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RESIDUAL CONTENTS OF SELECTED HEAVY METALS IN COMMERCIAL CANNED FISH IN EGYPT: DIETARY INTAKES AND HEALTH RISK ASSESSMENT

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Abstract: Canned fish is considered as an important fish product that can supply humans with part of their needs from essential amino acids, polyunsaturated fatty acids, vitamins, and minerals. However, canned fish might become a source of human exposure to xenobiotics such as heavy metals. This study aimed to estimate the residual contents of six heavy metals, namely lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg), aluminum (Al), and tin (Sn) in four kinds of commercially purchased canned fish products from Zagazig city, Egypt. Eighty imported samples including canned anchovies, canned mackerel, canned sardine, and canned tuna (20 samples/each canned fish product) were examined. Besides, we calculated the estimated daily intakes (EDI), and potential health risks, hazard quotient (HQ), and hazard index (HI), of the examined heavy metals associated with the consumption of such fish products among Egyptian population. The obtained results revealed residual concentrations of the tested heavy metals higher than the established maximum permissible limits (MPL) by European Commission for Pb in 20%, 95%, and 100% of the examined canned anchovies, sardine, and tuna, respectively. Whereas, 80%, 100%, 100%, and 100% of the examined canned anchovies, mackerel, sardine, and tuna, respectively exceeded MPL of As. None of the examined samples exceeded MPL of Cd, Hg, Al, and Sn. The calculated HQ and HI for the examined heavy metals based on the daily intakes revealed values greater than 1.0 indicating potential human health risks. Therefore, it is highly advisable to reduce our daily intake of canned fish.

Key words: heavy metals; canned fish; estimated daily intakes; health risk assessment; Egypt

Introduction

Fish is considered as an essential source for protein with high biological values, polyunsaturated fatty acids, vitamins, and minerals such as calcium, and phosphorus. In Egypt, the consumption of fish is increasing because of the shortage in the red meat, and their lower price compared with chicken and meat (1).

Canning is considered as one method of fish preservation and development of new fish products, namely canned tuna, canned sardine, canned mackerel, and canned anchovies. The canning industry is developing in Egypt and canned fish products can be found in the Egyptian markets either imported from outside or from Egyptian origin. The canning process can preserve fish for long periods and improve the microbial quality of the fish. However, it has little effects on the chemical residues in the used fish. Chemical residues such as heavy metals can find their way into the human body via consumption of contaminated fish (2). Yet, few reports investigated the occurrence of heavy metals, particularly, arsenic (As), tin (Sn), and aluminum (Al) in the marketed canned fish in Egypt. For instances, Morshdy et al. (2) examined samples retailed in Zagazig city, Egypt, they detected lead (Pb) at 0.13 ± 0.02, 0.01 ± 0.004, and 0.02 ± 0.01 ppm in canned tuna, sardine, and mackerel, respectively; they also detected cadmium (Cd) at 0.02 ± 0.001, 0.05 ± 0.003, and 0.03 ± 0.003 ppm in canned tuna, sardine, and

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mackerel, respectively. While El-Dahman et al. (3) detected Pb at 0.90 ± 0.01 ppm, and mercury (Hg) at 1.03 ± 0.02 ppm from canned tuna retailed in El-Gharbia and Kafr-Elsheikh Governorates, Egypt.

Heavy metals are persistent chemicals that can get entry into the fish flesh from the contaminated aquatic environment (4). Heavy metals have several adverse effects on the human health. For instances, Pb is one of the heavy metals that is linked to many cases of deaths among children as in China (5), and Zambia (6). Furthermore, Pb has deleterious effects on the intellectual abilities, and mental health. Cadmium (Cd) is another heavy metal with no-known physiological function. Cadmium is the main cause for *itai-itai* disease that is recorded among Japanese people with heavy fish consumption. Such disease is characterized by kidney dysfunction and osteomalacia (7). Besides, Cd is recorded as a group B1 carcinogen (8). Arsenic is another heavy metal which is linked to multiple organ damage, carcinogenesis with unknown mechanisms, and skin irritation (9). Corrosion of the can material might lead to leaching of Sn into the fish flesh, and subsequently into the human body. Tin might cause abnormal metabolism of the essential elements such as zinc (Zn), copper (Cu), and iron (Fe). In addition, it reduces calcium content in bone, and leads to organ damage, particularly in the kidney (10). Mercury is another metal linked to fish consumption. Mercury toxicity was reported among Japanese people in Minamata bay, Japan during 1950s, and Iraq during 1970. Besides Hg is linked to deviations in the neurological behavior and autism (11). Aluminum is another metal that can be leached from the canning materials into the fish flesh and causes several health implications if consumed. Such health disorders involve anemia, dementia, osteomalacia and organ toxicity (12). Heavy metals can be measured in different food matrices using atomic absorption spectrometry or inductively coupled plasma mass spectrometry (4).

Insight of the previous facts, this study aimed to estimate the residual contents of six heavy metals including Pb, Cd, As, Hg, Al, and Sn in four kinds of canned fish (canned anchovies, canned mackerel, canned sardine, and canned tuna) retailed in Zagazig city, Egypt. Besides, the estimated daily intakes and the potential health risks associated with the consumption of canned fish among the Egyptian population were calculated.

Material and methods

This study was conducted according to the guidelines of Zagazig University, Egypt. All chemicals used in the current study were of HPLC grade, or the highest available purity.

Sample collection:

Eighty processed and imported canned fish samples including canned anchovies, mackerel, sardine, and tuna (20, each) were collected randomly and equally from grocery stores and hypermarkets in Zagazig city, Sharkia Governorate, Egypt. Protein, fat, and moisture contents in the collected canned fish samples were 18-24%, 1-4%, and 70-75%, respectively. Samples were collected during the period from April to October 2020. Samples were kept at room temperature in the laboratory of Meat Hygiene, Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Egypt till the time of metal extraction and measurements.

Sample preparation:

Sample preparation and metal measurements were done according to previous methods (1, 13). In brief, one gram from the muscular tissue of each canned fish sample was digested in 5 ml of a digestion solution contained 3 ml nitric acid 65% (Merk, Darmastadt, Germany), and 2 ml perchloric acid 70% (Merk, Darmastadt, Germany). The homogenate was kept at room temperature for 12 h. Then, the mixture was heated at 70°C for 3 h in water bath with swirling every 30 min. The digested mixture was allowed to cool to room temperature, and then diluted with 20 ml DDW, and filtered by using filter paper. The filtrate was kept at room temperature until heavy metal measurement.

Analytical procedures:

The graphite furnace atomic absorption spectroscopy (Perkin Elmer® PinAAcle™ 900T atomic absorption spectrophotometer (Shelton, CT, USA) was used for the measurement of Pb, Cd, Al, and Sn. While As and Hg were measured using hydride generation/cold vapor atomic absorption spectroscopy (Shelton, CT, USA). All the analyses were done at the central laboratory, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Quality assurance and quality control:

The reference material DORM-3 Fish protein prepared by the National Research Council; Canada was used for quality assurance of the analytical procedures. The detection limits (LOD) (μg/g) for the analyzed metals were 0.01 for Pb, 0.005 for Cd, 0.02 for As, 0.01 for Hg, and 0.02 for Sn, 0.10 for Al. The detected levels for the tested metals were expressed as μg/g wet weight (μg/g ww). The recovery rates for the detected metals ranged from 90 to 105%. The determination coefficient (R^2) ranged between 0.980 to 0.999 to all tested metals.

Estimated daily intake (EDI):

EDI values of the detected heavy metals via consumption of fish by Egyptian population was calculated based on the following equation:

EDI (μg/kg/day) = $C_m * F_{IR}/BW$ (14)

Where C_m is the concentration of the tested metal (μg/g ww); F_{IR} is the ingestion rate of fish by Egyptian population. F_{IR} was set at 48.57 g/day (15); BW is the body weight of Egyptian adults; this was set at 70 kg.

Health risk assessment:

The non-carcinogenic risk, hazard quotient (HQ), of the tested heavy metals was calculated based on the following equation:

$HQ = EDI/RFD * 10^{-3}$ (14)

where RFD is the recommended reference dose (RfD) (mg/kg/d) (0.004, 0.001, 0.0003, 0.0005, 0.00012, and 0.0003 for Pb, Cd, As, Hg, Al, and Sn, respectively) (14).

A hazard index (HI) was used to estimate the risk of mixed metals. HI was calculated from the following equation:

$HI = \sum HR_i$

where *i* represents each metal

Values of HQ or HI of more than one indicates a potential risk to human health.

Statistical analysis:

Statistical analysis was evaluated using One way analysis of variance (ANOVA) followed by the post-hoc test, Tukey-Kramer HSD difference test (JMP) (SAS Institute, Cary, NC, USA). A $P < 0.05$ was considered to be significant. Values were expressed as means ± standard deviation (SD).

Results

The obtained results revealed the detection of Pb in all the examined samples. Canned sardines had significantly ($p < 0.05$) the highest residual Pb content (μg/g ww) (1.17 ± 0.28), followed by canned tuna (0.72 ± 0.13), canned anchovies (0.32 ± 0.08), and canned mackerel (0.19 ± 0.05), respectively (Fig. 1A).

Cadmium was also detected in all canned fish samples. Similar to Pb, canned sardine had the highest Cd residues (μg/g ww) (0.09 ± 0.02), followed by canned tuna, canned anchovies, and canned mackerel with average Cd content of 0.05 ± 0.02, 0.04 ± 0.01, and 0.03 ± 0.01 μg/g ww, respectively (Fig. 1B).

Arsenic was detected in all the examined canned fish samples with a range of 1.69-3.45 μg/g ww in canned anchovies, 9.69-13.03 μg/g ww in canned mackerel, 4.23-7.79 μg/g ww in canned sardine, and 5.66-10.41 μg/g ww in canned tuna. Canned mackerel had the highest As content followed by canned tuna, sardine, and anchovies, respectively (Fig. 2A).

Mercury was also detected in all the examined canned fish samples. The average Hg contents (μg/g ww) were 0.05 ± 0.01, 0.04 ± 0.01, 0.03 ± 0.01, and 0.02 ± 0.01 in canned sardine, mackerel, tuna, and anchovies, respectively (Fig. 2B).

Aluminum was detected in all the examined samples. The average contents (μg/g ww) of Al in the examined samples were 7.22 ± 1.62 in canned tuna, 6.89 ± 1.03 in canned anchovies, 5.57 ± 1.75 in canned sardine, and 0.77 ± 0.13 in canned mackerel. Where, canned mackerel had significantly the lowest Al residues among the examined canned fish samples (Fig. 3A).

Tin was also detected in all the examined canned fish samples with relatively similar levels. The mean values of Sn contents (μg/g ww) among the examined samples were 0.07 ± 0.01, 0.07 ± 0.01, 0.06 ± 0.01, and 0.06 ± 0.01 in canned mackerel, tuna, anchovies. And sardine, respectively (Fig. 3B).

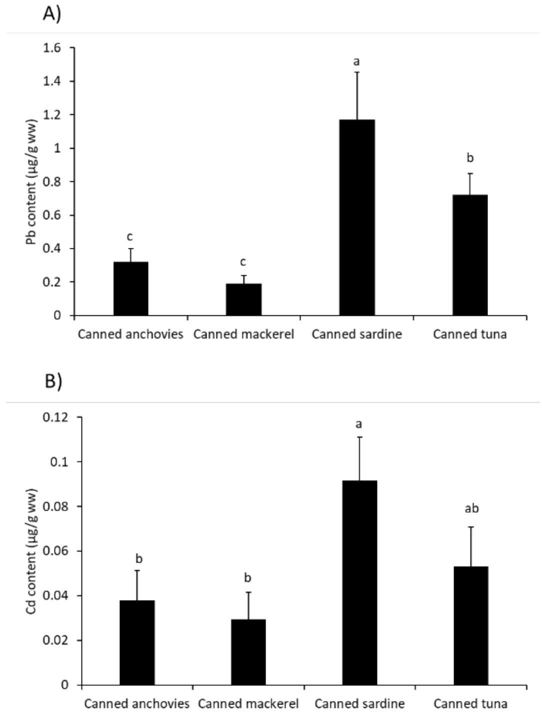

Figure 1: A) Lead (Pb), B) cadmium (Cd) residual contents (μg/g ww) in canned fish marketed in Egypt. Data represent means ± SD (n = 20 each product). Columns carrying different letter are statistically significant at *P* <0.05

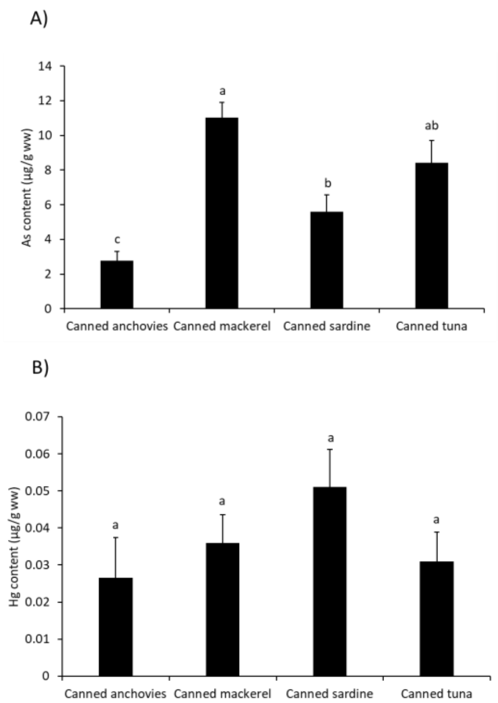

Figure 2: A) Arsenic (As), B**)** mercury (Hg) residual contents (μg/g ww) in canned fish marketed in Egypt. Data represent means ± SD (n = 20 each product). Columns carrying different letter are statistically significant at *P* <0.05

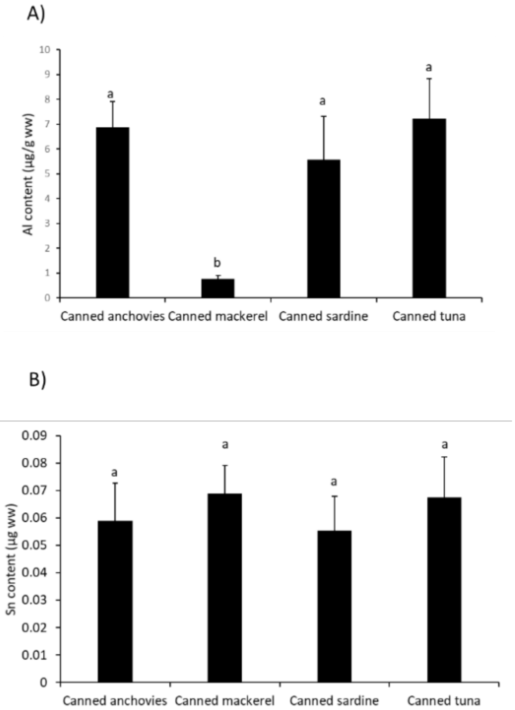

Figure 3: A) Aluminum (Al), B) tin (Sn) residual contents (μg/g ww) in canned fish marketed in Egypt. Data represent means ± SD (n = 20 each product). Columns carrying different letter are statistically significant at *P* <0.05.

Discussion

Heavy metals such as Pb, Cd, As, Hg, Sn, and Al are of no known physiological functions. Repetitive ingestion of such metals even at low concentrations might lead to several toxicological implications and organ damage because of their bioaccumulation, and biomagnification nature (4).

The recorded Pb concentrations in the presentstudy were relatively similar to that recorded in tuna fish retailed in China (0.03 to 8.62 μg/g ww) (19), while our values were higher than that reported in canned fish marketed in Saudi Arabia (0.03 to 0.51 μg/g ww) (20), Italy (0.16 ± 0.11 μg/g ww) (21), Iran (0.01 to 0.08 μg/g ww) (22), and Poland (0.01 ± 0.005 μg/g ww) (23).

The recorded Cd concentrations in the current study were in accordance with that recorded in tuna fish from China (0.02 to 0.06 μg/g ww) (19), and canned tuna retailed in Italy (0.01 to 0.06 μg/g ww) (21), and Poland (0.06 ± 0.01 μg/g ww) (23); but higher than that reported in Iran (0.001 to 0.006 μg/g ww) (22). Higher Cd concentrations were reported in canned tuna marketed in

Table 1: Dietary intakes and health risk assessment of heavy metals due to consumption of canned fish in Egypt

	Pb			Cd			As			Hg			Al			Sn			HI
	%	EDI	HQ	%	EDI	HQ	%	EDI	HQ	%	EDI	HQ	%	EDI	HQ	%	EDI	HQ	
Canned anchovies	20	0.223	0.891	0	0.026	0.026	80	1.934	0.580	0	0.018	0.009	0	4.779	0.573	0	0.041	0.012	**2.091**
Canned mackerel	0	0.132	0.529	0	0.020	0.020	100	7.659	**2.298**	0	0.025	0.012	0	0.536	0.064	0	0.048	0.014	**2.937**
Canned sardine	95	0.811	**3.244**	0	0.063	0.063	100	3.882	**1.164**	0	0.035	0.018	0	3.866	0.464	0	0.038	0.012	**4.965**
Canned tuna	100	0.502	**2.007**	0	0.037	0.037	100	5.846	**1.754**	0	0.025	0.012	0	5.009	0.601	0	0.047	0.014	**4.425**

%: referrers to percentage of samples exceeding the maximum permissible limits (MPL) of Pb, Cd, As, Hg, Al, and Sn (μg/g ww) are 0.3 **(16)**, 0.1 **(16)**, 2.0 **(17)**, 1.0 **(16)**, 250.0 **(18)**, and 200.0 **(16)**, respectively.
EDI: Estimated daily intake (μg/kg/day), HQ: Hazard quotient, HI: Hazard index

Saudi Arabia (0.07 to 0.64 μg/g ww) (20). Arsenic was also detected in canned fish produced in other countries but at lower levels as reported in canned fish retailed in China (0.03 to 1.53 μg/g ww) (19), Iran (0.25 to 1.42 μg/g ww) (22), and Poland (0.96 ± 0.29 μg/g ww) (23). Similarly, Hg was also detected in canned fish retailed in Italy (0.05 ± 0.05 μg/g ww) (21), and Poland (0.06 ± 0.009 μg/g ww) (23). Unlikely, higher Hg concentrations were recorded in canned fish retailed in Iran (0.06 to 0.16 μg/g ww) (22), and Brazil (0.41 μg/g ww) (24). Aluminum and tin are used in the packaging materials of canned fish; therefore, it is expected to detect Al, and Sn in all the examined samples. Aluminum was also detected at similar levels (4.76 ± 4.37 μg/g ww) in canned tuna from Lebanon (25). Similar to Al, Sn was detected at variable concentrations in all the examined samples in the present study. The recorded Sn concentrations in the present study agrees with Sn levels reported in canned fish from Poland (0.12 ± 0.01 μg/g ww) (23), but higher levels were recorded in Lebanon (3.34 ± 1.42 μg/g ww) (25).

It is clear from the obtained results that the inter-species variations in their accumulation of heavy metals, for instances, sardine and tuna had the highest residual contents of the different metals, while anchovies had the lowest concentrations. This might depend on the position of the fish in the food chain as predatory fishes as tuna fish accumulate higher levels of the heavy metals (4). The high levels of Pb, and As among the examined canned fish products reflects either the heavy exposure of the fish to such metals during their lifetime or post-processing contamination or contamination of the canning materials (1, 2, 4).

The recorded concentrations of the examined heavy metals in the present study were within the acceptable MPL, except for Pb, and As. Therefore, this study was extended to estimate the potential health risks associated with the consumption of such fish products via calculation of EDI, HQ, and HI. The calculated EDI values (μg/kg/day) for Pb were 0.223, 0.132, 0.811, and 0.502; for Cd were 0.026, 0.020, 0.063, and 0.037; for As were 1.934, 7.659, 3.882, and 5.846; for Hg were 0.018, 0.025, 0.035, and 0.025; for Al were 4.779, 0.536, 3.866, and 5.009; for Sn were 0.041, 0.048, 0.038. and 0.047 via ingestion of canned anchovies, mackerel, sardine, and tuna respectively. Such values were within the provisional tolerable weekly intakes of the examined heavy metals as established by the World Health Organization (26). Further calculation of HQ for the examined metals revealed values far below one for Cd, Hg, Al, and Sn indicating that canned fish would not pose health risks associated with these metals. Similar safe HQ values were reported for Al, and Sn in Lebanon (25). However, HQ values for Pb, and As were higher than one. Besides, calculation of HI for mixed contaminants revealed values higher than one for all the examined canned fish. This indicates that excessive consumption of canned fish might pose potential health risks for Egyptian consumers. Similar high HI values were reported in Greece after consumption of albacore (27).

Conclusion

The obtained results in the present study revealed the detection of Pb, Cd, As, Hg, Al, and Sn in all examined samples at variable levels. Several samples had residual levels higher than the recommended MPL, particularly for Pb, and As.

The calculated EDI, HQ, and HI revealed that excessive consumption of canned fish might pose health risks for Egyptian population, and therefore, it is advisable to reduce consumption of such fish products.

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The authors declare that there is no conflict of interest.

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