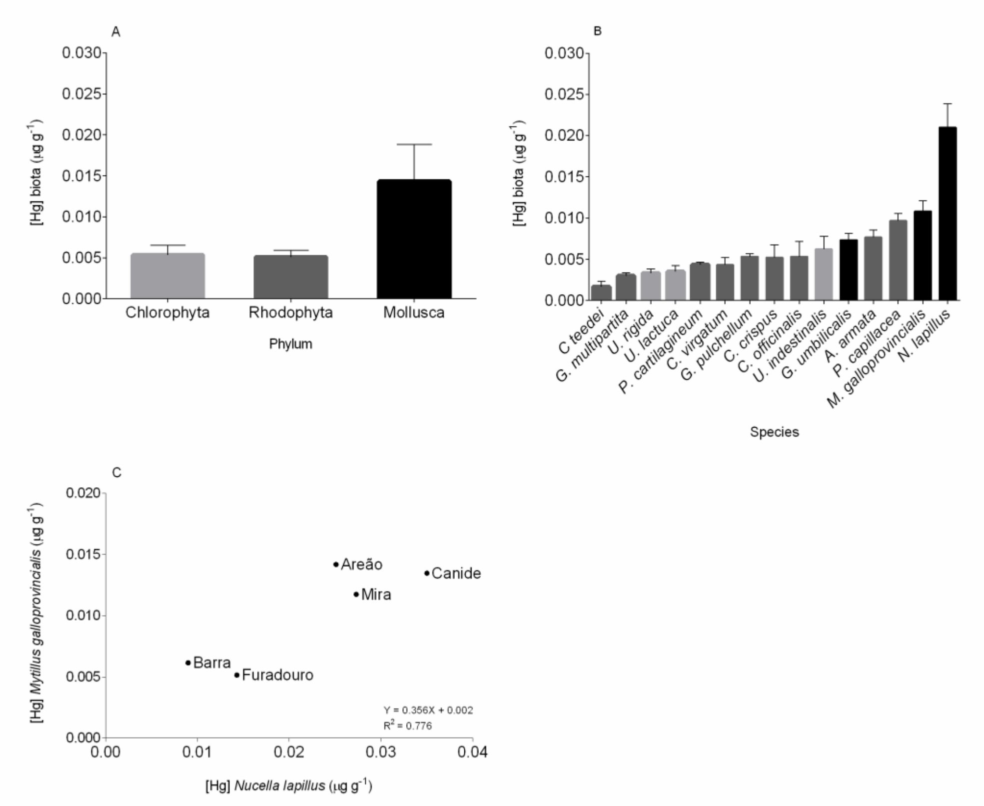


Fig. 4.3 Average Hg concentration (µg g^{-1}) per Phylum (A); per species (B) and relationship between the Hg concentration present in *Mytillus galloprovincialis* and *Nucella lapillus* (C).

The Hg biomagnification factor (BMF) was calculated for *G. umbilicalis* since it feeds on macroalgae (Sampaio et al. 2017) and for N. lapillus considering the Hg levels of this species and its prey (*M. galloprovincialis*). BMF value ranged from 1 to 4 for *G. umbilicalis* and 1 and 3 for *N. lapillus*. Thus, the present study provides evidence of Hg biomagnification. Most of the species present in this study have much lower [Hg] than that established for biota (EQS) and for human consumption, which means that, in terms of risk assessment, the consumption of these species has no negative effects on human health or wildlife. The only exception is the species *N. napillus*, which has a [Hg] equal to the EQS.

The collected species do not have a homogeneous distribution throughout the sampling sites, so it was not possible to verify the existence of a correlation between the [Hg] found in the species and the [Hg] of superficial sediments of the respective sampling site for all species. The macroalgae *U. rigida* (Fig. 4.4A), seem to have a positive correlation with the [Hg] found in the superficial sediments; however, this correlation is not significant ($p>0.05$) probably due to the reduced number of sampling points. On the other hand, *N. lapillus* (Fig. 4.4B) and *M. galloprovincialis* (Fig. 4.4C) did not show any correlation, despite being found in more sampling sites. However, since *M. galloprovincialis* is a filter-feeder, the Hg accumulation is this species was positively correlated with the SPM Hg levels (Fig. 4.4D), which is in accordance with previous studies focusing on the transfer of Hg from SPM to filter-feeder species (Jędruch et al. 2019).

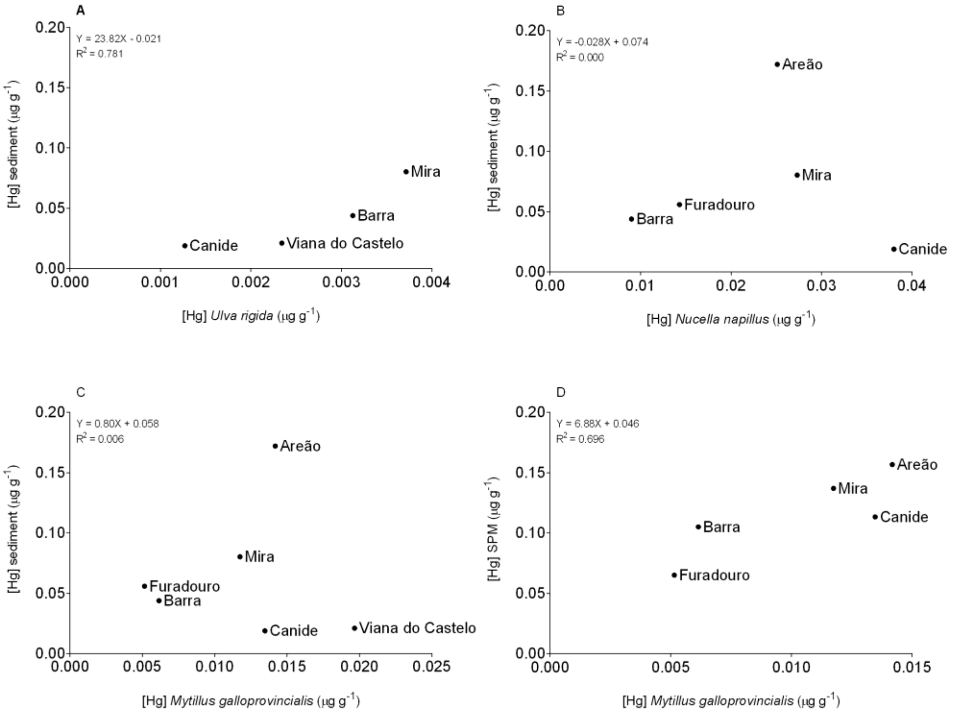


Fig. 4.4 Relationship between Hg concentration (µg g^{-1}) in sediments and collected species (A, B and C) and correlation between Hg concentration (µg g^{-1}) in SPM and *Mytilus galloprovincialis* (D).

The ability of the collected species to accumulate Hg from the associated superficial sediments was assessed by BSAF (Fig. 4.5). Bioaccumulation and biomagnification along the trophic chain might lead to high concentration of Hg, sometimes exceeding legislation limits (0.5µg g^{-1} or 1.0µg g^{-1} in the exception list (EU 2006). Although, aquatic organisms can be exposed to Hg by several routes, such as the direct contact with Hg contaminated water and sediments and through ingestion of Hg contaminated food (USEPA 1997). In addition, toxicity of metals to marine organisms does not solely depend on total accumulated tissue concentrations but is related to a threshold concentration of internal metabolically available metal (Rainbow 2007). The mean value of BSAF oscillated between 0.05 (*C. crispus*) and 0.5 (*N. lapillus*). The results referring to the BSAF obtained for the collected species and the results considering the feeding habits of *N. lapillus*, *M. galloprovincialis* and *G. umbilicalis* suggest that food intake is of greater importance bioaccumulation of Hg than their contact with sediments.

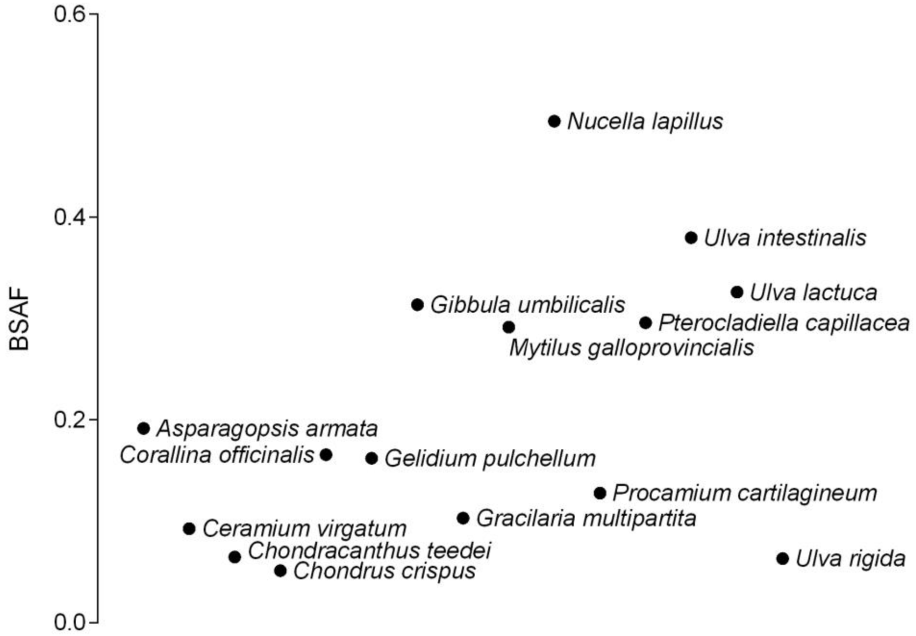


Fig. 4.5 Mean values of BSAF in the different intertidal species.

4. Conclusion

This study demonstrates that the [Hg] present in the surface sediments along the Portuguese Northwest coast is classified as uncontaminated and is not capable of promoting adverse effects on biota. At the same time, the results of this study reinforce the theory of the affinity of Hg for organic matter. In addition, the [Hg] present in all species is not likely to cause adverse health problems in humans and wildlife through the ingestion of these species and the differences in Hg levels found between producers and consumers as well as the predator-prey correlation suggest a potential biomagnification of Hg along the food chain. However, although the Hg concentration in some species increases in sampling sites that have higher levels of Hg in the sediment, these species are not considered accumulators which means that their Hg levels do not depend on the concentration found in the sediments but on the [Hg] in food.

Conflict of interest

The authors declare that they have no conflict of interests (financial or non-financial).

Acknowledgements

Thanks are due for the financial support to CESAM (UIDB/50017/2020+UIDP/50017/2020), to FCT/MEC through national funds. The authors also thank FCT (Fundação para a Ciência e Tecnologia) for the PhD grant of Vieira, H. C. (PD/BD/127808/2016). Bordalo M.D. is funded by national funds (OE), through FCT – Fundação para a Ciência e a Tecnologia, I.P., in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19. We are thankful for the financial support by the project INSIDER (PTDC/CTA-AMB/30495/2017 and POCI-01-0145-FEDER-030495) funded by FEDER, through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES.

References

Álvarez-Salgado, X. A., F. G. Figueiras, F. F. Pérez, S. Groom, E. Nogueira, A. V. Borges, L. Chou, C. G. Castro, G. Moncoiffé, A. F. Ríos, A. E. J. Miller, M. Frankignoulle, G. Savidge, and R. Wollast. 2003. The Portugal coastal counter current off NW Spain: new insights on its biogeochemical variability. Progress in Oceanography 56:281-321.

Arcagni, M., R. Juncos, A. Rizzo, M. Pavlin, V. Fajon, M. A. Arribére, M. Horvat, and S. Ribeiro Guevara. 2018. Species- and habitat-specific bioaccumulation of total mercury and methylmercury in the food web of a deep oligotrophic lake. Science of The Total Environment 612:1311-1319.

Barbaro, A., A. Francescon, B. Polo, and M. Bilio. 1978. Balanus amphitrite (Cirripedia: Thoracica) — A potential indicator of fluoride, copper, lead, chromium and mercury in North Adriatic lagoons. Marine Biology 46:247-257.

Bargagli, R., F. Monaci, J. Sanchez-Hernandez, and D. Cateni. 1998. Biomagnification of mercury in an Antarctic marine coastal food web. Marine Ecology Progress Series 169:65-76.

Belabed, B.-E., X. Laffray, A. Dhib, M. Fertouna-Belakhal, S. Turki, and L. Aleya. 2013. Factors contributing to heavy metal accumulation in sediments and in the intertidal mussel *Perna perna* in the Gulf of Annaba (Algeria). Marine Pollution Bulletin 74:477-489.

Bignert, A., D. Cossa, R. Emmerson, R. Fryer, C. Füll, J. Fumega, R. Laane, H. Calls, B. McHugh, and B. Miller. 2004. OSPAR/ICES workshop on the evaluation and update of background reference concentrations (B/RCs) and ecotoxicological assessment criteria (EACs) and how these assessment tools should be used in assessing contaminants in water, sediment, and biota: Workshop The Hague, 9-13 February 2004. Final Report.

Bonsignore, M., D. Salvagio Manta, S. Mirto, E. M. Quinci, F. Ape, V. Montalto, M. Gristina, A. Traina, and M. Sprovieri. 2018. Bioaccumulation of heavy metals in fish, crustaceans, molluscs and echinoderms from the Tuscany coast. Ecotoxicology and Environmental Safety 162:554-562.

Bu-Olayan, A. H., and B. V. Thomas. 2017. Effect of Mercury Transfer from Producer to Consumer in a Marine Environment. Polish Journal of Environmental Studies 26:2487-2494.

Burkhard, L. 2009. Estimation of biota sediment accumulation factor (BSAF) from paired observations of chemical concentrations in biota and sediment.

Burkhard, L. P. 2003. Factors influencing the design of bioaccumulation factor and biota-sediment accumulation factor field studies. Environmental Toxicology and Chemistry: An International Journal 22:351-360.

Cairrão, E., M. J. Pereira, M. R. Pastorinho, F. Morgado, A. M. V. M. Soares, and L. Guilhermino. 2007. *Fucus* spp. as a Mercury Contamination Bioindicator in Costal Areas (Northwestern Portugal). Bull Environ Contam Toxicol 79:388-395.

Chakraborty, P., P. V. Raghunadh Babu, K. Vudamala, D. Ramteke, and K. Chennuri. 2014. Mercury speciation in coastal sediments from the central east coast of India by modified BCR method. Marine Pollution Bulletin 81:282-288.

Chen, J., P. Chakravarty, G. R. Davidson, D. G. Wren, M. A. Locke, Y. Zhou, G. Brown Jr, and J. V. Cizdziel. 2015. Simultaneous determination of mercury and organic carbon in sediment and soils using a direct mercury analyzer based on thermal decomposition–atomic absorption spectrophotometry. Analytica Chimica Acta 871:9-17.

Coelho, J. P., J. Pimenta, R. Gomes, C. M. Barroso, M. E. Pereira, M. A. Pardal, and A. Duarte. 2006. Can *Nassarius reticulatus* be used as a bioindicator for Hg contamination? Results from a longitudinal study of the Portuguese coastline. Marine Pollution Bulletin 52:674-680.

Commission, O. 2013. OSPAR List of Chemicals for Priority Action (Revised 2013)(Reference Number 2004-12).in OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic, London, UK.

Costa, S., D. Crespo, B. M. G. Henriques, E. Pereira, A. C. Duarte, and M. A. Pardal. 2011. Kinetics of Mercury Accumulation and Its Effects on *Ulva lactuca* Growth Rate at Two Salinities and Exposure Conditions. Water, Air, & Soil Pollution 217:689-699.

Costley, C. T., K. F. Mossop, J. R. Dean, L. M. Garden, J. Marshall, and J. Carroll. 2000. Determination of mercury in environmental and biological samples using pyrolysis atomic absorption spectrometry with gold amalgamation. Analytica Chimica Acta 405:179-183.

Cranston, R. E., and D. E. Buckley. 1972. Mercury pathways in a river and estuary. Environ Sci Technol 6:274-278.

Cruz, J. 2007. Ocean wave energy: current status and future prespectives. Springer Science & Business Media.

EU. 2006. Commission Regulation (EC) No 1881/2006. JO L364, 19 December 2006 (pp. 5-24).

EU. 2013. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy

Off. J. Eur. Union, L226 (2013), pp. 1-17.

Fitzgerald, W. F., C. H. Lamborg, and C. R. Hammerschmidt. 2007. Marine biogeochemical cycling of mercury. Chemical Reviews 107:641-662.

Fostier, A. H., F. do N. Costa, and M. d. G. A. Korn. 2016. Assessment of mercury contamination based on mercury distribution in sediment, macroalgae, and seagrass in the Todos os

Santos bay, Bahia, Brazil. Environmental Science and Pollution Research 23:19686-19695.

Gagnon, C., É. Pelletier, and A. Mucci. 1997. Behaviour of anthropogenic mercury in coastal marine sediments. Marine Chemistry 59:159-176.

Giltrap, M., A. Macken, M. Davoren, E. McGovern, B. Foley, M. Larsen, J. White, and B. McHugh. 2013. Utilising caging techniques to investigate metal assimilation in Nucella lapillus, *Mytilus edulis* and *Crassostrea gigas* at three Irish coastal locations. Estuarine, Coastal and Shelf Science 132:77-86.

Hammerschmidt, C. R., and W. F. Fitzgerald. 2006. Bioaccumulation and trophic transfer of methylmercury in Long Island Sound. Archives of Environmental Contamination and Toxicology 51:416-424.

Hassan, H., A. A. Elezz, M. Abuasali, and H. AlSaadi. 2019. Baseline concentrations of mercury species within sediments from Qatar's coastal marine zone. Marine Pollution Bulletin 142:595-602.

Intergovernmental Panel on Climate Change. 2014. Climate Change 2014-Impacts, Adaptation and Vulnerability: Part A: Global and Sectoral Aspects: Volume 1, Global and Sectoral Aspects: Working Group II Contribution to the IPCC Fifth Assessment Report. Cambridge University Press.

Jędruch, A., M. Bełdowska, and M. Ziółkowska. 2019. The role of benthic macrofauna in the trophic transfer of mercury in a low-diversity temperate coastal ecosystem (Puck Lagoon, southern Baltic Sea). Environ Monit Assess 191:137-137.

Kainz, M., M. Lucotte, and C. C. Parrish. 2003. Relationships between organic matter composition and methyl mercury content of offshore and carbon-rich littoral sediments in an oligotrophic lake. Canadian Journal of Fisheries and Aquatic Sciences 60:888-896.

Knightes, C. D. 2008. Development and test application of a screening-level mercury fate model and tool for evaluating wildlife exposure risk for surface waters with mercury-contaminated sediments (SERAFM). Environmental Modelling & Software 23:495-510.

Liu, J., L. Cao, and S. Dou. 2019a. Trophic transfer, biomagnification and risk assessments of four common heavy metals in the food web of Laizhou Bay, the Bohai Sea. Science of The Total Environment 670:508-522.

Liu, Y., X. Luo, Y. Zeng, M. Deng, W. Tu, Y. Wu, and B. Mai. 2019b. Bioaccumulation and biomagnification of hexabromocyclododecane (HBCDD) in insect-dominated food webs from a former e-waste recycling site in South China. Chemosphere:124813.

Matos, P., E. Sousa, M. A. Pardal, E. Pereira, and P. G. Cardoso. 2016. Structural and Functional Responses of Macrobenthic Communities to Mercury Contamination. Water, Air, & Soil Pollution 227:41.

McHugh, B., R. Berbee, E. Farmer, R. Fryer, N. Green, M. Larsen, L. Webster, P. Lepom, E. McGovern, and T. Maes. 2016. Mercury assessment in the marine environment: assessment criteria comparison (EAC/EQS) for mercury. 1911458094, OSPAR Commission.

Mohsen, M., Q. Wang, L. Zhang, L. Sun, C. Lin, and H. Yang. 2019. Heavy metals in sediment, microplastic and sea cucumber *Apostichopus japonicus* from farms in China. Marine Pollution Bulletin 143:42-49.

Moreno-Sierra, D., M. E. Bergés-Tiznado, F. Márquez-Farías, Y. E. Torres-Rojas, J. R. Ruelas-Inzunza, and F. Páez-Osuna. 2016. Trace metals in target tissues and stomach contents of the top predator sailfish *Istiophorus platypterus* from the Eastern Pacific: concentrations and contrasting behavior of biomagnification. Environmental Science and Pollution Research 23:23791-23803.

Ortiz, V. L., R. P. Mason, and J. Evan Ward. 2015. An examination of the factors influencing mercury and methylmercury particulate distributions, methylation and demethylation rates in laboratory-generated marine snow. Marine Chemistry 177:753-762.

Pato, P., M. Válega, E. Pereira, C. Vale, and A. C. Duarte. 2008. Inputs from a Mercury-Contaminated Lagoon: Impact on the Nearshore Waters of the Atlantic Ocean. Journal of Coastal Research:28-38.

Pereira, M. E., A. I. Lillebø, P. Pato, M. Válega, J. P. Coelho, C. B. Lopes, S. Rodrigues, A. Cachada, M. Otero, M. A. Pardal, and A. C. Duarte. 2009. Mercury pollution in Ria de Aveiro (Portugal): a review of the system assessment. Environ Monit Assess 155:39-49.

Pirrone, N., S. Cinnirella, X. Feng, R. Finkelman, H. Friedli, J. Leaner, R. Mason, A. Mukherjee, G. Stracher, and D. Streets. 2010. Global mercury emissions to the atmosphere from anthropogenic and natural sources. Atmospheric Chemistry and Physics 10:5951-5964.

Rainbow, P. S. 2007. Trace metal bioaccumulation: models, metabolic availability and toxicity. Environment International 33:576-582.

Rubal, M., P. Veiga, E. Cacabelos, J. Moreira, and I. Sousa-Pinto. 2013. Increasing sea surface temperature and range shifts of intertidal gastropods along the Iberian Peninsula. Journal of Sea Research 77:1-10.

Sampaio, E., I. F. Rodil, F. Vaz-Pinto, A. Fernández, and F. Arenas. 2017. Interaction strength between different grazers and macroalgae mediated by ocean acidification over warming gradients. Marine Environmental Research 125:25-33.

Sanei, H., and F. Goodarzi. 2006. Relationship between organic matter and mercury in recent lake sediment: The physical–geochemical aspects. Applied Geochemistry 21:1900-1912.

Santos, R. S., R. D. Nash, and S. J. Hawkins. 1994. Fish assemblages on intertidal shores of the island of Faial, Azores. ARQUIPÉLAGO. Ciências Biológicas e Marinhas= Life and Marine Sciences 12:87-100.

Scanu, S., D. Piazzolla, F. M. Frattarelli, E. Mancini, F. Tiralongo, M. V. Brundo, D. Tibullo, R. Pecoraro, C. Copat, M. Ferrante, and M. Marcelli. 2016. Mercury Enrichment in Sediments of the Coastal Area of Northern Latium, Italy. Bull Environ Contam Toxicol 96:630-637.

Scheuhammer, A. M., M. W. Meyer, M. B. Sandheinrich, and M. W. Murray. 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. AMBIO: a Journal of the Human Environment 36:12-19.

Shore, R., M. Pereira, L. Walker, and D. Thompson. 2011. Mercury in nonmarine birds and mammals.

Sinkus, W., V. Shervette, J. Ballenger, L. A. Reed, C. Plante, and B. White. 2017. Mercury bioaccumulation in offshore reef fishes from waters of the Southeastern USA. Environmental Pollution 228:222-233.

Szefer, P., A. A. Ali, A. A. Ba-Haroon, A. A. Rajeh, J. Gełdon, and M. Nabrzyski. 1999. Distribution and relationships of selected trace metals in molluscs and associated sediments from the Gulf of Aden, Yemen. Environmental Pollution 106:299-314.

Tchounwou, P. B., W. K. Ayensu, N. Ninashvili, and D. Sutton. 2003. Environmental exposure to mercury and its toxicopathologic implications for public health. Environmental Toxicology: An International Journal 18:149-175.

Torossian, J. L., K. E. Hosek, S. C. Donelan, G. C. Trussell, B. S. Helmuth, and M. L. Zippay. 2020. Physiological and biochemical responses to acute environmental stress and predation risk in the blue mussel, *Mytilus edulis*. Journal of Sea Research 159:101891.

USEPA. 1997. Mercury Study Report to Congress Volume VI: An Ecological Assessment for Anthropogenic Mercury Emissions in the United States. U.S. Environmental Protection Agency.

Vieira, H. C., F. Morgado, A. M. V. M. Soares, and S. N. Abreu. 2015. Fish consumption recommendations to conform to current advice in regard to mercury intake. Environmental Science and Pollution Research 22:9595-9602.

Vignati, D. A., S. Polesello, R. Bettinetti, and M. S. Bank. 2013. Mercury environmental quality standard for biota in Europe: Opportunities and challenges. Integrated Environmental Assessment and Management 9:167-168.

White, G. E., G. C. Hose, and C. Brown. 2015. Influence of rock-pool characteristics on the distribution and abundance of inter-tidal fishes. Marine Ecology 36:1332-1344.

Wiener, J. G., D. P. Krabbenhoft, G. H. Heinz, and A. M. Scheuhammer. 2003. Ecotoxicology of mercury. Handbook of ecotoxicology 2:409-463.

Chapter V:

Water temperature modulates mercury accumulation and oxidative stress status of Common Goby (*Pomatoschistus microps*)

Water temperature modulates mercury accumulation and oxidative stress status of Common Goby (*Pomatoschistus microps*)

Vieira, H.C.[1], Bordalo, M.D.[1], Soares, A.M.V.M.[1], Rodrigues, A.C.M.[1], Pires, S.F.S.[1], Rocha, R.J.M.[1], Rendón-von Osten, J.[2], Abreu, S. N.[1], Morgado, F.[1]

[1] CESAM - Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

[2] EPOMEX Institute, Autonomous University of Campeche, Campeche, Mexico

Abstract

Mercury (Hg) is a widespread pollutant across estuarine and coastal areas, raising concern on its potential impact on aquatic organisms. Hg may origin from natural and anthropogenic sources, being persistent and potentially toxic to biota, ultimately representing a serious risk to human health. Hg bioaccumulation and toxicity may also induce reactive oxygen species (ROS) production in marine organisms, responsible for cell and tissue damage. Additionally, the temperature is undoubtedly an important environmental factor to consider regarding bioaccumulation, due to its marked influence on the physiology and ecology of aquatic organisms. This study aimed to investigate the effect of different temperature scenarios (15, 20 and 25ºC) on the Hg bioaccumulation in *Pomatoschistus microps* (Krøyer, 1838) liver and muscle, as well as on oxidative stress responses and energy metabolism, after short-term exposure to a naturally contaminated sediment with an environmentally relevant [Hg] (1.2 µg g^{-1}). Our results showed that Hg bioaccumulation tends to increase along the temperature gradient with higher values of Hg accumulated in liver than in muscle tissue. The action of antioxidant enzymes and stress proteins seems to be effective in combating oxidative stress in the liver. Despite the action of antioxidant defenses in the muscle, oxidative damage was observed at the protein level concomitantly with a decrease in aerobic energy production after exposure to Hg at higher temperatures.

These findings are ecologically relevant and highlight the importance of further investigation of combined effects of Hg and other stressors, especially in a scenario of a changing climate where events leading to rapid alterations on water parameters are more frequent.

Keywords: Estuarine fish; temperature gradient; metal accumulation; biochemical biomarkers

1. Introduction

Mercury (Hg) is recognized as one of the most toxic elements present in the aquatic environment (Ullrich et al. 2001), due to its frequency, toxicity, and potential for human exposure. The Government Agency for Toxic Substances and Disease Registry of the United States, ranked Hg as the 3rd element on their substances priority list in 2017 (ATSDR 2017). Hg is a naturally occurring element released to the environment through a number of natural processes (e.g. weathering of rocks, degassing of the earth's crust and volcanism – Jinadasa and Fowler 2019) and re-emitted through biomass burning (e.g., forest fires – Wang et al. 2004). However, the extensive applications over the years by industry (e.g. chlor-alkali plants; as a catalyst), mining process (e.g. use of Hg in precious metal extraction) and technological applications (e.g. paint, electronic devices), highly increased the Hg amount released into to environment (Driscoll et al. 2013, Bonsignore et al. 2018).

In aquatic ecosystems, sediments are repositories of some metals (for example, Hg) contributing to increased contamination as these metals tend to bind to the fine particles of the sediment (Ioannides et al. 2014), having a pivotal role in the bioavailability of Hg to biota. The Portuguese law's guidelines for sediment quality (Portaria n.º 1450/2007 de 12 de Novembro) classifies marine sediment quality into five classes. Marine sediments having [Hg] <0.5 μg g^{-1} are classified as class 1 (Clean dredged material - can be deposited in the aquatic environment or replaced in places subject to erosion or used for feeding beaches without restrictive standards). [Hg] in the range 0.5 – 1.5 μg g^{-1} are classified as class 2 (Dredged material with trace contamination - may be immersed in the aquatic environment taking into account the characteristics of the receiving environment and the legitimate use of it). [Hg] between 1.5 – 3 μg g^{-1} are classified as Class 3 (Lightly contaminated dredged material - can be used for embankments or, in the case of immersion requires a thorough study of the deposition site and subsequent monitoring of the site). [Hg] in the range 3 – 10 μg g^{-1} are classified as class 4 (Contaminated dredged material - preposition on land, in a waterproofed place, with the recommendation of later covering of impermeable soils.) Marine sediments having a [Hg] > 10 μg g^{-1} are classified as class 5 (highly contaminated material - ideally not to be dredged and in case of imperative, the dredges should be sent for prior treatment and/or duly authorized waste disposal, being forbidden to be immersed).

Hg can result in serious environmental and health problems, since its exposure can promote adverse biological and behavioral effects (e.g. reproduction, feeding and locomotion – Chan et al. 2003) due to its toxicity even at low concentrations (Bonsignore et al. 2018). This toxicity can be explained by the high affinity of Hg to thiol groups in proteins, including enzymes

(Waheed et al. 2020), which also promotes its ability to bioaccumulate and biomagnify efficiently in the aquatic food chain, reaching higher Hg levels in top predators (Carrasco et al. 2011, Slemr et al. 2011, Vieira et al. 2020b). Different taxonomic groups such as macroalgae (Costa et al. 2011), zooplankton, molluscs (Arcagni et al. 2018) and fish (Sinkus et al. 2017, Maury-Brachet et al. 2019) are used to monitor the impact of pollution in the marine environment, through the analysis of pollutant content in the tissue of species belonging to these groups. Hg accumulation in fish tissues can thus be influenced by the concentration and bioavailability of the Hg in the surrounding environment (Chen et al. 2018), abiotic factors such as water pH, organic matter richness, oxygen saturation, salinity and temperature (de Almeida Rodrigues et al. 2019), and biotic factors such as fish length, age, trophic position and rate of metabolism or elimination from the tissue (Ribeiro et al. 1996, Bonsignore et al. 2018, Mills et al. 2019). The common goby *Pomatoschistus microps*, Krøyer (1838) is an abundant epibenthic fish species with a crucial role in estuarine ecosystems, since it is an intermediary predator in estuarine food webs (Vieira et al. 2008). This species has a wide geographical distribution, high abundance and great fecundity, which are important characteristics for being considered a bioindicator species in monitoring studies (Quintaneiro et al. 2008). *P. microps* has been widely used as a test organism (Fonte et al. 2016, Miranda et al. 2019, Vieira et al. 2020a), holds a key role in estuarine food webs as intermediary predator and is found in a large range of temperatures (8 – 24 ºC) (Moreira et al. 1992).

Changes in seawater temperature have been observed in most of the global oceans over the years; however, these changes are not homogeneous (Deser et al. 2010, Alexander et al. 2018). Water temperature is a particularly relevant factor for Hg accumulation studies, as it plays an important role in Hg accumulation in fish (Ahonen et al. 2018, Chen et al. 2018), promoting changes in fish metabolism, prey species composition and Hg bioavailability (Dijkstra et al. 2013). Moreover, not only Hg exposure can induce the formation of reactive oxygen species (ROS) [e.g. superoxide anion ($O2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\cdot}$)] (Shanker and Aschner 2003) but also an increase in water temperature (Pinto et al. 2019). ROS presence in high concentrations is responsible for cell and tissue damage (Monteiro et al. 2013). To prevent damage caused by ROS, fish have several different types of cellular antioxidant defense mechanisms (Larose et al. 2008), enzymatic antioxidant defenses such as catalase (CAT) and glutathione-*S*-transferase (GST); and also non-enzymatic antioxidant defenses, such as total glutathione (tGSH – Mieiro et al. 2011). These enzymatic and non-enzymatic antioxidants are essential in maintaining the redox status of fish cells and serving as an important biological defenses against oxidative stress (Monteiro et al. 2009). In the last few decades, the use of biomarkers has been increasing in both biomonitoring programs and in toxicity bioassays (De Marchi et al. 2020). The use of different biomarkers that can signal exposure to contaminants and quantify its effects on the health of organisms, enables a more comprehensive and integrative assessment of environmental quality (Solé et al., 2010).

Although there are some studies that relate temperature to Hg bioaccumulation, they lack environmental relevance in terms of [Hg] and exposure, often using very high concentrations and neglecting sediment as a route of contamination. In this context, this study aims to fill these gaps by assessing the effects of a naturally contaminated sediment as an environmentally relevant route of Hg exposure in the native benthic fish *P. microps*. Furthermore, considering that aquatic organisms are frequently exposed to temperature fluctuations, it is intended to evaluate the combined effects of Hg exposure with three distinct water temperatures (15 ºC, 20 ºC, 25 ºC) not only on the Hg bioaccumulation but also on its toxicity in terms of biochemical and cellular responses. We, therefore, hypothesize that: i) Hg accumulation in the fish tissues is influenced by temperature, ii) Hg may alter the antioxidant status and energy allocation of the exposed organisms, iii) Hg toxicity to fish is modulated by the water temperature.

2. Materials and Methods

2.1. Sediment sampling

All sediment samples were collected in Ria de Aveiro (Portugal) during low tide, a coastal lagoon that received an effluent rich in Hg from a chlor-alkali plant located in an industrial chemical complex near of Estarreja for more than four decades, which resulted in an environmental Hg contamination gradient inside the lagoon (Vieira et al. 2020a). The contaminated sediment was collected in Laranjo bay (40°43'13.4"N 8°37'15.4"W), which is the most Hg impacted area of the Ria de Aveiro (Vieira et al. 2019). On the other hand, the control sediment was collected in a sampling site called Barra (40°37'50.40"N, 8°44'39.34"), with low Hg levels in sediments (Pereira et al. 2006), considered by Portuguese law an uncontaminated area by Hg. In the laboratory, the sediments were oven dried at 35ºC for 3 days and subsequently homogenized and sieved (shaken continuously at amplitude 8) with stainless steel sieves with meshes sizes 1 mm.

2.2.Fish sampling and maintenance in the laboratory

Eighty *P. microps* adult specimens (> 3 cm long) were captured during low tide using a hand-operated small trawl net in the same sampling site where the reference sediment was collected. The sampling area presented salinity values of 32, pH 8, temperature of 21 ºC and O_2 saturation levels of 95%. After being collected, specimens were immediately transported alive to the laboratory in 30 L containers with aeration. At the laboratory, fish were allocated to three different recirculated aquatic systems composed of six tanks of 5 L each (four fish per tank). All systems presented similar conditions of salinity (25±1), pH (7.8±0.5), O_2 saturation (89%), photoperiod (14h light/10h dark) but different temperatures (15±1 ºC, 20±1 ºC and 25±1 ºC) for an acclimation period of one week. After this acclimatization period, fish were transferred to the bioaccumulation assay tanks.

Fish were fed daily with commercial fish food (TetraMin®) containing traces of Hg (0.050±0.001 $\mu g\ g^{-1}$), the daily amount of food was estimated to correspond to approximately 2% of the fish weight (17mg on average). In addition, the liver and muscle (collected between the dorsal fin and caudal fin) of five *P. microps* specimens were removed to measure baseline levels of Hg. These samples were frozen at -20 ºC for later determination of Hg.

2.3. Bioaccumulation assay

The bioaccumulation assay was carried out to evaluate the influence of mean annual water surface temperature from three different locations in the northern Atlantic Ocean on Hg bioaccumulation (15±1 ºC, 20±1 ºC and 25±1 ºC) on *P. microps* Hg accumulation. The highest assay temperature (25ºC) was chosen based on the annual mean sea surface temperature in the southern Gulf of Mexico (Campeche coast, Mexico) ranging from 23.9 ºC to 29.4 ºC and an annual temperature average of 26.9±0.5 ºC. The temperature of 20 ºC was based on the annual sea surface temperature from Terceira Island (one of the nine volcanic islands of the Azores archipelago, Portugal, located in the middle of the North Atlantic Ocean), which presents an annual variation from 16.3 ºC to 23.8 ºC with an annual temperature average of 18.7±0.8 ºC. The lowest temperature of the experiment (15 ºC) was based on the annual temperature from Aveiro (North-western coast of Portugal) where the average of sea surface temperature is 16.1±0.5 ºC.

The experimental design (Fig. 5.1) included 2 treatments for each temperature: 1 control (uncontaminated sediment) and 1 treatment containing sediment with low [Hg], with 12 P. microps specimens each (4 specimens per aquarium). The fish from the control treatments at the different temperatures (15ºC, 20ºC and 25ºC) had an average size of 3.80±0.02, 3.73±0.09, and 3.81±0.05, respectively. In the contaminated treatments *P. microps* average size was 3.90±0.09 at 15 ºC, 3.70±0.06 at 20 ºC and 3.81±0.05 at 25 ºC. No significant differences (p>0.05) were found in the size of the fish in the different treatments.

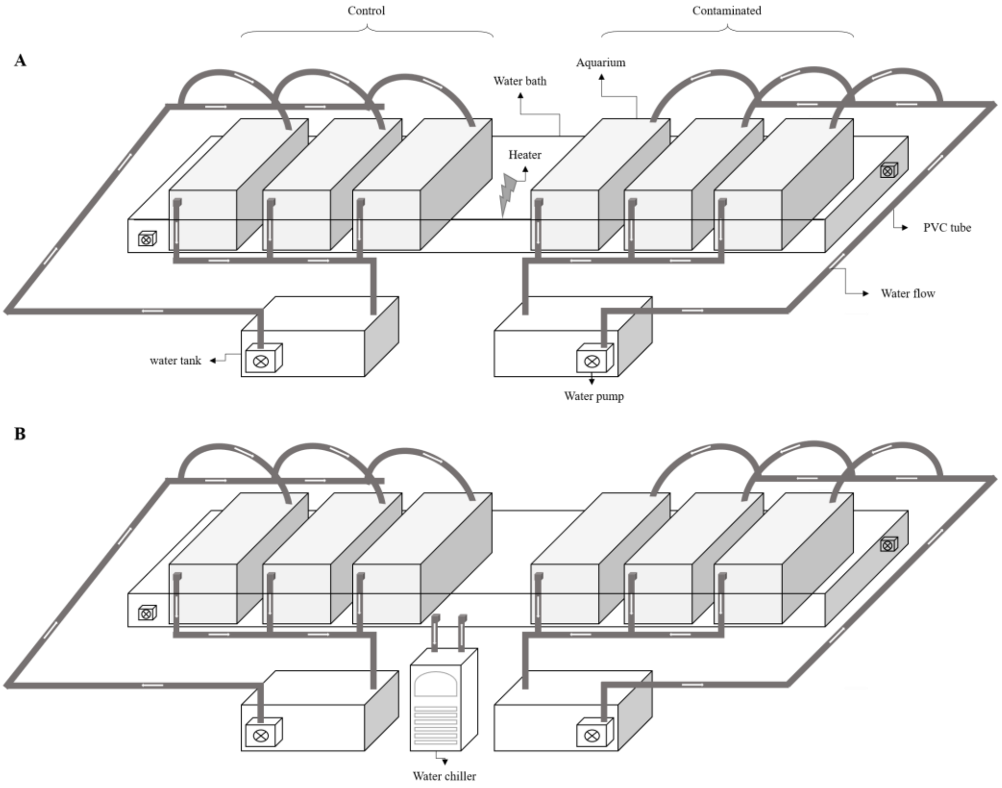


Fig. 5.1 Scheme of the recirculated test systems used in the bioaccumulation test. Two systems A) were used to obtain the temperature of 20ºC and 25ºC and one system B) was used for the temperature of 15ºC.

The amount of sediment added to each aquarium was calculated based on OECD 315 guideline (OECD 2008) which recommends a sediment–water ratio of 1:4. At the beginning of the experiment, 1000 g of uncontaminated sediment was added to each aquarium for the control treatment, while 1000 g of contaminated sediment was added to each aquarium for the Hg contaminated treatments.

The exposure period lasted for 7 days and the water physicochemical parameters (salinity (24.9±0.04), pH (8.01±0.02) and O_2 saturation (86.59±0.79%) were monitored at the beginning and at the end of the experiment and no significant differences ($p>0.05$) were found between the physicochemical parameters of acclimation period and the exposure period. After the 7-day exposure, *P. microps* were collected and euthanized by decapitation under ice-cold induced anesthesia. From each fish, the liver and the muscle were isolated on ice. The liver and muscle samples used in biomarkers analysis were frozen at – 80ºC and the liver and muscle samples used for Hg quantification were frozen at -20 ºC for later Hg determination.

2.4. Hg quantification

Hg quantification was performed using the Advanced Mercury Analyzer (AMA-254, made by ALTEC and distributed by LECO). According to Costley et al. (2000), this kind of Hg quantification does not require previous digestion of the sample; the procedure is based in a pyrolysis process of the sample using a combustion tube heated at 750ºC under an oxygen atmosphere, the released Hg is trapped in a gold amalgamator and subsequently detected and quantified by atomic absorption spectrometry.

Analytical quality of the procedure was checked using the reference material TORT-3 (Lobster Hepatopancreas Reference Material for Trace Metals, National Research Council of Canada). Obtained data (0.281±0.003 μg g^{-1} of Hg) and reference (0.29±0.02 μg g^{-1} of Hg) values were not statistically different ($p>0.05$).

2.5. Biomarker analysis

2.5.1. Sample preparation for biomarkers analysis

Fish tissue samples were individually homogenized on ice using ultra-pure water (1000 μl per liver sample, 13.9±5.6 mg mean weight; 1600μl per muscle sample, 28.7±7.2 mg mean weight), using the sonicator (pulsed mode of 10% for 30s, 250 Sonifier, Branson Ultrasonics). From each liver sample, the homogenate was diluted 1:1 with 0.2 M K-phosphate buffer, pH 7.4, and centrifuged for 10 min at 10000 g (4 ºC). The post-mitochondrial supernatant (PMS) was divided into microtubes and kept in −80ºC until further analyses of oxidative stress-related biomarkers. One aliquot containing 4% butylated hydroxytoluene (BHT) in methanol was used for the determination of lipid peroxidation (LPO).

From each sample of muscle 3 aliquots were taken for the analysis of lipid, sugar and protein contents, and electron transport system (ETS) activity. One aliquot containing 4% BHT in methanol was also used for the determination of LPO. Another two aliquots were stored for lactate dehydrogenase (LDH) and protein carbonylation (PC) determination.

All biomarkers determinations are performed spectrophotometrically (Microplate reader MultiSkan Spectrum, Thermo Fisher Scientific, USA), in micro-assays set up in 96 well flat bottom plates.

2.5.2. Oxidative stress biomarkers

Protein concentration of PMS was determined according to the Bradford method (Bradford, 1976), using bovine γ-globulin as a standard. Catalase (CAT) activity was determined in PMS by measuring decomposition of the substrate H_2O_2 at 240 nm (Clairborne, 1985). Glutathione-*S*-transferases (GST) activity was determined in PMS following the conjugation of

GSH with 1-chloro-2,4- dinitrobenzene (CDNB) at 340 nm (Habig et al., 1974). Total glutathione (tGSH) content was determined with PMS fraction at 412 nm using a recycling reaction of reduced glutathione (GSH) with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) in the presence of glutathione reductase (GR) excess (Baker et al., 1990; Tietze, 1969). TG content was calculated as the rate of TNB2- formation with an extinction coefficient of DTNB chromophore formed, ε = 14.1 x 103$M^{-1}cm^{-1}$ (Baker et al., 1990; Rodrigues et al., 2017). Endogenous lipid peroxidation (LPO) was determined by measuring thiobarbituric acid-reactive substances (TBARS) at 535 nm (Bird and Draper, 1984). PC was measured by the quantification of carbonyl groups based on the reaction of 2,4-dinitrophenylhydrazine (DNPH) with carbonyl groups, according to the DNPH alkaline method described by Mesquita et al. (2014). The amount of carbonyl groups was quantified spectrophotometrically at 450 nm (22,308 $mM^{-1}cm^{-1}$extinction coefficient) and results were expressed in nmol carbonyl per mg protein. Tris/NaCl phosphate buffer (pH 7.2, Tris 81.3 mM; NaCl 203.3 mM) was added to the sample and centrifuged at 3300 × g for 3 min at 4 ºC. LDH activity was measured in the supernatant by following the decrease of absorbance at 340 nm, due to NADH oxidation caused by pyruvate consumption according to (Vassault 1983) adapted to microplate (Diamantino et al. 2001).

2.5.3. Heat shock proteins (HSP70)

HSP70/HSC70 content was assessed by ELISA, adapted from (Rosa et al., 2014). 50 µl of the sample was added to a 96 well microplate and allowed to incubate overnight at 4 ºC. The next day, the microplates were washed (3×) in 0.05% PBS-Tween-20. A 100 µl sample of blocking solution (1% BSA) was added to each well and left to incubate at room temperature for 2 h. Microplates were washed and 5 µg ml^{-1} primary antibody (1º Anti-HSP70 mouse mAB (C92F3A-5) Millipore), detecting 72 and 73 kDa proteins corresponding to the molecular mass of inducible hsp and hsc70, was added and were incubated overnight at 4 ºC. The non-linked antibodies were removed by rewashing the microplates, to which were then added 1 µg ml^{-1} of the secondary antibody, anti-mouse IgC (2º Anti-mouse IgC (fab specific) Sigma) and incubate overnight at 4 ºC. After another wash, 100 µl of substrate p-nitrophenyl phosphate was added to each well and incubated for 30 min at room temperature. Then, 50 µl of stop solution (3 M NaOH) was added to each well, and the absorbance was read at 405 nm. The amount of HSP70/Hsc70 in the samples was calculated using a purified HSP70 active protein as a standard (HSP70 protein Millipore) to a range from 0 to 1500 ng ml^{-1}.

2.5.4. Cellular Energy Allocation (CEA)

Energy available (Ea – the sum of sugars, lipids and proteins) and aerobic energy production (Ec – estimated as ETS activity) were determined by the methods described by De Coen & Janssen (1997) with slight modifications for microplate (Rodrigues et al., 2015). The final CEA value was calculated as: CEA = Ea/ Ec (Verslycke et al., 2004).

Total lipid content of muscle tissue was determined adding chloroform, methanol and ultra-pure water in a 2:2:1 proportion to 300 µl of the homogenate. After centrifugation, the organic phase of each sample was transferred to clean glass tubes and H2SO4 was added prior to incubation (15 min at 200 ºC). Absorbance was measured at 375 nm and tripalmitin was used as a lipid standard. The fraction for carbohydrate content was obtained by adding 15% TCA to 300 µL of homogenate (incubated for 10 min at -20 ºC). After centrifugation (1000 g for 10 min at 4 ºC), carbohydrates quantification were performed in the supernatant by adding 5% phenol and H_2SO_4 to the samples, with glucose as a standard, the absorbance read at 492 nm. The remaining pellet was used for total protein content quantification after resuspendion with 1M NaOH (incubated for 30 min at 60 ºC), and the neutralization with 1.67 HCL. The Bradford's method (Bradford, 1976) was used for total protein content quantification using bovine serum albumin as a standard and absorbance measured at 520 nm. Fractions of energy available were converted into energetic equivalent values using the corresponding energy of combustion: 39500 mJ/ g lipid, 17500 mJ/ g glycogen, 24000 mJ/ g protein (Gnaiger, 1983).

Electron transport system (ETS) activity was measured using the INT (Iodonitrotetrazolium) reduction assay, in which ETS is measured as the rate of INT reduction in the presence of the nonionic detergent Triton X-100, with the absorbance read at 490 nm. Cellular oxygen consumption rate was calculated based on the stoichiometrical relationship in which for 2 µmol of formazan formed, 1 µmol of oxygen is consumed. Ec value was obtained by the conversion to energetic values using the specific oxyenthalpic equivalent for an average lipid, protein and carbohydrate mixture of 480 kJ/ mol O_2 (Gnaiger, 1983).

2.6. Statistical analysis

The values in all determinations are presented as mean±SE. All data were assessed for normality by using Kolmogorov-Smirnov test. The parametric statistical t-test was used to compare the [Hg] between sediments (control and Hg contaminated) and two-way ANOVA was used to test the significance of differences among Hg, temperatures and their interactions, followed by all pairwise multiple comparison procedures (Tukey test) to test differences between treatments (control and Hg contaminated) for all temperatures. All statistical analysis was performed using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla California USA) and statistically significant differences were considered when $p<0.05$.

3. Results and Discussion

The [Hg] in the sediments to which the fish were exposed during the 7-day trial are shown in Fig. 5.2. In the sediment control conditions, the average of [Hg] was 0.0023±0.0003 μg g^{-1} (15ºC), 0.0020±0.000 μg g^{-1} (20 ºC) and 0.0023±0.0003 μg g^{-1} (25ºC). The average of [Hg] in the sediment-contaminated treatments was 1.140±0.07 μg g^{-1} (15ºC), 1.150±0.047 μg g^{-1} (20ºC) and 1.144±0.06 μg g^{-1} (25ºC). Sediment [Hg] showed a significant difference (t=303.2, df=4; $p<0.05$) between the sediment contaminated treatments and respective controls for all tested temperatures.

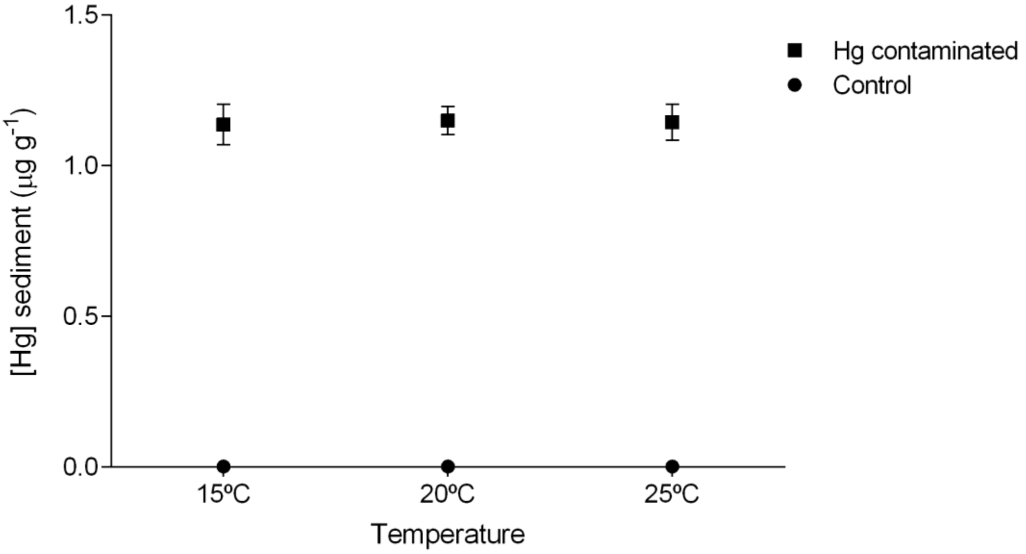


Fig. 5.2 Hg concentration (μg g^{-1}) present in the sediment of each treatment (control and Hg contaminated) used in the bioaccumulation test of *P. microps* exposed during 7 days at 15ºC, 20ºC and 25ºC.

Therefore, considering the average [Hg] used in the present exposure experiment, the controls of all temperatures are classified as class 1, while the sediment contaminated treatments are classified as class 2. Furthermore, according to USEPA (1997), the [Hg] from the contaminated sediments is 5 and 2 times lower than the highest concentrations measured along the shores of the North Atlantic and Pacific (5.00 and 2.20 μg g^{-1}, respectively).

Considering the [Hg] in *P. microps* tissues after the 7-day exposure (Fig. 5.3), a significant effect of Hg exposure and its interaction with temperature was observed (Annexes – Table A1).

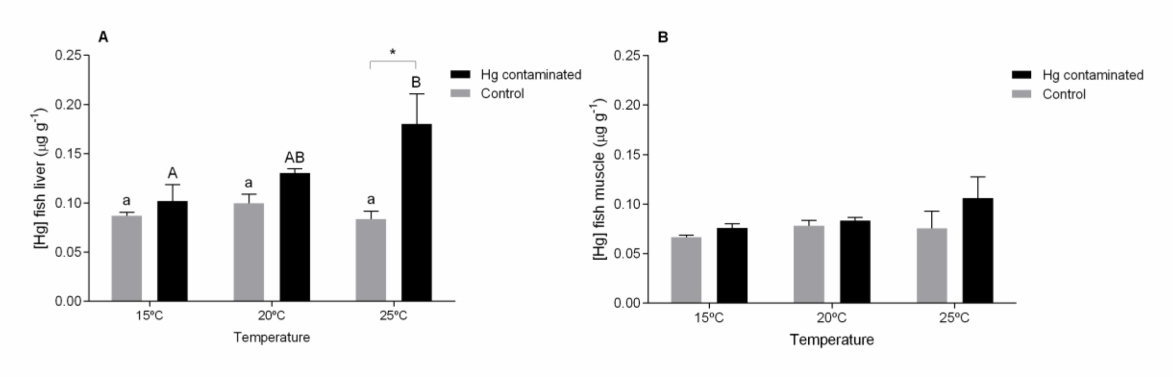


Fig. 5.3 Hg concentration (mean±SE) in *P. microps* liver (A) and muscle (B) after the short-term exposure. Asterisk (*) denotes a significant difference between the control and Hg treatment at the same temperature and the different lower-case letters.

The average [Hg] found in the liver (Fig. 5.3A) of organisms exposed to uncontaminated sediment (control) at the different temperatures was 0.087±0.004 μg g^{-1} (15 ºC), 0.099±0.009 μg g^{-1} (20 ºC) and 0.0833±0.0084 μg g^{-1} (25 ºC). No significant differences ($p<0.05$) were observed between these values, nor regarding the basal [Hg] in the liver (0.09±0.006 μg g^{-1}). When considering [Hg] in the liver of organisms exposed to contaminated sediment (treatment) at different temperatures (15, 20 and 25 ºC), a gradual significant increasing concentration ($p<0.05$) was observed from the lower (15 ºC, 0.105±0.009 μg g^{-1}) to the higher temperature (25 ºC, 0.197±0.260 μg g^{-1}) (Fig. 5.3A).

Regarding the muscle tissue, no significant differences were found between the basal [Hg] levels (0.06±0.007 μg g^{-1}) and the [Hg] present in muscle at different temperatures. The accumulation of Hg in the muscle of exposed fish shows a small rise with increasing temperature 0.075±0.004 μg g^{-1} at 15 ºC, 0.083±0.003 μg g^{-1} at 20 ºC and 0.10±0.02 μg g^{-1} (25 ºC); however, no significant differences ($p>0.05$) were found in the [Hg], considering the temperatures tested and the [Hg] of sediments (Annexes – Table A1, Fig. 5.3B).

The observed Hg bioaccumulation results between tissues are in accordance with Abreu et al. (2000) and Havelková et al. (2008), who stated that the distribution of Hg in muscle tissue and internal organs of fish depends on the degree of contamination. In heavily contaminated areas, [Hg] in internal organs are usually significantly higher than the [Hg] found in the muscle. Several studies have demonstrated that the liver is the target organ for Hg accumulation in fish from heavily contaminated localities, leading to a higher [Hg] in the liver than in muscle (Kennedy 2003, Gonzalez et al. 2005, Havelková et al. 2008), as observed in our study. Liver is a metabolically active tissue, which primarily tends to concentrate metals (e.g. Hg) before being metabolized and excreted (Storelli et al. 2005). The binding and immobilization of Hg to metallothionines and other proteins containing sulfhydryl groups that are produced in the liver could result in increased liver concentration relative to muscle (Cizdziel et al. 2003, Mieiro et al. 2009). Thus, the difference in the [Hg] between these tissues can be explained by differences in their physiological functions (Storelli et al. 2005). Therefore, after a short-term exposure, it is possible to quantify this higher accumulation in the liver, before occurring redistribution to other tissues (e.g. muscle – Costley et al. 2000).

Moreover, the effect of temperature on the Hg bioaccumulation in aquatic organisms is a topic that is still far from being fully understood, since there are several factors that can influence the bioaccumulation of metals in the aquatic environments. Other studies such as Chowdhury and Blust (2001), Jezierska and Witeska (2006) and Baykan et al. (2007) state that the temperature can stimulate biochemical and physiological processes, changes in the membrane permeability, alterations of metal transport systems, and binding to several ligands. Using different fish species (*Gambusia affinis* and *Sparus aurata*), Boudou et al. (1979) and Guinot et al. (2012) have demonstrated that the increase of temperature and the Hg accumulation have a strong correlation. However, unlike our work, Hg exposure in these studies was carried out through water contamination and via dietary sources, respectively, and the mechanisms used to counteract Hg toxicity have not been evaluated.

The generation of ROS, such as H_2O_2 and $O2^{\cdot-}$, has been identified as one of the causes of Hg-induced neurotoxicity (Roos et al. 2009). It is also known that environmental temperature increases may promote the ROS generation (Braz-Mota et al. 2017). Therefore, study the changes on oxidative stress status and energy allocation of fish under combined temperature and Hg exposure and their interactions were of primordial interest.

CAT activity decreased significantly with temperature increase (Annexes – Table A2, Fig. 5.4A), although differences between groups have not been identified. The ROS species induced by these stressors are converted into H_2O_2 by the activity of SOD, and posteriorly, CAT action can efficiently convert H_2O_2 into water and oxygen avoiding further cellular damage caused by these ROS (Kehrer et al. 2010, Prasad and Strzalka 2013). Therefore, the inhibition of CAT activity observed mainly at 25 ºC is consistent with higher ROS production in fish exposed to increased water temperatures (Banh et al. 2016). Although not statistically significant, it is also possible to observe a decreasing pattern for CAT activity with increased temperature and Hg exposures. Previous studies in fish (*Mugil cephalus* and *Dicentrarchus labrax*) have demonstrated a decrease in CAT activity under natural exposure to Hg (Maria et al. 2009, Padmini and Usha Rani 2009, Mieiro et al. 2011). Temperature also changed GST activity significantly (Annexes – Table A2, Fig 5.4B) and a significant difference between control and contaminated sediment treatments was found at 15 ºC.

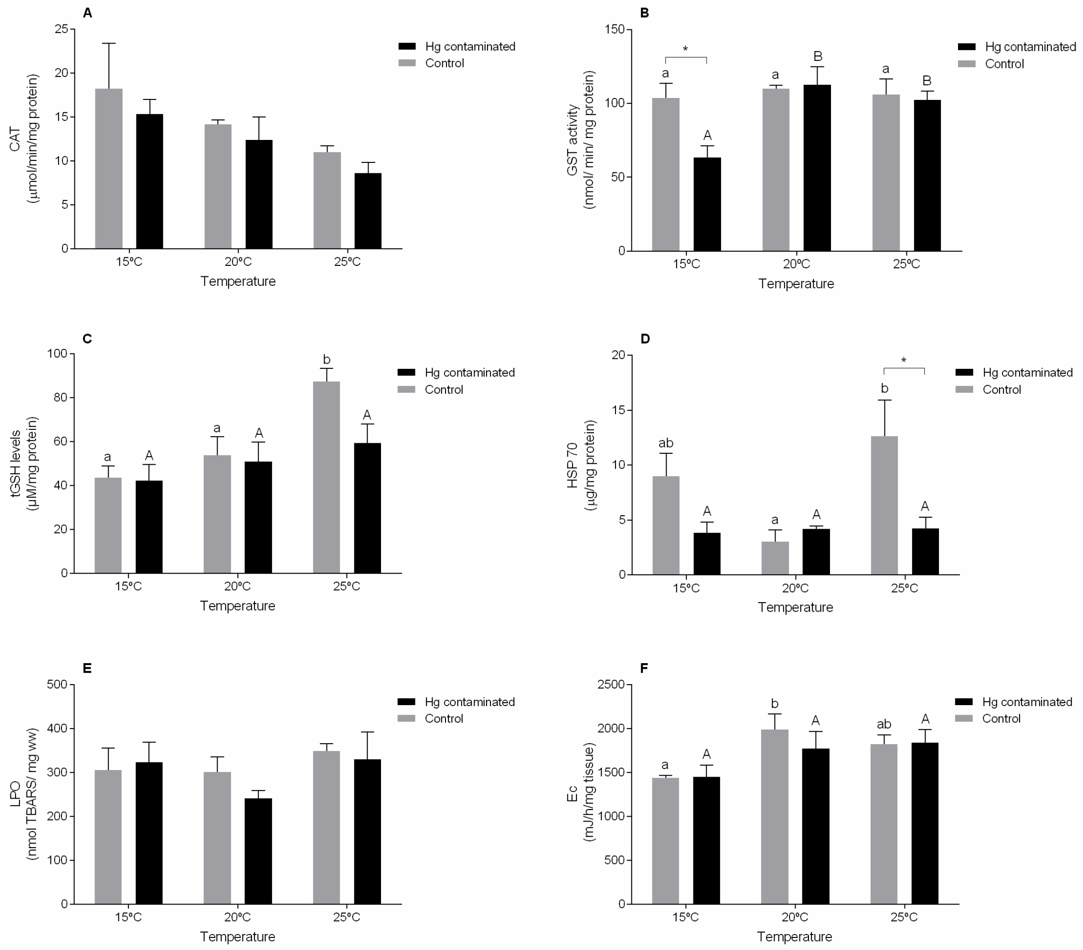


Fig. 5.4 Biomarker responses of *P. microps* liver after 7 days of exposure to low [Hg] at different temperatures. **(A)** Catalase (CAT), **(B)** Glutathione-S-transferase (GST), **(C)** Total glutathione (tGSH), **(D)** Heat Shock Proteins (HSP70), **(E)** Lipid Peroxidation (LPO) and **(F)** Energy consumption. Asterisk (*) denotes a significant difference compared with the control and Hg treatment from the same temperature and the different lower-case letters indicate differences in the controls at the different temperatures and the upper case letters indicate differences in the Hg treatments.

At the same time, a comparison between Hg contaminated treatments showed that the GST activity at 15 ºC was significantly lower (~50%) than at 20ºC and 25ºC (Fig. 5.4B). These results are in line with Mieiro et al. (2011) who observed a decrease in GST activity in the *Dicentrarchus labrax* caught in a site moderately contaminated with Hg when compared to specimens from a reference site, at winter time (lower temperature), and posterior increase in GST activity during summer (higher temperature). When looking to the biomarker tGSH, no interactions were found between temperature and Hg exposures (Annexes – Table A2, Fig. 5.3D). Still, higher tGSH levels were observed at 25 ºC in sediment control treatments (Fig. 5.4C). These results are in accordance with elevated tGSH levels observed in the killifish (*Fundulus heteroclitus macrolepidotus*) liver with increasing temperature by Leggatt et al.

(2007). Glutathione (GSH) is a protein that is essential for oxidative stress enzymes detoxification pathway. This pathway includes the glutathione-*S*-transferases (GST), the glutathione peroxidation (GPx) and the glutathione reductase (GR). For GST to function, it requires the conjugation with GSH. The same happens with GPx that transforms GSH into glutathione reduced (GSSH) to function and deal with reactive oxygen species. As the GSSH is being formed, the enzyme GR is responsible for the conversion of GSSH back into GSH. Although GPx and GR were not measured, a possible explanation for the significant decrease of tGSH in the liver of fish exposed to Hg at 25 ºC may be related not to the previously described pathway, but with the conjugation of the glutathione with the metal itself (Quig 1998, Lushchak 2012).

Concerning the stress protein HSP70 in the liver, significant effects of Hg exposure, temperature and their interaction were observed (Annexes – Table A3). Significant differences between HSP70 levels in liver of fish from control and contaminated sediment treatments were found at 25ºC. At the same time, a comparison between control treatments showed that the HSP70 levels at 25 ºC were significantly higher than at 20 ºC (Fig. 5.4D). The higher levels of HSP70 observed at higher water temperatures are in accordance with their function as chaperones, thus, HSP production is increased to deal with the higher levels of protein denaturation due to high temperatures, as a mechanism of thermotolerance (Tomanek 2010, Madeira et al. 2012). Furthermore, it has also been observed a higher production of HSPs in species from highly variable and warmer habitats such as intertidal/supratidal zone (Tomanek 2010, Madeira et al. 2012), which is the case of P. microps. The higher levels of HSP70 at 25 ºC are also in accordance with the inhibition of CAT activity and increase in tGSH levels that suggest higher levels of ROS and need to an increase of the detoxification mechanisms in the liver of these fish. Thus, HSP70 and its co-chaperones can also have a crucial role in the detoxification of damaged molecules by sort and direct them to the proteasome or lysosomes for degradation, contributing to the lack of oxidative damage observed. On the other hand, the inhibition of HSP70 in treatments contaminated with mercury may be related to the fact that the sensitivity of HSP70 decreases in the presence of Hg, which implies that the induction of HSP70 may be dependent on concentration and time. Inhibition of HSP70 generally indicates a more toxic response than its up-regulation (Zhang et al. 2017).

Finally, no significant oxidative damage (measured as LPO) was found in fish liver due to the studied conditions (Annexes – Table A2, Fig. 5.4E). LPO has been identified as one of the main reasons for the loss of cell function under oxidative stress conditions since ROS have the ability to oxidize membrane lipid components, mainly phospholipids. The peroxidation of membrane lipids can disturb the assembly of cell membranes, which inevitably will impact membrane fluidity, membrane permeability, physicochemical properties, nutrient transport and metabolic processes, ultimately leading to cell death (Storey 1996, Monteiro et al. 2009, Catalá and Díaz 2016). Considering that LPO is a valuable indicator of oxidative damage of cellular components, our results suggest that the activation of cellular detoxification mechanisms was indeed successful in preventing oxidative damage.

To counteract oxidative stress, the cell requires energy, and the aerobic respiratory system produces highly energetic molecules by the movement of electrons from oxidizable substracts to O_2. Ec (estimated as ETS activity) in the liver was significantly affected by water temperature (Annexes – Table A2, Fig. 5.4F) with a significant increase in Ec from 15 to 20 ºC. According to Sokolova et al. (2012), the production of ROS above baseline levels, due to thermal stress or exposure to metals requires energy-costly upregulation, leading to increased ETS activity.

Similarly to the liver, no significant changes on LPO levels were observed in the fish muscle tissue under exposure to Hg and different temperatures (Annexes – Table A3, Fig. 5.5A). However, oxidative damage of proteins (measured as protein carbonylation – PC) increased significantly with temperature (Annexes – Table A3, Fig. 5.5B). Higher values of PC were observed at 25ºC with or without Hg ($p<0.05$), when compared to the respective 15ºC treatments. The protein oxidation measured as protein carbonyl content can be also evaluated as a potential biomarker of Hg-mediated oxidative damage (Monteiro et al. 2009). The formation of carbonyl groups results from the direct oxidation of amino acid side chains by metals or ROS (Pantke et al. 1999). Oxidation of proteins was observed in response to Hg exposure in cucumber seedlings (*Cucumis sativus L.*) germinated in glass recipients containing different [Hg] (Cargnelutti et al. 2006), in the tropical freshwater fish matrinxã (*Brycon amazonicus*) exposed to 150 µg Hg L^{-1} during 96 h in a static system (Monteiro et al. 2009) and in *Azotobacter vinelandii* exposed to 2.5 mmol l^{-1} during 48h (Onwurah 1999).

Furthermore, no significant alterations were observed on the LDH activity in fish muscle tissue (Annexes – Table A3, Fig. 5.5C); thus, no shifting of aerobic glycolysis to anaerobiosis is suggested (Sokolova and Lannig 2008).

Concerning the energy reserves in the muscle tissue, no significant effects of Hg exposure, temperature or their interaction were observed (Annexes – Table A2). These are not surprising results since fish were fed during the experimental period. Nevertheless, it is possible to observe a decreasing pattern for sugars with increased temperature (Fig. 5.5D). This suggests the use of this fast metabolized energy reserve to energy costs of protection and detoxification mechanisms (Sokolova and Lannig 2008, Rodrigues et al. 2017) to counteract the levels of oxidative stress imposed by the tested conditions.

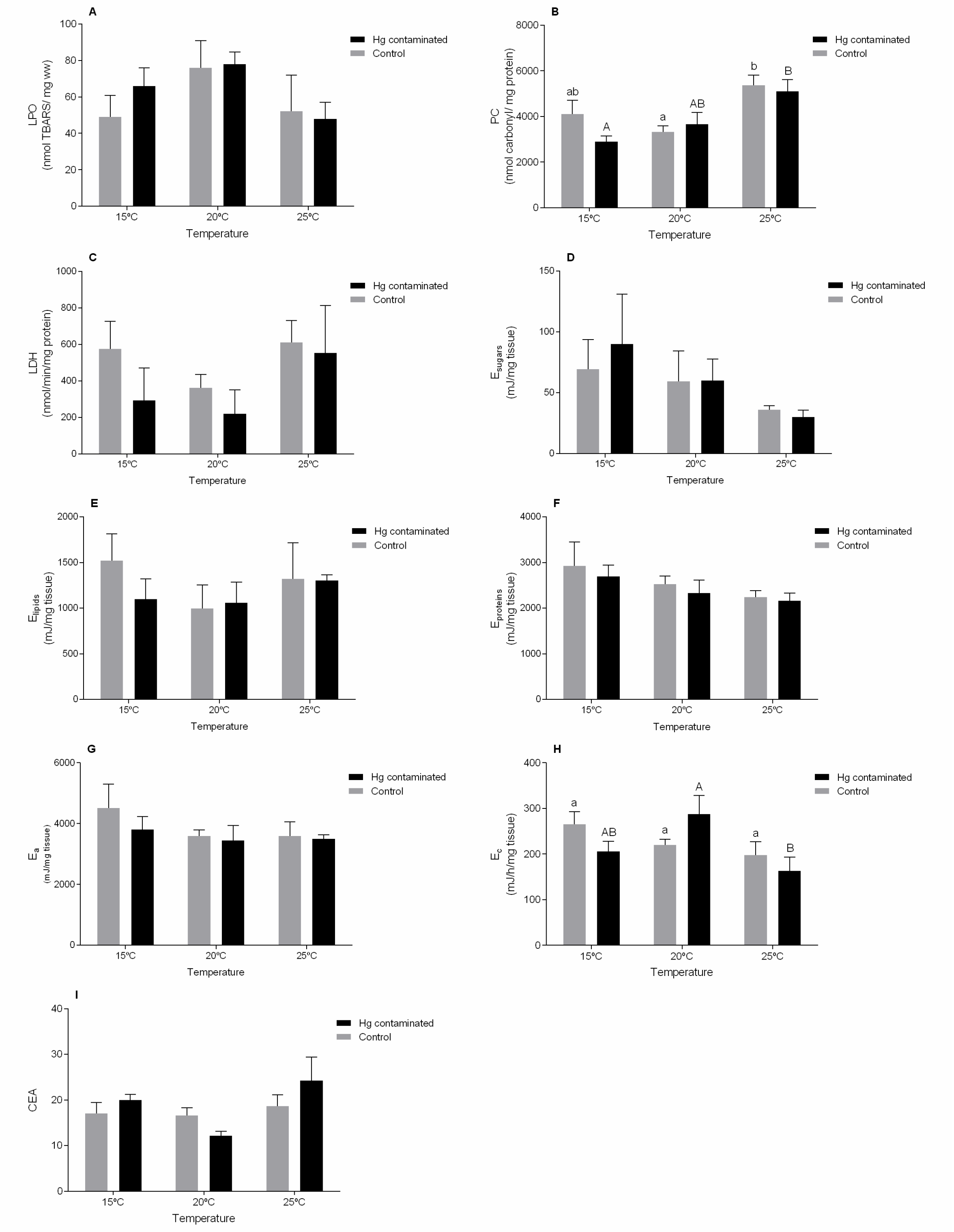


Fig. 5.5 Biomarker responses of *P. microps* muscle after 7 d exposure to low [Hg] at different temperatures. (A) Lipid Peroxidation (LPO), (B) Protein carboxylation (PC), (C) Lactate dehydrogenase (LDH), (D) Sugar content (Esugar), (E) Lipid content (Elipids), (F) Protein content (Eproteins), (G) Energy available (Ea), (H) Energy consumption (Ec) and (I) Cellular energy allocation (CEA). The different lower-case letters indicate differences in the controls at the different temperatures and the upper case letters indicate differences in the Hg treatments.

Since no significant changes were observed for sugars, lipids and protein contents, no significant differences were also observed for their sum, the available energy (Ea – Annexes – Table A2, Fig. 5.5G). Ec was significantly affected by temperature (Annexes – Table A2, Fig. 5.5H), with a significant decreased on Ec observed at the Hg treatments from 20 ºC to 25 ºC (Fig. 5.5H). The observed decreased in aerobic energy production when fish were exposed to Hg at 25 ºC may be due to the fact that the conjugation of the Hg with the temperature increases the production of ROS, which can increase damage of mitochondrial proteins (corroborated by our data in Fig. 5.5B) resulting in a malfunction of the electron transport system (ETS). This hypothesis is also proposed by Choksi et al (2004) who suggests that the damage caused by oxidative stress in key proteins from bovine hearts can lead to dysfunctions in the mitochondria, as well as negatively affecting specific components of the electron transport chain. Despite the significant difference found for Ec, the integration of this parameter with the Ea into the CEA index did not show any significant differences (Annexes – Table A2, Fig. 5.5I).

Conclusion

The changes on the bioaccumulation and toxic effects of the exposure to Hg natural contaminated sediments with different water temperature scenarios was investigated in the native widely distributed *P. microps*. This species was found to be a good model organism to be used in studies using sediment as an exposure route of contamination due to its benthonic behaviour and effective response to short exposures. The present study also highlights that liver is the organ that first reflects environmental contamination, presenting the highest accumulation rates after 7 days of exposure in relation to muscle. Despite the greater accumulation of Hg in the liver, the antioxidant defenses seem to act efficiently, avoiding cellular damage in the liver caused by the increased ROS production due to the stress caused by temperature and by the metal itself. On the other hand, the increased water temperatures lead to oxidative damage in the muscle observed at the levels of proteins, most likely due to the proteins susceptibility to higher temperatures. Additionally, energy consumption appears to be affected in different ways in the liver or muscle. In the liver, the increase in energy consumption at higher temperatures seems to be related to the activation of defense mechanisms to counteract ROS, while in the muscle the decrease in energy consumption seems to be related to the damage caused by oxidative stress due to ROS and by the Hg itself in proteins, resulting in a malfunction of the electron transport chain.

Thus, the present study emphasizes that changes on environmental factors, such as water temperature, play an important role in the Hg accumulation in aquatic organisms and might impair their capability to deal with chemical contamination. Further, the combined exposure to an abiotic and chemical stressors lead to increased oxidative stress and energy alterations even at low [Hg] in a short period of time.

Ethical issues

Animal handling and the cold-induced anesthesia were performed by the researchers Vieira, H.C and Rodrigues, A.C.M., who are accredited researchers by the DGAV (Portuguese National Authority for Animal Health) as competent person for experiments with animals (equivalent to FELASA category B).

Conflict of interest

The authors declare that they have no conflict of interests (financial or non-financial).

Acknowledgements

Thanks are due for the financial support to CESAM (UIDB/50017/2020+UIDP/50017/2020), to FCT/MEC through national funds. FCT also financed a doctoral grant to Vieira, H.C. (PD/BD/127808/2016). Bordalo M.D. and Rocha, R.J.M are funded by national funds (OE), through FCT – Fundação para a Ciência e a Tecnologia, I.P., in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19.

References

Abreu, S. N., E. Pereira, C. Vale, and A. C. Duarte. 2000. Accumulation of Mercury in Sea Bass from a Contaminated Lagoon (Ria de Aveiro, Portugal). Marine Pollution Bulletin 40:293-297.

Ahonen, S. A., B. Hayden, J. J. Leppänen, and K. K. Kahilainen. 2018. Climate and productivity affect total mercury concentration and bioaccumulation rate of fish along a spatial gradient of subarctic lakes. Science of The Total Environment 637-638:1586-1596.

Alexander, M. A., J. D. Scott, K. D. Friedland, K. E. Mills, J. A. Nye, A. J. Pershing, and A. C. Thomas. 2018. Projected sea surface temperatures over the 21st century: Changes in the mean, variability and extremes for large marine ecosystem regions of Northern Oceans.

Arcagni, M., R. Juncos, A. Rizzo, M. Pavlin, V. Fajon, M. A. Arribére, M. Horvat, and S. Ribeiro Guevara. 2018. Species- and habitat-specific bioaccumulation of total mercury and methylmercury in the food web of a deep oligotrophic lake. Science of The Total Environment 612:1311-1319.

ATSDR. 2017. ATSDR's Substance Priority List. Agency for Toxic Substances and Disease Registry, Atlanta, GA. Available at https://www.atsdr.cdc.gov/SPL/# [accessed on December 2019].

Banh, S., L. Wiens, E. Sotiri, and J. R. Treberg. 2016. Mitochondrial reactive oxygen species production by fish muscle mitochondria: Potential role in acute heat-induced oxidative stress. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 191:99-107.

Baykan, U., G. Atli, and M. Canli. 2007. The effects of temperature and metal exposures on the profiles of metallothionein-like proteins in *Oreochromis niloticus*. Environmental Toxicology and Pharmacology 23:33-38.

Bonsignore, M., D. Salvagio Manta, S. Mirto, E. M. Quinci, F. Ape, V. Montalto, M. Gristina, A. Traina, and M. Sprovieri. 2018. Bioaccumulation of heavy metals in fish, crustaceans, molluscs and echinoderms from the Tuscany coast. Ecotoxicology and Environmental Safety 162:554-562.

Boudou, A., A. Delarche, F. Ribeyre, and R. Marty. 1979. Bioaccumulation and bioamplification of mercury compounds in a second level consumer, *Gambusia affinis*-temperature effects. Bull Environ Contam Toxicol 22:813-818.

Braz-Mota, S., L. M. L. Fé, F. A. C. Delunardo, H. Sadauskas-Henrique, V. M. F. de Almeida-Val, and A. L. Val. 2017. Exposure to waterborne copper and high temperature induces the formation of reactive oxygen species and causes mortality in the Amazonian fish *Hoplosternum littorale*. Hydrobiologia 789:157-166.

Cargnelutti, D., L. A. Tabaldi, R. M. Spanevello, G. de Oliveira Jucoski, V. Battisti, M. Redin, C. E. B. Linares, V. L. Dressler, É. M. de Moraes Flores, F. T. Nicoloso, V. M. Morsch, and M. R. C. Schetinger. 2006. Mercury toxicity induces oxidative stress in growing cucumber seedlings. Chemosphere 65:999-1006.

Carrasco, L., L. Benejam, J. Benito, J. M. Bayona, and S. Díez. 2011. Methylmercury levels and bioaccumulation in the aquatic food web of a highly mercury-contaminated reservoir. Environment International 37:1213-1218.

Catalá, A., and M. Díaz. 2016. Editorial: Impact of Lipid Peroxidation on the Physiology and Pathophysiology of Cell Membranes. Frontiers in Physiology 7.

Chan, H. M., A. M. Scheuhammer, A. Ferran, C. Loupelle, J. Holloway, and S. Weech. 2003. Impacts of Mercury on Freshwater Fish-Eating Wildlife and Humans. Human and Ecological Risk Assessment: An International Journal 9:867-883.

Chen, M. M., L. Lopez, S. P. Bhavsar, and S. Sharma. 2018. What's hot about mercury? Examining the influence of climate on mercury levels in Ontario top predator fishes. Environmental Research 162:63-73.

Choksi, K. B., W. H. Boylston, J. P. Rabek, W. R. Widger, and J. Papaconstantinou. 2004. Oxidatively damaged proteins of heart mitochondrial electron transport complexes. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1688:95-101.

Chowdhury, M. J., and R. Blust. 2001. Effect of temperature on the uptake of waterborne strontium in the common carp, *Cyprinus carpio* (L.). Aquatic Toxicology 54:151-160.

Cizdziel, J., T. Hinners, C. Cross, and J. Pollard. 2003. Distribution of mercury in the tissues of five species of freshwater fish from Lake Mead, USA. Journal of Environmental Monitoring 5:802-807.

Costa, S., D. Crespo, B. M. G. Henriques, E. Pereira, A. C. Duarte, and M. A. Pardal. 2011. Kinetics of Mercury Accumulation and Its Effects on *Ulva lactuca* Growth Rate at Two Salinities and Exposure Conditions. Water, Air, & Soil Pollution 217:689-699.

Costley, C. T., K. F. Mossop, J. R. Dean, L. M. Garden, J. Marshall, and J. Carroll. 2000. Determination of mercury in environmental and biological samples using pyrolysis atomic absorption spectrometry with gold amalgamation. Analytica Chimica Acta 405:179-183.

de Almeida Rodrigues, P., R. G. Ferrari, L. N. dos Santos, and C. A. Conte Junior. 2019. Mercury in aquatic fauna contamination: A systematic review on its dynamics and potential health risks. Journal of Environmental Sciences 84:205-218.

De Marchi, L., R. J. M. Rocha, A. Rodrigues, A. M. Soares, C. Pretti, F. Chiellini, and R. Freitas. 2020. Environmental Fate of Multistressors on Carpet Shell Clam *Ruditapes decussatus*: Carbon Nanoparticles and Temperature Variation. Sustainability 12:4939.

Deser, C., A. S. Phillips, and M. A. Alexander. 2010. Twentieth century tropical sea surface temperature trends revisited. Geophysical Research Letters 37.

Diamantino, T. C., E. Almeida, A. M. Soares, and L. Guilhermino. 2001. Lactate dehydrogenase activity as an effect criterion in toxicity tests with Daphnia magna straus. Chemosphere 45:553-560.

Dijkstra, J. A., K. L. Buckman, D. Ward, D. W. Evans, M. Dionne, and C. Y. Chen. 2013. Experimental and natural warming elevates mercury concentrations in estuarine fish. PLoS ONE 8:e58401.

Driscoll, C. T., R. P. Mason, H. M. Chan, D. J. Jacob, and N. Pirrone. 2013. Mercury as a Global Pollutant: Sources, Pathways, and Effects. Environ Sci Technol 47:4967-4983.

Fonte, E., P. Ferreira, and L. Guilhermino. 2016. Temperature rise and microplastics interact with the toxicity of the antibiotic cefalexin to juveniles of the common goby (*Pomatoschistus microps*): post-exposure predatory behaviour, acetylcholinesterase activity and lipid peroxidation. Aquatic Toxicology 180:173-185.

Gonzalez, P., Y. Dominique, J. C. Massabuau, A. Boudou, and J. P. Bourdineaud. 2005. Comparative Effects of Dietary Methylmercury on Gene Expression in Liver, Skeletal Muscle, and Brain of the Zebrafish (Danio rerio). Environ Sci Technol 39:3972-3980.

Guinot, D., R. Ureña, A. Pastor, I. Varó, J. d. Ramo, and A. Torreblanca. 2012. Long-term effect of temperature on bioaccumulation of dietary metals and metallothionein induction in Sparus aurata. Chemosphere 87:1215-1221.

Havelková, M., L. Dušek, D. Némethová, G. Poleszczuk, and Z. Svobodová. 2008. Comparison of Mercury Distribution Between Liver and Muscle – A Biomonitoring of Fish from Lightly and Heavily Contaminated Localities. Sensors 8:4095.

Ioannides, K., K. Stamoulis, C. Papachristodoulou, E. Tziamou, C. Markantonaki, and I. Tsodoulos. 2014. Distribution of heavy metals in sediment cores of Lake Pamvotis (Greece): a pollution and potential risk assessment. Environ Monit Assess 187:4209.

Jezierska, B., and M. Witeska. 2006. The metal uptake and accumulation in fish living in polluted waters. Pages 107-114 Soil and water pollution monitoring, protection and remediation. Springer.

Jinadasa, B. K. K. K., and S. W. Fowler. 2019. Critical review of mercury contamination in Sri Lankan fish and aquatic products. Marine Pollution Bulletin 149:110526.

Kehrer, J. P., J. D. Robertson, and C. V. Smith. 2010. 1.14 - Free Radicals and Reactive Oxygen Species. Pages 277-307 in C. A. McQueen, editor. Comprehensive Toxicology (Second Edition). Elsevier, Oxford.

Kennedy, C. J. 2003. Uptake and accumulation of mercury from dental amalgam in the common goldfish, Carassius auratus. Environmental Pollution 121:321-326.

Larose, C., R. Canuel, M. Lucotte, and R. T. Di Giulio. 2008. Toxicological effects of methylmercury on walleye (*Sander vitreus*) and perch (*Perca flavescens*) from lakes of the boreal forest. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 147:139-149.

Leggatt, R. A., C. J. Brauner, P. M. Schulte, and G. K. Iwama. 2007. Effects of acclimation and incubation temperature on the glutathione antioxidant system in killifish and RTH-149 cells. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 146:317-326.

Lushchak, V. I. 2012. Glutathione homeostasis and functions: potential targets for medical interventions. Journal of amino acids 2012.

Madeira, D., L. Narciso, H. Cabral, C. Vinagre, and M. Diniz. 2012. HSP70 production patterns in coastal and estuarine organisms facing increasing temperatures. Journal of Sea Research 73:137-147.

Maria, V. L., I. Ahmad, M. Oliveira, A. Serafim, M. J. Bebianno, M. Pacheco, and M. A. Santos. 2009. Wild juvenile *Dicentrarchus labrax* L. liver antioxidant and damage responses at Aveiro Lagoon, Portugal. Ecotoxicology and Environmental Safety 72:1861-1870.

Maury-Brachet, R., S. Gentes, E. P. Dassié, A. Feurtet-Mazel, R. Vigouroux, V. Laperche, P. Gonzalez, V. Hanquiez, N. Mesmer-Dudons, G. Durrieu, and A. Legeay. 2019. Mercury contamination levels in the bioindicator piscivorous fish Hoplias aïmara in French Guiana rivers: mapping for risk assessment. Environmental Science and Pollution Research.

Mieiro, C. L., M. Pacheco, M. E. Pereira, and A. C. Duarte. 2009. Mercury distribution in key tissues of fish (*Liza aurata*) inhabiting a contaminated estuary—implications for human and ecosystem health risk assessment. Journal of Environmental Monitoring 11:1004-1012.

Mieiro, C. L., M. E. Pereira, A. C. Duarte, and M. Pacheco. 2011. Brain as a critical target of mercury in environmentally exposed fish (*Dicentrarchus labrax*)—Bioaccumulation and oxidative stress profiles. Aquatic Toxicology 103:233-240.

Mills, N., M. J. Weber, C. L. Pierce, and D. Cashatt. 2019. Factors influencing fish mercury concentrations in Iowa rivers. Ecotoxicology 28:229-241.

Miranda, T., L. R. Vieira, and L. Guilhermino. 2019. Neurotoxicity, Behavior, and Lethal Effects of Cadmium, Microplastics, and Their Mixtures on *Pomatoschistus microps* Juveniles from Two Wild Populations Exposed under Laboratory Conditions—Implications to Environmental and Human Risk Assessment. International journal of environmental research and public health 16:2857.

Monteiro, D. A., F. T. Rantin, and A. L. Kalinin. 2009. Inorganic mercury exposure: toxicological effects, oxidative stress biomarkers and bioaccumulation in the tropical freshwater fish matrinxã, Brycon amazonicus (Spix and Agassiz, 1829). Ecotoxicology 19:105.

Monteiro, D. A., F. T. Rantin, and A. L. Kalinin. 2013. Dietary intake of inorganic mercury: bioaccumulation and oxidative stress parameters in the neotropical fish *Hoplias malabaricus*. Ecotoxicology 22:446-456.

Moreira, F., C. A. Assis, P. R. Almeida, J. L. Costa, and M. J. Costa. 1992. Trophic relationships in the community of the upper Tagus estuary (Portugal): A preliminary approach. Estuarine, Coastal and Shelf Science 34:617-623.

OECD. 2008. Test No. 315: Bioaccumulation in Sediment-dwelling Benthic Oligochaetes.

Onwurah, I. N. 1999. Lipid peroxidation and protein oxidation in Azotobacter vinelandii exposed to mercury, silver, crude oil, and fenton reagent. Toxic Substance Mechanisms 18:167-176.

Padmini, E., and M. Usha Rani. 2009. Evaluation of oxidative stress biomarkers in hepatocytes of grey mullet inhabiting natural and polluted estuaries. Science of The Total Environment 407:4533-4541.

Pantke, U., T. Volk, M. Schmutzler, W. J. Kox, N. Sitte, and T. Grune. 1999. Oxidized proteins as a marker of oxidative stress during coronary heart surgery. Free Radical Biology and Medicine 27:1080-1086.

Pereira, E., S. N. Abreu, J. P. Coelho, C. B. Lopes, M. A. Pardal, C. Vale, and A. C. Duarte. 2006. Seasonal fluctuations of tissue mercury contents in the European shore crab *Carcinus* *maenas* from low and high contamination areas (Ria de Aveiro, Portugal). Marine Pollution Bulletin 52:1450-1457.

Pinto, D., L. Pellegrin, L. F. Nitz, S. T. da Costa, J. M. Monserrat, and L. Garcia. 2019. Haematological and oxidative stress responses in *Piaractus mesopotamicus* under temperature variations in water. Aquaculture Research 50:3017-3027.

Prasad, M., and K. Strzalka. 2013. Physiology and biochemistry of metal toxicity and tolerance in plants. Springer Science & Business Media.

Quig, D. 1998. Cysteine metabolism and metal toxicity. Alternative Medicine Review 3:262-270.

Quintaneiro, C., D. Querido, M. Monteiro, L. Guilhermino, F. Morgado, and A. M. V. M. Soares. 2008. Transport and acclimation conditions for the use of an estuarine fish (*Pomatoschistus microps*) in ecotoxicity bioassays: Effects on enzymatic biomarkers. Chemosphere 71:1803-1808.

Ribeiro, C. A. O., J. R. D. Guimarães, and W. C. Pfeiffer. 1996. Accumulation and Distribution of Inorganic Mercury in a Tropical Fish (*Trichomycterus zonatus*). Ecotoxicology and Environmental Safety 34:190-195.

Rodrigues, A. C., C. Gravato, C. Quintaneiro, M. D. Bordalo, C. Barata, A. M. Soares, and J. L. Pestana. 2017. Energetic costs and biochemical biomarkers associated with esfenvalerate exposure in *Sericostoma vittatum*. Chemosphere 189:445-453.

Roos, D. H., R. L. Puntel, M. M. Santos, D. O. G. Souza, M. Farina, C. W. Nogueira, M. Aschner, M. E. Burger, N. B. V. Barbosa, and J. B. T. Rocha. 2009. Guanosine and synthetic organoselenium compounds modulate methylmercury-induced oxidative stress in rat brain cortical slices: Involvement of oxidative stress and glutamatergic system. Toxicology in Vitro 23:302-307.

Shanker, G., and M. Aschner. 2003. Methylmercury-induced reactive oxygen species formation in neonatal cerebral astrocytic cultures is attenuated by antioxidants. Molecular Brain Research 110:85-91.

Sinkus, W., V. Shervette, J. Ballenger, L. A. Reed, C. Plante, and B. White. 2017. Mercury bioaccumulation in offshore reef fishes from waters of the Southeastern USA. Environmental Pollution 228:222-233.

Slemr, F., E.-G. Brunke, R. Ebinghaus, and J. Kuss. 2011. Worldwide trend of atmospheric mercury since 1995. Atmospheric Chemistry and Physics 11:4779-4787.

Sokolova, I. M., M. Frederich, R. Bagwe, G. Lannig, and A. A. Sukhotin. 2012. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Marine Environmental Research 79:1-15.

Sokolova, I. M., and G. Lannig. 2008. Interactive effects of metal pollution and temperature on metabolism in aquatic ectotherms: implications of global climate change. Climate Research 37:181-201.

Storelli, M., R. Giacominelli-Stuffler, A. Storelli, and G. Marcotrigiano. 2005. Accumulation of mercury, cadmium, lead and arsenic in swordfish and bluefin tuna from the Mediterranean Sea: a comparative study. Marine Pollution Bulletin 50:1004-1007.

Storey, K. B. 1996. Oxidative stress: animal adaptations in nature. Brazilian Journal of Medical and Biological Research 29:1715-1733.

Tomanek, L. 2010. Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. Journal of Experimental Biology 213:971-979.

Ullrich, S. M., T. W. Tanton, and S. A. Abdrashitova. 2001. Mercury in the Aquatic Environment: A Review of Factors Affecting Methylation. Critical Reviews in Environmental Science and Technology 31:241-293.

USEPA. 1997. Mercury Study Report to Congress Volume III: Fate and Transport of Mercury in the Environment. Office of Air Quality Planning & Standards and Office of Research and Development.

Vassault, A. 1983. Lactate dehydrogenase, in: Bergmyer, M.O. (Ed.), Methods of Enzymatic Analysis, Enzymes: Oxireductases, Transferase. Academic Press, New York, pp. 118–126.

Vieira, H., A. Soares, F. Morgado, and S. Abreu. 2019. 8 Mercury Exposure, Fish Consumption and Provisional Tolerable Weekly Intake. Ecotoxicology of Marine Organisms:185.

Vieira, H. C., S. N. de Abreu, and F. Morgado. 2020a. Field Caging Transplants of Fish for Assessment and Monitoring in Aquatic Toxicology. Pages 1-15 in W. Leal Filho, A. M. Azul, L. Brandli, A. Lange Salvia, and T. Wall, editors. Life Below Water. Springer International Publishing, Cham.

Vieira, H. C., J. Rendón-von Osten, A. M. V. M. Soares, F. Morgado, and S. N. Abreu. 2020b. Mercury bioaccumulation in the long-fin squid *Loligo forbesi* near the Mid-Atlantic Ridge: Implications to human exposure. Ecotoxicology and Environmental Safety 203:110957.

Vieira, L. R., A. Sousa, M. F. Frasco, I. Lima, F. Morgado, and L. Guilhermino. 2008. Acute effects of Benzo[a]pyrene, anthracene and a fuel oil on biomarkers of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). Science of The Total Environment 395:87-100.

Waheed, R., A. M. El Asely, H. Bakery, R. El-Shawarby, M. Abuo-Salem, N. Abdel-Aleem, F. Malhat, A. Khafaga, and A. Abdeen. 2020. Thermal stress accelerates mercury chloride toxicity in *Oreochromis niloticus* via up-regulation of mercury bioaccumulation and HSP70 mRNA expression. Science of The Total Environment 718:137326.

Wang, Q., D. Kim, D. D. Dionysiou, G. A. Sorial, and D. Timberlake. 2004. Sources and remediation for mercury contamination in aquatic systems—a literature review. Environmental Pollution 131:323-336.

Zhang, T., Q. Lu, C. Su, Y. Yang, D. Hu, and Q. Xu. 2017. Mercury induced oxidative stress, DNA damage, and activation of antioxidative system and Hsp70 induction in duckweed (*Lemna minor*). Ecotoxicology and Environmental Safety 143:46-56.

Chapter VI:

Total and organic mercury in fish from different geographical areas in the North Atlantic Ocean and health risk assessment

Total and organic mercury in fish from different geographical areas in the North Atlantic Ocean and health risk assessment

Vieira, H.C.[1], Ramirez, M.M.B.[2], Bordalo, M.D.[1], Rodrigues, A.C.M.[1], Soares, A.M.V.M.[1], Abreu, S. N.[1], Morgado, F.[1], Rendón-von Osten, J.[2]

[1] CESAM - Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

[2] EPOMEX Institute, Autonomous University of Campeche, Campeche, Mexico

Abstract

Fish consumption is considered the main route of exposure to mercury for the human population. In this sense, this study analyzed the total and organic mercury content present in the muscle tissue of 38 fish species caught in different geographical areas of the North Atlantic Ocean (included in FAO fishing areas 27 and 31). The potential risk to human health through fish consumption was assessed by using the maximum limit for mercury intake of 1.3 µg methylmercury Kg body weight^{-1} week^{-1}. The results show that carnivorous species presented higher mercury content (range 0.03–0.88 µg g^{-1}) when compared to omnivorous (range 0.003–0.19 µg g^{-1}) in all sampling sites. Furthermore, demersal fish exhibited higher mercury levels (range 0.01–0.88 µg g^{-1}) than the pelagic species (range 0.003–0.38 µg g^{-1}) did. From the 38 species analyzed only Zeus faber presented mercury levels (0.68±0.07 µg g^{-1}) above the maximum limit (0.5 µg g^{-1}) established for human consumption. On the other hand, the consumption of 13 species from fishing area 27 (Azores archipelago and Northwest Portuguese coast) and 1 species (*Cynoscion nebulosus*) from fishing area 31 (Southeast Mexican coast) may lead to a mercury intake higher than the recommended. However, only the consumption of *Zeus faber* and *Aphanopus carbo* from the fishing area 27 is discouraged. Thus, the fish consumption per capita in a specific area can result in exceeding the limits of mercury ingestion, even in cases where the mercury content in the fish is below the recommended for consumption.

Keywords: Mercury bioaccumulation; organic mercury; commercial fish species; estimated daily intake; target hazard quotient

1. Introduction

The marine fisheries sector plays both an important economic and social role in the world (Teh and Sumaila 2013). Global total marine catches increased from 81.2 million tonnes in 2017 to 84.4 million tonnes in 2018, with 21.33 million tonnes being caught in the Atlantic Ocean. For statistical purposes, 19 major marine fishing areas have been created covering all adjacent oceans and seas. The Atlantic Ocean was thus divided into 6 major fishing areas: area 21 (Atlantic, Northwest), area 27 (Atlantic, Northeast), area 31 (Atlantic, Western Central), area 34 (Atlantic, Eastern Central), area 41 (Atlantic, Southwest) and area 47 (Atlantic, Southeast). According to FAO (2020), the contribution of each of these fishing areas to the total caught in the Atlantic Ocean in 2018 ranged between 7 and 44%, with the fishing area 27 being the largest contributor for the total caught in the Atlantic Ocean.

Fish consumption has well-known human health benefits due to the presence of a variety of essential nutrients, such as omega-3 polyunsaturated fatty acids (n-3 PUFAs), protein, iodine, selenium, vitamin D, and others essential elements (WHO 2003, Marrugo-Negrete et al. 2020). For example, the long-chain omega-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) prevent heart diseases (Innes and Calder 2018), improve neurological functions in childhood (Koletzko et al. 2010), reduce cholesterol levels (Mason et al. 2016) and contribute to the development and fetal growth (Marrugo-Negrete et al. 2020). Unfortunately, fish consumption is also considered as the major pathway of mercury (Hg) exposure to humans (Moriarity et al. 2020). Due to its high toxicity even in small amounts (Chakraborty 2017), the consumption of certain fish species can pose a potential risk to consumers' health, especially those belonging to vulnerable groups such as fetuses and newborns (Burger and Gochfeld 2009), since Hg can pass through the placenta and crosses the blood-brain barrier and can have harmful effects, especially on the brain of the developing fetus (Pugach and Clarkson 2009)

Mercury is predominantly found in the inorganic form (Lee et al. 2020a); however, this inorganic Hg can be transformed into an organic form (e.g. methylmercury – MeHg) through a bacterial process (Vieira et al. 2017). MeHg can bioaccumulate and biomagnify in the aquatic trophic chain, from plankton to the largest predatory fish (Custódio et al. 2020). In addition, MeHg has high affinity for sulfhydryl groups in proteins, and for this reason, the Hg contained in the muscle tissue of fish is found mainly as MeHg (Bebianno et al. 2007, Polak-Juszczak 2018).

After ingestion, more than 90% of the MeHg is absorbed by the gastrointestinal tract (Lee et al. 2020b) and then transported to the blood where it may cross the blood-brain barrier

(Moriarity et al. 2020). MeHg is a potent neurotoxin that affects particularly the brain and nervous system of human beings (Unoki et al. 2018). MeHg poisoning may cause a neurological disease (Minamata disease) whose clinical manifestation includes dysarthria, postural and action tremor, cognitive impairment and hearing loss and disequilibrium (Jackson 2018). In recognition of the risks associated with exposure to Hg through the diet, the international community has taken action to protect human health (Buck et al. 2019). The European Union and Food and Agriculture Organization (FAO)/ World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) has determined the maximum permitted level of 0.5 µg g^{-1} Hg (ww) in the muscle of the most fish species and 1 µg g^{-1} Hg (ww) in the muscle of some predatory fish species (EU 2008, Codex Alimentarius Commission 2018). Furthermore, JECFA has established a reference dose (RfD) for MeHg Called rovisional tolerable weekly intake (PTWI) of 1.3 µg kg body weight $(bw)^{-1}$ $week^{-1}$ (EFSA Scientific Committee 2015). This RfD is an estimate of the maximum daily exposure value for the human population that is unlikely to have an appreciable risk of harmful effects over a lifetime (Milatou et al. 2020).

This study aims to: i) evaluate the total [Hg] ([THg]) and organic [Hg] ([OHg]) in the muscle of fish species of commercial interest in two different fishing areas in the North Atlantic Ocean; ii) compare the [THg] in the different species according to their trophic level, habits and lifestyle; iii) estimate the contribution of each species to the OHg intake for human consumers; iv) evaluate the human health implications of such OHg exposure using the non-carcinogenic target hazard quotient (THQ) and v) calculate the number of meals that can be taken taking into account the reference values established by JECFA in order to establish recommendations for fish consumption.

2. Materials and Methods

2.1. Fish sampling and preparation

A total of 180 individuals, corresponding to 38 fish species, were obtained through recreational fishing and in local markets in two FAO fishing areas of the North Atlantic Ocean (Fig. 6.1). Twenty-nine of these species were caught in two distinct locations belonging to the FAO fishing area 27, more precisely, 18 from the Azores Archipelago, Portugal (Aa) and the other 9 from the Northwest coast of Portugal (NPc). The remaining 11 species were caught on the Southeast Mexican coast (SMc), which belongs to the FAO fishing area 31. Due to the natural species distribution and the characteristics of the coastal areas, it was not possible to collect the same species of fish in all locations. However, all species present in this study are widely consumed by the population of the respective sampling areas.

Fig. 6.1 Map of fishing areas 27 and 31 established by FAO showing the sampling areas: Azores archipelago (**Aa**), Northwest Portuguese coast (**NPc**) and Southeast Mexican coast (**SMc**).

Fish specimens were identified, measured, and weighted. Then, a portion of the dorsal muscle tissue without skin or bones was collected and stored at -20 ºC, in individual and properly identified plastic bags, for later Hg quantification. Trophic levels, food preferences and lifestyle for each species were assessed electronically at www.fishbase.org (Froese and Pauly 2016) and presented in Table 6.1.

Table 6.1 Information of fish species regarding sampling site, scientific and common names, trophic level, feeding group, food preferences and lifestyle.

Site	Scientific name	Common name	Trophic level	Feeding group	Food preferences	Lifestyle
Aa	*Phycis phycis*	Forkbeard	4.3	Carnivores	Nekton	Demersal
Aa	*Pagellus acarne*	Axillary seabream	3.8	Carnivores	Zoobenthos	Demersal
Aa	*Sphyraena sphyraena*	European barracuda	4	Carnivores	Nekton	Pelagic
Aa	*Pagellus bogaraveo*	Blackspot seabream	4.2	Carnivores	Nekton	Demersal
Aa	*Scomber scombrus*	Atlantic mackerel	3.4	Omnivores	Zooplankton	Pelagic
Aa	*Trachurus trachurus*	Horse mackerel	3.7	Omnivores	Zooplankton	Pelagic
Aa	*Serranus atricauda*	Blacktail comber	4.3	Carnivores	Nekton	Demersal
Aa	*seriola dumerili*	Greater amberjack	4.5	Carnivores	Nekton	Pelagic
Aa	*Pagrus pagrus*	Red porgy	3.9	Carnivores	Nekton	Demersal
Aa	*Zeus faber*	John dory	4.5	Carnivores	Nekton	Demersal

Aa	*Balistes carolinensis*	Grey triggerfish	4.1	Carnivores	Zoobenthos	Demersal
Aa	*Mullus surmuletus*	Surmullet	3.5	Omnivores	Zoobenthos	Demersal
Aa	*Sardina pilchardus*	European pilchard	3.1	Omnivores	Zooplankton	Pelagic
Aa	*Diplodus sargus*	White seabream	3.1	Omnivores	Zoobenthos	Demersal
Aa	*Chelon labrosus*	Thicklip grey mullet	2.6	Herbivores	Plants	Demersal
Aa	*Sparisoma cretense*	Parrotfish	2.9	Omnivores	Zoobenthos	Demersal
Aa	*Pontinus kuhlii*	Offshore rockfish	4.1	Carnivores	Nekton	Demersal
Aa	*Helicolenus dactylopterus*	Blackbelly rosefish	3.5	Carnivores	Zoobenthos	Demersal
NPc	*Trachurus* trachurus	Horse mackerel	3.7	Omnivores	Zooplankton	Pelagic
NPc	*Trachurus picturatus*	Blue jack mackerel	3.3	Omnivores	Zoobenthos	Demersal
NPc	*Sardina pilchardus*	European pilchard	3.1	Omnivores	Zooplankton	Pelagic
NPc	*Merluccius merluccius*	European hake	4.4	Carnivores	Nekton	Demersal
NPc	*Micromesistius poutassou*	Blue whiting	4.1	Carnivores	Zoobenthos	Pelagic
NPc	*Aphanopus carbo*	Black scabbardfish	4.5	Carnivores	Nekton	Demersal
NPc	*Trisopterus luscus*	Pouting	3.7	Omnivores	Zoobenthos	Demersal
NPc	*Scomber scombrus*	Atlantic mackerel	3.6	Omnivores	Zooplankton	Pelagic
NPc	*Sarda sarda*	Atlantic bonito	4.5	Carnivores	Nekton	Pelagic
SMc	*Haemulon plumierii*	White grunt	3.8	Carnivores	Zoobenthos	Demersal
SMc	*Caranx crysos*	Blue runner	4.1	Carnivores	Nekton	Demersal
SMc	*Ocyurus chrysurus*	Yellowtail snapper	4	Carnivores	Nekton	Demersal
SMc	*Archosargus probatocephalus*	Sheepshead	3.5	Omnivores	Zoobenthos	Demersal
SMc	*Lagodon rhomboides*	Pinfish	4.4	Carnivores	Zoobenthos	Demersal
SMc	*Centropomus undecimalis*	Common snook	4.2	Carnivores	Nekton	Demersal
SMc	*Diapterus auratus*	Irish mojarra	2.4	Omnivores	Detritus	Demersal
SMc	*Peprilus paru*	American harvestfish	4.5	Carnivores	Zooplankton	Demersal
SMc	*Umbrina roncador*	Yellowfin drum	3.5	Omnivores	Zoobenthos	Demersal
SMc	*Lachnolaimus maximus*	Hogfish	4.2	Carnivores	Zoobenthos	Demersal
SMc	*Cynoscion nebulosus*	Spotted weakfish	4	Carnivores	Nekton	Demersal

Based on the trophic level, the species were divided into two feeding categories (omnivores and carnivores), according to Stergiou and Karpouzi (2002). These authors' classification was based on the eating habits of 146 species in the Mediterranean Sea and considered omnivores those species with a trophic level greater than 2.1 and lower than 3.7 while carnivorous presented trophic levels between 3.7 and 4.5.

According to their lifestyle fish species were also classified as demersal or pelagic. Demersal fish species are those who live near the sea substrate and may be dependent of the bottom (benthic) or dwell in the interface between the bottom and the water column (benthopelagic) (Pinho and Menezes 2009). The pelagic fish species are those that spend much of their lives swimming in open water away from the bottom (Castro and Huber 2008).

2.2. Total Hg and Organic Hg quantification

The quantification of THg in fish samples was carried out by placing approximately 50 mg of fish tissue in the combustion chamber of the Advanced Mercury Analyzer (AMA-254, made by ALTEC and distributed by LECO). This process does not require a previous digestion of the sample; the procedure is based on a pyrolysis process of the tissue using a combustion tube heated at 750 °C under an oxygen atmosphere and the released Hg is trapped in a gold amalgamator and subsequently detected and quantified by atomic absorption spectrometry (Costley et al. 2000). On the other hand, the concentration of OHg (quantified as total organic Hg) was determined according to the method described by Valega et al. (2006). Briefly, the procedure was as follows: 200 to 400 mg of tissue were placed in 50 ml test tubes for centrifugation; 5 ml of a solution of KBr (18%) in H_2SO_4 (5%) and 1 ml of CuSO4 (1 mol L^{-1}) were added to the tubes, which were kept at room temperature for 15 minutes. After this period, 5 ml of toluene was added. The tubes were then shaken vigorously for 15 minutes and centrifuged for another 15 min. After centrifugation, 5 ml of $Na_2S_2O_3$ was added to 3 ml of the organic fraction previously separated into clean scintillation vials. Then, the vials were shaken vigorously for 5 min. For organic Hg measurements, 500 µL of the aqueous phase were placed in the combustion chamber of the AMA 254 analyzer.

All Sample analysis were triplicated to check the reproducibility of the results, and three blank analyses (analysis without sample) were performed between samples to verify that Hg was not being accumulated over the samples. In this study, blank readings typically correspond to values < 0.02 ng of Hg. Analytical quality for each procedure was checked using the reference material DOLT-5 (Dogfish Liver Reference Material for Trace Metals, National Research Council of Canada). Obtained data (0.41±0.03 µg g^{-1} of Hg for THg and 0.11 ± 0.02 µg g^{-1} of Hg for OHg) and reference values (0.44±0.18 µg g^{-1} of Hg for THg and 0.119 ± 0.058 µg g^{-1} of Hg for OHg) were not statistically different ($p>0.05$).

2.3. Risk assessment in human population

To evaluate the risk for human health through fish consumption, the Hg estimated daily intake (EDI) was calculated by the following formula (WHO 2008):

$$EDI = \frac{amount\ of\ fish\ ingested\ (g\ week^{-1}) \times [Hg]\ in\ fish\ ingested\ (\mu g\ g^{-1})}{Kilogram\ body\ weight\ (kg\ bw)}$$

For this calculation, the amount of consumed fish per week was based on the average annual fish consumption per capita (2015 – 2017) published by FAO in the 2020 edition of The State of World Fisheries and Aquaculture. Thus, for Portugal (Azores archipelago and Northwest

Portuguese coast) the average annual consumption per capita is 60 kg whereas for Mexico (Southern Mexican coast) an average of 20 kg was considered (FAO 2020).

The target hazard quotient (THQ) is used to determine the non-carcinogenic risk level due to pollutant exposure (Sarkar et al. 2016). THQ indicates the ratio between exposure to Hg and the JECFA RfD. If the ratio is less than 1, it means that the level of exposure is smaller than the RfD, suggesting that a daily exposure at this level is not likely to cause any deleterious effects during the lifetime of a human consumer. In other words, a THQ below 1 means that the adverse effects are negligible. The THQ is based on the following equation (Vieira et al. 2020a):

$$THQ = \frac{EF \times ED \times FIR \times C}{RfD \times ABW \times AT} \times 10^{-3}$$

where EF is the exposure frequency (365 days year^{-1});

ED is the exposure duration (adults, 70 years, equivalent to the average lifetime);

FIR is the food ingestion rate (g person-1 day^{-1});

C is the metal concentration in fish (µg g-1, wet weight);

RfD is the oral reference dose (µg g^{-1} day^{-1});

ABW is the average body weight (60kg);

AT is the averaging exposure time for non-carcinogens (365 days year^{-1} × ED).

Recommendations for fish consumption (choices to avoid, good choices and best choices) were based on the maximum values of fish meals per week without exceeding the established RfD established by JECFA can be calculated according to Vieira et al. (2015), using the following formula:

$$\text{Meals per week} = \frac{(\text{RfD} \times \text{Bw}) \times 7\text{days}}{(\text{Fish meal size (g)} \times [\text{Hg}]) \div 1000}$$

The size of the fish meal was based on the 2015-2020 Dietary Guidelines for Americans who recommend at least 226.8g of fish per week based on a 2000 calories diet (Dietary Guidelines Advisory Committee 2015) and a body weight (Bw) of 60 kg based on World Health Organization guidelines (WHO 2008) was used.

2.4. Statistical analysis

The normality of the data was verified using the Shapiro-Wilk test. Since the data were not normally distributed, non-parametric statistical method Kruskal-Wallis test was used to compare [THg] of different food preferences from each sampling site and [THg] of different feeding groups between sampling sites. Non-parametric statistical method Mann Whitney test was also used to compare the [THg] of the feeding groups of each site and [THg] present in pelagic species of sampling sites from fishing area 27. Statistical analyses were performed using GraphPad Prism (version 6.01). Statistical significance was defined as $p < 0.05$. [Hg] data are presented as mean value ± standard error value (mean ± SE).

3. Results and Discussion

3.1. Total [Hg] according to feeding strategy and lifestyle

Total [Hg] present in different fish species can be significantly influenced by their eating habits (Ethier et al. 2008), since Hg can be transferred from prey to predators (Vieira et al. 2020b). Considering the feeding groups, it was possible to observe a similar pattern of Hg bioaccumulation on all sampling stations (Fig. 6.2), with carnivorous species presenting significantly higher [THg] ($p<0.05$) than the omnivorous ones.

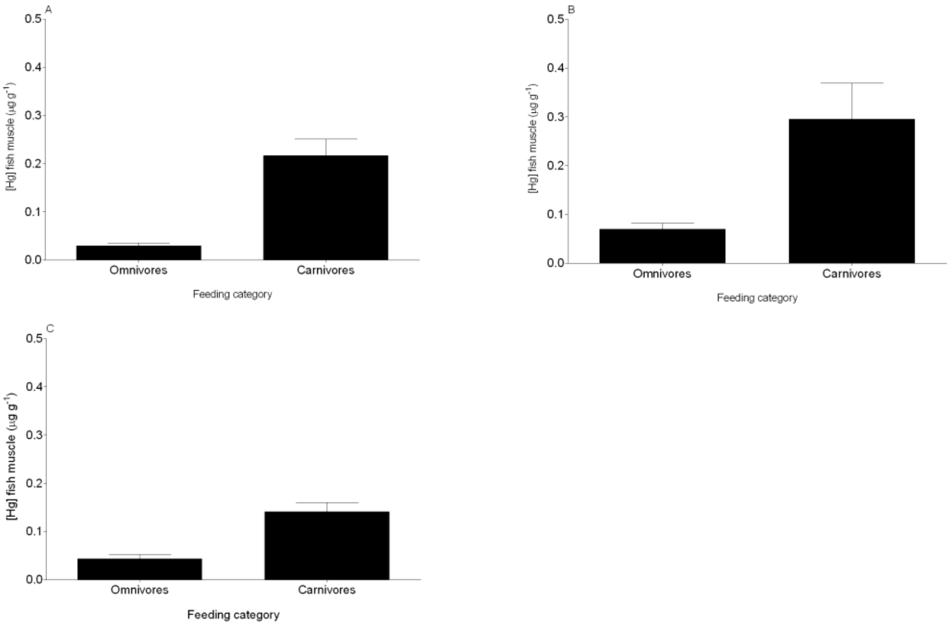


Fig. 6.2 Hg concentration (µg g^{-1}, ww) present in the muscle of carnivores and omnivores fish species from Azores archipelago (A) Northwest Portuguese coast (B) and Southeast Mexican coast (C).

Total [Hg] in species caught in the Azores archipelago (Fig. 6.2A) ranged from 0.003 µg g^{-1} and 0.17 µg g^{-1} in omnivorous species and between 0.03 µg g^{-1} and 0.89 µg g^{-1} in the carnivorous. The variation in [Hg] of species obtained on the Northwest Portuguese coast (Fig. 6.2B) was 0.01 µg g^{-1} – 0.19 µg g^{-1} and 0.04 µg g^{-1} – 0.88 µg g^{-1} for omnivorous and carnivorous species, respectively. In the Southeast Mexican coast (Fig. 6.2C), the [Hg] presented lower values (omnivores: 0.01 μg g^{-1} – 0.12 μg g^{-1} and carnivorous: 0.03 μg g^{-1} – 0.42 μg g^{-1}). No statistical differences ($p>0.05$) were found between the [THg] in the carnivorous from both sampling sites located in FAO fishing area 27 and the carnivorous species present in FAO fishing area 31. The same result was obtained for omnivorous species; however, in FAO fishing zone 27, omnivorous caught in the Azores archipelago have a significantly lower [THg] the ones caught on the Northwest Portuguese coast.

Increased Hg levels in carnivorous fish species, when compared to omnivorous, have also been observed by Xu et al. (2017) in fish species from two cage-cultured farms in Southern China, by Buck et al. (2019) in fish species from 40 different waterbodies in 26 countries and by Custódio et al. (2020) in fish species from Brazil. Fish species from higher trophic levels tend to accumulate greater amounts of Hg in their tissues (Zhang et al. 2020) since this metal is efficiently assimilated by the tissues of aquatic organisms, being subsequently transferred from levels lower trophic levels for higher trophic levels through diet (Kershaw and Hall 2019).

The differences in the [THg] found in omnivorous species from the different sampling sites (Azores archipelago and Northwest Portuguese coast) in FAO fishing area 27 may be related to the fact that the Northwest Portuguese coast sampling site is located in the limit of the Eastern North Atlantic Upwelling System (Sousa et al. 2020). The coastal upwelling environment is biologically rich and plays an important role in many of the world's fisheries; however, these areas can represent a significant source of Hg for marine food chains, since they contribute to the presence of Hg in surface waters (Conaway et al. 2009) through the transfer of Hg from the deepest areas to shallow areas (Silva et al. 2011).

Specifically, omnivorous and carnivorous fish species may have different food preferences (Table 6.1). Considering the FAO fishing area 27, the species caught in the Azores archipelago show preference for algae, zooplankton, zoobenthos and nekton, while the species obtained on the Northwest Portuguese coast prefer zooplankton and nekton. On the other hand, the fish species sampled in the FAO fishing zone 31 preferentially feed on detritus, zooplankton, zoobenthos and nekton.

In all sampling sites, fish with the highest [THg] were those that have a food preference for nekton (Table 6.2), which were significantly different ($p<0.05$) from species with other feeding choices. Food items from higher trophic levels such as nekton usually result in greater exposure to Hg, as observed by Azevedo et al. (2019), Wang et al. (2019) and Vieira at al. (2020a). Accordingly, the second highest value of [THg] was attributed to species feeding on zoobenthos followed by zooplankton whereas the detritus- and plant-feeding species presented the lowest Hg values.

Table 6.2 Total Hg concentration (μg g⁻¹, ww) present in the muscle of fish species from Azores archipelago, Northwest Portuguese coast and Southeast Mexican coast according to their food preferences.

Food preferences	Azores archipelago	Northwest Portuguese coast	Southeast Mexican coast
Plants	0.02±0.003	–	–
Detritus	–	–	0.04±0.02
Zooplankton	0.01±0.002	0.03±0.01	0.04±0.01
Zoobenthos	0.05±0.01	0.13±0.01	0.08±0.02
Nekton	0.22±0.04	0.28±0.06	0.18±0.03

Regarding the lifestyle (Fig. 6.3), distinct scenarios were observed in relation to the average [THg] of the fish species caught in the 2 locations of the FAO fishing area 27. In the Azores archipelago, [THg] was 0.06±0.02 μg g^{-1} in pelagic fish and 0.13±0.2 μg g^{-1} in the demersal. On the other hand, in the Northwest Portuguese coast pelagic presented [THg] of 0.07±0.02 μg g^{-1} while in the demersal species an [Hg] of 0.26±0.06 μg g^{-1} was detected. Significant differences ($p<0.05$) were found between the [THg] of pelagic species and the [THg] of demersal species in both sampling sites. Unfortunately, pelagic fish species were not obtained in the fishing area 31; however, an [THg] of 0.11±0.01 μg g^{-1} was found in the demersal species.

When comparing the [THg] present in pelagic fish from different sampling sites, species obtained on the Northwest Portuguese coast showed [THg] significantly higher ($p<0.05$) than species caught in the Azores archipelago. Moreover, it was observed that the demersal species from the Northwest Portuguese coast presented a significantly higher [THg] when compared to species obtained in the Azores archipelago and Southeast Mexican coast.

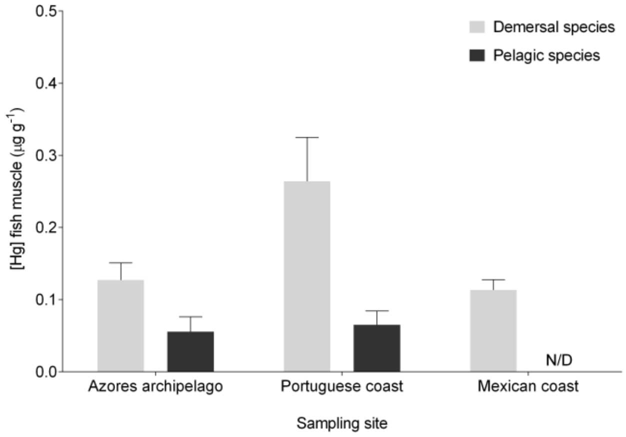


Fig. 6.3 Hg concentration (μg g⁻¹, ww) present in the muscle of Pelagic and Demersal fish species from different sampling sites

Other studies such as Azevedo et al. (2019), Sulimanec Grgec et al (2020) and Vieira et al. (2020a) have also reported differences in [Hg] between pelagic and demersal species. Choy et al, (2009) analyzed stomach contents and observed that the prey of fish species that live at greater depths present higher levels of Hg than the prey in shallower waters, suggesting that deeper water species tend to be more exposed to Hg because they feed on species with higher [Hg]. This could be the reason for the differences between pelagic and demersal species found in the Azores archipelago and Northwest Portuguese coast, since demersal species live in deeper waters than the pelagic. On the other hand, the differences in the [THg] of pelagic species between the Portuguese coast and the Azores archipelago, may be related to the fact that the species of the Portuguese coast may have been captured in an upwelling area. The main source of Hg in the open ocean is the deep water column (Choy et al. 2009), and in the upwelling areas the deep water layers are displaced to the surface, which means that pelagic species in these upwelling areas may be more exposed to Hg.

3.2. Interspecific differences of [Hg]

Considering the FAO fishing area 27, more specifically in the Azores archipelago, the average [THg] varied from 0.007±0.001 μg g^{-1} in *Trachurus trachurus* to 0.682±0.07 μg g^{-1} in Zeus faber (Fig. 6.4A). [THg] present in the species *Mullus surmuletus* (0.06±0.01 μg g^{-1}), *Helicolenus dactylopterus* (0.35±0.05 μg g^{-1}), *Phycis phycis* (0.06±0.01 μg g^{-1}), *Diplodus sargus* (0.11±0.02 μg g^{-1}), *Chelon labrosus* (0.02±0.002 μg g^{-1}) and *Pagellus bogaraveo* (0.06±0.02 μg g^{-1}) are within the [Hg] ranges reported by Andersen and Depledge (1997) for *Mullus surmuletus* (0.03 to 0.07 μg g^{-1}), *Helicolenus dactylopterus* (0.30 to 0.41 μg g^{-1}), Phycis phycis (0.06 to 0.07 μg g^{-1}), *Diplodus sargus* (0.03 to 0.17 μg g^{-1}), *Chelon labrosus* (0.01 to 0.03μg g^{-1}) and *Pagellus bogaraveo* (0.03 to 0.11μg g^{-1}), and are similar with the [THg] reported by Monteiro et al (1991) for *Helicolenus dactylopterus* (0.29±0.02 μg g^{-1}) and *Pontinus kuhlii* (0.16±0.01 μg g^{-1}). In the Northwest Portuguese coast (Fig. 6.4B), the species with the lowest [Hg] was *Sardina pilchardus* (0.014±0.002 μg g^{-1}) and the one with the highest [Hg] was the *Aphanopus carbo* (0.68 ± 0.07 μg g^{-1}). The [THg] observed in *Aphanopus carbo* is consistent with the values reported by Afonso et al. (2008 – 0.63 ± 0.27 μg g^{-1}). When comparing the fish species that were common to both sampling stations of FAO fishing area 27, specimens of *Scomber scombrus* and *Trachurus trachurus* from the Northwest Portuguese coast presented significantly higher [THg] than those from the Azores archipelago. This difference may be related to the characteristics of the sampling sites (as mentioned above for the pelagic species) or since [THg] may be influenced by abiotic factors such as fish length (Backstrom et al. 2020), the size of the individuals analyzed in the Azores archipelago (12.5±0.6 cm for

Trachurus trachurus and 17.7±0.6 cm for *Scomber scombrus*), which were smaller than the individuals sampled in the Northwest Portuguese coast (16.4±0.9 cm for *Trachurus trachurus* and 24.5±0.5 for *Scomber scombrus*). On the other hand, no significant differences ($p>0.05$) were observed in the [Hg] of *Sardina pilchardus*, although the specimens captured in the Azores archipelago are statistically smaller than the specimens of the Northwest Portuguese coast (12.4±0.9 cm against 16.04±0.4 cm). In the FAO fishing area 31 (Fig. 6.4C), [Hg] ranged between 0.02±0.005 µg g^{-1} (*Umbrina roncador*) and 0.34±0.03 µg g^{-1} (*Cynoscion nebulosus*). Similar [THg] for *Cynoscion nebulosus* (0.33±0.05 µg g^{-1}) have been found by Adams et al. (2010) in specimens collected from South Florida waters (FAO fishing area 31).

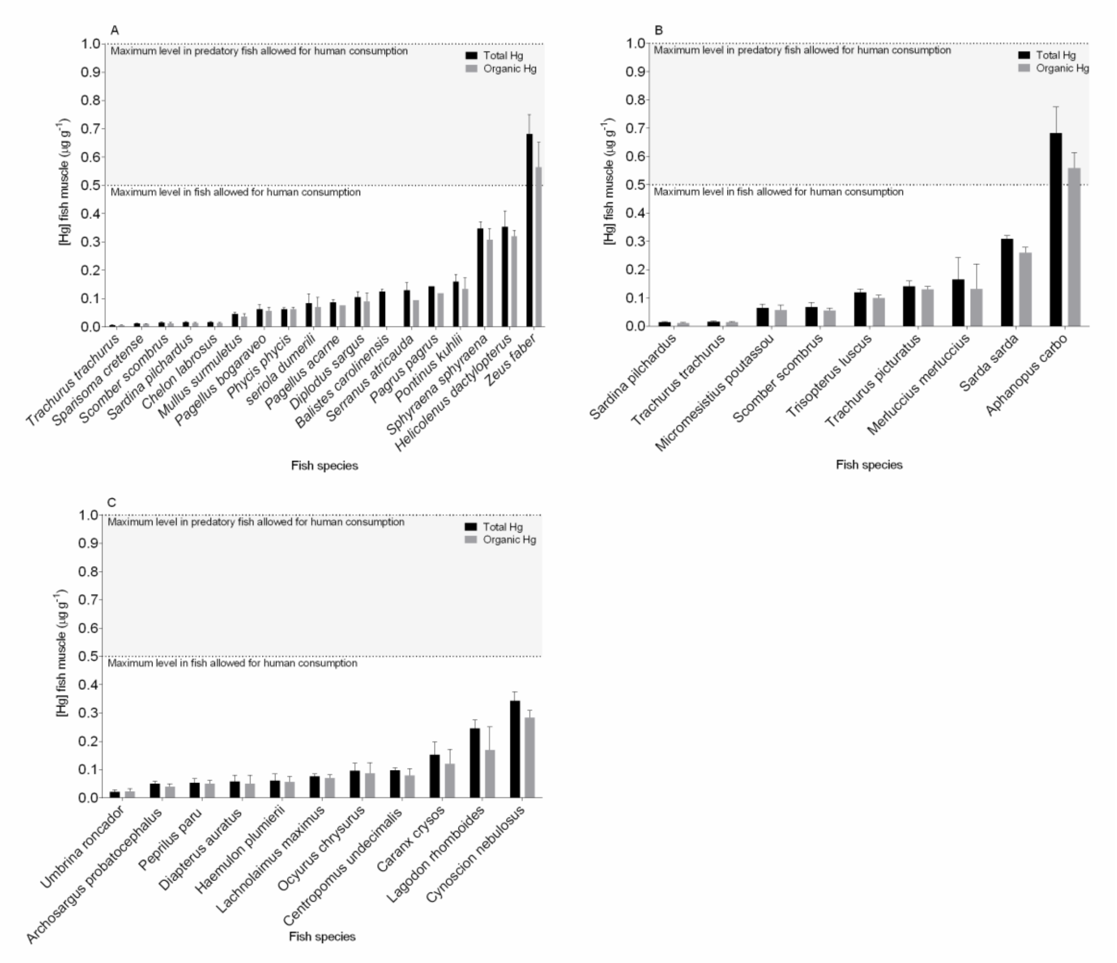


Fig. 6.4 Total and Organic Hg concentration (µg g^{-1}, ww) present in the muscle of fish species from Azores archipelago (A), Northwest Portuguese coast (B) and Southeast Mexican coast (C).

Of the 38 fish species analyzed in this study, only *Zeus faber* presented a [Hg] above the FAO/WHO and EU regulatory limit (0.50 µg g^{-1} ww) and all were below the limit established for predatory species [e.g. bonito (*Sarda sarda*), scabbard fish (*Aphanopus carbo*), seabream (*Pagellus spp.*)] (1.0 µg g^{-1} ww – EU 2008, Codex Alimentarius Commission 2018). Junqué et al. (2018) observed [Hg] above the limit of 0.5 µg g^{-1} ww in *Zeus faber* specimens from the

Western Mediterranean Sea. A [Hg] of 0.75 µg g^{-1} was also found by Di Lena et al. (2017) in the Central Adriatic (CA) and Central Tyrrhenian Seas (CT), Italy. On the other hand, although *Aphanopus carbo* presents the highest [Hg] (0.68 ± 0.07 µg g^{-1}), this species belongs to the abovementioned list of predatory species and therefore does not exceed the permitted limit (1 µg g^{-1}).

3.3. Risk assessment for the human fish consumers

Most of the Hg present in the fish muscle is found in its organic form (Ferreira da Silva and de Oliveira Lima 2020, Sulimanec Grgec et al. 2020), as observed by the present study, where the majority (> 80%) of Hg present in the muscle tissue of the 38 fish species was organic (Table 6.3).

Table 6.3 Hg concentration (µg g^{-1}, ww), estimated Daily Intake (EDI) and the estimated target hazard quotient (THQ) for each fish species from Azores archipelago Northwest Portuguese coast and Southeast Mexican coast.

Site	Scientif name	Organic Hg (µg g^{-1})	Organic Hg (%)	EDI	THQ
Azores archipelago	*Phycis phycis*	0.06	98	1.2	*0.9*
	Pagellus acarne	0.08	87	1.5	*1.2*
	Sphyraena sphyraena	0.31	89	5.9	*4.7*
	Pagellus bogaraveo	0.06	89	1.1	*0.9*
	Scomber scombrus	0.01	87	0.2	*0.2*
	Trachurus trachurus	0.01	83	0.1	*0.1*
	Serranus atricauda	0.09	85	1.8	*1.4*
	seriola dumerili	0.07	84	1.3	*1.1*
	Pagrus pagrus	0.12	83	2.3	*1.8*
	Zeus faber	0.56	83	10.8	*8.6*
	Mullus surmuletus	0.04	84	0.7	*0.6*
	Sardina pilchardus	0.01	83	0.3	*0.2*
	Diplodus sargus	0.09	86	1.7	*1.4*
	Chelon labrosus	0.01	81	0.3	*0.2*
	Sparisoma cretense	0.01	87	0.2	*0.2*
	Pontinus kuhlii	0.13	84	2.6	*2.0*
	Helicolenus dactylopterus	0.32	82	6.1	*4.9*
Northwest Portuguese coast	*Trachurus trachurus*	0.01	86	0.3	0.2
	Trachurus picturatus	0.13	90	2.4	1.9
	Sardina pilchardus	0.01	86	0.2	0.2
	Merluccius merluccius	0.13	80	2.6	2.0
	Micromesistius poutassou	0.06	89	1.1	0.9
	Aphanopus carbo	0.56	82	10.7	8.5
	Trisopterus luscus	0.10	85	2.0	1.6
	Scomber scombrus	0.05	81	1.0	0.8
	Sarda sarda	0.26	83	5.0	3.9

Southeast Mexican coast	*Haemulon plumierii*	0.10	91	0.7	*0.5*
	Caranx crysos	0.12	96	0.8	*0.6*
	Ocyurus chrysurus	0.08	88	0.5	*0.4*
	Archosargus probatocephalus	0.04	85	0.3	*0.2*
	Lagodon rhomboides	0.17	92	1.1	*0.9*
	Centropomus undecimalis	0.08	82	0.5	*0.4*
	Diapterus auratus	0.05	88	0.3	*0.3*
	Peprilus paru	0.05	89	0.3	*0.3*
	Umbrina roncador	0.02	94	0.1	*0.1*
	Lachnolaimus maximus	0.07	88	0.4	*0.4*
	Cynoscion nebulosus	0.28	82	1.8	1.4

Considering a weekly fish consumption of 1150.1 g in the sampling locations of FAO fishing Area 27 and a consumption of 383.5g per week in the sampling location located in FAO fishing area 31, the intake of Hg (Table 6.3) through fish consumption in these areas ranged between 0.1 and 10.8 µg MeHg kg bw^{-1} week^{-1} in the Azores archipelago, between 0.2 and 10.7 µg MeHg kg bw^{-1} week^{-1} on the Northwest Portuguese coast and between 0.13 and 1.8 µg MeHg kg bw^{-1} week^{-1} on the Southeast Mexican coast. The consumption of 8 of the 17 fish species caught in the Azores archipelago (*Pagellus acarne*, *Sphyraena sphyraena*, *Serranus atricauda*, *Seriola dumerili*, *Pagrus pagrus*, *Zeus faber*, *Pontinus kuhlii*, *Helicolenus dactylopterus*) leads to an intake of Hg above the levels recommended by the JECFA. On the Northwest Portuguese coast, the consumption of *Trachurus picturatus*, *Merluccius merluccius*, *Trisopterus luscus* and *Sarda sarda* also contributes to a Hg intake exceeding the recommended levels. On the other hand, in Southeast Mexican coast only the consumption of 1 species (*Cynoscion nebulosus*) leads to a higher Hg intake than the recommended. The majority of species that contribute to a Hg intake above the reference dose were caught in fishing area 27 due to the fact that the consumption of fish per capita in the sampling areas of fishing area 27 is 3 times higher than whereas the consumption of the sampling area of the fishing area 31.

THQ values (Table 6.3) ranged between 0.1 and 8.6 in the Azores archipelago, between 0.2 and 8.5 on the Northwest Portuguese coast and between 0.1 and 1.4 on the Southeast Mexican coast, which means that the consumption of some species analyzed in this study can be considered potentially hazardous to human health.

According to the 2015-2020 Dietary Guidelines for Americans, consumption of about 226.7 g per week of fish can provide an average of 250 mg per day of fatty acids (EPA and DHA) (Dietary Guidelines Advisory Committee 2015). Based on this quantity of fish consumed and the reference dose established by JECFA, the maximum number of weekly meals were grouped into three categories (Fig. 6.5): choices to avoid (less than 1 meal per week), good choices (1 meal per week) and best choices (two or more meals per week).

Considering the number of meals per week that a person should have to obtain the recommended amounts of fatty acids, only the consumption of 2 species (*Zeus faber* and *Aphanopus carbo*) is discouraged. These species were both captured in fishing zone 27 and are the species that have the highest levels of Hg in this study.

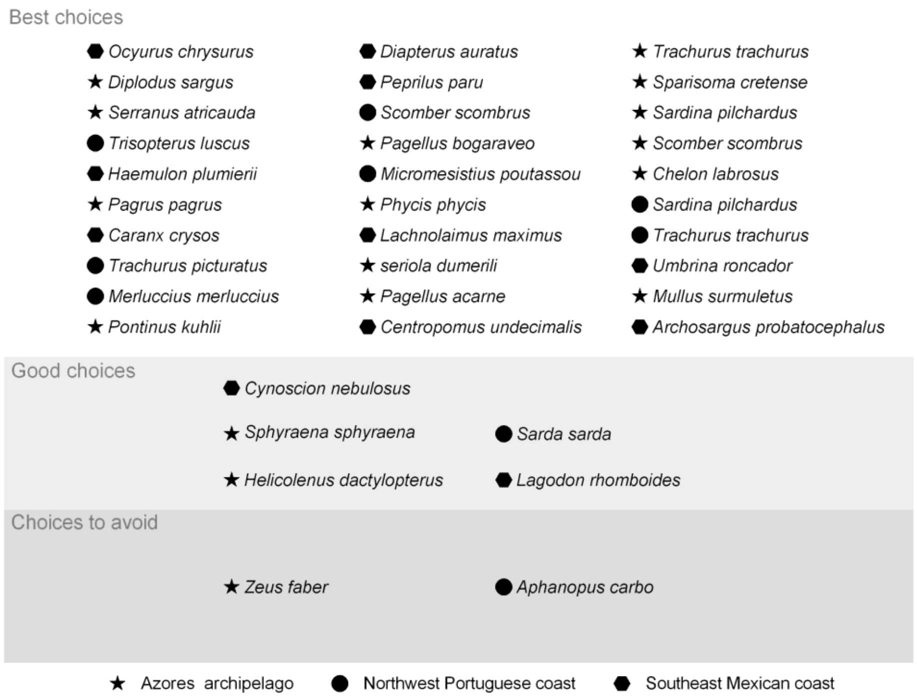


Fig. 6.5 Recommendations for fish consumption considering the [Hg] present in each fish species and the fish consumption established in the Dietary Guidelines for Americans.

4. Conclusion

Not surprisingly, the [THg] present in the carnivorous species is significantly higher than the [Hg] measured in the omnivorous in all sampling sites; however, no significant differences were found between FAO fishing areas. Furthermore, fish species that feed on organisms from higher trophic levels (nekton) exhibit significantly higher [THg] than fish species that feed on lower trophic levels suggesting trends of biomagnification. Considering the lifestyle of fish species in FAO fishing area 27, it was possible to observe that demersal species contain higher levels of Hg than pelagic species. The present study also demonstrates that only one of the 38 analyzed fish species exceeded the permissible limits for fish consumption (*Zeus faber*) and that more than 80% of the THg present in the muscle is in organic form.

The consumption of nine species of fish caught in the Azores Archipelago, four species caught on the Northwest Portuguese coast and 1 species from the Southeast Mexican coast contribute to a Hg intake higher than the recommended by JECFA. This indicates that a frequent consumption of these species, without respecting the permitted meals per week, can be considered dangerous for human health. Finally, only the consumption of two species is discouraged (*Zeus faber* and *Aphanopus carbo*), considering the consumption of a portion of 226.7g per week.

Although the majority of fish species analyzed in this study have [OHg] below the recommended limit for fish consumption, some of them can be considered potentially dangerous to human health, especially in areas that have a higher consumption of fish per capita.

Conflict of interest

The authors declare that they have no conflict of interests (financial or non-financial).

Acknowledgements

Thanks are due for the financial support to CESAM (UIDB/50017/2020+UIDP/50017/2020), to FCT/MEC through national funds. The authors also thank FCT (Fundação para a Ciência e Tecnologia) for the PhD grant of Vieira, H. C. (PD/BD/127808/2016). Bordalo, M.D. is funded by national funds (OE), through FCT, I.P., in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19.

References

Adams, D. H., C. Sonne, N. Basu, R. Dietz, D.-H. Nam, P. S. Leifsson, and A. L. Jensen. 2010. Mercury contamination in spotted seatrout, *Cynoscion nebulosus*: An assessment of liver, kidney, blood, and nervous system health. Science of The Total Environment 408:5808-5816.

Afonso, C., H. M. Lourenço, C. Pereira, M. F. Martins, M. L. Carvalho, M. Castro, and M. L. Nunes. 2008. Total and organic mercury, selenium and α-tocopherol in some deep-water fish species. Journal of the Science of Food and Agriculture 88:2543-2550.

Andersen, J. L., and M. H. Depledge. 1997. A survey of total mercury and methylmercury in edible fish and invertebrates from Azorean waters. Marine Environmental Research 44:331-350.

Azevedo, L. S., I. A. Pestana, A. F. da Costa Nery, W. R. Bastos, and C. M. M. Souza. 2019. Variation in Hg accumulation between demersal and pelagic fish from Puruzinho Lake, Brazilian Amazon. Ecotoxicology.

Backstrom, C. H., K. Buckman, E. Molden, and C. Y. Chen. 2020. Mercury Levels in Freshwater Fish: Estimating Concentration with Fish Length to Determine Exposures Through Fish Consumption. Archives of Environmental Contamination and Toxicology:1-18.

Bebianno, M. J., C. Santos, J. Canário, N. Gouveia, D. Sena-Carvalho, and C. Vale. 2007. Hg and metallothionein-like proteins in the black scabbardfish *Aphanopus carbo*. Food and Chemical Toxicology 45:1443-1452.

Buck, D. G., D. C. Evers, E. Adams, J. DiGangi, B. Beeler, J. Samánek, J. Petrlik, M. A. Turnquist, O. Speranskaya, K. Regan, and S. Johnson. 2019. A global-scale assessment of fish mercury concentrations and the identification of biological hotspots. Science of The Total Environment 687:956-966.

Burger, J., and M. Gochfeld. 2009. Perceptions of the risks and benefits of fish consumption: Individual choices to reduce risk and increase health benefits. Environmental Research 109:343-349.

Castro, P., and M. Huber. 2008. Marine Biology: Seventh Edition. McGraw-Hill Higher Education.

Chakraborty, P. 2017. Mercury exposure and Alzheimer's disease in India - An imminent threat? Science of The Total Environment 589:232-235.

Choy, C. A., B. N. Popp, J. J. Kaneko, and J. C. Drazen. 2009. The influence of depth on mercury levels in pelagic fishes and their prey. Proceedings of the National Academy of Sciences 106:13865-13869.

Codex Alimentarius Commission. 2018. Joint FAO/WHO food standards programme codex committee on contaminants in foods. 12th Session. Working document for information and use in discussions related to contaminants and toxins in the GSCTFF, CF/12 INF/1. Utrecht, The Netherlands, 12 - 16 March 2018, FAO/WHO.

Conaway, C. H., F. J. Black, M. Gault-Ringold, J. T. Pennington, F. P. Chavez, and A. R. Flegal. 2009. Dimethylmercury in Coastal Upwelling Waters, Monterey Bay, California. Environ Sci Technol 43:1305-1309.

Costley, C. T., K. F. Mossop, J. R. Dean, L. M. Garden, J. Marshall, and J. Carroll. 2000. Determination of mercury in environmental and biological samples using pyrolysis atomic absorption spectrometry with gold amalgamation. Analytica Chimica Acta 405:179-183.

Custódio, F. B., A. M. G. F. Andrade, L. R. Guidi, C. A. G. Leal, and M. B. A. Gloria. 2020. Total mercury in commercial fishes and estimation of Brazilian dietary exposure to methylmercury. Journal of Trace Elements in Medicine and Biology 62:126641.

Di Lena, G., I. Casini, R. Caproni, A. Fusari, and E. Orban. 2017. Total mercury levels in commercial fish species from Italian fishery and aquaculture. Food Additives & Contaminants: Part B 10:118-127.

Dietary Guidelines Advisory Committee. 2015. Dietary guidelines for Americans 2015-2020. Government Printing Office.

EFSA Scientific Committee. 2015. Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood. 1831-4732.

EU. 2008. Commission Regulation (EC) No 629/2008. OJ L 173, 2 July 2008, (p. 6–9).

FAO. 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229e.

Ferreira da Silva, S., and M. de Oliveira Lima. 2020. Mercury in fish marketed in the Amazon Triple Frontier and Health Risk Assessment. Chemosphere 248:125989.

Froese, R., and D. Pauly. 2016. FishBase: world wide web electronic publication version.

Innes, J. K., and P. C. Calder. 2018. The differential effects of eicosapentaenoic acid and docosahexaenoic acid on cardiometabolic risk factors: a systematic review. International journal of molecular sciences 19:532.

Jackson, A. C. 2018. Chronic Neurological Disease Due to Methylmercury Poisoning. Canadian Journal of Neurological Sciences / Journal Canadien des Sciences Neurologiques 45:620-623.

Junqué, E., M. Garí, R. M. Llull, and J. O. Grimalt. 2018. Drivers of the accumulation of mercury and organochlorine pollutants in Mediterranean lean fish and dietary significance. Science of The Total Environment 634:170-180.

Kershaw, J. L., and A. J. Hall. 2019. Mercury in cetaceans: Exposure, bioaccumulation and toxicity. Science of The Total Environment 694:133683.

Koletzko, B., R. Uauy, A. Palou, F. Kok, G. Hornstra, A. Eilander, D. Moretti, S. Osendarp, P. Zock, and S. Innis. 2010. Dietary intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in children – a workshop report. British Journal of Nutrition 103:923-928.

Lee, B. J., S. Y. Kwon, R. Yin, M. Li, S. Jung, S. H. Lim, J. H. Lee, K. W. Kim, K. D. Kim, and J. W. Jang. 2020a. Internal dynamics of inorganic and methylmercury in a marine fish: Insights from mercury stable isotopes. Environmental Pollution 267:115588.

Lee, J.-Y., G.-W. Hwang, A. Naganuma, and M. Satoh. 2020b. Methylmercury toxic mechanism related to protein degradation and chemokine transcription. Environmental Health and Preventive Medicine 25:30.

Marrugo-Negrete, J., S. Vargas-Licona, J. A. Ruiz-Guzmán, S. Marrugo-Madrid, A. G. Bravo, and S. Díez. 2020. Human health risk of methylmercury from fish consumption at the largest floodplain in Colombia. Environmental Research 182:109050.

Mason, R. P., R. F. Jacob, S. Shrivastava, S. C. R. Sherratt, and A. Chattopadhyay. 2016. Eicosapentaenoic acid reduces membrane fluidity, inhibits cholesterol domain formation, and normalizes bilayer width in atherosclerotic-like model membranes. Biochimica et Biophysica Acta (BBA) - Biomembranes 1858:3131-3140.

Milatou, N., M. Dassenakis, and P. Megalofonou. 2020. Mercury concentrations in reared Atlantic bluefin tuna and risk assessment for the consumers: To eat or not to eat? Food Chemistry 331:127267.

Monteiro, L., E. Isidro, and H. Lopes. 1991. Mercury content in relation to sex, size, age and growth in two scorpionfish (*Helicolenus dactylopterus* and *Pontinus kuhlii*) from Azorean waters. Water, Air, & Soil Pollution 56:359-367.

Moriarity, R. J., E. N. Liberda, and L. J. S. Tsuji. 2020. Subsistence fishing in the Eeyou Istchee (James Bay, Quebec, Canada): A regional investigation of fish consumption as a route of exposure to methylmercury. Chemosphere 258:127413.

Pinho, M. R., and G. Menezes. 2009. Demersal fishery off the Azores. Boletim do Núcleo Cultural da Horta, 18: 85-102.

Polak-Juszczak, L. 2018. Distribution of organic and inorganic mercury in the tissues and organs of fish from the southern Baltic Sea. Environmental Science and Pollution Research 25:34181-34189.

Pugach, S., and T. Clarkson. 2009. Prenatal mercury exposure and postnatal outcome: clinical case report and analysis. Clinical Toxicology 47:366-370.

Sarkar, T., M. M. Alam, N. Parvin, Z. Fardous, A. Z. Chowdhury, S. Hossain, M. E. Haque, and N. Biswas. 2016. Assessment of heavy metals contamination and human health risk in shrimp collected from different farms and rivers at Khulna-Satkhira region, Bangladesh. Toxicology Reports 3:346-350.

Silva, C. A. d., E. Tessier, V. T. Kütter, J. C. Wasserman, O. F. Donard, and E. V. Silva-Filho. 2011. Mercury speciation in fish of the Cabo Frio upwelling region, SE-Brazil. Brazilian Journal of Oceanography 59:259-266.

Sousa, M. C., A. Ribeiro, M. Des, M. Gomez-Gesteira, M. deCastro, and J. M. Dias. 2020. NW Iberian Peninsula coastal upwelling future weakening: Competition between wind intensification and surface heating. Science of The Total Environment 703:134808.

Stergiou, K. I., and V. S. Karpouzi. 2002. Feeding habits and trophic levels of Mediterranean fish. Reviews in Fish Biology and Fisheries 11:217-254.

Sulimanec Grgec, A., Z. Kljaković-Gašpić, T. Orct, V. Tičina, A. Sekovanić, J. Jurasović, and M. Piasek. 2020. Mercury and selenium in fish from the eastern part of the Adriatic Sea: A risk-benefit assessment in vulnerable population groups. Chemosphere 261:127742.

Teh, L. C. L., and U. R. Sumaila. 2013. Contribution of marine fisheries to worldwide employment. Fish and Fisheries 14:77-88.

Unoki, T., M. Akiyama, Y. Kumagai, F. M. Gonçalves, M. Farina, J. B. T. da Rocha, and M. Aschner. 2018. Molecular Pathways Associated With Methylmercury-Induced Nrf2 Modulation. Frontiers in Genetics 9.

Válega, M., S. Abreu, P. Pato, L. Rocha, A. R. Gomes, M. E. Pereira, and A. C. Duarte. 2006. Determination of Organic Mercury in Biota, Plants and Contaminated Sediments Using a Thermal Atomic Absorption Spectrometry Technique. Water, Air, and Soil Pollution 174:223-234.

Vieira, H. C., M. D. Bordalo, F. Morgado, A. M. V. M. Soares, and S. N. Abreu. 2017. Mercury content in the white and dark muscle of Skipjack tuna (*Katsuwonus pelamis*) along the canning process: Implications to the consumers. Journal of Food Composition and Analysis 56:67-72.

Vieira, H. C., J. Rendón-von Osten, A. M. V. M. Soares, F. Morgado, and S. N. Abreu. 2020a. Contribution of commercial fish species to human mercury exposure: an evaluation near the Mid-Atlantic Ridge. Journal of Food Composition and Analysis:103688.

Vieira, H. C., J. Rendón-von Osten, A. M. V. M. Soares, F. Morgado, and S. N. Abreu. 2020b. Mercury bioaccumulation in the long-fin squid *Loligo forbesi* near the Mid-Atlantic Ridge: Implications to human exposure. Ecotoxicology and Environmental Safety 203:110957.

Wang, Y., Q. Xie, Q. Xu, J. Xue, C. Zhang, and D. Wang. 2019. Mercury bioaccumulation in fish in an artificial lake used to carry out cage culture. Journal of Environmental Sciences 78:352-359.

WHO. 2003. Diet, nutrition and the prevention of chronic diseases: Report of a joint WHO/FAO expert consultation, 28 January–1 February 2002. Geneva: World Heath Organization/Food and Agricultural Organization.

WHO. 2008. Guidance for identifying populations at risk from mercury exposure. Geneva, Switzerland.

Xu, X., and W.-X. Wang. 2017. Mercury exposure and source tracking in distinct marine-caged fish farm in southern China. Environmental Pollution 220:1138-1146.

Zhang, H., C. Guo, H. Feng, Y. Shen, Y. Wang, T. Zeng, and S. Song. 2020. Total mercury, methylmercury, and selenium in aquatic products from coastal cities of China: Distribution characteristics and risk assessment. Science of The Total Environment 739:140034.

Chapter VII:

Contribution of commercial fish species to human mercury exposure: an evaluation near the Mid-Atlantic Ridge

Contribution of commercial fish species to human mercury exposure: an evaluation near the Mid-Atlantic Ridge

Vieira, H.C.[1], Rendón-von Osten, J.[2], Soares, A.M.V.M.[1], Morgado, F.[1], Abreu, S. N.[1]

[1] CESAM - Centre for Environmental and Marine Studies, Department of Biology,
University of Aveiro, 3810-193 Aveiro, Portugal
[2] EPOMEX Institute, Autonomous University of Campeche, Campeche, Mexico

Abstract

Fish consumption is frequently associated with the prevention of some human diseases, being simultaneously a major pathway of mercury (Hg) exposure. Therefore, the aim of the study was to evaluate the contribution of 28 commercial fish species to the human Hg exposure in the Azores archipelago (Portuguese region with highest fish consumption per capita). These species potentially contributed on average to 7.47 mg of Hg per capita, although low Hg levels had been detected in fish. Mora moro and Zeus faber exceeded the maximum permitted for fish consumption (> 0.5 μg g^{-1}, ww) even though they were not the species contributing the most to human Hg exposure. On the other hand, Katsuwonus pelamis was the main contributor due to increased fish landings. Furthermore, an increase in Hg content with trophic level has been suggested, as carnivore fish exhibited higher Hg levels than omnivores. In addition, demersal fish generally presented higher Hg concentration (although non-significant) than pelagic ones, possibly related with increased Hg values of their prey at this depth. Notwithstanding, THQ (Target Hazard Quotient) being < 1 for all species indicates that the daily human exposure to Hg via fish consumption is not likely to cause any negative health risks.

Keywords: Fish consumption; Food composition; Target Hazard Quotient; Risk assessment

1. Introduction

Fish consumption is frequently linked to the prevention of some human diseases, especially regarding cardiac and circulatory disorders, being also associated with the reduction of mortality in patients with coronary diseases (Kris-Etherton et al., 2002; Mozaffarian et al., 2003). These advantages are mainly owing to their contents of high-quality proteins, vitamins, content on n-3 fatty acids, such as docosahexaenoic acid (22:6, n-3, DHA), eicosapentaenoic acid (20:5, n-3, EPA) and other essential nutrients (Egeland and Middaugh, 1997; WHO, 2003; FAO, 2012). At the same time, in contrast with the health benefits, there is a risk derived from exposure to chemical pollutants contained in fish and shellfish that also needs to be considered (Domingo et al., 2007). For instance, fish consumption is considered as the major pathway of mercury (Hg) exposure in humans, being more than 90% of total Hg present in fish tissue found essentially in its organic form (methylmercury – MeHg) (Hall et al., 1997; Liang et al., 2013), the most toxic form of Hg (Storelli et al., 2002).

Most of the Hg released into the marine environment is inorganic (Storelli et al., 2002) from either natural or anthropogenic sources (Steenhuisen and Wilson, 2015). Natural emissions include volcano eruptions and geothermal sources, whereas anthropogenic releases are mostly from chloro-alkali production and fossil fuels combustion (Pirrone et al., 2010). Once in the aquatic systems, the inorganic Hg can be converted into MeHg by anaerobic bacteria in sediments (Baeyens et al., 2003; Forsyth et al., 2004).

A great percentage of MeHg ingested by human consumers is absorbed by the body (Hightower and Moore, 2003) potentially reflecting in serious health problems, when chronically exposed to this heavy metal. These problems include a variety of symptoms such as headaches, emotional changes, insomnia, and cognitive function deficits (Hanna et al., 2015). Furthermore, due to its potential to biomagnify along trophic chains, Hg may achieve hazardous concentrations in fish and shellfish species, fish-eating wildlife and human populations (Southworth et al., 2000; Chan et al., 2003). For this reason, the European Union has determined limits for fish and fish products consumption, concerning Hg exposure. The European Commission Regulation (EC) No 466/2001 of 8th March 2001 established maximum levels of 0.5 μg g^{-1} ww Hg permitted for human consumption in foodstuffs, excluding an “exception list” where the accepted tolerance level raises to 1 μg g-1 ww, comprising some species with high trophic level (EU, 2001). On the other hand, Food and Agriculture Organization (FAO)/ World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) and US Environmental Protection Agency (USEPA) have established reference doses (RfD) for MeHg intake based on epidemiological studies conducted in New Zealand, Seychelles and the Faeroe (Rice, 2004; Li et al., 2015). At last, JECFA considered a RfD named “provisional tolerable weekly intake” (PTWI) for MeHg of 1.3 μg kg body weight $(bw)^{-1}$ $week^{-1}$ (EFSA Scientific Committee, 2015) and USEPA set a RfD in 0.7 μg MeHg kg bw^{-1} $week^{-1}$ (USEPA, 1997).

The Azores archipelago located near the Mid-Atlantic Ridge (MAR) is an area of high fish consumption rate “per capita” as each Azorean consumes around 80 Kg of fish per year

(Megapesca, 2007). Through a search in Web of Science (May 2019), using the keywords "Mercury AND Azores", several studies were found reporting the Hg concentration in the muscle of fish captured in this area (Monteiro et al., 1991; Monteiro et al., 1996; Andersen and Depledge, 1997; Afonso et al., 2007; Magalhães et al., 2007; Costa, 2009; Torres et al., 2015). However, none of them take into account the dietary guidelines of international agencies for human Hg exposure. Such analysis may provide the necessary information about the Hg availability in commercial fish, allowing the consumers to make informed decisions about which fish to ingest. Taking this into consideration, the present work aims to: i) report the Hg concentration in different commercial fish species (landing from 1994 to 2018) in an area with high fish consumption near the MAR, ii) determine the contribution of these fish species to the Hg ingestion for human consumers, and iii) evaluate the human health implications of such Hg exposure using the non-carcinogenic target hazard quotient (THQ).

2. Materials and Methods

2.1. Study area

The study area (Fig. 7.1) is located near MAR, North Atlantic Ocean, where an isolated group of nine volcanic islands, the Azores archipelago, extends along the south east–north–west strip near the triple junction of Eurasian, African, and North American plates. As a result, the archipelago has a complex tectonic setting, where seismic–volcanic phenomena are common, being responsible for natural inputs of Hg to the aquatic environment (Depledge et al., 1992; Vieira et al., 2013).

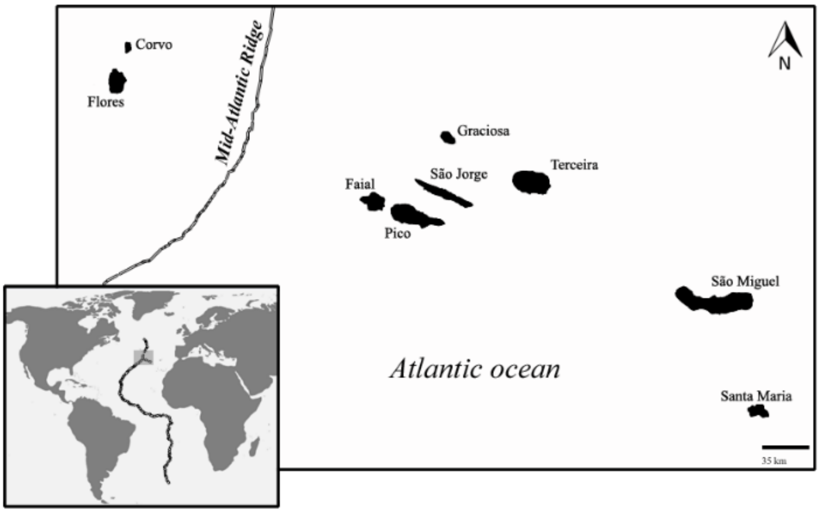


Fig. 7.1 Map of study area (Azores archipelago) enhancing the proximity of the archipelago to the MAR

2.2. Data analysis

Hg has been quantified in samples from 28 fish species caught in the Azorean Exclusive Economic Zone (AzEEZ). The present review was based on all found published papers presenting an evaluation of Hg concentrations in fish muscle (Table. 7.1). Table 7.1 also includes additional data resulting from Hg quantification in samples from recreational fishing, performed in "This study" in species where no previous data was available. Altogether, 28 commercial fish species were considered.

Table 7.1 Average of Hg concentrations (μg g^{-1}), number of sample (n) individual per study and reference of the 28 fish species caught near the Azores archipelago

Scientific name	Common name	n	Average$_{[Hg]}$ (μg g-1, ww)	References
Aphanopus carbo	Black scabbard fish	20; 135	0.80	Afonso et al. (2007); Costa et al. (2009)
Balistes carolinensis	Triggerfish	5	0.13	This study
Chelon labrosus	Thick-lipped grey mullet	10	0.20	Andersen and Depledge (1997)
Conger conger	Conger eel	5; 39	0.33	Andersen and Depledge (1997); Magalhães et al. (2007)
Diplodus sargus cadenati	White sea bream	55	0.30	Andersen et al. (1997)
Helicolenus dactylopterus	Blue-mouth	105; 31	0.28	Monteiro et al. (1991); Andersen and Depledge (1997)
Katsuwonus pelamis	skipjack tuna	53; 15	0.12	Andersen and Depledge (1997); Torres et al. (2015)
Lepidopus caudatus	silver scabbard fish	24; 55	0.30	Andersen and Depledge (1997); Magalhães et al. (2007)
Mora moro	Common mora	42	0.81	Magalhães et al. (2007)
Mullus surmuletus	Red mullet	13; 6	0.09	Andersen and Depledge (1997); This study
Muraena helena	Moray eel	1	0.05	Andersen and Depledge (1997)
Pagellus acarne	Axillary sea bream	24	0.20	Magalhães et al. (2007)
Pagellus bogaraveo	Red sea bream	11	0.26	Andersen and Depledge (1997)
Pagrus pagrus	red porgy	1	0.47	Andersen and Depledge (1997)
Phycis blennoides	Greater forkbeard	17	0.15	Magalhães et al. (2007)
Phycis phycis	Forkbeard	31; 56	0.11	Andersen and Depledge (1997); Magalhães et al. (2007)
Polyprion americanus	Wreckfish	14	0.27	Magalhães et al. (2007)
Pontinus kuhlii	Offshore rockfish	99	0.16	Monteiro et al. (1991)
Sardina pilchardus	Sardine	6	0.02	This study
Scomber japonicus	Chub mackerel	4	0.03	Monteiro et al. (1996)
Seriola dumerili	Greater amberjack	2	0.05	This study
Serranus atricauda	Blacktail Comber	8	0.17	This study
Sparisoma cretense	Parrotfish	9	0.01	This study
Sphyraena viridensis	Yellowmouth barracuda	3	0.37	This study
Thunnus alalunga	Albacore	46	0.37	Andersen and Depledge (1997)

Thunnus obesus	bigeye tuna	15	0.14	Torres et al. (2015)
Trachurus picturatus	Blue jack mackrel	20; 39; 48; 7	0.07	Monteiro et al. (1996); Andersen and Depledge (1997); Magalhães et al. (2007); This study
Zeus faber	Jonh dory	*4*	*0.68*	This study

2.3. Lifestyle and trophic level

The 28 fish species present in this work were grouped according to their lifestyle and trophic level (Fig. 7.2). Regarding their lifestyle, fish species can be classified as demersal or pelagic fish. Demersal fish species are those who live near the sea substrate, and may have dependent behaviour of the bottom (benthic) or dwell in the interface between the bottom and the water column (benthopelagic) (Pinho and Menezes, 2009). On the other hand, pelagic fish species are those that spend much of their lives swimming in open water away from the bottom (Castro and Huber, 2008). Accordingly, the lifestyle of the fish species was determined conforming to Menezes et al. (2006) and Almada et al. (2015). The trophic level for each species was determined using FishBase information. Furthermore, the identification of the functional trophic groups was performed according to Stergiou and Karpouzi (2002), who established five trophic groups: pure herbivores, omnivores with a preference for vegetable material, omnivores with a preference for animal material, carnivores with a preference for decapods and fish and carnivores with a preference for cephalopods and fish, based on the dietary habits of 332 Mediterranean Sea individuals belonging to 146 species, 59 families and 21 orders, these groups have estimated fractional trophic levels (TROPHs) ranging from 2.0 to 4.5.

2.4. Hg quantification

Hg quantification in fish samples from recreational fishing was performed with the Advanced Mercury Analyzer (AMA-254, made by ALTEC and distributed by LECO). This process does not require a previous digestion of the sample; the procedure is based on a pyrolysis process of the tissue using a combustion tube heated at 750 ºC under an oxygen atmosphere and the released Hg is trapped in a gold amalgamator and subsequently detected and quantified by atomic absorption spectrometry (Costley et al., 2000).

Sample analysis were triplicated to check the reproducibility of the results and three blank analyses (analysis without sample) were performed between samples to verify that Hg was not being accumulated over the samples. In this study, blank readings typically correspond to values < 0.02 ng of Hg. Analytical quality of the procedure was checked using the reference material TORT-2 (Lobster Hepatopancreas Reference Material for Trace Metals, National

Research Council of Canada). Obtained data (0.251±0.001 μg g^{-1} of Hg) and reference (0.27±0.06 μg g^{-1} of Hg) values were not statistically different ($p>0.05$).

2.5. Risk assessment in human population

As stated by Groth III (2010), the average Hg concentration may be combined with the market share percentages of each fish species to generate Hg input factors. These factors are not precise measures of exposure; however, they indicate the relative contributions of each fish species in the overall population's Hg intake (Groth III, 2010).

Moreover, there are two main methods of estimating risks: one is based on carcinogenic effects, and the other is based on non-carcinogenic effects (Chien et al., 2002). In accordance with Barone et al. (2015), for non-carcinogenic effects, the risk can be expressed as a target hazard quotient (THQ). THQ indicates the ratio between exposure and the reference dose. If the ratio is less than 1, it means that the level of exposure is smaller than the reference dose, suggesting that a daily exposure at this level is not likely to cause any deleterious effects during the lifetime of a human consumer. In other words, a THQ below 1 means that the adverse effects are negligible. The THQ is based on the following equation (Chien et al., 2002; Barone et al., 2015; Bortey-Sam et al., 2015):

$$THQ = \frac{EF \times ED \times FIR \times C}{RfD \times ABW \times AT} \times 10^{-3}$$

where EF is the exposure frequency (365 days $year^{-1}$);

ED is the exposure duration (adults, 70 years, equivalent to the average lifetime);

FIR is the food ingestion rate (g $person^{-1}$ day^{-1});

C is the metal concentration in fish (μg g^{-1}, wet weight);

RfD is the oral reference dose (μg g^{-1} day^{-1}); ABW is the average body weight (60kg);

AT is the averaging exposure time for non-carcinogens (365 days $year^{-1}$ × ED).

2.6.Statistical analysis

Data normality was tested using Kolmogorov-Smirnov test. Data did not follow a normal distribution, and the normality was established after log-transformation; therefore, the parametric statistical t-tests were used to compare the mean Hg concentration between functional trophic (omnivores and carnivores) and lifestyle (pelagic and demersal fish species). Statistical analyses were performed using Sigmaplot (version 11.0). Statistical significance was defined as $p<0.05$. Hg concentration data is presented as mean value ± standard error value (mean ± SE).

3. Results and Discussion

3.1. Interspecific differences of Hg in commercial fish

Hg concentration present in the muscle from the 28 commercial fish species ranged from 0.01 µg g^{-1} (ww) in the parrot fish *Sparisoma cretense* to 0.81 µg g^{-1} (ww) in the common mora *Mora moro* (Fig. 7.2).

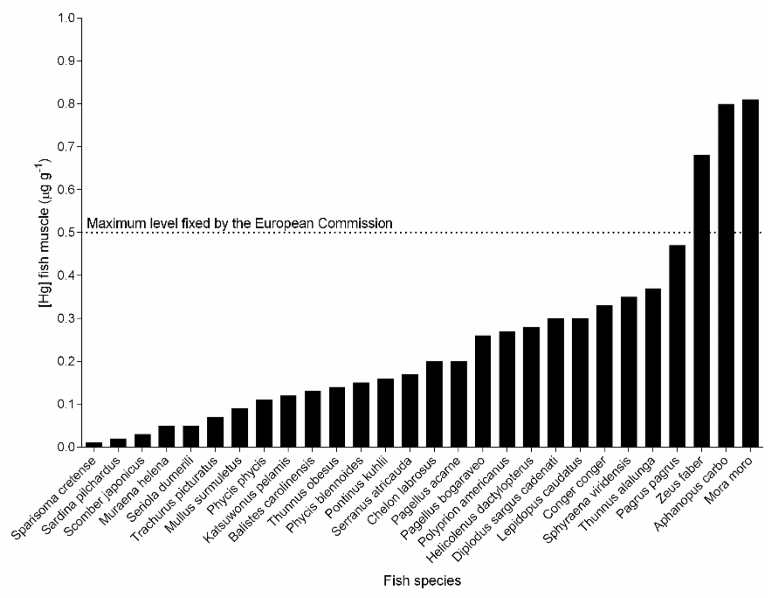


Fig. 7.2 Hg concentration (µg g^{-1}, ww) present in the muscle from 28 commercial fish species.

Hg concentration of each species was compared with the permissible limits established in Commission Regulation (EC) no. 629/2008 of July 2008 (EU, 2008). This regulation establishes, for most fish species, the value of 0.5 µg g^{-1} as the maximum Hg concentration for consumption, except for the species present in the "exception list" where the maximum allowed concentration raises to 1 µg g^{-1}. Regarding the permissible limits for fish consumption, only *Mora moro* and the *Zeus faber* exhibited higher values than the permitted (0.81 µg g^{-1} and 0.68 µg g^{-1}, respectively, exceeding the maximum value of 0.5 µg g^{-1}) but were lower than the values reported in previous studies for *Mora moro* (2.40 µg g^{-1}) from the NW Mediterranean (Koenig et al., 2013) and for *Zeus faber* (0.75 µg g^{-1}) captured in the Central Adriatic (CA) and Central Tyrrhenian (CT) Seas, Italy (Di Lena et al., 2017). Although the Hg concentration in *Aphanopus carbo* was the second highest in this study (0.80 µg g^{-1}), and similar with the Hg concentration (0.89±0.27 µg g^{-1}) reported by Afonso et al. (2008), this species belongs to the "exception list" and thus does not exceeds the permitted limit (1 µg g^{-1}).

The interspecific Hg variation in fish is the result of trophic position, growth rate, fish age and food web complexity (Magalhães et al., 2007; Koenig et al., 2013), thus; different fish species contain distinct Hg contents. Based on the combined fish and shellfish Hg content of 51 different commercially important varieties in the US seafood supply (U.S. Food and Drug Administration, 2009), a classification system of six categories was created: “very low” < “below average” < “above average” < “moderately high” < “high” < “very high” (Groth III, 2010). Taking as a starting point the value of 0.086 µg g^{-1} (weighted average Hg level in the US seafood supply as a whole), each limit between groups is the result of multiplying the weighted average Hg level per 0.5, 1, 2, 4 and 8 times, respectively.

This mathematical scheme is simple and should be relatively easy for consumers to grasp, facilitating risk communication (Groth III, 2010). In this way, each fish species present in this study was grouped (Table 7.2) based on those six categories.

Table 7.2 Hg classification system of the studied fish species in six categories based on Hg content.

Hg category	Hg range (µg g^{-1})	Fish species
Very low	≤0.043	*Scomber japonicus, Sparisoma cretense, Sardina pilchardus*
Below average	0.044-0.086	*Muraena helena, Trachurus picturatus, Seriola dumerili*
Above average	0.087-0.172	*Balistes carolinensis, Katsuwonus pelamis, Phycis blennoides, Phycis phycis, Pontinus kuhlii, Serranus atricauda, Thunnus obesus, Mullus surmuletus*
Moderately high	0.173-0.344	*Chelon labrosus, Conger conger, Diplodus sargus cadenati, Helicolenus dactylopterus, Lepidopus caudatus, Pagellus acarne, Pagellus bogaraveo, Polyprion americanus*
High	0.345-0.688	*Pagrus pagrus, Thunnus alalunga, Sphyraena viridensis, Zeus faber*
Very high	>0.688	*Aphanopus carbo, Mora moro*

Consequently, the first category classified as “very low” Hg category comprises only three fish species and represents 4.9% of the total landing per year. The maximum Hg concentration (0.03 µg g^{-1}) found in this category belongs to the *Scomber japonicus.* The category “below average” also includes three species: the Hg concentration determined in these species was 0.05 µg g^{-1} in the *Muraena helena* and the *Seriola dumerili* and 0.07 µg g^{-1} for the *Trachurus picturatus*. This category contributes to 10.3% of the landings. Seven other species have Hg concentration “above average”. In this group, Hg concentration ranged between 0.09 µg g^{-1} and 0.17 µg g^{-1}, being the higher Hg concentration attributed to the *Serranus atricauda*. These seven species correspond to 48.4% of landings in the Azorean ports, 32.7% of which belong to the *Katsuwonus pelamis*. The vast majority of fish species belongs to the “moderately high” category. Eight fish species contribute to 20.2% for the total landing and the maximum Hg concentration (0.33 µg g^{-1}) in this category belongs to the *Conger conger*. The last two Hg categories “high” and “very high” represent 3.6% and 1.5% of landings in the Azorean ports per year, respectively. For these categories the *Zeus faber* and *Mora moro* are the species with higher Hg concentration with 0.68 µg g^{-1} and 0.81 µg g^{-1}, respectively.

3.2. Hg concentration according to lifestyle and trophic level

According to Stergiou and Karpouzi (2002), the TROPHs calculations based on prey items in the diet, express the position of organisms within the food webs that largely defines aquatic ecosystems. In the present study, the 28 fish species were classified and distributed to five trophic groups based on their trophic level (Fig. 7.3). The group of "pure herbivores" with the TROPH values between 2.0 and 2.1 was not represented by any species. The group of omnivores with a preference for vegetable material (2.1 < TROPH < 2.9) was represented by a single species (*Chelon labrosus*), whereas the category of omnivores with a preference for animal material (2.9 < TROPH < 3.7) was represented by 7 fish species (*Diplodus sargus cadenati*, *Helicolenus dactylopterus*, *Mullus surmuletus*, *Scomber japonicus*, *Sparisoma cretense*, *Sardina pilchardus* and *Trachurus picturatus*). On the other hand, the group of carnivores (3.7 < TROPH < 4.5) was subdivided according to the preference either for decapods and fish (3.7 < TROPH < 4.0) or preference for fish and cephalopods (4.0 < TROPH < 4.5). The first subgroup of carnivores was represented by five fish species (*Lepidopus caudatus*, *Mora moro*, *Pagellus acarne*, *Pagrus pagrus* and *Phycis blennoides*) while the majority of species (15) belonged to the second subgroup (*Aphanopus carbo*, *Balistes carolinensis*, *Conger conger*, *Katsuwonus pelamis*, *Muraena helena*, *Pagellus bogaraveo*, *Phycis phycis*, *Polyprion americanus*, *Pontinus kuhlii*, *Serranus atricauda*, *Thunnus alalonga*, *Zeus faber*, *Seriola dumerili*, *Sphyraena viridensis* and *Thunnus obesus*), respectively.

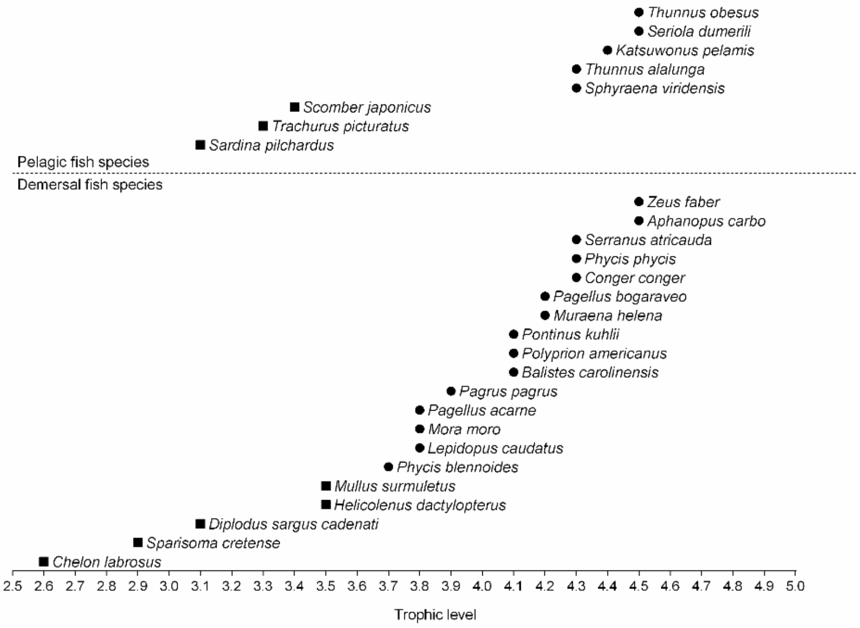


Fig. 7.3 Trophic level, functional trophic groups (■ Omnivores and ● Carnivores) and lifestyle information (pelagic and demersal fish species) of the 28 fish species evaluated in this study.

Due to the large variation in the number of species present in each trophic group, the five groups were reduced to two major ones: "omnivores" including fish species with a trophic level greater than 2.1 and less than 3.7, and "carnivores" consisting of fish species with TROPH values between 3.7 and 4.5.

On average, the Hg concentration found in carnivores fish species (0.30±0.04 µg g^{-1}) was significantly higher ($p<0.05$) than the Hg concentration present in omnivorous fish species (0.13±0.04), which goes in line with previous studies (Li et al., 2009; Kasper et al., 2012; Costa and Lacerda, 2014; Bastos et al., 2015). The increased Hg concentration with the trophic level (omnivores vs carnivores fish species) reinforces the notion that Hg biomagnifies along the trophic chain resulting in higher Hg concentrations in fish with more predatory feeding habits than those feeding at lower trophic levels (Ruelas-Inzunza et al., 2008; Li et al., 2009).

Regarding lifestyle, the average Hg concentrations of pelagic and demersal fish species were 0.14±0.08 and 0.29±0.05 µg g^{-1}, respectively. Despite the higher Hg levels found in demersal fish species, no significant difference ($p>0.05$) was found between the Hg concentration in pelagic and demersal fish species. Other authors have also reported higher Hg concentrations in demersal species when compared with pelagic species. For instance, Saei-Dehkordi et al. (2010) showed that demersal fish from the Persian Gulf had higher Hg quantities than those measured in pelagic species. Choy et al. (2009) have also reported that the increase of Hg concentration can be explained by the depth at which the species inhabit. In fact, the foraging habitat is a determining factor influencing the accumulation of Hg (Azevedo et al., 2019), since higher Hg levels are expected in prey that inhabit deeper environments when compared to shallower environments. Therefore, species with a predatory behaviour obtaining Hg essentially from their prey and living in deeper (demersal) zones are also expected to accumulate higher Hg levels through biomagnification than pelagic species (Choy et al., 2009).

3.3. Hg exposure and risk assessment of human fish consumption

The 28 commercial fish species present in this study contribute to 75.9% of the total fish landings in the Azorean ports. According to the fish landing reports (1994-2018) of Azores Fisheries Statistics (SREA – http://tiny.cc/3q33tz), an average of 10289 tons of these species is discharged every year, which indicates that each year these species provide approximately 1.8 kg of Hg for the fish consumers.

Despite being represented by a small number of species (8), the pelagic fish contribute to half (~50%) of the Hg exposure risk to humans, due to the high fish landing of these species. Furthermore, an average of about 75% of *Katsuwonus pelamis* discharges are directed for the canning industry (unpublished Azores Regional Government data), meaning that muscle tissue from this species will also become available to the population in the form of canned tuna. Therefore, raw or canned food, *Katsuwonus pelamis* is responsible for about 22.8% of the Hg exposure (Fig. 7.4). However, considering that the canning process leads to an increase in the [Hg] in the muscle due to the loss of moisture by the tissue (Vieira et al., 2017), the referred percentage may be even greater.

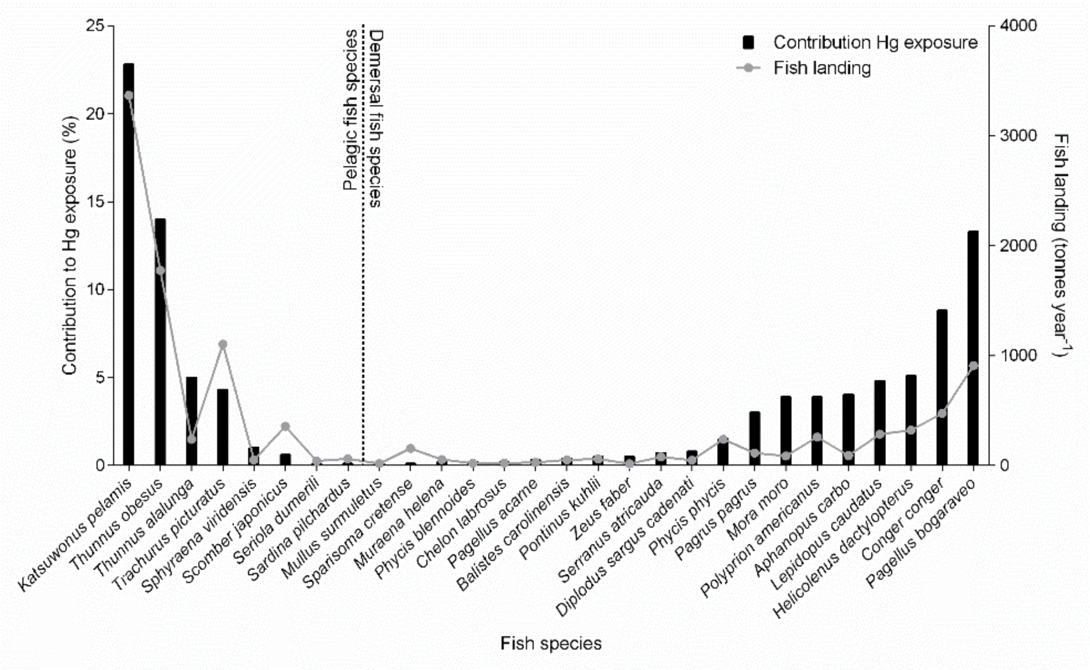


Fig. 7.4 Contribution to Hg exposure and fish landing of each fish species

Encompassing all data, and according to the Hg input factor (Fig. 7.5), the species that most contribute to Hg exposure are not those with the highest Hg concentrations. Considering the 4 fish species with the highest inputs of Hg, the first two (*Katsuwonus pelamis* and *Thunnus obesus*) belong to the "above average" category (ranging between 0.087 and 0.172 µg g^{-1}) whereas the other two (*Pagellus bogaraveo* and *Conger conger*) belong to the "moderately high" category (Hg concentration between 0.173 and 0.344 µg g^{-1}). A very large cumulative fraction (58.9%) of the Hg inputs is therefore represented by a relatively small number of fish species with lower Hg concentration, which, on the other hand has a significant percentage of the total fish landing in the Azorean ports. It is thus suggested that the quantity of fish landed plays an important role in human exposure to Hg.

In marine fish, about 90-95% of the Hg concentration present in fish muscle exists in the methylated form (Fitzgerald et al., 2007; WHO, 2008); however, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain uses the worst case scenario hypothesis to calculate the MeHg dietary exposure; therefore they assume that 100% of Hg present in fish is in its methylated form (Ceccatto et al., 2015; Di Lena et al., 2018). Consequently, the THQ was calculated assuming the Hg concentration as 100% MeHg for both RfD's (USEPA RfD and JECFA RfD).

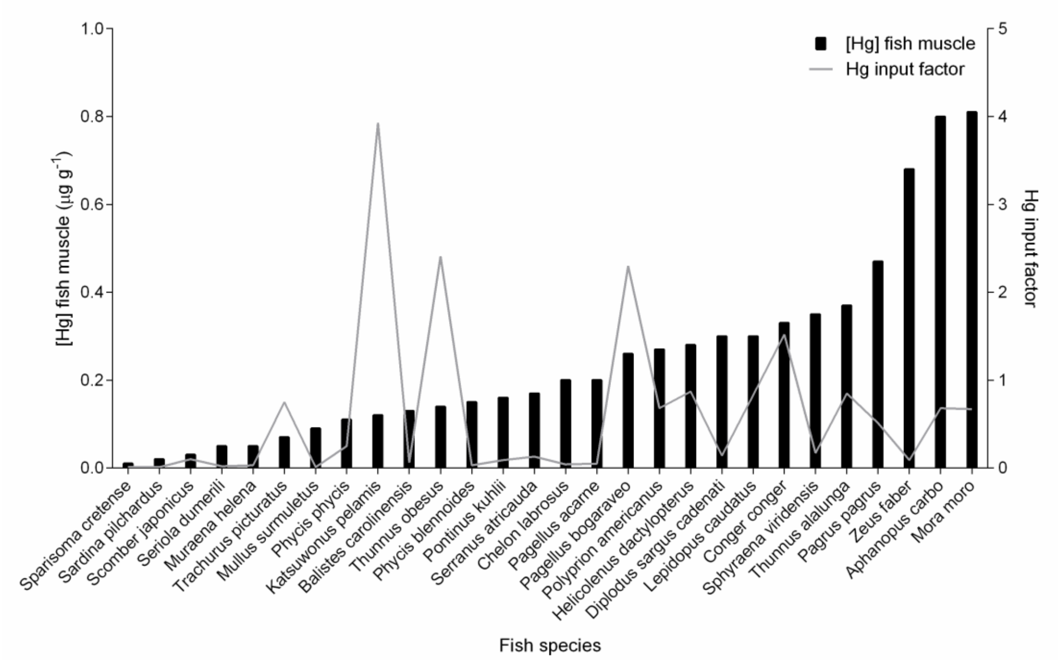


Fig. 7.5 Estimated Hg input factor for each fish species and comparison with the Hg concentration

Hg THQ values (Table 7.3) ranged between 0.02 and 0.13 for USEPA RfD and 0.001 and 0.007 for JECFA RfD. These THQ values were much lower than one for all fish species regarding both RfD's, indicating that the consumers are unlikely to develop health problems due to consumption of these fish species.

Table 7.3 Estimated target hazard quotient (THQ) for each fish species for both RfD's (USEPA RfD and JECFA RfD)

Scientific name	Mercury level (µg g^{-1})	Fish landing (kg year^{-1})	Fish available for consumption per capita (g day^{-1})	THQ	
				USEPA	JECFA
Aphanopus carbo	0.8	88021	0.98	0.1303	0.0072
Balistes carolinensis	0.13	50341	0.56	0.0121	0.0007
Chelon labrosus	0.2	21839	0.24	0.0081	0.0004
Conger conger	0.33	474060	5.26	0.2895	0.0161
Diplodus sargus cadenati	0.3	46533	0.52	0.0258	0.0014
Helicolenus	0.28	320020	3.55	0.1658	0.0092

dactylopterus					
Katsuwonus pelamis	0.12	3367006	37.38	0.7476	0.0415
Lepidopus caudatus	0.3	285083	3.17	0.1583	0.0088
Mora moro	0.81	84685	0.94	0.1269	0.0071
Mullus surmuletus	0.09	15919	0.18	0.0027	0.0001
Muraena helena	0.05	53369	0.59	0.0049	0.0003
Pagellus acarne	0.2	25832	0.29	0.0096	0.0005
Pagellus bogaraveo	0.26	908611	10.09	0.4371	0.0243
Pagrus pagrus	0.47	111761	1.24	0.0972	0.0054
Phycis blennoides	0.15	18724	0.21	0.0052	0.0003
Phycis phycis	0.11	237268	2.63	0.0483	0.0027
Polyprion americanus	0.27	257825	2.86	0.1288	0.0072
Pontinus kuhlii	0.16	58948	0.65	0.0175	0.0010
Sardina pilchardus	0.02	59713	0.66	0.0022	0.0001
Scomber japonicus	0.03	354450	3.94	0.0197	0.0011
Seriola dumerili	0.05	40357	0.45	0.0037	0.0002
Serranus atricauda	0.17	76213	0.85	0.0240	0.0013
Sparisoma cretense	0.01	154196	1.71	0.0029	0.0002
Sphyraena viridensis	0.35	50565	0.56	0.0327	0.0018
Thunnus alalunga	0.37	237402	2.64	0.1625	0.0090
Thunnus obesus	0.14	1774389	19.70	0.4597	0.0255
Trachurus picturatus	0.07	1101999	12.23	0.1427	0.0079
Zeus faber	0.68	13878	0.15	0.0175	0.0010

4. Conclusion

The evaluation of the contribution of commercial fish species to human Hg exposure (mostly urged in high fish consumption area) requires the review of published literature, and complementary quantifications to fulfil the gaps. Additional data on fish captures and landings, plus fish consumptions habits, including knowing the most representative species are also essential.

Encompassing all data will provide the base tools to assess the potential risk to human health in an area with higher rates of fish consumption per capita such as near the MAR.

The present study demonstrates that only two of the 28 analysed fish species exceeded the permissible limits for fish consumption. Despite the low Hg levels detected in fish, every year the population of this area is exposed to about 1.8 Kg of Hg (7.47mg of Hg per capita) via fish consumption. Carnivores fish species exhibited higher concentration of Hg than omnivores fish species suggesting trends of biomagnification. Furthermore, demersal species generally presented higher Hg concentration than pelagic ones. Fish species containing the highest Hg concentration in their tissues are not those that contribute the most to higher levels of human exposure. Despite having increased Hg levels, these species are discharged in smaller quantities explaining a lower contribution to human Hg exposure. Thus, fish landing seems to have a greater role in the human exposure than the Hg concentration found in the fish species.

Despite being included in an area near the MAR, where seismic–volcanic phenomena are common, and which is responsible for natural inputs of Hg to the aquatic environment, the THQ was < 1 for all fish species caught in the AzEEZ. This means that the level of exposure is lower than the reference dose, indicating that a daily exposure to fish at this level is not likely to cause any negative health effects during a lifetime in a human population.

This kind of study have some limitations; however, they can provide useful information for future health risk assessments.

Conflict of interest

The authors declare that they have no conflict of interests (financial or non-financial).

Acknowledgements

Thanks are due for the financial support to CESAM (UIDB/50017/2020+UIDP/50017/2020), to FCT/MEC through national funds. The authors also thank FCT (Fundação para a Ciência e Tecnologia) for the PhD grant of Vieira, H. C. (PD/BD/127808/2016).

References

Afonso, C., Lourenço, H.M., Dias, A., Nunes, M.L., Castro, M., (2007). Contaminant metals in black scabbard fish (*Aphanopus carbo*) caught off Madeira and the Azores. Food Chemistry 101(1), 120-125.

Afonso, C., Lourenço, H.M., Pereira, C., Martins, M.F., Carvalho, M.L., Castro, M., Nunes, M.L., (2008). Total and organic mercury, selenium and α-tocopherol in some deep-water fish species. Journal of the Science of Food and Agriculture 88(14), 2543-2550.

Almada, F., Abecasis, D., Villegas-Ríos, D., Henriques, S., Pais, M.P., Batista, M., Costa, B.H.e., Martins, J., Tojeira, I., Rodrigues, N.V., Araújo, R., Souto, M., Alonso, H., Falcón, J.M., Henriques, F., Catry, P., Cabral, H., Biscoito, M., Almada, V.C., (2015). Ichthyofauna of the Selvagens Islands. Do small coastal areas show high species richness in the northeastern Atlantic? Marine Biology Research 11(1), 49-61.

Andersen, J.L., Depledge, M.H., (1997). A survey of total mercury and methylmercury in edible fish and invertebrates from Azorean waters. Marine Environmental Research 44(3), 331-350.

Azevedo, L.S., Pestana, I.A., da Costa Nery, A.F., Bastos, W.R., Souza, C.M.M., (2019). Variation in Hg accumulation between demersal and pelagic fish from Puruzinho Lake, Brazilian Amazon. Ecotoxicology.

Baeyens, W., Leermakers, M., Papina, T., Saprykin, A., Brion, N., Noyen, J., De Gieter, M., Elskens, M., Goeyens, L., (2003). Bioconcentration and Biomagnification of Mercury and Methylmercury in North Sea and Scheldt Estuary Fish. Archives of Environmental Contamination and Toxicology 45(4), 498-508.

Barone, G., Storelli, A., Garofalo, R., Busco, V.P., Quaglia, N.C., Centrone, G., Storelli, M.M., (2015). Assessment of mercury and cadmium via seafood consumption in Italy: estimated dietary intake (EWI) and target hazard quotient (THQ). Food Additives & Contaminants: Part A 32(8), 1277-1286.

Bastos, W.R., Dórea, J.G., Bernardi, J.V.E., Lauthartte, L.C., Mussy, M.H., Lacerda, L.D., Malm, O., (2015). Mercury in fish of the Madeira river (temporal and spatial assessment), Brazilian Amazon. Environmental Research 140, 191-197.

Bortey-Sam, N., Nakayama, S.M.M., Ikenaka, Y., Akoto, O., Baidoo, E., Yohannes, Y.B., Mizukawa, H., Ishizuka, M., (2015). Human health risks from metals and metalloid via consumption of food animals near gold mines in Tarkwa, Ghana: Estimation of the daily intakes and target hazard quotients (THQs). Ecotoxicology and Environmental Safety 111, 160-167.

Castro, P., Huber, M., (2008). *Marine Biology: Seventh Edition*. McGraw-Hill Higher Education.

Ceccatto, A.P.S., Testoni, M.C., Ignácio, A.R.A., Santos-Filho, M., Malm, O., Díez, S., (2015). Mercury distribution in organs of fish species and the associated risk in traditional subsistence villagers of the Pantanal wetland. Environ Geochem Health, 1-10.

Chan, H., Scheuhammer, A., Ferran, A., Loupelle, C., Holloway, J., Weech, S., (2003). Impacts of mercury on freshwater fish-eating wildlife and humans. Human and Ecological Risk Assessment 9(4), 867-883.

Chien, L.-C., Hung, T.-C., Choang, K.-Y., Yeh, C.-Y., Meng, P.-J., Shieh, M.-J., Han, B.-C., (2002). Daily intake of TBT, Cu, Zn, Cd and As for fishermen in Taiwan. Science of The Total Environment 285(1–3), 177-185.

Choy, C.A., Popp, B.N., Kaneko, J.J., Drazen, J.C., (2009). The influence of depth on mercury levels in pelagic fishes and their prey. Proceedings of the National Academy of Sciences 106(33), 13865-13869.

Costa, B.G.B., Lacerda, L.D., (2014). Mercury (Hg) in fish consumed by the local population of the Jaguaribe River lower basin, Northeast Brazil. Environmental Science and Pollution Research 21(23), 13335-13341.

Costa, V.L.H., Figueiredo, I., Carvalho, L., Lopes, H., Farias, I., Pires, L., Afonso, C., Vieira, A.R., Nunes, M.L., Gordo, L.S., (2009). Mercury, cadmium and lead in black scabbardfish (*Aphanopus carbo* Lowe, 1839) from mainland Portugal and the Azores and Madeira archipelagos. Gordo, L.S. (Ed.) (2009). Stock structure and quality of black scabbardfish in the southern NE Atlantic. Scientia Marina (Barcelona) 73(Suppl. 2), 77-88.

Costley, C.T., Mossop, K.F., Dean, J.R., Garden, L.M., Marshall, J., Carroll, J., (2000). Determination of mercury in environmental and biological samples using pyrolysis atomic absorption spectrometry with gold amalgamation. Analytica Chimica Acta 405(1–2), 179-183.

Depledge, M.H., Weeks, J.M., Frias Martins, A., Tristao Da Cunha, R., Costa, A., (1992). The Azores Exploitation and pollution of the coastal ecosystem. Marine Pollution Bulletin 24(9), 433-435.

Di Lena, G., Casini, I., Caproni, R., Fusari, A., Orban, E., (2017). Total mercury levels in commercial fish species from Italian fishery and aquaculture. Food Additives & Contaminants: Part B 10(2), 118-127.

Di Lena, G., Casini, I., Caproni, R., Orban, E., (2018). Total mercury levels in crustacean species from Italian fishery. Food Additives & Contaminants: Part B 11(3), 175-182.

Domingo, J.L., Bocio, A., Falcó, G., Llobet, J.M., (2007). Benefits and risks of fish consumption: Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants. Toxicology 230(2–3), 219-226.

EFSA Scientific Committee, (2015). Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood, *EFSA Journal*, p. 3982.

Egeland, G.M., Middaugh, J.P., (1997). Balancing Fish Consumption Benefits with Mercury Exposure. Science 278(5345), 1904-1905.

EU, (2001). Regulation (EC) No 466/2001. JO L77, 16.03.01 (pp. 01-13).

EU, (2008). Commission Regulation (EC) No 629/2008. OJ L 173, 2 July 2008, (p. 6–9).

FAO, (2012). *The State of World Fisheries and Aquaculture 2012*. Food & Agriculture Organization.

Fitzgerald, W.F., Lamborg, C.H., Hammerschmidt, C.R., (2007). Marine Biogeochemical Cycling of Mercury. Chemical Reviews 107(2), 641-662.

Forsyth, D.S., Casey, V., Dabeka, R.W., McKenzie, A., (2004). Methylmercury levels in predatory fish species marketed in Canada. Food Additives & Contaminants 21(9), 849-856.

Groth III, E., (2010). Ranking the contributions of commercial fish and shellfish varieties to mercury exposure in the United States: Implications for risk communication. Environmental Research 110(3), 226-236.

Hall, B., Bodaly, R., Fudge, R., Rudd, J., Rosenberg, D., (1997). Food as the dominant pathway of methylmercury uptake by fish. Water, Air, and Soil Pollution 100(1-2), 13-24.

Hanna, D.E., Solomon, C.T., Poste, A.E., Buck, D.G., Chapman, L.J., (2015). A review of mercury concentrations in freshwater fishes of Africa: Patterns and predictors. Environmental Toxicology and Chemistry 34(2), 215-223.

Hightower, J.M., Moore, D., (2003). Mercury levels in high-end consumers of fish. Environmental health perspectives 111(4), 604-608.

Kasper, D., Palermo, E.F.A., Branco, C.W.C., Malm, O., (2012). Evidence of elevated mercury levels in carnivorous and omnivorous fishes downstream from an Amazon reservoir. Hydrobiologia 694(1), 87-98.

Koenig, S., Solé, M., Fernández-Gómez, C., Díez, S., (2013). New insights into mercury bioaccumulation in deep-sea organisms from the NW Mediterranean and their human health implications. Science of The Total Environment 442(0), 329-335.

Kris-Etherton, P.M., Harris, W.S., Appel, L.J., Committee, f.t.N., (2002). Fish Consumption, Fish Oil, Omega-3 Fatty Acids, and Cardiovascular Disease. Circulation 106(21), 2747-2757.

Li, P., Feng, X., Chan, H.-M., Zhang, X., Du, B., (2015). Human Body Burden and Dietary Methylmercury Intake: The Relationship in a Rice-Consuming Population. Environ Sci Technol 49(16), 9682-9689.

Li, S., Zhou, L., Wang, H., Liang, Y., Hu, J., Chang, J., (2009). Feeding habits and habitats preferences affecting mercury bioaccumulation in 37 subtropical fish species from Wujiang River, China. Ecotoxicology 18(2), 204-210.

Liang, P., Qin, Y.-Y., Zhang, C., Zhang, J., Cao, Y., Wu, S.-C., Wong, C.K.C., Wong, M.H., (2013). Plasma mercury levels in Hong Kong residents: In relation to fish consumption. Science of The Total Environment 463–464, 1225-1229.

Magalhães, M.C., Costa, V., Menezes, G.M., Pinho, M.R., Santos, R.S., Monteiro, L.R., (2007). Intra- and inter-specific variability in total and methylmercury bioaccumulation by eight marine fish species from the Azores. Marine Pollution Bulletin 54(10), 1654-1662.

Megapesca, (2007). Relatório Estatístico - Caracterização do consumo de Pescado nos Açores. Megapesca, Alfeizerão, Portugal.

Menezes, G.M., Sigler, M.F., Silva, H.M., Pinho, M.R., (2006). Structure and zonation of demersal fish assemblages off the Azores Archipelago (mid-Atlantic). Marine Ecology Progress Series 324, 241-260.

Monteiro, L., Isidro, E., Lopes, H., (1991). Mercury content in relation to sex, size, age and growth in two scorpionfish (*Helicolenus dactylopterus* and *Pontinus kuhlii*) from Azorean waters. Water, Air, & Soil Pollution 56(1), 359-367.

Monteiro, L.R., Costa, V., Furness, R.W., Santos, R.S., (1996). Mercury concentrations in prey fish indicate enhanced bioaccumulation in mesopelagic environments. Marine Ecology Progress Series 141, 21-25.

Mozaffarian, D., Lemaitre, R.N., Kuller, L.H., Burke, G.L., Tracy, R.P., Siscovick, D.S., (2003). Cardiac Benefits of Fish Consumption May Depend on the Type of Fish Meal Consumed. Circulation 107(10), 1372.

Pinho, M.R., Menezes, G., (2009). Demersal fishery off the Azores. Boletim do Núcleo Cultural da Horta, 18: 85-102.

Pirrone, N., Cinnirella, S., Feng, X., Finkelman, R., Friedli, H., Leaner, J., Mason, R., Mukherjee, A., Stracher, G., Streets, D., (2010). Global mercury emissions to the atmosphere from anthropogenic and natural sources. Atmospheric Chemistry and Physics 10(13), 5951-5964.

Rice, D.C., (2004). The US EPA reference dose for methylmercury: sources of uncertainty. Environmental Research 95(3), 406-413.

Ruelas-Inzunza, J., Meza-López, G., Páez-Osuna, F., (2008). Mercury in fish that are of dietary importance from the coasts of Sinaloa (SE Gulf of California). Journal of Food Composition and Analysis 21(3), 211-218.

Saei-Dehkordi, S.S., Fallah, A.A., Nematollahi, A., (2010). Arsenic and mercury in commercially valuable fish species from the Persian Gulf: Influence of season and habitat. Food and Chemical Toxicology 48(10), 2945-2950.

Southworth, G.R., Peterson, M.J., Ryon, M.G., (2000). Long-term increased bioaccumulation of mercury in largemouth bass follows reduction of waterborne selenium. Chemosphere 41(7), 1101-1105.

Steenhuisen, F., Wilson, S.J., (2015). Identifying and characterizing major emission point sources as a basis for geospatial distribution of mercury emissions inventories. Atmospheric Environment 112, 167-177.

Stergiou, K.I., Karpouzi, V.S., (2002). Feeding habits and trophic levels of Mediterranean fish. Reviews in Fish Biology and Fisheries 11(3), 217-254.

Storelli, M.M., Stuffler, R.G., Marcotrigiano, G.O., (2002). Total and methylmercury residues in tuna-fish from the Mediterranean sea. Food Additives & Contaminants 19(8), 715-720.

Torres, P., Rodrigues, A., Soares, L., Garcia, P., (2015). Metal Concentrations in Two Commercial Tuna Species from an Active Volcanic Region in the Mid-Atlantic Ocean. Archives of Environmental Contamination and Toxicology 70(2), 341-347.

U.S. Food and Drug Administration, (2009). Draft Report of Quantitative Risk and Benefit Assessment of Consumption of Commercial Fish, Focusing on Fetal Neurodevelopmental Effects (Measured by Verbal Development in Children) and on Coronary Heart Disease and Stroke in the General Population: Appendix A, Technical Description of the Risk and Benefit Assessment Methodology

USEPA, (1997). Mercury Study Report to Congress Volume IV. An Assessment of Exposure to Mercury in the United States, *EPA-452/R-97-006*. Office of Air Quality Planning and Standards and Office of Research and Development, Washington, DC.

Vieira, H.C., Bordalo, M.D., Morgado, F., Soares, A.M.V.M., Abreu, S.N., (2017). Mercury content in the white and dark muscle of Skipjack tuna (Katsuwonus pelamis) along the canning process: Implications to the consumers. Journal of Food Composition and Analysis 56, 67-72.

Vieira, H.C., Morgado, F., Soares, A.M.V.M., Abreu, S.N., (2013). Mercury in Scalp Hair Near the Mid-Atlantic Ridge (MAR) in Relation to High Fish Consumption. Biological Trace Element Research 156(1-3), 29-35.

WHO, (2003). Diet, nutrition and the prevention of chronic diseases: Report of a joint WHO/FAO expert consultation, 28 January–1 February 2002. Geneva: World Heath Organization/Food and Agricultural Organization.

WHO, (2008). Guidance for identifying populations at risk from mercury exposure, Geneva, Switzerland.

Chapter VIII:

Mercury bioaccumulation in the long-fin squid *Loligo forbesi* near the Mid-Atlantic Ridge: implications to human exposure

Mercury bioaccumulation in the long-fin squid *Loligo forbesi* near the Mid-Atlantic Ridge: implications to human exposure

Vieira, H.C.[1], Rendón-von Osten, J.[2], Soares, A.M.V.M.[1], Morgado, F.[1], Abreu, S. N.[1]

[1] CESAM - Centre for Environmental and Marine Studies, Department of Biology,
University of Aveiro, 3810-193 Aveiro, Portugal
[2] EPOMEX Institute, Autonomous University of Campeche, Campeche, Mexico

Abstract

Due to growing commercial interest as a fishing resource and its intermediate position in the marine trophic chains as both prey and predator, cephalopods can play an important role in mercury (Hg) transfer along the marine food webs, since they can bioaccumulate this metal in its tissues.

Our study aims to analyze Hg accumulation in *Loligo forbesi* caught in the Azores Archipelago (Portugal) quantifying Hg in different tissues (mantle and stomach), as well in the squid stomach content, in order to evaluate the efficiency of Hg transfer from prey to predator. Hg data from the tissues was used to estimate the weekly tolerable Hg intake due to squid consumption.

Overall data indicate that Hg measured in the stomach tissue (0.1±0.01 $\mu g\ g^{-1}$) was significantly higher than Hg levels found in the mantle (0.04±0.001 $\mu g\ g^{-1}$) and stomach contents (0.01±0.001 $\mu g\ g^{-1}$). BMF (bioaccumulation factor) was > 1 for all the samples, indicating a biomagnification process from prey to predator. Hg concentration in the mantle tissue was correlated with mantle size; although females present higher Hg levels than males, the difference was found to be not related to gender but rather to the fact that females had larger bodies.

Finally, considering the Hg concentration found in the mantle and the permitted Hg levels, it is advisable to consume up to 1050-1890g of squid per week, according to the regulatory agencies. Thus, our results indicate that, since these doses are respected, consumption of squids from the Azorean waters do not pose a risk to humans.

Keywords: Mercury; Azores archipelago; cephalopods; bioaccumulation factor; food safety

1. Introduction

Cephalopods play a determinant role in marine ecosystems (Hastie et al. 2016, Xavier et al. 2016), as consumers of crustaceans, small fish and other cephalopods (Xavier et al. 2018), but also as prey for several marine mammals, large predatory fish and sea birds (de la Chesnais et al. 2019, Judkins and Vecchione 2020).

Furthermore, cephalopods are becoming an increasingly important fisheries resource in the north-eastern Atlantic (Hastie et al. 2016). Two species of the genus Loligo (Cephalopoda: Loliginidae) occur in the northeast Atlantic, *Loligo forbesi* and *Loligo vulgaris* (Pierce et al. 1994). However, *L. forbesi* is the only species of loliginid squid living in the Azores waters (Porteiro and Martins 1994).

Due to its position in the marine food webs, cephalopods may be determinant actors in the transfer of pollutants (Bustamante et al. 1998), having the ability to rapidly bioaccumulate and concentrate contaminants in the tissues as a result of a strict carnivorous regime. They present high prey consumption rates and efficient assimilation of nutrients (Penicaud et al. 2017).

Mercury (Hg) is one of the contaminants already reported in the tissues of different cephalopod species captured around the world (Pierce et al. 2008, Ahmad et al. 2015, Seco et al. 2020). Released to the environment through natural and anthropogenic sources (Moreno-Brush et al. 2019), Hg is a global pollutant (Andreoli and Sprovieri 2017) that occupies the third position in the priority list of hazardous substances according to the US Agency for toxic substances and disease registry (ATSDR 2017). Hg occurs in the environment in several chemical forms, including inorganic forms [elemental form (Hg^0)] and organic forms (e.g. methylmercury – MeHg – Pereira et al. 2019]. Depending on the physical, chemical and biological conditions of the environment, inorganic Hg forms may be transformed to methylated species that are much more toxic to the organisms (Ullrich et al. 2001, Pereira et al. 2019). Furthermore, its high affinity to proteins (Bloom 1992) may result in Hg biomagnification along aquatic food chains, often reaching hazardous concentrations in fish-eating wildlife and human populations (Lavoie et al. 2018). In order to prevent possible risks and adverse health effects of Hg associated to seafood consumption, the European Union has determined limits for fish and fish products consumption, concerning Hg exposure. The European Commission Regulation (EC) No 466/2001 of 8th March 2001 established maximum levels of 0.5 $\mu g\ g^{-1}$ ww Hg permitted for human consumption in foodstuffs, excluding an "exception list" where the accepted tolerance level raises to 1 $\mu g\ g^{-1}$ ww, comprising some species with high trophic level (EU 2005). At the same time, the agencies

Food and Agriculture Organization (FAO)/ World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) and US Environmental Protection Agency (USEPA) have determined reference doses (RfD) regarding the Hg intake (Vieira et al. 2015). JECFA established their Reference dose RfD for MeHg of 1.3 μg kg body weight^{-1} week^{-1} (EFSA Scientific Committee 2015) and USEPA set a RfD of 0.7 μg MeHg kg body weight^{-1} week^{-1} (USEPA 1997a).

This work aims: i) to evaluate Hg accumulation in different tissues of the squid *Loligo forbesi*, ii) to evaluate the efficiency of Hg transfer from prey to predators and iii) to estimate the safe amount of squid that can be ingested per week based on the recommendations for Hg intake from the international agencies JECFA and USEPA.

2. Materials and Methods

2.1. Sampling area & sampling preparation

The sampling area is located near the MAR (Mid-Atlantic Ridge), North Atlantic Ocean, where an isolated group of nine volcanic islands, the Azores archipelago, extends along the southeast – northwest strip near the triple junction of Eurasian, African, and North American plates. As result, the archipelago has a complex tectonic setting, where seismic–volcanic phenomena are common, being responsible for natural inputs of Hg to the aquatic environment (Vieira et al. 2013).

Dorsal mantle length (cm) and wet weight (g) were recorded from 113 squids of the species *Loligo forbesi* collected in the Terceira Island (Azores) using artisanal jigging fishery. Each specimen was dissected, the stomach was extracted and the sex was recorded. After that, the stomach was dissected to remove the stomach contents and a portion of the clean stomach (without traces of stomach contents) and mantle tissue was taken. All collected samples (mantle, stomach, and stomach contents) were stored in individual plastic bags properly identified and stored at -20 ºC for later Hg quantification. From the 113 dissected squids it was only possible to register the sex of 43 specimens (Table 8.1).

Tabela 8.1 Number of female and male squid with corresponding mean mantle length and mean total body weight, standard error (SE) and range.

	n	Mantle Length$_{(cm)}$			Weight$_{(g)}$		
		Average	SE	Min – Max	Average	SE	Min – Max
Female	23	31.5	1.7	17 – 44.2	672	79.3	146 – 1400
Male	20	24.2	1.6	14.5 – 46.5	380.3	65	100 – 1430

2.2. Hg quantification

Hg quantification was performed using the Advanced Mercury Analyzer (AMA-254, made by ALTEC and distributed by LECO). Hg quantification does not requires a previous digestion of the sample (Costley et al. 2000); the procedure is based in a pyrolysis process of the sample using a combustion tube heated at 750 ºC under an oxygen atmosphere and the Hg released is trapped in a gold amalgamator and subsequently detected and quantified by atomic absorption spectrometry. The AMA–LECO 254 analyzer quantifies the amount of Hg present in the sample with a detection limit of is 0.01 ng Hg.

Analytical quality of the procedure was validated using certified reference materials (CRM), namely TORT-3 (Lobster Hepatopancreas Reference Material for Trace Metals, National Research Council of Canada). Obtained data (0.28±0.02 µg g^{-1} of Hg for TORT-3) and reference values of Hg (0.29±0.02 µg g^{-1} of Hg) were not statistically different ($p>0.05$).

2.3. Biomagnification factors (BMF)

The biomagnification factor (BMF) provides information about the Hg biomagnification process from prey to predators (Liu et al. 2019a). The BMF was calculated according to Moreno-Sierra et al. (2016) using the equation:

$$\mathrm{BMF} = \frac{[\mathrm{Hg}]_{\mathrm{predator}}}{[\mathrm{Hg}]_{\mathrm{prey}}}$$

A BMF value >1 indicates that the Hg is biomagnifying, whereas a BMF <1 implies that a Hg is not accumulated by the organism being metabolized and eliminated. (Liu et al. 2019b).

2.4. Hg exposure and consumption per week

According to Vieira et al. (2015), the maximum values of fish meals per week without exceeding the established RfD by international agencies can be calculated using the following formula:

$$\text{meals per week } = \frac{(\mathrm{RfD} \times \mathrm{Bw}) \times 7\mathrm{days}}{(\text{Squid meal size (g)} \times [\mathrm{Hg}] \text{ mantle}) \div 1000}$$

In both calculations, a body weight (Bw) of 60 kg based on USEPA guidelines was used (USEPA 1997b) was used.

2.5. Statistical analysis

After data was checked for normality (Shapiro-Wilk test), parametric statistical tests were used. Linear regressions analyses were used to examine the association between mantle length and Bw and Hg concentration ([Hg]) and mantle length. One-way ANOVA was used to compare [Hg] of the different tissues and the comparison of [Hg] between males and females were tested by t-test. In addition, a covariance analysis was used, to analyze differences in Hg content between males and females, considering sex as a factor and the mantle length of each animal as a covariable. The analysis allowed to control for size.

Statistical analyses were performed using GraphPad Prism (version 6.01). Statistical significance was defined as $p<0.05$. [Hg] data are presented as mean value ± standard error value (mean ± SE).

3. Results and Discussion

3.1. Hg concentrations in the long-fin squid

Considering all squid specimens that composed the present study, the mantle length ranged from 12.1 to 46.5cm and the weight ranged from 63 to 1430g. As for the length-weight ratio, linear increase to the mantle size of the squids was observed with a significant and positive correlation (R2=0.95, $p<0.05$ – Fig. 8.1). Similarly, a significant correlation with a high coefficient of determination between weight and length for specimens of the Loliginidae family (*Loligo forbesi* and *Loligo vulgaris*) in different areas of the Portuguese coastal zone was also described by Moreno et al. (1994).

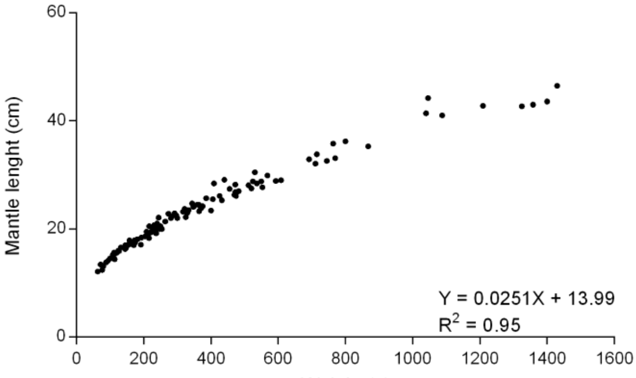


Fig. 8.1 Relationships between mantle length and weight of 113 specimens from the squid species *L. forbesi* caught in the Azores archipelago.

Regarding the mantle tissue, the present work indicates low [Hg] in the specimens of *L. forbesi* [ranging between 0.01 μg g^{-1} and 0.07 μg g^{-1} (mean of 0.04±0.001 μg g^{-1})] in accordance with previous studies. For instance, Pierce et al. (2008) reported an average [Hg] of 0.035±0.019 µg g^{-1} in the mantle tissue of *L. forbesi* fished in UK waters, while Bustamante et al. (2006) presented similar values for *L. forbesi* specimens captured in the Bay of Biscay and Celtic Sea (0.04±0.01 µg g^{-1}, and 0.06±0.02 µg g^{-1}, respectively). Nonetheless, the same study also reported lower values in specimens from the Faroe Islands (0.01±0.002 µg g^{-1}). On the contrary, Bonsignore et al. (2013) found higher levels of Hg (0.147±0.024 µg g^{-1}) in the muscle of *L. forbesi* specimens caught in Augusta Bay, Italy. Higher [Hg] were also reported by Monteiro et al. (1992) with 0.11±0.01 µg g^{-1} in mantle tissue from *L. forbesi* specimens captured in several islands of the Azorean archipelago. Nevertheless, these concentrations are still considered low when compared to the maximum levels of Hg permitted for human consumption in foodstuffs (0.5 µg g^{-1}) established by European Commission Regulation (EC – EU 2005).

The Hg accumulation in the squids' tissues is expected to be influenced by several biotic and abiotic factors, including size and sex. As expected, a positive and significant correlation ($R^2 = 0.63$, $p < 0.05$) was observed between the [Hg] quantified in the mantle tissue and the mantle length, meaning that the contamination level increases with the size (mantle length) of the squids (Fig. 8.2). These findings are in line with previous studies from Monteiro et al. (1992), Bustamante et al. (2006), Pierce et al. (2008), and support the theory that Hg is very efficiently bioaccumulated and retained in squid's mantle over their lifetime (Pierce et al. 2008, Penicaud et al. 2017).

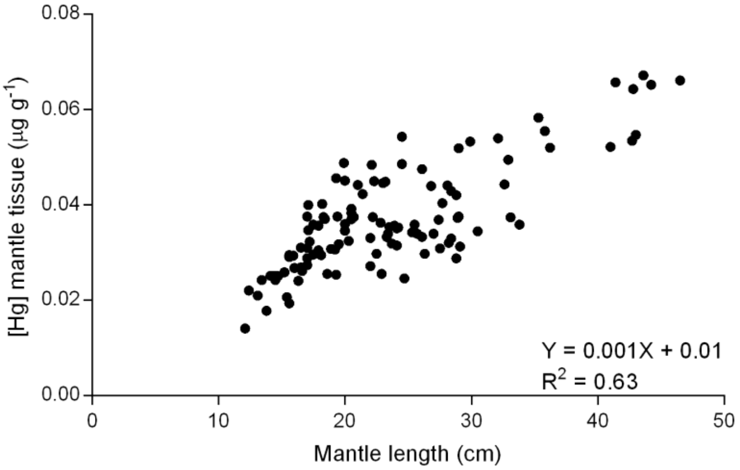


Fig. 8.2 Relationships between [Hg] quantified in the mantle tissue and size (mantle length) in squid specimens from the species *L. forbesi* collected in the Azores archipelago.

[Hg] was also found to increase with mantle size in other squid species, such as *Alluroteuthis antarcticus* and *Psychroteuthis glacialis* collected from the Scotia Sea (Seco et al. 2020), and also in *Gonatus fabricii* caught in Disko Island, Greenland (Lischka et al. 2020) and *Sthenoteuthis pteropus* collected around the Republic of Cape Verde in the eastern tropical Atlantic (Lischka et al. 2018)

Bustamante et al. (2006) and Monteiro et al. (1992) observed higher bioaccumulation rates of Hg in *L. forbesi* females than males. In the present study, females were also found to accumulate more Hg than males (0.04±0.002 and 0.03±0.001 μg g^{-1}, respectively). Nevertheless, according to the analysis of covariance, sex (when controlled for the mantle length) did not influence the observed difference in [Hg] between males and females ($p>0.05$), being [Hg] explained by the mantle length ($p<0.05$). The differences in the [Hg] between males and females may be related to the existence of physiological and/or ecological differences between sexes (Monteiro et al. 1992). However, the lack of data on the influence of such intra-specific factors on Hg bioaccumulation, it is generally admitted that Hg concentrations in cephalopod tissues are positively correlated with size (Bustamante et al. 2006). Although males of the species may be larger and grow faster than females (Rosa et al. 2013), in this study females are larger than males (Table 8.1), which reinforces the idea that the [Hg] in this species increases with size.

When looking into the different tissues, [Hg] increased according to the following order: stomach tissue (0.1±0.01 μg g^{-1}) > mantle (0.04±0.002 μg g^{-1}) > stomach contents (0.01±0.001 μg g^{-1} – Fig. 8.3), all being statistically different ($p<0.05$), and suggesting a differential distribution of Hg among tissues.

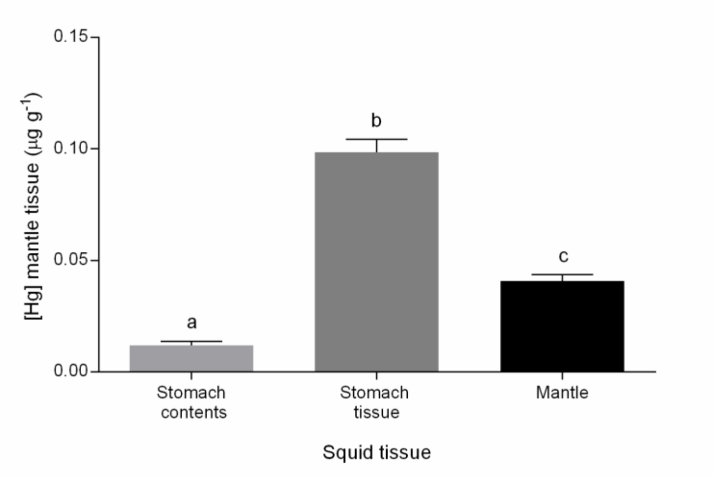


Fig. 8.3 Hg concentration (μg g^{-1}, ww) present in three different tissues of *L. forbesi* squid. Different lower-case letters indicate significant differences between tissues.

However, such pattern was not observed by Seco et al. (2020) that did not find any differences between different tissues in four of the five studied species (*Alluroteuthis antarcticus*, *Gonatus antarcticus*, *Kondakovia longimana* and *Psychroteuthis glacialis*). This indicates some degree of equilibrium between contaminant accumulation and excretion rates, as well as an efficient Hg redistribution of Hg between tissues in these species.

Most studies available in the literature focus on the Hg accumulation in the digestive gland and mantle, being the digestive gland the organ with the higher concentrations [e.g. Bustamante et al. (2006), Pierce et al. (2008), Bonsignore et al. (2013), Ahmad et al. (2015), Lischka et al. (2018)]. The digestive gland of cephalopods has been identified as the main metal accumulation organ being particularly efficient in the retention of these elements, likely as a function of chelating agents, especially proteins (Rodrigo and Costa 2017). Furthermore, the digestive gland of cephalopods is a multitasking organ, having not only a role in the digestion and in the storage and detoxification of xenobiotics (Bustamante et al. 2006, Penicaud et al. 2017).

To our knowledge, this is the first study that focus on the potential of Hg accumulation in the cephalopod's stomach epithelium. The high [Hg] observed in this tissue may be explained by the stomach lining of *Loligo sp.* to be composed by a chitin protein complex (Bidder 1950). Chitin is recognized as an excellent metal-ligand, forming stable complexes with many metal ions (Chui et al. 1996, Camci-Unal and Pohl 2010). This way, the presence of chitin in the stomach tissue can promote the existence of preferential Hg-binding sites, leading to a more significant [Hg] in the stomach tissue and reducing Hg remobilization to other tissues (e.g. mantle). This difference in [Hg] between the stomach and the muscle suggests that the stomach (such as the digestive gland) plays an important role in the redistribution of Hg.

The BMF was calculated based on the [Hg] in the stomach contents (prey) and the [Hg] present in the mantle (predator) of 16% of the total number of squids (only 18 individuals presented a full stomach). Since [Hg] had a mean of 0.012 µg g^{-1} (ranged from 0.001 to 0.027 µg g^{-1}) in the stomach contents and an average of 0.048 µg g^{-1} (ranged between 0.026 and 0.067 µg g^{-1}) in the mantle, all specimens showed BMF > 1 (ranging from 1.1 to 44.2), suggesting that Hg biomagnification occurs from prey to predator. The high variation of the BMF's found in this study is related to the variability of [Hg] found in the prey (stomach contents), which ranged between 0.001 and 0.024 µg g^{-1}, as well as in the mantle tissue that presented a variation between 0.026 and 0.067 µg g^{-1}. Since the uptake of Hg can be influenced by their prey preferences (Chouvelon et al. 2011), it would be interesting to discriminate the preyed species within the gut contents. Unfortunately, due to the squid's feeding mechanism, in which larger prey are cut into chunks by the beak before ingestion, coupled with rapid digestion, the visual identification and quantification of prey species from gut contents is particularly difficult (Pierce et al. 1994) without using cost expensive techniques for example based on eDNA. Notwithstanding, some studies were able to identify scad (probably *Trachurus picturatus*), board fish (*Capros aper*), scabbard fish (*Aphanopsus carbo* or *Lepidopus caudatus*) and mackerel (probably *Scomber japonicus*) as the main species in the diet of *L. forbesi* in the Azores (Boyle and Pierce 1994, Pierce et al. 1994). All of these species identified as the main prey of *L. forbesi*, are species with low [Hg] (Monteiro et al. 1996, Andersen and Depledge 1997), which may explain the low levels of Hg observed in the squid specimens of this study.

3.2. Human health perspective

The consumption of seafood is recommended due to the high nutritional value (protein and unsaturated fatty acids – Vieira et al 2017). However, there are possible risks and adverse effects associated with seafood consumption, for example the ingestion of Hg. Bustamante et al. 2006 showed that about 80% of the Hg present in edible tissues of *L. forbesi* caught in the North Eastern Atlantic (Faroe Islands, Celtic Sea and Bay of Biscay) corresponds to MeHg. However, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain uses the worst case scenario hypothesis to calculate the MeHg dietary exposure; therefore they assume that 100% of Hg present in fish is in its methylated form (Ceccatto et al. 2015, Di Lena et al. 2018).

Considering the [Hg] found in the mantle (0.04±0.001 µg g^{-1}), which is generally the edible part of the squid, and the RfD established by international agencies, our analyses advise a consumption up to 1890g of squid per week (RfD established by JECFA). Since the RfD established by USEPA is more conservative than JECFA, the consumption limit decrease to 1050g per week (Fig. 8.4).

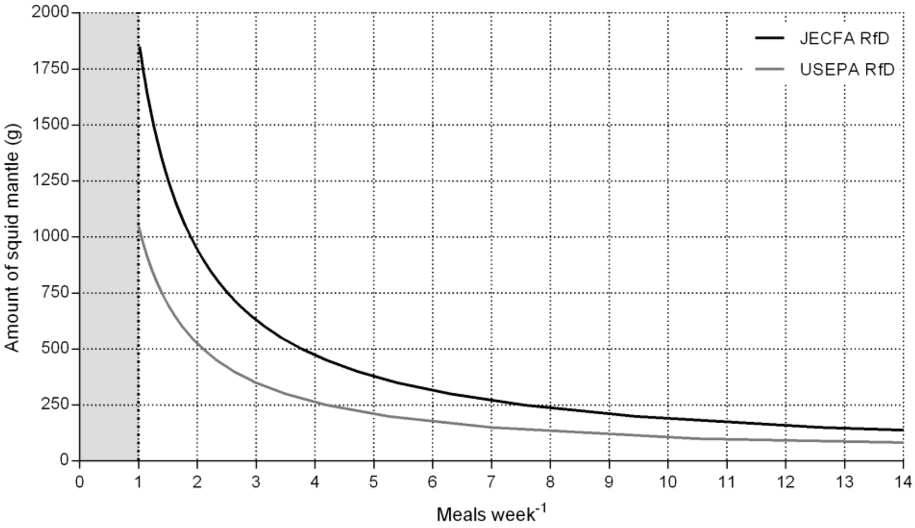


Fig. 8.4 Number of squid meals per week and amount of mantle to be consumed without exceeding the MeHg RfD established by JECFA and USEPA.

According to the fish landing reports (1994-2018) of Azores Fisheries Statistics (SREA – https://tinyurl.com/ycq8j8g7), on average 325 tons of squids were discharged in the Azorean ports in the last 25 years (Fig. 5) and the 2018 report from the Azores Regional Statistics Service (SREA), the population in the Azores Archipelago ascends to 243356 persons.

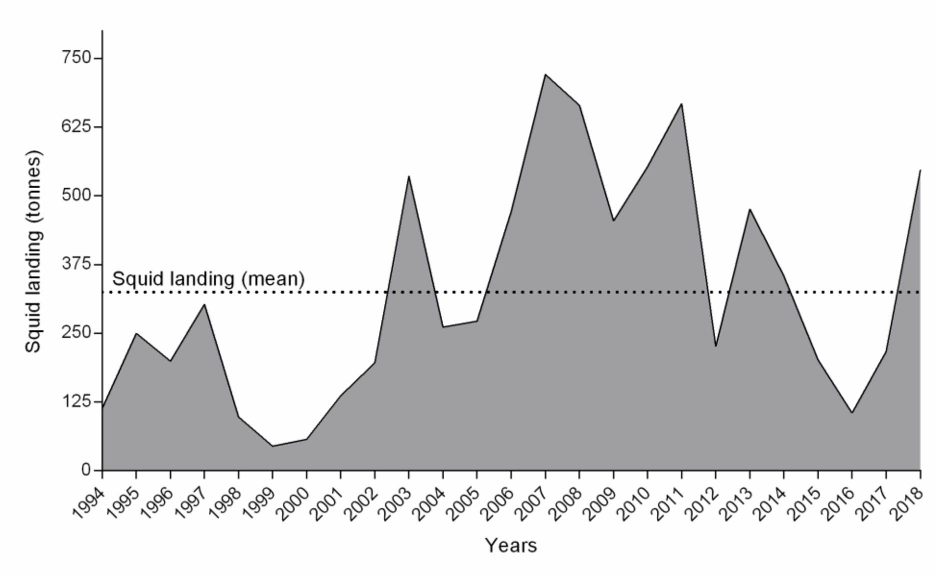


Fig. 8.5 Squid landing in Azorean ports in the last 25 years according to Azores Fisheries Statistics (SREA – https://tinyurl.com/ycq8j8g7)

Thus, considering the population and the quantity of squid discharged in the ports of the Azores, the squid consumption per capita is of 27.75g per week. These numbers indicate that squid consumption is 68 times lower than the JECFA and 38 times lower than the USEPA RfD values.

4. Conclusion

Hg concentration found in *L. forbesi* caught in the Azorean coast was found to be relatively low when compared to the maximum Hg amount permitted in cephalopods for human consumption (0.5 µg g^{-1}). The difference between [Hg] of stomach tissue and mantle tissue suggests a self-protection response, minimizing Hg assimilation probably as a result of detoxification mechanisms. The BMF between the stomach contents and mantle tissue reinforces the theory that Hg can biomagnify along the trophic chain. Intra-specific differences in the [Hg] were found between males and females; however, this difference is related to the fact that sampled females were larger than males. Finally, with regard to public health, a risk alert for exposure to Hg should not be considered if the consumer ingests the recommended amount of squid per week.

Conflict of interest

The authors declare that they have no conflict of interests (financial or non-financial).

Acknowledgements

Thanks are due for the financial support to CESAM (UIDB/50017/2020+UIDP/50017/2020), to FCT/MEC through national funds. The authors also thank FCT (Fundação para a Ciência e Tecnologia) for the PhD grant of Vieira, H. C. (PD/BD/127808/2016).

References

Ahmad, N. I., M. F. M. Noh, W. R. W. Mahiyuddin, H. Jaafar, I. Ishak, W. N. F. W. Azmi, Y. Veloo, and F. A. Mokhtar. 2015. The mercury levels in crustaceans and cephalopods from Peninsular Malaysia. Environmental Science and Pollution Research 22:12960-12974.

Andersen, J. L., and M. H. Depledge. 1997. A survey of total mercury and methylmercury in edible fish and invertebrates from Azorean waters. Marine Environmental Research 44:331-350.

Andreoli, V., and F. Sprovieri. 2017. Genetic aspects of susceptibility to mercury toxicity: an overview. International journal of environmental research and public health 14:93.

ATSDR. 2017. ATSDR's Substance Priority List. Agency for Toxic Substances and Disease Registry, Atlanta, GA. Available at https://www.atsdr.cdc.gov/SPL/# [accessed on December 2019].

Bidder, A. M. 1950. The digestive mechanism of the European squids *Loligo vulgaris*, *Loligo forbesii*, *Alloteuthis media* and *Alloteutihis subulata*. Journal of Cell Science 3:1-43.

Bloom, N. S. 1992. On the Chemical Form of Mercury in Edible Fish and Marine Invertebrate Tissue. Canadian Journal of Fisheries and Aquatic Sciences 49:1010-1017.

Bonsignore, M., D. Salvagio Manta, E. Oliveri, M. Sprovieri, G. Basilone, A. Bonanno, F. Falco, A. Traina, and S. Mazzola. 2013. Mercury in fishes from Augusta Bay (southern Italy): Risk assessment and health implication. Food and Chemical Toxicology 56:184-194.

Boyle, P. R., and G. J. Pierce. 1994. Fishery biology of northeast Atlantic squid: an overview. Fisheries Research 21:1-15.

Bustamante, P., F. Caurant, S. Fowler, and P. Miramand. 1998. Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. Science of The Total Environment 220:71-80.

Bustamante, P., V. Lahaye, C. Durnez, C. Churlaud, and F. Caurant. 2006. Total and organic Hg concentrations in cephalopods from the North Eastern Atlantic waters: Influence of geographical origin and feeding ecology. Science of The Total Environment 368:585-596.

Camci-Unal, G., and N. L. B. Pohl. 2010. Quantitative Determination of Heavy Metal Contaminant Complexation by the Carbohydrate Polymer Chitin. Journal of Chemical & Engineering Data 55:1117-1121.

Ceccatto, A. P. S., M. C. Testoni, A. R. A. Ignácio, M. Santos-Filho, O. Malm, and S. Díez. 2015. Mercury distribution in organs of fish species and the associated risk in traditional subsistence villagers of the Pantanal wetland. Environ Geochem Health:1-10.

Chouvelon, T., J. Spitz, Y. Cherel, F. Caurant, R. Sirmel, P. Mèndez-Fernandez, and P. Bustamante. 2011. Inter-specific and ontogenic differences in δ13C and δ15N values and Hg and Cd concentrations in cephalopods. Marine Ecology Progress Series 433:107-120.

Chui, V. W. D., K. W. Mok, C. Y. Ng, B. P. Luong, and K. K. Ma. 1996. Removal and recovery of copper(II), chromium(III), and nickel(II) from solutions using crude shrimp chitin packed in small columns. Environment International 22:463-468.

Costley, C. T., K. F. Mossop, J. R. Dean, L. M. Garden, J. Marshall, and J. Carroll. 2000. Determination of mercury in environmental and biological samples using pyrolysis atomic absorption spectrometry with gold amalgamation. Analytica Chimica Acta 405:179-183.

de la Chesnais, T., E. A. Fulton, S. R. Tracey, and G. T. Pecl. 2019. The ecological role of cephalopods and their representation in ecosystem models. Reviews in Fish Biology and Fisheries 29:313-334.

Di Lena, G., I. Casini, R. Caproni, and E. Orban. 2018. Total mercury levels in crustacean species from Italian fishery. Food Additives & Contaminants: Part B 11:175-182.

EFSA Scientific Committee. 2015. Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood. 1831-4732.

EU. 2005. Regulation (EC) No. 78/2005. JO L16, 19.01.05 (pp. 43–45).

Hastie, L. C., G. J. Pierce, J. Wang, I. Bruno, A. Moreno, U. Piatkowski, and J.-P. Robin. 2016. Cephalopods in the north-eastern Atlantic: species, biogeography, ecology, exploitation and conservation. Pages 123-202 Oceanography and marine biology. CRC Press.

Judkins, H., and M. Vecchione. 2020. Vertical distribution patterns of cephalopods in the northern Gulf of Mexico. Frontiers in Marine Science 7:47.

Lavoie, R. A., A. Bouffard, R. Maranger, and M. Amyot. 2018. Mercury transport and human exposure from global marine fisheries. Scientific reports 8:6705.

Lischka, A., T. Lacoue-Labarthe, P. Bustamante, U. Piatkowski, and H. J. T. Hoving. 2020. Trace element analysis reveals bioaccumulation in the squid *Gonatus fabricii* from polar regions of the Atlantic Ocean. Environmental Pollution 256:113389.

Lischka, A., T. Lacoue-Labarthe, H. J. T. Hoving, J. JavidPour, J. L. Pannell, V. Merten, C. Churlaud, and P. Bustamante. 2018. High cadmium and mercury concentrations in the tissues of the orange-back flying squid, *Sthenoteuthis pteropus*, from the tropical Eastern Atlantic. Ecotoxicology and Environmental Safety 163:323-330.

Liu, J., L. Cao, and S. Dou. 2019a. Trophic transfer, biomagnification and risk assessments of four common heavy metals in the food web of Laizhou Bay, the Bohai Sea. Science of The Total Environment 670:508-522.

Liu, Y., X. Luo, Y. Zeng, M. Deng, W. Tu, Y. Wu, and B. Mai. 2019b. Bioaccumulation and biomagnification of hexabromocyclododecane (HBCDD) in insect-dominated food webs from a former e-waste recycling site in South China. Chemosphere:124813.

Monteiro, L., F. Porteiro, and J. Gonçalves. 1992. Inter- and intra-specific variation of mercury levels in muscle of cephalopods from the Azores. Arquipelago. Serie Ciencias da Natureza:13-22.

Monteiro, L. R., V. Costa, R. W. Furness, and R. S. Santos. 1996. Mercury concentrations in prey fish indicate enhanced bioaccumulation in mesopelagic environments. Marine Ecology Progress Series 141:21-25.

Moreno-Brush, M., D. S. McLagan, and H. Biester. 2019. Fate of mercury from artisanal and small-scale gold mining in tropical rivers: Hydrological and biogeochemical controls. A critical review. Critical Reviews in Environmental Science and Technology:1-39.

Moreno-Sierra, D., M. E. Bergés-Tiznado, F. Márquez-Farías, Y. E. Torres-Rojas, J. R. Ruelas-Inzunza, and F. Páez-Osuna. 2016. Trace metals in target tissues and stomach contents of the top predator sailfish *Istiophorus platypterus* from the Eastern Pacific: concentrations and contrasting behavior of biomagnification. Environmental Science and Pollution Research 23:23791-23803.

Moreno, A., M. Morais da Cunha, and J. M. F. Pereira. 1994. Population biology of veined squid (*Loligo forbesi*) and European squid (*Loligo vulgaris*) from the Portuguese coast. Fisheries Research 21:71-86.

Penicaud, V., T. Lacoue-Labarthe, and P. Bustamante. 2017. Metal bioaccumulation and detoxification processes in cephalopods: a review. Environmental Research 155:123-133.

Pereira, P., M. Korbas, V. Pereira, T. Cappello, M. Maisano, J. Canário, A. Almeida, and M. Pacheco. 2019. A multidimensional concept for mercury neuronal and sensory toxicity in fish - From toxicokinetics and biochemistry to morphometry and behavior. Biochimica et Biophysica Acta (BBA) - General Subjects 1863:129298.

Pierce, G. J., P. R. Boyle, L. C. Hastie, and M. B. Santos. 1994. Diets of squid Loligo forbesi and Loligo vulgaris in the northeast Atlantic. Fisheries Research 21:149-163.

Pierce, G. J., G. Stowasser, L. C. Hastie, and P. Bustamante. 2008. Geographic, seasonal and ontogenetic variation in cadmium and mercury concentrations in squid (Cephalopoda: Teuthoidea) from UK waters. Ecotoxicology and Environmental Safety 70:422-432.

Porteiro, F., and H. Martins. 1994. Biology of *Loligo forbesi* Steenstrup, 1856 (Mollusca: Cephalopoda) in the Azores: sample composition and maturation of squid caught by jigging. Fisheries Research 21:103-114.

Rodrigo, A. P., and P. M. Costa. 2017. The Role of the Cephalopod Digestive Gland in the Storage and Detoxification of Marine Pollutants. Frontiers in Physiology 8.

Rosa, R., G. Pierce, and R. O'Dor. 2013. Advances in squid biology, ecology and fisheries. Nova Biomedical.

Seco, J., J. C. Xavier, A. S. Brierley, P. Bustamante, J. P. Coelho, S. Gregory, S. Fielding, M. A. Pardal, B. Pereira, G. Stowasser, G. A. Tarling, and E. Pereira. 2020. Mercury levels in Southern Ocean squid: Variability over the last decade. Chemosphere 239:124785.

Ullrich, S. M., T. W. Tanton, and S. A. Abdrashitova. 2001. Mercury in the Aquatic Environment: A Review of Factors Affecting Methylation. Critical Reviews in Environmental Science and Technology 31:241-293.

USEPA. 1997a. Mercury Study Report to Congress Volume IV. An Assessment of Exposure to Mercury in the United States. Office of Air Quality Planning and Standards and Office of Research and Development, Washington, DC.

USEPA. 1997b. Mercury Study Report to Congress Volume V: Health Effects of Mercury and Mercury Compounds.

Vieira, H. C., M. D. Bordalo, F. Morgado, A. M. V. M. Soares, and S. N. Abreu. 2017. Mercury content in the white and dark muscle of Skipjack tuna (*Katsuwonus pelamis*) along the canning process: Implications to the consumers. Journal of Food Composition and Analysis 56:67-72.

Vieira, H. C., F. Morgado, A. M. V. M. Soares, and S. N. Abreu. 2013. Mercury in Scalp Hair Near the Mid-Atlantic Ridge (MAR) in Relation to High Fish Consumption. Biological Trace Element Research 156:29-35.

Vieira, H. C., F. Morgado, A. M. V. M. Soares, and S. N. Abreu. 2015. Fish consumption recommendations to conform to current advice in regard to mercury intake. Environmental Science and Pollution Research:1-8.

Xavier, J. C., Y. Cherel, L. Allcock, R. Rosa, R. M. Sabirov, M. E. Blicher, and A. V. Golikov. 2018. A review on the biodiversity, distribution and trophic role of cephalopods in the Arctic and Antarctic marine ecosystems under a changing ocean. Marine Biology 165:93.

Xavier, J. C., S. Ferreira, S. Tavares, N. Santos, C. L. Mieiro, P. N. Trathan, S. Lourenço, F. Martinho, D. Steinke, J. Seco, E. Pereira, M. Pardal, and Y. Cherel. 2016. The significance of cephalopod beaks in marine ecology studies: Can we use beaks for DNA analyses and mercury contamination assessment? Marine Pollution Bulletin 103:220-226.

Chapter IX:

Final Considerations

1. Final considerations

The present thesis provides an integrative approach on the Hg contamination in sediments, intertidal species and marine species with commercial interest from different coastal areas of the North Atlantic (Azores archipelago, Northwest Portuguese coast and Southeast Mexican coast) while assessing human exposure to the Hg through the consumption of these species.

Studies related to the presence of Hg in coastal sediments from distinct areas in the North Atlantic (chapters I, II and III) revealed that the amount of Hg present in those sediments is dependent on factors such as the fraction of sediment analyzed, percentage of organic matter present in the fraction sediment analyzed, as well as the distance to potential sources of Hg contamination. Despite the heterogeneity between the studied sites (urban areas, remote areas, exposed beaches, unexposed beaches), **the coarser sediment fraction (sand) tends to have lower [Hg] than the finer sediment fraction**. The differences in the distribution of Hg between the fractions can be explained by the fact that the coarser sediment fractions usually have less organic matter load than the finer fraction of the sediment. Furthermore, **the [Hg] present in the fine sediment fraction tends to increase with the proximity of Hg contamination hotspots or places with historical Hg contamination**, leading to some sampling sites presenting higher [Hg] than the concentration established in the guidelines for sediment quality (SQG). However, since **the fine fraction represents less than 1% of the total sediment fraction in most of the sampling points**, even if the fine fraction presents a high [Hg], this concentration decreases when we analyze the sediment as the sum of all fractions. Therefore, from an ecotoxicological point of view, **adverse biological effects on biota are not expected considering the [Hg] present in sediments in the analyzed sampling areas** (chapter II).

Furthermore, although coastal sediments are generally considered a source of Hg for the marine food chains, results from these sampling sites suggested that **there is no transfer of mercury from the sediments to biota** (chapter III and IV). Exposure to Hg can occur through several routes such as inhalation, absorption and ingestion. The **higher [Hg] found in intertidal species from higher trophic levels when compared to species of lower trophic levels** (consumers versus producers), together with the obtained correlation between the [Hg] of the predator and the prey, suggest that **the exposure to Hg is not dependent on the [Hg] present in the sediment but rather occurs through the diet.**

In addition to the levels of Hg contamination to which the organisms are exposed, other factors may have an influence on both the Hg accumulation and toxicity in aquatic organisms.

Through the present findings (chapter V) it is possible to observe that **the increase in water temperature promotes the accumulation of Hg** even in a short period of exposure. Furthermore, the Hg accumulation differs between tissues, and after seven days of exposure **it is in the liver that the greatest accumulation of Hg is observed.** The presence of Hg in the organism and the increase in water temperature can induce the production of ROS that cause cell damage. Despite this, **no cellular damage was observed in the liver due to oxidative stress**, probably due to the action of antioxidant defenses to combat ROS. However, **damage by oxidative stress was observed in the level of proteins and energy consumption in the muscle promoted by the increase in temperature.** The damage observed in muscle proteins may be related to the fact that Hg has a high affinity with the thiol groups of proteins and due the proteins susceptibility to higher temperatures.

Chapters VI and VII indicate that **Hg accumulation in the studied fish species can be influenced by their feeding habits and food preference**, which was common to all sampling sites. For instance, Hg accumulation in carnivorous fish species was found to be higher than in omnivorous. Moreover, fish feeding on prey from higher trophic levels presented increased [Hg] than those feeding on lower trophic levels (biomagnification process). Additionally, in chapter VIII a **correlation between the [Hg] in the squid species *Loligo forbesi* and their size** was observed. Interesting aspects related to Hg accumulation were also verified as the fact that the stomach tissue has higher [Hg] in relation to the mantle suggests that **the squid's stomach may play an important role in the Hg detoxification.**

Considering the fish species from chapter VI, **more than 80% of the Hg analyzed in fish muscle was present in organic form** (the most toxic form). Due to the harmful effects of Hg on human health, the maximum limit of Hg present in fish and seafood was set at 0.5 $\mu g\ g^{-1}$ for most fish species and 1 $\mu g\ g^{-1}$ for a restricted group of predatory fish species. Taking into account these limits, from all the studied species (chapter VI, VII and VIII) **only 2 species presented [Hg] above the limit established for consumption**.

The human exposure to Hg depends on both the type and the amount of eaten fish. On the other hand, the quantity of consumed fish depends on several factors such as fish availability, access to fishing resources, as well as food traditions. Although the majority of the fish in Chapter VI displayed [Hg] below the abovementioned maximum limit for consumption, 13 of those species (8 from the Azores archipelago, 4 from the Northeast Portuguese coast and 1 from the Southwest Mexican coast) exceeded the provisional tolerable weekly intake (PTWI) of 1.3 μg kg body weight $(bw)^{-1}$ $week^{-1}$ suggested by JECFA. The Azores archipelago and the Northwest Portuguese coast have a high fish consumption per capita when compared to the Southeast Mexican coast, suggesting that the **fish consumption in a specific area can result**

in exceeding the limits of Hg ingestion, even when the [Hg] in the fish is below the recommended. In particular, data from the Azores archipelago (chapter VII) showed that the species with the highest [Hg] are not those that contribute the most to human Hg exposure, with the greatest contribution being made by species with lower [Hg] but captured in greater quantities. This means that **in terms of risk assessment the amount of fish consumed/captured is as important as the [Hg] present in fish muscle.**

ANNEXES

Table A1 Results for the two-way ANOVA testing for the Hg accumulation in liver and muscle of *P. microps* with [Hg] in sediment and water temperatures as factors.

	Tissue	Factor	df	F	*p*	% of total variation
Accumulation	Liver	Hg	1, 12	14.64	**0.002**	35.84
		Temperature	2, 12	3.02	0.08	14.77
		Hg x Temperature	2, 12	4.09	**0.04**	20.02
	Muscle	Hg	1, 12	2.49	0.14	13.31
		Temperature	2, 12	1.42	0.28	15.17
		Hg x Temperature	2, 12	0.68	0.52	7.32

Table A2 Results for two-way ANOVA analysis on biochemical biomarkers responses in the liver of *P. microps* with Hg exposure and water temperature as factors.

		Biomarker	Factor	df	F	*p*	% of total variation
Liver	**Oxidative stress**	CAT	Hg	1, 18	1.29	0.27	4.79
			Temperature	2, 18	3.76	**0.04**	28.02
			Hg x Temperature	2, 18	0.02	0.97	0.19
		GST	Hg	1, 18	3.66	0.07	9.35
			Temperature	2, 18	5.32	**0.01**	27.18
			Hg x Temperature	2, 18	3.43	0.05	17.52
		tGSH	Hg	1, 18	2.94	0.63	0.96
			Temperature	2, 18	8.44	0.12	20.04
			Hg x Temperature	2, 18	1.94	0.70	3.03
		LPO	Hg	1, 18	0.23	0.64	0.96
			Temperature	2, 18	2.37	0.12	20.04
			Hg x Temperature	2, 18	0.36	0.70	3.03
	Stress proteins	HSP 70	Hg	1, 18	8.49	**0.04**	20.26
			Temperature	2, 18	3.86	**0.04**	18.39
			Hg x Temperature	2, 18	3.86	**<0.01**	18.42
	Cellular energy allocation	Ec	Hg	1, 18	0.30	0.59	0.97
			Temperature	2, 18	5.76	**0.01**	37.55
			Hg x Temperature	2, 18	0.43	0.65	2.83

Table A3 Results from two-way ANOVA for biomarkers analysis in muscle of *P. microps* with Hg exposure and water temperature as factors.

		Biomarker	Factor	df	F	*p*	% of total variation
Muscle	**Oxidative stress**	LPO	Hg	1, 18	0.23	0.64	0.95
			Temperature	2, 18	2.37	0.12	20.04
			Hg x Temperature	2, 18	0.36	0.70	3.03
		PC	Hg	1, 18	1.07	0.31	2.60
			Temperature	2, 18	9.67	**0.001**	46.77
			Hg x Temperature	2, 18	1.47	0.26	7.09
		LDH	Hg	1, 18	1.47	0.24	6.35
			Temperature	2, 18	1.60	0.23	13.85
			Hg x Temperature	2, 18	0.24	0.79	2.10
	Cellular energy allocation	Sugar	Hg	1, 18	0.18	0.79	0.32
			Temperature	2, 18	2.04	0.16	18.12
			Hg x Temperature	2, 18	0.07	0.84	1.57
		Lipids	Hg	1, 17	0.33	0.58	1.64
			Temperature	2, 17	0.75	0.48	7.60
			Hg x Temperature	2, 17	0.45	0.64	4.55
		Proteins	Hg	1, 18	0.50	0.49	2.15
			Temperature	2, 18	2.25	0.13	19.51
			Hg x Temperature	2, 18	0.04	0.96	0.31
		Ea	Hg	1, 17	0.66	0.43	3.02
			Temperature	2, 17	1.11	0.35	10.67
			Hg x Temperature	2, 17	0.25	0.78	2.41
		Ec	Hg	1, 18	0.16	0.69	0.50
			Temperature	2, 18	3.67	**0.04**	23.53
			Hg x Temperature	2, 18	2.84	0.08	18.22
		CEA	Hg	1, 17	0.36	0.56	1.31
			Temperature	2, 17	3.36	0.06	24.40
			Hg x Temperature	2, 17	1.77	0.20	12.85