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Metals and arsenic in fish from a Ramsar site under past and present human pressures: Consumption risk factors to the local population

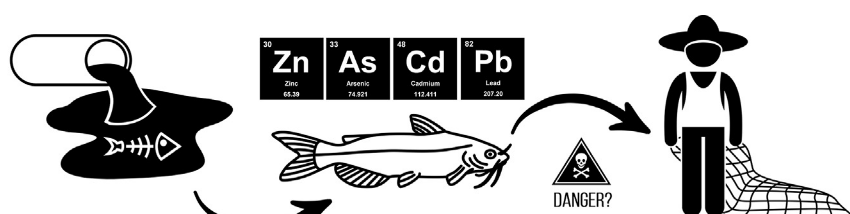
Paloma Kachel Gusso-Choueri [a,b,c,*], Giuliana Seraphim de Araújo [c], Ana Carolina Feitosa Cruz [c], Tatiana Roselena de Oliveira Stremel [d], Sandro Xavier de Campos [d], Denis Moledo de Souza Abessa [c], Ciro Alberto de Oliveira Ribeiro [a,b], Rodrigo Brasil Choueri [e]

[a] Post-Graduation Program in Ecology and Conservation, Universidade Federal do Paraná, P.O. Box 19031, 81531-990 Curitiba, PR, Brazil
[b] Laboratório de Toxicologia Celular, Departamento de Biologia Celular, Universidade Federal do Paraná, CP19031, 81531-990 Curitiba, PR, Brazil
[c] NEPEA, Campus do Litoral Paulista, Universidade Estadual Paulista Júlio de Mesquita Filho, Praça Infante Dom Henrique, s/n, 11330-900 São Vicente, SP, Brazil
[d] Post-Graduation Program in Applied Chemistry, Universidade Estadual de Ponta Grossa, Av. General Carlos Cavalcanti, 4748, 84030-900, Uvaranas, Ponta Grossa, PR, Brazil
[e] Departamento de Ciências do Mar, Universidade Federal de São Paulo, Rua Carvalho de Mendonça, 144, 11070-100 Santos, SP, Brazil

HIGHLIGHTS

- The risk of human health through the ingestion of fish was estimated.
- The study area is a Ramsar site polluted by mining activities and urbanization.
- The consumption of *C. spixii* pose risk to human health due to Cd, Pb and As.
- As in *C. spixii* showed high levels of cancer risk in the surroundings of the city.
- Traditional populations living in Marine Protected Areas may be under risk.

GRAPHICAL ABSTRACT



Credits: Icons from the Noun Project Inc. (licensed under Creative Commons Attribution). Bakunetsu Kaito (pipe); Arthur Shlain (catfish); Muhammad Ilyas (toxic sign); Gan Khoon Lay (fisherman); Ersin (arrows). Creation by R.Choueri.

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ABSTRACT

The risk of metals and As in seafood for traditional populations living in a Marine Protected Areas (MPA) is seldom assessed, although the risk of human exposure to contaminants is one of the indicators associated with the socioeconomic goals of MPAs. The current study aimed to estimate the potential risk of some metals (Cd, Pb, and Zn) and arsenic (As) for human health through the ingestion of fish locally harvested in a Ramsar site, the Cananéia-Iguape-Peruíbe Environmental Protected Area (APA-CIP). Previous studies showed environmental impacts in this area due to former mining activities and urbanization. *Cathorops spixii*, a catfish largely consumed by the local population, was collected along the estuary in three seasons with different rain regimes. Metals and As loads in muscle tissue were quantified and it was estimated (i) the target hazard quotient (THQ) and (ii) the daily intake (EDI) for metals and As, (iii) the cancer risk (CRisk) only for As, and (iv) the number of eligible meals per month. Cd, Pb, and As were found at concentrations above action levels for human consumption. Depending on the level of exposure of the local population, the consumption of *C. spixii* may pose risk to human health. Highest THQs were estimated for fish collected in sites closer to the main contamination sources in the APA-CIP, i.e. the mouth of Ribeira de Iguape River (P1) and the city of Cananéia (P4, P5, and P6). Arsenic showed high levels of cancer risk, although restricted to the area close to the city. The exposure of the local population to metal and As contaminated seafood cannot be disregarded in environmental studies and management of the APA-CIP.

* Corresponding author at: Laboratory for the Study of Aquatic Pollution and Ecotoxicology (NEPEA), São Paulo State University, São Vicente Campus (UNESP Campus do Litoral Paulista), Praça Infante Dom Henrique, s/n, CP 11330-900 São Vicente, SP, Brazil.
E-mail address: pgusso@yahoo.com.br (P.K. Gusso-Choueri).

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1. Introduction

Fish is an important protein source to the human population, providing around 37% of the total animal protein consumed by the human population worldwide (FAO, 2000). However, toxic substances released through human activities into aquatic ecosystems have often increased the bioavailability of contaminants to fish and biota in general. Seafood is a natural vehicle of metals to the human population (Copat et al., 2012; Begum et al., 2013).

Mining and smelting activities are serious threats to the aquatic environment in many countries (Kroll et al., 2005; Avellan et al., 2017). Mining contamination can occur mainly through acid drainage, atmospheric deposition, wind-blown particulate matter and mining waste disposal (e.g. Riba et al., 2005a; Taylor et al., 2014; Camizuli et al., 2014; Molina-Villalba et al., 2015). In some instances, such activities can affect more the surrounding areas than the local of the mining operations itself (Fernández-Caliani et al., 2008) since pollutants may be transported by water and air. In the environment, metals can accumulate in different environmental compartments (Riba et al., 2005b; Ruelas-Inzunza et al., 2011; Camizuli et al., 2014) including fish (e.g. Park and Curtis, 1997; Moiseenko and Kudryavtseva, 2001; Riba et al., 2005b; Jordanova et al., 2016). Metals accumulated in edible tissues of fish pose health risks to consumers (Rabitto et al., 2011; Tang et al., 2013; Sow et al., 2013). Indeed, the diet can be an important route of exposure in the case of populations indirectly exposed to mining activities (Fréry et al., 2001; Castro-González and Méndez-Armenta, 2008; Marrugo-Negrete et al., 2008; Zhuang et al., 2014). Therefore, studies focused on the quality of edible fish are relevant for characterizing human health risks in areas under the influence of mining activities (Subotić et al., 2013).

The Environmental Protected Area of Cananéia-Iguape-Peruíbe (hereafter referred as "APA-CIP", an acronym for the *Área de Proteção Ambiental de Cananéia-Iguape-Peruíbe*), Southeastern Brazil, is recognized as a World Natural Heritage Site by UNESCO (2000) and is part of the UNESCO's Biosphere Reserve of the Atlantic Rainforest. The area was recently included in the Ramsar's List of Wetlands of International Importance (https://rsis.ramsar.org/ris/2310). Apart from its ecological relevance, one of the objectives of the APA-CIP is protecting its cultural and historical value for traditional people, such as the traditional fishermen (known as *Caiçaras*), Maroons (known as *Quilombolas*), and native americans.

Small-scale fishery (primarily artisanal fishery) is among the most relevant economic activities in this area (Mendonça and Katsuragawa, 2001). Drag seines, gillnets, vertical longlines, surface drift gillnet, *gerival* (a type of beam trawl for shrimp fishing), dip net, covered pots (for capturing lobsters and prawn), and *iriko* (a small-mesh size net for catching anchovies) are the most common fishing gear in the area of the APA-CIP (Mendonça and Katsuragawa, 2001). More recently, the significance of recreational fishing (especially anglers) has been increasing as well (Barcellini et al., 2013). The madamango sea catfish (*Cathorops spixii*) is abundant in the APA-CIP and is one of the most fished species by both *Caiçaras* and recreational anglers (Mendonça and Katsuragawa, 2001; Motta et al., 2016). Despite not being the target specie neither for artisanal or for recreational fisheries, this catfish is largely consumed by local population (Favaro et al., 2005).

In spite of the legal protection of the APA-CIP (corresponding to category V of IUCN), this estuarine-lagoon environment has experienced increased contamination by metals from former mining activities located in the Ribeira de Iguape River basin (RIR). Additionally, the construction of an artificial navigational channel connecting the river with the lagoon favored the increasing input of metals toward the estuarine lagoon (Guimarães and Sígolo, 2008; Mahiques et al., 2009; Abessa et al., 2014). Recent studies showed that pollution is affecting the biota (Cruz et al., 2014; Gusso-Choueri et al., 2015, 2016).

Previous studies have also provided some evidences that metals and As body burdens in catfish from the APA-CIP could pose a risk to human health (Azevedo et al., 2012a; Gusso-Choueri et al., 2015). Cd, Pb, and Zn were found in the epaxial muscle tissue of *C. spixii* and *Genidens genidens* specimens at levels comparable to the specimens collected at a highly polluted estuary in Southeastern Brazil (Santos Estuarine System) (Azevedo et al., 2012a, 2012b). Hg was studied as well but the measured concentrations showed low levels in catfish tissue, which was an evidence of the low anthropogenic input of this substance in the APA-CIP (Azevedo et al., 2011, 2012b). Moreover, Hg is not related to the mining activities along the Ribeira de Iguape River basin (Guimarães and Sígolo, 2008; Melo et al., 2012; Piedade et al., 2014), thus it does not represent an element of concern for the region. Pb is the main element related to the mining activities in the RIR watershed. Previous studies reported high levels of Pb in the blood of children and adults living nearby the closed Pb refinery (Paoliello et al., 2002), and increased levels of As (compared to the reference area) in the urine of adults and children population (Figueiredo et al., 2007). Sakuma et al. (2010) also conducted studies of arsenic exposure of children from some sites across the RIR watershed and concluded that mining activities contributed to the presence of arsenic in their urine. Gusso-Choueri et al. (2015) made a very preliminary human health risk assessment, focused on *C. spixii* from the APA-CIP, by simply comparing the concentration levels of metals found in the edible part (the axial muscle) of the catfish with consumption limits provided by legal documents such as USEPA (2000), EC (2006), Mercosul (2011), FAO/WHO (2014), and the Brazilian Sanitary Vigilance Authority (ANVISA, 2013). Although this analysis already showed some exceedances, the approach was too simple and may not reveal, for example, the specific risk for sensitive subpopulations or people with increased susceptibility to toxicological effects, such as pregnant women and children (USEPA, 2000). In addition, the legal documents do not establish reference values for some potentially toxic metals (e.g. Zn). The calculation of risk factors to a certain human population is considered a more reliable approach.

The aim of the current study was to assess the potential risk of metals (Cd, Pb, and Zn) and arsenic (As) to human health due to the ingestion of catfish *C. spixii* fished in the APA-CIP. To achieve such a goal, *C. spixii* was sampled along the estuary in three seasons with different rainfall regimes. The human risk assessment was estimated by means of the daily intake (EDI) and the target hazard quotient (THQ) for metals and As, and the cancer risk (CRisk) for As (USEPA, 2000). Lastly, the number of eligible meals per month was estimated in order to subsidize management actions aiming to reduce the risk of chronic systemic effects.

We hypothesize that the consumption of *C. spixii* from some sites of the APA-CIP can pose risk to human health. The quality of human health has been suggested as one of the indicators associated with socioeconomic goals of Marine Protected Areas (MPA) (Pomeroy et al., 2005). However, the risk of dietary metals and As for people living in a MPA is rarely assessed. The current results will provide information that would be useful for the protection of the traditional population living in Marine Protected Areas affected by toxic metals pollution.

2. Material and methods

2.1. Study area

The Cananéia-Iguape-Peruíbe Environmental Protected Area (APA-CIP) (24°40′S and 25°05′S) presents two main climate seasons: a drier winter (mean temperature of 20 °C and mean pluviosity of 95.3 mm $month^{-1}$) and a rainier summer (mean temperature of 28 °C and mean pluviosity of 266.9 mm $month^{-1}$). The largest freshwater contributor to the estuarine lagoon is the Ribeira de Iguape River (RIR) (Fig. 1). The river flows into the lagoon through the *Valo Grande* channel, an artificial connection built in 1852 for navigational purposes which significantly altered the natural physicochemical and sediment characteristics of the estuary (Mahiques et al., 2013).

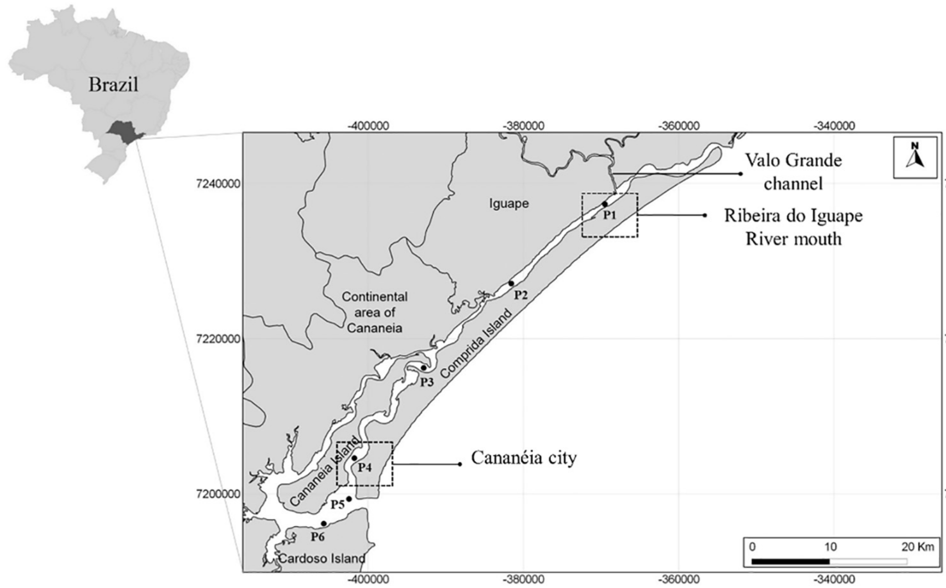

Fig. 1. Sampling stations located within the APA-CIP, Brazil.

Natural geological deposits of Pb and Zn are found in the RIR basin (Moraes et al., 2003). Former mining activities (ceased in the 1990s) have contributed to the high levels of metals (Pb, Zn, Cu, Cr) and arsenic (As) recorded in the river waters, bottom and suspended sediments (Eysink et al., 1988; Corsi and Landim, 2003; Moraes et al., 2003; Guimarães and Sígolo, 2008). The metal contamination of lagoonal-estuarine sediments has increased substantially after the *Valo Grande* channel opening (Mahiques et al., 2009).

Three cities are situated within the APA-CIP (Iguape, with a population of 30,259 inhabitants; Ilha Comprida, with 9025 inhabitants; and Cananéia, with 12,601 inhabitants) (IBGE, 2017). These cities lack proper sewage treatment or even basic sanitation infrastructure (Morais and Abessa, 2014) and previous studies have showed their contribution as a source of pollution to the APA-CIP, in addition to the RIR discharge (Cruz et al., 2014; Gusso-Choueri et al., 2015, 2016).

2.2. Fish collection and sample preparation

Cathorops spixii (madamango sea catfish) is a demersal species that spends its whole life cycle in muddy-bottom estuaries (Azevedo et al., 1998). This species has been considered as an important artisanal fishing resource in tropical and sub-tropical South American Atlantic coasts (Reis, 1986; Melo and Teixeira, 1992; Álvarez-León and Rey-Carrasco, 2003), and it is largely consumed by the local population.

The sampling sites were set with the aim of encompassing the main potential contaminant sources along the APA-CIP (Fig. 1). Thus, the site P1 is the closest to the RIR mouth and site P4 is the closest to the Cananéia city. The details on fish collection can be found in Gusso-Choueri et al. (2015, 2016), but in general the specimens were collected with a bottom otter trawl during three seasons with different pluviosity: (i) the partially dry season (P); (ii) the dry season (D); and (iii) the rainy season (R). The average rainfall volumes during these three seasons of sampling was performed were 192 mm, 111 mm and 390 mm, respectively (CEPAGRI, 2014). Sampling was performed from P2 to P5 in the first sampling campaign (P) and from P1 to P6 in the subsequent campaigns (D and R). This was done to include samplings sites closer to the influence area of the RIR and the *Cananéia* city in the later campaigns.

After collection, specimens were kept in containers with local water, under aeration until transportation to the laboratory. In the laboratory, the specimens were anesthetized with benzocaine (10%) in water, then weighted and measured before euthanasia by the section of the spinal cord. The axial muscle used in metal body burden analyses was stored in plastic vessels at −20 °C until analyses.

2.3. Analyses of metal body burdens

Details on the measurements of the concentrations of metals and As in the axial muscle of *C. spixii* are found in Gusso-Choueri et al. (2015). In brief, As was measured by AAS-GF with Zeeman background correction. Metals were measured by FAAS following the standard method 200.9 (USEPA, 1994). The limit of detection for As was 0.0059 mg kg^{-1}, 0.04 mg kg^{-1} for Cd, 0.06 mg kg^{-1} for Pb, and 0.05 mg kg^{-1} for Zn. The limits of quantification were 0.1780 mg kg^{-1} for As, 0.06 mg kg^{-1} for Cd, 0.55 mg kg^{-1} for Pb, and 0.07 mg kg^{-1} for Zn. Reference material (Qhemis High Purity®) was used to prepare standard curves and the quality control of the analyses was based on the Method of Standard Additions (MSA) following the USEPA method 200.9 (USEPA, 1994). Duplicate and blank samples were done as well. Recovery rates varied within the range from 80% to 120% in all analyses. All glassware was acid washed and rinsed with Milli-Q water.

2.4. Human risk assessment

The mean results of Cd, Pb, Zn and As body burdens in the axial muscle tissue (i.e. the edible part of the fish) were used to estimate the daily consumption (estimated daily intake) of the chemicals of interest; the potential health risks (target hazard quotient and cancer risk) and the maximum allowable fish consumption rate to the population at each sampling site. Cu and Cr were not included in the current study since they did not show tissue concentration values above the limit of detection (Gusso-Choueri et al., 2015). The risk was estimated considering exposure of adults and children.

The As consumption limits calculations were performed as inorganic arsenic contents (3% of the total organic arsenic) (FSA, 2004; Rose et al., 2010). The concentration of the metals and As per mass of wet weight of the tissue samples were based on the concentrations per mass of dry weight reported by Gusso-Choueri et al. (2015) assuming water content of 80% in the muscle tissues (Begum et al., 2013).

2.4.1. Estimated daily intake (EDI)

One of the approaches that comprise the evaluation of human health risk due to the consumption of contaminated fish is the estimation of the dietary daily intakes (EDI) (USEPA, 2000). The EDI was quantified according to the Eq. (1):

$$EDI = (C \times MS) \times BW^{-1} \quad (1)$$

where C is the annual mean concentration (mg kg^{-1} wet weight) of the chemical; MS is the food meal size (0.227 kg for adults and 0.114 kg for children, according to USEPA (2000)); and BW is the body weight (70 kg for adults and 16 kg for children). The EDI values were presented as the mass of the chemical (μg) per unit of mass of body (kg of BW) per unit of time (day) (μg kg^{-1} bw day^{-1}).

The results were compared with reference values provided by the FAO/WHO joint committee (Provisional Maximum Tolerable Intake, CODEX STAN 193–1995). This reference values are presented in three different contexts: i) Provisional Maximum Tolerable Daily Intake, which includes contaminants with no cumulative properties and represents the allowable human exposure resultant of the natural occurrence of the metal in food and drinking water; ii) Provisional Tolerable Weekly Intake, a reference value for contaminants with cumulative properties, which represents a value of permissible weekly human exposure unavoidably associated with the consumption of otherwise non-contaminated and nutritious food; iii) Provisional Tolerable Monthly Intake, used as a reference value for contaminants with higher cumulative properties (those with a longer half-life in the human body). It represents a value of permissible human monthly exposure inevitably associated with the consumption of otherwise non-contaminated and nutritious food.

2.4.2. Estimation of target hazard quotient (THQ) and cancer risk (CRisk)

Risk factors (THQ and CRisk) were calculated to standardize fish and sea food consumption advisories for minimizing the risk of both cancer and non-cancer endpoints (USEPA, 1989; USEPA, 2000). Health risk to adults and children was simulated in 3 different scenarios of fish consumption: monthly, weakly and daily intake.

In the current study, THQ and CRisk were calculated as recommended by USEPA (2000) which is conservative. It was assumed that no portion of the metal in the muscle was lost or magnified during the cooking process, and consequently the ingestion dose is equal to the absorbed contaminant dose (Moreau et al., 2007). The estimation of the THQ for metals and As, calculated according to Eq. (2), considers the ratio between the exposure and the reference dose. Values of exposure higher than the reference dose (i.e. THQ above 1) suggest that systemic effects may occur (USEPA, 1989).

$$THQ = (EF \times ED \times MS \times C) \times (RfD \times BW \times AT)^{-1} \quad (2)$$

where EF is the exposure frequency (365 days year^{-1} for people who eat fish seven times a week, 52 days year^{-1} for people who eat fish once a week, and 12 days year^{-1} for people who eat fish once a month); ED is the exposure duration (70 years for adults and 6 years for children); MS is the food meal size (0.227 kg for adults and 0.114 kg for children according to USEPA, 2000); C is the annual mean concentration of the metal in fish (mg kg^{-1}, wet weight); RfD is the oral reference dose (Cd $= 1 \times 10^{-3}$ mg kg^{-1} day^{-1}, As $= 3 \times 10^{-4}$ mg kg^{-1} day^{-1} (USEPA, 2017) and Pb $= 4 \times 10^{-3}$ mg kg^{-1} day^{-1} (Storelli and Barone, 2013)); BW is the body weight (70 kg for adults and 16 kg for children); AT is the average time of exposure (days) to the chemical (365 days year^{-1} × ED). The average fish ingestion rates, body weight, and lifetime of the target population were set in accordance with the values provided by USEPA (1989); USEPA (2000).

Once the inorganic As is classified by the USEPA as a human carcinogen (USEPA, 1989; USEPA, 2002), the lifetime cancer risk (CRisk) (Eq. (3)) was estimated for this compound in the current study. CRisk was estimated by using the cancer slope factor (CSF) provided by the USEPA's Integrated Risk Information System (IRIS) (2017). CRisk above the acceptable lifetime risk value (10^{-5}) indicates a probability > 1 chance over 100,000 of an individual develop cancer (USEPA, 1989; USEPA, 2000).

$$CRisk = (EF \times ED \times MS \times C \times CSF) \times (BW \times AT)^{-1} \quad (3)$$

The RfD, used to estimate the THQ (see Eq. (2)), is defined as an estimate of daily exposure to the human population, including sensitive subgroups, that is likely to be without an appreciable risk of deleterious effects during a lifetime (USEPA, 1989). CSF, in turn, is defined as the "cancer potency". Since USEPA assumes that carcinogens do not have safe thresholds, the CSF is usually the upper 95% confidence limit on the linear term in the multistage model used by USEPA (1996) based on data obtained in an epidemiological study or a chronic animal bioassay (USEPA, 2000).

2.4.3. Estimation of safe monthly consumption rates (CRmm)

The consumption rate (i.e. maximum allowable number of fish meals that could be consumed over a month which would not be expected to cause any chronic systemic effects) (CR_{mm}) was evaluated for Pb, Cd and As according to USEPA (2000). First, the consumption rate limit (CR_{lim}) (maximum safe consumption rate) was estimated according to Eq. (4), and expressed in kg fish d^{-1}. It was assumed that no other source of metals or As exists in the diet of consumers.

$$CR_{lim} = (RfD \times BW) \times C^{-1} \quad (4)$$

where RfD is the reference dose for each metal or As (mg kg^{-1} day^{-1}); BW is the body weight (kg); C is the annual mean concentration of a chemical measured in the sample (mg kg^{-1}). The safe fish intake in a weekly basis (CR^{*}_{lim}) was subsequently calculated by simply multiplying the CR_{lim} by 7. Lastly, the maximum safe number of fish meals in a monthly basis (according to Moreau et al., 2007) was calculated (see Eq. (5)):

$$CR_{mm} = \left(CR^{*}_{lim} \times T_{ap}\right) \times MS^{-1} \quad (5)$$

where CR_{mm} is the maximum allowable consumption rate (meals month^{-1}), CR^{*}_{lim} is the maximum weekly consumption rate of fish (kg week^{-1}), T_{ap} is the average time period in a month (4.3 week month^{-1}), and MS is the meal size. Number of meals higher than 16 month^{-1} was considered with no obvious human health risk (USEPA, 2000).

3. Results and discussion

3.1. Cd, Pb, Zn and as in the axial muscle

Table 1 shows the results of concentrations of Cd, Pb, Zn, and As in the axial muscle tissues of *C. spixii* on a wet weight basis (mg kg^{-1} w. w.) of specimens sampled during the partially dry season, the dry season (D), and the rainy season (R) in different sites along the APA-CIP. A full discussion on the temporal and spatial patterns of metals (Cu, Mn, Zn, Cr, Co, Ni, Cd, and Pb) and As burdens in the axial muscle and liver tissues of *C. spixii* (in the context of an environmental quality assessment) was done by Gusso-Choueri et al. (2015). The results specifically for Cd, Pb and As loads in the axial muscle of *C. spixii*, the focus of the current study, showed a seasonal difference between dry and rainy season in all sampling sites, similarly to the other metals as discussed by Gusso-Choueri et al. (2015). The exception was P1, where the levels of Pb varied only marginally between seasons, suggesting a constant influence of the RIR throughout the year. In the other sites, the levels of Cd, Pb, and As were consistently higher during the dry season. This can be a result of the increase of metals concentration in coastal environments

Table 1
Cd, Pb, Zn and As mean (and standard deviations) concentrations (mg kg^{-1} w.w.) in muscle tissues of *Cathorops spixii* sampled during three seasons with different pluviometric regimes the partially dry season (P); the dry season (D); and the rainy season (R) in the APA-CIP. Action levels for human consumption established by different health agencies are also given (mg kg^{-1} w.w.) (the exceedances are presented in bold). LOQ values were used in annual means in case of detection below the LOQ.

Sampling station-season	Cd	Pb	Zn	As
P1 – D	**2.73 ± 1.03**	**6.48 ± 1.48**	2.23 ± 0.33	0.0004 ± 0.0000
P1 – R	<0.11	**6.31 ± 1.08**	6.70 ± 4.01	0.0005 ± 0.0000
P1 -annual mean	**1.42 ± 1.07**	**6.39 ± 0.07**	4.61 ± 3.37	0.0005 ± 0.0000
P2 – P	**0.03 ± 0.00**	**7.34 ± 0.35**	2.20 ± 0.15	0.0005 ± 0.0006
P2 – R	**0.01 ± 0.00**	**1.61 ± 0.61**	4.80 ± 0.95	0.0008 ± 0.0002
P2 -annual mean	**0.07 ± 0.03**	**4.48 ± 2.34**	3.50 ± 1.843	0.0007 ± 0.0000
P3 - P	**1.66 ± 0.00**	**7.06 ± 0.24**	0.83 ± 0.01	0.0013 ± 0.0006
P3 - D	**1.81 ± 0.76**	**4.33 ± 1.07**	1.84 ± 0.27	0.0021 ± 0.0014
P3 - R	<0.11	<0.11	2.42 ± 0.42	0.0005 ± 0.0002
P3 -annual mean	**1.19 ± 0.70**	**3.84 ± 1.88**	1.70 ± 0.81	0.0013 ± 0.0006
P4 - P	**3.09 ± 0.48**	**7.30 ± 0.55**	3.34 ± 1,73	0.0233 ± 0.0156
P4 - D	**2.11 ± 1.63**	**9.58 ± 2.54**	6.90 ± 5.47	**1.1451 ± 0.0666**
P4 - R	<0.11	<0.11	4.50 ± 2.08	0.0015 ± 0.0000
P4 -annual mean	**1.77 ± 0.87**	**5.66 ± 3.89**	4.93 ± 1.819	0.3900 ± 0.5340
P5 - P	**4.54 ± 2.38**	**6.17 ± 2.08**	1.55 ± 0.64	0.0116 ± 0.0026
P5 - D	**0.22 ± 0.31**	**14.64 ± 2.14**	2.50 ± 3.29	0.0044 ± 0.0666
P5 - R	<0.11	<0.11	13.12 ± 1.47	0.0006 ± 0.0002
P5 -annual mean	**1.63 ± 0.69**	**6.97 ± 5.94**	5.71 ± 6.43	0.0055 ± 0.0046
P6 - D	<0.11	**9.97 ± 1.29**	3.07 ± 0.29	0.0298 ± 0.0005
P6 - R	<0.11	<0.11	2.42 ± 0.71	0.0019 ± 0.0002
P6 -annual mean	<0.11	**5.04 ± 4.03**	2.75 ± 0.46	0.0149 ± 0.0140
Action levels				
ANVISA (2013)	0.05 to 0.3	0.3	–	1.0
FAO/WHO (2014)	1.0	2.0	–	1.0
EC (2006)	0.1 to 1.0	0.2 to 2.0	–	–

*Metals and As concentrations were converted from dry weight (d.w.) (as reported by Gusso-Choueri et al., 2015) to wet weight (w.w.).

during the dry season due to the diminished freshwater inflow (Sainz et al., 2004; Fianko et al., 2007; Costas et al., 2011). Zn, in turn, did not show a clear seasonal pattern.

Cd, Pb, Zn, and As loads in fish also show some spatial pattern. The rank order of the annual means of Cd body burdens was: P4 > P5 > P1 > P3 > P2 > P6. For Pb, the rank order was: P5 > P1 > P4 > P6 > P2 > P3. For Zn: P5 > P4 > P1 > P2 > P6 > P3, and for As: P4 > P6 > P5 > P3 > P2 > P1. In general, higher loads were found at the sampling sites closer to the Cananéia city (mainly P4 and P5). Pb in fish tissue was high during all sampled seasons in the sampling site closer to the RIR (P1), again suggesting a perennial influence of the river in the levels of Pb in fish tissues.

The levels of metals and As found in the axial muscle of *C. spixii* in the current study are, in general, higher than those found in muscle of estuarine fishes as reported by other studies performed at polluted sites (e.g. Souza et al., 2013; Vasanthi et al., 2013). In addition, as discussed by Gusso-Choueri et al. (2015) in a study which included a preliminary human health risk assessment, the levels of Cd, Pb and total As were higher than the guidelines set by the Brazilian Sanitary Vigilance Authority (ANVISA, 2013) and Mercosul (2011), as well as the guidelines provided by EC (2002); EC (2006) and FAO/WHO (2014) (Table 1). The high levels of As and Cd found in *C. spixii* from APA-CIP are concerning because these contaminants have a known carcinogenic potential while Pb is known to cause neurotoxicity and other disorders (USEPA, 2000; Squadrone et al., 2013).

Higher levels of metals and As in species that live and feed in direct contact with sediments (like *C. spixii*) are expected since such organisms are directly exposed to sediment-associated contamination (Storelli, 2008; Storelli and Barone, 2013). Liu et al. (2015) measured metals loads in water, fish, plant and soil samples and assessed exposure risk to migratory birds. The authors found that soil consumption had a greater influence on birds than water exposure and argued that this was because of the tendency of metals to adsorb onto surface sediment. Similarly to the current research, a study of bioaccumulation in Ariidae catfish from the APA-CIP also concluded that the levels of Cd and Pb in the muscle tissue of *C. spixii* and *Genidens genidens* indicated the influence of a source of metals in the area (Azevedo et al., 2012b).

Levels of As found in the axial muscle of *C. spixii* from the APA-CIP are relatively high in the sampling sites close to the urban site (Cananéia city). The levels of the semimetal found in the current study were within the range reported in a study with seafood (fish, shellfish, and cephalopods) from local fish markets in a place in SE Spain (levels ranging from 0.04 to 20.02 mg kg^{-1} w.w.) (Delgado-Andrade et al., 2003). In the APA-CIP, a previous study also reported quantities of As in the muscle tissue of *C. spixii* similar to those found in the current study (Kuniyoshi et al., 2011). In addition, the same authors showed that higher levels of As in *C. spixii* was found in the vicinities of Cananéia city compared to the mouth of the RIR, which corroborates the findings of the current study (Kuniyoshi et al., 2011).

The enrichment of metal and As in the RIR basin and downstream may have a contribution from non-anthropogenic sources, since this is a geochemically anomalous environment (Figueiredo et al., 2007). Despite that, the activities of metal mining and Pb smelting have been accounted for Ag, Ba, Cd, Cu, Pb, and Zn contamination in the river and the lagoon-estuary (Moraes et al., 2003; Figueiredo et al., 2007; Guimarães and Sígolo, 2008; Mahiques et al., 2009). Mahiques et al. (2009) performed a historical analysis of the sediments of the APA-CIP and reported that the levels of Pb, Cu, Zn and Cr increased significantly after the RIR flow was deviated into the estuary through the Valo Grande channel.

The amount of contaminants in aquatic organisms potentially responds to different factors, such as the exposure period, the concentration of the chemical, environmental temperature, salinity, pH and seasonal changes (Terra et al., 2008; Copat et al., 2012; Greenfield et al., 2013). Such variables can influence in the geochemical process of metallic ions as well as in the physiology of aquatic organisms, with implication on the bioavailability, uptake, metabolism, and excretion of metals (Chapman et al., 1998; Paquin et al., 2000; Chapman and Wang, 2001; Vijver et al., 2004). Salinity is especially important to modulate As body burdens in marine fish. It has been observed that the accumulation and retention of As (specifically arsenobetaine) is related

Table 2
EDI of Cd, Pb, Zn and inorganic As (μg kg^{-1} bw day^{-1}) for adults and children in the different sites of the APA-CIP.

Sampling station	Cd		Pb		Zn		As	
	Adult	Child	Adult	Child	Adult	Child	Adult	Child
P1	4.61	10.12	20.74	45.56	14.96	32.87	0	0
P2	0.24	0.52	14.52	31.90	11.35	24.94	0	0
P3	3.87	8.50	12.43	27.32	5.50	12.09	0	0
P4	5.74	12.61	18.35	40.32	15.97	35.09	0.04	0.08
P5	5.27	11.59	22.61	49.68	18.50	40.65	0	0
P6	0.37	0.81	16.34	35.90	8.91	19.58	0	0

to the osmotic regulation (Clowes and Francesconi, 2004; Amlund and Berntssen, 2004), since arsenobetaine has a chemical structure similar to a well-known organic osmolyte, the glycine betaine. For marine fish, a positive correlation between salinity and arsenic burdens in three fish species (*Clupea harengus*, *Gadus morhua*, *Platichthys flesus*) from the Baltic and the North Sea was shown (Larsen and Francesconi, 2003). In the current study, *C. spixii* were collected in salinities ranging from 0 to 20 in the sites under higher influence of the RIR (P1, P2, and P3) and from 26 to 32 in the sites closer to the city (P4, P5, and P6), thus the highest levels of As were found in fish from the sites with the highest salinity. Although the influence of urban wastewater as a point source of As in the APA-CIP is probable due to the presence in many household products (Carbonell-Barrachina et al., 2000), the natural background and the retention of As as arsenobetaine for osmotic regulation may have a contribution to the levels in fishes from the vicinities of the Cananéia city.

3.2. Human risk assessment

3.2.1. Dietary daily intakes

Results of the Estimated Daily Intake (EDI) of the current research were presented in Table 2.

The EDI of Cd to adults ranged from 0.24 (P2) to 5.27 (P5) μg kg^{-1} bw day^{-1}, whereas the EDI of Cd to children was found between 0.52 (P2) and 12.61 (P5) μg kg^{-1} bw day^{-1}. The rank order of the intake levels was P4 > P5 > P1 > P3. Those values are concerning because they violate the Provisional Tolerable Monthly Intake (25 μg kg^{-1} bw month^{-1}, equivalent to 0.83 μg kg^{-1} bw day^{-1}) provided the FAO/WHO joint committee (CODEX STAN 193-1995, 2015).

The values of EDI for Pb ranged from 12.43 (P3) to 22.61 (P5) μg kg^{-1} bw day^{-1} to adults and 27.32 (P3) to 49.68 (P5) μg kg^{-1} bw day^{-1} to children. In terms of ranking, the order of the EDI was P5 > P1 > P4 > P6 > P2 > P3. According to FAO/WHO joint committee (CODEX STAN 193-1995, 2015), the intake of such contaminant at these concentrations may cause risk to the population (both adults and children) in all sampling sites.

EDI values of Zn ranged from 5.50 (P3) to 18.50 (P5) μg kg^{-1} bw day^{-1} to adults and 12.09 (P3) and 40.65 (P5) μg kg^{-1} bw day^{-1} to children. These values were below the levels established by Joint FAO/WHO Expert Committee on Food Additive (JECFA) that is 300 to 1000 μg kg^{-1} bw day^{-1}.

EDI values of inorganic As were 0.04 μg kg^{-1} bw day^{-1} to adults and 0.08 μg kg^{-1} bw day^{-1} to children (both in P4). Only fish sampled in P4 pose a risk to the consumers due to As contamination.

3.2.2. Estimation of target hazard quotient (THQ) and cancer risk (CRisk)

Risk factors (THQ and CRisk) of Cd, Pb, Zn, and inorganic As in *C. spixii* collected at the sampling sites along the APA-CIP are presented in Table 3.

It is assumed that THQ values greater than one, and CRisk above 1 × 10^{-5}, are of concern because there is a high risk of developing chronic systemic effects (assessed by THQ) or cancer (assessed by CRisk) due to the intake of the evaluated contaminants (USEPA, 2000). In general, every-day consumption (365 days year^{-1}) of *C. spixii* presents potential risks of chronic systemic effects to children and adults due to the presence of Cd and Pb in fish collected all along the estuary. The estimation also indicates that children may be at risk of chronic systemic effects even in the scenario of eating fish once a week (56 days year^{-1}). The THQ values of Zn and As do not violate the reference values.

Although THQ values for As are not concerning, the cancer risk is above the acceptable lifetime risk to children in the estimated exposure levels of 365 and 56 days year^{-1} for fish sampled in P4. This means that the human populations who consume this fish in any of these scenarios have a probability of developing cancer that is >1 over 100,000 individuals (USEPA, 1989; USEPA, 2000).

It is important to emphasize the CRisk of As is calculated over an estimation of the amount of inorganic As from the measured levels of total As. Previous studies in other places associated chronic inorganic As exposure via contaminated food with an increasing of the number of diseases, such as cancer in the skin, lung, bladder and kidney (Castro-González and Méndez-Armenta, 2008; Sirot et al., 2009). Organic As

Table 3
Target hazard quotient in the different sites of the APA-CIP for Cd, Pb, Zn and inorganic As, and cancer risk estimate for inorganic As, estimated for different levels of exposure. THQ > 1 and CR > 10^{-5} are showed in bold.

Sampling station	Exposure level	Cd		Pb		Zn		As		As cancer risk	
	(days year^{-1})	THQ adult	THQ child	THQ adult	THQ child	THQ adult	THQ child	THQ adult	THQ child	CRisk adult	CRisk child
P1	365	**4.6**	**10.1**	**5.2**	**11.4**	<1	<1	<1	<1	1. 21 × 10^{-13}	2.82 × 10^{-7}
	56	<1	**1.4**	<1	**1.6**	<1	<1	<1	<1	1.73 × 10^{-14}	4.02 × 10^{-8}
	12	<1	<1	<1	<1	<1	<1	<1	<1	3.99 × 10^{-15}	9.29 × 10^{-9}
P2	365	<1	<1	**3.6**	**8.0**	<1	<1	<1	<1	2.84 × 10^{-13}	4.32 × 10^{-7}
	56	<1	<1	<1	**1.1**	<1	<1	<1	<1	4.05 × 10^{-14}	6.16 × 10^{-8}
	12	<1	<1	<1	<1	<1	<1	<1	<1	9.34 × 10^{-15}	1.42 × 10^{-8}
P3	365	**3.9**	**8.5**	**3.1**	**6.8**	<1	<1	<1	<1	9.89 × 10^{-13}	8.07 × 10^{-7}
	56	<1	**1.2**	<1	**1.0**	<1	<1	<1	<1	1.41 × 10^{-13}	1.15 × 10^{-7}
	12	<1	<1	<1	<1	<1	<1	<1	<1	3.25 × 10^{-14}	2.65 × 10^{-8}
P4	365	**5.7**	**12.6**	**4.6**	**10.1**	<1	<1	<1	<1	8.28 × 10^{-8}	**2.33 × 10^{-4}**
	56	<1	**1.8**	<1	**1.4**	<1	<1	<1	<1	1.18 × 10^{-8}	**3.33 × 10^{-5}**
	12	<1	<1	<1	<1	<1	<1	<1	<1	2.72 × 10^{-9}	7.67 × 10^{-6}
P5	365	**5.3**	**11.6**	**5.7**	**12.4**	<1	<1	<1	<1	1.67 × 10^{-11}	3.31 × 10^{-6}
	56	<1	**1.7**	<1	**1.8**	<1	<1	<1	<1	2.38 × 10^{-12}	4.72 × 10^{-7}
	12	<1	<1	<1	<1	<1	<1	<1	<1	5.48 × 10^{-13}	1.09 × 10^{-7}
P6	365	<1	<1	**4.1**	**9.0**	<1	<1	<1	<1	1.21 × 10^{-10}	8.93 × 10^{-6}
	56	<1	<1	<1	**1.3**	<1	<1	<1	<1	1.73 × 10^{-11}	1.27 × 10^{-6}
	12	<1	<1	<1	<1	<1	<1	<1	<1	3.99 × 10^{-12}	2.94 × 10^{-7}

compounds (such as the arsenobetaine, discussed above) are considered to be nontoxic and therefore not a threat to human health (ATSDR, 1998).

Although no studies have been carried out specifically in the APA-CIP to assess the relationship between contaminated seafood and cancer incidence, higher cancer rates were reported in the region (*Vale do Ribeira*) compared with other regions in the state of São Paulo (Luizaga, 2015). It is worth pointing out that a direct cause-effect relationship cannot be established since this region also presents low social and economic indicators, but the current study suggests that the role of contamination via seafood must be further investigated in the APA-CIP.

3.2.3. Estimation of the allowable monthly consumption

An important aspect of the assessment of risks to human health through exposure to potentially harmful substances in fish is the estimation of the allowable daily consumption of such substances (Moreau et al., 2007). This information is given in terms of maximum safe number of meals in a certain period of time, which is more suitable to communicate with local people and decision-makers since it is easy to understand. The maximum safe number of fish meals month^{-1} (monthly consumption rate, CR_{mm}) is presented in the Table 4 for Cd, Pb and inorganic As at each sampling site along the APA-CIP. An integrated CR_{mm} value considering the lowest CR_{mm} among all chemicals at each sampling station is also presented. The maximum safe number of fish meals ranged from 5 to 8 meals month^{-1} to adults, and 2–4 meals month^{-1} to children. The maximum safe number of meals is lowest for *C. spixii* collected in the sites closer to the Cananéia city, intermediate for the site closer to the RIR, and highest to the sites between far from main contamination sources in the APA-CIP. It is noteworthy that the CR_{mm} only takes into account the reference dose of the chemical for systemic effects; if the cancer slope was taken into account, the number of maximum number of fish meals would be considerably smaller because of the levels of As in the fish muscle tissue.

In the current study, the value for fish meal size used to estimate EDI, THQ, CRisk, and safe consumption rates was established by USEPA (2000) (one meal day^{-1} of 0.227 kg for adults and 0.114 kg for children). This is because there is no specific data about the consumption rate of fish by *Caiçaras* from the APA-CIP. However, this value is within the range of variation of a typical fish meal size of traditional communities in the Santos Estuarine System (located about 200 km N from the APA-CIP), which ranges from 0.150 to 0.300 kg of fish (CETESB, 2006).

Although the value established by USEPA is commonly used in studies concerning consumption risk factors of fish (Hardell et al., 2010; Mansilla-Rivera and Rodríguez-Sierra, 2011; Copat et al., 2013; Taweel et al., 2013; Yu et al., 2014), one may argue that the estimation of consumption risks would be more precise if the data of consumption rate was specific to the country. In the case of the current study, even if it was used the consumption of fish per capita in Brazil as a parameter to estimate the meal size (14.4 kg year^{-1}, or a consumption of 0.039 kg day^{-1} for adults and 0.020 kg day^{-1} for children) (Brazilian Minister of Agriculture, 2017), still there would be risk to both adults and children due to Pb levels in fish from all sampling sites in the APA-CIP, Cd in fish from P1, P3, P4, and P5, and As in fish from P4.

In the current study, the use of the USEPA's reference value to estimate the consumption risk was preferred to the mean national consumption of fish in Brazil because the last one may lead to an underestimation of the consumption of *C. spixii* by traditional fishermen in the APA-CIP. Previous studies have shown the importance of fish in the *Caiçara*'s diet: data from Mourão (1971) show that 85% of the meals contained fish (apart from rice, beans, manioc flour, banana, and potato), while beef, poultry or bush meat were consumed in 8.5% of the meals of *Caiçaras* from Southern São Paulo. More recently, Hanazaki (2001) and Hanazaki and Begossi (2004) showed the increased importance of poultry and beef in the diet of this *Caiçara* people, although fish was still the main protein source (32% against 24% and 23% for poultry and beef, respectively). According to these publications, "Catfish" (including *C. spixii*) was the second most consumed group of fish, following "White mullet" (Hanazaki, 2001). Hanazaki (2001) also draws attention to the fact that most of the consumed fish is locally harvested.

4. Final remarks

The results of the current study pointed out that the levels of metals and As in edible parts of a widely consumed fish have a potential risk of affecting human health through the consume of contaminated fish. It is important to bear in mind that this study considered only one fish species, which showed metals and As in muscle at levels considerably higher than other species as reported by previous studies. People often eat several species of fish and other seafood in their diets, and therefore further studies are required to measure the contamination levels in edible parts of different organisms used for local consumption, as well as to detail the consumption patterns. Other uncertainty, although referring specifically to As, is that the risk was calculated based not on a direct measure of inorganic As, but on an estimation of inorganic As from the total As in the axial muscle. A direct measure of inorganic As in edible parts of *C. spixii* would improve the risk assessment. Additionally, the lack of data about levels of contaminants other than metals and As (such as hydrocarbons and pesticides) in edible tissues of fish and seafood from APA-CIP also add uncertainty in the evaluation of risk to human health of consuming fish harvested in this area. In a broader sense, a more accurate assessment of human health risk in this area must consider also nonfish sources of exposure to contaminants (other food items, drinking and bathing water, soil, air).

Nonetheless, the estimated risk factors presented in the current study indicated that there is potential risk of people who consume *C. spixii* developing chronic systemic effects and/or cancer in the APA-CIP. Small-scale fisheries are one of the main economic and subsistence activity in this area (Mendonça and Katsuragawa, 2001; Barcellini et al., 2013). *Caiçaras* (traditional fishermen), *Quilombolas* (maroons), and Native Americans frequently consume their own fish catch as an important source of protein in their diets, and *C. spixii* is widely consumed in the region. Furthermore, it is important to have in mind that, for traditional people, eating fish is not only a dietary choice, but it integrates their lifestyle and culture (USEPA, 2000). Therefore, metals and As contents found in the edible part of *C. spixii* are a potential health issue for the traditional population, especially for children who are even more susceptible than adults.

Table 4
Maximum allowable fish consumption rate (meals month^{-1}) (CR_{mm}) for adults (meal size = 227 g) and children (meal size = 114 g) in the different sites of the APA-CIP (P1 to P6).

Sampling station	Cd		Pb		Zn		As		Integrated CR_{mm}	
	Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child
P1	7	3	6	3	>16	>16	>16	>16	6	3
P2	>16	>16	8	4	>16	>16	>16	>16	8	4
P3	8	4	10	4	>16	>16	>16	>16	8	4
P4	5	2	7	3	>16	>16	>16	>16	5	2
P5	6	3	5	2	>16	>16	>16	>16	5	2
P6	>16	>16	7	3	>16	>16	>16	>16	6	2

5. Conclusions

The current study showed that the concentrations of Cd, Pb and, more limitedly, inorganic As in the axial muscle tissue of *C. spixii*, may pose a risk to human health in the APA-CIP depending on the number of days year^{-1} that a fish meal is consumed (i.e. the level of exposure). Higher Target Hazard Quocients were estimated for the population who consume fish harvested in sites closer to the major sources of contamination in the APA-CIP, i.e. the mouth of the Ribeira de Iguape River (P1) and the city of Cananéia (P4, P5, and P6). In addition, the current results suggest a high cancer risk restricted to the area under the influence of the Cananéia city due to the presence inorganic As in *C. spixii* muscle.

The current findings raise concerns about the local population's health. This study showed that health risks for the population living in MPAs affected by chemical pollution cannot be overlooked in environmental studies and specifically in the management of the APA-CIP Ramsar site. Further studies are needed (e.g. feeding habits of the exposed population, levels of contaminants in other food items, levels of contaminants in the adult and child population) to detail at what extent such population are exposed to metal contamination in food and possibly subsidize management actions.

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