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Abstract:	Being on the top of the food chain, tunas are subjected to significative phenomena of bioaccumulation of conservative contaminants such as Potential Toxic Elements (PTEs). In this study, Cd, Pb and Fe levels in muscle of Mediterranean bluefin tuna (Thunnus thynnus) were determined by atomic absorption spectroscopy (GFAAS), both in wild and farmed groups, to investigate the safety and the quality of this fish as seafood. A total of 68 samples were collected, wild samples (n = 30) from Sardinia island (Italy) and farmed samples (n = 38) from an aquaculture fish farm in Malta. Mean values, expressed as mg kg $^{-1}$ wet weight, were found as 0.014 (wild) and 0.02 (farmed) for Cd; 0.11 (wild) and 0.03 (farmed) for Pb and 13 (wild) and 7 (farmed) for Fe. Relationships between metal concentrations and biometric parameters were evaluated and a comparison between the levels of metals of wild and farmed groups has also been conducted. No statistically significant difference between the two groups was found for Cd, with 99% of samples below the EU limit. The difference for Pb levels were statistically significant, with wild samples showing concentrations more than four times higher than the farmed ones, but with 98% of samples below the EU limit. The levels of Fe were significantly lower in the farmed group with respect to wild specimens, although samples of both groups could be considered good products for the intake of this element. On the base of the recommended tolerable weekly intakes, samples of this study can be considered a safe seafood.

Potential toxic elements (PTEs) in wild and farmed Atlantic Bluefin Tuna (*Thunnus thynnus*) from Mediterranean Sea: risks and benefits for human consumption

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Abstract

Being on the top of the food chain, tunas are subjected to significative phenomena of bioaccumulation of conservative contaminants such as Potential Toxic Elements (PTEs). In this study, Cd, Pb and Fe levels in muscle of Mediterranean bluefin tuna (*Thunnus thynnus*) were determined by atomic absorption spectroscopy (GFAAS), both in wild and farmed groups, to investigate the safety and the quality of this fish as seafood. A total of 68 samples were collected, wild samples (*n* = 30) from Sardinia island (Italy) and farmed samples (*n* = 38) from an aquaculture fish farm in Malta. Mean values, expressed as mg kg^{-1} wet weight, were found as 0.014 (wild) and 0.02 (farmed) for Cd; 0.11 (wild) and 0.03 (farmed) for Pb and 13 (wild) and 7 (farmed) for Fe. Relationships between metal concentrations and biometric parameters were evaluated and a comparison between the levels of metals of wild and farmed groups has also been conducted. No statistically significant difference between the two groups was found for Cd, with 99% of samples below the EU limit. The difference for Pb levels were statistically significant, with wild samples showing concentrations more than four times higher than the farmed ones, but with 98% of samples below the EU limit. The levels of Fe were significantly lower in the farmed group with respect to wild specimens, although samples of both groups could be considered good products for the intake of this element. On the base of the recommended tolerable weekly intakes, samples of this study can be considered a safe seafood.

Keywords: Atlantic bluefin tuna; Mediterranean Sea; cadmium; lead; iron; food risks/benefits.

1. Introduction

Over the past two decades, the interest on potential toxic elements (PTEs), in association with aquatic contamination and food safety, has grown. These elements can be dangerous because of their high environmental persistence and potential ecological risks as many aquatic organisms can bioaccumulate them or their compounds in different body tissues. While some elements are recognized to be essential for organisms (Cu, Zn, Fe, Ni, Co, Se, Mo, Cr), some others have no biological role and are considered highly toxic (Ag, Al, Cd, Hg, Pb, As, Sr, U).

PTEs are introduced in the marine environment through various anthropogenic and natural sources. Examples of anthropogenic sources are smelting processes, fuel combustion and industrialization (Forstner and Wittman, 2012). Natural erosion, volcanic activity and wind-blown dust represent most of the natural sources. Moreover, PTEs can reach the aquatic environment through atmospheric fallout, waste dumping, accidental leaks, and runoff of terrestrial systems (industrial and domestic effluents) (Eisler, 1981).

Among these, Cd and Pb are listed as priority substances for water, sediments and biota and their maximum levels are fixed by the Marine Strategy Framework Directive (Directive 2008/56/EC). In particular, MSFD descriptor 9 reports: “Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards”.

Cadmium (Cd) is classified as a non-essential element and it is toxic to multiple tissues both for acute and chronic exposure (Faroon *et al.*, 2012; Liu *et al.*, 2007; Liu *et al.*, 2008). Cd and its inorganic compounds are classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (IARC Group 1) (IARC, 1997). The Europe Food Safety Agency (EFSA) specified a tolerable weekly intake (TWI) of 2.5 μg kg^{-1} body weight (b.w.) (Alexander *et al.*, 2009).

Lead (Pb) is a cumulative toxic element that affects multiple body systems. Both natural and anthropogenic sources contribute to Pb dispersal to the environment, but the atmospheric one, primarily from its past use as a fuel additive, has made it a pervasive and persistent pollutant worldwide (Martín *et al.*, 2015; Barbaro *et al*. 2016). In 2010, the Joint FAO/WHO Expert Committee on Food Additives meeting (JECFA) confirmed that Pb reduces children’s IQ and increases adults’ systolic blood pressure by approximately 3 mmHg. Thus, it was concluded that the provisional tolerable weekly intake (PTWI) standard was no longer appropriate, and it was accordingly withdrawn (JECFA, 2010). Pb inorganic compounds are classified as IARC Group 2A, while Pb organic compounds as IARC Group 3 (IARC, 2020).

Iron (Fe) is an essential element as it is an important cofactor for a wide variety of cellular processes, such as oxygen transport, respiration, the tricarboxylic acid cycle, lipid metabolism, gene regulation and DNA synthesis (Cairo *et al*., 2006). Regulation (EU) Nº1169/2011 sets a reference daily intake corresponding to 14 mg of this mineral (Annex XIII). However, high tissue Fe concentrations have been associated with the development and progression of several pathological conditions (Fraga and Oteiza, 2002).

Many surveys have been carried out to detect the presence of PTEs in the aquatic biota, including different species of fishes, to evaluate the food safety in accordance with limits set by legislation (Annibaldi *et al*., 2019; Araújo et al., 2016; Bosch *et al*., 2016; Chouvelon *et al*., 2017). Regulation (EC) N°1881/2006 and amending regulation 488/2014/EU set the maximum levels for certain contaminants foodstuff, including metals such as lead, cadmium, mercury, inorganic tin, and inorganic arsenic.

Atlantic bluefin tuna *Thunnus thynnus* (ABFT) is a high-performance fish located at the top the food chain, with very high metabolic rates. It is exposed to a large amount of toxic substances and accumulates significant concentrations of metals in its muscle tissues (Licata *et al*. 2005; Storelli *et al*. 2010). According to the United Nations Food and Agriculture Organization (FAO), aquaculture is growing faster than other major food production sectors, with 5.8 percent annual growth rate since 2010 (FAO, 2018) and the increase of tuna demand for sushi market in the last decades, made the aquaculture of ABFT follow a similar trend (De la Gándara, 2015).

Although there are several studies detecting toxic metals accumulation in ABFT muscle tissues (Hellou *et al*., 1992; Di Bella *et al*.; 2015; Ugarte *et al*., 2011), scientific publications on the levels of metals in farmed tunas and comparisons with wild samples are rather limited (Vizzini *et al*., 2010; Annibaldi *et al*., 2019; Özden *et al*., 2018). In this study, levels of Cd, Pb and Fe in wild and farmed ABFTs were determined to address the following questions: (1) can ABFT be considered a safe food for human consumption considering the legislative limits for these elements?; (2) are there differences in PTEs levels between wild and farmed groups?; (3) are there relationships with PTEs levels and the size/gender factors and (4) which is the possible role of feed conditions and environment in the presence of PTEs?

2. Materials and methods

2.1 Samples collection

The animals were sampled under the guidelines Art 36, par.1 Regulation (EU) N°508/2014. The procedures did not include animal experimentation, so ethics approval is not necessary for accordance with the Italian legislation.

Wild samples were collected as indicated in Annibaldi *et al.* (2019). Briefly, wild samples of ABFT were caught by traps in Carloforte Tonnare (Sardinia Island, Italy) (**Figure 1, A**) during the period of May-June 2017. A total of 30 samples were caught. Sex of fishes (17 males and 13 females) were determined by examining gonads under a dissecting microscope. The overall mean curved fork length (from the tip of the upper jaw to the fork of caudal fin) was 130 ± 10 cm (males 130 ± 11 cm, females 131 ± 9 cm) and the overall mean body weight was 43 ± 9 kg (males 42 ± 10 kg, females 43 ± 8 kg). Muscle samples were taken from the dorsal region near the tail.

Farmed ABFT samples were caught in the spawning sites of Mediterranean Sea during the reproductive season (May-June 2015), as indicated in Truzzi *et al.* (2018), and then transported by towed cages to a fish farm located in the South-East area of Malta (**Figure 1, B**) where they were fed for a period of about five months with defrosted raw fish coming from Pacific and Atlantic Ocean (Annibaldi *et al.* 2019). In November 2015, during the post reproductive phase, 38 samples (18 males, 20 females) were collected. The overall mean curved fork length was 226 ± 33 cm (males 237 ± 31 cm, females 216 ± 31 cm). The overall body weight was 233 ± 92 kg (males 272 ± 88 kg, females 198 ± 83 kg). The sex of the fish was determined by examining gonads under a dissecting microscope. All fish were in the adult stage. For each tuna, three independent samples of muscle were taken (about 10 g each) from the top of the dorsal region.

2.2 Laboratory and apparatus

Samples were prepared and analysed in a clean room laboratory ISO 14644-1 Class 6, with areas at ISO Class 5 under laminar flow. The acid-cleaning procedures, used for all the laboratory materials, were performed as described by Illuminati *et al*. (2014). Samples were weighted in the analytical balance AT261 Mettler Toledo (Greifensee, Switzerland, readability 0.01 mg, repeatability SD = 0.015 mg). Variable volume micropipettes and neutral tips were from Brand (Wertheim, Germany, Transferpette). Scalpels with sterile stainless-steel blades were from Granton (Mod. 91021, Sheffield, England).

2.3 Chemicals and reagents

A two-stage system Midi (Elix and Milli-Q) from Millipore (Bedford, MA, USA) was used to produce ultrapure water. Working standard solutions were prepared by appropriate dilution from inorganic atomic absorption 1.0 g L^{-1} Cd, Pb and Fe standard solutions from Carlo Erba (Milan, Italy) and stored in a refrigerator at +4°C protected from light. Citric acid powder was purchased from Sigma Aldrich. Superpure nitric acid (67-69%) and hydrogen peroxide (30%) were purchased from Carlo Erba (Milan, Italy). Dogfish muscle DORM-2 (NRCC, Ottawa, ON, Canada) was used as certified reference material (CRM).

2.4 Samples treatment

Samples were treated in the same way as described by Annibaldi *et al*. (2019). About 0.5 g of tissue for each sample was minced and homogenized (homogenizer MZ 4110, DCG Eltronic, Monza, Italy). After the homogenization, tissues were accurately weighed and freeze-dried (Edwards EF4 modulyo, Crawley, Sussex, England) until constant weight (± 0.2 mg), then put into Teflon PFA vessels (HP-500 plus, CEM, Mathews, NC, USA) of a Microwave Accelerated Reaction System, MARS-5, 1500 W (CEM, Mathews, NC, USA) and digested without any pretreatment with a mixture of 3 mL of HNO_3 and 3 mL of H_2O_2. An HP-500 control vessel containing the same matrix of samples was used to control temperature and pressure during the process. The system makes possible to operate in four modalities: standard control, power/time control, ramp to temperature, ramp to pressure. The program used for tissue digestion is reported in **Table 1**.

2.5 Analytical methodology

Metals quantitative determinations were carried as in Truzzi *et al*. (2019, 2020). Briefly, an atomic absorption spectrophotometer 240Z AA-GTA120 Graphite Tube Atomizer (Agilent Technologies, Santa Clara, California, USA) equipped with Zeeman background correction was used. Argon with a purity of 99.999% was used as the carrier gas. Multi-element hollow cathode lamps were used as a light source. Cd, Pb and Fe were measured at wavelengths of 228.8, 283.3 and 248.3 nm. To improve the analytical measurements a 0.2% Pd matrix modifier in citric acid was used. Procedural blanks accounted for less than 1% of the total element concentrations in samples.

2.6 Accuracy

To assess the accuracy of the data obtained from the instrumental analyses Cd, Pb and Fe were determined in DORM-2 certified reference material. Certified mean values and experimental mean values obtained from the analysis of DORM-2, expressed in mg kg^{-1} dry weight (d.w.), are shown in **Table 2**. No statistically significant differences ($p > 0.05$) between certified and measured values were detected.

2.7 Statistical analysis

Data are expressed as arithmetic mean ± standard deviation (SD) of the performed replications. Statistical analyses were performed as indicated in Roveta *et al.*, 2020, using the analysis of variance (one-way ANOVA) after testing the homogeneity of the variance with Levene's test

(Wayne 2005). In case of heteroscedasticity, the non-parametric Kruskal-Wallis analysis of variance was applied. Depending on the resulting statistics, post-hoc comparison was eventually performed with the Bonferroni correction, always considering a significant level of 0.05. Statistical analyses were performed using STATGRAPHICS (STATGRAPHICS Centurion 2018, Statgraphics Technologies Inc., The Plains, VA, USA). All graphs were created using Systat SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA).

3. Results

Table 3 shows the mean concentrations of Cd, Pb and Fe, expressed in mg kg^{-1} of wet weight (w.w.), together with biometric parameters of wild and farmed groups divided by sex. Metals concentrations were evaluated as a function of body weight, having this latter a statistically significant correlation with length parameter ($p < 0.05$, $r = 0.9730$).

3.1 Cd content

Overall mean concentration of Cd was 0.014 ± 0.006 mg kg^{-1} w.w. (from 0.0041 to 0.0270 mg kg^{-1} w.w.) and 0.021 ± 0.020 mg kg^{-1} w.w. (from 0.0007 to 0.0857 mg kg^{-1} w.w.) for wild and farmed samples, respectively. No statistically significant difference was evidenced between the two groups of samples ($p > 0.05$) (**Figure 2, a**). In relation to sex, there was not a statistically significant difference ($p > 0.05$) between Cd levels of the two sexes both in wild and in farmed group, with farmed males showing a Cd content higher than farmed females (0.028 ± 0.025 and 0.014 ± 0.008 mg kg^{-1} w.w. respectively) (**Figure 2, b**).

Concerning the body weight of analyzed specimens, no statistically significant correlations were found between Cd content and body weight in wild males ($r = 0.1095$; $p > 0.05$), and in farmed males ($r = 0.1546$; $p > 0.05$). No statistically significant correlations were also revealed in females, but a specific trend can be found. In particular, with the weight increase, Cd content decreased in wild females ($r = 0.4518$; $p > 0.05$) (**Figure 3, a**) and increased in farmed females ($r = 0.4576$; $p > 0.05$) (**Figure 3, b**).

3.2 Pb content

Overall mean concentration of Pb was 0.11 ± 0.08 mg kg^{-1} w.w. (from 0.0048 to 0.3551 mg kg^{-1} w.w.) and 0.03 ± 0.02 mg kg^{-1} w.w. (from 0.0037 to 0.0774 mg kg^{-1} w.w.) for wild and farmed samples, respectively. A statistically significant difference was found between the two groups of samples ($p < 0.05$) (**Figure 4, a**): Pb in wild samples was more than four times higher compared to farmed tunas. In relation to sex, there was not a statistically significant difference ($p > 0.05$) of Pb levels between males (0.09 ± 0.06 mg kg^{-1} w.w.) and females (0.13 ± 0.09 mg kg^{-1} w.w.) of wild group nor in males and females of farmed group (0.02 ± 0.02 and 0.03 ± 0.02 mg kg^{-1} w.w. respectively) (**Figure 4, b**), with females showing an higher Pb content in both groups.

There was not a statistically significant correlation between concentration of Pb and body weight neither in males (r = 0.143; $p > 0.05$) nor in females (r = 0.196; $p > 0.05$) wild samples. As for the wild group, body weight of the samples did not affect the concentrations of Pb neither in males (r = 0.126; $p > 0.05$) nor in females (r = 0.1122; $p > 0.05$) farmed samples. Therefore, no particular trend of Pb content in relation to body weight has been detected.

3.3 Fe content

Overall mean concentration of Fe was 13 ± 7 mg kg^{-1} w.w. (from 4 to 31 mg kg^{-1} w.w.) and 7 ± 3 mg kg^{-1} w.w. (from 4 to 16 mg kg^{-1} w.w.) for wild and farmed samples, respectively. A statistically significant difference was found between the two groups of samples ($p < 0.05$) (**Figure 5, a**), with a higher Fe concentration in wild specimens. Wild females showed a statistically higher Fe levels (17±8 mg kg^{-1} w.w.) with respect to farmed males and farmed females (8±3 and 7±2 mg kg^{-1} w.w., respectively) ($p < 0.05$), whereas no statistically significant difference of Fe levels was found between wild females and wild males (10±4 mg kg^{-1} w.w.) ($p > 0.05$) (**Figure 5, b**).

Concerning the body weight of analyzed specimens, no statistically significant correlations were found between Fe content and body weight in wild females (r = 0.0001; $p > 0.05$), and in farmed males (r = 0.0257; $p > 0.05$). No statistically significant correlations ($p > 0.05$) were also revealed in farmed females and in wild males, but a specific trend can be found. In particular, in wild males Fe levels decreased with the increasing of body weight, (r = -0.4883; $p > 0.05$) (**Figure 6, a**), while in farmed females an opposite trend was detected, with Fe content increasing with the increase of body weight (r = 0.3290; $p > 0.05$) (**Figure 6, b**).

4. Discussion

4.1 PTEs levels

Cd, Pb and Fe in muscle of ABFT has been detected with very low concentrations compared to other biological tissues such as the liver (Licata *et al.*, 2005).

The relatively low concentrations of Cd in the ABFT muscle can be explained by the fact that Cd accumulates mostly in liver, kidney, and gill while the concentration in muscle tissue is generally much lower (Karaytug *et al.*, 2007), considering the levels of dissolved Cd range from 0.062 nmol L^{-1} in open Mediterranean Sea (Tankere and Statham, 1996) to 0.1 - 0.6 nmol L^{-1} in oceans (Aparicio-González *et al.*, 2012).

The difference between the concentrations of the two groups is probably related to the different feeding conditions as farmed tunas were fed with fish at low Pb content such as Pacific mackerel (*Scomber japonicus*) and Atlantic mackerel (*Scomber scombrus*) (Keskin *et al.*, 2007). A difference in levels between sexes was detected, with female samples showing higher Pb concentration than males, both in wild and farmed groups. A similar condition in muscle sample of wild tunas was identified by Di Bella *et al.*, 2015. Levels of dissolved Pb ranges from a mean of 0.062 nmol L^{-1} in open Mediterranean Sea (Tankere and Statham, 1996) to 0.03 - 0.1 nmol L^{-1} (Aparicio-González *et al.*, 2012).

The difference between Fe concentrations in the two groups of samples can be probably related to the different feeding condition, as observed by Percın *et al.*, 2011. As for Pb, Fe levels were higher in female samples of wild group as detected by Di Bella *et al.*, 2015 in wild samples with a similar body weight of the ones on the present study.

A statistically analyses was carried out to evaluate the possible relationship between the bioaccumulation of these metals in ABFT muscle tissue. A positive statistically significant linear correlation was found between Cd and Fe levels, both in wild ($r = 0.5230$; $p = 0.0036$) and farmed ($r = 0.4852$; $p = 0.0049$) specimens (**Figure 7**). There are not previous studies on possible relationship between these two elements in *T. thynnus*, however a positive statistically significant linear correlation was recorded in muscle tissue of different marine fishes such as *Coryphaena hippurus* (Kojadinovic *et al.*, 2007) and *Rutilus rutilus* (Alipour *et al.*, 2015), while interesting synergic modulation has been documented for *Danio rerio* (Cooper et al., 2006), rats and humans (Flanagan *et al.*, 1978; Åkesson *et al.*, 2002). No statistically significant correlations were found between Cd-Pb and Pb-Fe ($p > 0.05$).

4.2 Comparison with literature data

The results obtained in this work were compared with scientific literature with reference to the tuna specimens belonging to the *Thunnus* species (**Table 4)**. Since only one paper dealt on farmed specimens the discussion for farmed group is limited.

Levels of Cd in *T. thynnus* recorded in this study were of the same order of magnitude compared to those of specimens of the same species caught in the same area and also in different geographical sites, while they were lower than that found in *T. obesus* and *T. albacares* (Torres *et al.*, 2016, Araújo *et al.*, 2016, Chouvelon *et al.*, 2017). In this study, levels of Pb found in the wild group were higher than those found in literature, while the farmed samples showed a Pb content lower than that found in other specimens both in the *T. thynnus* species and in other species (Falcò *et al.*, 2006; Storelli *et al.*, 2005; Ugarte *et al.*, 2012; Di Bella *et al.*, 2015; Ashraf and Jaffar, 1988; Yusa *et al.*, 2008; Besada *et al*., 2006; Torres *et al*., 2016; Araújo *et al*., 2016; Ruelas-Inzunza *et al*., 2012; Burger and Gochfeld, 2005; Chouvelon *et al*., 2017). Concerning Fe, values ranged from 3.649 mg kg^{-1} ww (Di Bella *et al*., 2015) to over 130 mg kg^{-1} ww (Topçuoğlu *et al*., 1990). In a study conducted in Turkey (Percın *et al*., 2011), *T. thynnus* wild specimens are richer in Fe than the farmed ones, a condition similar to the one of the present study.

4.3 Risks assessment and benefits for consumers

Concerning Cd, with an overall mean value of 0.0174 ± 0.0152 mg kg^{-1} w.w., both wild and farmed specimens had a concentration well below the legal limit (0.10 mg kg^{-1} w.w., Commission Regulation (EC) N°1881/2006 and amending regulation 488/2014/EU).

Pb was detected with an overall mean value of 0.06 ± 0.07 mg kg^{-1} w.w. In accordance with current European legislation, which establishes the limit of Pb contained in tuna muscle of 0.30 mg kg^{-1} w.w. (Commission Regulation (EC) N°1881/2006 and amending regulation 488/2014/EU), only two samples had a Pb concentration beyond that limit: male sample code 89, with a concentration of 1.18 ± 0.01 mg kg^{-1} w.w., considered a statistical outlier, and female sample code 354, with a concentration of 0.36 ± 0.02 mg kg^{-1} w.w. Therefore, 93.33% of wild samples and all farmed samples are below the legal limit, even if there is no longer a dose that is considered protective for humans (JECFA, 2010).

Tuna is a food rich in Omega-3. The International Society for the Study of Fatty Acids and Lipids recommends a daily intake (RDA) of at least 500 mg of Omega-3 (Harris *et al*., 2008). Farmed tuna samples analyzed in this study contained 24 - 27 mg g^{-1} of Omega-3 EPA+DHA (eicosa-5,8,11,14,17-pentaenoic acid and docosa-4,7,10,3,16,19-hexaenoic acid) (Truzzi *et al.*, 2018), then a portion of 200 g of these tunas satisfies the RDA. This amount of muscle contains extremely low quantity of Cd and Pb (3.48 μg of Cd and 12.72 μg of Pb). Considering that the TWI for Cd is 2.5 μg kg^{-1} b.w. (JECFA, 2010), both wild and farmed ABFT resulted safe as seafood.

Tunas are considered a good food as source of Fe, containing approximately 1.2 mg 100 g^{-1} of edible portion (HealthLinkBC, 2020), a value that is similar to the results of this study (overall mean 10 ± 6 mg kg^{-1}). According to Kalogeropoulos *et al*. (2012), tunas of this study have a Fe levels similar to Mediterranean raw seafood such as anchovy, bogue, hake, picarel, sand smelt and striped mullet.

5. Conclusions

This study provides informations on the concentration of Cd, Pb and Fe in the muscle of wild and farmed Atlantic bluefin tuna (*Thunnus thynnus*) specimens caught in the Mediterranean Sea. About

Cd, there were no statistically significant differences between wild and farmed specimens, and 99% of samples showed a Cd content below the limit allowed by EU legislation. Wild samples showed Pb concentrations more than four times higher than the farmed ones, despite having a considerably smaller size. Moreover, this metal seems to bioaccumulate more in female samples. 98.4% of samples had a concentration of Pb below the limit allowed by EU legislation. Fe intake is slightly lower in the case of farmed tunas’ consumption, but samples of both groups could be considered good products for the intake of this element.

According to the results, the consumption of farmed ABFT provides more protection to the consumer against Pb toxic effects, while they did not show differences for Cd and Fe intake with respect to the wild specimens. Moreover, a relationship was found between Cd and Fe content while the size did not affect bioaccumulation phenomena for these elements. However, within the framework of the Marine Strategy that aims to a good environmental status both for Descriptor 3 (the population of commercial fish species is healthy) and Descriptor 9 (contaminants in seafood are below safe levels), the limited availability of scientific data, especially on farmed tunas in the area of Mediterranean Sea, makes further studies necessary to support the increasing demand of this food in our diet.

Conflicts of Interest: The authors declare no conflict of interest.

Author Contributions:
O.C. and C.T. conceived and designed the experiment;
F.G. wrote the paper;
F.G. and A.A. analysed the data;
C.T. and S.I. revised the manuscript;
P.P. provided the logistic support.

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Figure 1. ABFT sampling areas in Mediterranean Sea: (A) Carloforte tonnare, Sardinia, Italy (wild samples); (B) Fish farm, Malta (farmed samples). © 2019 Microsoft

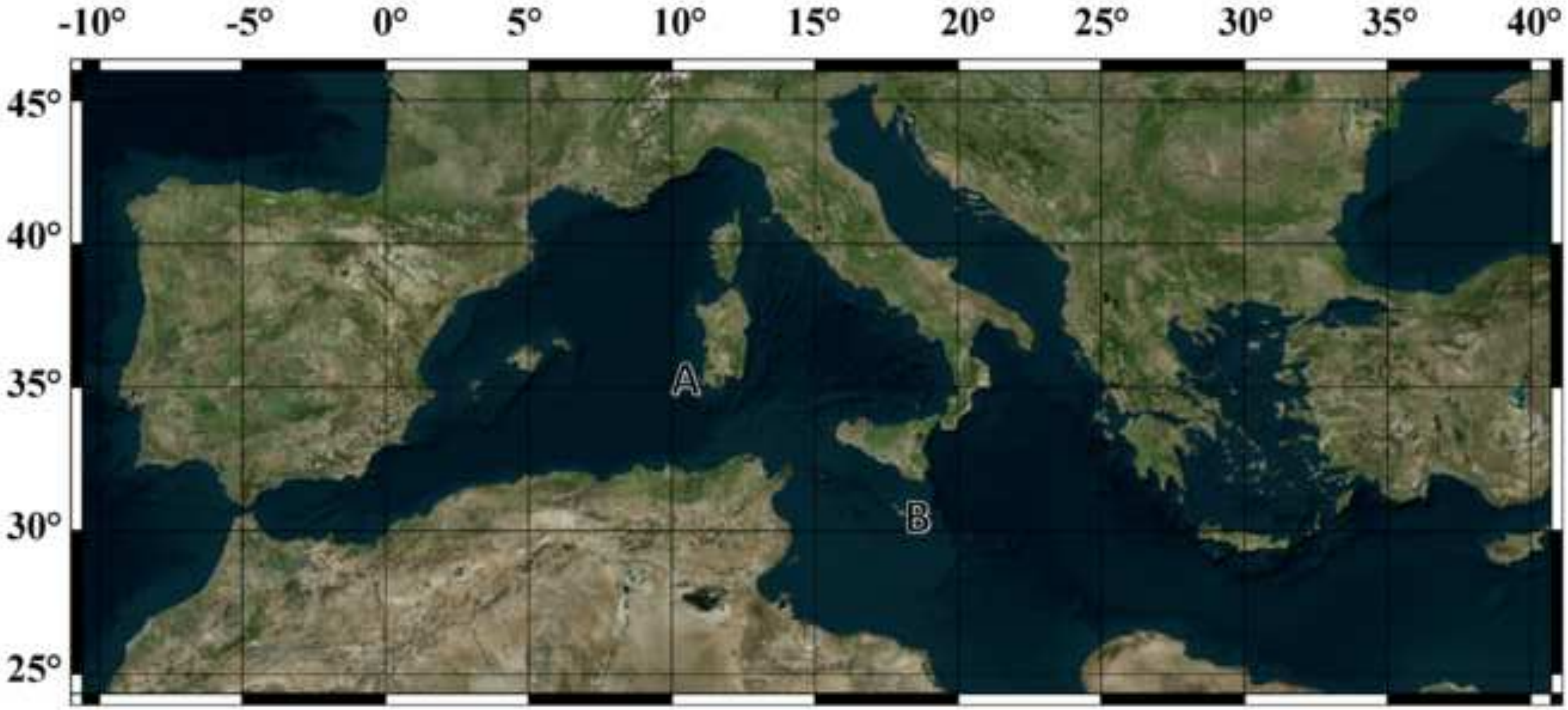

Figure 2. Mean Cd concentration (mg kg-1 w.w.) in muscle tissue of a) wild and farmed ABFT; b) males and females of wild and farmed ABFT. Bars bearing different letters

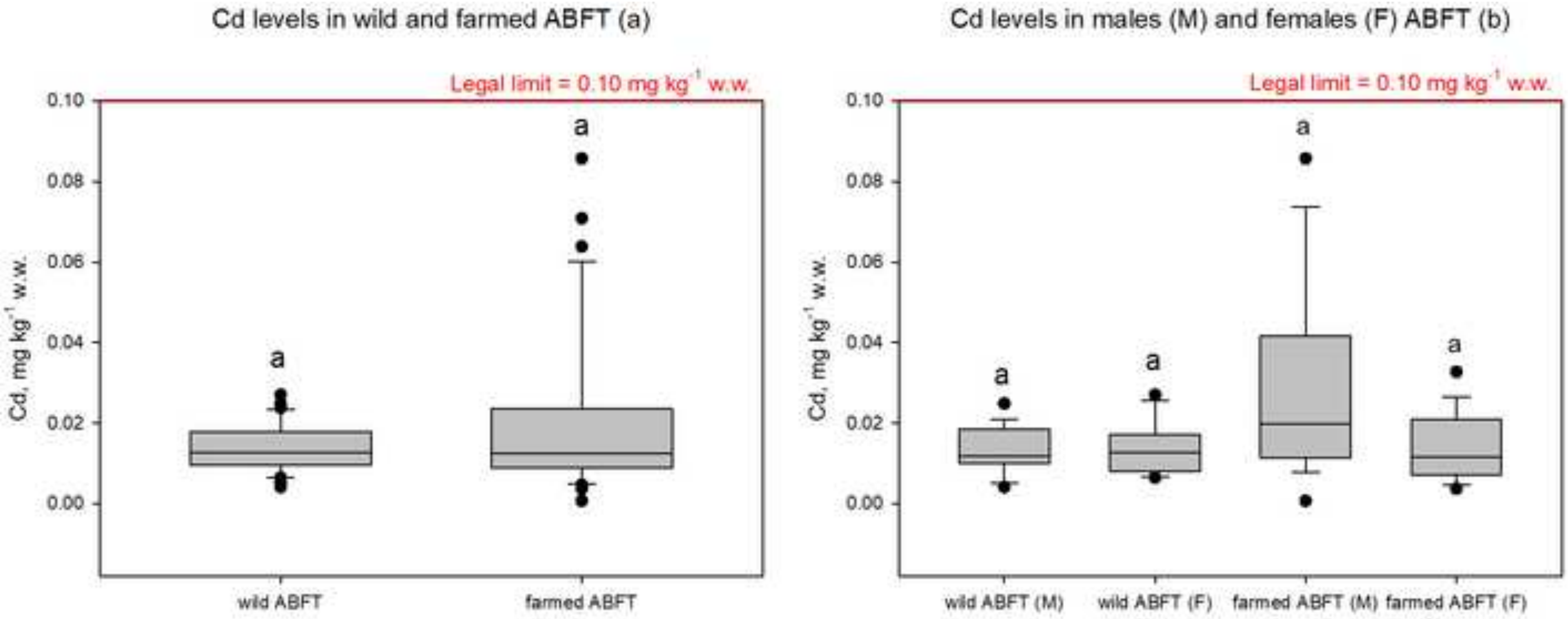

Figure 3. Relation between Cd levels (mg kg-1 w.w.) and weight (kg) in female specimens: a) wild samples; b) farmed samples.

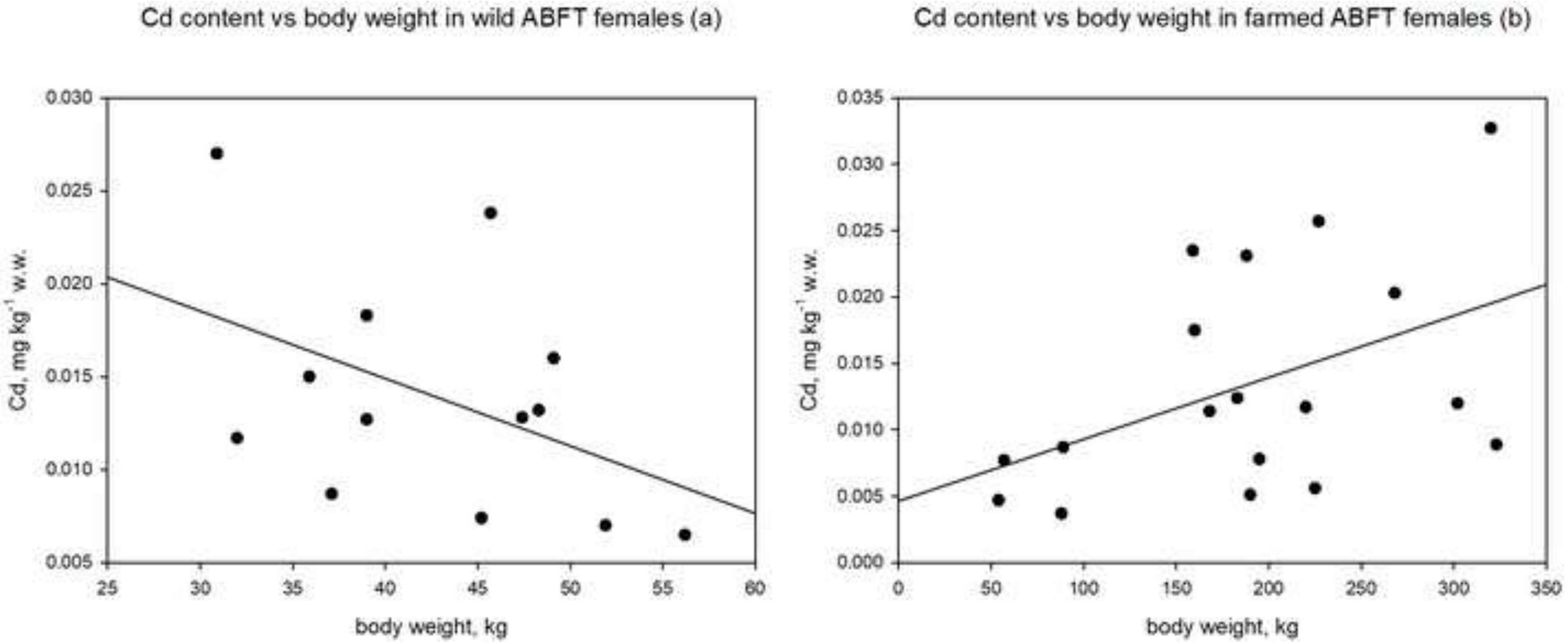

Figure 4. Mean Pb concentration (mg kg-1 w.w.) in muscle tissue of a) wild and farmed ABFT; b) males and females of wild and farmed ABFT. Bars bearing different letters

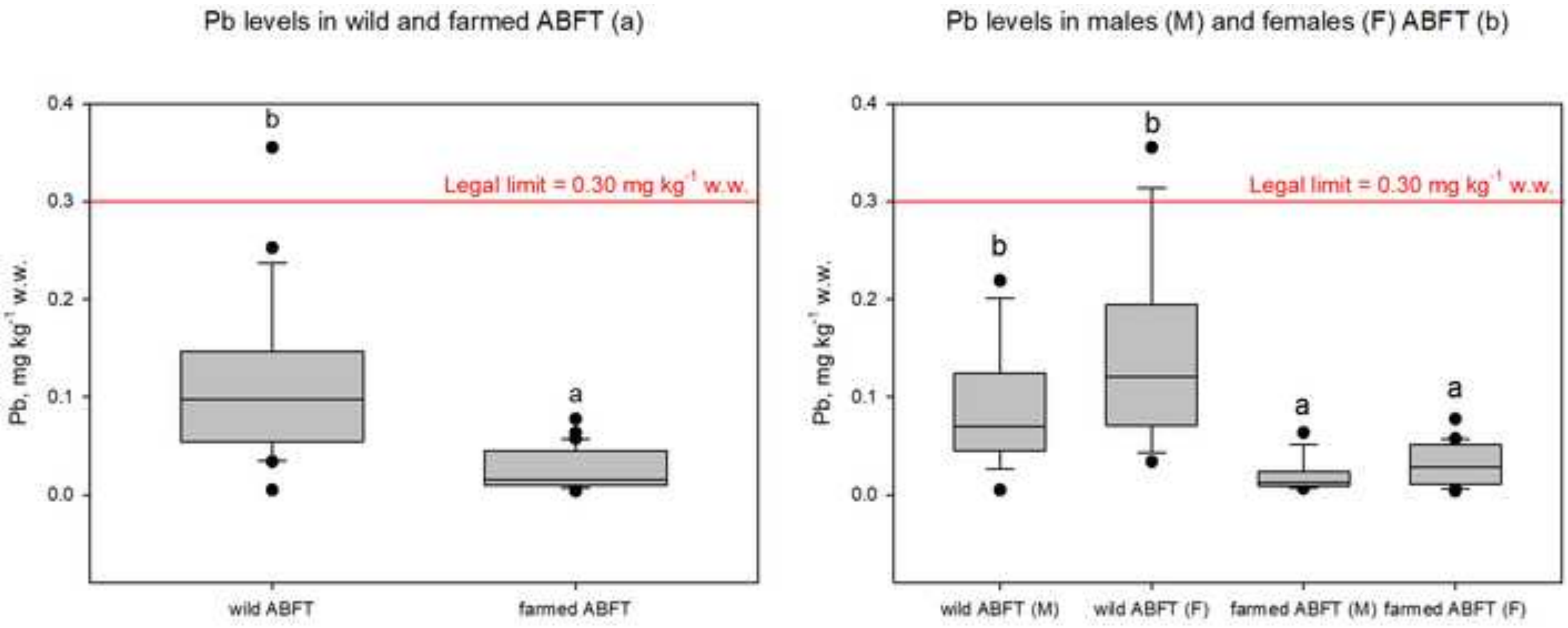

Figure 5. Mean Fe concentration (mg kg-1 w.w.) in muscle tissue of a) wild and farmed ABFT; b) males and females of wild and farmed ABFT. Bars bearing different letters

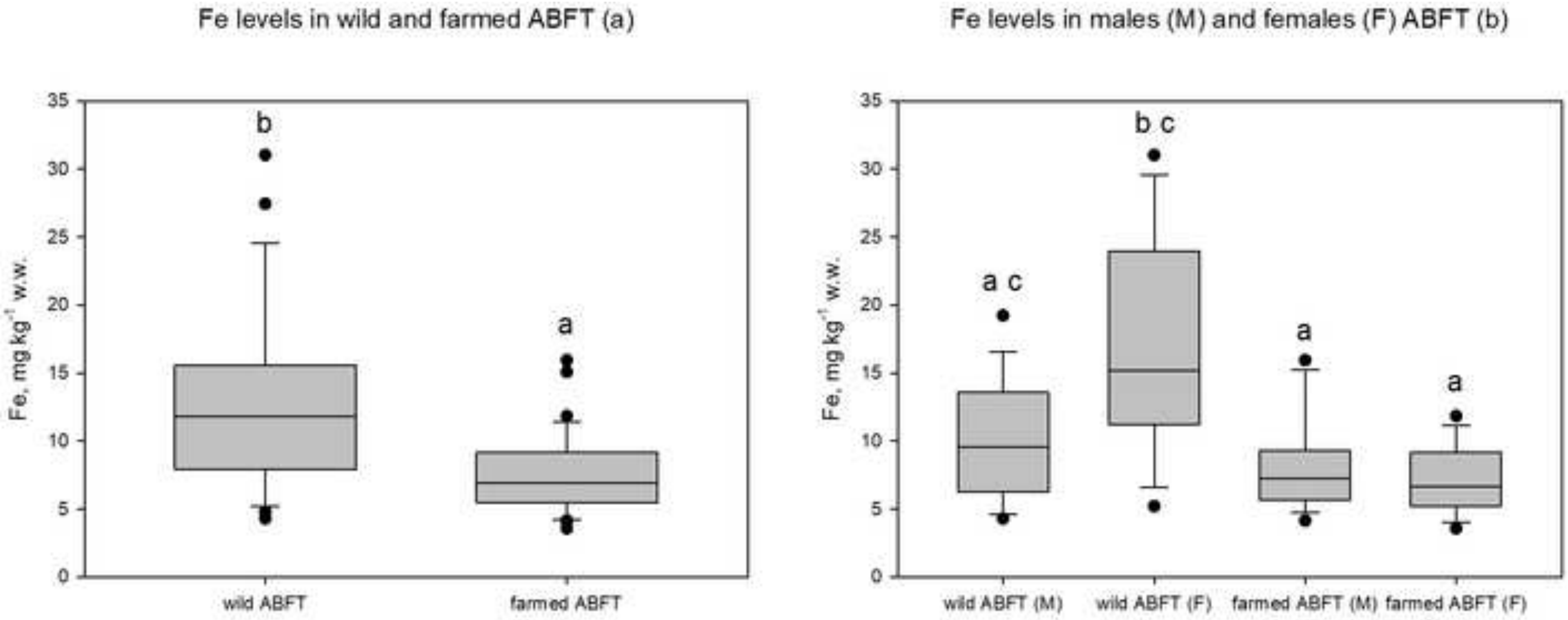

Figure 6. Relation between Fe levels (mg kg-1 w.w.) and weight (kg) in a) males of wild samples; b) females of farmed samples.

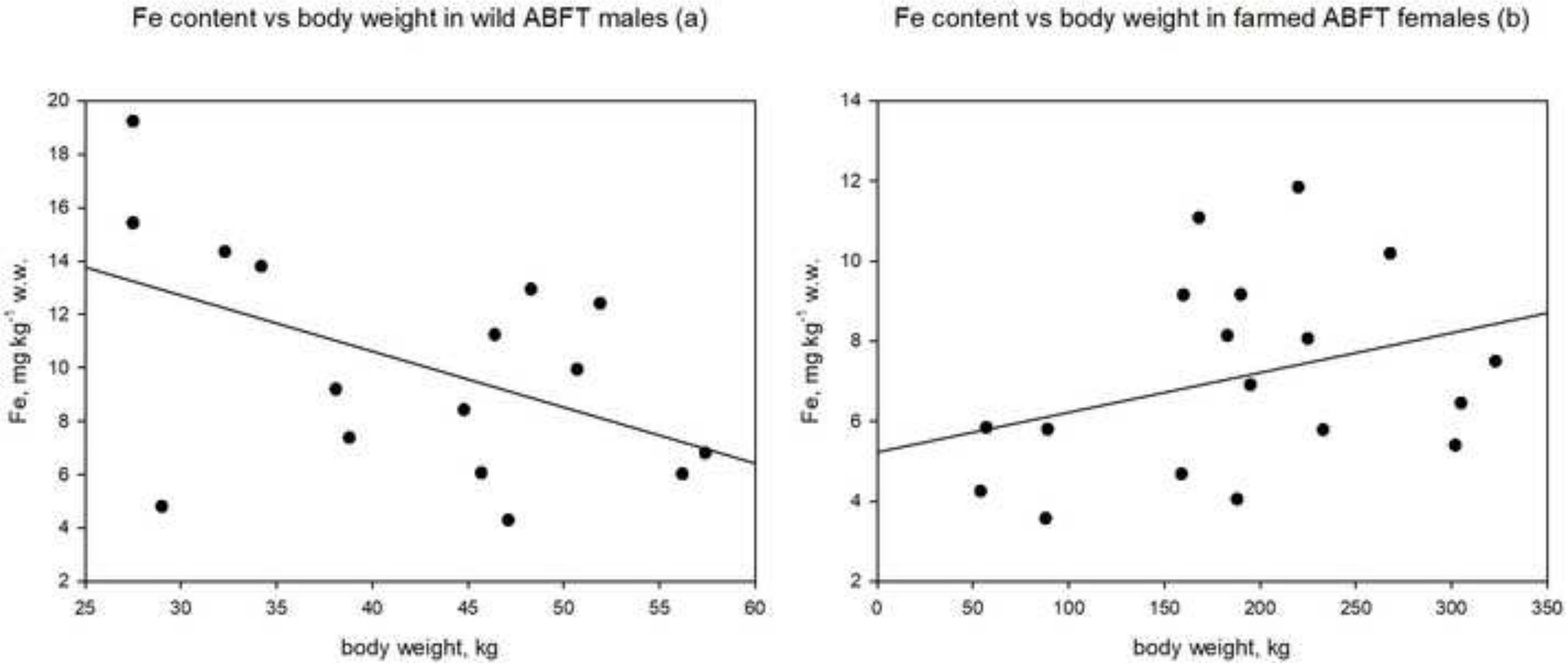

Figure 7. Relation between Cd and Fe levels (mg kg-1 w.w.) in a) wild and b) farmed ABFT.

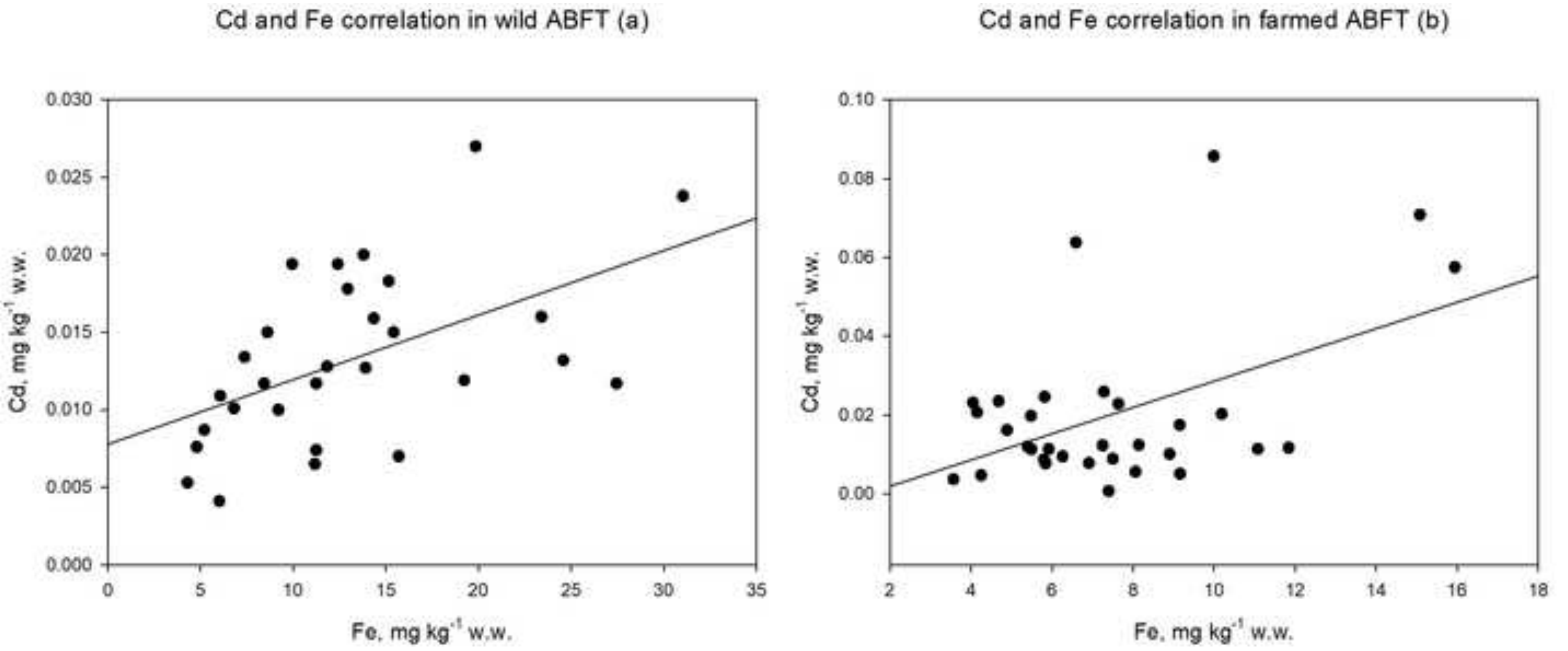

Table 1. Parameter of Microwave Assisted Digestion for tuna tissues.

Step	**Oven Power (W)**	**Power (%)**	**Time (min)**	**Pressure (psi)**	**Temperature (°C)**	**Hold time (min)**
1	800	100	10	50	150	5
2	800	100	10	90	160	5
3	800	100	10	150	175	5

Table 2. Certified mean values of DORM-2 vs experimental mean values.

Element	**Certified mean values (mg kg^{-1} d.w.)**	**Experimental mean values (mg kg^{-1} d.w.)**
Cd	0.043 ± 0.008	0.047 ± 0.004
Pb	0.065 ± 0.007	0.071 ± 0.004
Fe	142 ± 10	132 ± 1

Table 3. Biometric parameters and Cd, Pb and Fe concentration (mg kg^{-1} w.w.) in ABFT.

				Metal, Mean ± SD (min-max), mg kg^{-1} w.w.		
	n	**Weight (kg)**	**Length (cm)**	**Cd**	**Pb**	**Fe**
Wild (*Sardinia*)	**30**	**130 ± 10**	**43 ± 9**	**0.014 ± 0.006 (0.004-0.027)**	**0.11 ± 0.08 (0.005-0.355)**	**13 ± 7 (4-31)**
Males	17	130 ± 11	42 ± 10	0.016 ± 0.007 (0.004-0.025)	0.09 ± 0.06 (0.005-0.219)	10 ± 4 (4-19)
Females	13	131 ± 9	43 ± 8	0.014 ± 0.006 (0.006-0.027)	0.13 ± 0.09 (0.034-0.355)	17 ± 8 (5-31)
Farmed (*Malta*)	**38**	**226 ± 33**	**233 ± 92**	**0.021 ± 0.020 (0.0007-0.086)**	**0.03 ± 0.02 (0.0037-0.077)**	**7 ± 3 (4-16)**
Males	18	237 ± 31	272 ± 88	0.028 ± 0.025 (0.0007-0.086)	0.02 ± 0.02 (0.0062-0.063)	8 ± 3 (4-16)
Females	20	216 ± 31	198 ± 83	0.014 ± 0.008 (0.0037-0.033)	0.03 ± 0.02 (0.0037-0.077)	7 ± 2 (4-12)

Table 4. Concentration of Cd, Pb and Fe in *Thunnus spp*.

Sampling area (*sampling*)	**Weight (kg) Lenght (cm)**	**Cd (mg kg^{-1} w.w.) Mean ± SD (min – max)**	**Pb (mg kg^{-1} w.w.) Mean ± SD (min – max)**	**Fe (mg kg^{-1} w.w.) Mean ± SD (min – max)**	**References**
T. thynnus					
Sardinia (*wild*)	43 ± 9 (kg)	0.014 ± 0.006	0.11 ± 0.08	13 ± 7	This study
Malta (*farmed*)	233 ± 92 (kg)	0.02 ± 0.02	0.03 ± 0.02	7 ± 3	This study
Spain (*wild*)		(0.01 - 0.02)	(0.01 - 0.02)		Falcò *et al*., 2006
Sicily (*wild*)	50 - 190 (kg)	(n.d. - 0.26)	(n.d. - 0.24)		Licata *et al*., 2005
Turkey (*wild* and *farmed*)	53 - 56 (kg)			8.456 ± 0.548 (*wild*) 6.057 ± 0.457 (*farmed*)	Percın *et al*., 2011
Mediterranean Sea and North Atlantic (*canned*)		0.014	0.013		Suppin *et al*., 2005
Ionian Sea (*wild*)	3.6 (kg)	(0.01 - 0.04)	(0.07 - 0.18)		Storelli *et al*., 2005
Tyrrhenian Sea (*wild*)	13 - 161 (kg)	(0.00 - 0.03)	(n.d. - 0.33)		Storelli *et al*., 2010
North Atlantic (*wild*)	> 50 (kg)	(0.008 - 0.02)	(0.01 - 0.03)		Ugarte *et al*., 2012
Canada (*wild*)	200 (kg)	(0.02 - 0.05)	< 0.03	29.00 (d.w.)	Hellou *et al*., 1992
Mediterranean Sea (*wild*)	130 - 190 (kg)	(0.012 - 0.025)	(< 0.010 - 0.083)	(3.649 - 21.138)	Di Bella *et al*., 2015
Spain (*wild*)		(n.d. - 0.0127)	n.d.		Olmedo *et al*., 2013
Arabic Sea (*wild*)			(0.065 - 0.089)	(1.769 - 2.591)	Ashraf and Jaffar, 1988
Black Sea (*wild*)	~ 150 (kg)	0.45 ± 0.10	< 0.5	130 ± 40	Topçuoğlu *et al*., 1990
Mediterranean Sea (*wild*)	1.2 (kg)	(0.003 - 0.020)	(0.02 - 0.085)		Yusa *et al*., 2008
Ionian Sea (*farmed*)	80 - 540 (kg)			19.30 ± 6.53	Milatou *et al*., 2015
T. orientalis					
Mexico (*farmed*)	7 - 25 (kg)	(0.010 - 0.0158)			Lares *et al*., 2012
T. obesus					
Spain (*wild*)		(0.002 - 0.039)	(0.002 - 0.048)		Besada *et al*., 2006
Portugal (*wild*)	10.6 ± 0.8 (kg)	0.186 ± 0.058	0.036 ± 0.001		Torres *et al*., 2016
T. albacares					
Ecuador (*wild*)	74 - 163 (cm)	2.4 ± 5.1	0.07 ± 0.06		Araújo *et al*., 2016
California (*wild*)	10 - 30 (kg)	(0.01 - 0.86)	(0.01 - 0.4)		Ruelas-Inzunza *et al*., 2012
USA (not specified)		0.03 ± 0.005	0.04 ± 0.01		Burger and Gochfeld, 2005
Réunion (*wild*)	102 ± 5 (cm)	0.07 ± 0.03	0.01 ± 0.03		Chouvelon *et al*., 2017
Seychelles (*wild*)	96 ± 5 (cm)	0.09 ± 0.16	n.d.		Chouvelon *et al*., 2017
South Africa (*wild*)	87 ± 8 (cm)	0.34 ± 0.26	0.01 ± 0.02		Chouvelon *et al*., 2017