http://dx.doi.org/10.5935/0103-5053.20130209

J. Braz. Chem. Soc., Vol. 24, No. 10, 1642-1648, 2013.
Printed in Brazil - ©2013 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Bioaccumulation of Tributyltin by Blue Crabs

Monizze Vannuci-Silva,[a] Amauri A. Menegario,*[,a] Mariana Franchi,[a] Ana L. Brossi-Garcia,[a,b] João M. de Souza,[c] Marcus A. G. de Araújo Jr.,[c] Monica A. F. S. Bindes G. Lopes[d] and José S. Govone[e]

[a]Centro de Estudos Ambientais (CEA) and [b]Departamento de Zoologia, Universidade Estadual Paulista, 13506-900 Rio Claro-SP, Brazil

[c]Gerência de Avaliação e Monitoramento Ambiental, Centro de Pesquisas da Petrobras (CENPES), 21941-915 Rio de Janeiro-RJ, Brazil

[d]Pontifícia Universidade Católica do Rio de Janeiro, 22451-900 Rio de Janeiro-RJ, Brazil

[e]Matemática Aplicada e Computação (DEMAC), Universidade Estadual Paulista, Departamento de Estatística, 13506-900 Rio Claro-SP, Brazil

O presente estudo avaliou a bioacumulação de tributilestanho (TBT) pelo siri azul (*Callinectes sapidus*). Os animais foram alimentados com comida contaminada com 30 μg g^{-1} de TBT, expresso como Sn. Os analitos foram determinados nas brânquias, hepatopâncreas e músculo. Realizou-se uma digestão ácida para determinação da concentração total de Sn, e a técnica de extração em fase sólida foi utilizada para determinação seletiva de TBT. Obteve-se limites de detecção de 44,6 e 4,46 ng g^{-1} para HG-ICP OES (geração de hidretos (HG) por espectrometria de emissão óptica com plasma indutivamente acoplado) e ICP-MS (ICP-espectrometria de massas), respectivamente. Os resultados para os tecidos dos animais não contaminados foram inferiores a 50 ng g^{-1}, enquanto os submetidos à alimentação contaminada mostraram elevadas concentrações de Sn (até 6229 ng g^{-1}) e TBT (até 3357 ng g^{-1}) relacionadas aos dias de exposição. De acordo com os resultados, Sn é acumulado pelo siri azul em elevadas concentrações no hepatopâncreas. Para a maioria dos animais, os resultados sugerem que o Sn é bioacumulado como TBT.

This study evaluated the bioaccumulation of tributyltin (TBT) by the blue crab (*Callinectes sapidus*). Animals were fed with contaminated food containing 30 μg g^{-1} of TBT expressed as Sn. The analytes were determined in the gills, hepatopancreas and muscle. Acid digestion was used in the total Sn determination, and a solid-phase extraction technique was used for the selective determination of TBT. Limits of detection of 44.6 and 4.46 ng g^{-1} were found for HG-ICP OES (hydride generation-inductively coupled plasma optical emission spectroscopy) and ICP-MS (ICP-mass spectrometry), respectively. The results for non-contaminated animals were below 50 ng g^{-1}, while the animals subjected to the contaminated food showed higher tissue concentrations of Sn (until 6229 ng g^{-1}) and TBT (until 3357 ng g^{-1}) related to the number of exposure days. According to the results, Sn is bioaccumulated by the blue crab in higher concentrations in the hepatopancreas. For most of these animals, the results suggest that Sn is bioaccumulated as TBT.

Keywords: tin bioaccumulation, speciation, HG-ICP OES, ICP-MS

Introduction

Bioaccumulation is the result of absorption (by surface, breathing and diet) and the excretion processes (by breathing, defecation, metabolic biotransformation and dilution) of substances in organisms. For crustaceans and marine bivalves, accumulation can occur from an aquatic environment, ingested food and sediment.[1]

The presence of antifouling paints and biocides containing tributyltin (TBT) in coastal ecosystems is of great environmental concern. Although TBT can persist in sediments for years[2,3] and be accumulated by benthonic biota,[4] little material is known about its accumulation through food in high trophic level benthonic organisms.

*e-mail: amenega@rc.unesp.br

Animals exposed to TBT in marine waters can accumulate the compound in their bodies, resulting in morphologic, physiologic and ecologic problems. TBT can be very toxic to marine invertebrates.[5,6]

Metallic elements, organochloride pesticides and organometallic compounds of tin have been found in *Tachypleus tridentatus* crabs from coastal waters near Japan.[7] The results showed high absorption rates for this species, which can be a serious threat to their survival.[7]

Many studies attempting to evaluate the TBT bioaccumulation process and its effects using amphipods,[8] fish[9-11] and molluscs[12-17] can be found in the literature. Even at low concentration level in sediments, TBT causes imposex in molluscs.[18] For fish, TBT can lead to a bias of sex toward males.[19] However, few studies using crabs exposed to TBT have been reported.

Weis and Kim[20] studied *U. pugilator* crabs exposed to 0.5 mg L^{-1} TBT and concluded that the presence of the compound in the organism can delay tissue regeneration and ecdysis. In addition, anatomical abnormalities were found during tissue regeneration and basal growing, indicating that the compound has teratogenic effects on tissue development.

Botton *et al.*[21] investigated acute and chronic TBT exposure effects on *Limulus polyphemus* embryos and larval stages. It was concluded that these animals are highly resistant to TBT when compared with the first developmental stages of other marine arthropods. The survival of *Limulus polyphemus* embryos and larval stages in the presence of tin organometallic compounds could suggest the possibility of its contamination and circulation through the food chain to seabirds and fish.

Rouleau *et al.*[22] conducted a study on the distribution and pharmacokinetic effects of one dose of dietetic (5 μg) TBT radio-marked with ^{113}Sn and ^{203}Hg methylmercury (MeHg). These compounds were studied for 154 days in *Chionoecetes opilio* crabs. Both compounds showed high uptake by the crabs (80-100%). The autoradiography and dissection data showed that the distribution of TBT was less homogeneous compared with MeHg, and the data showed higher lumen intestinal radioactivity for TBT. In these animals, the bioaccumulation factor (BCF) resulting from chronic exposure to the TBT-contaminated food was estimated to be 0.1-0.6. Although these values were lower than those estimated for MeHg (1.8-2.4), they are not negligible and indicate that TBT absorption through food is an important route of bioaccumulation.

Although there have been several studies evaluating metallic elements bioaccumulation (Cd, Cr, Cu, Hg, Pb and Zn) by the *Callinectes sapidus* crab,[23-32] no study has investigated TBT.

The main objective of this work was to investigate the TBT bioaccumulation process in *C. sapidus*. Additionally, the objectives focused on evaluating which form (organic or inorganic) the metallic element is stored and in which tissue the bioaccumulation predominates (gill, hepatopancreas or muscle). Thus, the animals were subjected to TBT-contaminated food, and after exposure, the total Sn and TBT concentrations in different tissues were determined. Several variables, such as time of exposure (days) and amount of ingested food, were evaluated.

Experimental

Materials

Instruments

In this study, an Agilent 7500ce inductively coupled plasma mass spectrometer (ICP-MS) and a GBC model Integra XL inductively coupled plasma optical emission spectrometer (ICP OES) were used. Operations conditions for the instruments and for hydride generation (HG) are described below:

ICP-MS condition: plasma RF power of 1500 W; sample depth from load coil of 7.5 mm; carrier gas flow of 0.8 L min^{-1}; makeup gas flow of 0.1 L min^{-1}; spray chamber temperature at 2 °C; sample flow rate of 0.6 μL min^{-1}; concentric micromist nebulizer; nickel sample and skimmer cones interface; *m/z* 118; 120; integration time of 0.1000 s; reaction/collision cell without gas; detector mode in pulse HV.

HG-ICP OES condition: forward power of 1200 W; plasma gas flow of 10 L min^{-1}; auxiliary gas flow of 0.5 L min^{-1}; radial view of 10 mm; Sn emission line at 189.926; borohydride flow of 1.4 mL min^{-1}; sample flow of 2.6 mL min^{-1}; and carrier gas flow of 0.6 L min^{-1}.

Reagents and solutions

Deionized water (18.2 MW cm) was produced in a Milli-Q system (Millipore, Bedford, MA, EUA). The nitric acid used for the ICP-MS analysis was purified by distillation below its boiling point. The other reagents were analytical grade.

Solutions of 3% $NaBH_4$ (m/v) (MP Biomedicals, EUA) in 0.05 mol L^{-1} NaOH (Synth, São Paulo, Brazil) were used in the hydride generation system and prepared immediately before analysis.

All solid phase extraction (SPE) tests for the TBT determination were conducted using commercially obtained dehydrated *Saccharomyces cerevisiae* yeast (Fermix, São José dos Campos, Brazil).

The working solutions were prepared from stock solutions of 1000 mg L^{-1} Sn (IV) made from Sn^0 (Aldrich, Milwaukee, EUA) and 1000 mg L^{-1} TBT made from TBTCl (tributyltin chloride, Aldrich).

Methods

Field work

Callinectes sapidus, known as blue crab, were caught in the Santos city (São Paulo, Brazil) ocean coast (S 23º 54' 750" WO 45º 25' 460") using traps made of plastic mesh and by manual catching. All of the collected individuals were carried to the laboratory (Centro de Estudos Ambientais, Universidade Estadual Paulista).

Exposure

Four crabs were kept for 14 days and fed non-contaminated food (controls). Nine other crabs were incubated for 40 days and fed contaminated food.

The crabs were individually kept in plastic bottles. The seawater used in the experiments was collected at the same place and in the same period in which the crabs were caught. This water was analyzed and it showed no significant amount of tin. The water was cleaned daily by suction of the eventual residue and renewed every 5 days.

The concentration of TBT in contaminated food (30 μg g^{-1}) was stated by considering a previously related exposure experiment.[22] Hake (*Merluccius hubbsi*) fillet was cut into pieces and 5 g samples were then separated into flasks. The food was contaminated by adding 150 μL of a TBT stock solution to the 5 g fish samples, resulting in a final TBT concentration of 30 μg g^{-1}. After this addition, the mixture was mixed into the fish meat using a vortex agitator for 5 min. The mixture visually seemed very homogeneous slurry. The flasks were then stored at 4 ºC. The contamination of the food with Sn from TBT was prepared one day before use.

Each animal was fed 3 times a week with small pieces of fish (with or without contamination). The food was given to the animals until they refused to ingest it, and the eaten fish mass was registered for each animal. The mass of ingested food for each contaminated animal is shown in Table 1.

After exposure, all of the animals were euthanized by chilling at −10 ºC and classified by sex (6 females and 10 males), mass (average of 55.9 g), carapace length (3.9-5.2 cm) and width (8.1-11.4 cm). Using stainless materials, the gills, hepatopancreas and muscle tissues were removed from each individual, weighed and stored in 2 mL Eppendorf tubes at −10 ºC until analysis.

All of the glassware and plastics used during exposure, tissue dissection and determination of Sn concentrations were decontaminated with 20% HNO_3 (v/v) and rinsed with deionized water before use.

Table 1. Crab exposure

Animal	Sex	Exposure / day	Ingested food / g
1A	female	0	0.00
2A	female	2	0.09
3A	female	3	0.00
4A	female	3	0.34
5A	female	5	0.21
6A	female	7	0.64
7A	male	18	2.33
8A	male	40	10.07
9A	male	40	13.61

Total Sn determination

The collected tissues were digested using a nitro-perchloric digestion as previously reported.[33,34] The digestion process was conducted with blank samples to evaluate any occasional contamination due to the reagents and/or the flasks.

To evaluate the accuracy of the method, the total Sn concentrations in the digested samples were determined by hydride generation combined with inductively coupled plasma optical emission spectrometry (HG-ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS). The experimental conditions for total Sn determination by HG-ICP OES were previously evaluated.[35]

TBT determination

TBT determination (and other tin organic compounds) in sediments and biological tissues has been effectively performed by coupling gas chromatography and inductively coupled plasma mass spectrometric (CG-ICP-MS).[36,37] Although these methods are sensitive and selective, coupling between gas chromatography (CG) and ICP-MS is complex and expensive. Also, sample preparation for CG analysis requires a tedious derivatization procedure, which considerably reduces the analytical frequency of CG-ICP-MS methods. As the goal is only the selective tributyltin determination in the presence of Sn(IV), these drawbacks can be easily overcame by using a method combining hydride generation inductively coupled plasma optical emission spectrometry and SPE using baker yeast. This procedure is based on selective retention of TBT by the yeast at pH 6.[35]

An essential requirement for selective retention of TBT by the yeast is the pH adjustment, since at pH near to 5, inorganic tin is significantly retained by the yeast, while at

pH near to 7, the TBT is not retained quantitatively. Also, for analysis of biological tissues, the removal of methanol in the extracts is mandatory because this compound interferes on the retention of TBT by the yeast.[35]

The tin extraction from biological material was performed with a procedure described by Silva *et al.*[35] that consists of homogenizing and ultrasounding a mixture of tissues (0.33 g) in methanol (10 mL) and HCl (0.33 mL). Afterwards, this mixture was centrifuged, and the liquid phase was evaporated to 1 mL using a rotary evaporator. The 1 mL residue was recovered in 13 mL of water, in a 15 mL Falcon tube, and the sample pH was adjusted to 6.

After the extraction of Sn from the tissues, a SPE was performed to separate the organic Sn compound (TBT) from the inorganic forms potentially present in the samples.[35] Then, 10 mL of sample were added to a 15 mL Falcon tube containing 0.625 g *Saccharomyces cerevisiae*. The resulting suspensions were vigorously agitated and centrifuged. During this procedure, TBT is retained by the yeast (solid phase), while any inorganic Sn remains in the liquid phase. Finally, the solid phase was treated with nitric acid and analyzed by HG-ICP OES[35] and ICP-MS.

Results and Discussion

Non-contaminated blue crabs

HG-ICP OES and ICP-MS techniques were used to determine the total amount of Sn in the gills, hepatopancreas and muscle of the non-contaminated crabs. The results were similar for both techniques. The limit of detection (LOD) for Sn using ICP-MS (0.055 μg L^{-1}) was 10 times lower than the one obtained using HG-ICP OES (0.55 μg L^{-1}) and allowed for analyte determination in the majority of samples (Table 2). In this test, mass available for determination of total Sn changed from 0.7 to 1.4 g for gills, from 0.35 to 0.7 g for hepatopancreas and from 0.6 to 1.5 g for muscle. When a 0.35 g of tissue (minimum mass) was available for determination of total Sn (and considering 30 mL final volume of the digest), LOD (wet basis) (expressed in ng g^{-1}) were 4.46 for ICP-MS and 44.6 for HG-ICP OES.

The accuracy of the results obtained by both techniques was evaluated through recovery tests (by spiking Sn (IV) before the digestion step). The determinations made by ICP-MS presented better recoveries than those obtained by HG-ICP OES, with average values between 73 to 89% and 64 to 87%, respectively.

All tissues showed relatively low Sn concentrations (highest value of 45.18 ng g^{-1} for the hepatopancreas sample), except for the concentration obtained from muscle sample 2 (Table 2). This sample may have been contaminated by the analyte during the digestion procedure. Therefore, it was not expected to interfere with the Sn concentration previously observed in the bioaccumulation experiments performed during the next step.

Contaminated blue crabs

Total Sn concentration

Figures 1 and 2 show the total Sn concentrations found in each animal in relation to the number of days they were exposed to the contaminated food determined by HG-ICP OES and ICP-MS, respectively. The limits of detection (wet basis) for total Sn (Figures 1 and 2) were slightly higher than previous experiments (non-contaminated

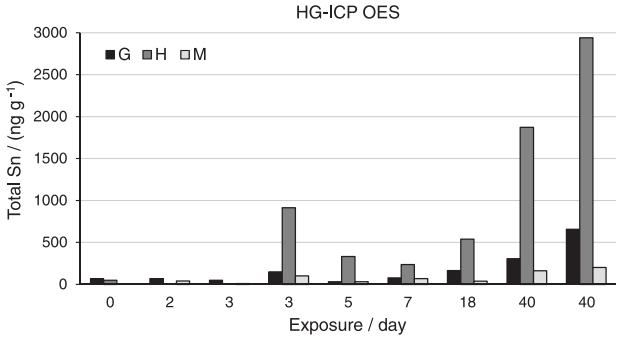

Figure 1. Total Sn concentrations after different days of exposure determined by HG-ICP OES: G = gills, H = hepatopancreas and M = muscle. Limits of detection (wet basis): 30 ng g^{-1} for the gills and muscle and 60 ng g^{-1} for the hepatopancreas.

Table 2. Total Sn concentrations determined by HG-ICP OES (OES) and ICP-MS (MS) for non-contaminated crabs

Animal	Total Sn concentration / (ng g^{-1})					
	Gills		Hepatopancreas		Muscle	
	MS	OES	MS	OES	MS	OES
1B	< 2.36	< 23.57	31.65	< 44.59	NA	NA
2B	21.47	< 23.57	< 4.46	< 44.59	117.14	97.86
3B	29.67	< 23.57	32.03	< 44.59	14.7	< 26.61
4B	24.00	< 23.57	45.18	< 44.59	25.16	< 26.61

NA: not analyzed.

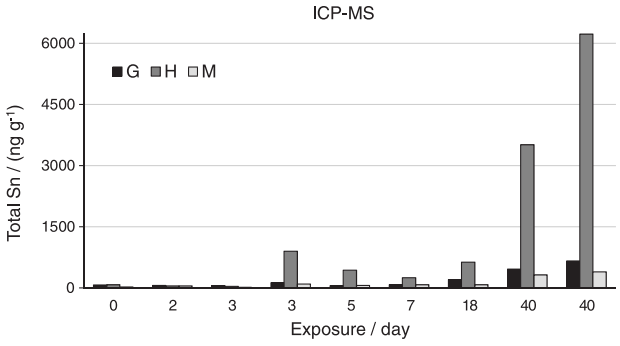

Figure 2. Total Sn concentrations after different days of exposure determined by ICP-MS: G = gills, H = hepatopancreas and M = muscle. Limits of detection: 3 ng g^{-1} for the gills and muscle and 6 ng g^{-1} for the hepatopancreas.

blue crabs) because available mass of tissues was lower (part of the tissues was used for TBT determination).

As observed in the figures, the results from both techniques were similar and yielded graphs with similar patterns. However, higher Sn concentrations (mainly for two hepatopancreas samples) were observed when ICP-MS determinations were used. Signal suppression due to change on stannane generation by the presence of organic material in these samples is a possible interference in HG-ICP OES determination. Due to the large variations observed in both data sets, the results could only be compared statistically by performing a logarithmic standardization of the data. After standardization, an *F*-test was applied to determine if it was possible to compare the samples. A paired *t*-test was performed after the *F*-test (significance level 95%). The *t*-test showed that both data sets were not significantly different (significance level 95%). Therefore, despite the observed increase in Sn concentration values, a significant difference between the results obtained by the HG-ICP OES and ICP-MS techniques was not statistically confirmed.

Figures 1 and 2 show an increase in the total Sn concentrations for all tissues as incubation time increased, except for one case (hepatopancreas, 3rd day). Thus, to establish the bioaccumulation process (to evaluate the relationship between the days of exposure and the amount of tin in the tissues), the Pearson´s correlation test was used considering data set from ICP-MS measurements. Correlations ($\sqrt{R^2}$) of 0.87 ($p = 0.002$), 0.91 ($p = 0.0007$) and 0.86 ($p = 0.002$) were obtained for the gills, hepatopancreas and muscles, respectively, showing a strong trend between increases in Sn concentrations for all tissues as the number of exposure days increased. Therefore, we can assume that the Sn bioaccumulation process occurred throughout the entire experimental period (40 days).

By studying the mass of the contaminated food eaten by the crabs, an identical pattern was observed compared with the data obtained for the number of exposure days (Figures 1 and 2). The correlation between tin concentrations and amount of food consumed was also high: 0.95 ($p = 0.0001$), 0.96 ($p = 0.0001$) and 0.90 ($p = 0.0008$) for the gills, hepatopancreas and muscles, respectively. This high correlation level indicates that the tin bioaccumulation source is directly related to the amount of tin contaminated food which was ingested.

To evaluate the differences between the Sn concentrations in the tissues, a Friedman statistic test was used. This test allows for comparisons between dependent variables (different tissues from the same animal).

The results showed significant statistical differences ($p < 0.05$), i.e., there were differences between the total Sn concentrations found in the different tissues. When comparing the gills and hepatopancreas separately, there was no significant statistical difference between the tissues. In addition, the same behavior (no significant statistical difference) occurred when the gills and muscle were compared. However, the hepatopancreas and muscle samples showed a statistically significant ($p < 0.05$) difference (12), suggesting that the hepatopancreas bioaccumulated more Sn than the muscle. This characteristic suggests that these animals preferentially store the metallic element in the hepatopancreas. When the differences between the gills and muscle (9), gills and hepatopancreas (3) and hepatopancreas and muscle (12) were analyzed, the data showed the following sequence for Sn concentrations in contaminated crabs: muscle < gills < hepatopancreas.

The factors for the total amount of Sn bioaccumulation were calculated for all of the samples by dividing the final concentration of Sn (obtained for each tissue from each animal) by the Sn concentration of the food given during the experiment (30 μg g^{-1}).[38] The bioaccumulation factors are shown in Figure 3.

It was observed that BCF increased as the time of exposure increased for all tissues. The highest values for the bioaccumulation factors were found in the

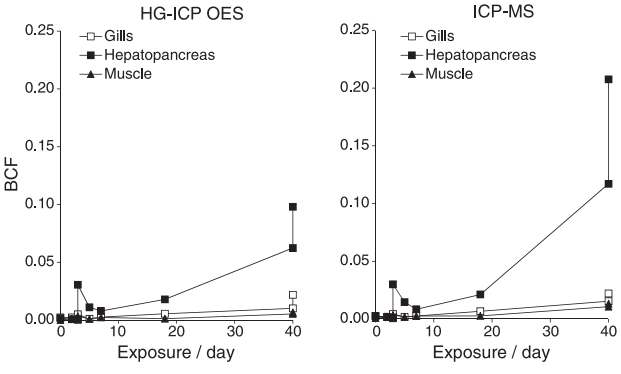

Figure 3. Bioaccumulation factor (BCF) for the total Sn in crab tissues determined by HG-ICP OES and ICP-MS.

hepatopancreas. BCF values were similar to those reported by Rouleau *et al.*[22] (0.1-0.6) for TBT. There are no values reported in the literature for total Sn BCF concerning crab/food, crab/seawater or crab/sediment. As a comparison, the following BCF values of other metallic elements have been previously reported: for crab/seawater 15.019, 145, 525, 98, 20.647 and 380 for Zn, Pb, Cd, Ni, Cu and Cr, respectively; for crab/sediment 0.158, 0.002, 0.020, 0.003, 0.438 and 0.002 for Zn, Pb, Cd, Ni, Cu and Cr, respectively.[38] The Sn BCF (hepatopancreas, after 18 exposure days) obtained is comparable to highest crab/sediment BCF reported (for Zn, Cd and Cu).

TBT concentration

The relationships found between the TBT concentrations and number of exposure days determined by HG-ICP OES and ICP-MS are shown in Figures 4 and 5, respectively. By observing the patterns of these graphs for TBT concentrations, it is possible to infer that it is similar to the patterns obtained for the total Sn concentrations (Figures 1 and 2).

Figures 4 and 5 indicate that the results for the total Sn and TBT concentrations are consistent. The highest TBT concentrations were found in the samples that presented the highest total Sn concentrations. For almost all samples, the TBT concentrations represent a significant part of the total Sn concentrations (especially considering the results in Figure 5), suggesting that the metallic element is primarily stored as TBT (or other organic species of tin, e.g., DBT, MBT). For example, the concentration of TBT after 40 days of exposure represents 68 ± 36, 51 ± 63 and 32% (only one measurement) of total Sn concentration for gills, hepatopancreas and muscle, respectively (data set from ICP-MS measurements).

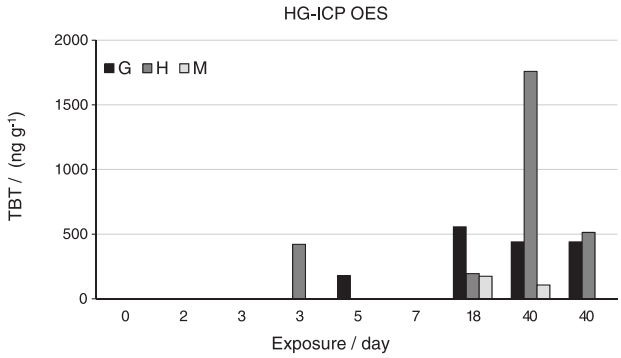

Figure 4. TBT concentrations after different days of exposure determined by HG-ICP OES: G = gills, H = hepatopancreas and M = muscle. Limits of detection (wet basis): 140 ng g^{-1}.

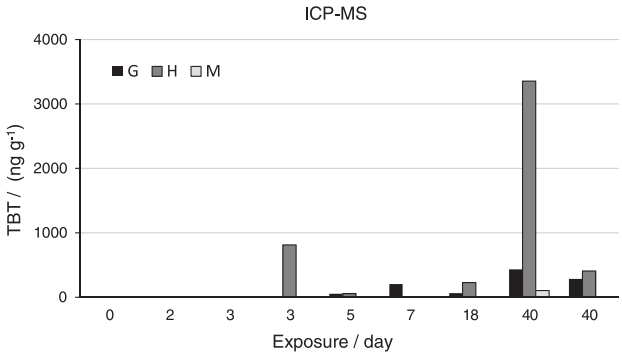

Figure 5. TBT concentrations after different days of exposure determined by ICP-MS: G = gills, H = hepatopancreas and M = muscle. Limits of detection (wet basis): 6 ng g^{-1}.

The results from this study suggest that *Callinectes sapidus* is a potentially good bioindicator for the presence of TBT in an environment. Therefore, further studies with this species are of great importance because the deleterious effects of Sn on ecosystems, especially in its organic form (TBT), are well defined and related to many research studies.[2-22]

Conclusion

HG-ICP OES and ICP-MS techniques can be effectively used to evaluate total Sn concentration in contaminated crabs. However, the limit of detection for HG-ICP OES method does not allow the determination of Sn in non-contaminated crabs. Determinations of Sn in crab tissue digest by ICP-MS presented better recoveries values as compared with HG-ICP OES. Also, the results found by HG-ICP OES in some samples were lower than those found by ICP-MS. However, the two data sets were not significantly different (significance level 95%).

The analysis of tissue samples from crabs subjected to contaminated food with TBT showed that these animals accumulate Sn in their tissues (gills, hepatopancreas and muscle). According to BCF, it appears that there is no mechanism for the regulation or excretion of TBT.

Among the tissues analyzed in this work, the hepatopancreas showed the highest capacity for TBT bioaccumulation. By comparing the total Sn and TBT concentrations found in the tissues, we inferred that most of the accumulated Sn was present as TBT. In this sense, Sn could be used as a biomarker for TBT exposure in environment.

Acknowledgments

The authors thank FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial support.

References

1. Arnot, J. A.; Gobas, A. P. C.; *Environ. Rev.* **2006**, *14*, 257.
2. Adelman, D.; Hinga, K. R.; Pilson, M. E.; *Environ. Sci. Technol.* **1990**, *24*, 1027.
3. Demora, S. J.; Stewart, C.; Phillips, D.; *Mar. Pollut. Bull.* **1995**, *30*, 50.
4. Bryan, G. W.; Langston, W. J.; *Environ. Pollut.* **1992**, *76*, 89.
5. Bryan, G. W.; Gibbs, P. E.; Hummerstone, L. G.; Burt, G. R.; *J. Mar. Biol. Assoc. U. K.* **1986**, *66*, 611.
6. Weis, J. S.; Cristini, A.; Rao, K. R.; *Am. Zool.* **1992**, *32*, 495.
7. Kannan, K.; Yasunaga, Y.; Iwata, H.; Ichihashi, H.; Tanabe, S.; Tatsukawa, R.; *Arch. Environ. Contam. Toxicol.* **1995**, *28*, 40.
8. Ohji, M.; Arai, T.; Miyazaki, N.; *J. Mar. Biol. Assoc. U. K.* **2003**, *83*, 111.
9. Shimasaki, Y.; Oshima, Y.; Inoue, Y.; Shibata, H.; Nakayama, K.; Inoue, S.; Imoto, H.; Kang, I. J.; Honjo, T.; *J. Fac. Agric., Kyushu Univ.* **2008**, *53*, 67.
10. Meador, J. P.; Sommers, F. C.; Cooper, K. A.; Yanagida, G.; *Environ. Res.* **2011**, *111*, 50.
11. Ohji, M.; Harino, H.; Arai, T.; *Mar. Environ. Res.* **2011**, *62*, 318.
12. Hyun, M. H.; Jae, R. O.; Sung-Hyun, K.; Kwang, W. L.; *Mar. Environ. Res.* **1999**, *47*, 61.
13. Jacobsen, J. A.; Asmund, G.; *Sci. Total Environ.* **2000**, *245*, 131.
14. Coelho, M. R.; Bebianno, M. J.; Langston, W. J.; *Mar. Environ. Res.* **2002**, *54*, 193.
15. Meng-Pei, H.; Shiu-Mei, L.; *Sci. Total Environ.* **2003**, *313*, 41.
16. Tang, C. H.; Lin, C. S.; Wang, W. H.; *Environ. Toxicol. Chem.* **2009**, *28*, 2333.
17. Tang, C. H.; Hsu, C. H.; Wang, W. H.; *Mar. Environ. Res.* **2010**, *70*, 125.
18. Chiavarini, S.; Massanisso, P.; Nicolai, P.; Nobili, C.; Morabito, R.; *Chemosphere* **2003**, *50*, 311.
19. Santos, M. M.; Micael, J.; Carvalho, A. P.; Morabito, R.; Booy, P.; Massanisso, P.; Lamoree, M.; Reis-Henriques, M. A.; *Comp. Biochem. Physiol. C: Pharmacol. Toxicol*. **2006**, *142*, 151.
20. Weis, J. S.; Kim, K.; *Arch. Environ. Contam. Toxicol.* **1998**, *17*, 583.
21. Botton, M. L.; Hodge, M.; Gonzalez, T. I.; *Estuaries* **1998**, *21*, 340.
22. Rouleau, C.; Gobeil, C.; Tjalve, H.; *Environ. Sci. Technol.* **1999**, *33*, 3451.
23. Evans, D. W.; Kathman, R. D.; Walker, W. W.; *Mar. Environ. Res.* **2000**, 49, 419.
24. Reichmuth, J. M.; Weis, P.; Weis, J.; *Environ. Pollut.* **2010**, *158*, 361.
25. Martins, C. M. G.; Barcarolli, I. F.; Menezes, E. J.; Giacomin, M. M.; Wood, C. M.; Bianchini, A.; *Aquat. Toxicol*. **2011**, *101*, 88.
26. Laporte J.-M.; Andres, S.; Mason, R. P.; *Comp. Biochem. Physiol. C: Pharmacol. Toxicol*. **2002**, *131*, 185.
27. Andres, S.; Laporte, J.-M.; Mason, R. P.; *Aquat. Toxicol*. **2002**, *56*, 303.
28. Ciftci, N.; Cicik, B.; Erdem, C.; Ay, O.; Karayakar, F.; Karaytug, S.; *Fresenius Environ. Bull.* **2011**, *20*, 1089.
29. Paganini, C. L.; Bianchini, A.; *Environ. Toxicol. Chem.* **2009**, *28*, 1200.
30. Sastre, M. P.; Reyes, P.; Ramos, H.; Romero, R.; Rivera, J.; *Bull. Mar. Sci*. **1999**, *64*, 209.
31. Brouwer, M.; Engel, D.; Bonaventura, J.; Johnson, Ga.; *J. Exp. Zool*. **1992**, *263*, 32.
32. Rosas, C.; Castaneda, T.; Barrera, G.; Negron, G.; Takagui, S.; Maubert, M.; *Rev. Invest. Mar.* **1989**, *10*, 71.
33. Chagas, G. C.; Brossi-Garcia, A. L.; Menegário, A. A.; Franchi, M.; Pião, A. C. S.; Govone, J. S.; *Holos Environ.* **2009**, *9*, 289.
34. Franchi, M.; Menegário, A. A.; Brossi-Garcia, A. L.; Chagas, G. C.; Silva, M. V.; Pião, A. C. S.; Govone, J. S.; *J. Braz. Chem. Soc.* **2011**, *22*, 230.
35. Silva, M. V.; Menegário, A. A.; Franchi, M.; Tonello, P. S.; Brossi-Garcia, A. L.; *Quim. Nova* **2010**, *33*, 1529.
36. Monperrus, M.; Zuloaga, O.; Krupp, E.; Amouroux, D.; Wahlen, R.; Fairman, B.; Donard, O. F. X.; *J. Anal. At. Spectrom.* **2003**, *18*, 247.
37. Point, D.; Davis, W. C.; Christopher, S. J.; Ellisor, M. B.; Pugh, R. S.; Becker, P. R.; Donard, O. F. X.; Porter, B. J.; Wise, S. A.; *Anal. Bioanal. Chem*. **2007**, *387*, 2343.
38. Young-Tack, K.; Chan-Won, L.; *Microchem. J.* **2001**, *70*, 255.

Submitted: May 22, 2013
Published online: August 28, 2013

FAPESP has sponsored the publication of this article.