Determination of cadmium, lead and mercury residual levels in meat of canned light tuna (Katsuwonus pelamis and Thunnus albacares) and fresh little tunny (Euthynnus alletteratus) in Libya

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Abstract
Surveillance for mercury (Hg), lead (Pb) and cadmium (Cd) contamination in tuna products is crucial for consumer food safety. Hg, Pb and Cd contaminants were monitored in a total of 60 specimens of fresh little tunny (Euthynnus alletteratus) and popular brands of skipjack and yellowfin (Katsuwonus pelamis and Thunnus albacares) canned tuna commercially available in Tripoli, Libya. Direct Mercury Analyzer (DMA-80) was implemented for determination of total Hg level and graphite furnace atomic absorption spectrometry (GFAAS) was employed for determination of Cd and Pb concentrations. The results indicated that Hg had the highest concentration level and Cd had the lowest concentration level either in tested canned tuna or fresh little tunny samples. The average concentration of Hg in fresh little tunny samples was 1.185 ± 0.968 mg kg⁻¹ wet weight (ww) and often exceeded the standard permissible limit. In addition, canned yellowfin tuna had the lowest levels of Cd (0.027 ± 0.026 mg kg⁻¹ ww), Pb (0.075 ± 0.071) and Hg (0.163 ± 0.122 mg kg⁻¹ ww). Results of the current surveillance indicated that canned skipjack and yellowfin tuna sold in Tripoli markets show contaminant levels well under the European thresholds adopted for Cd, Pb and Hg. However, consumption of large quantities of Mediterranean little tunny products significantly increases human exposure to the risk of Hg toxicity.

Keywords: Cadmium, Canned tuna, Lead, Little tunny, Mercury.
and people who consume large amounts of certain tuna may increase the risk of being affected by Hg toxicity (Morgano et al., 2014).

Increasing ingestion of Hg-contaminated food surpassing the permissible limit established by World Health Organization (WHO) was associated with frequent intake of large size fish of tuna species. Canned tuna consists of large species of tuna (e.g. albacore and yellowfin tuna), contains moderate amounts of Hg, whereas, canned tuna consists of smaller species (e.g. skipjack), contains around one-third the Hg concentrations of albacore and yellowfin tuna (Bratt, 2010). Interestingly, Hg, Pb and Cd contaminants were investigated in fresh bluefin tuna (T. thynnus) and canned tuna commercially available in Italy. None of the tested samples exceeded the European control limits fixed for Cd and Pb, however 8.9% of the canned tuna and 20% of fresh tuna samples surpassed permissible limit of Hg (Storelli et al., 2010). In addition, tuna caught off Libya and canned in Misurata cannery was considered of low concentration of Hg, Pb and Cd trace elements, well below the permissible levels recommended by the FAO/WHO (Voegborlo et al., 1999).

Trace elements content of tuna fish needs to be well documented as tuna products are widely consumed by humans. As a part of the national monitoring programme in Libya, Cd, Pb and Hg are frequently investigated in imported canned tuna by the National Centre for Food and Drug Control (FAO/WHO, 2005). However, published articles on the levels of Cd, Pb and Hg trace elements in fresh tuna (E. alletteratus) from offshore waters of the west Libyan coast are lacking. Also for commercially available canned tuna distributed in Tripoli, publications on this topic are rather limited. Therefore, the objective of this investigation was to estimate the levels of Cd, Pb and Hg trace elements to determine probable toxicological relations and to warrant agreement with reference values established by European legislations (European-Commission, 2006).

**Materials and Methods**

**Collection of samples**

A total of 60 random tuna samples consisted of 20 chunk meat samples (weight approximately 100 g) of fresh little tunny and 40 canned light tuna samples (20 skipjack and 20 yellowfin) of different brand origin and country of manufacture (Italy, Libya, Spain and Thailand) were collected from retail markets at Tripoli city (Libya) with a valid shelf life. The fresh little tunny samples were carefully picked with a clean stainless steel scalpel from the axial muscle anterior to the origin of the first dorsal fin (Adams, 2004). Fresh samples were immediately transferred to sterile polyethylene bags and kept at -20 °C until analyzed.

**Analytical procedures for estimation of total Hg**

Direct thermal decomposition amalgamation atomic absorption spectrometry (TDA/AAS) was implemented for the determination of Hg using DMA-80 (Milestone Srl, Sorisole (BG) - Italy). DMA-80 is fully compliant with USEPA test method 7473 (USEPA, 2007). Canned tuna were opened; oil and brine were drained off carefully. The chunk meat samples were homogenized thoroughly in a food blender with stainless steel cutters (Voegborlo et al., 1999). Approximately 125 mg of each undigested sample homogenate was loaded directly into a Quartz sample boat, and then the sample boat was delivered into a decomposition furnace. In brief, the working principles in operation with the DMA-80 system are as follows: (1) Samples drying and thermal decomposing in an oxygen-rich stream at 750 °C with the consequent sublimation of Hg; (2) Catalytic reduction of Hg; (3) Hg is selectively trapped by gold amalgamation; and (4) The amalgamation furnace is heated and Hg is quickly introduced into multiple measuring cells situated along the optical path of the spectrophotometer, then Hg is quantitatively determined by atomic absorption spectrometry using a low-pressure Hg lamp at a wavelength of 253.65 nm.

**Analytical procedures for estimation of Cd and Pb**

Approximately 1 g of homogenized samples was transferred to high pressure 100 mL TFM vessels (SK-10, Milestone Inc.) that were carefully cleaned and soaked for 24 h in 15% HNO₃ then thoroughly rinsed with Milli-Q® water and dried at 80°C for about 2 h before use. The samples were subjected for microwave-assisted digestion in closed quartz vessels (ETHOS One, Milestone Inc.) with 3 mL of H₂O₂ (≥37%, TraceSELECT®), 6 mL of HNO₃ (≥69.0%, TraceSELECT®) and 1 mL of HCl (≥37%, TraceSELECT®) for 30 min (Olmedo et al., 2013). The digested solution was then transferred to a sterile polyethylene vials for later analysis. Graphite furnace atomic absorption spectrometry (GFAAS) was used for determination of Cd, Pb levels using Varian 240Z Zeeman atomic absorption spectrophotometer (Varian, Inc., Walnut Creek, CA) equipped with a Varian GTA-120 graphite tube atomizer and a programmable sample dispenser (PSD-120). Cadmium standard for AAS (1000 mg/L, TraceCERT®) was diluted in 0.2% HNO₃ (TraceSELECT®) to prepare a calibration curve (0, 0.1, 0.5, 1 and 2 μg/L) and samples were also diluted 1:4 in 0.2% HNO₃. Aliquots of 20 μL of digested samples were introduced directly into graphite tube atomizer and a Zeeman background correction system (Varian GTA-120/240Z AA/PSD-120) with an equal volume of matrix modifier (a mixture of 3.3% Palladium, Pd and 0.03% Magnesium, Mg as nitrates in 0.2% HNO₃). Lead standard for AAS (1000 mg/L, TraceCERT®) was diluted in 0.2% HNO₃ (TraceSELECT®) to prepare a calibration curve (0, 0.1, 0.5, 1 and 2 μg/L) and samples were also diluted 14 in 0.2% HNO₃. Aliquots of 20 μL of digested samples were introduced directly into Varian
GTA-120/240Z AA/PSD-120 with an equal volume of matrix modifier (10 g/L of NH₄H₂PO₄ dissolved in 0.2% HNO₃ and 0.1% Triton X-100). A hollow cathode was operated at 228.8 nm with a current of 4 mA and a slit width of 0.5 nm for Cd and at 283.3 nm, 10 mA and 0.5 nm for Pb.

**Quality control**
Quality control for each analytical group contained a sample spiked with 2 mg kg⁻¹ of each trace element. The quality control specimens were prepared according to the procedure used in the digestion of tuna samples for the Cd and Pb analysis. Recovery rates were 88 ± 15%, 127 ± 33% and 101 ± 2% for Cd, Pb and Hg, respectively.

**Statistical analysis**
One-way Analysis of Variance (ANOVA) was implemented using SPSS version 16 software to test the significance of contaminating trace elements level variations among the tuna samples. The statistical significance level was set at \( p < 0.05 \) for all tests. Tukey’s procedure was used as a *post hoc* test.

**Results**

**Determination of Cd level**
The average value of Cd in canned yellowfin samples (0.027 ± 0.026 mg kg⁻¹ wet weight (ww)) was significantly lower than canned skipjack samples (0.079 ± 0.058 mg kg⁻¹ ww) and fresh little tunny samples (0.072 ± 0.027 mg kg⁻¹ ww) (Table 1). However, no significant difference was found between mean values of Cd in canned skipjack and fresh little tunny samples (Fig. 1).

The maximum range of Cd in the examined fresh little tunny and canned skipjack samples was 0.11 and 0.19 mg kg⁻¹ ww, respectively (Table 1). Among tested samples, only 5% of fresh little tunny and 30% of canned skipjack specimens were barely surpassing the maximum level (0.1 mg kg⁻¹ ww) adopted for Cd in tuna (European-Commission, 2006) (Fig. 2).

**Determination of Pb level**
The average value of Pb concentration in canned yellowfin (0.075 ± 0.071 mg kg⁻¹ ww) was significantly lower than Pb values in both fresh little tunny (0.228 ± 0.136 mg kg⁻¹ ww) and canned skipjack (0.239 ± 0.205 mg kg⁻¹ ww) (Table 2 and Fig. 1). Furthermore, mean values of Pb estimated in current study were not exceeding the maximum level (0.3 mg kg⁻¹ ww) adopted for Pb in fish muscle (European-Commission, 2006), except 5 specimens out of 20 (25%) fresh little tunny samples and 7 out of 20 (35%) of canned skipjack tuna samples were exceeding the Pb maximum limit (Fig. 2).

**Determination of Hg level**
In the current study, 20 specimens each of fresh little tunny caught off Libya, canned skipjack and yellowfin tuna were analyzed for total Hg using TDA/AAS technology which is developed as a preferred direct technique for the analysis of total Hg in fish tissue because it is rapid (5 min per sample) and easy to use and requires no sample preparation (Lasrado et al., 2005; Carbonell et al., 2009). DMA-80 was employed to measure total Hg contamination in canned seafood. Canned tuna showed more frequent detection and higher content of total Hg than other canned fishery products (Yang et al., 2015). Hg content in all tuna specimens was found at different measurable levels (Table 3), the average value of Hg in fresh little tunny (1.185 ± 0.968 mg kg⁻¹ ww) was significantly higher than canned skipjack (0.373 ± 0.249 mg kg⁻¹ ww) and canned yellowfin (0.163 ± 0.122 mg kg⁻¹ ww) (Table 3 and Fig. 1). However, no significance difference was found between Hg mean levels in canned skipjack or yellowfin tuna (Fig. 1).

**Table 1.** Descriptive statistical analysis of estimated values of Cd contaminant level (mg kg⁻¹ ww) in fresh and canned tuna samples (No.=20).

| Type of tuna samples | Mean  | Standard deviation | Standard error | Minimum | Maximum |
|----------------------|-------|--------------------|---------------|---------|---------|
| Fresh little tunny   | 0.072 | 0.027              | 0.006         | <LOQ    | 0.11    |
| Canned skipjack      | 0.079 | 0.058              | 0.013         | 0.01    | 0.19    |
| Canned yellowfin     | 0.027*| 0.026              | 0.006         | <LOQ    | 0.09    |

*Statistical significance: \( p < 0.05 \). LOQ: Limit of Quantitation.
The mean value of Hg in fresh little tunny barely surpassed the maximum level (1 mg kg\(^{-1}\) ww) adopted for Hg in tuna (European-Commission, 2006). Nevertheless, Hg levels surpassing the maximum limit were detected in 9 out of 20 (45%) of the analyzed samples, in addition box blot analysis of individual values in fresh little tunny samples revealed disparities in Hg content (Fig. 2).

**Discussion**

According to Eisler (2010), whole finfishes in general contain Cd 0.1 - 0.3 mg kg\(^{-1}\) ww, whereas fish muscle usually contains Cd less than 0.1 mg kg\(^{-1}\) ww and fish liver Cd concentration is greater and reaches up to 24.7 mg mg kg\(^{-1}\) ww. High Cd concentration in the water sediments is reflected in Cd content of prey (Eisler, 2010). The trophic position of yellowfin (4.60 ± 0.67), is considered more elevated than in skipjack (3.94 ± 1.06) (Ruelas-Inzunza et al., 2014).

Biomagnification of Cd in muscle of predator fish such as Eastern pacific yellowfin (average concentration 0.18 ± 0.15 mg kg\(^{-1}\) dry base) and skipjack (average concentration 0.23 ± 0.22 mg kg\(^{-1}\) dry base) is significantly depending on the function of the trophic position (Ruelas-Inzunza et al., 2014). Muscle tissue of *E. alletteratus* and skipjack obtained from fish landing sites in Tema, Ghana, West Africa, contained less than 0.1 mg kg\(^{-1}\) ww of Cd (Asmah and Biney, 2014). Meanwhile, North Atlantic *E. alletteratus* contained Cd 0.2 mg kg\(^{-1}\) on dry base (which corresponds to an estimated 0.25 mg kg\(^{-1}\) ww) (Windom et al., 1973). In Western Indian Ocean, the means of Cd content in yellowfin and skipjack tuna caught off Mozambique Channel and Reunion Island were 0.06 ± 0.05 and 0.18 ± 0.11 mg kg\(^{-1}\) ww, respectively (Kojadinovic et al., 2007). On the other hand, mean Cd concentration

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**Table 2.** Descriptive statistical analysis of estimated values of Pb contaminant level (mg kg\(^{-1}\) ww) in fresh and canned tuna samples (No.=20).

| Type of tuna samples   | Mean  | Standard deviation | Standard error | Minimum | Maximum |
|------------------------|-------|--------------------|----------------|---------|---------|
| Fresh little tunny     | 0.228 | 0.136              | 0.03           | 0.06    | 0.56    |
| Canned skipjack        | 0.239 | 0.205              | 0.045          | 0.03    | 0.60    |
| Canned yellowfin       | 0.075*| 0.071              | < 0.016        | <LOQ    | 0.22    |

*Statistical significance: \(p<0.05\). LOQ: Limit of Quantitation.

**Table 3.** Descriptive statistical analysis of estimated values of total Hg contaminant level (mg kg\(^{-1}\) ww) in fresh and canned tuna samples (No.=20).

| Type of tuna samples   | Mean  | Standard deviation | Standard error | Minimum | Maximum |
|------------------------|-------|--------------------|----------------|---------|---------|
| Fresh little tunny     | 1.185*| 0.968              | 0.216          | 0.08    | 3.30    |
| Canned skipjack        | 0.373 | 0.249              | 0.055          | 0.08    | 0.75    |
| Canned yellowfin       | 0.163 | 0.122              | 0.027          | 0.03    | 0.47    |

*Statistical significance: \(p<0.05\).

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Fig. 2. Graphical representation of box blots show data sets distribution of Cd, Pb and Hg levels in tested tuna samples. Whiskers represent lower and upper quartiles; Cross band inside the box indicates median, the boxes represent the interquartile range, which covers the central 50% of the data. Open circle indicates outlier data. 95% confidence interval.

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was magnified up to 0.61 ± 0.37 mg kg⁻¹ on dry base of skipjack (which corresponds to an estimated 0.75 mg kg⁻¹ ww) caught off Reunion Island (Kojadinovic et al., 2007). However, skipjack caught off Eastern Pacific Ocean and in front of the Baja California Peninsula had much less Cd (0.055 mg kg⁻¹ ww) (Ruelas-Inzunza et al., 2012).

In yellowfin caught off New South Wales waters, Australia, the average Cd concentration in muscle was 0.04 mg kg⁻¹ ww (Bebbington et al., 1977). However in yellowfin caught off Taiwan, Cd concentration is much lower (0.02 mg kg⁻¹ ww) (Han et al., 1998). Much less concentrations of Cd (0.004 - 0.006 mg kg⁻¹ ww) were reported in yellowfin caught off Eastern Pacific and Atlantic Oceans (Besada et al., 2006; Ruelas-Inzunza et al., 2012).

To safeguard human consumers of tuna fish, the European Union recommends less than 0.1 mg kg⁻¹ ww; the same level of protection is recommended in Turkey, and China (European-Commission, 2006; Eisler, 2010). Increased anthropogenic Pb concentrations in marine fish are reported in proximity to mining activities, metal finishing facilities, organic industries, areas where Pb arsenate pesticides are implemented and areas of Pb aerosol fallout (Eisler, 2010). The ability of tuna fish for bioaccumulation of Pb from seawater was reported elsewhere; concentration factors for whole tunas were about 100, being highest in liver and lowest in muscle (Eisler, 2010).

Apparently, yellowfin had a low tendency for Pb bioaccumulation, as the average muscular concentration of Pb in yellowfin caught off several waters worldwide (Atlantic Ocean, Western Indian Ocean, Eastern Pacific Ocean and Taiwan) ranged from less than 0.01 to 0.06 mg kg⁻¹ ww (Han et al., 1998; Besada et al., 2006; Kojadinovic et al., 2007; Ruelas-Inzunza et al., 2012, 2014).

The estimated mean values of Pb concentration in fresh little tunny and canned skipjack samples of current study were closely similar to Pb concentrations in E. alletteratus (0.20 ± 0.03 mg kg⁻¹ ww) and skipjack (0.32 ± 0.1 mg kg⁻¹ ww) obtained from fish landing sites in Tema, Ghana, West Africa (Asmah and Biney, 2014). Much lower Pb values were estimated in skipjack caught off Eastern Pacific and Western Indian Oceans (0.03 mg kg⁻¹ ww and 0.07 ± 0.08 mg kg⁻¹ dry base, respectively) (Ruelas-Inzunza et al., 2012). On the other hand, Pb content reached 0.55 - 0.59 mg kg⁻¹ on dry base of skipjack (which corresponds to an estimated 0.7 - 0.75 mg kg⁻¹ ww) specimens sold in Ibaraki Prefecture, Japan (Bassari, 1994). The effects of canning on Pb content of tuna muscle have been misjudged for decades. This was supposedly due to analytical errors which resulted in reduced Pb concentrations in canned tuna by factors up to 10,000 times. However, the link between Pb content in canned tuna and adverse impacts on human health remains unproven (Eisler, 2010). Canned tuna samples consumed in Libya contained Pb with a mean value of 0.28 mg kg⁻¹ ww (Voegborlo et al., 1999). Likewise, samples of canned tuna purchased locally from Saudi Arabia contained Pb 0.03 - 0.51 mg kg⁻¹ ww, with a mean value of 0.23 mg kg⁻¹ ww (Ashraf et al., 2006).

To safeguard human consumers of fish muscle in Turkey, the maximum allowable concentration of Pb is 1.0 mg kg⁻¹ ww and for China, it is 0.5 mg kg⁻¹ ww (Eisler, 2010). Regular monitoring the Hg contamination in fish and fishery products is essential to protect vulnerable population such as children (Yang et al., 2015). The Hg body burden in fishes caught off Atlantic Ocean is higher than conspecific species of equal size caught off Mediterranean Sea. It is possible that the higher Hg body burdens were due to the greater natural geochemical Hg levels in the Mediterranean Sea (Eisler, 2010). Data on Hg accumulations in several types of tuna fish are particularly abundant; however there is lack of data reports on Hg levels in little tunny (E. alletteratus), especially in Libya.

The variation in Hg concentration in tuna represents a critical point during risk analysis for consumers (Storelli et al., 2010). Elevated Hg levels can be harmful, especially if excess quantities of tuna are consumed by pregnant women and young children, as bioaccumulation of Hg can damage the developing brain of a fetus or a child (Bratt, 2010).

Geographical areas of concern where Hg concentrations in marine fish muscle exceed current regulations for human consumers include Italy, Spain, Taiwan, Florida, and Oregon (Eisler, 2010). E. alletteratus is one of the pelagic fish in several tropical and subtropical waters, including the Mediterranean Sea, Gulf of Guinea, Black Sea, Caribbean Sea and Gulf of Mexico. In North Atlantic Ocean, E. alletteratus caught off Sargasso Sea contained Hg 1.4 mg kg⁻¹ on dry base (which corresponds to an estimated 1.75 mg kg⁻¹ ww) (Windom et al., 1973). Furthermore, total Hg in its muscle varied from 0.11 to 3.4 with a mean value of 0.94 ± 0.60 mg kg⁻¹ ww in Florida (Adams, 2004), and from 2.1 to 2.3 with a mean value of 2.2 ± 0.14 mg kg⁻¹ ww in Bermuda (Dewailly et al., 2008). In Gulf of Mexico, E. alletteratus contained total Hg from 0.24 to 2.52 with an average value of 1.08 ± 0.72 mg kg⁻¹ ww (Cai et al., 2007).

The concentration of Hg in skipjack tuna caught off Eastern Pacific Ocean and in front of the Baja California Peninsula was 0.104 mg kg⁻¹ ww (Ruelas-Inzunza et al., 2012). In addition canned tuna commercially available in California, USA and Genova, Italy contained 0.16 – 0.27 mg kg⁻¹ ww (Cugurra and Maura, 1976; Cappon and Smith, 1982).

Recently, low traces of Hg were recorded in yellowfin obtained from Eastern Pacific water, Brazil, Gulf
of California and Gulf of Guinea with a range 0.03 - 0.09 mg kg⁻¹ ww (Voegborlo et al., 2006; García-Hernández et al., 2007; Ruelas-Inzunza et al., 2012; Schmidt et al., 2015). Furthermore, the Hg range increased up to 0.3 mg kg⁻¹ ww in yellowfin caught off Atlantic and Pacific Oceans, Equatorial zone and Hawaii coast (Kraepiel et al., 2003; Adams, 2004; Besada et al., 2006; Kaneko and Ralston, 2007; Ordiano-Flores et al., 2011). On the other hand, unusual high Hg level of 2.93 mg kg⁻¹ Hg ww was reported in yellowfin in Taiwan (Han et al., 1998). Frozen fillet of yellowfin tuna collected from different local markets in Brazil contained Hg 0.058 ± 0.001 mg kg⁻¹ ww. After different culinary treatments, no significant Hg loss was observed for uncooked and boiled fish. However, 4 to 25% Hg loss was observed in yellowfin tuna after roasting (175 °C for 20 min) or frying (180 °C for 5 min) (Schmidt et al., 2015).

Canned tuna fish from the Mediterranean coast of Libya had Hg levels well below the permissible limits at range from 0.20 to 0.66 with an average value of 0.29 mg kg⁻¹ ww. (Voegborlo et al., 1999). However, there is significant effect of canning on total Hg content in trawl-caught albacore tuna (T. alalunga); as the Hg concentration is significantly increased from 0.17 mg kg⁻¹ ww (range 0.09 - 0.24 mg kg⁻¹) in the pre-canned samples to 0.21 mg kg⁻¹ ww (range 0.10 - 0.33 mg kg⁻¹) ww in the post-canned samples (Rasmussen and Morrissey, 2007).

Current study revealed that Hg had the highest concentrations among tested trace elements, followed by Pb and Cd either in fresh little tunny or canned skipjack and yellowfin tuna. This order of measured trace elements concentrations is in agreement with occurrence of such toxic metals in Italian fresh and canned tuna (Storelli et al., 2010).

The maximum level for tuna products (Thunnus species, Euthynus species, skipjack) is set at 1 mg kg⁻¹ ww for Hg by the Regulation (EC) No 1881/2006 (European-Commission, 2006). Tuna intake with Hg levels more than 1 mg kg⁻¹ ww, exposes the consumer to Hg toxic effect (Storelli et al., 2010). Unfortunately, about half the number of the tested fresh little tuna samples exceeded this limit. Therefore, a continuous monitoring of Hg concentration in little tunny products is essential for food safety. On the other hand, Cd and Pb concentrations either in fresh little tunny caught off Libya or canned tuna sold in Tripoli city did not surpass the toxicological standard levels (European-Commission, 2006), and accordingly there was minor food safety concerns of Cd or Pb toxicity from eating tuna therein.

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Conflict of interest
The authors hereby declare that there is no conflict of interest related to the present study.

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