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As, Cd, Cr, Pb and Hg in seafood species used for sashimi and evaluation of dietary exposure

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The presence of inorganic contaminants As, Cd, Cr, Hg and Pb was evaluated in 116 samples of muscles of seafood used for sashimi from Japanese restaurants in Campinas, SP, Brazil. Tuna (*Thunnus thynnus*), salmon (*Salmo salar*), mullet (*Mugil platanus*) and octopus (*Octopus vulgaris*) were the most common seafood species in these restaurants. Samples were digested in a microwave assisted system and the contaminants were determined by inductively coupled plasma (ICP OES). The percentage of samples above the maximum limit permitted by MERCOSUL and European regulations were: As (90% tuna, 48% salmon, 31% mullet and 100% octopus); Cd (61% octopus); Hg (7% tuna); Pb (6% mullet). Octopus and tuna were the sashimi that most contributed to arsenic and mercury intake, respectively.
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1. Introduction

Japanese cuisine may be considered one of the most representative source for consumption of seafood (Moura Filho et al., 2007). The increase in the consumption may bring health benefits as well as associated risks. Toxicologists believe that seafood is one of the primary vectors of toxic substances, such as inorganic contaminants, organic pollutants and microorganisms (Guérin et al., 2011).

Seafood may accumulate various inorganic contaminants, with mercury, cadmium, lead and arsenic being the most frequently studied elements. These elements present properties of bioaccumulation, bio magnification in the food chain, persistence in the environment and disturbances in metabolic processes. Bioaccumulations and bio magnifications are used to determine the toxicity for different species (Morgano et al., 2007; Tavares & Carvalho, 1992) and factors such as sex, size and physiology may affect the accumulation of inorganic contaminants in fishes' tissues (Canli & Atli, 2003). The persistence of these elements in the environment has effects in the long term, even after emissions cease (Tavares & Carvalho, 1992).

Some researchers propose that fish and other aquatic life forms may be considered good environmental indicators of heavy metal contamination (Burger et al., 2002): tuna, for example, is known to be the species that accumulates mercury more than any other, and people who consume large amounts of tuna may increase the risk of being affected by the toxicity of inorganic mercury and of methylmercury (EPA & FDA, 2004).

Morgano, Rabonato, Milani, Miyagusku, and Balian (2011) studied the species of salmon, tuna, red snapper and bass commercialized in São Paulo (Brazil) and found high levels of arsenic and chromium in these species. Lowenstein et al. (2010) studied sushi prepared with five different species of tuna sold in restaurants and supermarkets in the USA and found mercury levels above those allowed by the regulations of Canada, European Union, Japan, and those recommended by the World Health Organization. Ikem and Egiebor (2005) found inorganic contaminants in samples of canned tuna and salmon from Georgia and Alabama (USA), and calculated the contribution to the weekly ingestion of the contaminants Hg, As, Cd and Pb, indicating that the consumption of 350 g week^{-1} of canned tuna and salmon results in a weekly contribution lower than the provisional tolerable weekly ingestion (PTWI).

While the consumption of Japanese cuisine has been growing rapidly in recent years, the study of the presence of inorganic contaminants in sashimi is still limited. Therefore, the purpose of

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this study was to validate a method and to evaluate the presence of the As, Cd, Cr, Pb and total Hg over a year, in muscles of seafood species used in the most consumed sashimi from Japanese restaurants in the city of Campinas, SP, Brazil.

2. Material and methods

2.1. Samples

Over a year, four samples of sashimi were bimonthly acquired from five Japanese restaurants in the city of Campinas, São Paulo, Brazil (total of 116 samples). Wasabi, shiso and vegetables were removed and the muscles were homogenized and stored in a freezer at −21 °C.

Seafood species studied were: Tuna (*Thunnus thynnus*, $n = 29$), Salmon (*Salmo salar*, $n = 29$), Mullet (*Mugil platanus*, $n = 16$), Octopus (*Octopus vulgaris*, $n = 18$), Black anchovy (*Ruvettus pretiosus*, $n = 6$), Amberjack (*Seriola lalandi*, $n = 2$), Dorado (*Coryphaena hippurus*, $n = 3$), Crab stick ($n = 7$), Namorado sandperch (*Pseudopercis numida*, $n = 1$), Snook (*Centropomus* sp., $n = 4$) and Frigate Tuna (*Spanish mackerel*, $n = 1$).

2.2. Reagents

Analytical grade reagents were utilized (Merck, Darmstadt, Germany), standard reference solutions of 1.000 mg L^{-1} (Merck, Darmstadt, Germany) were used for analytical curves of As, Cd, Cr, Pb and Hg, and water purified by reverse osmosis (18.2 MΩ) was used for analysis.

2.3. Analytical methodology

Digestion was performed in a closed microwave digestion system (Start D, Milestone, Sorisole, Italy): about 900 mg of the samples were placed into a PTFE digestion vessel, and 7 mL of concentrated HNO_3 and 3 mL of concentrated H_2O_2 were added. Decomposition of the samples was carried at $T_{max} = 180$ °C for 40 min and the resulting solutions were transferred to 25 mL volumetric flasks with 5% HCl (v/v). For Hg, 1 mL of 7% potassium permanganate solution (m/v) and 0.6 mL of 20% (m/v) hydroxylamine hydrochloride were added, and then transferred to 25 mL volumetric flasks with 5% HNO_3 (v/v). The analytical curves were from 0.0025 to 0.5 mg L^{-1} for As, Cd, Cr and Pb; and 0.0005–0.05 mg L^{-1} for Hg.

Quantification of inorganic contaminants was performed by ICP OES, using a Varian Vista MPX model (Mulgrave Victoria, Australia), with axial vision. Operating conditions: radiofrequency power of 1000 W; nebulizer rate of 0.9 L min^{-1}; plasma gas flow rate (Ar) of 15 L min^{-1}; auxiliary gas flow rate (Ar) of 1.5 L min^{-1}; integration and reading times of 10 and 3 s and 3 replicates. The analytical line wavelengths were: As (193.696 nm); Cd (214.439 nm); Cr (267.716 nm); Pb (220.353 nm) and Hg (194.164 nm). For hydride generator of total mercury (VGA 77, Varian, Mulgrave, Australia), solutions of HCl 10 mol L^{-1} and 25% $SnCl_2$ (m/v) in 20% HCl (v/v) were used.

2.4. Quality control

The analytical method was validated according to the Inmetro (2011) and the Mapa (Brasil, 2009) guidelines, and the uncertainty was calculated according to the Eurachem Guide (2002). For accuracy, a certified reference material TORT-2 (*Lobster Hepatopancreas*) from the National Research Council (Canada) was used. Correlation coefficients with ($r^2 \geq 0.999$) were obtained and blanks and triplicates were used in sample preparation.

Table 1
Results of method validation using CRM TORT-2 (*Lobster Hepatopancreas*) ($n = 7$).

Element	Measured value (mg kg^{-1})[a]	Certified value (mg kg^{-1})[b]	Rec (%)[c]	LOD (mg L^{-1})[d]	LOQ (mg L^{-1})[e]	Precision (%)[f]
Arsenic	21.4 ± 2.1	21.6 ± 1.8	99	0.074	0.25	3
Cadmium	24.6 ± 2.4	26.7 ± 0.6	92	0.006	0.019	1
Chromium	0.77 ± 0.08	0.77 ± 0.15	100	0.004	0.015	2
Lead	0.37 ± 0.17	0.35 ± 0.13	106	0.018	0.062	22
Mercury	0.21 ± 0.04	0.27 ± 0.06	78	0.0013	0.0042	9

[a] Expanded standard uncertainty with $k = 2$ ($p = 95\%$).
[b] Certified reference material TORT-2 (*Lobster Hepatopancreas*) do National Research Council (Canada).
[c] Rec = recovery.
[d] LOD = limit of detection.
[e] LOQ = limit of quantification.
[f] Precision = coefficient of variation.

2.5. Assessing dietary exposure

The estimated weekly intake of As, Cd, Cr, Hg and Pb was calculated assuming the consumption of one portion of sashimi (~50 g day^{-1}) by a 60 kg adult. Concentrations below the limit of quantification (LOQ) assumed these respective values: As = 0.25 mg kg^{-1}; Cd = 0.019 mg kg^{-1}; Cr = 0.015 mg kg^{-1}; Hg = 0.0042 mg kg^{-1}; Pb = 0.062 mg kg^{-1}.

3. Results and discussion

3.1. Validation of method and uncertainty estimative

Experimental results for the CRM were concordant with certified values as presented in Table 1. The limits of detection (LOD) and quantification (LOQ) were calculated to be 3 and 10 times the standard deviation of the analytical blank, multiplied by the dilution factor used in sample preparation. For the uncertainty estimative of the method, uncertainties associated with weighing, dilution, analytic standard, curve and method precision were considered and the expanded uncertainty was calculated using $k = 2$ ($p = 95\%$).

3.2. Inorganic contaminants present in the sashimi samples

The results obtained are presented in Table 2 and were analyzed considering the maximum limits established by the Mercosul (2011) and European Union (Commission Regulation, 2008) regulations: 1.0 mg kg^{-1} for As; 0.05 mg kg^{-1} for Cd (0.1 mg kg^{-1} for tuna); 0.3 mg kg^{-1} for Pb (Mercosul, 2011) and 0.2 mg kg^{-1} for Pb (Commission Regulation, 2008); 0.5 and 1.0 mg kg^{-1} for Hg (for non-predator and predator fish, respectively). For chromium, Brazilian regulation set 0.10 mg kg^{-1} as maximum tolerance level for any type of food (Brasil, 1965).

In Japanese restaurants, the availability of seafood used in sashimi is seasonal, with tuna and salmon sashimi present for the whole year.

High levels of total As were detected in the analyzed samples, particularly in octopus, amberjack and tuna fish, 14.66, 10.95 and 4.36 mg kg^{-1}, respectively. In this study, the element arsenic was quantified in its total form, nevertheless arsenic might be present in numerous organic and inorganic forms. The total arsenic values contains less than 10% of inorganic forms (arsenite and arsenate), while its organic form, arsenobetaine, a nontoxic compound, is usually the predominant form of As in fish and crustaceans, representing up to 80% (Francesconi & Edmonds, 1997; Francesconi &

Table 2
Levels of As, Cd, Cr, Pb and Hg in samples of muscles of seafood species used for sashimi and percentage of samples above limits set by Mercosul, Brazilian and European regulation.

Sashimi	*n*		Inorganic contaminants (mg kg^{-1}, wet base)				
			Total As	Cd	Cr	Pb	Total Hg
Tuna	29	Mean	1.83	0.005	0.037	0.035	0.409
			(0.43–4.36)	(<0.019–0.035)	(<0.015–0.077)	(<0.062–0.149)	(0.049–1.289)
		Median	1.56	<0.019	0.036	<0.062	0.277
		% >Allowed[a]	*90*	*0*	*0*	*0*	*7*
Salmon	29	Mean	0.92	0.007	0.043	0.089	0.010
			(0.27–1.77)	(<0.019–0.038)	(<0.015–0.078)	(<0.062–0.262)	(0.006–0.014)
		Median	0.94	<0.019	0.043	0.095	0.009
		% >Allowed[a]	*48*	*0*	*0*	*0*	*0*
Mullet	16	Mean	0.69	0.007	0.038	0.171	0.036
			(<0.25–1.44)	(<0.019–0.031)	(0.018–0.075)	(<0.062–0.405)	(<0.004–0.494)
		Median	0.71	<0.019	0.032	0.180	0.006
		% >Allowed[a]	*31*	*0*	*0*	*6*	*0*
Octopus	18	Mean	6.24	0.072	0.083	0.058	0.089
			(2.17–14.66)	(<0.019–0.233)	(<0.062–0.228)	(<0.062–0.250)	(0.019–0.235)
		Median	5.66	0.055	0.080	<0.062	0.074
		% >Allowed[a]	*100*	*61*	*33*	*0*	*0*
Black anchovy	6	Mean	0.63	0.016	0.040	0.083	0.301
			(<0.25–1.21)	(<0.019–0.035)	(0.024–0.068)	(<0.062–0.194)	(0.176–0.430)
		Median	0.73	0.020	0.036	0.074	0.282
		% >Allowed[a]	*17*	*0*	*0*	*0*	*0*
Amberjack	2	Mean	6.27	0.031	0.038	0.146	0.169
			(1.59–10.94)	(0.031–0.031)	(0.028–0.048)	(0.110–0.181)	(0.079–0.258)
		Median	6.27	0.031	0.038	0.146	0.169
		% >Allowed[a]	*100*	*0*	*0*	*0*	*0*
Dorado	3	Mean	1.39	0.006	0.041	0.055	0.079
			(1.07–1.64)	(<0.019–0.019)	(0.032–0.051)	(<0.062–0.164)	(0.059–0.099)
		Median	1.47	<0.019	0.039	<0.062	0.078
		% >Allowed[a]	100	0	0	0	0
Crab stick	7	Mean	0.11	<0.019	0.042	0.029	0.020
			(<0.25–0.47)	<0.019	(0.017–0.062)	(<0.062–0.126)	(0.013–0.027)
		Median	<0.25	<0.019	0.038	<0.062	0.019
		% >Allowed[a]	*0*	*0*	*0*	*0*	*0*
Namorado sandperch	1	Mean	2.18	0.021	0.044	0.101	0.454
		% >Allowed[a]	*100*	*0*	*0*	*0*	*0*
Snook	4	Mean	0.86	0.005	0.038	0.099	0.017
			(0.53–1.24)	(<0.019–0.020)	(0.027–0.046)	(0.077–0.149)	(0.005–0.024)
		Median	0.84	<0.019	0.039	0.084	0.020
		% >Allowed[a]	*25*	*0*	*0*	*0*	*0*
Frigate tuna	1	Mean	1.18	0.025	0.0627	0.105	0.163
		% >Allowed[a]	*100*	*0*	*0*	*0*	*0*

[a] Limits set by Brazilian and European regulation: As = 1.0 mg kg^{-1}; Pb = 0.3 mg kg^{-1} (0.2 mg kg^{-1} European); Cd = 0.05 mg kg^{-1} (tuna = 0.1 mg kg^{-1}); Hg = 0.5 mg kg^{-1} (1.0 mg kg^{-1} predators); Cr = 0.1 mg kg^{-1}. SD = standard deviation.

Kuehnelt, 2004; Hedeggard & Sloth, 2011; Uneyama, Toda, Yamamoto, & Morikawa, 2007).

High levels of mercury were found in tuna sashimi (0.049–1.289 mg kg^{-1}) and black anchovy (0.176–0.430 mg kg^{-1}) and for tuna, 7% of the samples analyzed presented levels above the maximum allowed by the Mercosul and European regulation (1 mg kg^{-1}, predator species). This contaminant has been targeted for regulation by international agencies and organizations, with methylmercury being considered the most toxic species for humans (Hedeggard & Sloth, 2011).

For octopus sashimi, 61% of the total analyzed samples ($n = 18$) contained Cd levels above 0.05 mg kg^{-1} with maximum concentrations up to 0.233 mg kg^{-1}. Chronic exposure to this element has been largely associated with development of pulmonary, renal, cardiovascular and bone damage (Castro-Gonzalez & Mendez-Armenta, 2008).

In general, lead presence in samples was low and only mullet sashimi showed levels above the maximum allowed by the Mercosul (0.3 mg kg^{-1}) and European (0.2 mg kg^{-1}) regulations, while the highest levels of Cr were presented in octopus (maximum of 0.228 mg kg^{-1}). Tuna, salmon, mullet, black anchovy, amberjack, dorado and snook presented very similar values, with Cr mean concentrations between 0.03 and 0.04 mg kg^{-1}.

3.3. *Evaluation of the presence of contaminants during the period of sample collection*

Graphs presented in Fig. 1 show mean concentrations of As, Cd, Cr, Pb and Hg in mullet, octopus, salmon and tuna samples gathered over one year. Levels of these contaminants were variable over the year and, in most of cases, higher between March and August (autumn and winter seasons).

Saei-Dehkordi, Fallah, and Nematollahi (2010) monitored As and Hg in species of fish from the Persian Gulf, assessing the effect of season and habitat. Among the analyzed species, 6 samples of longfin tuna (*Thunnus tonggol*) presented As levels of 0.131 ± 0.033 and 0.310 ± 0.092 mg kg^{-1} in summer and winter respectively, while for Hg the variation in concentration was 0.506 ± 0.132 mg kg^{-1} to 0.548 ± 0.247 mg kg^{-1}.

However, in the present study the origin of seafood is unknown and, although weather may represent an important factor for the concentration of contaminants in biological organisms, other variables such as the metabolism of the animals and the environmental aspects also could be related. To provide conclusive data, physiologic and environmental studies are required.

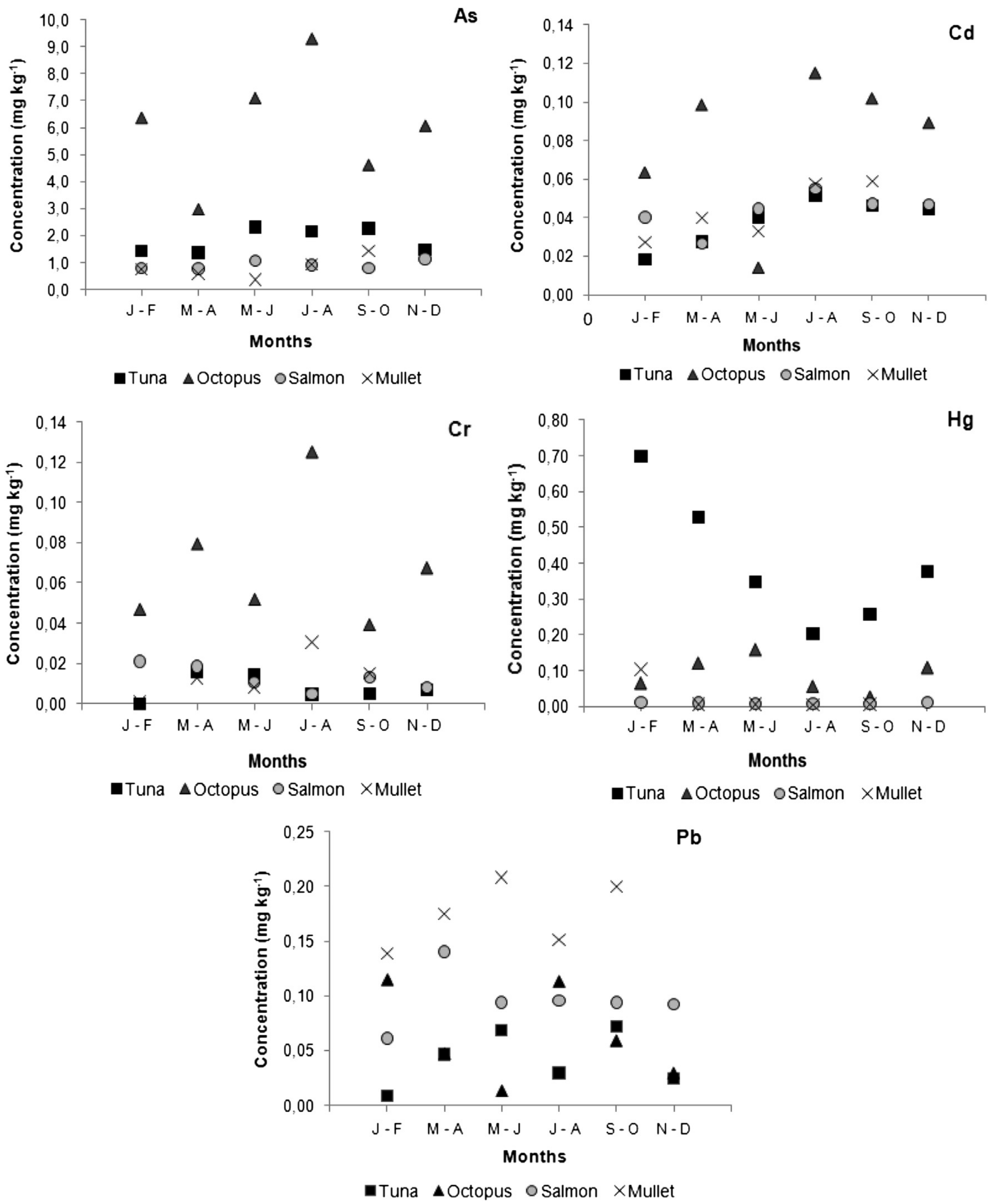

Fig. 1. Mean concentrations of As, Cd, Cr, Pb and Hg in mullet, octopus, salmon and tuna samples gathered over a year.

3.4. As, Cd, Cr, Pb and Hg contaminants: data in literature

Results of some studies in the literature about the presence of inorganic contaminants in fish are presented in Table 3.

In general, samples analyzed in this study presented higher levels than those found in literature. For tuna samples, results were similar to Morgano et al. (2011) and lower for Cr compared to Guérin et al. (2011) and Burger and Gochfeld (2005).

For salmon and black anchovy, Cr levels were also lower than ones from previous studies, while other contaminants were presented in high levels, especially As, with a maximum concentration of 1.77 mg kg^{-1} for salmon samples. Snook and mullet Hg maximum levels were higher than the study of Curcho et al. (2009) which found a maximum concentration 0.178 mg kg^{-1} for snook.

3.5. Estimated intake of inorganic contaminants from seafood consumption

The contribution from seafood consumption to the estimated weekly intake of As, Cd, Cr, Pb and total Hg was calculated considering an adult (60 kg) who consumed 50 g of fish day^{-1} (half-portion of sashimi and equivalent to the consumption recommendation of

Table 3
Comparative among contaminants evaluated in this study and in literature data.

Species	This study	Literature data		
		Value	n	Reference
Tuna ($n = 29$)	As: 0.43–4.36 Cd: <0.019–0.035 Cr: <0.015–0.077 Pb: <0.062–0.149 Hg: 0.049–1.289	Cr: 0.294 Pb: 0.007	8	Guérin et al., 2011
		Cd: <0.0001–0.09 Pb: <0.0001–4.13 Hg: <0.0002–1.14	60	Mol, 2011
		As: 0.18–3.67 Hg: 0.025–0.968	21	Morgano et al., 2011
		Cd: 0.003–0.020 Pb: 0.02–0.09 Hg: 0.02–2.50	26	Yusà et al., 2008
		Cd: 0.010 Pb: 0.016 Hg: 0.48	14	Domingo, Bocio, Falcó, & Llobet, 2007
		As: 1.0 ± 0.1 Cd: 0.03 ± 0.005 Cr: 0.20 ± 0.05 Pb: 0.04 ± 0.01 Hg: 0.65 ± 0.1	50	Burger & Gochfeld, 2005
Salmon ($n = 29$)	As: 0.27–1.77 Cd: <0.019–0.038 Cr: <0.015–0.078 Pb: <0.062–0.262 Hg: 0.006–0.014	Cr: 0.314 Pb: 0.005	8	Guérin et al., 2011
		Cd: 0.012 Pb: 0.103 Hg: 0.05	14	Domingo et al., 2007
Mullet ($n = 16$)	As: <0.25–1.44 Cd: <0.019–0.031 Cr: 0.018–0.075 Pb: <0.062–0.405 Hg: <0.004–0.494	Hg: <0.010–0.025	14	Curcho et al., 2009
Black anchovy ($n = 6$)	As: <0.25–1.21 Cd: <0.019–0.035 Cr: 0.024–0.068 Pb: <0.062–0.194 Hg: 0.176–0.430	Cr: 0.450 Pb: 0.047	3	Guérin et al., 2011
		Cd: 0.010 Pb: 0.014 Hg: 0.080	–	Domingo et al., 2007
		As: 0.26 ± 0.04 Cd: 0.006 ± 0.002 Cr: 0.25 ± 0.06 Pb: 0.06 ± 0.01 Hg: 0.26 ± 0.02	51	Burger & Gochfeld, 2005
		Cd: 0.17 Pb: 0.16 Hg: 0.18	31	Blanco, González, & Vieites, 2008
		Cd: 0.009 ± 0.001 Cr: 0.086 ± 0.020 Pb: 0.118 ± 0.017	–	Demirezen & Uruç, 2006
Snook ($n = 4$)	As: 0.53–1.24 Cd: <0.019–0.020 Cr: 0.027–0.046 Pb: 0.077–0.149 Hg: 0.005–0.024	As: 1.7 ± 0.3 Cd: 0.004 ± 0.001 Cr: 0.08 ± 0.02 Pb: 0.11 ± 0.01 Hg: 0.38 ± 0.06	7	Burger & Gochfeld, 2005
		Hg: 0.015–0.178	12	Curcho et al., 2009

United States Environmental Protection Agency (US EPA): 340 g week^{-1} or ~50 g day^{-1} of seafood) (EPA & FDA, 2004).

From Table 4, the species that mostly contributed to the estimated intake of As were: amberjack, octopus and tuna (36.58, 36.40, 10.68 μg kg^{-1} body weight, respectively). The contribution to the intake of Cd was negligible in most of the species of seafood studied, with octopus having the greatest contribution (Cd = 0.43 μg kg^{-1} body weight).

The greatest contributions to the intake of Pb were found in mullet (1.02 μg kg^{-1} body weight) and amberjack (0.85 μg kg^{-1} body weight). The greatest contribution to Hg intake was found in tuna (2.39 μg kg^{-1} body weight) and black anchovies (1.76 μg kg^{-1} body weight), while the species of seafood that the most contributed to the intake of Cr was octopus (Cr = 0.49 μg kg^{-1} body weight).

4. Conclusion

The samples analyzed from different muscles of seafood species used in sashimi from Japanese restaurants presented levels of As, Cr, Hg and Pb above the maximum levels allowed by Mercosul and European regulations. However, variable concentrations were observed during a one-year period suggesting higher concentrations in autumn and winter. The estimated weekly intake shows that tuna, amberjack and octopus sashimi were the highest contributors for As, mullet and amberjack for Pb; tuna and black anchovy for Hg and octopus for Cd and Cr intake.

Conflict of interest

The authors declare that there are no conflicts of interest.

Table 4
Estimated weekly ingestion for consumption of 50 g day^{-1} of seafood (1/2 portion of sashimi) by 60 kg.

Sashimi	Contaminant	As	Cd	Cr	Hg	Pb
Tuna	Mean (mg kg^{-1})	1.83	0.02	0.038	0.409	0.075
	Ingestion (μg kg^{-1} body weight)[a]	10.68	0.12	0.22	2.39	0.44
Salmon	Mean (mg kg^{-1})	0.92	0.021	0.043	0.01	0.106
	Ingestion (μg kg^{-1} body weight)[a]	5.37	0.12	0.25	0.06	0.62
Mullet	Mean (mg kg^{-1})	0.73	0.021	0.038	0.036	0.174
	Ingestion (μg kg^{-1} body weight)[a]	4.26	0.12	0.22	0.21	1.02
Octopus	Mean (mg kg^{-1})	6.24	0.073	0.084	0.089	0.093
	Ingestion (μg kg^{-1} body weight)[a]	36.40	0.43	0.49	0.52	0.54
Black anchovy	Mean (mg kg^{-1})	0.67	0.022	0.04	0.301	0.114
	Ingestion (μg kg^{-1} body weight)[a]	3.68	0.09	0.48	1.76	0.23
Amberjack	Mean (mg kg^{-1})	6.27	0.031	0.038	0.169	0.146
	Ingestion (μg kg^{-1} body weight)[a]	36.58	0.18	0.22	0.99	0.85
Dorado	Mean (mg kg^{-1})	1.39	0.019	0.041	0.079	0.096
	Ingestion (μg kg^{-1} body weight)[a]	8.11	0.11	0.24	0.46	0.56
Snook	Mean (mg kg^{-1})	0.86	0.019	0.038	0.017	0.099
	Ingestion (μg kg^{-1} body weight)[a]	5.02	0.11	0.22	0.10	0.58

[a] Estimated weekly ingestion for 50 g day^{-1} of sashimi by 60 kg adult.

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