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Toxic Metals in Tuna and Swordfish:

Literature Review and Meta-Analysis

UNIVERSIDADE DO ALGARVE

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a Literature Review and Meta-Analysis

Mestrado em Tecnologia de Alimentos

Trabalho realizado sob a orientação de:
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Declaration of authorship of the work

I hereby declare to be the author of this work, which is original and unpublished. Authors and works consulted are properly cited in the text and included in the reference list.

(Full Name of the Autor)

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RESUMO

O pescado é uma das principais fontes de alimentação, nutrição, rendimento e meios de subsistência. O consumo de pescado está associado não só por ser fonte de proteínas, às vitaminas D e A ou aos minerais, mas também de ácidos gordos polinsaturados (*poli-unsaturated fatty acids*, ou PUFA) ómega-3, que desempenham um papel importante na prevenção da maioria das patologias cardiovasculares. Foi demonstrado que o pescado tem vários benefícios para a saúde, tais como propriedades antioxidantes, anti-inflamatórias, de cicatrização de feridas, neuroprotetoras e cardioprotetoras.

A produção de pescado a nível mundial aumentou muito, passando de 19 milhões de toneladas (peso vivo equivalente) na década de 1950 para um recorde de cerca de 179 milhões de toneladas em 2018 (FAO, 2020). O pescado tem registado uma maior procura (Delgado et al., 2004), e o consumo está a aumentar globalmente 2,5% ao ano (Peterson & Fronc, 2007; Banco Mundial, 2013). Os peixes marinhos mais importantes do ponto de vista comercial são incluem salmão, arenque, bacalhau, cantarilho, a cavala, o atum, a sardinha e o espadarte. Devido ao seu elevado valor comercial, espécies como o espadarte e o atum são comercializadas em quantidades relativamente mais pequenas com um valor de mercado mais elevado, enquanto outras espécies são comercializadas em maiores quantidades, mas com valores mais baixos. Os atuns têm sido um importante produto económico de elevado valor comparativo entre vários recursos aquáticos com uma ampla quota de mercado. Incluem cerca de >10 espécies que ocorrem nos oceanos Atlântico, Índico e Pacífico e no mar Mediterrâneo. Em 2021, foram capturadas cerca de 4,8 milhões de toneladas das principais populações comerciais de atum, com gaiado (*Katsuwonus pelamis*) a representar 56 % das capturas, albacora (*Thunnus albacares*) 31 %, o patudo (*Thunnus obesus*) 8 % e o atum voador (*Thunnus alalunga*) 4 %. O espadarte (Xiphias gladius) é um peixe meso-pelágico altamente migratório (Fig. 5) e um predador de topo, amplamente distribuído no Oceano Atlântico e no Mar Mediterrâneo. A captura anual total de espadarte registada em 2021 foi de 712 000 t (ISSF, 2023). A nível mundial, estima-se que a biomassa do espadarte tenha diminuído pelo menos 22% nos últimos 20 anos.

Apesar dos benefícios comprovados para a saúde, o consumo de pescado pode ocasionalmente constituir um risco para a saúde devido à contaminação, nomeadamente com determinados metais pesados ou tóxicos no caso de algumas espécies como são o espadarte e o atum. As funções e características químicas do grupo heterogéneo de elementos que se designam metais pesados ou

tóxicos são diversas. Manganês (Mn), cobre (Cu), níquel (Ni), ferro (Fe) e zinco (Zn) são metais (pesados) necessários, enquanto cádmio (Cd), arsénio (As), mercúrio (Hg) e chumbo (Pb) são metais (pesados) não-essenciais. Os metais essenciais apoiam o metabolismo humano, mas os metais não-essenciais são prejudiciais mesmo em pequenas quantidades e não se decompõem no ambiente nem formam outras moléculas intermediárias – metais tóxicos. As fontes primárias destes metais são antropogénicas, e.g. a indústria, a agricultura, as aplicações médicas, assim como ocorrências naturais, incluindo erupções vulcânicas, desgaste de rochas e liberação de metais pesados no meio ambiente. Os metais tóxicos são perigosos, não-biodegradáveis e persistentes no meio ambiente. A vida selvagem, especialmente os peixes, são particularmente afetados, acumulando os metais tóxicos através do seu aparelho respiratório, bem como do consumo de outros pequenos peixes e plantas marinhas contaminados. A (bio)acumulação destes metais nocivos nos tecidos corporais dos peixes decorre ao longo do tempo de vida. Assim, o consumo de peixe pode constituir uma fonte consistente de acumulação ao longo da cadeia alimentar – biomagnificação. Na mesma cadeia alimentar, a biomagnificação refere-se a um aumento da concentração num organismo de um nível trófico inferior para um nível trófico superior devido à alimentação, resultando numa maior concentração dessas substâncias nos organismos das espécies que ocupam os níveis superiores da cadeia. Os metais (pesados) tóxicos podem entrar no corpo humano de várias maneiras, inclusive por meio de alimentos, neste caso pelo consumo de pescado contaminado, pela água, pele ou respiração. Esses metais são geralmente solúveis em água e são absorvidos pelo cólon antes de circularem para outros órgãos. No entanto, os metais (pesados) tóxicos têm impacto no sistema respiratório e em diversas células, incluindo endotélio e células epiteliais, em doses baixas. O risco não está na quantidade de peixe consumido, mas sim no tipo de peixe consumido; sendo que o consumo de espécies de pescado que foram expostas à poluição por metais pesados pode ter um efeito nocivo duradouro nos seres humanos.

O presente estudo visa preencher uma lacuna na literatura científica relativa aos níveis de metais tóxicos em atum e espadarte frescos, os quais foram avaliados e reportados em diversos estudos, porém ainda não foram compilados e analisados de forma abrangente. Assim, os objetivos deste trabalho consistem em: realizar uma revisão da literatura académica publicada desde o ano 2000 e compilar e contextualizar a informação sobre a prevalência e os riscos para a saúde dos metais tóxicos presentes no atum e espadarte pescados e consumidos em todo o mundo; fazer uma meta-análise para examinar os eventuais efeitos de variáveis moderadoras ("explicativas") sobre a variabilidade observadas nas concentrações de metais tóxicos; e discutir os riscos para a saúde associados ao consumo destas espécies de peixes em Portugal.

Para a revisão da literatura, foram obtidos dados de estudos primários, incluindo artigos científicos, dissertações/teses académicas e relatórios técnicos, publicados em inglês desde o ano 2000. Bases de dados académicas como ScienceDirect da Elsevier, B-On e Google Scholar foram utilizadas para encontrar artigos sobre metais tóxicos em atum e espadarte. Os critérios de inclusão dos estudos foram baseados na disponibilidade do texto completo, relato da concentração de metais tóxicos em atum e/ou espadarte, publicação completa ou resumo, originalidade (novos dados experimentais) e pesquisa transversal (revisão), e publicação desde o ano 2000, com inclusão exclusiva de artigos publicados em inglês para evitar erros de tradução. O procedimento adotado seguiu a metodologia *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA). Para a meta-análise, a média (e o desvio padrão) das concentrações de metais tóxicos, Hg, Cd, Pb e As, em cada estudo primário foram obtidos para cada combinação das variáveis moderadoras (ver acima), o que resultou em k=23 estudos no caso do espadarte e k=38 estudos para o atum. Para realizar a meta-análise das médias, assumimos um modelo de efeitos aleatórios, uma vez que antecipámos uma heterogeneidade considerável entre estudos. Um conjunto de estatísticas foi calculado para quantificar a heterogeneidade entre os estudos. A análise de subgrupos, também conhecidas como análise de moderadores, é uma forma de identificar a razão pela qual um padrão de heterogeneidade específico pode ser encontrado entre os estudos. Realizaram-se análises de subgrupos seguindo um modelo de efeitos mistos, utilizando um teste baseado em Q para diferenças de subgrupo. Por último, foram utilizados gráficos derivados da meta-análise para ilustrar os resultados da meta-análise (*forest plots*) e para avaliar a heterogeneidade dos efeitos e os enviesamentos nos estudos primários, um indicador do enviesamento da publicação (*funnel plots*). No que toca à avaliação de riscos, estimou-se inicialmente a ingestão (média) diária (*estimated daily intake*, EDI) para os metais analisados considerando vários cenários alternativos de consumo (*daily (sea)food consumption*, DFC). Para as avaliações de risco não-carcinogénico, utilizou-se o quociente de perigo (*target hazard quotient*, THQ) e o quociente de perigo total/acumulado (*total target hazard quotient*, TTHQ). No caso da avaliação do risco carcinogénico (RC), para a exposição ao Pb e ao As ao longo da vida foi obtido utilizando o fator de inclinação do cancro (*cancer slope factor*, CSF).

Neste trabalho, foram compilados e sintetizados os níveis de metais tóxicos, nomeadamente mercúrio (Hg), cádmio (Cd), chumbo (Pb) e arsénio (As), no espadarte e atum frescos pescados e consumidos em todo o mundo, a partir da literatura académica publicada desde o ano 2000. Não temos conhecimento de estudos de síntese publicados sobre os níveis de metais tóxicos e os riscos para a saúde dos consumidores associados às espécies estudadas.

Foram registadas na literatura concentrações apreciáveis de Hg, Cd, Pb e As nos tecidos musculares das duas espécies de peixe aqui estudadas, o atum e o espadarte.

No caso do espadarte, os níveis de metais tóxicos na literatura publicada variaram entre 0,15 mg/kg e 3,97 mg/kg para o Hg, 0,01 mg/kg e 1,04 mg/kg para o Cd e 0,01 mg/kg e 3,90 mg/kg para o Pb. Comparando as localizações, os níveis mais elevados de Hg, 3,97 mg/kg, foram encontrados no Oceano Índico Ocidental e a concentração mais baixa, 0,07 mg/kg, foi determinada para amostras obtidas no Mar Mediterrâneo. No que respeita aos níveis de Cd, os níveis mais elevados foram de 1,04 mg/kg e a concentração mais baixa, de 0,01 mg/kg, no Oceano Índico e no Mar Tirreno. As concentrações de Pb no espadarte variaram entre um mínimo de 0,01 mg/kg, na Ilha da Reunião, e um máximo de 3,90 mg/kg no mar da Argélia. Não foi encontrada qualquer relação entre o tamanho e as concentrações de metais no espadarte. Num total de 18 estudos, os dados relativos ao espadarte revelaram concentrações elevadas de Hg e Pb acima dos limites admissíveis (1 mg/kg), o que pode indicar um risco acrescido para a saúde.

No caso do atum, as concentrações de metais tóxicos variaram entre 0,04 mg/kg e 3 mg/kg para o Hg, 0,01 mg/kg e 2 mg/kg para o Cd, 0,01 mg/kg e 0,92 mg/kg para o Pb e 0,9 mg/kg e 30,5 mg/kg para o As. Comparando os locais, o mar Mediterrâneo e o mar da China foram os dois locais com concentrações elevadas de Hg, 3 mg/kg, e de As, 30,5 mg/kg, respetivamente. Quase todas as espécies apresentaram níveis elevados de Hg, >1 mg/kg, mas o atum de cauda longa também apresentou níveis elevados de As, superiores a 30,5 mg/kg. No total, os dados de 19 estudos relativos ao atum ultrapassaram os limites admissíveis (1 mg/kg) para o Hg e o As, aumentando assim o risco para a saúde do consumidor.

A meta-análise após uma revisão sistemática da literatura é uma forma eficaz de fazer compilar e analisar a informação publicada sobre metais tóxicos no espadarte e atum. As concentrações médias estimadas para os metais tóxicos, para o espadarte, 1,13 mg/kg para o mercúrio, 0,14 mg/kg para o cádmio e 0,70 mg/kg para o chumbo e, no caso do atum, foram de 0,72 mg/kg para o mercúrio, 0,15 mg/kg para o cádmio, 0,11 mg/kg para o chumbo, 7,19 mg/kg para o arsénio. Registaram-se diferenças significativas (teste Q, $p<0,0001$) entre espécies (no caso do atum), anos e locais para todos os metais tóxicos aqui considerados. Esta considerável heterogeneidade está de acordo com os resultados descritos acima na revisão narrativa da literatura. Não obstante, os resultados da meta-análise devem ser considerados com cuidado devido a sinais de enviesamento de publicação.

A avaliação do risco para a saúde humana decorrente da exposição a metais pesados através do consumo de peixe revelou um risco adverso significativo para a saúde humana, uma vez que os

valores calculados estavam acima dos limites de referência para os dois metais Hg e As. No entanto, os valores do quociente de perigo (THQ) calculados para o arsénio e o mercúrio foram >1, o que indica uma causa provável de efeitos adversos durante a vida de uma pessoa devido ao consumo de atum e de espadarte. O risco carcinogénico máximo no cenário alternativo 3 (consumos de 0,62 g/pessoa/dia para o espadarte e 5 g/pessoa/dia para o atum) tendo valores de consumo mais realistas em relação aos outros dois cenários alternativos, não representa risco acrescido para a saúde dos consumidores em Portugal, exceto devido ao elevado teor de As no atum.

Em conclusão, este trabalho realça a importância de compreender e monitorizar os metais tóxicos no pescado, no caso em espadarte e em atum, para a segurança alimentar do pescado e a saúde humana. No entanto, é necessária mais investigação para aperfeiçoar as análises.

Palavras-Chave: Atum, Espadarte, Metais tóxicos, Riscos para saúde, Revisão da literatura, Meta-análise

ABSTRACT

Mercury (Hg), cadmium (Cd), lead (Pb) and Arsenic (As) are toxic metals that continue to attract much attention because they are prone to be accumulated in fish tissues and can harm human health if taken up with food. The aim of the work was to explore the concentration levels of toxic metals and associated risk assessment in commercially, widely available tuna and swordfish thru a literature review and then using meta-analysis. Data was extracted from papers on the bioaccumulation of these metals in the tuna and swordfish from various water bodies Mediterranean, Pacific, Indian and Atlantic Ocean are utilized to identify general tendencies and specifics in the accumulation of toxic metals depending on the moderator ("explanatory") variables such as species, location, fish size. Literature results show that the mean concentrations in muscle samples of swordfish and tuna were Hg>Pb>Cd and As>Hg>Cd=Pb, respectively. Meta-analysis is a statistical examination of data from several studies that results in a quantitative synthesis of study findings. It has the potential to be an important tool for accelerating development in fishing industry by identifying known and unknown facts (toxic metals). Toxic metal toxicity because of fish consumption with high metal concentrations can result in damage or reduced mental and central nervous system function, lower energy levels, and damage to blood composition, lungs, kidneys, bones, liver, and other vital organs. The mean concentration of As is 7.19 mg/kg in Tuna and 1.13 mg/kg in Hg for swordfish, including muscle, exceeding the permitted levels set by EU regulations, Codex Alimentarius general standards, FDA standards, and WHO. Moreover, noncancer risk using the target hazard quotient (THQ) of each of the four toxic metals was assessed, and cancer risk using the Carcinogenic risk (CR) for Portugal was evaluated. The THQ value of Hg is 2.36 in swordfish and 6.57 for As in tuna, were higher than the limits of safe (THQ <1) and CR showed the high carcinogenic risk for As (1.77) for tuna consumers. The total target hazard quotient (TTHQ) was also high, 10.13 for Hg in swordfish and 6.32 for Hg and 10.96 for As in tuna, which is 6 to 10 folds higher than safe limits (TTHQ <1). The Estimated Daily Intakes (EDI) as well as THQ for Hg, Cd and As indicated that swordfish and tuna consumption pose a high risk to human health in Portugal. These data suggest a need for continuous monitoring to avoid health risk associated with consumption of top predator fishes, which bioaccumulate and bio amplify toxic metals.

Keywords: Tuna, Swordfish, Toxic metals, Health risk, literature review, meta-analysis.

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1 INTRODUCTION

1.1 RELATIVE IMPORTANCE OF TUNAS AND SWORDFISH IN SEAFOOD PRODUCTION

Seafoods are one of the key sources for food, nutrition, income, and livelihood (Rahmaniya & Sekharan, 2018). Seafood including mollusks, crustaceans, cephalopods, fish, and others are important part of the human diet across the world that can be naturally rich or contaminated with some toxic metals (Torres et al., 2015; Yi et al., 2011). Fish consumption is associated not only with proteins, vitamins D and A, or minerals but also with omega-3-polyunsaturated fatty acids (PUFA) that play an important role in the prevention of most cardiovascular pathologies. Fish provides adequate nutrition for humans as one of the food sources. The variety of nutrients in fish makes it an important source of nutrition that is readily available around the world. Fish has been shown to have several health benefits such as antioxidant, anti-inflammatory, wound healing, neuroprotective and cardioprotective properties (Chen et al., 2022). Thus, the European Food Safety Authority (EFSA) has recently revalued the role of seafood in European diets, assessing the beneficial effects of its consumption on health outcomes, and recommending a fish consumption of 1–2 fish-based meals per week (Panel, 2015); a similar recommendation was proposed by the World Health Organization and Food and Agriculture Organization (FAO/WHO, 2011).

The production of seafood worldwide has increased greatly from 19 million tones (live weight equivalent) in the 1950s to a record of about 179 million tons in 2018 (FAO, 2020). Total seafood production and wild fish catch by country is represented by Fig. 1 and Fig. 2, it is very clear that China is the world’s largest seafood producer and fish catcher, producing more than

60 million tons in 2019. This is followed by Indonesia, India, Vietnam, and the United States (Roser et al., 2023).

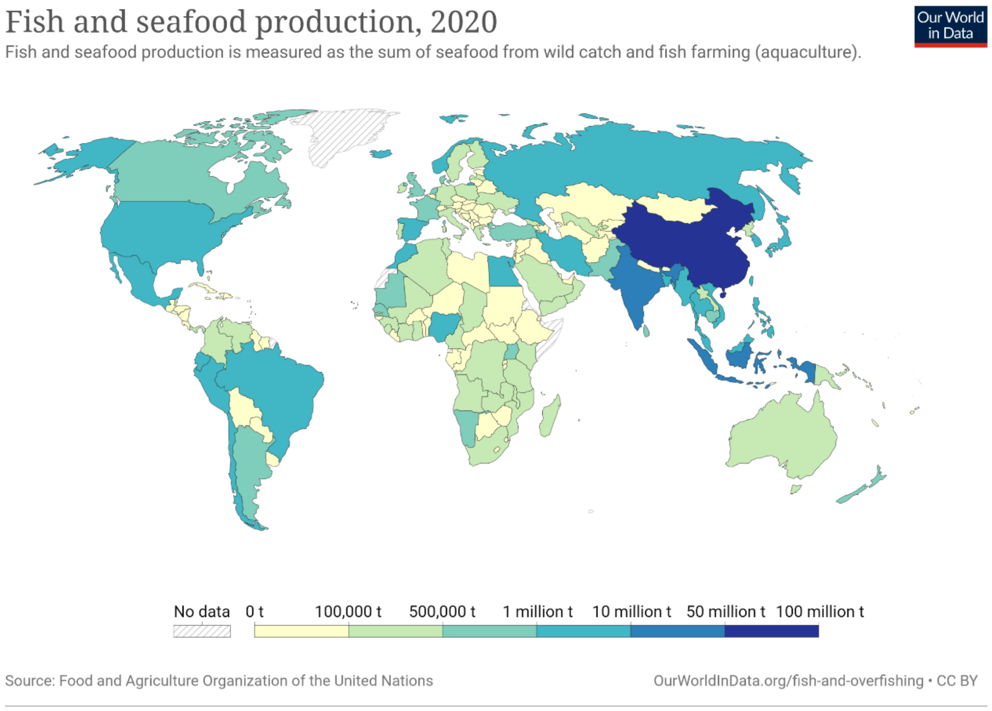


Fig. 1. Fish and Seafood production by different countries (adapted from Roser & Ritchie, 2023)

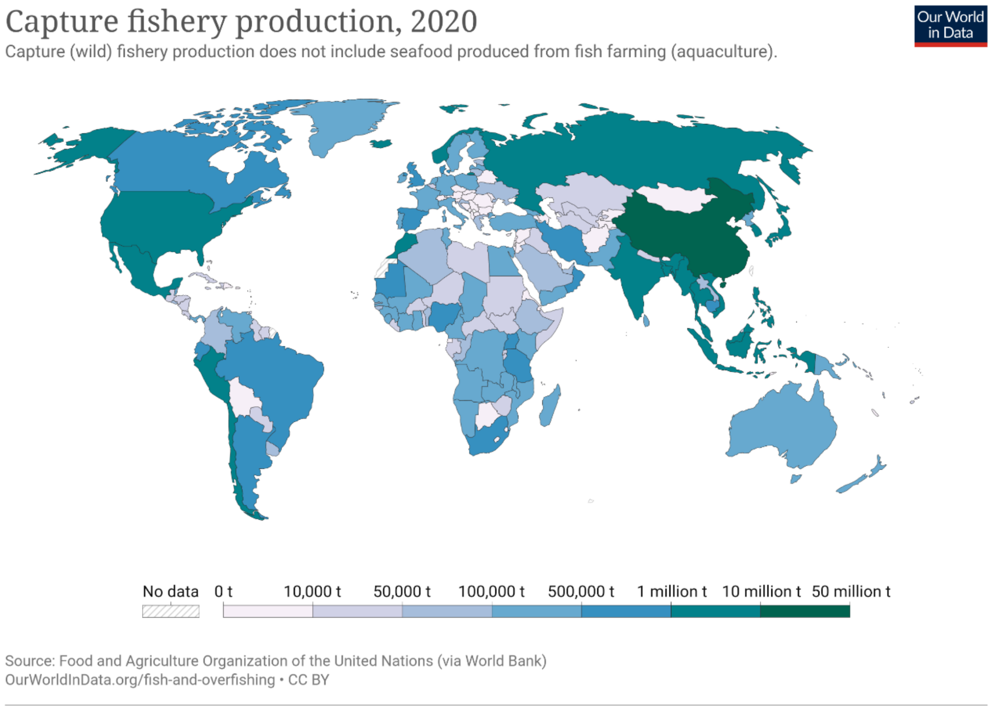


Fig. 2. Captured fisheries production by different countries (adapted from Roser & Ritchie, 2023)

The trade of seafood value surpasses that of sugar, maize, coffee, rice, and cocoa combined, making it one of the most traded food commodities in the world (Asche et al., 2015). Seafood is in higher demand (Delgado et al., 2004), and consumption is rising globally by 2.5% yearly (Peterson & Fronc, 2007; World Bank, 2023). Today, fish is an important component in the diet of 3.2 billion people, providing nearly 20% of the average intake of animal protein sources (FAO, 2018). Seemingly, the fastest-growing food category globally, after drinkable yogurt and fresh soup, is fresh fish and shellfish, which ranks third overall (Nagarajarao, 2016). The demand for fish will rise quickly because of population growth and seafood continues to be an essential source of nutrients in terms of food security and to be perceived as healthy.

The most commercially important marine fishes are salmon, herring, codfish, flatfish, redfish, mackerel, tuna, sardine, and swordfish (FAO, 2022). Due to their commercial high value, species including swordfish, abalone, lobster, and tuna are traded in relatively smaller quantities with higher market value (Fabinyi & Liu, 2014; Norman-López et al., 2013), whereas other species are traded in larger quantities, but at lower values.

1.1.1 TUNAS

The family Scombridae includes some of the world's most popular food and sport fishes, e.g., mackerels, tunas, and bonitos. The family consists of 51 species in 15 genera and two subfamilies (Thunnini and Sardini). The 15 species of Thunnini (Fig. 3) are albacore, bigeye, black skipjack, blackfin, bluefin (three species: Atlantic, Pacific, and Southern), bullet, frigate, kawakawa, little tunny, longtail, skipjack, slender and yellowfin. All species are in the subfamily Scombridae, except the butterfly kingfish (Nelson, 2007). Scombrids are, for the most part, pelagic fishes living in tropical and subtropical seas. Tunas are large pelagic fish, living in groups and migrating over long distances, that inhabit water surfaces in tropical and subtropical areas around the world (Pruzinsky, 2018).

About 4.8 million tons of main commercial tuna populations were caught in 2021, with skipjack tuna (*Katsuwonus pelamis*) accounting for 56% of the catch, yellowfin (*Thunnus albacares*) for 31%, bigeye *(Thunnus obesus)* for 8%, and albacore (*Thunnus alalunga*) for 4%. The capture of bluefin tuna (*Thunnus thynnus*) made about 1% of the total. The greatest tropical tuna fisheries are found in the Pacific Ocean, which is followed by the Indian Ocean (21% of global tropical tuna captures) (Galland et al., 2016).

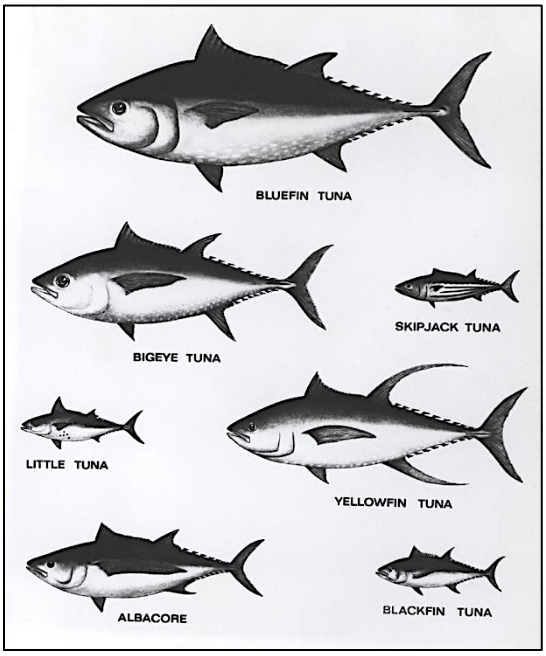


Fig. 3. Illustrations of the main species of tuna (adapted from Wikipedia contributors, 2023).

Tunas have been an important economic commodity and high comparative value among various aquatic resources with a wide market share. They include approximately >10 species occurring in the Atlantic, Indian and Pacific Oceans and in the Mediterranean Sea. Their global production (Fig. 4) has tended to increase continuously from less than 0.6 million tons in 1950 to above 1.9 million tons in 2018 (Alcala-Orozco et al., 2021). The global tuna market size reached 42.2 billion US$ in 2022 which is further estimated to be 50.2 billion US$ by 2028, exhibiting a Compound Annual Growth Rate (CAGR) of 2.94% during 2022-2028.

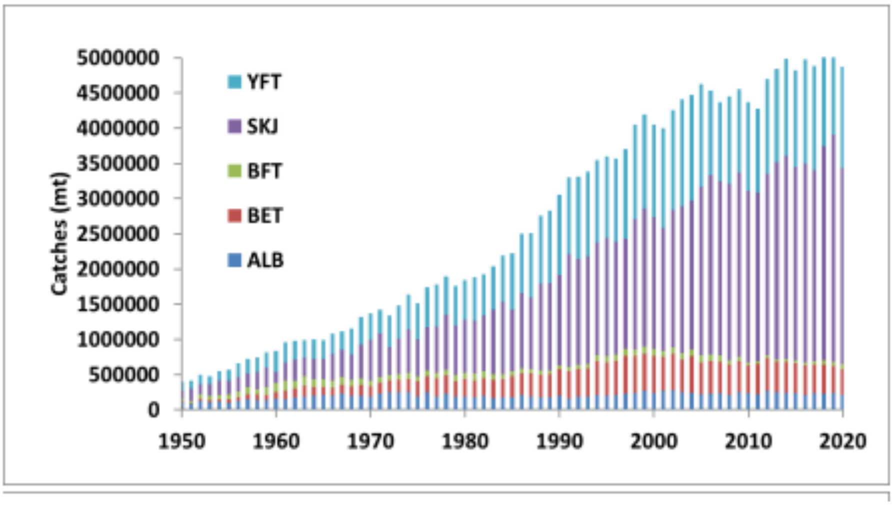


Fig. 4. Catches (1950-2020) of different tuna species, Yellowfin tuna (YFT), Skipjack tuna (SKJ), Bluefin tuna (BFT), Bigeye tuna (BET) and Albacore tuna (ALB) (adapted from ISSF, 2022).

Almost 60% of the total tuna catches comes from the West and Central Pacific Ocean (WPCO). Since all the tuna catches from this region is under the custody of the Pacific Island Countries (PICs), these countries use their tuna resources for economic development. It is widely valued and purchased item by consumers, restaurants, and canning industries as well. Such socio-economic importance is closely related to its fishing and consumption, including some of the most exploited (Collette et al., 2011; FAO/WHO, 2011), commercialized, and consumed species on the planet (MSC, 2020).

The widespread use of fish aggregating devices, i.e., rafts of material that attract schools of fish, has led to an alarming increase in the catch of juvenile tuna, jeopardizing the chances that some stocks can recover. This situation is the result of decades of overfishing and mismanagement exacerbated by illegal fishing, which is rampant in some regions. With the huge global demand for tuna, governments, and Fisheries management (EFSA, WHO, USEPA, or FAO) bodies must act now to reverse these species decline. According to IUCN red list five out of eight major tuna species are in the threatened or near endangered (IUCN, 2023). These includes Southern bluefin, which is critically endangered, Atlantic bluefin, that is endangered, bigeye, and albacore and yellowfin, which are considered vulnerable.

1.1.2 SWORDFISH

Swordfish (*Xiphias gladius* L., 1758) is an apex predator, highly migratory meso-pelagic fish (Fig. 5) widely distributed in the Atlantic Ocean and in the Mediterranean Sea (Araújo & Cedeño-Macias, 2016). It is a large species of high commercial value, reaching a maximum length of 445 cm, weighing up to 540 kg and it can live for 25 years (Nakamura & Yasumoto, 1985). Swordfish are opportunistic predators that feed primarily on pelagic fishes and invertebrates, particularly squid. Swordfish typically forage in deep water during the day and stay in the mixed layer at night (Abascal et al., 2010). Based on stomach contents from *X. gladius*, it is likely that the swordfish uses its sword to kill some of its prey, as is shown by the slashes on the bodies of prey found in swordfish stomachs (Collette et al., 2011). These fishes, typically migratory and predatory, live in the Atlantic, Pacific, and Indian Oceans, and in the temperate Mediterranean Sea (de Azevedo e Silva et al., 2007).

The total annual swordfish catch recorded for 2021 was 712,000 t (ISSF, 2023). Globally, swordfish biomass is estimated to have declined by at least 22% over the past 20 years (1994/1995–2014/2015; 3 generation lengths). Global decline was calculated based on estimated decreases of 40% in the Indian Ocean, 45% decrease in the South Atlantic, 58% decrease in the Southwest Pacific, and a 16% decrease in the Mediterranean, accompanied by an estimated 3–18% increase in the North Atlantic and a 30% increase in the Western and Central North Pacific stocks (Collette et al., 2011). Overfishing is occurring in the Mediterranean and the South Atlantic stocks, which are estimated to account for at least 25% of this species' global population, therefore ICCAT imposed a Mediterranean-wide one month fishery closure for all gears targeting swordfish in 2008, followed by a two-month closure since 2009 (ICCAT, 2015). Successive years of good recruitment in the North Atlantic region, and a combination of international management efforts including individual country quotas, gear requirements, effort reductions, and minimum sizes, as well as domestic measures implementing time-area closures off the U.S. East coast to protect juvenile swordfish, resulted in a relatively rapid rebuilding of the fishery (Neilson et al., 2013).

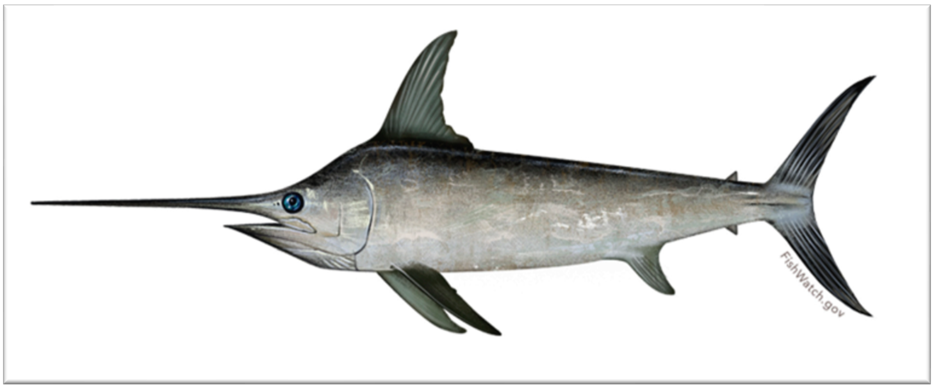


Fig. 5. Illustration of an adult swordfish, *X. gladius* (adapted from https://www.fisheries.noaa.gov/species/north-atlantic-swordfish)

In 2022, the prices in the international market for swordfish increased to 5.50-7.50 US$/lb. (11.47-15.65 EUR/kg) proving an opportunity to increase exports. Swordfish support significant commercial fisheries worldwide, with recent global annual catches of around 110,000-120,000 t (FAO, 2020) (Fig. 6). The species is also important to many subsistence and artisanal fisheries and is a highly prized recreational sportfish (Moore, 2020). In 2021, 77.7 million US$ worth swordfish frozen fillets were traded. The catches in 2017 (10,046 t) represents a 50.4% decrease since 1987 peak in North Atlantic landings (20,238 t). These reductions have been attributed to ICCAT regulatory recommendations. In 2021 (Fig. 7), the top importers of swordfish, frozen fillets were Japan (US$ 23.3M), Italy (US$ 20.8M), United States (US$ 16.2M), France (US$ 3.92M), and South Korea (US$ 3.14M).

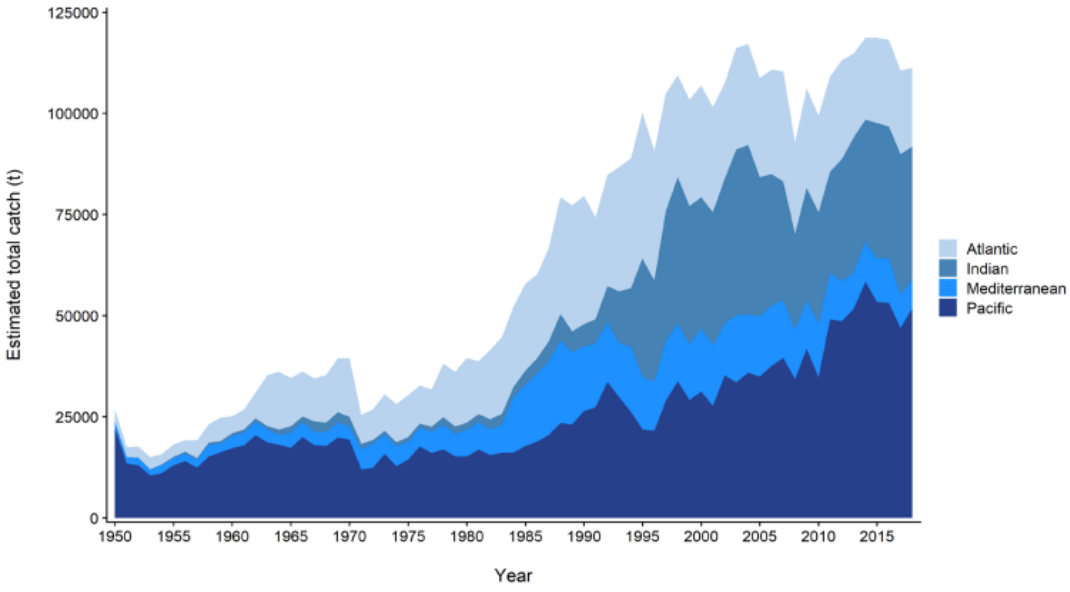


Fig. 6. Estimated global landings of Swordfish by region 1950-2018 (adapted from FAO, 2020).

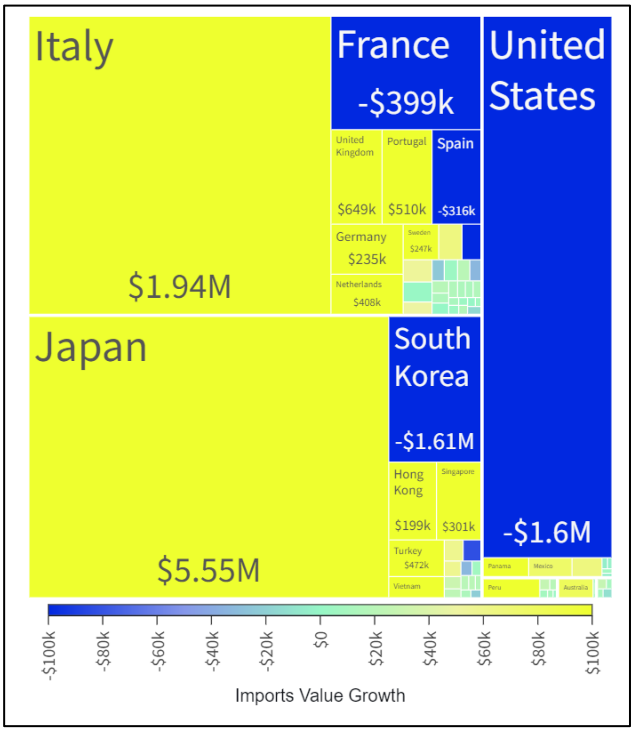


Fig. 7. Importing countries of Swordfish frozen fillets (2020-2021) (adapted from OEC, 2023).

1.2 NUTRITIONAL COMPOSITION OF FISH AND PROBLEMS OF TOXIC METALS CONTAMINATION IN FISH

In terms of nutritional composition, fish and fishery products have a high-water content (50-85%), rich in protein (12-24%) but poor in carbohydrates (0.1-3%), and their lipid content is quite variable (0.1-22%). Moreover, fish and fishery products constitute important sources (0.8-2%) of minerals (K, P, Na, Mg, Ca, Zn, Cu) and vitamins B that is water soluble, and A, D and E that are fat-soluble, thus occurring in fatty fish and mollusks. On the other hand, lipid content is quite variable even in the same species, depending on reproductive cycle stage sexual maturity, growth, water temperature, food abundance and quality, stress, etc. Fat (or blue) fish, which have >10% fat content, such as sardine, tuna, and salmon, are rich in long-chain, polyunsaturated fatty acids (PUFA). These PUFAs, namely eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), are nutritionally valuable (Esteves et al., 2016). In contrast, swordfish is a lean (white) fish, since its fat content is <5%, but a good source of protein, is naturally low in sodium content, and is rich in many vitamins, minerals (e.g., selenium), and nutrients, e.g., omega (n-3) fatty acids. As a food source, fish provides adequate nutrition and has been shown to have several health benefits such as antioxidant, anti-inflammatory, wound healing, neuroprotective and cardioprotective. Fishes' diverse nutrients make it an important and readily available source worldwide (Chen et al., 2022)

However, the indisputable benefits deriving from fish consumption may, in occasions, be offset by the presence of environmental contaminants at elevated level (Dadar et al., 2016; Storelli et al., 2005). In recent years, environmental concerns have shifted to the marine environment since the ocean is the destination for most contaminants produced and spilled by humans (Banday et al., 2020).

Toxic metals are inorganic elements that are naturally present on Earth in small but measurable amounts. Of the 70 metals and metalloids found in the environment, 23 fall into the category of toxic metals or trace metals, some of which are powerful biological poisons (Wood, 2011). According to Munoz-Olivas and Cámara (2001), toxic elements are naturally occurring elements that are a part of the marine environment. They can be essential (such as copper, zinc, iron, and manganese), probably essential (such as nickel, vanadium, and cobalt), or potentially toxic (such as arsenic, cadmium, lead, and mercury). Some, e.g., arsenic, cadmium, lead, and mercury, are necessary for many biological processes and enzymatic activity at low concentrations but become toxic when consumed in large amounts or over extended periods of time (Bat et al., 2012; Uluozlu et al., 2007). These metals enter the aquatic environment from both natural and anthropogenic sources and can be (bio)accumulated by marine organisms through a variety of pathways, including respiration, adsorption, and ingestion (Türkmen et al., 2008). These metals can be biomagnified in the food chain and then be ingested by people who consume marine foods, posing health hazards (Baeyens et al., 2005). The survival of living organisms is greatly threatened by the indiscriminate discharge of toxic metals into the environment without proper or no treatment, which includes industrial discharge, contamination in agrochemicals, traffic, domestic, and other areas (Tracey et al., 2023). Adverse health effects on humans from their use/consumption include renal dysfunction, lung illness, liver failure, kidney malfunction, and long-term harm to the central and peripheral neurological systems (Dadar et al., 2016). Compared to other forms of marine pollution, trace metal contamination is less obvious and direct, but its consequences on human health and marine ecosystems are severe and widespread (Shahjahan at al., 2022).

The fish, several of which make up the top of the marine food chain and include tuna and swordfish, are known to be impacted by anthropogenic activities, fishing pressure, pollution, and climate change (Romeo et al., 2015; Woodworth-Jefcoats et al., 2019). Presence of toxic metals is a threat on safe consumption of fish because of its non-biodegradable nature, stability, tendency to accumulate in the sediments and long half-life period in the environment making them difficult to manage. The sources of toxic metal contamination are diverse and their long-lasting impact on humans by the consumption of toxic metal contaminated fish species has been widely reported in literature (Saha et al., 2016). One of the unfortunate examples is Minamata disease, caused by the consumption of seafood polluted by methyl mercury in Minamata bay, Japan, discovered in 1956, which affected more than 50,000 people with brain damage, paralysis, incoherent speech and delirium, and even now nearly 2 - 4% of the total Hg discharged by the Chisso factory between 1932 and 1968 remains in the bay sediments with flow access to Yatsushiro Sea (WHO, 2003).

1.3 TOXIC METALS AND THEIR SOURCES

In the last 10 years, metals have drawn a lot of attention as possible (probable) environmental toxins. The list includes numerous metals' toxicity thresholds for humans: cobalt, aluminum, chromium, lead, nickel, zinc, copper, cadmium, and mercury (Cappello et al., 2018). The various sources of toxic metals can be seen in Fig. 8. The main class of pollutants present in the environment are toxic metals, which are created both naturally and by human activity (Briffa et al.,2020). Toxic metals are difficult to manage because they are stable molecules that are not biodegradable, have a propensity to collect in sediments, and have a lengthy half-life period in the environment. The traits like bioaccumulation and biomagnification in living tissues, and inability to eliminate by oxidation, precipitation, or bioremediation like organic contaminants, make elements of particular concern. The ultimate recipients of these contaminants and toxic metals are aquatic environments, particularly rivers and the sea, and even small changes in the environment's physical and chemical characteristics can have an adverse effect on fish's normal physiology because they are particularly sensitive to such changes (Akter et al., 2021; Lakra & Nagpure, 2009). They either directly harm aquatic habitats by contaminating living things or indirectly harm them by disrupting the food chain (Cappello et al., 2018; Merola et al., 2021; Shiry et al., 2021). According to Delahaut et al. (2020), several variables affect the bioavailability and uptake of toxic metals, including their concentration, their exposure time, their interactions with other metals, the age and size of the fish, their detoxification systems, their metabolic processes, how they feed, and the physicochemical parameters of their environment.

Fig. 8. Natural and Anthropogenic sources of toxic metals (adapted from Nnaji et al., 2023)

Since these metals are stable molecules that are not biodegradable, have a propensity to collect in sediments, and have a prolonged half-life period in the environment, they tend to accumulate in the living tissues of organisms over time and to increase their concentration as they move up the food chain.

1.4 BIOACCUMULATION AND BIOMAGNIFICATION (OF TOXIC METALS)

The term **bioaccumulation** refers to the net build-up of a toxic element following exposure in a tissue of interest or an entire organism. Toxic element bioaccumulation can refer to an organism's complete body (including trace elements that have been ingested by the organism or adsorbed to surfaces) or just a single tissue. Several environmental factors, such as air, water, solid phases, and nutrition, can contribute to the bioaccumulation of trace elements (McGeer et al., 2003).

In the same food chain, **biomagnification** refers to a rise in concentration in an organism from a lower trophic level to a higher trophic level because of bioaccumulation from the meal. Biological magnification often refers to the process whereby substances such as pesticides or heavy metals work their way into lakes, rivers and the ocean, and then move up the food chain in progressively greater concentrations as they are incorporated into the diet of aquatic organisms such as zooplankton. As the base of the aquatic food chain is phytoplankton, they are consumed by zooplankton or benthic invertebrates, such as shellfish, which then feed fish other animals, eventually humans. The substances (toxic metals) become increasingly concentrated in tissues or internal organs as they move up the chain, because the substances are very slowly metabolized or excreted (Ciesielski et al., 2006).

The bioavailability and the uptake of toxic metals depend on many factors such as concentration of toxic metals, its exposure period, interaction with other metal, age and size of the fish, detoxifying mechanisms, metabolic processes of fish, feeding behavior, physicochemical parameters of the environment etc. (Delahaut et al., 2020). At low concentrations of metals in water, their toxic properties are dependent on such ecological factors as pH and concentrations of Ca and organic ligands. Along with other factors, temperature influences the biogeochemical cycles of elements. For example, it has been proved that the Hg content increases with age in large fish (Woodworth-Jefcoats et al., 2019). Another factor that intensifies Hg methylation is low pH of the waters (Jardine, 2016). Further, Cd concentrations in natural waters increase with a decrease in their pH during the high-water season. For Pb, accumulation depends on both the overall concentrations of Pb in the water and other factors like pH and Ca concentration. Low Ca concentrations and low pH levels in the waters cause Pb to be deposited in fish organisms more actively, meaning that Pb accumulation is more dependent on the concentration of the water and is accelerated in waters with lower pH levels (Wood, 2011). A temperature increase will lead to an increase in the salt concentrations, and the penetration abilities of metals such as Cd and Pb will decrease in more strongly mineralized waters (Moiseenko & Gashkina, 2020).

1.5 TOXIC METALS IN TUNA AND SWORDFISH

Among the different types of pollutants, toxic metals such as mercury (Hg), cadmium (Cd), arsenic (As) lead (Pb), aluminum (Al), tin (Sn), etc. are of greatest concern because of their persistent and bio accumulative nature, responsible for deleterious effects both on the aquatic flora and fauna and to their consumers including humans (Damiano et al. 2011). Extensive studies have been carried out by several researchers on toxic metals contamination in fresh samples of swordfish and tuna including bluefin tuna (*Thunnus thynnus*), yellowfin tuna (*Thunuus albacares*) and skipjack tuna (*Katsuwonus pelamis*). For example, Bosch et al., (2016) reported the average total mercury (Hg) concentration in yellowfin tuna based on the muscle type, muscle position and fish size caught from the South Atlantic off the coast of South Africa was 0.77 µg/g. Ruelas-Inzunza et al. (2018) conducted a study on the distribution and biomagnification of cadmium (Cd) and lead (Pb) in muscle and stomach content in yellowfin and skipjack tuna and found that the level of Cd and Pb were higher in stomach content compared to the muscle samples. Damiano et al. (2011) in his research reported that samples from Mediterranean and Atlantic Sea for swordfish need to be monitored as they have high values of toxic metals Hg, Cd and Pb (0.66–2.41, 0.04–0.16, and 0.97–1.36 mg/kg), respectively.

1.6 HEALTH EFFECTS OF TOXIC METALS

Considering that fish is a good source of critical nutrients, it is crucial for a balanced diet. The harmful metals, on the other hand, enter the human body and cause a variety of ailments, when fish tissues collect metals in varied concentrations and when that surpasses the safety thresholds. Fish intake may thus turn out to be a significant source of metal exposure and a threat to human health as a result (Rahmaniya & Sekharan, 2018).

According to the International Agency for Research on Cancer (IARC, 2009), toxic metals including cadmium, mercury, lead, and arsenic provide a multitude of risks to people. Lowered energy levels altered blood chemistry and other crucial organs are all possible effects of toxic metal poisoning. It can also cause damage to the brain and central nervous system. Toxicity can cause serious sickness and a worse quality of life if it is not diagnosed or treated properly. Acute toxic metal intoxications can cause harm to the bones, lungs, kidneys, liver, endocrine glands, and central nervous system (Shahjahan et al., 2022). Metals' toxic effects on human health are illustrate in Fig. 9.

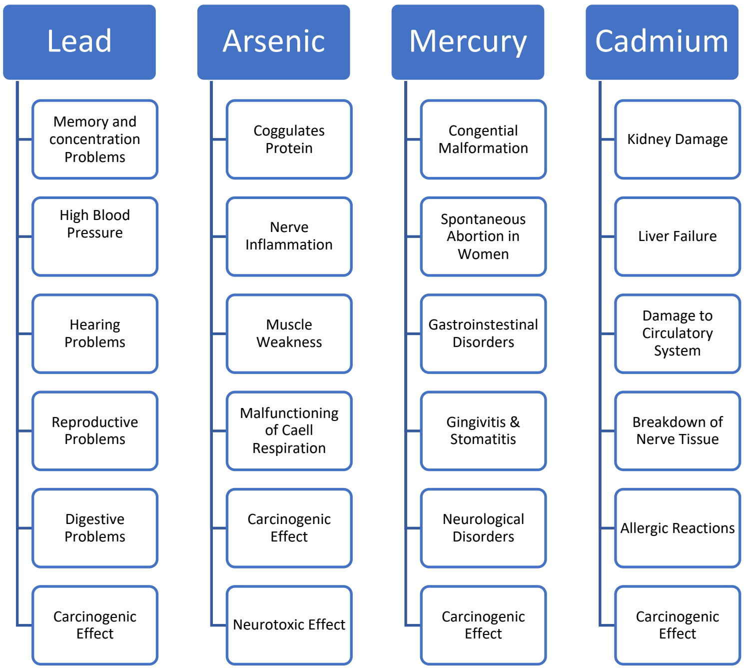


Fig. 9. Toxic metal toxic effects on human health (adapted from Sonone et al.,2020)

Metals cannot be broken down and are non-biodegradable. Organisms may detoxify metal ions by hiding the active element within a protein or depositing them in intracellular granules in an insoluble form to be excreted in the organism's faces or for long-term storage. When the toxic metals are swallowed or inhaled into our bodies, they bioaccumulate in our system (Briffa et al., 2020). Thus, they are classified as dangerous. This bioaccumulation causes biological and physiological complications. Some of these (toxic) metals are necessary for life and are called essential elements which are required for a variety of biochemical and physiological functions (Lenntech et al., 2018). However, the relation between the person and metal concentration can be seen in Fig. 10.

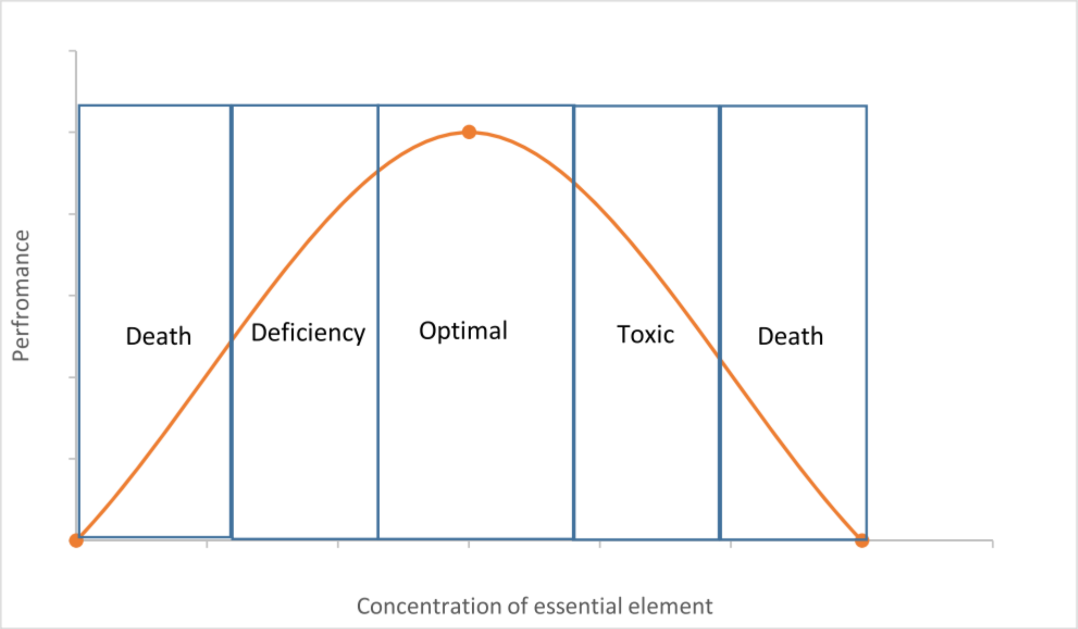


Fig. 10. The relationship between the person's performances concerning the concentration of the essential elements in the diet (adapted from Briffa et al., 2020).

1.7 LITERATURE (SYSTEMATIC) REVIEWS AND META-ANALYSIS FOR SYNTHETIZING RESEARCH

The question of how to bring together and interpret research studies that are independent from one another is a basic and important question in all sciences. The eventual inadequacy of the results of a single study and the need to synthesize findings by scientists have led to the development of methodologies that allow for combining the results of many independent studies. Many methods have been used to synthesize the findings of multiple studies.

Literature reviews and meta-analysis are two approaches aimed at synthesizing different studies that are independent of one another but also compatible. A systematic literature review (SLR) is a process of identifying, evaluating, and synthesizing all relevant research evidence on a specific topic. It is a rigorous method of reviewing the literature that helps to ensure that the findings are accurate and unbiased. By systematically searching for all relevant research, SLRs can help to ensure that no important studies are overlooked. A meta-analysis is a statistical technique that combines the results of multiple studies to provide a more precise estimate of the effect of an intervention or exposure. Meta-analyses can be used to synthesize the findings of SLRs, but they can also be used to synthesize the findings of individual studies (White et al., 2012). Gene Glass used the term "meta-analysis" in 1976 to describe the statistical examination of a sizable group of analytical findings with a view to integrating the results. Complementarily to conventional methods of literature (or narrative) review, meta-analysis provides the statistical foundation for synthesizing and analysing the findings of primary, experimental research, all of which have investigated a specific hypothesis. Additionally, utilizing moderator factors to try and explain this variability, meta-analysis enables assessing the amount of (experimentally) generated change in the dependent variable (Harrison, 2010). Meta-analyses are being utilized more often in a variety of other academic subjects, including those pertaining to food, such as food safety, nutritional/health impacts, food composition and analysis, and consumer perception of food qualities and uses. Meta-analysis studies can be a follow-on a systematic review, but this is not true of all meta-analyses (Littell et al., 2008).

When both methods are used together, it is possible to compile the quantitative evidence, analysis, and scientific approaches. This approach makes it possible to obtain a large(r) sample size and to provide new perspectives on developing social policies based upon data analysis and scientific approach.

1.8 OBJECTIVES

The levels of toxic metals in fresh tuna and swordfish have been assessed and reported in several studies but have not yet been comprehensively compiled and analyzed. Moreover, we are not aware of published syntheses studies on the levels of toxic metals and related health risk for consumers in fresh swordfish and tuna other than in canned tuna in Iran using meta-analysis (Rahmani et al., 2018).

Thus, the objectives of this work were to:

1. Review the scholarly published since the year 2000 and compile and contextualize the information about the prevalence and health risks of toxic metals present in tuna and swordfish (and eventually other billfishes) fished and consumed worldwide.
2. Carry out a meta-analysis to examine the eventual effects of moderator (“explanatory”) variables upon the changes observed in the concentrations of toxic metals.
3. Discuss the health risks incurred with the consumption of these fish species.

2 MATERIAL AND METHODS

2.1 LITERATURE REVIEW: SEARCH AND SELECTION OF STUDIES

For the literature review, the data was obtained from expected 38 primaries studies (for simplicity, named papers hereafter), among research papers, academic dissertations/thesis, and technical reports, published in English since the year 2000.

Scholarly databases such as Elsevier's ScienceDirect, B-On and Google Scholar were used to find articles regarding toxic metals in tuna and swordfish. Search terms (and combinations of terms) were: "toxic metals" or "potential toxic elements" or "risk assessment" or "bioaccumulation" or "biomagnification" or "tuna" or "swordfish", or "billfishes". The reference list of articles was explored further to find additional information in the references' list.

Inclusion criteria for the studies were (i) full text available; (ii) report of concentration of toxic metals in tuna and/or swordfish; (iii) published full article or abstract; (iv) original (experimental new data) and cross-sectional (review) research; and (v) published since the year 2000. To avoid any mistake in the translation process and for the clarity of the reports, only those articles published in the "English language" were included.

In essence, the procedure used herein followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) methodology (Page et al., 2021). It is illustrated in Fig. 11. PRISMA is an evidence-based minimum set of items for reporting in systematic reviews and meta-analyses (Page et al., 2021; Prisma, 2023).

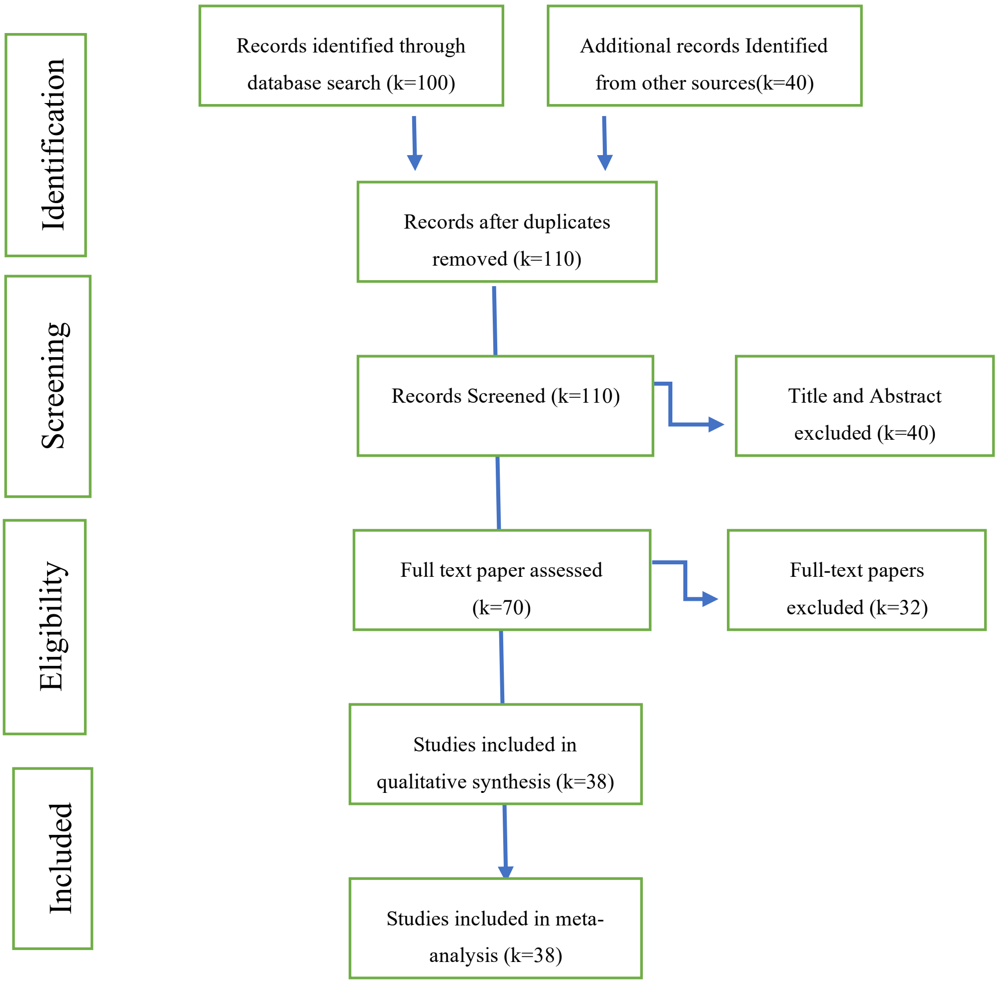


Fig. 11. Flowchart of the procedure used in this study to carry out the literature review and meta-analysis, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (or PRISMA).

Each paper was examined following a framework to retrieve the information of variables, specifically the concentration of toxic metals, namely mercury (Hg), cadmium (Cd), lead (Pb), arsenic (As), that constitutes the response-variable or the effect, and moderator ("explanatory") variables such as species, location, size/age of specimens (which can be considered a proxy of bioaccumulation period),-year of assessment, number of samples with concentration of metals above the limits recommended/regulated by the European Commission (2006), FAO/WHO (2011) and US EPA (1989). Only studies where the concentrations were from the muscle part of the fish were considered as it is one of the most indicative biomarkers for the estimation of trace metals pollution in water systems because fishes are highly sensitive to any kind of environmental alterations that make them suitable bioindicators for aquatic ecosystem monitoring because they readily metabolize, detoxify and accumulate heavy metals within the body (Shahjahan et al., 2022).

All data were compiled in a spreadsheet, allowing for description and further (statistical) analyses.

2.2 META-ANALYSIS

For the meta-analysis, following Harrer et al. (2021), the average (and standard deviation) of toxic metals' concentrations in each paper were retrieved for every combination of the moderator variables (see above) which resulted in k=23 studies in the case of swordfish and k=38 studies for tuna. The mean, $\bar{x}$, constituted the outcome measure and for each study the standard error, $se = s/\sqrt{n}$, was calculated from the standard deviation s and the sample size n. The se was used to estimate the sampling error ϵ_k in the following equation that mathematically describes the relationship between the observed effect (the mean) in study k, $\hat{\theta}_k$, as an estimate of the true effect size, θ_k, plus some sampling error, ϵ_k:

$$\hat{\theta}_k = \theta_k + \epsilon_k$$

To conduct the meta-analysis of the means, we assumed a random-effects model since we anticipated considerable between-study heterogeneity in the true effects. Moreover, the goal of the random-effects model is therefore not to estimate the one true effect size of all studies, but the mean of the distribution of true effects. We can express the random-effects model as (Borenstein et al., 2009):

$$\hat{\theta}_k = \mu + \zeta_k + \epsilon_k$$

where the observed effect size, $\hat{\theta}_k$, deviates from the pooled effect μ because of two error terms, ζ_k and ϵ_k. A crucial assumption of the random-effects model is that the size of ζ_k, i.e. the error caused by between-study heterogeneity that is associated with the distribution of true effect sizes with mean μ , $\theta_k = \mu + \zeta_k$, is independent of k (i.e. we presuppose that the size of ζ_k is a product of chance and nothing which indicates a priori that ζ_k in one study is higher than in another). The aim of both the fixed- and random-effects model is to synthesize the effects of many different studies into one single number.

A set of statistics was calculated to quantify heterogeneity among studies. The Cochran's Q is used to distinguish studies' sampling error from actual between-study heterogeneity and is commonly used to test if the variation in a meta-analysis significantly exceeds the amount we would expect under the null hypothesis of no heterogeneity, i.e. to check if there is excess variation in our data, thus being a test of heterogeneity. In addition, the I^2 statistic is the percentage of the variation in effect sizes due to between-study heterogeneity and not caused by sampling error. As a "rule of thumb", we can interpret I^2 following (Higgins and Thompson 2002): 25%=low heterogeneity; 50%=moderate heterogeneity; and 75%=substantial heterogeneity. If $I^2 > 25\%$ of the total variance, the variance between studies can be deemed as large enough to attempt to model it using available study characteristics (i.e., moderator variables) in a subgroup analysis (Higgins et al., 2003). The H^2 statistic, also derived from Cochran's Q and like I^2, describes the ratio of the observed variation, measured by Q, and the expected variance due to sampling error. Finally, τ^2 quantifies the variance of the true effect sizes underlying our data. This allows to calculate the pooled effect, defined as the mean of the true effect size distribution.

High heterogeneity can also be caused by the fact that there are two or more subgroups of studies in our data that have a different true effect, i.e. mean content. Subgroup analyses, also known as moderator analyses, are one way to identify why a specific heterogeneity pattern can be found in our data. They allow us to test specific hypotheses, describing why some type of study produces lower or higher effects than another. Herein, we conducted subgroup analyses following a fixed-effects (plural) model, also designated mixed-effects model, using a Q-based test for subgroup differences (Harrer et al., 2021).

Finally, meta-analytical derived graphs were used to illustrate meta-analysis results (forest plots) and to assess heterogeneity in the effects and biases in primary studies, a proxy of publication bias (funnel plots). Herein, contour-enhanced funnel plots were used to help distinguish publication bias from other causes of asymmetry in the results (Peters et al., 2008).

The meta-analysis described above as well as the crafting of plots were carried out in R-Studio using package **meta** (Balduzzi et al., 2019) developed for R (R Core Team, 2023). The significance level was set at $\alpha = 0.05$.

2.3 HEALTH RISKS

Estimated daily intake

The estimated daily intakes (EDI) for the analyzed metals were calculated by multiplying the respective mean concentration of the metal determined in the targeted fish samples by the weight of fish consumed by an average individual in Portugal which was obtained from the survey by Golden et al. (2022) and calculated by using the formula proposed by Ahmed et al. (2015):

$$EDI = DFC \times MC$$

where DFC is the daily food (fish) consumption (g/person/day) and MC is the mean metal concentration of metal in fish sample (mg/kg).

In this work, to compare the health risks several, alternative scenarios of DFC were studied:

- Current fish consumption of 62.7 g/person/day, as per the Portuguese Food Balance sheet (Instituto Nacional de Estatística, 2021), which is considered the reference scenario (SC.0).
- According to the National Food, Nutrition, and Physical Activity Survey of the Portuguese General Population 2015-2016 (Lopes et al., 2018), fish/seafood consumption was 42.0 g/person/day of fish (SC.1).
- Considering that the consumption of fisheries and aquaculture products in 2019 in Portugal was 59.91 kg/person/year (in live weight) (European Commission, 2024), gives a DFC 164.1 g/person/day of fish (SC.2).
- Taking into account the data on commercial fishery landings in Portugal (DGRM, 2024), swordfish represents only 1% of the landings and tunas 8%; thus, considering a DFC of fish of 62.7 g/person/day /SC.0), we obtain a DFC of ca. 0.6 g/person/day for swordfish and 5.0 g/person/day for tuna. This was used as the last alternative, “more realistic” scenario (SC.3).

Non-carcinogenic risk

The non-carcinogenic risk assessments are typically conducted to estimate the potential health risks of pollutants using the target hazard quotient (THQ). The THQ values through the consumption of fish species by local inhabitants can therefore be assessed for each heavy metal and calculations were made using the standard assumption for an integrate US EPA risk analysis as follows (US EPA, 1989):

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

where, EF is the exposure frequency (365 d year^{-1}), ED is the exposure duration (70 years) equivalent to the average human life time, FIR is the food ingestion rate (62.7 g person^{-1} d^{-1}) (Instituto Nacional de Estatística, 2021), C is the metal concentration in samples (mg kg^{-1} , wet weight), BW average body weight (adult: 60 kg), AT is the averaging time for non-carcinogens (365 d year^{-1} × number of exposure years, assuming 70 years), RfD is the oral reference dose (mg kg^{-1} d^{-1}). The RfDs are based on 0.004, 0.001, 1.5, 0.003, and 0.0005 mg kg^{-1} BW d^{-1} for Pb, Cd, Cr, As and Hg respectively (Ullah et al., 2017; US EPA, 2016). If the THQ value is less than 1, the exposed population is unlikely to experience any adverse health hazard. Conversely, if the THQ is equal to or higher than 1, there is a potential health risk (US EPA, 2016), and related interventions and protective measurements should be taken.

It has been reported that exposure to two or more pollutants may result in additive and/or interactive effects (Hallenbeck, 1993). Thus, in this study, cumulative health risk was evaluated by summing THQ value of individual metals (toxicants) and expressed as Total THQ (TTHQ) as follows:

$$TTHQ = THQ_{toxicant\ 1} + THQ_{toxicant\ 2} + \cdots + THQ_{toxicant\ n}$$

The greater the value of TTHQ, the greater the level of concern.

Carcinogenic risk

Carcinogenic risk (CR) indicates an incremental probability of an individual of developing cancer over a lifetime due to exposure to a potential carcinogen. Cancer risk over a lifetime exposure to Pb and As were obtained using cancer slope factor (CSF), provided by US EPA (US EPA, 2016). The equation used for estimation of the cancer risk is:

$$CR = CSF \times EDI$$

where CSF is the carcinogenic slope factor of 0.0085 (mg/kg/day)$^{-1}$ for Pb and 1.5 (mg/kg/day)$^{-1}$ for As, set by USPEA (USPEA, 2010). EDI is the estimated daily intake of heavy metal. Acceptable risk levels for carcinogens range from 10^{-4} (risk of developing cancer over a human lifetime is 1 in 10000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1000000) (Ullah et al., 2017).

3 RESULTS AND DISCUSSION

Considering the objectives of this work, the study's findings correspond, on one hand, to a systematic literature review of scholarly works published since the year 2000, compiling and contextualizing information about the prevalence and health risks of toxic metals present in tuna and swordfish fished and consumed worldwide. This constitutes Section 3.1. Following that, a meta-analysis was carried out to assess the overall concentrations of metals in swordfish and tuna and to check eventual effects of moderator ("explanatory") variables on the observed changes in the levels of toxic, harmful metals in swordfish and tuna. Section 3.2. comprises this latter part of the work. Finally, Section 3.3 comprises the health risk assessment to have an insight of the potential hazardous effect that toxic metals have on consumers.

3.1 LITERATURE REVIEW

3.1.1 SWORDFISH

Tables 1 describes the published papers retrieved and Table 2 compiles the information extracted from the 20 published papers about biometry and toxic metals in swordfish that fulfilled the stipulated requirements for inclusion in this review. Mercury (Hg), cadmium (Cd) and lead (Pb), are three of the most frequently studied elements in swordfish (respectively, 18, 17, and 16 out of 20 published papers) and the most important form of pollution of the aquatic environment because of their toxicity and accumulation by marine organisms (Okyere et al., 2015).

Table 1. Published papers retrieved and analyzed in this study for swordfish with information about the year and location of sampling.

Paper	Citation	DOI	Year (sampling)	Location
1	Damiano et al., 2011	https://doi.org/10.1016/j.marpolbul.2011.04.028	2011	Ionian Sea
1	Damiano et al., 2011	https://doi.org/10.1016/j.marpolbul.2011.04.028	2011	Southern Tyrrhenian Sea
1	Damiano et al., 2011	https://doi.org/10.1016/j.marpolbul.2011.04.028	2011	Central Tyrrhenian Sea
1	Damiano et al., 2011	https://doi.org/10.1016/j.marpolbul.2011.04.028	2011	North-Western Atlantic (Azores Oriental)
1	Damiano et al., 2011	https://doi.org/10.1016/j.marpolbul.2011.04.028	2011	North-Central Atlantic (Azores Occidental)
2	Gobert et al., 2017	http://dx.doi.org/10.1016/j.marpolbul.2017.05.029	2011	Mediterranean Sea
3	Di Bella et al., 2020	https://doi.org/10.1016/j.marpolbul.2020.111512	2017	Mediterranean Sea
4	Monteiro et al., 2021	https://doi.org/10.1016/j.foodchem.2020.128438	2019	Eastern Indian Ocean
5	Galimberti et al., 2016	http://dx.doi.org/10.1016/j.foodcont.2015.08.009	2010	extra-EU Countries
6	Storelli et al., 2005	https://doi.org/10.1016/j.marpolbul.2005.06.041	2003	Ionian Sea
7	Jinadasa et al., 2018	https://doi.org/10.1080/19393210.2018.1551247	2017	Waters off Sri Lanka
8	Jinadasa et al., 2014	http://pubs.sciepub.com/jfs/2/1/3/index.html#	2009	Indian Ocean off Sri Lanka
9	Pragnya et al., 2021	https://doi.org/10.1016/j.marpolbul.2021.112162	2020	Visakhapatnam, India
10	Jinadasa et al., 2010	http://dx.doi.org/10.4038/sljas.v15i0.5481	2009	Waters off Sri Lanka
11	Barone et al., 2018	https://doi.org/10.3390/toxics6020022	2017	Mediterranean Sea
12	Chouvelon et al., 2017	https://doi.org/10.1016/j.scitotenv.2017.04.048	2013	Reunion Island
12	Chouvelon et al., 2017	https://doi.org/10.1016/j.scitotenv.2017.04.048	2014	Reunion Island
12	Chouvelon et al., 2017	https://doi.org/10.1016/j.scitotenv.2017.04.048	2013	Seychelles
12	Chouvelon et al., 2017	https://doi.org/10.1016/j.scitotenv.2017.04.048	2014	Seychelles
12	Chouvelon et al., 2017	https://doi.org/10.1016/j.scitotenv.2017.04.048	2013	South-Eastern Atlantic, South Africa
12	Chouvelon et al., 2017	https://doi.org/10.1016/j.scitotenv.2017.04.048	2014	South-Eastern Atlantic, South Africa

13	Rodrigues et al., 2013	https://doi.org/10.1007/s00128-013-0989-4	2009	South Atlantic off the southern coast of Brazil
13	Rodrigues et al., 2013	https://doi.org/10.1007/s00128-013-0989-4	2012	South Atlantic off the northern coast of Brazil
14	Jinadasa et al., 2013	https://doi.org/10.1080/19393210.2013.807521	2009	Sri Lanka
15	Bodin et al., 2017	http://dx.doi.org/10.1016/j.chemosphere.2017.01.099	2014	Western-Central Indian Ocean
16	Kojadinovic et al., 2007	https://doi.org/10.1016/j.envpol.2006.07.015	2006	Western Indian Ocean (Mozambique Channel)
16	Kojadinovic et al., 2007	https://doi.org/10.1016/j.envpol.2006.07.015	2006	Western Indian Ocean (Réunion Island)
17	Mehouel et al., 2019	http://www.veterinaryworld.org/Vol.12/January-2019/2.pdf	2015	Waters off Algiers
18	Falcó et al., 2006	https://doi.org/10.1021/jf0610110	2015	Local markets, Italy
19	Desideri et al., 2010	https://doi.org/10.1007/s10967-010-0541-5	2007	Central Adriatic Sea, Italy
20	Papetti and Rossi, 2009	https://doi.org/10.1007/s10661-008-0725-4	2006	Central Tyrrhenian Sea

Table 2. Information retrieved from the published papers considered in this study: sample size, year, biometric results (TL, cm, and BW, kg) and toxic metals concentrations (mg/kg wet weight, presented as mean±standard deviation (X±SD)) in the muscle tissues of swordfish.

Paper	**Citation**	**Study no.**	**Sample size (n)**	**Year (sampling)**	**TL (cm)**	**BW (kg)**	**Location**	**Hg**	**Cd**	**Pb**
1	Damiano et al., 2011	1	5	2011			Ionian Sea	1.58±0.71	0.16±0.08	1.05±0.36
1	Damiano et al., 2011	2	5	2011			Southern Tyrrhenian Sea	2.41±1.62	0.10±0.05	1.16±0.75
1	Damiano et al., 2011	3	5	2011			Central Tyrrhenian Sea	1.04±0.61	0.12±0.13	1.36±0.92
1	Damiano et al., 2011	4	5	2011			North-Western Atlantic (Azores Oriental)	0.89±0.19	0.05±0.03	1.08±0.50
1	Damiano et al., 2011	5	5	2011			North-Central Atlantic (Azores Ocidental)	0.66±0.33	0.04±0.04	0.97±0.65
2	Gobert et al., 2017	6	33	2011	114±31	18±7	Mediterranean Sea		0.03±0.01	0.08±0.07
3	Di Bella et al., 2020	7	28	2017			Mediterranean Sea	0.87±0.42		0.02±0.01
4	Monteiro et al., 2021	8	16	2019			Eastern Indian Ocean	1.77±0.52	0.14±0.06	0.25±0.26
5	Galimberti et al., 2016	9	26	2010			extra-EU Countries	0.60±0.40	0.50±22.5	20.0±37.50
6	Storelli et al., 2005	10	58	2003	64±5	3±1	Ionian Sea	0.07±0.04	0.01±0.00	0.05±0.01
7	Jinadasa et al., 2018	11	75	2017	104±40	42±20	Waters off Sri Lanka	0.62±1.06	0.04±0.04	
8	Jinadasa et al., 2014	12	176	2009	137±58	45±25	Indian Ocean off Sri Lanka	0.90±0.60		
9	Pragnya et al., 2021	13	NA	2020	250±18	182±17	Visakhapatnam, India		0.01±0.00	0,.14±0.03
10	Jinadasa et al., 2010	14	35	2009	141±28	51±25	Waters off Sri Lanka	1.24±0.60	0.13±0.08	0.03±0.04
11	Barone et al., 2018	15	30	2017	153±34	53±35	Mediterranean Sea	0.77±0.48	0.16±0.05	0.11±0.07

12	Chouvelon et al., 2017	16	64	2013	104±4		Reunion Island	1.77±0.68	0.06±0.02	0.01±0.01
12	Chouvelon et al., 2017	17	64	2014	101±5		Reunion Island	1.64±0.57	0.08±0.03	0.01±0.04
12	Chouvelon et al., 2017	18	50	2013	96±5		Seychelles	1.31±0.31	0.16±0.22	0.01±0.00
12	Chouvelon et al., 2017	19	68	2014	96±5		Seychelles	1.47±0.46	0.04±0.01	0.00±0.00
12	Chouvelon et al., 2017	20	98	2013	83±4		South-Eastern Atantic, South Africa	0.80±0.15	0.37±0.25	0.01±0.02
12	Chouvelon et al., 2017	21	99	2014	90±9		South-Eastern Atantic, South Africa	1.12±0.56	0.30±0.26	0.02±0.02
13	Rodrigues et al., 2013	22	107	2009			South Atlantic off the southern coast of Brazil	0.63±0.02		
13	Rodrigues et al., 2013	23	585	2012			South Atlantic off the nothern coast of Brazil	0.55±0.01		
14	Jinadasa et al., 2013	24	176	2009			Sri Lanka (Galle, Mutwal, Negombo and Trincomalee)	0.90±0.52		
15	Bodin et al., 2017	25	33	2014	150±31		Western-Central Indian Ocean	0.99±0.45	0.16±0.05	0.11±0.07
16	Kojadinovic et al., 2007	26	42	2006	122±29	21±18	Western Indian Ocean (Mozambique Channel)	1.61±1.1	1.04±1.09	0.12±0.12
16	Kojadinovic et al., 2007	27	14	2006	127±24	22±16	Western Indian Ocean (Réunion Island)	3.97±2.67	0.60±0.45	0.01±0.04
17	Mehouel et al., 2019	28	30	2015			Waters off Algiers	0.56±0.15	0.57±1.34	3.90±2.79
18	Falcó et al., 2006	29	20	2015			Local markets, Italy	1.59±0.22	0.02±0.10	0.01±0.02
19	Desideri et al., 2010	30	19	2007			Central Adriatic Sea, Italy	0.46±0.16	0.15±0.00	2.29±0.76
20	Papetti and Rossi, 2009	31	5	2006	100	5	Central Tyrrhenian Sea	0.15±0.02	0.01±0.00	0.97±0.01

Legend: TL-total length (cm), BW-body weight (kg), sd-standard deviation.

In the next subsections, concentration levels of the toxic metals in swordfish are broken-down by the factors that were considered to affect the observed changes (i.e., moderator ("exploratory") variables *sensu* meta-analysis), namely location, fish size, and weight.

Location

Habitat of fishes plays an important role in bioaccumulation of toxic metals in fishes (Wu et al., 2023). Swordfish is highly migratory pelagic fish which increases the risk of bioaccumulation of toxic metals in various parts of the body (muscle, liver). Swordfish are mainly caught by the long liners targeting them (e.g., Brazil and Uruguay) and tunas (e.g., Japan and Taiwan), although small catches of swordfish are also taken by other surface gear and driftnet fisheries (Chang et al., 2012).

Extensive research has been carried out every year to monitor the toxic metals in seas with the help of fishes. Papers reporting concentrations levels of toxic metals in swordfish published since the year 2000 studied different areas in the Mediterranean Sea, e.g., the Ionian Sea, the Tyrrhenian Sea and the waters off Algeria, areas in the Atlantic Ocean, e.g. North-Central Atlantic (Azores Islands) and South Atlantic off coasts of Brazil, and areas in the Indian Ocean, e.g. Mozambique channel and waters off Sri Lanka.

In the Mediterranean Sea, Hg ranges from 0.07 mg/kg (Storelli et al., 2005) to 2.41 mg/kg (Damiano et al., 2011), between 0.01 mg/kg (Papetti & Rossi, 2009; Pragnya et al., 2021) and 0.16 mg/kg (Barone et al., 2018) for Cd and between 0.02 mg/kg (Di Bella et al., 2020) and 3.90 mg/kg for Pb (Mehouel et al., 2019).

The reported concentrations of Hg in swordfish in the Atlantic Ocean ranged from 0.55 mg/kg (Rodrigues et al., 2013) to 1.12 mg/kg (Chouvelon et al., 2017), 0.04 mg/kg (Damiano et al., 2011) to 0.37 mg/kg (Chouvelon et al., 2017) for Cd and 0.01 mg/kg (Chouvelon et al., 2017) to 1.08 mg/kg for Pb (Damiano et al., 2011).

In the Indian Ocean, the concentration of Hg lies between 0.62 mg/kg (Jinadasa et al., 2018) to 3.97 mg/kg (Kojadinovic et al., 2007), 0.04 mg/kg (Chouvelon et al., 2017; Jinadasa et al., 2018) to 1.04 mg/kg (Kojadinovic et al., 2007), for Cd, and ranges from 0.01 mg/kg (Kojadinovic et al., 2007) to 0.25 mg/kg for Pb (Monteiro et al., 2021). From table 2 it can be observed that Hg in Indian Ocean was present in much higher levels as compared to other oceans.

Comparing locations, the highest levels of Hg, 3.97 mg/kg, were found in Western Indian Ocean (Kojadinovic et al., 2007) and the lowest concentration, 1.04 mg/kg, was determined for samples obtained from Mediterranean Sea by Damiano et al. (2011). When looking at levels of Cd, the highest levels were 1.4 mg/kg in Western Indian Ocean (Mozambique Channel) (Kojadinovic et al., 2007) and the lowest concentration, 0.01 mg/kg, in the Indian Ocean (Pragnya et al., 2021) and Mediterranean Sea (Storelli et al., 2005). The concentrations of Pb in swordfish ranged from a minimum of 0.01 mg/kg in the Atlantic Ocean (Chouvelon et al., 2017) to a maximum of 3.90 mg/kg in the waters of Algeria (Mehouel et al.,2019).

Swordfish feeds in mesopelagic waters, not only in epipelagic waters, wherein the process of methylation also occurs, which is the main form of accumulating in organisms, because they are poorly oxygenated (Kojadinovic et al., 2007). The interspecific variations in toxic element levels confirm that bioaccumulation depended on the species considered, therefore, on its physiological capacity for assimilation and excretion of the ingested toxic element and its anatomy (size, nature of the integuments, surface of contact with water, etc.) (Ouro-Sama et al., 2014). Although the Western Indian Ocean (Réunion Island) has the highest mercury concentrations in the present study, it is generally accepted that fish from the Indian Ocean have higher mercury levels than fish from other seas or oceans because of the region's extensive deposits of metallic mercury and mercury ores (Storelli et al., 2005; Salvo et al., 2014).

Data from Kojadinovic et al. (2007) had the greatest amount of Cd and Hg for swordfish, 1.04 mg/kg, and 3.97 mg/kg from Western Indian Ocean. Lower values for Hg concentration in swordfish were found in the works by Damiano et al. (2011) from Mediterranean Sea that was 1.24 mg/kg and by Chouvelon et al. (2017) on Hg concentration in swordfish from Indian and Atlantic Ocean, 1.47 and 1.7 mg/kg, respectively. When comparing the results for swordfish, those of Jinadasa et al. (2010) from the Indian Ocean (0.13 mg/kg and 0.03 mg/kg for Cd and Pb) and Storelli et al. (2005) from the Ionian Sea (0.01 mg/kg and 0.05 mg/kg for Cd and Pb) were lower than the study by Mehouel et al. (2019) on the Algerian coast (0.57 mg/kg Cd, and 3.90 mg/kg Pb).

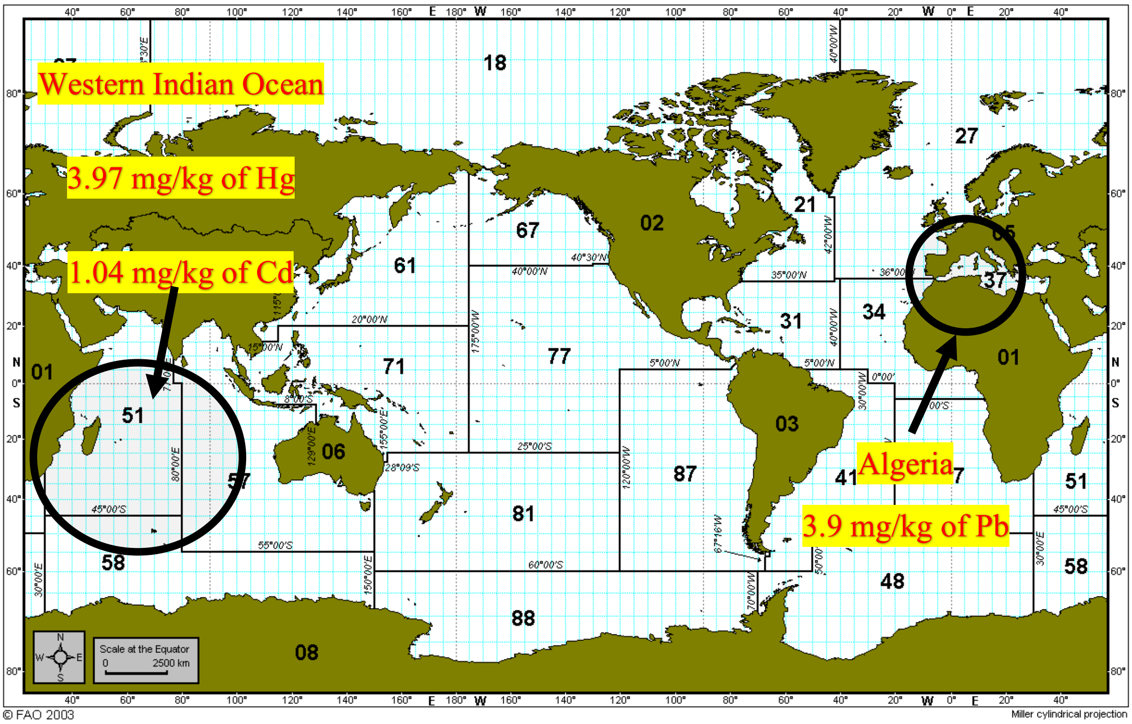


Fig. 12. Areas marked with circles have reportedly high concentration of toxic metals, Hg, Cd and Pb, in swordfish (map adapted from FAO., 2022)

Jinadasa et al. (2010) reported that Hg concentration was higher in predatory fish, 1.2 mg/kg in swordfish (Western Indian Ocean), and the same was observed by Kojadinovic et al. (2007), 3.97 mg/kg. This is because swordfish feeds in mesopelagic waters and not only in epipelagic waters: mesopelagic waters house the process of methylation, which is the main form of accumulating in organisms, because they are poorly oxygenated. Higher values for Hg in swordfish were reported both by Chen et al. in 2014 about the Hg concentration in swordfish from Indian and Atlantic Ocean which was 1.47 and 1.2 mg/kg, respectively. All the values are nearly 3-fold less when compared to data in Kojadinovic et al. (2007) wherein the highest the Hg concentration was 3.97 mg/kg in Western Indian Ocean.

Cd concentrations in swordfish from the Mediterranean Sea of 1.7 mg/kg (Zaza at al., 2015) and 1.04 mg/kg (Kojadinovic et al., 2007) were higher in comparison to other studies, namely Storelli et al. (2005) and Pragnya et al. (2021) around the Reunion Island (0.01 and 0.01 mg/kg, respectively).

The Pb concentrations in swordfish reported by Damiano et al. (2011) in the Tyrrhenian and Ionian Sea (Mediterranean Sea) and Desideri et al. (2010) in the Central Adriatic Sea were found to be higher in concentartions. This is in line with the data in the study by Mehouel et al. (2019) which recorded a Pb concentration of 3.97 mg/kg in the Algerian Coast (Mediterranean Sea) (Fig. 12).

Size (length and/or weight)

Seemingly, no apparent relation was found between length of swordfish and toxic metals' concentration (Fig. 13). Hg and Pb were found to be highest in value, 3.97 mg/kg, in fishes averaging 127 cm long (Kojadinovc et al., 2007), 3.90 mg/kg in undefined length as compared to Pb, 1.48 mg/kg with mean length of 122 cm for Cd, respectively (Kojadinovic et al., 2007).

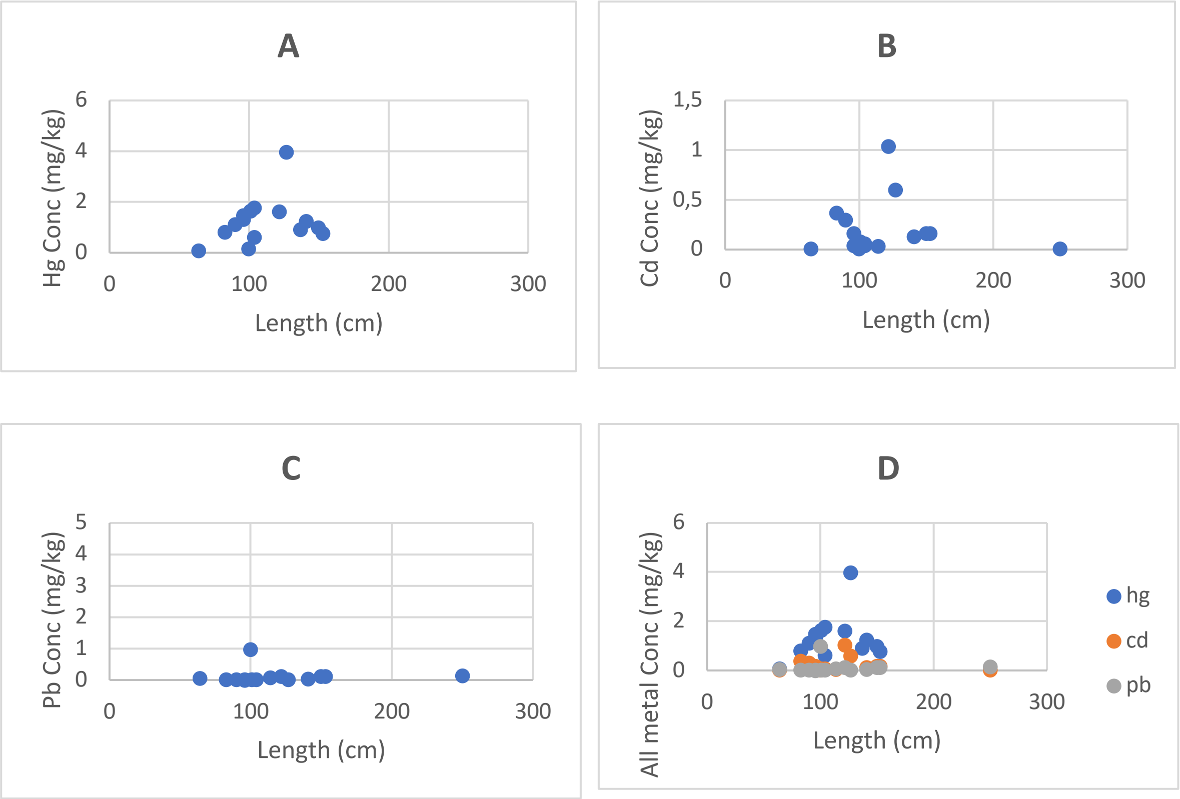


Fig. 13. Concentration levels of toxic metals (mg/kg) vs. length (cm) of swordfish. A-Hg, B-Cd, C-Pb, D-All metals.

In terms of weight, there was no pattern observed between weight and toxic metals also (Fig. 14). Again, Hg was present in high value 3.97 mg/kg in 22 kg fish (Kojadinovc et al., 2007). Cd and Pb concentrations were found to be between 1.5-3.9 mg/kg in 21-25 kg swordfish (Kojadinovic et al., 2007; Mehouel et al., 2019).

Mercury (Hg) and cadmium (Cd) were found in high concentrations in swordfish according to Galimberti et al. (2016). They found that Hg content was higher in predatory fish, reaching 580 µg/kg in swordfish. Due to their predatory nature and position at the top of the food chain in marine pelagic environments, swordfish tend to acquire large amounts of mercury (Hg), which can be biomagnified along the food chain. Additionally, pelagic fishes have growth and metabolic rates that are two to five times higher than those of other species (Storelli et al., 2005). Additionally, compared to other predatory species, it feeds on larger prey, where Hg accumulates higher than smaller organisms (Noël et al., 2013; Perugini et al., 2013).

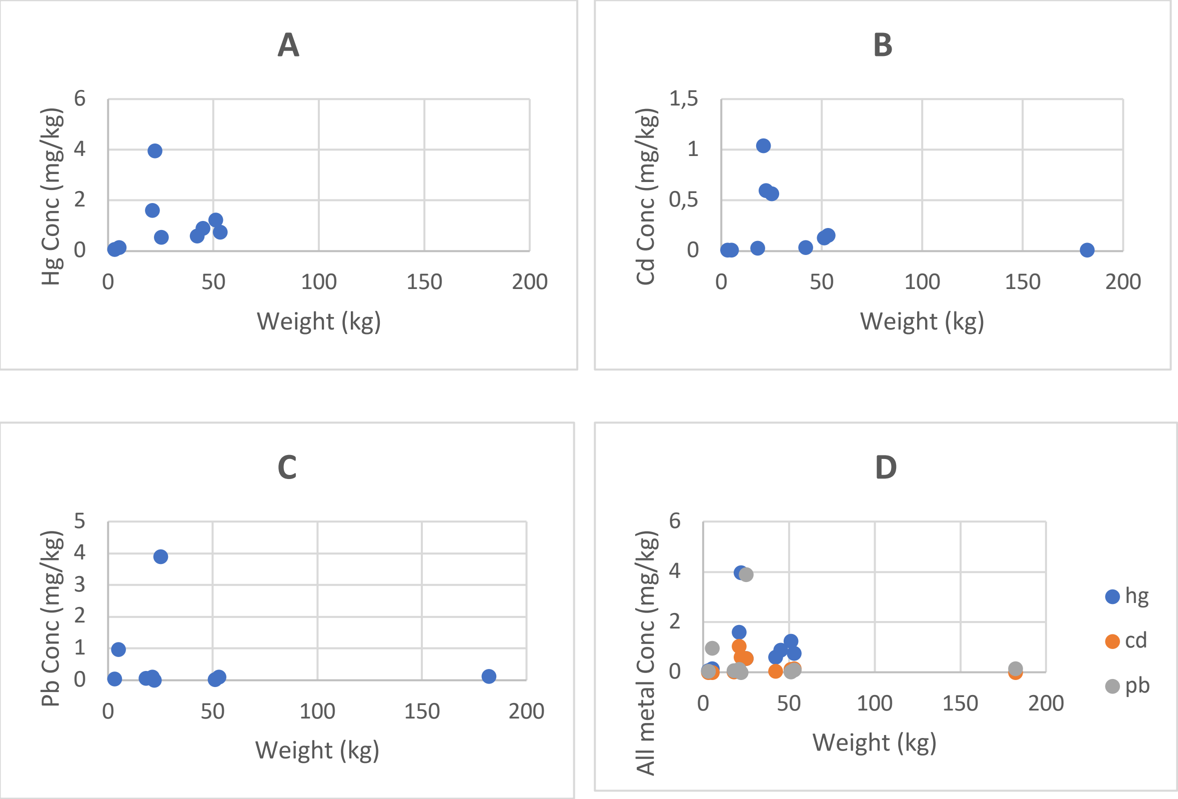


Fig. 14. Concentration levels of toxic metals (mg/kg) vs. Weight (kg) of swordfish. A-Hg, B- Cd, C-Pb, D-All metals.

The longevity of this species—whose average life span is over 25 years—also contributes to the high Hg concentration in swordfish (Monteiro & Lopes, 1990). Swordfish feeds in mesopelagic waters rather than exclusively epipelagic seas like yellowfin tuna because these waters are weakly oxygenated and thus host the process of methylation, which is the major form of accumulating in organisms (Kojadinovic et al., 2007). According to Di Bella et al. (2020), swordfishes longer than 125 cm were noted to have greater levels of Hg. The same was observed for swordfish, with a 127 cm length and 3.97 mg/kg of Hg. Also, in the works of Mehouel et al. (2019) and Bodin et al. (2017), Cd and Pb showed no relationship between size and harmful metal accumulation.

3.1.2 TUNA

The information extracted from the 19 published papers about biometry and toxic metals in tunas that fulfilled the stipulated requirements for inclusion in this review is compiled in Table 3 and Table 4. Mercury (Hg), cadmium (Cd), lead (Pb) and arsenic (As) were studied in tunas and reported since the year 2000 (respectively, 15, 14, and 7 out of the published papers). These metals are the most important form of pollution of the aquatic environment because of their toxicity and accumulation by marine organisms (Okyere et al., 2015).

In the next subsections, the concentrations of the toxic metals in tunas are examined considering the factors that expectedly affect the observed changes (i.e., moderator ("exploratory") variables *sensu* meta-analysis), namely location, fish size and species.

Location

As tunas are highly migratory species the risk of bioaccumulation of large amounts of toxicants, such as the toxic metals mercury (Hg), cadmium (Cd), lead (Pb) and Arsenic (As) increases. Hg, Cd, Pb and As are non-essential elements that can be very harmful even at low concentrations, occupying top positions in the lists of toxicants and recognized for their toxicity towards most organisms (Copat et al., 2015).

Extensive research has been carried out every year to monitor the toxic metals in seas with the help of fishes. Papers reporting concentrations levels of toxic metals in tuna published since the year 2000 studied different areas in the Mediterranean Sea, e.g. Tyrrhenian Sea, north and southeastern Aegean Sea, areas in the Atlantic Ocean, e.g. Mid-Atlantic Ocean, Bay of Biscay, and areas in the Indian Ocean, e.g. Sultanate of Oman, Western Indian Ocean, Red Sea, North Indian Ocean (Arabian Sea), South China Sea, e.g. Kuala Besut, Kuala Terengganu, Dungun, Kemaman, Pacific Ocean, e.g. Ecuador.

In the Mediterranean Sea, Hg ranged from minimum of 0.03 mg/kg (Al-Busaidi et al., 2011) to 3 mg/kg (Licata et al., 2005), while contents in Cd ranged 0.08 mg/kg to 0.1 mg/kg (Stamatis et al., 2019) and in Pb they from 0.02 mg/kg (Licata et al., 2005) to 0.04 mg/kg (Stamatis et al., 2019). No data was available for As in this region. Among all the three oceans it was the only ocean with high Hg concentration because this region has a long-lasting legacy of mercury mining activities and a high density of submarine volcanoes that has strongly contributed to its mercury budget (Cinnirella et al., 2019).

The reported concentrations of Hg in tuna in the Atlantic Ocean ranged from 0.1 mg/kg (Torres et al., 2015) to 1.7 mg/kg (Chouvelon et al., 2017), 0.01 mg/kg (Ugarte et al., 2012) to 0.3 mg/kg (Chouvelon et al., 2017) for Cd, 0.01 mg/kg (Chouvelon et al., 2017) to 0.1 mg/kg (Torres et al., 2015) for Pb, and for As, 0.0 mg/kg to 1.5 mg/kg (Ugarte et al., 2012). Chouvelon et al. (2017) reported high Hg concentration in the Atlantic Ocean as compared to other authors in the same region (Torres et al., 2015; Ugarte et al., 2012).

In the Indian Ocean, Hg ranged from 1.1 mg/kg to 0.5 mg/kg (Kojadinovic et al.,2007). For Cd, values between 0.1 mg/kg (Al-Shwafi, 2002) and 0.7 mg/kg (Ahmed et al., 2015) were observed. For Pb it ranged between 0.02 mg/kg (Kojadinovic et al., 2007) to 0.27 mg/kg (Ahmed et al., 2015) and for As no data was available for this ocean.

Comparing locations, the highest levels of Hg, 3 mg/kg, were found in Tyrrhenian Sea (Italy) by Licata et al. (2005) and the lowest concentration, 0.03 mg/kg, was determined in samples obtained from Indian Ocean by Al-Busaidi et al. (2011). When looking at levels of Cd, the highest levels were 2.4 mg/kg in the Pacific Ocean (Araújo & Cedeño-Macias, 2016) and the lowest concentration, 0.01 mg/kg, in the Atlantic Ocean (Ugarte et al., 2012). In the Atlantic Ocean, the concentrations of Pb in tuna fish ranged from a minimum of 0.01 mg/kg (Chouvelon et al., 2017) to a maximum of 0.1 mg/kg (Torres et al., 2015). The concentrations of As in tuna fish varied from a minimum of 0.92 mg/kg in the Pacific Ocean (Ruelas-Inzunza et al., 2018) to a maximum of 30.5 mg/kg, in the Red China Sea (Aziz et al., 2022).

Results show that the South China Sea fleet (in the Terengganu waters) has the highest As content (30.5 mg/kg) (Aziz et al., 2022), followed by tuna fish from Straits of Messina (Italy) which has the highest Hg concentration, 3.03 mg/kg (Licata et al., 2005). According to Araújo & Cedeño-Macias (2016), Kojadinovic et al. (2007), Ruelas-Inzunza et al. (2018) and Chouvelon et al. (2017), tunas collected in Eastern Pacific, Atlantic Ocean and Indian ocean waters have high mercury values (>1 mg/kg), and all the individual samples had concentrations that are greater than the permitted ingestion limit: 1 ppm or 1 mg/kg (European Commission, 2006).

Araújo & Cedeño-Macias (2016) found elevated amounts of Hg in Ecuador, though, with a mean of 1.4 mg/kg and a range of (<0.011–17 mg/kg). This might be because of East Pacific volcanic activity and emissions from Asian industrialized nations (Oktariani et al., 2023). Similar amounts of Hg were reported in the literature for tuna. Chouvelon et al. (2017)

conducted many studies on predatory fishes collected in the same FAO fishing areas obtained values ranging 0.90 mg/kg in tuna caught in Atlantic Ocean to 1 mg/kg in tuna caught in Atlantic Ocean around Reunion Island. For instance, the current systems in the central Pacific Ocean may transport mercury (Hg) from developed nations like Japan and China to the center Pacific, where it precipitates to the bottom (Goldberg, 1975). Low concentrations of lead and cadmium were found by Ruelas-Inzunza et al. (2018), 0.09-0.03 mg/kg Pb and 0.04-0.10 mg/kg Cd, and by Falco et al. (2006), respectively 0.02-0.09 mg/kg Cd and 0.23-0.25 mg/kg Pb, in the Western Indian Ocean.

Except for the high amounts of Hg and As, the metal concentrations in the different species were below the established limits throughout the research.

Table 3. Published papers retrieved and analysed in this with information about the species of tuna, year and location of sampling.

Paper	Citation	DOI	Species	Year (sampling)	Location
1	Al-Busaidi et al., 2011	http://dx.doi.org/10.1016/j.chemosphere.2011.05.057	Skipjack tuna°	2007	Sultanate of Oman
1	Al-Busaidi et al., 2011	http://dx.doi.org/10.1016/j.chemosphere.2011.05.057	Yellowfin tuna•	2007	Sultanate of Oman
2	Araújo & Cedeño-Macias, 2016	http://dx.doi.org/10.1016/j.scitotenv.2015.09.090	Yellowfin tuna•	2013	Pacific Ocean[1]
3	Chouvelon et al., 2017	http://dx.doi.org/10.1016/j.scitotenv.2017.04.048	Albacore tuna^	2013	Atlantic Ocean[2]
3	Chouvelon et al., 2017	http://dx.doi.org/10.1016/j.scitotenv.2017.04.048	Albacore tuna^	2013	Western Indian and South-eastern Atlantic Oceans[3]
3	Chouvelon et al., 2017	http://dx.doi.org/10.1016/j.scitotenv.2017.04.048	Albacore tuna^	2013	Western Indian and South-eastern Atlantic Oceans[3]
4	Miedico et al., 2020	https://doi.org/10.1016/j.jfca.2020.103638	Skipjack tuna°	2014	
4	Miedico et al., 2020	https://doi.org/10.1016/j.jfca.2020.103638	Unspecified tuna	2014	
4	Miedico et al., 2020	https://doi.org/10.1016/j.jfca.2020.103638	Yellowfin tuna•	2014	
5	Burger et al., 2005	https://doi.org/10.1016/j.envres.2005.02.001	Yellowfin tuna•	2005	New Jersey[4]
6	Aziz et al., 2022	https://doi.org/10.47853/FAS.2022.e15	Longtail tuna©	2022	Kuala Besut [5]
6	Aziz et al., 2022	https://doi.org/10.47853/FAS.2022.e15	Longtail tuna©	,2022	Kuala Terengganu[5]
6	Aziz et al., 2022	https://doi.org/10.47853/FAS.2022.e15	Longtail tuna©	2022	Dungun[5]

6	Aziz et al., 2022	https://doi.org/10.47853/FAS.2022.e15	Longtail tuna©	2022	Kemaman[5]
7	Torres et al., 2015	https://doi.org/10.1007/s00244-015-0249-1	Bigeye§	2011	Mid-Atlantic region[6]
7	Torres et al., 2015	https://doi.org/10.1007/s00244-015-0249-1	Skipjack tuna◦	2011	Mid-Atlantic region[6]
8	Licata et al., 2005	https://doi.org/10.1007/s10661-005-2382-1	Bluefin tuna֍	2003	Tyrrhenian Sea[7]
9	Ruelas-Inzunza et al., 2018	https://doi.org/10.1007/s11356-018-2166-0	Skipjack tuna◦	2008	Eastern Pacific Ocean
9	Ruelas-Inzunza et al., 2018	https://doi.org/10.1007/s11356-018-2166-0	Yellowfin tuna•	2008	Eastern Pacific Ocean
9	Ruelas-Inzunza et al., 2018	https://doi.org/10.1007/s11356-018-2166-0	Skipjack tuna◦	2008	Eastern Pacific Ocean
9	Ruelas-Inzunza et al., 2018	https://doi.org/10.1007/s11356-018-2166-0	Yellowfin tuna•	2008	Eastern Pacific Ocean
10	Kojadinovic et al., 2007	https://doi.org/10.1016/j.envpol.2006.07.015	Yellowfin tuna•	2004	Western Indian Ocean[8]
10	Kojadinovic et al., 2007	https://doi.org/10.1016/j.envpol.2006.07.015	Yellowfin tuna•	2004	Western Indian Ocean[8]
11	Al-Shwafi, 2002	http://hdl.handle.net/10576/9621	Yellowfin tuna•	1998	Red Sea[9]
11	Al-Shwafi, 2002	http://hdl.handle.net/10576/9621	Yellowfin tuna•	1998	Red Sea[9]
12	Ugarte et al., 2012	https://doi.org/10.1080/03067319.2011.603078	Albacore tuna^	2012	Cantabrian Sea coastal fleet (Bay of Biscay)
12	Ugarte et al., 2012	https://doi.org/10.1080/03067319.2011.603078	Bluefin tuna֍	2012	North Atlantic
13	Falcó et al., 2006	https://doi.org/10.1021/jf0610110	Tuna	2005	Spain
14	Stamatis et al., 2019	http://dx.doi.org/10.3390/ijerph16050821	Albacore tuna^	2015	North Aegean Sea
14	Stamatis et al., 2019	http://dx.doi.org/10.3390/ijerph16050821	Albacore tuna^	2015	Southeastern Aegean Sea

15	Ahmed et al., 2015	http://dx.doi.org/10.5296/ast.v3i1.6814	Longtail tuna©	2006	Arabian Sea[10]
15	Ahmed et al., 2015	http://dx.doi.org/10.5296/ast.v3i1.6814	Longtail tuna©	2007	Arabian Sea[10]
15	Ahmed et al., 2015	http://dx.doi.org/10.5296/ast.v3i1.6814	Longtail tuna©	2008	Arabian Sea[10]
15	Ahmed et al., 2015	http://dx.doi.org/10.5296/ast.v3i1.6814	Longtail tuna©	2009	Arabian Sea[10]
15	Ahmed et al., 2015	http://dx.doi.org/10.5296/ast.v3i1.6814	Longtail tuna©	2010	Arabian Sea[10]
16	Asmah et al., 2014	https://www.fisheriesjournal.com/archives/2014/vol1issue6/PartB/136.pdf	Frigate Tuna	2013	West Africa, Ghana (local fishermen)
16	Asmah et al., 2014	https://www.fisheriesjournal.com/archives/2014/vol1issue6/PartB/136.pdf	Atlantic Little Tuna	2013	West Africa, Ghana (local fishermen)
16	Asmah et al., 2014	https://www.fisheriesjournal.com/archives/2014/vol1issue6/PartB/136.pdf	Skipjack Tuna	2013	West Africa, Ghana (local fishermen)
17	Jinadasa et al., 2014	http://dx.doi.org/10.1080/19393210.2014.938131	Yellowfin tuna	2009	Sri Lankan main exporting companies[5]
18	Jinadasa et al., 2010	http://dx.doi.org/10.4038/sljas.v15i0.5481	Yellowfin tuna	2009	Sri Lanka local fish market

1 Playita Mía (Ecuador), 2 Reunion Island (REU), 3 South Africa, 4 fish market, 5 Terengganu waters (South China Sea) (local market), 6 Azores (Portugal), 7 Straits of Messina (Italy), 8 (Mozambique Channel) (Réunion Island), 9 Gulf of Aden (Yemen), 10 North Eastern border of Pakistan, 11 Local market. ○ Katsuwonus pelamis, ● Thunnus albacares, ^ Thunnus alalunga, + Auxis thazard, ¤ Euthynnus alletteratus, © Thunnus tonggol, § Thunnus obesus, ֍ Thunnus thynnus

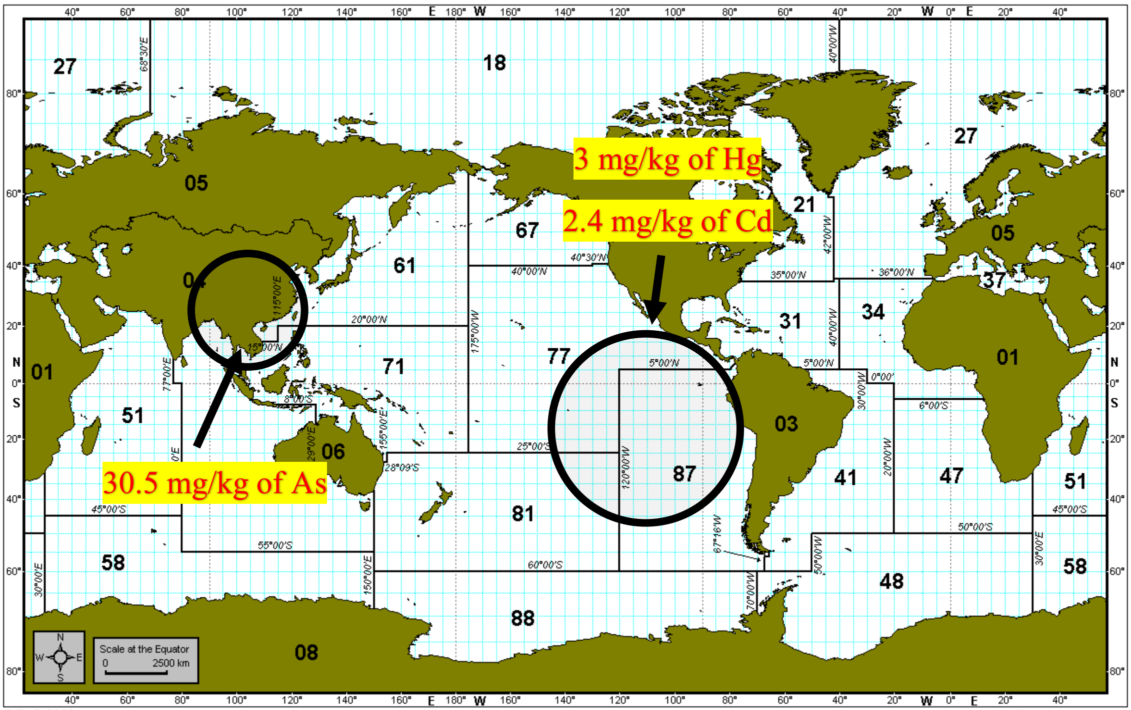
Table 4. Information retrieved from the published papers considered in this study: sample size, year, species of tuna, biometric results (TL, cm, and BW, kg) and toxic metals concentrations (mg/kg wet weight, presented as mean±standard deviation (X±SD)) in the muscle tissues of tuna.

Paper	Citation	Study no.	Sample size (n)	Sample year	Species	TL	BW	Location	Hg	Cd	Pb	As
1	Al-Busaidi et al., 2011	1	102	2007	Skipjack tuna[○]			Sultanate of Oman	0.06±0.1	0.004±0.01	0.04±0.1	
1	Al-Busaidi et al., 2011	2	346	2007	Yellowfin tuna[•]			Sultanate of Oman	0.03±0.08	0.009±0.06	0.02±0.1	
2	Araújo & Cedeño-Macias, 2016	3	44	2013	Yellowfin tuna[•]			Pacific Ocean[1]	1.4±1.3	2.4±5.1	0.07±0.06	
3	Chouvelon et al., 2017	4	443	2013	Albacore tuna[^]	102 ± 5		Atlantic Ocean[2]	1.7±0.6	0.07±0.03	0.01±0.03	
3	Chouvelon et al., 2017	5	443	2013	Albacore tuna[^]	96 ± 5		Atlantic Ocean[3]	1.4±0.4	0.09±0.1		
3	Chouvelon et al., 2017	6	443	2013	Albacore tuna[^]	87 ± 8		Atlantic Ocean[3]	0.9±0.4	0.3±0.2	0.01±0.02	
4	Miedico et al., 2020	7	3		Skipjack tuna[○]				0.3±0.3	0.02±0.01		
4	Miedico et al., 2020	8	33		unspecified tuna				0.4±0.5	0.02±0.02	0.02±0.04	
4	Miedico et al., 2020	9	45		Yellowfin tuna[•]				0.5±0.4	0.01±0.01	0.01±0.02	
5	Burger et al., 2005	13	50	2005	Yellowfin tuna[•]			New Jersey[4]	0.6±0.1	0.03±0.05	0.04±0.01	1.0±0.1
6	Aziz et al., 2022	14	25	2022	Longtail tuna[©]	51.5±1.8	1.6±0.1	Kuala Besut[5]	0.4±0.09			17.8±4.1
6	Aziz et al., 2022	15	25	2022	Longtail tuna[©]	44.3±3.3	1.1±0.2	Kuala Terengganu[5]	0.1±0.03			8.7±0.1
6	Aziz et al., 2022	16	25	2022	Longtail tuna[©]	49.7±3.8	1.7±0.4	Dungun[5]	1.5±0.3			30.5±6.8
6	Aziz et al., 2022	17	25	2022	Longtail tuna[©]	38.1±5.1	0.8±0.3	Kemaman[5]	0.4±0.1			16.7±5.1
7	Torres et al., 2015	18	15	2011	Bigeye[§]		10.6±0.8	Mid-Atlantic region[6]	0.1±0.02	0.1±0.05	0.03±0.001	

7	Torres et al., 2015	19	15	2011	Skipjack tuna○		3.3±0.2	Mid-Atlantic region[6]	0.04±0.01	0.1±0.05	0.1±0.02	
8	Licata et al., 2005	20	14	2003	Bluefin tuna❁			Tyrrhenian Sea[7]	3±0.5	0.03±0.07	0.02±0.06	
9	Ruelas-Inzunza et al., 2018	21	24	2008	Skipjack tuna○	43±66	3.6±1.3	Eastern Pacific Ocean	0.9±0.7			3.7±0.8
9	Ruelas-Inzunza et al., 2018	22	22	2008	Yellowfin tuna•	46±185	21.7±21.3	Eastern Pacific Ocean	0.7±0.6			2.2±0.6
9	Ruelas-Inzunza et al., 2018	23	6	2008	Skipjack tuna○		4.3±0.5	Eastern Pacific Ocean	1.1±0.1	0.03±0.005	0.05±0.02	1.5±0.04
9	Ruelas-Inzunza et al., 2018	24	14	2005	Yellowfin tuna•		15.7±0.5	Eastern Pacific Ocean	0.8±0.2	0.09±0.2	0.1±0.09	0.9±0.1
10	Kojadinovic et al., 2007	25	24	2006	Yellowfin tuna•	107±22.7	21±12.5	Western Indian Ocean[8]	0.5±0.3	0.2±0.2	0.09±0.1	
10	Kojadinovic et al., 2007	26	24	2006	Yellowfin tuna•	104±30.2	24±18.2	Western Indian Ocean[8]	1.1±2.3	0.2±0.2	0.02±0.07	
11	Al-Shwafi, 2002	27	7	1998	Yellowfin tuna•	80±05	0.63±0.06	Indian Ocean[9]		0.1±0.02	0.2±0.09	
11	Al-Shwafi, 2002	28	7	1998	Yellowfin tuna•	74.5±7.2	0.58±0.06	Indian Ocean[9]		0.2±0.03	0.3±0.04	
12	Ugarte et al., 2012	29	22	2012	Albacore tuna^			Atlantic Ocean[13]	0.4±0.2	0.01±0.006	0.02±0.007	1.5±0.3
12	Ugarte et al., 2012	30	12	2012	Bluefin tuna❁			North Atlantic Ocean[13]	0.5±0.1	0.01±0.003	0.02±0.005	1.2±0.3
13	Falco et al., 2006	31	20	2005	Tuna			Spain	0.4±0.05	0.01±0.02	0.01±0.02	0.99±1.2
14	Stamatis et al., 2019	32	82	2015	Albacore tuna^	87.6±5.2	9.8±1.1	Mediterranean Sea[12]	0.4±0.1	0.1±0.1	0.04±0.1	
14	Stamatis et al., 2019	33	82	2015	Albacore tuna^	70.8±5.3	9.8±1.1	Mediterranean Sea[12]	0.4±0.1	0.08±0.1	0.04±0.07	
15	Ahmed et al., 2015	34	52	2006	Longtail tuna©	62±2.2	2.5±0.2	Indian Ocean[10]		0.2±0.1	0.18±0.1	

15	Ahmed et al., 2015	35	63	2007	Longtail tuna©	62±2.1	2.2±0.2	Indian Ocean[10]		0.6±0.1	0.23±0.14	
15	Ahmed et al., 2015	36	65	2008	Longtail tuna©	60±1.7	2.4±0.3	Indian Ocean[10]		0.4±0.2	0.19±0.11	
15	Ahmed et al., 2015	37	62	2009	Longtail tuna©	61±1.7	2.4±0.3	Indian Ocean[10]		0.2±0.1	0.27±0.12	
15	Ahmed et al., 2015	38	58	2010	Longtail tuna©	61±3.6	2.6±0.5	Indian Ocean[10]		0.7±0.1	0.22±0.18	
16	Jinadasa et al., 2014	10	140	2009	Yellowfin tuna•	123.4±118.5	45.3±50.7	Indian Ocean[14]	0.90±0.52	0.1±0.05	0.11±0.45	
17	Jinadasa et al., 2010	10	25	2009	Yellowfin tuna•	128±125.5	46.3±50.75	Sri Lanka[4]	0.39±0.51	0.02±0.04	0.06±0.12	
18	Asmah et al., 2014	10	6	2013	Frigate Tuna	34.2±2.48	0.60±0.11	West Africa		0.24±0.2	0.92±0.4	
18	Asmah et al., 2014	11	6	2013	Atlantic Little Tuna	45±1.41	1±0.04	West Africa		<0.10	0.20±0.02	
18	Asmah et al. ,2014	12	7	2013	Skipjack Tuna	44.8±5.57	1.54±0.004	West Africa		0.13±0.09	0.39±0.2	
19	Ordiano-Flores et al., 2011	39	68	2010	Yellowfin tuna	74.3±11.4		Baja California Sur, Mexico	0.51±0.32			
19	Ordiano-Flores et al., 2011	40	200	2010	Yellowfin tuna	92.2±19.5		Equatorial Zone, Mexico	0.98±0.68			

Legend: TL-total length (cm), BW-body weight (kg), sd-standard deviation. 1 Playita Mía (Ecuador), 2 Reunion Island (REU), 3 South Africa (western Indian and south-eastern), 4 fish market, 5 Terengganu waters (South China Sea) (local market), 6 Azores (Portugal), 7 Straits of Messina (Italy), 8 (Mozambique Channel) (Réunion Island), 9 Red Sea (Yemen), 10 North Eastern border of Pakistan, 11 Local market, 12 North and Southeastern Aegean Sea, 13 Cantabrian Sea coastal fleet (Bay of Biscay), 14 Sri Lanka ○ Katsuwonus pelamis, ● Thunnus albacares, ^ Thunnus alalunga, + Auxis thazard, ¤ Euthynnus alletteratus, © Thunnus tonggol, § Thunnus obesus, ֍ Thunnus thynnus.



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Miller cylindrical projection

Fig. 15. Areas marked with circles have reportedly high concentration of toxic metals, Hg, Cd, Pb and As, in Tuna (map adapted from FAO, 2022)

In the Eastern Pacific Ocean, particularly in Mexico and USA, some authors have verified relatively high metal levels in yellowfin tuna, although the risk to human health has been considered low (Ordiano-Flores et al., 2011; Ralston et al., 2007). Yellowfin tuna from Mediterranean Sea (Fig. 15) have the highest mercury content, 3 mg/kg (Licata et al., 2005), much higher than data in Ferriss and Essington (2011), viz. mean of 0.3 µg/g with a maximum of 0.7 µg/g for yellowfin tuna, in the same region. In the case of albacore, *T. alalunga*, Chouvelon et al. (2017) reported 0.09 mg/kg, while for albacore, *T. albacares,* Miedico et al. (2020) found 0.5 mg/kg and for bigeye Torres et al. (2015) reported 0.1 mg/kg. All values are under the permissible limit (1 mg/kg, European Commission, 2006). Drevnick et al. (2015) reported that an annual rate of 3.8% increase in Hg in Eastern Pacific Ocean was observed from 1998 to 2008. This indicates that mercury is becoming the most dominating toxic metal among others. Considering the mean values, 65.5% of the sampled muscle of yellowfin tuna presented values of Cd and Pb below the permissible limits (in the EU, ≤0.10 mg/kg and ≤0.30 mg/kg, respectively). In *T. albacares*, the highest concentration of As was reported in specimens from Eastern Pacific Ocean, 2.2 mg/kg (Ruelas-Inzunza et al., 2018). The most elevated levels of Hg (1.40 mg/kg, Araújo & Cedeño-Macias, 2016) were found in individuals from the Eastern Pacific (Ecuador). In the case of *K. pelamis*, has the most elevated concentrations of As (3.7 mg/kg) found in study (Ruelas-Inzunza at al., 2018, from Eastern Pacific) which is similar to values reported by Núñez et al. (2018), 1.43 mg/kg in the Mediterranean Sea off Spain (Fig. 15).

Near the Sultanate of Oman, the highest cadmium concentration was detected in yellowfin tuna with 2.4 mg/kg and it could be due to their feeding habits, as big species feed at higher tropical levels (Araújo & Cedeño-Macias.,2016). Highest lead concentration was detected in 0.92 mg/kg in Frigate Tuna, followed by skipjack tuna at a level of 0.39 mg/kg as reported by Asmah et al. (2014). The highest mercury concentration was found in bluefin tuna with a value of 3 mg/kg (Licata et al.,2005). Cd concentrations in muscle tissue of all the reported tunas did not exceed the EC maximum limits for edible tissues except for one, yellowfin tuna from Araújo & Cedeño-Macias.,2016 (2.4 mg/kg) . However, skipjack caught off Eastern Pacific Ocean and in front of the Baja California Peninsula had much less Cd (0.03 & 0.09 mg/kg) (Ruelas-Inzunza et al.,2018). Given that volcanic activity is an important natural input of Pb in the Azores and given the low Pb content in both species, it appears that the volcanic activity in the Azores has no measurable effect on the surrounding marine ecosystem Pb levels where these tuna species occur (Torres et al.,2015). In our data base the concentrations of Cd and Pb were found to be under limits.

Size (length and/or weight)

In the graphs of length versus concentrations of the various metals considered (Fig. 16), no pattern was observable relating toxic metals accumulation and fish size. Mercury (Hg) (3 mg/kg in unknown length) and cadmium (Cd) (0.82 mg/kg~70 cm long specimen) were found to be present in more amounts as compared to the other studied metals. Arsenic (As) was found to be in amount which is 30.5 mg/kg for 47.8 cm.

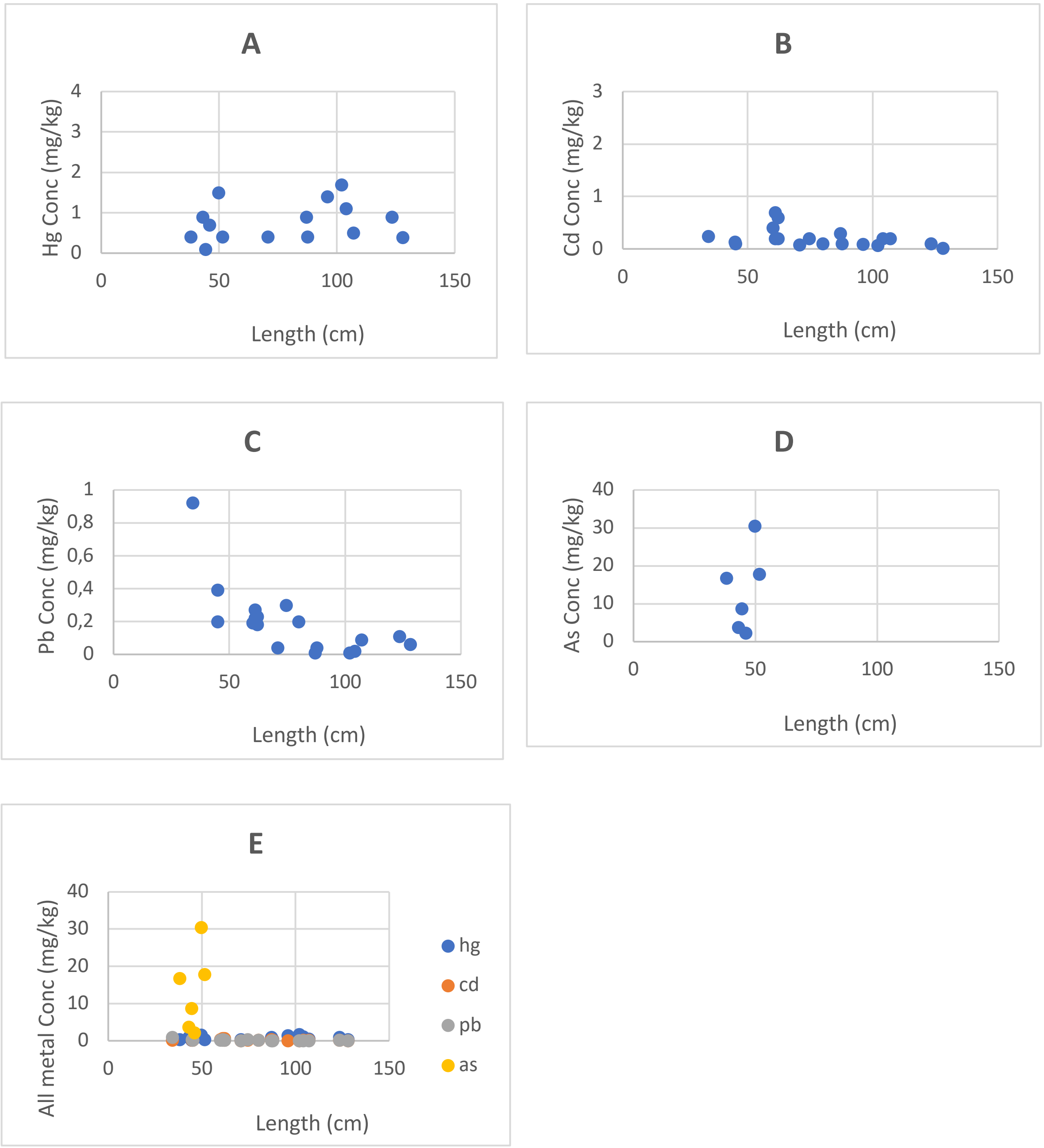


Fig. 16. Concentration levels of toxic metals (mg/kg) vs. length (cm) of Tuna.
A-Hg, B- Cd, C-Pb, D-As, E-All metals.

In terms of weight, 5 kg of bluefin tuna contains ca. 3 mg/kg of mercury making it the most accumulated metals in tuna as represented in (Fig. 17 A) (Licata et al., 2005). About 2 mg/kg of cadmium was found in tuna fish with 9 kg weight (Fig. 17 C). Lead was found to be in the least concentration with respect to other metals (Fig. 17 B). One 1.7 kg of longtail tuna contained 30.5 mg/kg of arsenic (Fig. 17 D) (Aziz et al., 2022).

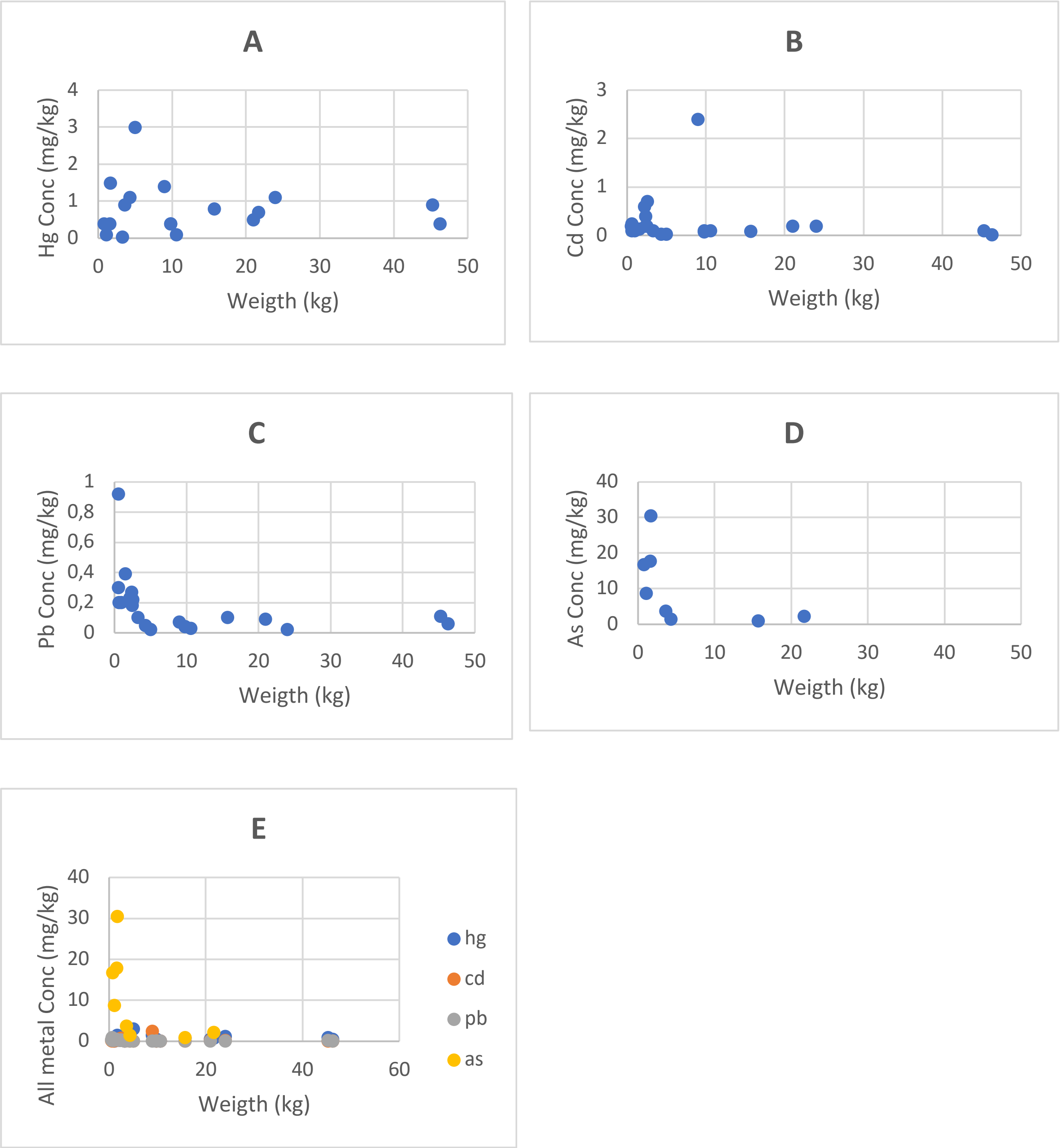


Fig. 17. Concentration levels of toxic metals (mg/kg) vs. weight (kg) of Tuna.
A-Hg, B- Cd, C-Pb, D-As, E-All metals.

According to Phillips et al. (1980), Braune (1987), Lange et al. (1994), Lacerda et al. (1993), Bidone et al. (1997), and Burger et al. (2001), certain metals bioaccumulate with fish size and age. In contrast, no significant relationships between concentrations and individual weight were found by Gobert et al. (2017), which is consistent with our results. Other authors have noted a positive correlation between the size of yellowfin (*T. albacares*) and skipjack tuna (*K. pelamis*) caught in the Atlantic Ocean, South China Sea, and Western Indian Ocean, respectively. These authors include, Agusa et al. (2005), Bosch et al. (2016), and Kojadinovic et al. (2007). Yunus et al. (2020) and Zaini et al. (2020) researched heavy metals in South China Sea water and found high As concentrations because of high natural and anthropogenic sources. As was found in high concentrations in *T. tonggol* compared to other metals, and to the best of our knowledge, there is very little information that would support a link between metal accumulation in this species, size, and weight.

Tuna Species

Several species of tuna are subjected to fishing and eventually become food products for human consumption. Concentration levels published for the different species of tuna are presented and discussed next together with a short description of the species' biology and ecology.

***Thunnus albacares* (yellowfin tuna)**

Yellowfin tuna, *T. albacares*, is a fusiform fish that can reach >200 cm long (commonly 180 cm) and 200 kg live weight and inhabits the mixed surface layer of the ocean above the thermocline, living in the upper portion of the water column. They are found worldwide in tropical and subtropical waters, from latitudes of approximately 40°N to 35°S and can be found in both pelagic and coastal waters, typically in schools. Yellowfin tunas are highly migratory their swimming speed 4-5 km/h (top at 48 km/h) and can reproduce year-round. Their diet generally includes fish, crustaceans, cephalopods and molluscs. They are known for their high-quality meat, which is often considered a delicacy, and their strong fighting ability when hooked. Yellowfin tuna is an important commercial tuna species, particularly the raw sashimi market. A 100 g serving of yellowfin tuna has 109 kcal, 24.4 g protein, and 0.49 g fat. Yellowfin tuna is a natural source of rich minerals including iodine, selenium, calcium, zinc, potassium, phosphorus, and magnesium. It is also a moderate source of polyunsaturated fatty acids (PUFA). Yellowfin tuna is caught using several different fishing gears, driftnets, gillnets, purse seines, net traps (e.g., armações/almadravas) handlines, and hand-operated pole-and-lines (Wright et al., 2021).

In the published papers on *T. albacares*, the concentration of toxic metals ranged 0.03 mg/kg (Al-Busaidi et al., 2011) to 1.4 mg/kg (Araújo & Cedeño-Macias, 2016) in the case of Hg, 0.009 mg/kg (Al-Busaidi et al., 2011) to 2.4 mg/kg (Araújo & Cedeño-Macias, 2016) for Cd, 0.01 mg/kg (Miedico et al., 2020) to 0.11 mg/kg (Jinadasa et al., 2014) for Pb, and 1 mg/kg (Burger et al., 2005) to 2.2 mg/kg (Ruelas-Inzunza et al., 2018) for As. Concentration of Hg was 1.4 mg/kg for a <163 cm in case of yellowfin tuna (Araújo & Cedeño-Macias., 2016). As per the European Commission Regulation No. 1881/2006 the amount of Pb and Cd presented for yellowfin tuna were below the allowable limits, 0.30 mg/kg FW and 0.1 mg/kg FW, respectively (European Commission, 2006).

***Katsuwonus pelamis* (skipjack tuna)**

Skipjack tuna (*K. pelamis*) is a medium-sized fish that grows up to 1 m in length (2-4 kg of weight). It is a cosmopolitan pelagic fish found in tropical and warm-temperate waters worldwide, and it is a very important species for fisheries. Skipjack tuna is a shoaling species that often forms groups of up to 50,000 individuals and is known to swim in schools, especially around floating objects, or marine mammals. It is a mid-trophic level, highly migratory fish species that is distributed throughout tropical and subtropical waters. They can swim with a speed of ~75 km/h and can dive down to 1200-1800 m depth. Skipjack tuna is an important prey species for open ocean sharks and large bony fishes, especially the billfishes, and plays an important intermediate role in pelagic food webs. They mostly prey on small fishes, squid, and crustaceans. It is also an important commercial tuna species, and most of these catches are destined for canning or pouching. Skipjack tuna is a lean meat that is a good source of protein, selenium, and other minerals. A 100 g serving of skipjack tuna has 132 kcal, 28 g protein, and 1.3 g fat. Skipjack tuna is a popular fish for human consumption. Skipjack tuna is a lean meat that is a good source of protein, selenium, and other minerals. It is also a good choice for consumption as it contains just 0.144 ppm of mercury in its flesh, and the US FDA categorizes skipjack in the “best choice” section considering mercury levels in its flesh (Arrate et al., 2021; Collette et al., 2011).

In the published papers on *K. pelamis*, the concentration of toxic metals ranged 0.04 mg/kg (Torres et al., 2015) to 1.1 mg/kg (Ruelas-Inzunza et al., 2018) in the case of Hg, 0.004 mg/kg (Al-Busaidi et al., 2011) to 0.13 mg/kg (Asmah et al., 2014) for Cd, 0.04 mg/kg (Al-Busaidi et al., 2011) to 0.39 mg/kg (Asmah et al., 2014) for Pb, and 1.5 mg/kg (Ruelas-Inzunza et al., 2018) to 3.7 mg/kg (Ruelas-Inzunza et al., 2018) for As.

***Thunnus tonggol* (longtail tuna)**

T. tonggol, known as longtail tuna or northern bluefin tuna, is a species of tuna that inhabits tropical Indo-West Pacific waters (Griffiths et al., 2019). It can grow up to 145 cm (commonly 81 cm) in length and 35.9 kg (normally about 9.1 kg) in weight and is more slender than other "true" bluefin tunas. Longtail tuna is a shoaling species that often forms groups of up to 50,000 individuals and is known to swim in schools, especially around floating objects, or marine mammals. It is a mid-trophic level, highly migratory fish species that is distributed throughout tropical and subtropical waters, with seasonal abundance in waters off Taiwan. They mostly prey on crustaceans, clupeid fishes, squids, tunicates, herring and sardines (Collette et al., 2011). Longtail tuna is an important commercial tuna species, and most of these catches are destined for canning or pouching. Longtail tuna is a good source of protein, selenium, and other minerals (Kazemi et al., 2022). A 100 g serving of longtail tuna has 266 kcal, 22.3 g protein, and 14.9 g fat. It is also a low mercury and safer food choice for human consumption as it is much further down the food chain than its albacore and blue fin tunas and contains about 0.03 mg/kg of mercury in its flesh (Abdussamad et al., 2012). However, it is important to be aware of the potential risks associated with consuming longtail tuna, particularly if they are caught in areas where contamination, is a concern. The mercury levels in longtail tuna can vary depending on the capture location.

In the published papers on *T. tonggol*, the concentration of toxic metals ranged 0.1 mg/kg to 1.5 mg/kg in the case of Hg (Aziz et al., 2022), 0.2 mg/kg to 0.7 mg/kg (Ahmed et al., 2015) for Cd, 0.18 mg/kg to 0.27 mg/kg (Ahmed et al., 2015) for Pb, and 8.7 mg/kg to 30.5 mg/kg (Aziz et al., 2022) for As. Arsenic was found to be in high concentrations in longtail tuna as compared to other metals, 30.5 mg/kg in a 49.7 cm length fish (Aziz et al., 2022). Cadmium was in least amount 0.2 mg/kg in a 62 cm length of longtail tuna (Ahmed et al., 2015).

In sum, the highest concentrations of the different metals were found in bluefin tuna 3 mg/kg, followed by yellowfin tuna, 1.4 mg/kg Hg and 2.4 mg/kg Cd, 0.92 mg/kg Pb in frigate tuna, and in longtail tuna, 30.5 mg/kg As. In research done over an active volcanic area in the Atlantic Ocean (Azores Islands), Torres et al. (2015) observed a similar bioaccumulation "distribution" for *K. pelamis*, i.e., greater Cd and Pb concentrations than Hg concentrations. It has been shown that the concentration of Pb is lower in deep ocean waters (0.01-0.02 g/L) than in surface ocean waters (0.3 g/L) (Sepe et al., 2003). This shows that species with shallower ranges are exposed to greater Pb concentrations than those with deeper ranges, and as a result, K. pelamis, a species with shorter ranges, can collect higher Pb levels in its tissues. In comparison to other larger tuna species like longtail tuna in the Mediterranean Sea, Torres et al. (2015) found that *K. pelamis* had a greater Cd content in its muscle tissue. Yellowfin tuna had the highest cadmium content, at 2.4 mg/kg, and this might be attributed to their eating patterns as large species tend to eat at higher trophic levels. Malakootian et al. (2016) and Ahmed et al. (2015) reported that longtail tuna from Persian Gulf had high Hg and Pb concentration among the fish. Andayesh et al. (2015) observed that As levels were very high and above the allowable range (1.99-2.47 mg/kg).

3.2 META-ANALYSIS

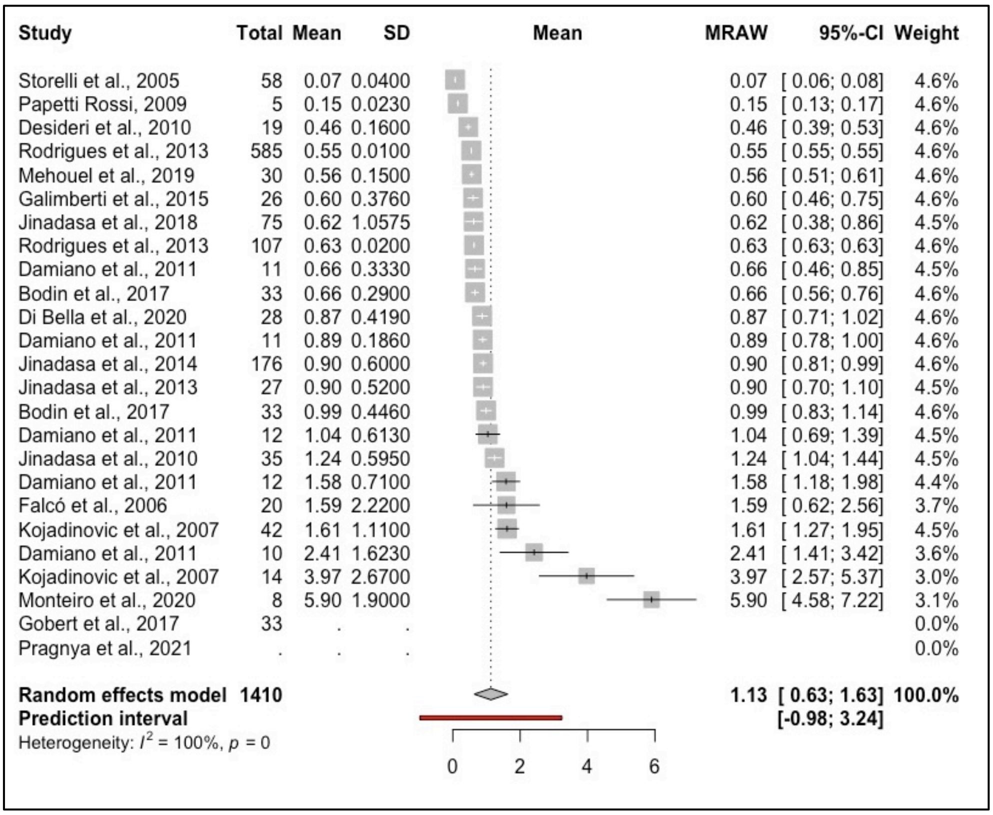
3.2.1. Swordfish

In the conducted metal-analysis of the 23 studies about toxic metals in swordfish, the estimated mean concentrations for three metals, Hg, Cd and Pb, are compiled in Table 5. Considering the studies retrieved, the mean concentrations of Hg, Cd and Pb were 1.13 mg/kg, 0.14 mg/kg, and 0.70 mg/kg, respectively. Even if the mean is just above the maximum limits (ML) for Hg and Cd, it is well above the ML in the case of Pb. When considering the 95% confidence intervals, all metals have studies with values above the MLs (Figs. 18, 19 and 20). It can also be observed in the forest plots (Figs. 18, 19 and 20) that there is quite variation in the results among studies. In each forest plot, the grey boxes represent the effect sizes (mean concentration of the toxic metal) found in each study and the whiskers their respective 95% confidence intervals. The bigger the box means the study weighted more (i.e., bigger sample size). The grey diamond shape at the bottom represents the pooled mean of the studies. The length of the diamond symbolizes the confidence interval of the pooled result on the x-axis. Typically, forest plots also include a vertical reference line, which indicates the point on the x-axis equal to no effect (set to 0), and another (dashed) line showing the pooled mean of the studies. When the grey diamond does not cross the vertical line, it indicates significance (i.e. the pooled effect is different from 0). We can confirm this also from the 95% confidence interval that does not include 0 (Tawfik et al., 2019).

Table 5. Estimated mean concentrations (mg/kg) and 95% confidence intervals of the toxic metals in swordfish derived from the meta-analysis of k=23 studies vs. Maximum limits.

Metal	**Mean concentration (mg/kg)**	**95% Confidence Interval**	**Maximum limits(1,2)**
Mercury	1.13	0.6338; 1.6275	0.5-1 mg/kg
Cadmium	0.14	0.0279; 0.2551	0.1 mg/kg
Lead	0.70	0.2196; 1.1726	0.2-0.5 mg/kg

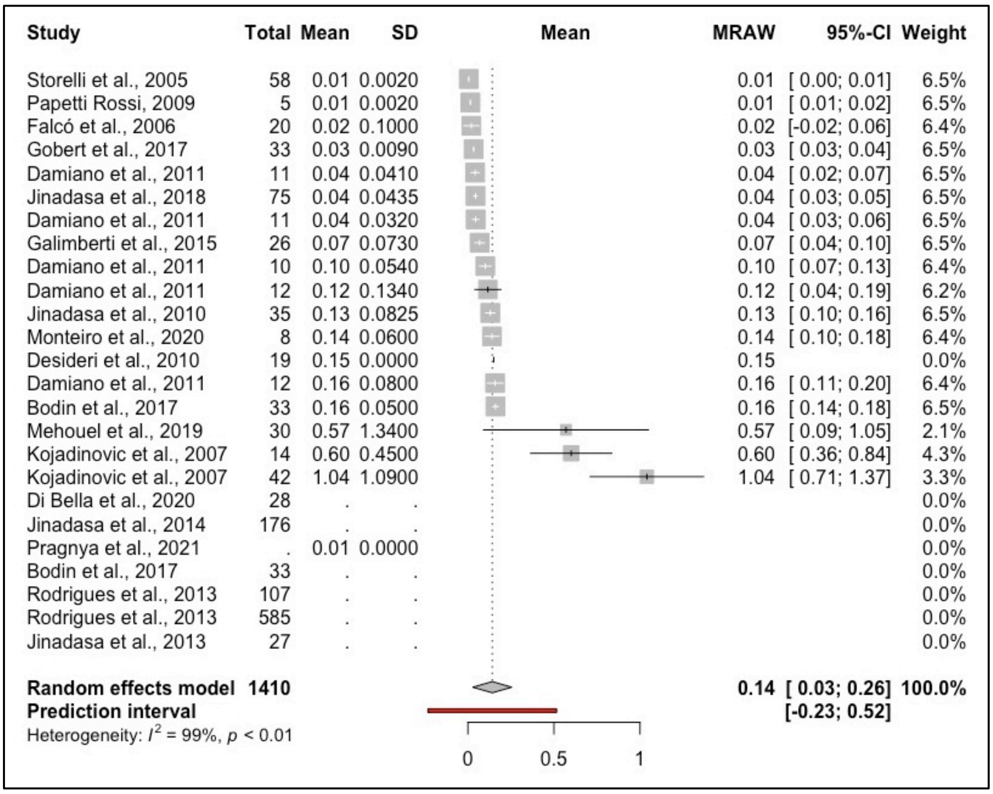
1. European Commission (2006), 2. FAO/WHO (2009)



Study	Total	Mean	SD	MRAW	95%-CI	Weight
Storelli et al., 2005	58	0.07	0.0400	0.07	[0.06; 0.08]	4.6%
Papetti Rossi, 2009	5	0.15	0.0230	0.15	[0.13; 0.17]	4.6%
Desideri et al., 2010	19	0.46	0.1600	0.46	[0.39; 0.53]	4.6%
Rodrigues et al., 2013	585	0.55	0.0100	0.55	[0.55; 0.55]	4.6%
Mehouel et al., 2019	30	0.56	0.1500	0.56	[0.51; 0.61]	4.6%
Galimberti et al., 2015	26	0.60	0.3760	0.60	[0.46; 0.75]	4.6%
Jinadasa et al., 2018	75	0.62	1.0575	0.62	[0.38; 0.86]	4.5%
Rodrigues et al., 2013	107	0.63	0.0200	0.63	[0.63; 0.63]	4.6%
Damiano et al., 2011	11	0.66	0.3330	0.66	[0.46; 0.85]	4.5%
Bodin et al., 2017	33	0.66	0.2900	0.66	[0.56; 0.76]	4.6%
Di Bella et al., 2020	28	0.87	0.4190	0.87	[0.71; 1.02]	4.6%
Damiano et al., 2011	11	0.89	0.1860	0.89	[0.78; 1.00]	4.6%
Jinadasa et al., 2014	176	0.90	0.6000	0.90	[0.81; 0.99]	4.6%
Jinadasa et al., 2013	27	0.90	0.5200	0.90	[0.70; 1.10]	4.5%
Bodin et al., 2017	33	0.99	0.4460	0.99	[0.83; 1.14]	4.6%
Damiano et al., 2011	12	1.04	0.6130	1.04	[0.69; 1.39]	4.5%
Jinadasa et al., 2010	35	1.24	0.5950	1.24	[1.04; 1.44]	4.5%
Damiano et al., 2011	12	1.58	0.7100	1.58	[1.18; 1.98]	4.4%
Falcó et al., 2006	20	1.59	2.2200	1.59	[0.62; 2.56]	3.7%
Kojadinovic et al., 2007	42	1.61	1.1100	1.61	[1.27; 1.95]	4.5%
Damiano et al., 2011	10	2.41	1.6230	2.41	[1.41; 3.42]	3.6%
Kojadinovic et al., 2007	14	3.97	2.6700	3.97	[2.57; 5.37]	3.0%
Monteiro et al., 2020	8	5.90	1.9000	5.90	[4.58; 7.22]	3.1%
Gobert et al., 2017	33	.	.			0.0%
Pragnya et al., 2021	.	.	.			0.0%
Random effects model	**1410**			**1.13**	**[0.63; 1.63]**	**100.0%**
Prediction interval					**[-0.98; 3.24]**	

Heterogeneity: $I^2 = 100\%$, $p = 0$

Fig. 18. Forest plot showing the meta-analysis for Hg in swordfish.



Study	Total	Mean	SD	MRAW	95%-CI	Weight
Storelli et al., 2005	58	0.01	0.0020	0.01	[0.00; 0.01]	6.5%
Papetti Rossi, 2009	5	0.01	0.0020	0.01	[0.01; 0.02]	6.5%
Falcó et al., 2006	20	0.02	0.1000	0.02	[-0.02; 0.06]	6.4%
Gobert et al., 2017	33	0.03	0.0090	0.03	[0.03; 0.04]	6.5%
Damiano et al., 2011	11	0.04	0.0410	0.04	[0.02; 0.07]	6.5%
Jinadasa et al., 2018	75	0.04	0.0435	0.04	[0.03; 0.05]	6.5%
Damiano et al., 2011	11	0.04	0.0320	0.04	[0.03; 0.06]	6.5%
Galimberti et al., 2015	26	0.07	0.0730	0.07	[0.04; 0.10]	6.5%
Damiano et al., 2011	10	0.10	0.0540	0.10	[0.07; 0.13]	6.4%
Damiano et al., 2011	12	0.12	0.1340	0.12	[0.04; 0.19]	6.2%
Jinadasa et al., 2010	35	0.13	0.0825	0.13	[0.10; 0.16]	6.5%
Monteiro et al., 2020	8	0.14	0.0600	0.14	[0.10; 0.18]	6.4%
Desideri et al., 2010	19	0.15	0.0000	0.15		0.0%
Damiano et al., 2011	12	0.16	0.0800	0.16	[0.11; 0.20]	6.4%
Bodin et al., 2017	33	0.16	0.0500	0.16	[0.14; 0.18]	6.5%
Mehouel et al., 2019	30	0.57	1.3400	0.57	[0.09; 1.05]	2.1%
Kojadinovic et al., 2007	14	0.60	0.4500	0.60	[0.36; 0.84]	4.3%
Kojadinovic et al., 2007	42	1.04	1.0900	1.04	[0.71; 1.37]	3.3%
Di Bella et al., 2020	28	.	.			0.0%
Jinadasa et al., 2014	176	.	.			0.0%
Pragnya et al., 2021	.	0.01	0.0000			0.0%
Bodin et al., 2017	33	.	.			0.0%
Rodrigues et al., 2013	107	.	.			0.0%
Rodrigues et al., 2013	585	.	.			0.0%
Jinadasa et al., 2013	27	.	.			0.0%
Random effects model	**1410**			**0.14**	**[0.03; 0.26]**	**100.0%**
Prediction interval					**[-0.23; 0.52]**	

Heterogeneity: $I^2 = 99\%$, $p < 0.01$

Fig. 19. Forest plot showing the meta-analysis for Cd in swordfish.

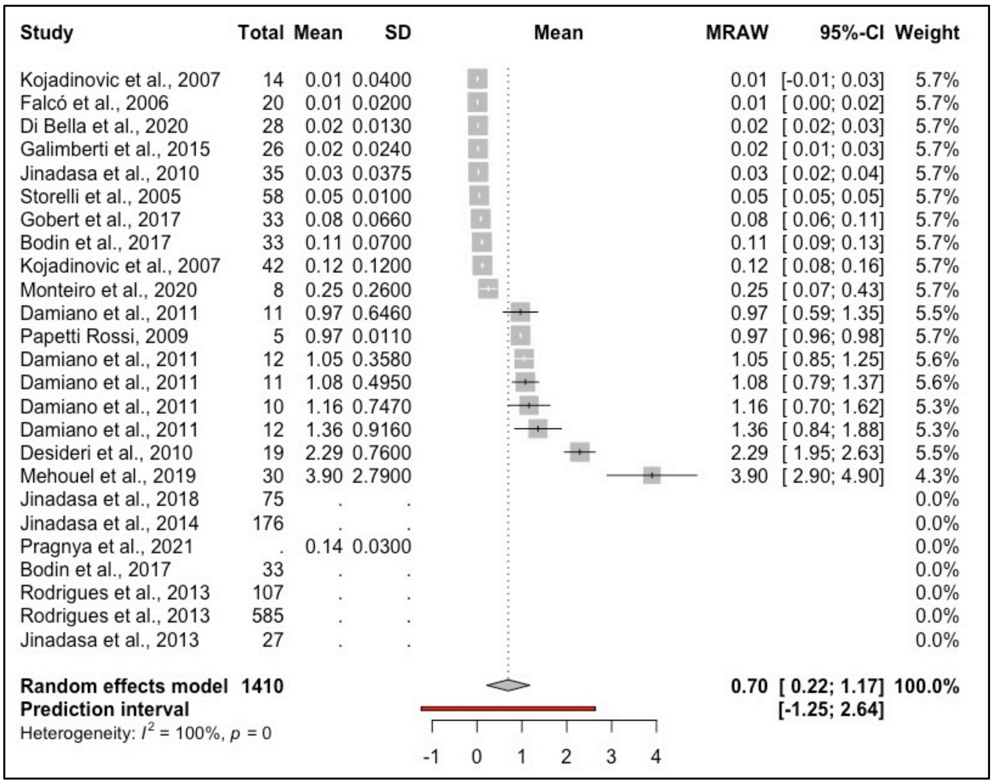


Fig. 20. Forest plot showing the meta-analysis for Pb in swordfish.

In fact, the variation observed is clearly describe by the heterogeneity statistics (Table 6). As a rule of thumb, the interpretation of I^2 in the context of meta-analyses [of randomized trials] is as follows (Jonathan et al., 2019): 0% to 40%: might not be important; 30% to 60%: may represent moderate heterogeneity; 50% to 90%: may represent substantial heterogeneity; and 75% to 100%: considerable heterogeneity. Comparing to our results for all the toxic metals, I^2 is ranging between 75% to 100% ($p<0.05$), so there is considerable heterogeneity among studies.

Table 6. Results for heterogeneity statistics (I^2, tau τ and tau^2) and Q test (Cochran's Q test) for the meta-analysis of studies about toxic metals in swordfish

Metal	I^2	τ	τ^2	Q-test	df	p-value
Mercury	99.8%	0.99	0.98	11966.70	22	< 0.0001
Lead	99.9%	9.08	82.50	35117.48	18	< 0.0001
Cadmium	98.5%	0.17	0.02	1072.01	16	< 0.0001

The observed high heterogeneity can also be caused by the fact that there are two or more subgroups of studies in our data that have a different true effect (i.e., mean concentration). When there's high heterogeneity, i.e. high variance between studies, one can attempt to model it using available study characteristics (i.e., moderator variables) in a subgroup analysis (Higgins et al., 2003). A subgroup analyses, following a mixed-effects model and using a Q-based test for subgroup differences (Harrer et al., 2021), was conducted with moderator variables/factors *year* and *location*. For both moderator variables, there are significant differences for every metal (Table 7). This is in line with the findings described above in Section 3.1.1.

Table 7. Results for the Q tests (Cochran's Q test) of heterogeneity for the meta-analysis of studies about toxic metals in swordfish considering moderator variables *year* and *location*.

Metal **Moderator variable**	**Q**	**df**	**p-value**
Hg			
Year	338.78	10	< 0.0001
Location	753.56	14	< 0.0001
Cd			
Year	636.89	9	< 0.0001
Location	866.36	11	< 0.0001
Pb			
Year	34209.27	10	< 0.0001
Location	22993.00	12	< 0.0001

Researchers are often restricted to published results, regardless of how comprehensive their search is. Study reporting bias, which can be influenced by the direction or strength of the results, is a clear cause of error. Several factors can impact whether, when, or how a study is published, all of which contribute to reporting bias (Andrel et al., 2009). On the other hand, missing studies can pose a threat to the validity of meta-analytic inferences. This caveat, also termed “File Drawer” problem, refers to the issue that not all relevant research findings are published and are therefore missing in a meta-analysis. It is widely believed that studies with negative or 'disappointing' results (i.e. non-confirming the initial hypothesis) are often underrepresented in published literature due to publication bias (e.g. Schmucker et al., 2014). Publication bias is one of several types of non-reporting bias. Several other factors can distort the evidence in meta-analyses (Page et al., 2020): citation bias, time-lag bias, multiple publication bias, language bias, and outcome reporting bias.

All these affect the meta-analysis. Funnel plots offer a graphical strategy for examining any potential biases in meta-analyses. These plots show the observed effect sizes of studies on the x-axis plotted against standard errors on the y-axis. Typically, the y-axis is inverted so that higher values represent lower standard errors (i.e. larger sample sizes). Ideally, an unbiased sample of studies will appear as a mostly symmetrical, upside-down funnel in the plots, centered on the population average effect. Funnel plots are used to assess heterogeneity in the effects and biases in primary studies, a proxy of publication bias (Andrel et al., 2009). The enhanced-contour funnel plots (Peters et al., 2008) for the metals' concentrations in swordfish are in Figures 21, 22 and 23. There is clear asymmetry in all the funnel plots and this may be caused by publication bias. In addition, since the perceived missing studies are in regions of the plots of low statistical significance (i.e. p>0.1), publication bias is probably the major cause of funnel asymmetry (Harrer et al., 2021; Peters et al., 2008).

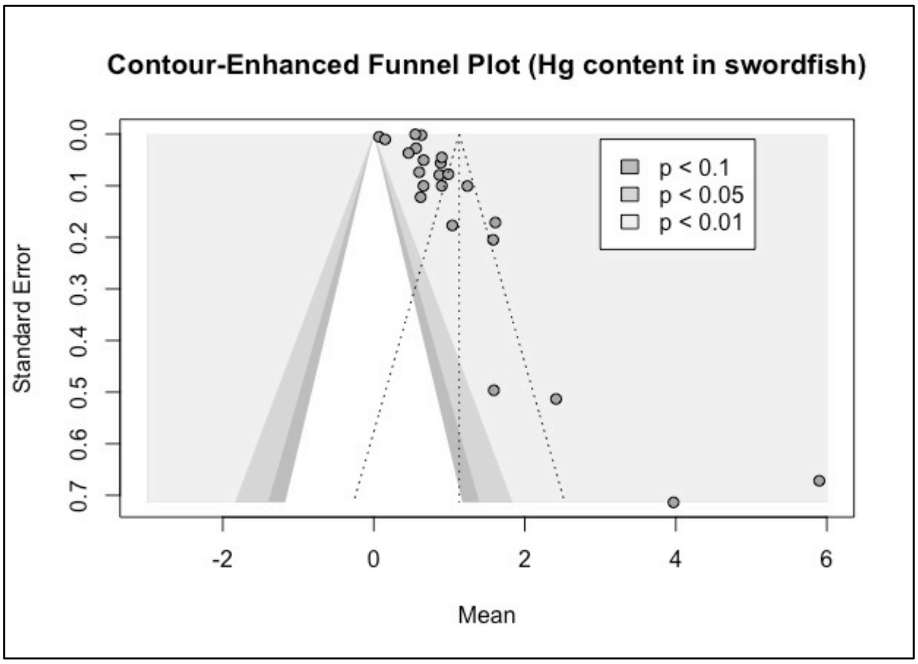


Fig. 21. Funnel plot of the meta-analysis for Hg in swordfish.

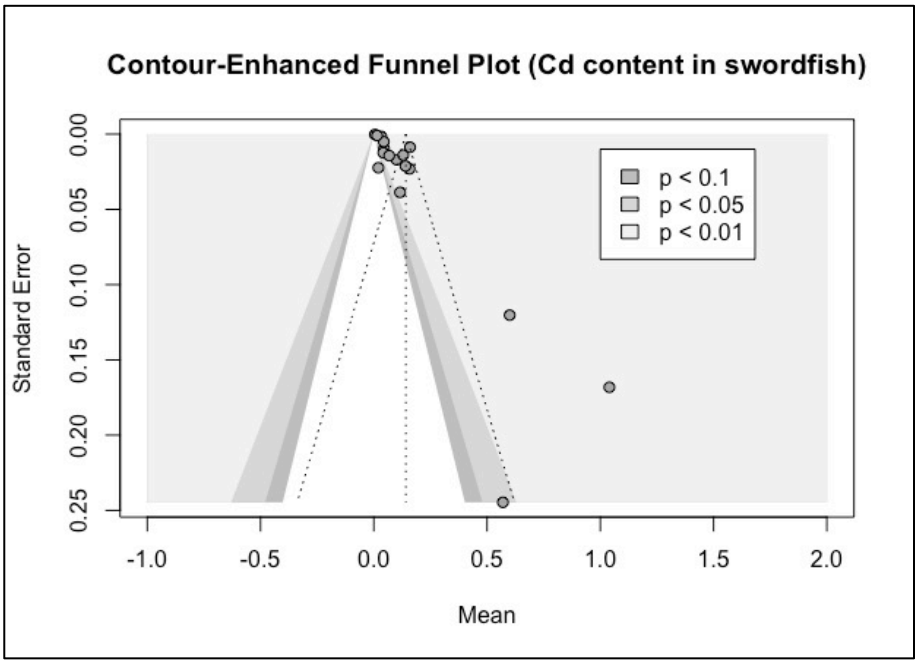


Fig. 22. Funnel plot for the meta-analysis for Cd in swordfish.

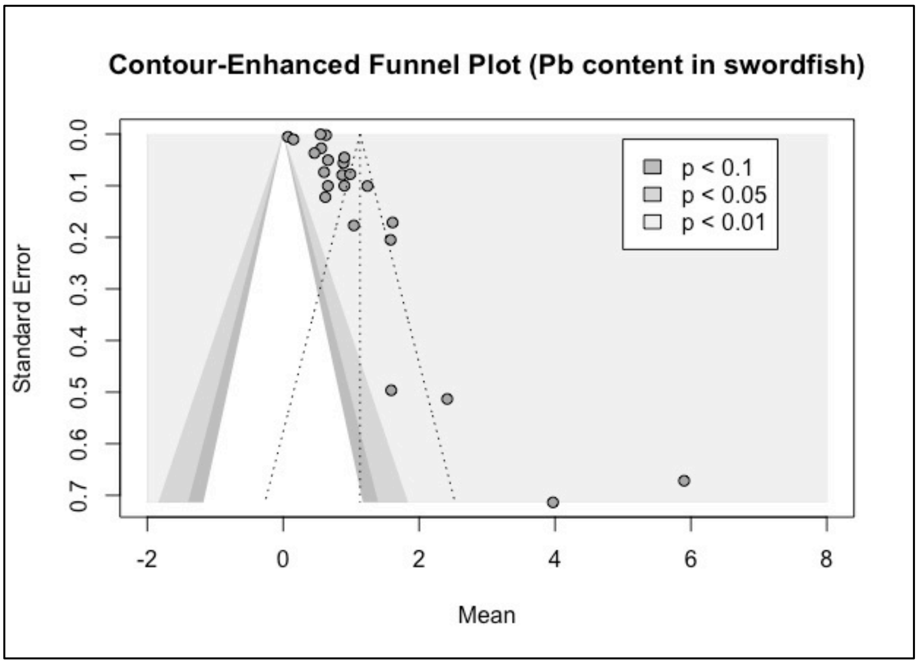


Fig. 23. Funnel plot for the meta-analysis of Pb in swordfish.

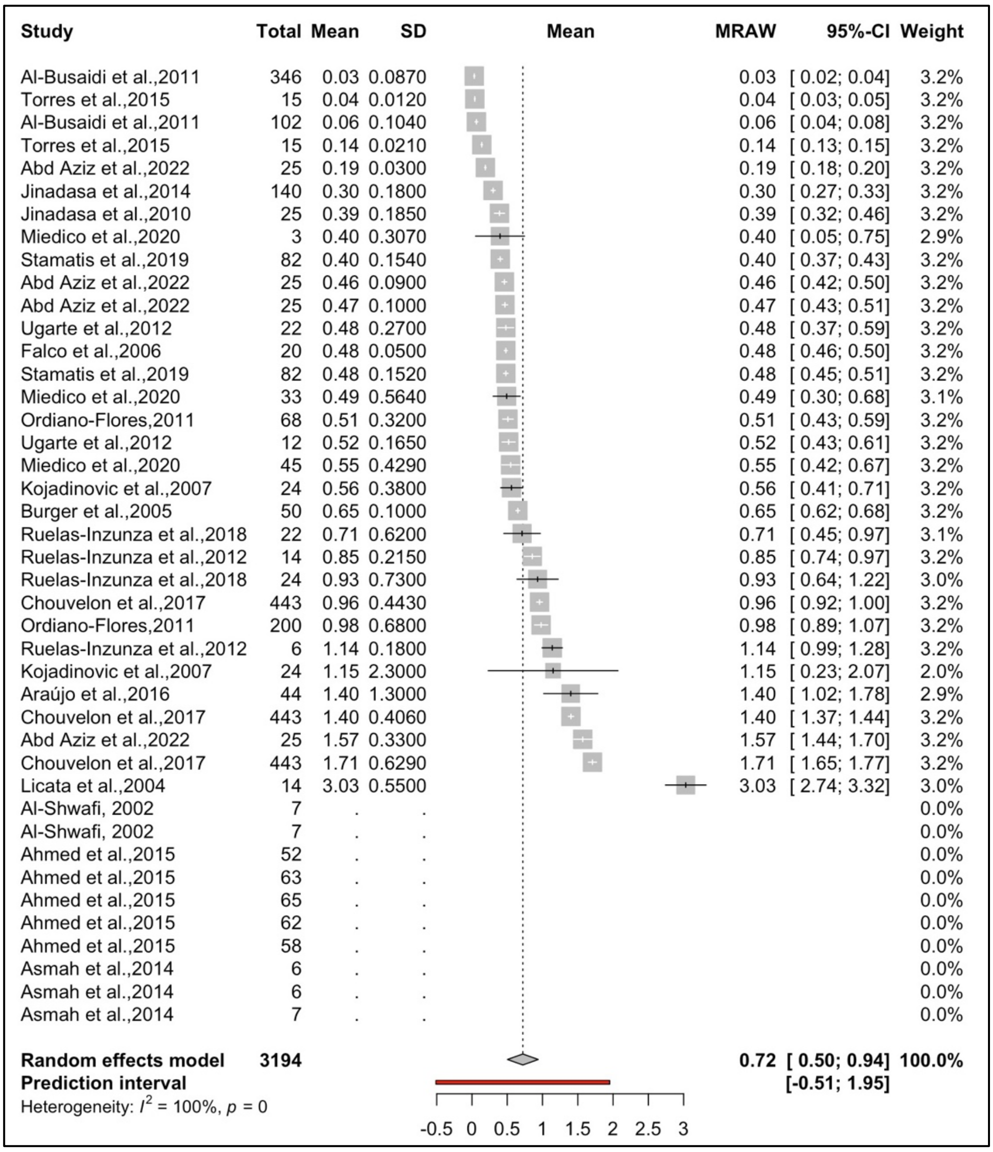
3.2.2. Tuna

In the conducted metal-analysis of k=40 studies (from 20 papers), the estimated mean concentrations for the four metals, mercury, cadmium, lead, and arsenic, found in tuna are in Table 8. When compared to the maximum limits (MLs), mercury (Hg, 0.72 mg/kg), cadmium (Cd, 0.15 mg/kg) and lead (Pb, 0.11 mg/kg) were under the limits, while arsenic (As, 7.19 mg/kg) presented levels seven times greater than the MLs. From the forest plots (Figures 24 to 27) that present the main results of the meta-analyses, we can see studies and their respective mean (and 95% confidence intervals, CI). Therein, the grey boxes represent the effect size of each study, i.e. mean concentration of the toxic metal, found in each study, and the whiskers represent their respective 95% CI. The size of the box means the study weighted more (i.e., bigger sample size). The pooled mean of the studies is represented by a grey diamond shape at the bottom. The diamond's length represents the 95% confidence interval of the pooled result on the x-axis. Since the grey diamond range does include 0, the pooled effects (i.e. the mean concentration) for each metal is significant.

Table 8. Estimated mean concentrations (mg/kg) and 95% confidence intervals of the toxic metals estimated in tuna derived from the meta-analysis of k=32 studies vs. maximum limits.

Metal	Mean concentration (mg/kg)	95% Confidence interval	Maximum limits(1,2)
Mercury	0.72	[0.5031; 0.9392]	0.5-1 mg/kg
Cadmium	0.15	[0.0798; 0.2291]	0.1 mg/kg
Lead	0.11	[0.0677; 0.1544]	0.2-0.5 mg/kg
Arsenic	7.19	[1.1564; 13.219]	1 mg/kg

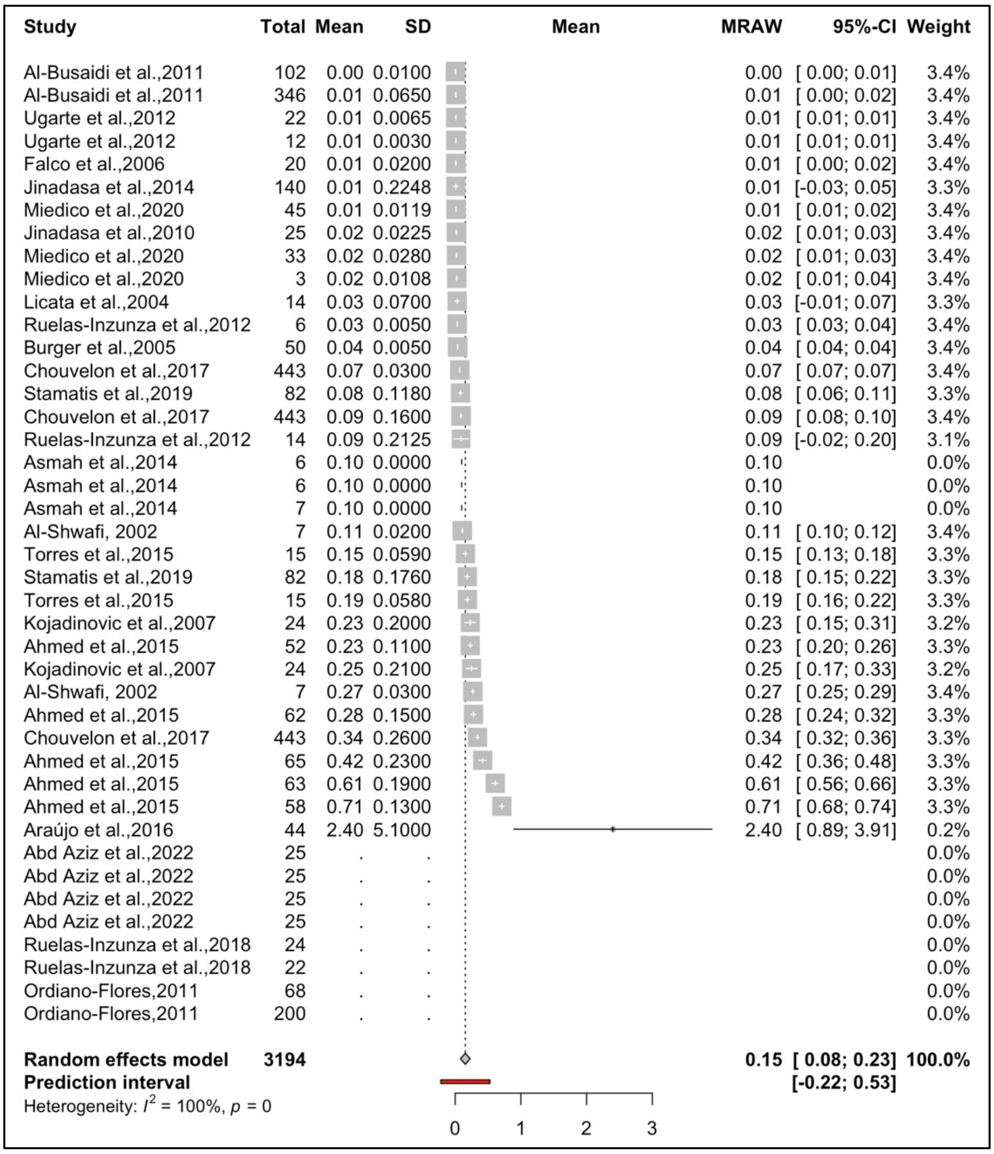
1. European Commission (2006), 2. FAO/WHO (2009)



Study	Total	Mean	SD	MRAW	95%-CI	Weight
Al-Busaidi et al.,2011	346	0.03	0.0870	0.03	[0.02; 0.04]	3.2%
Torres et al.,2015	15	0.04	0.0120	0.04	[0.03; 0.05]	3.2%
Al-Busaidi et al.,2011	102	0.06	0.1040	0.06	[0.04; 0.08]	3.2%
Torres et al.,2015	15	0.14	0.0210	0.14	[0.13; 0.15]	3.2%
Abd Aziz et al.,2022	25	0.19	0.0300	0.19	[0.18; 0.20]	3.2%
Jinadasa et al.,2014	140	0.30	0.1800	0.30	[0.27; 0.33]	3.2%
Jinadasa et al.,2010	25	0.39	0.1850	0.39	[0.32; 0.46]	3.2%
Miedico et al.,2020	3	0.40	0.3070	0.40	[0.05; 0.75]	2.9%
Stamatis et al.,2019	82	0.40	0.1540	0.40	[0.37; 0.43]	3.2%
Abd Aziz et al.,2022	25	0.46	0.0900	0.46	[0.42; 0.50]	3.2%
Abd Aziz et al.,2022	25	0.47	0.1000	0.47	[0.43; 0.51]	3.2%
Ugarte et al.,2012	22	0.48	0.2700	0.48	[0.37; 0.59]	3.2%
Falco et al.,2006	20	0.48	0.0500	0.48	[0.46; 0.50]	3.2%
Stamatis et al.,2019	82	0.48	0.1520	0.48	[0.45; 0.51]	3.2%
Miedico et al.,2020	33	0.49	0.5640	0.49	[0.30; 0.68]	3.1%
Ordiano-Flores,2011	68	0.51	0.3200	0.51	[0.43; 0.59]	3.2%
Ugarte et al.,2012	12	0.52	0.1650	0.52	[0.43; 0.61]	3.2%
Miedico et al.,2020	45	0.55	0.4290	0.55	[0.42; 0.67]	3.2%
Kojadinovic et al.,2007	24	0.56	0.3800	0.56	[0.41; 0.71]	3.2%
Burger et al.,2005	50	0.65	0.1000	0.65	[0.62; 0.68]	3.2%
Ruelas-Inzunza et al.,2018	22	0.71	0.6200	0.71	[0.45; 0.97]	3.1%
Ruelas-Inzunza et al.,2012	14	0.85	0.2150	0.85	[0.74; 0.97]	3.2%
Ruelas-Inzunza et al.,2018	24	0.93	0.7300	0.93	[0.64; 1.22]	3.0%
Chouvelon et al.,2017	443	0.96	0.4430	0.96	[0.92; 1.00]	3.2%
Ordiano-Flores,2011	200	0.98	0.6800	0.98	[0.89; 1.07]	3.2%
Ruelas-Inzunza et al.,2012	6	1.14	0.1800	1.14	[0.99; 1.28]	3.2%
Kojadinovic et al.,2007	24	1.15	2.3000	1.15	[0.23; 2.07]	2.0%
Araújo et al.,2016	44	1.40	1.3000	1.40	[1.02; 1.78]	2.9%
Chouvelon et al.,2017	443	1.40	0.4060	1.40	[1.37; 1.44]	3.2%
Abd Aziz et al.,2022	25	1.57	0.3300	1.57	[1.44; 1.70]	3.2%
Chouvelon et al.,2017	443	1.71	0.6290	1.71	[1.65; 1.77]	3.2%
Licata et al.,2004	14	3.03	0.5500	3.03	[2.74; 3.32]	3.0%
Al-Shwafi, 2002	7	.	.			0.0%
Al-Shwafi, 2002	7	.	.			0.0%
Ahmed et al.,2015	52	.	.			0.0%
Ahmed et al.,2015	63	.	.			0.0%
Ahmed et al.,2015	65	.	.			0.0%
Ahmed et al.,2015	62	.	.			0.0%
Ahmed et al.,2015	58	.	.			0.0%
Asmah et al.,2014	6	.	.			0.0%
Asmah et al.,2014	6	.	.			0.0%
Asmah et al.,2014	7	.	.			0.0%
Random effects model	**3194**			**0.72**	**[0.50; 0.94]**	**100.0%**
Prediction interval					**[-0.51; 1.95]**	

Heterogeneity: $I^2 = 100\%$, $p = 0$

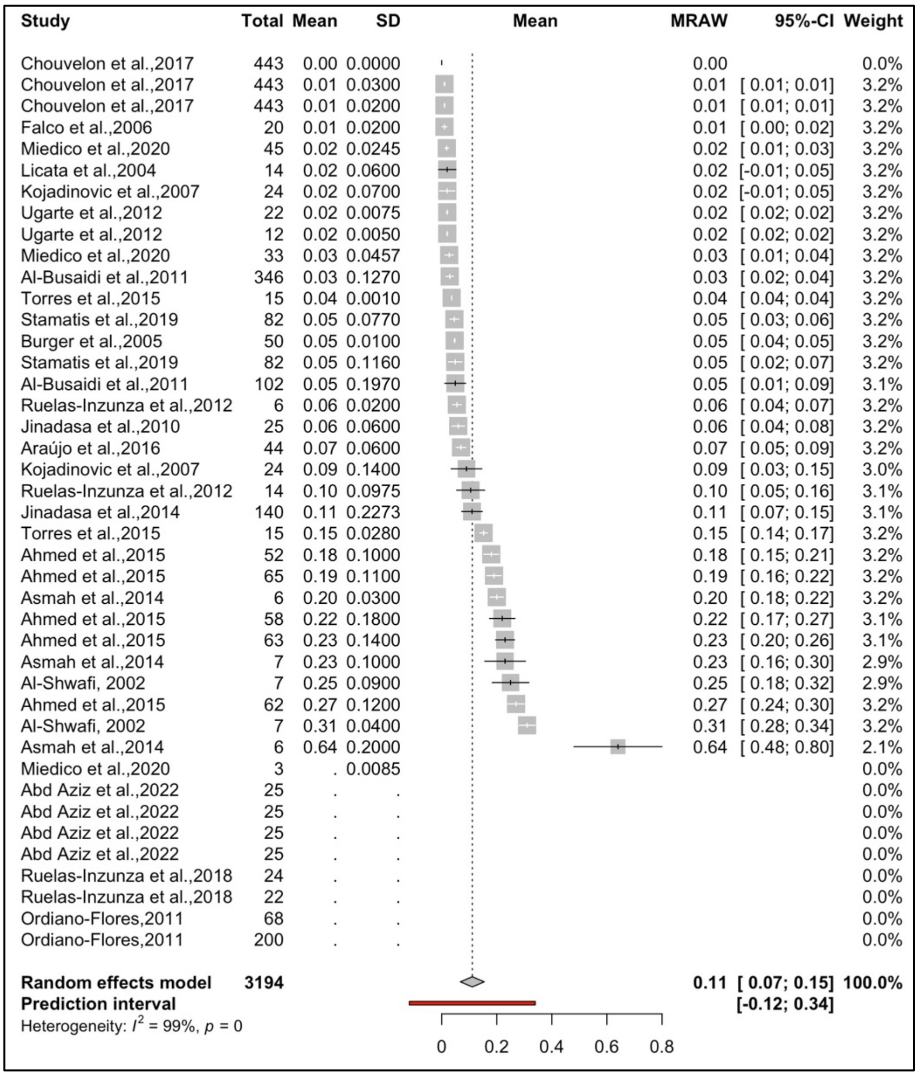
Fig. 24. Forest plot showing the meta-analysis for Hg in tuna.



Study	Total	Mean	SD	MRAW	95%-CI	Weight
Al-Busaidi et al.,2011	102	0.00	0.0100	0.00	[0.00; 0.01]	3.4%
Al-Busaidi et al.,2011	346	0.01	0.0650	0.01	[0.00; 0.02]	3.4%
Ugarte et al.,2012	22	0.01	0.0065	0.01	[0.01; 0.01]	3.4%
Ugarte et al.,2012	12	0.01	0.0030	0.01	[0.01; 0.01]	3.4%
Falco et al.,2006	20	0.01	0.0200	0.01	[0.00; 0.02]	3.4%
Jinadasa et al.,2014	140	0.01	0.2248	0.01	[-0.03; 0.05]	3.3%
Miedico et al.,2020	45	0.01	0.0119	0.01	[0.01; 0.02]	3.4%
Jinadasa et al.,2010	25	0.02	0.0225	0.02	[0.01; 0.03]	3.4%
Miedico et al.,2020	33	0.02	0.0280	0.02	[0.01; 0.03]	3.4%
Miedico et al.,2020	3	0.02	0.0108	0.02	[0.01; 0.04]	3.4%
Licata et al.,2004	14	0.03	0.0700	0.03	[-0.01; 0.07]	3.3%
Ruelas-Inzunza et al.,2012	6	0.03	0.0050	0.03	[0.03; 0.04]	3.4%
Burger et al.,2005	50	0.04	0.0050	0.04	[0.04; 0.04]	3.4%
Chouvelon et al.,2017	443	0.07	0.0300	0.07	[0.07; 0.07]	3.4%
Stamatis et al.,2019	82	0.08	0.1180	0.08	[0.06; 0.11]	3.3%
Chouvelon et al.,2017	443	0.09	0.1600	0.09	[0.08; 0.10]	3.4%
Ruelas-Inzunza et al.,2012	14	0.09	0.2125	0.09	[-0.02; 0.20]	3.1%
Asmah et al.,2014	6	0.10	0.0000	0.10		0.0%
Asmah et al.,2014	6	0.10	0.0000	0.10		0.0%
Asmah et al.,2014	7	0.10	0.0000	0.10		0.0%
Al-Shwafi, 2002	7	0.11	0.0200	0.11	[0.10; 0.12]	3.4%
Torres et al.,2015	15	0.15	0.0590	0.15	[0.13; 0.18]	3.3%
Stamatis et al.,2019	82	0.18	0.1760	0.18	[0.15; 0.22]	3.3%
Torres et al.,2015	15	0.19	0.0580	0.19	[0.16; 0.22]	3.3%
Kojadinovic et al.,2007	24	0.23	0.2000	0.23	[0.15; 0.31]	3.2%
Ahmed et al.,2015	52	0.23	0.1100	0.23	[0.20; 0.26]	3.3%
Kojadinovic et al.,2007	24	0.25	0.2100	0.25	[0.17; 0.33]	3.2%
Al-Shwafi, 2002	7	0.27	0.0300	0.27	[0.25; 0.29]	3.4%
Ahmed et al.,2015	62	0.28	0.1500	0.28	[0.24; 0.32]	3.3%
Chouvelon et al.,2017	443	0.34	0.2600	0.34	[0.32; 0.36]	3.3%
Ahmed et al.,2015	65	0.42	0.2300	0.42	[0.36; 0.48]	3.3%
Ahmed et al.,2015	63	0.61	0.1900	0.61	[0.56; 0.66]	3.3%
Ahmed et al.,2015	58	0.71	0.1300	0.71	[0.68; 0.74]	3.3%
Araújo et al.,2016	44	2.40	5.1000	2.40	[0.89; 3.91]	0.2%
Abd Aziz et al.,2022	25	.	.			0.0%
Abd Aziz et al.,2022	25	.	.			0.0%
Abd Aziz et al.,2022	25	.	.			0.0%
Abd Aziz et al.,2022	25	.	.			0.0%
Ruelas-Inzunza et al.,2018	24	.	.			0.0%
Ruelas-Inzunza et al.,2018	22	.	.			0.0%
Ordiano-Flores,2011	68	.	.			0.0%
Ordiano-Flores,2011	200	.	.			0.0%
Random effects model	**3194**			**0.15**	**[0.08; 0.23]**	**100.0%**
Prediction interval					**[-0.22; 0.53]**	

Heterogeneity: $I^2 = 100\%$, $p = 0$

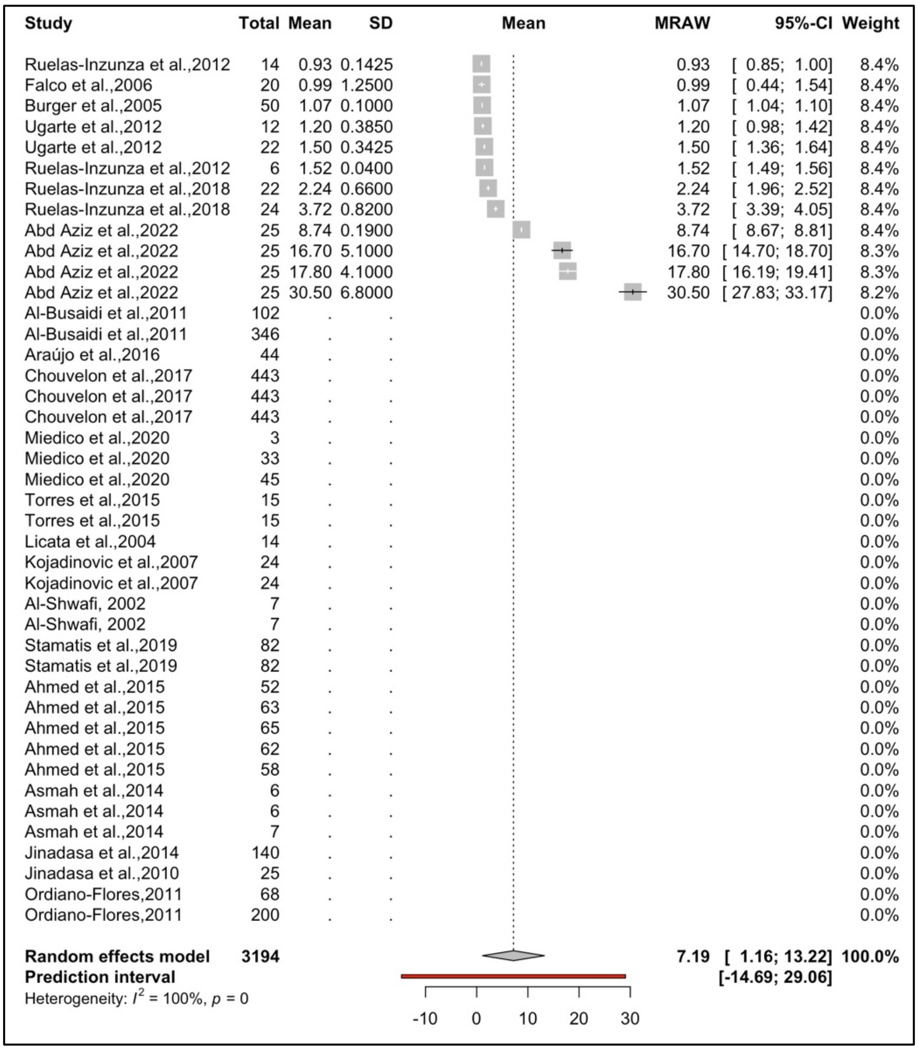
Fig. 25. Forest plot showing the meta-analysis for Cd in tuna.



Study	Total	Mean	SD	MRAW	95%-CI	Weight
Chouvelon et al.,2017	443	0.00	0.0000	0.00		0.0%
Chouvelon et al.,2017	443	0.01	0.0300	0.01	[0.01; 0.01]	3.2%
Chouvelon et al.,2017	443	0.01	0.0200	0.01	[0.01; 0.01]	3.2%
Falco et al.,2006	20	0.01	0.0200	0.01	[0.00; 0.02]	3.2%
Miedico et al.,2020	45	0.02	0.0245	0.02	[0.01; 0.03]	3.2%
Licata et al.,2004	14	0.02	0.0600	0.02	[-0.01; 0.05]	3.2%
Kojadinovic et al.,2007	24	0.02	0.0700	0.02	[-0.01; 0.05]	3.2%
Ugarte et al.,2012	22	0.02	0.0075	0.02	[0.02; 0.02]	3.2%
Ugarte et al.,2012	12	0.02	0.0050	0.02	[0.02; 0.02]	3.2%
Miedico et al.,2020	33	0.03	0.0457	0.03	[0.01; 0.04]	3.2%
Al-Busaidi et al.,2011	346	0.03	0.1270	0.03	[0.02; 0.04]	3.2%
Torres et al.,2015	15	0.04	0.0010	0.04	[0.04; 0.04]	3.2%
Stamatis et al.,2019	82	0.05	0.0770	0.05	[0.03; 0.06]	3.2%
Burger et al.,2005	50	0.05	0.0100	0.05	[0.04; 0.05]	3.2%
Stamatis et al.,2019	82	0.05	0.1160	0.05	[0.02; 0.07]	3.2%
Al-Busaidi et al.,2011	102	0.05	0.1970	0.05	[0.01; 0.09]	3.1%
Ruelas-Inzunza et al.,2012	6	0.06	0.0200	0.06	[0.04; 0.07]	3.2%
Jinadasa et al.,2010	25	0.06	0.0600	0.06	[0.04; 0.08]	3.2%
Araújo et al.,2016	44	0.07	0.0600	0.07	[0.05; 0.09]	3.2%
Kojadinovic et al.,2007	24	0.09	0.1400	0.09	[0.03; 0.15]	3.0%
Ruelas-Inzunza et al.,2012	14	0.10	0.0975	0.10	[0.05; 0.16]	3.1%
Jinadasa et al.,2014	140	0.11	0.2273	0.11	[0.07; 0.15]	3.1%
Torres et al.,2015	15	0.15	0.0280	0.15	[0.14; 0.17]	3.2%
Ahmed et al.,2015	52	0.18	0.1000	0.18	[0.15; 0.21]	3.2%
Ahmed et al.,2015	65	0.19	0.1100	0.19	[0.16; 0.22]	3.2%
Asmah et al.,2014	6	0.20	0.0300	0.20	[0.18; 0.22]	3.2%
Ahmed et al.,2015	58	0.22	0.1800	0.22	[0.17; 0.27]	3.1%
Ahmed et al.,2015	63	0.23	0.1400	0.23	[0.20; 0.26]	3.1%
Asmah et al.,2014	7	0.23	0.1000	0.23	[0.16; 0.30]	2.9%
Al-Shwafi, 2002	7	0.25	0.0900	0.25	[0.18; 0.32]	2.9%
Ahmed et al.,2015	62	0.27	0.1200	0.27	[0.24; 0.30]	3.2%
Al-Shwafi, 2002	7	0.31	0.0400	0.31	[0.28; 0.34]	3.2%
Asmah et al.,2014	6	0.64	0.2000	0.64	[0.48; 0.80]	2.1%
Miedico et al.,2020	3	.	0.0085			0.0%
Abd Aziz et al.,2022	25	.	.			0.0%
Abd Aziz et al.,2022	25	.	.			0.0%
Abd Aziz et al.,2022	25	.	.			0.0%
Abd Aziz et al.,2022	25	.	.			0.0%
Ruelas-Inzunza et al.,2018	24	.	.			0.0%
Ruelas-Inzunza et al.,2018	22	.	.			0.0%
Ordiano-Flores,2011	68	.	.			0.0%
Ordiano-Flores,2011	200	.	.			0.0%
Random effects model	**3194**			**0.11**	**[0.07; 0.15]**	**100.0%**
Prediction interval					**[-0.12; 0.34]**	

Heterogeneity: $I^2 = 99\%$, $p = 0$

Fig. 26. Forest plot showing the meta-analysis for Pb in tuna.



Study	Total	Mean	SD	MRAW	95%-CI	Weight
Ruelas-Inzunza et al.,2012	14	0.93	0.1425	0.93	[0.85; 1.00]	8.4%
Falco et al.,2006	20	0.99	1.2500	0.99	[0.44; 1.54]	8.4%
Burger et al.,2005	50	1.07	0.1000	1.07	[1.04; 1.10]	8.4%
Ugarte et al.,2012	12	1.20	0.3850	1.20	[0.98; 1.42]	8.4%
Ugarte et al.,2012	22	1.50	0.3425	1.50	[1.36; 1.64]	8.4%
Ruelas-Inzunza et al.,2012	6	1.52	0.0400	1.52	[1.49; 1.56]	8.4%
Ruelas-Inzunza et al.,2018	22	2.24	0.6600	2.24	[1.96; 2.52]	8.4%
Ruelas-Inzunza et al.,2018	24	3.72	0.8200	3.72	[3.39; 4.05]	8.4%
Abd Aziz et al.,2022	25	8.74	0.1900	8.74	[8.67; 8.81]	8.4%
Abd Aziz et al.,2022	25	16.70	5.1000	16.70	[14.70; 18.70]	8.3%
Abd Aziz et al.,2022	25	17.80	4.1000	17.80	[16.19; 19.41]	8.3%
Abd Aziz et al.,2022	25	30.50	6.8000	30.50	[27.83; 33.17]	8.2%
Al-Busaidi et al.,2011	102	.	.			0.0%
Al-Busaidi et al.,2011	346	.	.			0.0%
Araújo et al.,2016	44	.	.			0.0%
Chouvelon et al.,2017	443	.	.			0.0%
Chouvelon et al.,2017	443	.	.			0.0%
Chouvelon et al.,2017	443	.	.			0.0%
Miedico et al.,2020	3	.	.			0.0%
Miedico et al.,2020	33	.	.			0.0%
Miedico et al.,2020	45	.	.			0.0%
Torres et al.,2015	15	.	.			0.0%
Torres et al.,2015	15	.	.			0.0%
Licata et al.,2004	14	.	.			0.0%
Kojadinovic et al.,2007	24	.	.			0.0%
Kojadinovic et al.,2007	24	.	.			0.0%
Al-Shwafi, 2002	7	.	.			0.0%
Al-Shwafi, 2002	7	.	.			0.0%
Stamatis et al.,2019	82	.	.			0.0%
Stamatis et al.,2019	82	.	.			0.0%
Ahmed et al.,2015	52	.	.			0.0%
Ahmed et al.,2015	63	.	.			0.0%
Ahmed et al.,2015	65	.	.			0.0%
Ahmed et al.,2015	62	.	.			0.0%
Ahmed et al.,2015	58	.	.			0.0%
Asmah et al.,2014	6	.	.			0.0%
Asmah et al.,2014	6	.	.			0.0%
Asmah et al.,2014	7	.	.			0.0%
Jinadasa et al.,2014	140	.	.			0.0%
Jinadasa et al.,2010	25	.	.			0.0%
Ordiano-Flores,2011	68	.	.			0.0%
Ordiano-Flores,2011	200	.	.			0.0%
Random effects model	**3194**			**7.19**	**[1.16; 13.22]**	**100.0%**
Prediction interval					**[-14.69; 29.06]**	

Heterogeneity: $I^2 = 100\%$, $p = 0$

Fig. 27. Forest plot showing the meta-analysis for As in tuna.

In fact, the considerable variation observed in the forest plots among studies is clearly described via the heterogeneity statistics (Table 9). Comparing to our results for all the toxic metals, $I^2>90\%$ and p-value<0.05, so there is considerable heterogeneity among studies. When there's high heterogeneity, i.e. high variance between studies, one can attempt to model it using available study characteristics (i.e., moderator variables) in a subgroup analysis (Higgins et al., 2003).

Table 9. Results for heterogeneity statistics (I^2, tau and tau^2) and Q test (Cochran's Q test) for the meta-analysis of studies about toxic metals in Tuna

Metal	**I^2**	**tau**	**tau^2**	**Q-test**	**df**	**p-value**
Mercury	99.8%	0.59	0.35	15647.90	31	< 0.0001
Cadmium	99.5%	0.18	0.03	6604.45	30	< 0.0001
Lead	98.9%	0.11	0.01	2915.05	31	< 0.0001
Arsenic	100%	9.43	88.95	38049.75	11	< 0.0001

The observed high heterogeneity can be caused by the fact that there are two or more subgroups of studies in our data that have a different true effect (i.e., mean concentration). A subgroup analyses, following a mixed-effects model and using a Q-based test for subgroup differences (Harrer et al., 2021), was conducted with moderator variables/factors *species*, *year,* and *location.* There are significant differences among species, years, and locations for every toxic metal considered herein. All Q-test statistics, Q=20.90-4758.51 with df=3-17, were considered significant (p<0.0001) (Table 10). This considerable amount of heterogeneity is in line with the findings described above in Section 3.1.2.

In the case of Hg (Table 11), the highest significant (average) concentration of Hg was estimated to be for albacore (0.90 mg/kg). Yearly, the maximum concentration of Hg, 3.03 mg/kg, was found in 2003. Fishes caught/sampled in the Seychelles and waters off Ecuador presented the highest values, ca. 1.40 mg/kg. When looking at Cd (Table 12), the highest concentration was 0.45 mg/kg in longtail tuna. Considering the year, the maximum was 0.71 mg/kg Cd during 2010. On the other hand, fishes sampled off Ecuador had the highest concentration of Cd, 2.40 mg/kg. For Pb (Table 13), the highest average concentration was estimated for longtail at ~0.22 mg/kg. Yearly, the maximum concentration of Pb, 0.22 mg/kg, was found in 2010. The highest values of approximately 0.31 mg/kg were found in fishes caught/sampled in the Gulf of Aden. Finally, for As (Table 14) the highest concentration was 18.38 mg/kg in longtail tuna. That maximum concentration was found during 2022 in Malaysia.

Table 10. Results for the Q tests (Cochran's Q test) of heterogeneity for the meta-analysis of studies about toxic metals in tuna considering moderator variables *species*, *year,* and *location*.

Metal **Moderator variable**	**Q**	**df**	**p-value**
Hg			
Species	54.42	6	< 0.0001
Year	894.61	12	< 0.0001
Location	3748.08	16	< 0.0001
Cd			
Species	172.64	6	< 0.0001
Year	4758.51	12	< 0.0001
Location	2345.72	17	< 0.0001
Pb			
Species	402.54	8	< 0.0001
Year	706.90	12	< 0.0001
Location	1204.39	17	< 0.0001
As			
Species	26.01	4	< 0.0001
Year	20.90	3	< 0.0001
Location	52.19	5	< 0.0001

Table 11. Results for subgroup analysis (random effects model) following the meta-analysis of studies for Hg in tuna considering the moderator variable/factor *species*, *year,* and *location*.

Moderator variable	**k**	**Mean concentration (mg/kg)**	**95% CI**
Species			
Skipjack	5	0.5067	*[-0.1214; 1.1348]*
Yellowfin	12	0.6266	[0.4004; 0.8527]
Albacore	6	**0.9054**	[0.3285; 1.4823]
Unspecified	1	0.4920	[0.2996; 0.6844]
Longtail	4	0.6692	*[-0.3030; 1.6415]*
Bigeye	1	0.1390	[0.1284; 0.1496]
Bluefin	3	1.3379	*[-2.2852; 4.9610]*
Year			
2003	1	**3.0300**	[2.7419; 3.3181]
2005	4	0.7741	[0.3256; 1.2226]
2006	2	0.6733	*[-2.2797; 3.6263]*
2007	2	0.0465	*[-0.1471; 0.2401]*
2008	2	0.8092	*[-0.5817; 2.2002]*
2009	2	0.3387	*[-0.2274; 0.9048]*
2010	2	0.7441	*[-2.2418; 3.7301]*
2011	2	0.0894	*[-0.5396; 0.7184]*

2012	2	0.5037	[0.2541; 0.7534]
2013	4	1.3645	[0.8562; 1.8728]
2014	3	0.5202	[0.3853; 0.6550]
2015	2	0.4405	*[-0.0613; 0.9424]*
2022	4	0.6692	*[-0.3030; 1.6415]*
Location			
Sultanate of Oman	2	0.0465	*[-0.1471; 0.2401]*
Ecuador	1	**1.4000**	[1.0159; 1.7841]
Reunion Islands	2	1.6257	*[-0.8883; 4.1397]*
Seychelles	1	**1.4030**	[1.3652; 1.4408]
South Africa	1	0.9580	[0.9167; 0.9993]
Italy	5	0.9867	*[-0.4275; 2.4008]*
USA	1	0.6500	[0.6223; 0.6777]
Malaysia	4	0.6692	*[-0.3030; 1.6415]*
Azores Islands	2	0.0894	*[-0.5396; 0.7184]*
Pacific Ocean	5	0.9401	[0.7516; 1.1286]
Mozambique	1	0.5600	[0.4080; 0.7120]
Bay of Biscay	1	0.4800	[0.3672; 0.5928]
North Atlantic	1	0.5200	[0.4266; 0.6134]
North Aegean Sea	1	0.4800	[0.4471; 0.5129]
Southeastern Aegean Sea	1	0.4010	[0.3677; 0.4343]
Sri Lanka	2	0.3387	*[-0.2274; 0.9048]*
Mexico	1	0.5100	[0.4339; 0.5861]

Table 12. Results for subgroup analysis (random effects model) following the meta-analysis of studies for Cd in tuna considering the moderator variable/factor *species*, *year,* and *location*.

Moderator variable	**k**	**Mean concentration (mg/kg)**	**95% CI**
Species			
Skipjack	4	0.0527	*[-0.0548; 0.1601]*
Yellowfin	11	0.1049	[0.0044; 0.2055]
Albacore	6	0.1288	[0.0056; 0.2520]
Unspecified	1	0.0210	[0.0114; 0.0306]
Longtail	5	**0.4499**	[0.1925; 0.7073]
Bigeye	1	0.1860	[0.1566; 0.2154]
Bluefin	3	0.0103	[0.0034; 0.0171]
Year			
1998	2	0.1898	*[-0.8267; 1.2063]*
2003	1	0.0300	*[-0.0067; 0.0667]*
2005	4	0.0277	[0.0016; 0.0537]

2006	3	0.2320	[0.2137; 0.2503]
2007	3	0.2076	*[-0.6560; 1.0712]*
2008	1	0.4200	[0.3641; 0.4759]
2009	3	0.1029	*[-0.2768; 0.4827]*
2010	1	**0.7100**	[0.6765; 0.7435]
2011	2	0.1706	*[-0.0263; 0.3676]*
2012	2	0.0100	[0.0100; 0.0100]
2013	4	0.2003	*[-0.3488; 0.7494]*
2014	3	0.0179	[0.0030; 0.0327]
2015	2	0.1320	*[-0.5159; 0.7799]*
Location			
Sultanate of Oman	2	0.0063	*[-0.0221; 0.0347]*
Ecuador	1	**2.4000**	[0.8931; 3.9069]
Reunion Islands	2	0.1448	*[-0.8695; 1.1591]*
Seychelles	1	0.0900	[0.0751; 0.1049]
South Africa	1	0.3400	[0.3158; 0.3642]
Italy	5	0.0156	[0.0083; 0.0228]
USA	1	0.0370	[0.0356; 0.0384]
Azores Islands	2	0.1706	*[-0.0263; 0.3676]*
Pacific Ocean	2	0.0332	*[-0.1089; 0.1753]*
Mozambique	1	0.2500	[0.1660; 0.3340]
Red Sea off Yemen	1	0.1100	[0.0952; 0.1248]
Gulf of Aden	1	0.2700	[0.2478; 0.2922]
Bay of Biscay	1	0.0100	[0.0073; 0.0127]
North Atlantic	1	0.0100	[0.0083; 0.0117]
North Aegean Sea	1	0.1840	[0.1459; 0.2221]
Southeastern Aegean Sea	1	0.0820	[0.0565; 0.1075]
Pakistan	5	0.4499	[0.1925; 0.7073]
Sri Lanka	2	0.0195	*[-0.0090; 0.0480]*

Table 13. Results for subgroup analysis (random effects model) following the meta-analysis of studies for Pb in tuna considering the moderator variable/factor *species*, *year,* and *location*.

Moderator variable	**k**	**Mean concentration (mg/kg)**	**95% CI**
Species			
Skipjack	4	0.1174	*[-0.0162; 0.2509]*
Yellowfin	11	0.0990	[0.0355; 0.1625]
Albacore	5	0.0243	[0.0015; 0.0470]
Unspecified	1	0.0271	[0.0115; 0.0427]
Longtail	5	**0.2173**	[0.1720; 0.2627]

Bigeye	1	0.0360	[0.0355; 0.0365]
Bluefin	3	0.0164	[0.0018; 0.0310]
Frigate	1	0.6400	[0.4800; 0.8000]
Atlantic little tuna	1	0.2000	[0.1760; 0.2240]
Year			
1998	2	0.2877	*[-0.0805; 0.6560]*
2003	1	0.0200	*[-0.0114; 0.0514]*
2005	4	0.0486	*[-0.0085; 0.1058]*
2006	3	0.0969	*[-0.1052; 0.2990]*
2007	3	0.1023	*[-0.1724; 0.3770]*
2008	1	0.1900	[0.1633; 0.2167]
2009	3	0.1466	*[-0.1263; 0.4195]*
2010	1	**0.2200**	[0.1737; 0.2663]
2011	2	0.0938	*[-0.6432; 0.8307*]
2012	2	0.0200	[0.0200; 0.0200]
2013	6	0.1840	*[-0.0579; 0.4258]*
2014	2	0.0199	*[-0.0229; 0.0626]*
2015	2	0.0469	[0.0294; 0.0645]
Location			
Sultanate of Oman	2	0.0313	*[-0.0483; 0.1109]*
Ecuador	1	0.0700	[0.0523; 0.0877]
Reunion Islands	2	0.0101	*[-0.0025; 0.0226]*
South Africa	1	0.0100	[0.0081; 0.0119]
Italy	4	0.0169	[0.0059; 0.0279]
USA	1	0.0470	[0.0442; 0.0498]
Azores Islands	2	0.0938	*[-0.6432; 0.8307]*
Pacific Ocean	2	0.0739	*[-0.2341; 0.3819]*
Mozambique	1	0.0900	[0.0340; 0.1460]
Red Sea off Yemen	1	0.2500	[0.1833; 0.3167]
Gulf of Aden	1	**0.3100**	[0.2804; 0.3396]
Bay of Biscay	1	0.0200	[0.0169; 0.0231]
North Atlantic	1	0.0200	[0.0172; 0.0228]
North Aegean Sea	1	0.0490	[0.0239; 0.0741]
Southeastern Aegean Sea	1	0.0460	[0.0293; 0.0627]
Pakistan	5	0.2173	[0.1720; 0.2627]
Ghana	3	0.3470	*[-0.2529; 0.9470]*
Sri Lanka	2	0.0828	*[-0.2336; 0.3991]*

Table 14. Results for subgroup analysis (random effects model) following the meta-analysis of studies for As in tuna considering the moderator variable/factor *species*, *year,* and *location*.

Moderator variable	k	Mean concentration (mg/kg)	95% CI
Species			
Skipjack	2	2.6152	*[-11.3424; 16.5727]*
Yellowfin	3	1.4015	*[-0.3783; 3.1814]*
Albacore	1	1.5000	[1.3569; 1.6431]
Longtail	4	**18.3728**	[4.0733; 32.6724]
Bluefin	2	1.1713	[0.2552; 2.0875]
Year			
2005	4	1.1474	[0.6985; 1.5963]
2008	2	2.9772	*[-6.4253; 12.3797]*
2012	2	1.3617	[-0.5384; 3.2618]
2022	4	**18.3728**	[4.0733; 32.6724]
Location			
Italy	1	0.9900	[0.4422; 1.5378]
USA	1	1.0700	[1.0423; 1.0977]
Malaysia	4	**18.3728**	[4.0733; 32.6724]
Pacific Ocean	4	2.0940	[0.1796; 4.0084]
Bay of Biscay	1	1.5000	[1.3569; 1.6431]
North Atlantic	1	1.2000	[0.9822; 1.4178]

Researchers are often restricted to published results, regardless of how comprehensive their search is. Study reporting bias, which can be influenced by the direction or strength of the results, is a clear cause of error. Several factors can impact whether, when, or how a study is published, all of which contribute to reporting bias (Andrel et al., 2009). On the other hand, missing studies can pose a threat to the validity of meta-analytic inferences. This caveat, also termed “File Drawer” problem, refers to the issue that not all relevant research findings are published and are therefore missing in a meta-analysis. It is widely believed that studies with negative or 'disappointing' results (i.e. non-confirming the initial hypothesis) are often underrepresented in published literature due to publication bias (e.g. Schmucker et al. 2014). Publication bias is one of several types of non-reporting bias. Meta-analyses can be biased by several factors. These include citation bias, time-lag bias, multiple publication bias, language bias and outcome reporting bias (Page et al., 2020).

All these affects the meta-analysis. Funnel plots provide a graphical method for assessing potential biases in meta-analyses by displaying the observed effect sizes of studies on the x-axis plotted against standard errors on the y-axis (see previous subsection). A symmetrical, inverted funnel with a narrow top and a wider bottom should look like the plot if every study included is a random sample from the same population. However, research with non-significant results—especially smaller studies—will not be included if there is publication bias since they are not available in the literature, and the funnel plot will have a gap on one side. It's also important to remember that funnel plot asymmetry might have other causes besides publishing bias. According to Yang et al. (2021), studies with a smaller sample size are often more likely to have a different, frequently broader range of effect sizes than studies with a higher sample size. Therefore, it is more reasonable to see asymmetry in a funnel plot as a measurement of small study effect. The enhanced-contour funnel plots for the metals' concentrations in tuna are in Figures 28 to 31. There is some asymmetry in the funnel plot, but mostly the lack of small size (high standard error) studies (apart from unique, "outliers"). This may be caused by publication bias, since the perceived missing studies are in regions of the plots of low statistical significance (i.e. p>0.1) (Harrer et al., 2021; Peters et al., 2008). The results from the meta-analysis should be considered with care.

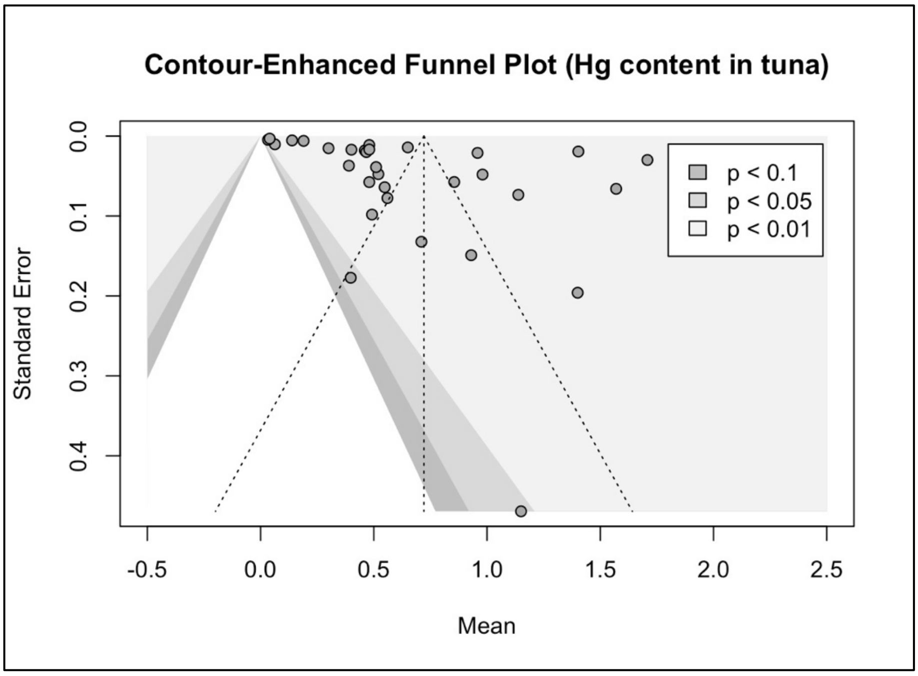


Fig. 28. Funnel plot for the meta-analysis for Hg in tuna.

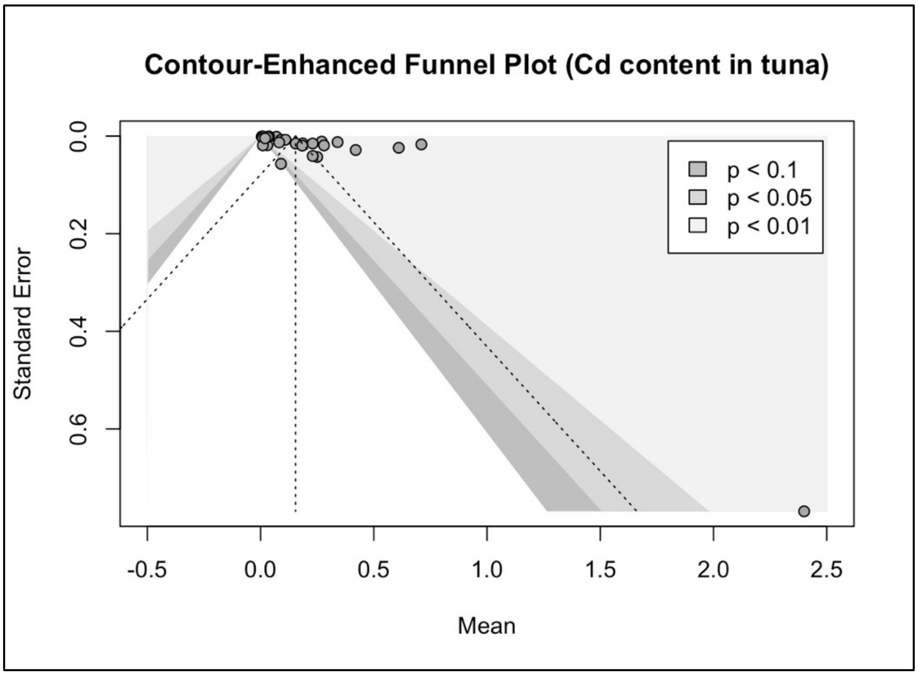


Fig. 29. Funnel plot for the meta-analysis for Cd in tuna.

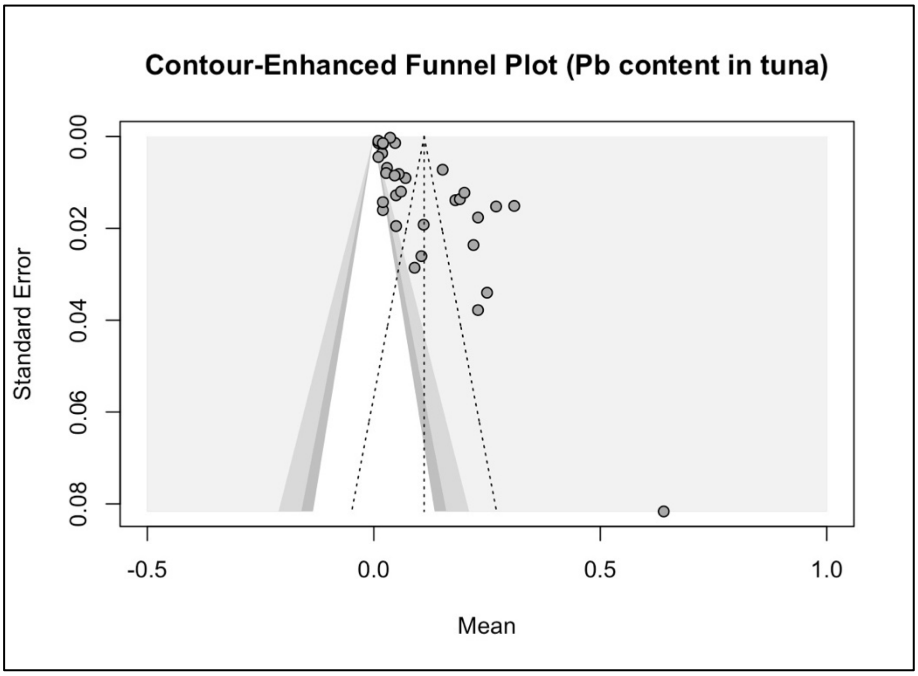


Fig. 30. Funnel plot for the meta-analysis for Pb in tuna.

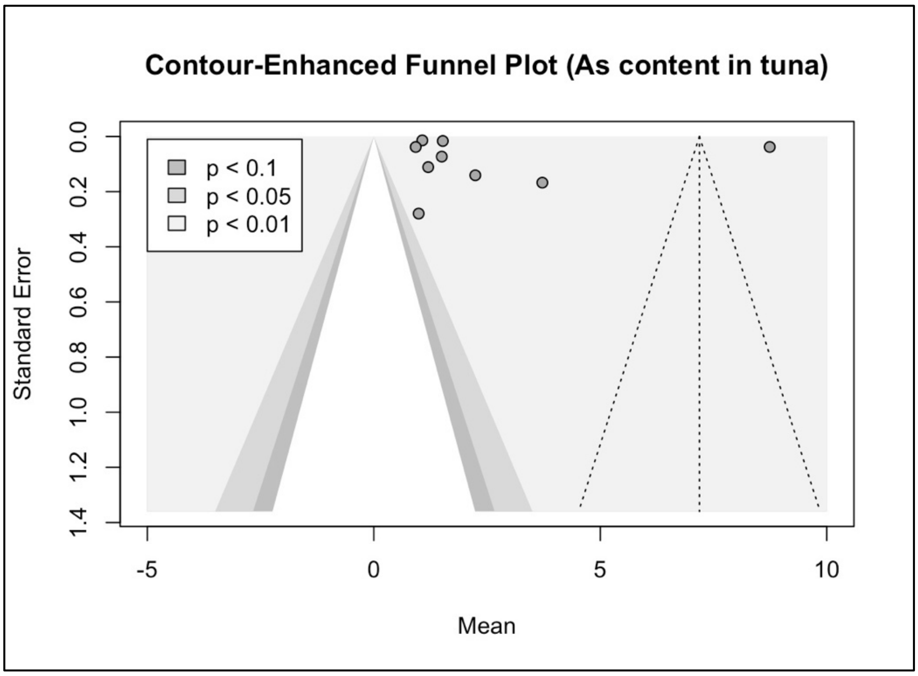


Fig. 31. Funnel plot for the meta-analysis for As in tuna.

3.3 HEALTH RISK ASSESSMENT

Aquatic ecosystems are the ultimate sinks for pollutants. Human activities such as agriculture urbanization, and industrialization are often the cause of water pollution (Bashir et al., 2020). Since the aquatic system is a *simple* solvent, most contaminants may easily dissolve in it and pollute it along with the species that live therein. Since many people rely heavily on aquatic products for their primary source of nutrition and since toxic metals in these seafood products have the potential to accumulate in both animals and humans, they pose a concern to human health. The many health benefits of fish consumption can be compromised by the presence of toxic metals and metalloids such as lead, cadmium, arsenic, and mercury, which can have harmful effects on the human body if consumed in toxic levels (Bosch et al., 2016).

While Cd, Pb, and Hg are poisonous and may negatively impact DNA and enzymatic activities, other (toxic) metals, such as Cu, Zn, and Fe, act as necessary components of enzymes and metabolic functions in fish (Jakimska et al., 2011). In addition to other issues, metal contamination may lead to the demise of several fish species. The maximum allowable limits (MALs) and provisional tolerable weekly intake (PTWI) for metals in foodstuffs are determined to protect consumers, and these limits vary depending on the type of metal and the country and can be species-specific (Bosch et al., 2016). To protect consumers from potential risks, food safety regulations are essential for public health. Official laboratories, such as those required by the European Commission (2006), Codex Alimentarius General Standards (FAO/WHO, 2009), FDA standards (CFR, 2023), and WHO/FAO (2011), are responsible for ensuring that food products adhere to quality requirements for hazardous toxic elements (refer to Table 15).

Table 15. Heavy Metal Regulations for Fishery Products (adapted from Digoarachchi, 2022)

Regulation Body	**Hg (ppm)**	**Cd (ppm)**	**Pb (ppm)**	**As (ppm)**
European Union regulation[1]	≤1	≤0.10	≤0.30	≤1
Codex General Standards[2]	≤0.5		≤0.3	
Australian/New Zealand Standards[3]	≤0.5		≤0.5	≤2
FDA Standards[4]	≤0.5			

1. European Commission (2006), 2. FAO/WHO (2009), 3. FSANZ (2023), 4. CFR (2023).
1 ppm=1 mg/kg.

Following the methods described in Subsection 2.3, the results of health risk assessment for humans derived from the consumption of swordfish and tuna in Portugal are presented in Tables 16 and 17, respectively.

Three alternative scenarios of fish/seafood consumption (SC.1 to SC.3) were considered to compare the health risks with the current fish consumption (62.7 g/person/day) as per Portuguese Food Balance sheet (Instituto Nacional de Estatística, 2021), which is considered as the reference scenario (SC.0). Alternative scenarios considered for daily food consumptions (DFC) of fish of 42.0 g/person/day (Lopes et al., 2018; SC.1), 164.0 g/person/day (European Commission, 2024; SC.2) and 0.6 g/person/day of swordfish plus 5.0 g/person/day of tunas (SC.3). The DFC and the results of the health risk assessments per scenario are in Tables 16 and 17, respectively for swordfish and tuna.

Table 16 presents the data for health risks due to the consumption of swordfish. From the data it is very much clear that Hg and Cd were the two metals to exceed the estimated daily intake (EDI) in comparison to daily dietary allowance. Only Hg was found to have a target hazard quotient (THQ) greater than 1, which means a potential health risk to the human body (US EPA, 2016). Individual metals like Pb and Cd seemingly posed no health risk from their THQ values (THQ<1). The Total Target Hazard Quotient (TTHQ) values were high (>1) in the case of Hg; whereas for Cd and Pb the TTHQ was less than 1. The Carcinogenic Risk (CR) of Pb was below the limits (between 10^{-4} to 10^{-6}), which suggests that cancer risk was not high among all the investigated metals.

Table 16. Health risk assessment for Portuguese population from fish/seafood consumption considering different scenarios with respect to consumption for swordfish.

Toxic Metal	Sce-nario	DFC (g/person/day)	Mean concentration (mg/kg)	EDI (mg/day /person)	Daily dietary allowance (mg/day/person)	THQ	TTHQ	CR
Hg	**SC.0**	62.7	1.13	0.06	0.003	2.36	10.13	
	SC.1	42.0		0.04		1.58		
	SC.2	164.0		0.18		6.17		
	SC.3	0.63		0.0007		0.02		
Cd	**SC.0**	62.7	0.17	0.01	0.006	0.17	0.74	
	SC.1	42.0		0.007		0.11		
	SC.2	164.0		0.02		0.46		
	SC.3	0.63		0.0001		0.001		
Pb	**SC.0**	62.7	0.53	0.03	0.21	0.13	0.56	$2.55 \cdot 10^{-4}$
	SC.1	42.0		0.02		0.07		$1.7 \cdot 10^{-4}$
	SC.2	164.0		0.08		0.36		$6.8 \cdot 10^{-4}$
	SC.3	0.63		0.0003		0.001		$2.5 \cdot 10^{-6}$

EDI - estimated daily intake, THQ - target hazard quotient, TTHQ - total target hazard quotient, CR - carcinogenic risk (refer to Subsection 2.3 for equations).

In Table 17 the estimated health risks of tuna consumption for consumers are presented. Hg and As were the two metals to exceed the estimated daily intake (EDI) in comparison to daily dietary allowance in all the three scenarios. Two metals, Hg and As had a target hazard quotient (THQ) greater than 1, which means a potential health risk to its consumers (US EPA, 2016). The other two metals, Pb and Cd seemingly pose no health risk from their THQ values (THQ<1). The Total Target Hazard Quotient (TTHQ) values were high (>1) in the case of Hg and As. Only the consumption of As poses high(er) Carcinogenic Risk (CR), which suggest cancer risk during consumption of tuna in Portugal.

Table 17. Health risk assessment for Portuguese population from fish/seafood consumption considering different scenarios with respect to consumption for tuna.

Metal	Scenario	DFC (g/person/day)	Mean concentration (mg/kg)	EDI (mg/day /person)	daily dietary allowance (mg/day/person)	THQ	TTHQ	CR
Hg	**SC.0**	62.7	0.70	0.04	0.003	1.43	6.32	
	SC.1	42.0		0.02		0.98		
	SC.2	164.0		0.11		3.82		
	SC.3	5.0		0.003		0.11		
Cd	**SC.0**	62.7	0.20	0.01	0.006	0.2	0.89	
	SC.1	42.0		0.008		0.14		
	SC.2	164.0		0.03		0.54		
	SC.3	5.0		0.001		0.01		
Pb	**SC.0**	62.7	0.12	0.007	0.21	0.03	0.13	$6.69\cdot10^{-5}$
	SC.1	42.0		0.005		0.02		$4.2\cdot10^{-5}$
	SC.2	164.0		0.01		0.08		$8.5\cdot10^{-5}$
	SC.3	5.0		0.0006		0.002		$5.1\cdot10^{-6}$
As	**SC.0**	62.7	7.22	0.44	0.13	2.51	10.96	0.66
	SC.1	42.0		0.30		1.68		0.45
	SC.2	164.0		1.18		6.57		1.77
	SC.3	5.0		0.03		0.2		0.045

EDI - estimated daily intake, THQ - target hazard quotient, TTHQ - total target hazard quotient, CR - carcinogenic risk (refer to Subsection 2.3 for equations).

When individual alternative scenarios were compared with the reference consumption, data revealed that alternative SC.3 was under the limits for both the fishes (tuna and swordfish), with the exception of As in tuna, namely in terms of CR. The other two alternative scenarios, SC.1 and SC.2, with fish/seafood consumption of 42 and 164 g/person/day, expectedly increased the values of EDI, THQ and TTHQ of three metals Hg, Cd and As.

The results were in line with studies from other researchers. Barone et al. (2018), studying swordfishes from Italy, found high THQ (>1) for Hg if swordfish consumed have a size of 167–233 cm with exposure ranging from 0.73 to 2.25 µg/kg bw/week. Storelli et al. (2020), also report similar results, with high estimated weekly intake (EWI) of Hg in children (5.88 μg/kg bw/week), resulting in high cancer risk (1.86×10^{-3}) from swordfish in Italy. In the case of tuna, the study by Kazemi et al. (2022) on *T. tonggol* reported high THQ & TTHQ in raw samples for arsenic (THQ-2.61, TTHQ-6.72) for adults and (THQ-12.18, TTHQ-12.34) for children. Furthermore, Milatou et al. (2020) reported high THQ (of 6.6) for Hg in reared Atlantic bluefin tuna. Overall, as tuna and swordfish production continue to increase and intensify there is a need to safeguard the health of humans through the reduction of the level of toxic elements (Hg, Cd, Pb and As) often associated with fish consumption.

4 CONCLUSIONS AND FUTURE WORK

In this work, the levels of toxic metals, namely mercury (Hg), cadmium (Cd), lead (Pb) and arsenic (As), in fresh swordfish and tuna fished and consumed worldwide were compiled and synthetized from the scholarly literature published since the year 2000. We are not aware of published syntheses studies on the levels of toxic metals and related health risk for consumers in fresh swordfish and tuna.

There were appreciable concentrations of Hg, Cd, Pb and As in the muscle tissues of the two fish species studied herein, tuna and swordfish, reported in the literature.

In the case of swordfish, the levels of toxic metals in the published literature ranged from 0.15 mg/kg to 3.97 mg/kg for Hg, 0.01 mg/kg to1.04 mg/kg for Cd, and 0.01 mg/kg to 3.90 mg/kg for Pb. Comparing locations, the highest levels of Hg, 3.97 mg/kg, were found in Western Indian Ocean and the lowest concentration, 0.07 mg/kg, was determined for samples obtained from Mediterranean Sea. When looking at levels of Cd, the highest levels were, 1.04 mg/kg in and the lowest concentration, 0.01 mg/kg, in Indian Ocean and Tyrrhenian Sea. The concentrations of Pb in swordfish ranged from a minimum of 0.01 mg/kg, in Reunion Island to a maximum of 3.90 mg/kg in Algerian Sea. No relation was found between size and the concentrations of metals in swordfish. A total of 18 studies data for swordfish depicted high Hg and Pb concentration above the permissible limits (1 mg/kg) which might indicate increased health risk.

In the case of tuna, concentrations of toxic metals ranged from 0.04 mg/kg to 3 mg/kg for Hg, 0.01 mg/kg to 2 mg/kg for Cd, 0.01 mg/kg to 0.92 mg/kg for Pb, and 0.9 mg/kg to 30.5 mg/kg for As. When comparing locations, the Mediterranean Sea and the China sea were the two locations with high concentrations of Hg, 3 mg/kg, and of As, 30.5 mg/kg, respectively. Almost all the species were detected to have high levels of Hg, >1 mg/kg, but longtail tuna also had high levels of As, above 30.5 mg/kg. A total of 19 studies data for tuna surpassed the permissible limits (1 mg/kg) for Hg and As and hence increases the health risk for the consumer.

Meta-analysis following a systematic literature review is an effective way to advance current knowledge about toxic metals in fishes. It was used to understand what the overall effect size was, i.e., average concentration, of toxic metals in tuna and swordfish across the published studies. Consequently, the three basic elements of the meta-analysis were discussed, the average effect sizes, the studies' heterogeneity (Q-statistic), and the effect of moderators (year, species and location) for swordfish (considering k=23 studies from 20 papers) and tuna (k=32 studies from 18 papers). The average concentration of the toxic metals retrieved from each paper constituted the outcome measure and a random-effects model was considered for the meta-analysis. The estimated mean concentrations for the toxic metals were 0.72 mg/kg for mercury, 0.15 mg/kg for cadmium, 0.11 mg/kg for lead, 7.19 mg/kg for arsenic and for swordfish it is, 1.13 mg/kg for mercury, 0.14 mg/kg for cadmium and 0.70 mg/kg for lead. There were significant differences (Q-test, $p<0.0001$) among species (in the case of tuna), years, and locations for every toxic metal considered herein. This considerable amount of heterogeneity is in line with the findings described above in the narrative literature review. Notwithstanding, the results from meta-analysis should be considered with care due to signs of publication bias. Systematic reviews can be very useful decision-making tools for monitoring toxic metals in fishes. They objectively summarize large amounts of information, identifying gaps in fish research, and identifying beneficial or harmful interventions which will be useful for researchers, and even for public and policymakers.

Human health risk assessment from heavy metal exposure through fish consumption showed significant carcinogenic adverse health risk to humans since calculated values were above the reference limits for two metal Hg and As. Nonetheless, Target Hazard Quotient (THQ) values calculated for Arsenic and mercury were >1 which implied a likely cause of adverse effects during a person's lifetime from tuna and swordfish consumption. The maximum carcinogenic risk was identified in arsenic from tuna (1.77) for alternative scenario 2 (164 g/person/day), but swordfish did not exceed the 10^{-6} threshold for any of the alternative scenarios. Thus, it was found that alternative scenario 3 (0.62 g/person/day for swordfish and 5 g/person/day for tuna) has the more realistic consumption values in regard to other two (1, 2), which poses no health risk to the Portuguese people, except with some cancer risk due to high As in tuna.

In conclusion, this work highlights the importance of understanding toxic metals in fish, swordfish, and tuna, for seafood safety and human health. However, further research is needed to refine analyses. Future studies should include non-English publications for a global perspective and improve meta-analysis methods. Exploring specific scenarios and countries will provide a more detailed assessment of health risks associated with toxic metals in fish, contributing to a comprehensive understanding of seafood quality, technology, and public health.

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