UNIVERSIDADE FEDERAL DE SÃO CARLOS
DEPARTAMENTO DE CIÊNCIAS FISIOLÓGICAS
LABORATÓRIO DE ZOOFISIOLOGIA E BIOQUÍMICA COMPARATIVA

ESTUDO DA ORIGEM E TRANSFERÊNCIA DE METAIS E METALÓIDES EM ÁREAS DE MANGUEZAL, POR MEIO DE ANÁLISES ISOTÓPICAS NA CADEIA TRÓFICA E EFEITOS BIOQUÍMICOS E MORFOLÓGICOS EM *Centropomus parallelus* Poey, 1860

IARA DA COSTA SOUZA

SÃO CARLOS – SP
2017

UNIVERSIDADE FEDERAL DE SÃO CARLOS
DEPARTAMENTO DE CIÊNCIAS FISIOLÓGICAS
LABORATÓRIO DE ZOOFISIOLOGIA E BIOQUÍMICA COMPARATIVA

ESTUDO DA ORIGEM E TRANSFERÊNCIA DE METAIS E METALÓIDES EM ÁREAS DE MANGUEZAL, POR MEIO DE ANÁLISES ISOTÓPICAS NA CADEIA TRÓFICA E EFEITOS BIOQUÍMICOS E MORFOLÓGICOS EM *Centropomus parallelus* Poey, 1860

Orientada: Iara da Costa Souza
Orientadora: Dra. Marisa Narciso Fernandes
Co-orientadora: Magdalena Monferrán

Tese apresentada ao Programa de Pós – Graduação em Ecologia e Recursos Naturais, do Centro de Ciências Biológicas e da Saúde – Universidade Federal de São Carlos, como parte dos requisitos para a obtenção do título de Doutor em Ciências.

Área de Concentração: Ecologia e Recursos Naturais

SÃO CARLOS – SP
2017

UNIVERSIDADE FEDERAL DE SÃO CARLOS

Centro de Ciências Biológicas e da Saúde
Programa de Pós-Graduação em Ecologia e Recursos Naturais

Folha de Aprovação

Assinaturas dos membros da comissão examinadora que avaliou e aprovou a Defesa de Tese de Doutorado da candidata Iara da Costa Souza, realizada em 10/03/2017:

Profa. Dra. Marisa Narciso Fernandes
UFSCar

Profa. Dra. Maria da Graça Gama Melão
UFSCar

Profa. Dra. Ana Teresa Lombardi
UFSCar

Prof. Dr. Camilo Dias Seabra Pereira
Unifesp

Prof. Dr. Denis Moledo de Souza Abessa
UNESP

Dedico esta Tese a minha mãe, minha mamis, minha Vandinha e meu incansável exemplo de amor sem limites.

...Eu não fui apenas aviador, mas que me foi necessário estudar, pensar, inventar, construir e só depois, voar...

Santos Dumont

AGRADECIMENTOS

À minha mãe, que foi meu porto seguro, minha apoiadora suprema, tanto de ideais, quanto de condições para seguir em frente. Agradeço ao colo, à casinha sempre limpa, à comida sempre maravilhosa, e principalmente ao seu grande amor por mim.

Ao meu pai, que sempre foi muito duro comigo, me empurrando quando o medo de cair era gigante, e me ensinando a levantar e seguir adiante, não importa o quão difícil seja.

Ao meu marido, Diego Ortega Romero Nunes, mais conhecido como minha paixão, meu grande amor, que me ajudou nas coletas, dirigiu quilômetros sem parar inúmeras vezes quando eu não conseguia mais. À sua ajuda na construção do meu escritório, na preparação de amostras e ao seu exemplo de imensa força e paciência quando a distância foi insuportável. Meu melhor companheiro, amigo, amante e melhor pessoa que eu poderia ter conhecido e que tenho a sorte de ter casado e construído uma família.

Ao Tarcísio, meu lindo bebê, que ainda não nasceu (mas está quase chegando), um bebê forte que não me deu trabalho e tem sido paciente nestes últimos meses, com a mamãe que vive sentada, escrevendo e deixando pouco espaço para ele.

À Prof. Dra. Marisa Narciso Fernandes que é um exemplo de conduta e profissionalismo, e que têm sido mais do que uma orientadora neste doutorado, mas um dos meus grandes pilares na construção da minha formação.

À Prof. Dra. Silvia Tamie Matsumoto que foi minha primeira orientadora e que tem me apoiado e caminhado comigo desde então. Ao seu incentivo e apoio com o GEMUT, sempre me socorrendo quando dois braços já não são suficientes.

Al Prof. Dr. Daniel Wunderlin, Prof. Dra Magdalena Monferrán y toda equipe del ICYTAC por acompañarme en todos estos años de trabajo, me apoyando y abriendo caminos para yo siga con mis sueños.

To Prof. Dr. Michael Elliott for believe in me since 2013 after a talk in Shanghai, China, and for receive me in IECS in 2014 and 2015. It was long talks with an improve in English along time, and the most important, improve in knowledge in estuarine ecosystem.

À Sabrina, mi gran amiga, mi mamá argentina que siempre me ha recibido en su casa, con todo amor y cariño, haciendo ñoquis, tarta de verduras, y claro con un mate para ponernos al día!

À Hiulana, minha parceira fiel de trabalhos de manguezal e companheira de vida nos mais diversos ensinamentos, desde força extrema, passando pela ética e moral e chegando ao amor e carinho natural em uma amizade com bases já tão sólidas!

Ao Vini, que foi meu braço direito na loucura de entender a oceanografia dos estuários, das análises bioquímicas e na vida. Uma parceria pessoal e profissional, baseada em muito respeito e carinho cultivado na nossa amizade.

À Marina e Mariana que estiveram comigo em muitos momentos deste doutorado, inclusive vivendo juntas! Ao apoio quase incondicional que eu tive não somente na vida acadêmica, mas na vida pessoal, que me auxiliou e me ensinou a ver a vida "fora da caixinha".

À Livinha e todo seu apoio e carinho, vendo unicórnios junto comigo nesta longa caminhada que é a pesquisa no Brasil; aos lanchinhos antes de 13 horas dirigindo de noite depois de uma semana de coleta! E por fim a companhia juntamente com o Ian em inúmeras horas de escrita de artigo que espero nos levar a Nature!

Ao André e Felipe que me receberam de novo no começo do doutorado na casa deles, mas que infelizmente não aceitaram fazer uma parede de drywall!

Ao Vitor que teve toda a paciência do mundo com meus sonhos de localizar metais em "sashimi" e que esteve comigo ao longo de um ano na batalha de analisar amostras na microscopia de transmissão.

À tia Eliza, tio Antônio, Adriano e Larissa, minha família paulista que esteve sempre me apoiando desde o mestrado e que foi essencial no começo do doutorado para ganhar forças e encarar este novo desafio.

À família do meu maridão, que me recebeu em casa, sempre com muito cuidado e paciência durante a semana de trabalho, com direito a caminhadas matinais com minha sogrinha e marmitinhas ou almoço sempre maravilhosos.

À equipe do laboratório LZBC que sempre me ajudou quando eu estava longe demais, em alguma viagem, ou quando eu estava perto mesmo, mais precisava de mais uma mão.

My acknowledgment for all support in Hull University, and the patient of Ann Lowry to teach me sample microscope preparation techniques and for all the talks between then; To Bob Mcknight and Michael Thompson for all the patient and help in chemical digestion samples, and also for all the talks and to teach me a little about the English culture.

My acknowledgment also for James Hutton Institute and Andy in first place for accept me and our collaboration, Carol-Ann, India and Barry for help me with all sample isotope preparation and analysis. Also for the long talks and the cold days that Aberdeen gives to me.

À AAPC Camburi e SOS Juntos Ambiental pelo apoio logístico nas coletas e inclusive ao alcance que este projeto teve na sociedade capixaba.

Aos pescadores, seu Jusceli e seu Ronaldo que auxiliaram na coleta do manguezal, sempre ensinando o que os livros não conseguem.

Ao Programa de Pós Graduação em Ecologia e Recursos Naturais da Universidade Federal de São Carlos (UFSCar).

Ao Cnpq pela concessão de bolsa no primeiro ano do doutorado.

À FAPESP pela concessão de bolsa de doutorado no país (Proc. FAPESP 2014/0832-3) e pela concessão da bolsa de estágio de pesquisa no experior (BEPE) na Inglaterra (Proc. FAPESP 2015/05258-1).

A todos que direta ou indiretamente estiveram envolvidos não somente nestes quatro anos de doutorado, mas neste sonho que é compreender o manguezal. Um ecossistema único e inspirador que ninguém sozinho consegue seguir adiante. Muito obrigada a todos que caminharam um dia, ou um ano; todos foram essenciais.

SUMÁRIO

RESUMO GERAL

SOUZA, IC. **ESTUDO DA ORIGEM E TRANSFERÊNCIA DE METAIS E METALÓIDES EM ÁREAS DE MANGUEZAL, POR MEIO DE ANÁLISES ISOTÓPICAS NA CADEIA TRÓFICA E EFEITOS BIOQUÍMICOS E MORFOLÓGICOS EM** *Centropomus parallelus* **Poey, 1860.** 2017. 100p. Tese (Doutorado em Ciências) – Departamento de ciências Fisiológicas, Universidade Federal de São Carlos, São Carlos, 2017.

Estuários e zonas costeiras são afetados por diferentes tipos de influências antrópicas derivadas de atividades portuárias, efluentes industriais e agrícolas. Duas áreas de manguezal em dois ecossistemas estuarinos: Baía de Vitória e Santa Cruz no Estado do Espírito Santo, Brasil, com diferentes níveis de contaminação por metais foram avaliadas para identificar a origem e transferência dos metais e metaloides, via cadeia trófica, a um organismo de topo de cadeia (o peixe, *Centropomus parallelus*). As concentrações de metais e metaloides foram analisadas nas matrizes abióticas (sedimento, água intersticial, água superficial e água de fundo) e bióticas (amostras de plâncton e camarão) para determinar a biomagnificação desses poluentes ao longo da cadeia trófica e a sua origem foi determinada via correlações isotópicas (carbono, nitrogênio e chumbo). Concomitantemente, as concentrações de metais e metaloides em brânquias, músculos, fígado e rim de *C. parallelus*, bem como biomarcadores bioquímicos de exposição e efeito nesses órgãos foram avaliados. Análises ultraestruturais foram realizadas em brânquias, músculos, fígado, gônada e rim de *C. parallelus* para avaliar a internalização e localização subcelular dos metais acumulados. Foi realizada regressão linear para os dados de metais acumulados dos diferentes indíviduos da cadeia trófica para avaliar a biomagnificação, biodiluição ou a não interferência da cadeia trófica na acumulação dos diferentes metais avaliados. Resultados dos isótopos estáveis foram interpretados com auxílio de um algoritmo criado (K-means; aprendizado de máquinas) para integrar dados da origem e transferência via cadeia trófica; Análise estatística multivariada foi aplicada na integração de dados físicos, químicos e biológicos avaliados em tecidos de *C. parallelus*. Resultados $\delta^{13}C$ e $\delta^{15}N$ estabelecem a estrutura da teia alimentar, confirmando o planctôn e as plantas de manguezal como base da cadeia, caranguejo e, camarão e ostra como níveis intermediários e o peixe, *C parallelus*, como o topo desta teia alimentar. Dados de $\delta^{15}N$ indicaram diferentes impactos antrópicos nos estuários, sendo Baía de Vitória provavelmente impactada por fertilizantes. A razão isotópica $^{87}Sr/^{86}Sr$ mostrou a influência marcante da água marinha neste ecossistema e em toda a cadeia trófica. A digital isotópica de chumbo sugere que o material particulado atmosférico é a fonte de contaminação de metais nestes ecossistemas, sendo fortemente influenciado por atividades metalúrgicas. A cadeia trófica planta-caranguejo-peixe apresentou maior potencial de biomagnificação, enquanto que a biodiluição foi mais observada nas cadeias planctôn-camarão-peixe e planctôn-ostra. Análises ultraestruturais das amostras abióticas e tecidos de *C. parallelus* mostraram a internalização de nanopartículas metálicas em estruturas celulares (vesículas, núcleo) de

todos os órgãos analisados. Análises nanocristalográficas mostraram que as nanoparticulas metálicas eram compatíveis com às encontradas no material particulado. Dados de biomarcadores corroboram as análises ultraestruturais, sugerindo que os metais compartimentalizados podem não estar biodisponíveis. As respostas e/ou a ausência deles em um determinado órgão mostram sensibilidade específica de órgãos e tecidos à acumulação de metais. Os níveis de metais nas brânquias indicam a contaminação da água e menor sensibilidade deste órgão para a maioria dos metais; sendo o músculo o tecido menos reativo. As respostas bioquímicas sugeriram que a via de eliminação de metal é através das brânquias e rim. O hepatopâncreas e os rins são importantes órgãos de desintoxicação, sendo *C. Parallelus* capaz de suportar as condições ambientais, no entanto, provavelmente com gasto energético que pode influenciar a taxa de crescimento e os processos de reprodução desta espécie.

Palavras-chave: estuário; metalografia; contaminantes; robalo-peva.

GENERAL ABSTRACT

SOUZA, IC. **ESTUDO DA ORIGEM E TRANSFERÊNCIA DE METAIS E METALÓIDES EM ÁREAS DE MANGUEZAL, POR MEIO DE ANÁLISES ISOTÓPICAS NA CADEIA TRÓFICA E EFEITOS BIOQUÍMICOS E MORFOLÓGICOS EM** *Centropomus parallelus* **Poey, 1860.** 2017. 100p. Tese (Doutorado em Ciências) – Departamento de ciências Fisiológicas, Universidade Federal de São Carlos, São Carlos, 2017.

Estuaries and coastal zones are affected by different types of anthropogenic influences derived from harbour activities, industrial and agriculture discharge. Two mangrove areas in two estuarine ecosystems: Bay of Vitória and Santa Cruz in the State of Espírito Santo, Brazil, with different levels of contamination by metals were evaluated to identify the origin and transfer of the metals and metalloids, through the trophic chain to a top-chain organism (the fish, *Centropomus parallelus*). The concentrations of metals and metalloids were analyzed in the abiotic matrix (sediment, interstitial water, surface water and bottom water) and biotic (samples of plankton and shrimp) to determine the biomagnification of these pollutants along the trophic chain and their origin was determined using isotopic correlations (carbon, nitrogen and lead). At the same time, metal and metalloid concentrations in gills, muscles, liver and kidney of *C. parallelus*, as well as biochemical biomarkers of exposure and effect in these organs were evaluated. Ultrastructural analyzes were performed on gills, muscles, liver, gonad and kidney of *C. parallelus* to evaluate metals internalization and subcellular localization. Linear regression was performed for metal accumulated data for all food web to evaluate biomagnification, biodilution or non-interference of the trophic chain in the accumulation of the different metals evaluated. Stable isotope results are interpreted using a new algorithm (K-means; machine learning) to integrate data from the origin and transfer trough the food web; Multivariate statistical analysis were performed to integrate physical, chemical and biological data for *C. parallelus* tissues. Results of δ13C and δ15N confirmed plankton and trees as the base of the food web; indicated the crab, shrimp and oyster as intermediate levels, and the fish, *C. parallelus*, was confirmed as the top of the food web. δ15N also indicated different anthropic impacts in the estuaries, with Bay of Victory being impacted by fertilizers. The 87Sr / 86So isotopic ratio showed a highlight influence of marine water on the ecosystems. The lead fingerprint suggests that the atmospheric particulate material is the source of metal contamination in the ecosystems, being strongly influenced by metallurgical activities. The plant-crab-fish trophic chain presented higher biomagnification potential, while biodilution was more observed in the plankton-shrimp-fish and plankton-oyster trophic chains. Ultrastructural analysis of the abiotic and tissues samples of *C. parallelus* showed the internalization of metallic nanoparticles in cellular structures (vesicles; nucleus) of all organs analyzed. Nanocrystallographic analyzes shown that metallic nanoparticles found were compatible with those found in atmospheric particulate matter. Biomarkers data corroborate as ultrastructural analyzes, suggesting that metal accumulated cannot be all available. Responses and/or absence of them in a given organ show specific sensitivity of organs and tissues to the accumulation of metals. Metal levels in the gills indicate water contamination and decreased organ sensitivity in most metals; muscle was the least reactive tissue. Biochemical responses suggested that the

route of metal elimination is through the gills and kidney. Hepatopancreas and kidneys are important organs of detoxification, being *C. Parallelus* able to support as environmental conditions, however, imposed with energy expenditure that can influence a growth rate and reproduction processes of this species.

Keywords: estuary; metallography; contaminants; fat snook.

CAPÍTULO 1

1 INTRODUÇÃO GERAL

Ecossistemas estuarinos são ambientes dinâmicos caracterizados como uma zona de transição entre os ambientes terrestre e marinho, típicos de regiões tropicais e subtropicais (Agoramoorthy et al., 2008; Cheng et al., 2012). Nesses ambientes, os manguezais desempenham um papel importante como fonte de produção primária, sendo constituídos por espécies vegetais adaptadas à salinidade e micro e macro algas que fornecem abrigo e alimentação para uma infinidade de organismos aquáticos (Shaeffer-Novelli, 1995). Associada à vegetação dos manguezais, existe uma fauna altamente diversificada, constituída por animais residentes, principalmente crustáceos e moluscos, e animais visitantes, que frequentam o ecossistema para alimentação, proteção e reprodução, tendo como componentes principais peixes, aves e mamíferos. Toda essa diversidade de fauna desempenha papel fundamental na reciclagem de nutrientes nas florestas do mangue, dando o primeiro passo na decomposição da matéria vegetal morta depositada no solo (Harbison, 1986). De acordo com a lei federal. 4771, de 15 de Setembro de 1965, os manguezais são considerados áreas de preservação permanente (APP). No entanto, na prática, não há aplicação da referida lei em muitas regiões do país e estes ecossistemas de importância ecológica e econômica têm passado por alterações progressivas nas últimas décadas (Siqueira et al, 2004). No passado, quase todo Porto de Vitória era ocupado por manguezais (Vale & Ferreira, 1998), no entanto, a expansão industrial, portuária e o turismo imobiliário levaram à degradação da maioria dos mangues da região e inclusive sua erradicação em algumas áreas (Ferreira et al, 2007).

Os metais e metalóides estão entre os principais contaminantes desses ambientes devido ao seu fácil transporte e acumulação. Os metais podem estar presentes no solo devido ao desgaste natural das rochas, à utilização de agrotóxicos ou deposição atmosférica, podendo alcançar os rios e lagos por meio do escoamento e descargas de efluentes pontuais e difusas e, consequentemente, os estuários (Newman e Unger, 2003; Yigit e Altindag, 2006). Em estudos ambientais, além de se medir os níveis de diferentes metais, se busca determinar a fonte destes poluentes, diferenciando suas origens naturais e antropogênicas, bem como determinar o seu transporte através da cadeia trófica (Loska et al., 2004; Imperato et al., 2003; Baize e Sterckeman, 2001).

As concentrações máximas de contaminantes permitidos em água e sedimentos são reguladas de acordo com sua toxicidade (WHO/UNEP, 1996; USEPA, 2009). Devido à sua persistência no meio ambiente, sua capacidade de bioacumulação e sua elevada toxicidade, os metais são considerados poluentes conservativos e representam uma ameaça para os manguezais, podendo atravessar todo o ciclo ecológico envolvido no ecossistema, por um longo espaço de tempo (Chen et al., 2011). O acúmulo de metais e metaloides na biota depende de suas características químicas, das características próprias de cada tecido (Chi et al., 2007) e do papel dos diferentes órgãos nos processos de absorção, regulação, armazenamento e excreção (Storelli et al., 2006).

O aumento de contaminantes no ambiente faz com que seja necessário incluir ferramentas novas e mais sensíveis para medir o impacto destes na biota desde seus estágios iniciais. A determinação dos níveis de contaminantes nos organismos permite verificar a exposição a um composto químico, mas não necessariamente, fornece informação sobre seu potencial toxicológico, ou os fatores que influenciam a acumulação. Uma abordagem complementar é a utilização de índices de estresse

subletal, biomarcadores ou sistemas de alerta precoce para avaliar a resposta de um organismo ou população aos contaminantes (Padinha et al., 2000) e detectar estágios iniciais de poluição. O uso de biomarcadores em estudos de campo, em geral, é realizado coletando organismos vivos na área investigada e os resultados obtidos podem fornecer uma visão integrada de como a fração biodisponível dos contaminantes presentes afeta os organismos expostos (Contardo-Jara e Wiegand, 2008; Amado et al., 2011).

Metais e metalóides que entram nos ecosistemas aquáticos e se acumulam em vários organismos podem ser transferidos a um nível trófico mais elevado através da cadeia alimentar. Os peixes são um exemplo importante que, uma vez expostos a vários contaminantes, podem bioacumular ou biomagnificar esses compostos que, em seguida, podem ser transferidos para os seres humanos por meio da alimentação, particularmente em populações onde são consumidos diariamente como parte da dieta (Jiang et al., 2010). O consumo de peixe proporciona muitos benefícios para a saúde humana devido ao seu alto teor de ácidos graxos essenciais poli-insaturados da família do ômega 3 (Gladyshev et al., 2009) que atua prevenindo doenças cardiovasculares e neurológicas (Silvers e Scott, 2002). No entanto, nos últimos anos, têm sido analisados os riscos e benefícios para a saúde humana no consumo desses animais que podem estar contaminados por poluentes presentes no ambiente aquático (Gladyshev et al., 2009; Jiang et al., 2010).

Medições de isótopos estáveis de carbono ($\delta^{13}C$), nitrogênio ($\delta^{15}N$) e chumbo ($\delta^{204}Pb$; $\delta^{206}Pb$; $\delta^{207}Pb$; $\delta^{208}Pb$) refletem o histórico alimentar de uma espécie, sendo comumente utilizados na quantificação de posições tróficas relativas de diversas espécies (Bond, 2010). A avaliação do ingresso e biomagnificação de contaminantes

antrópicos mediante a relação de isótopos estáveis como o carbono e o nitrogênio têm sido utilizadas como uma importante ferramenta em estudos de biomonitoramento em áreas com potencial de contaminação por metais e metalóides (Hoekstra et al., 2003; Betti et al., 2011). A medição dos níveis de isótopos de nitrogênio e sua correlação com os níveis de isótopos de carbono e chumbo, juntamente com a medição de outros metais e elementos traçáveis em um mesmo organismo tem sido utilizada no esclarecimento das posições tróficas dos organismos e na estimativa da biomagnificação destes elementos ao longo da cadeia alimentar (Peterson e Fry, 1987; Ikemoto et al., 2008).

Souza et al. (2013) detectaram níveis de metais e metaloides em manguezais do Espírito Santo e encontraram correlação entre a concentração deles nas águas superficiais e a acumulação no músculo do robalo, *Centropomus parallelus*. Considerando que a contaminação das águas superficiais pode ter origem tanto na poluição atmosférica quanto aquática como consequência dos processos industriais e os hábitos alimentares dessa espécie de peixe, que é topo de cadeia e mais suscetível aos processos de biomagnificação, as análises isotópicas são ferramentas úteis para a compreensão da origem da entrada de contaminantes na cadeia trófica. Além disso, o mecanismo de ação e a resposta de diferentes órgãos a bioacumulação de metais e metalóides permitem avaliar a saúde animal.

Desta forma, o presente projeto pretende responder as seguintes questões: 1) Qual a origem dos metais e metalóides presentes nos tecidos de *C. parallelus*; 2) Qual a importância da cadeia trófica na transferência e bioacumulação de metais e metalóides nos tecidos de *C. parallelus*? 3) Como os diferentes tecidos de *C. parallelus* respondem à bioacumulação de metais e metaloides? Para isso, este estudo visou identificar a origem de metais e metaloides e a passagem destes pela cadeia trófica por meio de

análises e correlações isotópicas. Em adição, os efeitos desses contaminantes em um organismo de topo de cadeia, no caso o peixe *C. parallelus*, foram avaliados utilizando marcadores químicos e biológicos que permite a distinção de regiões com diferentes graus e tipos de contaminação. O robalo peva, *C. parallelus* Poey 1860 (Centropomidae), é um peixe protândrico predador (Taylor et al., 2000), amplamente distribuído ao longo do Oceano Atlântico, de costas tropicais e subtropicais na Flórida (EUA) ao litoral sul brasileiro (Rivas, 1986), e possui desenvolvimento relativamente rápido, não apresentando grandes ciclos migratórios (Volpe, 1959). Essa espécie possui uma dieta carnívora, compondo níveis de topo de cadeia alimentar (Gilmore et al., 1983); durante seu estádio juvenil (até 45 cm de comprimento) apresenta hábitos bentônicos, alimentando-se de pequenos crustáceos (Cháves, 1963; Gilmore et al., 1983) e, no estágio adulto, tem hábitos bento-pelágicos alimentando-se principalmente de pequenos peixes (Carvajal, 1975). Devido a essas características, *C. parallelus* tem potencial para ser utilizado como modelo "topo de cadeia" em estudos ambientais como um bioindicador na avaliação dos efeitos de misturas complexas de poluentes presentes em estuários.

CAPÍTULO 2

2 OBJETIVOS

2.1 Geral

Identificar a origem de metais e metaloides e a transferência destes pela cadeia trófica por meio de análises e correlações isotópicas e seus efeitos em um organismo de topo de cadeia (*C. parallelus*) em dois ecossistemas estuarinos do Estado do Espirito Santo.

2.2 Específicos

- Analisar a concentração e transferência de metais e metaloides em matrizes abióticas (sedimento, água superficial e material particulado) e bióticas (plâncton, *R. Mangle, L. Racemosa, A. Schaueriana*, caranguejo, camarão e brânquias, fígado, rins e musculo de C. *parallelus*) em ecossistemas estuarinos neotropicais (Baía de Vitória e Santa Cruz), para determinar a transferência desses poluentes ao longo da cadeia trófica;
- Determinar exposição e efeito, via biomarcadores, a ação de metais e metaloides em brânquias, músculos, fígado e rim em *C. parallelus* e associando a bioacumulação nesses órgãos;
- Determinar a origem da contaminação por meio da análise dos isótopos de C, N, Sr e Pb, fornecendo uma ampla base de dados para o desenvolvimento de ferramentas e ações que minimizem os impactos causados por atividades antrópicas.
- Avaliar a internalização dos metais nas células de brânquias, músculos, fígado, rim e gonadas de *C. parallelus* utilizando microscopia eletrônica de transmissão;

CAPÍTULO 3

Use of Multiple Stable Isotopes to Assess Pollution Sources in a Mangrove Food Web

ABSTRACT

Metal contamination is a well-known problem in mangrove ecosystems however, the sources of such contamination have been less studied. The main goal of this study was to evaluate two mangrove food webs affected by different anthropic activities in order to identify the sources of pollution affecting these ecosystems. Stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $^{87}Sr/^{86}Sr$ and Pb signature) were analysed in sediments, mangrove trees (*Rhizophora mangle*, *Laguncularia racemosa*, *Avicennia schaueriana*), plankton, shrimps (*Macrobranchium sp.*), crabs (*Aratus sp.*), oysters (*Crassostrea rhizophorae*) and fish (*Centropomus parallelus*) from both areas. Strontium and lead isotopes were also analysed in water and atmospheric particulate matter (PM). $\delta^{13}C$ showed plankton and mangrove trees to be at the base of the food web, $\delta^{15}N$ indicated crab, shrimp and oyster at intermediate levels within this food web and the fish, *C parallelus*, was confirmed at the top level. $\delta^{15}N$ also indicates different anthropic pressures between both estuaries; Vitoria Bay close to intensive human activities, showed higher $\delta^{15}N$ for the food web, probably influenced by fertilizers and sewage. The ratio$^{87}Sr/^{86}Sr$ showed the greater influence of marine water rather than continental sediment throughout the entire food web. Pb isotope ratios suggest that all sources of contamination (PM, industrial effluents and solid wastes) are strongly influenced by metallurgical activities, showing values higher than those previously reported worldwide. Furthermore, the Pb isotopic signature corresponding to PM was also found in surface water, trees, plankton, crab, shrimp, oyster and fish from both estuaries, pointing out to PM as the main responsible for metal pollution at both mangrove ecosystems. To our knowledge, this is the first report demonstrating the effect of PM on the metal pollution at mangrove systems located close to metallurgical areas, and the influence of this kind of industrial activity on lead isotopic signature.

Keywords: carbon, lead, neotropical, nitrogen; strontium, trophic chain, metal pollution

1. Introduction

An estuary is a semi closed water body connecting freshwater with the sea and having influence from both environments (Wolanski and Elliott, 2015). Furthermore, urbanisation and industrialisation directly or indirectly expose estuaries to many pressures (Elliott and Whitfield, 2011). Amongst those pressures, metal contamination is a serious problem for mangrove ecosystems, once most sewage and industrial contaminants carried by rivers and other contamination sources derived from coastal engineering work may affect the health status of estuarine environment (Hogarth, 1999).

In tropical and subtropical climate zones, estuaries cover mangrove ecosystem with biota influenced by several physical and chemical changes (i.e. salinity, sea level, organic matter) in which the bioavailability of metal/metalloids in the aquatic ecosystem may change, favouring their transfer from the lower to the higher trophic levels through the food web up to the top level. Such trophic transfer may reach humans, particularly those who consume fish frequently (Jiang et al., 2010).

Metals transfer in the food web depends on contamination matrix (surface water, sediment or particulate matter). Understanding the ecosystem structure and its trophic relationships can provide information about contaminants uptake and their transfer throughout the trophic web. The analysis of stable isotopes, such as carbon (δ13C) and nitrogen (δ15N) are useful tools to establish trophic levels in a food web (Bond, 2010). δ13C is a valuable marker for identifying different primary producers, while δ15N is effective for the assessment of the trophic position, because the enrichment of the heavy nitrogen isotope occurs incrementally across trophic levels with a constant rate (3–4‰) (Hobson et al., 2002).

As metal contamination can originate from several sources, stable isotopes ratio has been used to identify the origin (source) of metals and metalloids in the food web (Peterson and Fry, 1987; Ikemoto et al., 2008; Podio et al., 2013). Strontium isotopic ratio ($^{87}Sr/^{86}Sr$) primarily reflects the

bedrock geology and marine signature; in estuarine waters, can determine if the freshwater or the seawater dominate the inputs. Lead isotopes ratios ($^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$) have been used as anthropogenic tracers (Chow and Johnstone, 1965; Chow and Earl, 1970, 1972; Chow et al., 1973, 1975) and can be used as 'fingerprints' of environmental pollution (Komárek et al., 2008; Cheng and Hu, 2010).

In the southeast coast of Brazil, the environment of the State of Espírito Santo has been negatively impacted by metallurgy, including mining complexes, steel and smelting industries. Previous studies showed environmental aerial contamination from metallic smoke particles (Arrivabene et al., 2015), surface water contamination with Fe, Pb, As, Hg, Cr and Cu, which was correlated with metal/metalloid accumulation in *Centropomus parallelus* (fish) muscles (Souza et al., 2013). Metal accumulation has been verified in different species of mangrove plants, such as *Laguncularia racemosa* (Souza et al., 2014a), *Rhizophora mangle* (Souza et al., 2014b) and *Avicennia schaueriana* (Souza et al., 2015). However, these studies did not establish neither the pollution source nor the probable pollutant pathway and transport through the estuarine mangrove food web. Moreover, mangrove ecosystem close to the industrial and domestic activities areas (Vitoria Bay) is more affected than that located north away from the main pollution sources (Santa Cruz) (Souza et al., 2013, 2014a, 2014b, 2015; Arrivabene et al., 2015).

Thus, the main goals of this study were: a) to establish the structure of both mangrove food webs using isotopic signatures, and b) to assess the source and pathway of metal contamination in both mangrove ecosystems (Vitória Bay and Santa Cruz; Espírito Santo State, Brazil). These two systems were used in order to give a contaminated and a reference area. We hypothesised that the evaluation of multiple stable isotopes in both mangrove ecosystems and in the Tubarão Complex should allow linking the industrial activity with differential pollution degrees observed at both estuaries, providing a reasonable tool-kit for the evaluation of other estuarine mangrove systems

affected by anthropic and industrial pollution worldwide.

2. Material and methods

2.1. Sampling areas:

This study was conducted in two mangrove areas located in the state of Espírito Santo, Brazil: Vitória Bay and Santa Cruz, which are located at 10 and 70 km, respectively, from the Tubarão Complex (Fig. 1). Sampling was carried out in March 2015, at the end of the summer.

The mangrove ecosystem in Santa Cruz (19°56′26.2″S and 40°12′87.0″W) covers approximately 12 km^2, it is formed by two rivers, being part of the "Piraquê-Açu" and "Piraquê-Mirim" Mangrove Ecological Reserve and has an extensive and well-preserved mangrove area, far from industries from Tubarão Complex that could introduce metal contamination (Fig. 1).

Vitória Bay (20°14′31.5″S and 40°19′84.7″W) is an estuarine complex formed by five rivers. Vitória Bay was originally an archipelago formed for 54 islands, which, after several landfills, resulted in current 14 islands. Landfills occurred between 1830 and 1996, totalling 12,000 m^2, from which half of this area were composed by mangroves. Salt marshes, coastal areas and reefs were covered using solid waste arising from harbour dredging or industrial activities. Since 70´s, landfills mainly unused industrial solid waste originated in the Tubarão Complex (Gazeta, 2016) in which Camburi Beach (adjacent to the Tubarão Complex) is an artificial beach constructed by deposited iron mining bags. Vitória Bay is also impacted by several pollution sources like domestic sewage, metallurgical, textile and paint industries, smelting and iron mining (Fig. 1).

The Tubarão Complex (20º17′03.8″S and 40º14′24.9″W) is located in a coastal populated area between Vitória City and Serra (Fig. 1), consists of iron pelletizing, steel industries and the harbour of each industry for exportation. The industries discharge solid waste in Camburi Beach, liquid effluent released directly into the sea or by an outfall and airborne particulate material from industrial process released in atmosphere together with iron storage and transported by open-air

courtyards to ships (Santos and Reis Jr, 2011).

2.2. Abiotic and biotic sampling:

Surface water (SW) and sediment (SD) samples were taken simultaneously with biological samples. On the Tubarão Complex, water were sampled approximately 100 meters from the four points located near to the complex: Surface Water 1 (SW1) - close to the discharge of the pelletizing iron industry; Surface Water 2 (SW2) - close to the pelletizing iron industry port; Surface Water 3 (SW3) - close to the steel industry effluent discharge and, Surface Water 4 (SW4) - close to the steel industry port (Fig. 1). Sediment were sampled in Camburi Beach in four points located near to the complex: Sediment 1(SD1) - adjacent to the iron pelleting industry in the infralittoral area; Sediment 2 (SD2) - adjacent to the iron pelleting industry in the overflow of the sea; Sediment 3 (SD3) - 1km from the Tubarão Complex; Sediment 4 (SD4) - 5km from the Tubarão Complex (Fig. 1). Atmospheric particulate matter (PM) was sampled in according to Arrivabene et al. (2015). Water samples were collected into pre-cleaned acid washed plastic bottles, 10-20 cm under water surface, acidified with ultrapure HNO_3 (sub-boiling grade) and stored at 4ºC until analysis. Prior to analyses, water samples were filtered using 0.45 µm nitrocellulose filter. Sediment samples (approx. 10-20 cm depth interval) were collected using a polypropylene spoon and quickly transferred into clean 1 L-polypropylene flasks (without head space). Then, they were dried at room temperature and sieved through nylon meshes (63 µm).

Samples of vascular trees leaves resident in mangrove area [*Rhizophora mangle* (RI), *Laguncularia racemosa* (LA), *Avicennia schaueriana* (AV)], plankton (PK), shrimp *Macrobranchium sp.* (SH), crab *Aratus sp.* (CR), oyster *Crassostrea rhizophorae* (OS) and juvenile fish *Centropomus parallelus* [muscle (MU), gill (GI), kidney (KI), hepatopancreas (HP) and gonad (GO)] were collected in each site. In Tubarão Complex, plankton, shrimp and oyster were not collected as they are not present in this area. Nine specimens from each biotic sample (plankton,

plants, oyster, crab, shrimp and fish) were collected. Three pools, consisting of three specimens each, were analysed in triplicate (n=3). Fully expanded leaves (3^{rd} to 5^{th} node) of the three plant species were collected and washed with ultrapure water. For plankton samples, it was traced a transect between the banks of the river, where 20 L surface water was collected and filtered with a plankton net of 20 μm, without distinction between phytoplankton, zooplankton and suspended particulate matter. Crabs were collected by hand directly from trees roots (where they are attached). Shrimps were collected with a pitfall trap. Fish were collected with hook and line, and organs were removed and separated in the field. All biological samples were freeze-dried until constant weight, grounded, homogenized with mortar and pestle and stored at room temperature until analysis.

2.3. Analysis of δ13C and δ15N

Sediment samples, atmospheric particulate matter, plankton, trees, oyster, crab, shrimp and fish organs (1.5 to 3 mg) were set in 5 × 3.5 mm tin capsules (CEI-003-57, CE Instruments) for analysis. The δ13C and δ15N natural isotopic ratios were determined using a Flash EA 1112 Series Elemental Analyser connected via a Conflo IV to a Delta V Advantage isotope ratio mass spectrometer (IRMS) (Thermo Scientific, Bremen, Germany), in dynamic auto dilution mode. The δ13C VPDB and δ15N Air-N_2 values were used to normalize values to their respective scales, using International Atomic Energy Agency (IAEA) certified reference materials (CRMs) USGS40 and USGS41 (both L-glutamic acid). Additionally the USGS40 was used as CRM for quality control for both carbon and nitrogen. For quality control, at batch of fifteen samples pairs of USGS40 and USGS41 were analysed. Long term precisions for a quality control standard (dried milled topsoil) were: total carbon 3.79 ± 0.13 %, δ13C -27.81 ± 0.18 ‰, total nitrogen 0.28 ± 0.02 % and δ15N 4.65 ± 0.61 ‰ (mean ± SD, n = 10). Data processing was performed using the Isodat 3.0 software (Thermo Fisher Scientific, Bremen, Germany) and exported into Excel.

2.4. Analysis of Sr ($^{87}Sr/^{86}Sr$) and Pb isotopes ($^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$, $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$)

Sediment and particulate matter (0.1 g dry weight each) were digested with 4 mL of nitric acid and 1 mL of hydrochloric acid (ultrapure, sub-boiling grade). Biotic samples (0.1 g dry weight each) were digested with 4 mL of nitric acid, 1mL of hydrochloric acid and 0.5 mL of hydrogen peroxide (ultrapure, sub-boiling grade) in pre-cleaned PTFE close-vessels, using a Microwave Accelerated Reaction System (MARS Xpress TM; CEM Corporation, USA) (Souza et al., 2013). Prior to measurement, all digested samples were filtered using 0.45 µm nitrocellulose filter to remove any remaining particles in according to method described by Usepa (2009). All glassware and polypropylene bottles/containers used in the digestion procedure were leached with a sulphuric/nitric acid solution overnight and further rinsed with ultrapure water.

All reagents used for the ion exchange columns and isotope analyses were trace grade. Glassware was cleaned with 10% Decon90, followed by 10% nitric acid soak. An aliquot of each digest was purified for Sr isotope analysis by using a cation exchange resin (Bio Rad AG 50W X 8 200-400 mesh hydrogen form) to isolate Sr from other elements. The majority of the purified samples were dried down and loaded on a tantalum filament. Samples known to have low Sr concentration were loaded on a rhenium filament with a tantalum activator solution. The $^{87}Sr/^{86}Sr$ ratio was determined using a VG Sector 54, Thermal Ionisation Mass Spectrometer (TIMS), running either a 1 or 2V three-cycle dynamic procedure. The mass fractionation was exponentially corrected using the naturally invariant $^{86}Sr/^{88}Sr$ ratio of 0.1194. A maximum of 150 ratios were collected. At least one of the certified reference material SRM987 (NIST, USA) was run in each batch of analysis for quality control, producing a mean value for this study of $^{87}Sr/^{86}Sr = 0.710249 \pm 0.000021$ (2SD), n=18. (Certificate $^{87}Sr/^{86}Sr = 0.71034 \pm 0.00026$). A typical column and instrumental procedure blank for this period was 177pg Sr.

Lead was purified from the matrix by passing an aliquot of the digested sample through an anion exchange resin (Bio Rad AG1-X8 200-400 mesh in chloride form). The purified samples were dried down and loaded onto a rhenium filament on top of a silica gel emitter solution. The $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ ratios were determined by using a static procedure on the TIMS instrument. The voltage of the signal was very dependent on the sample, ranging from 5V to 0.005V. A maximum of 250 ratios were collected. No mass bias correction was applied for these samples. At least one certified reference material CRM981 (NIST, USA) was run in each batch of analysis for quality control, producing a mean value for this study of $^{206}Pb/^{207}Pb$ 1.0947 ± 0.0005 (2SD), $^{208}Pb/^{207}Pb$ 2.3661 ± 0.0013 (2SD) n=29. ($^{206}Pb/^{207}Pb$ 1.09333 ± 0.00033 (2SD), $^{208}Pb/^{207}Pb$ 2.37044).

2.5. Statistical analysis

Data are reported as mean ± standard deviation (data were previously tested for normal distribution). One-way analysis of variance (ANOVA), followed by the Tukey's test or T-test (when there was two matrix for comparison) was applied with a significance level $P < 0.05$. K-means algorithm was created over the dataset to find the best clustering considering k=4, that means, 4 clusters for the data. The graph of this algorithm uses PCA to reduce the dimensionality of the original data for easing visualization with centroids for each group. The algorithm was executed using the Octave 4.0 software.

3. Results and discussion

3.1. Trophic relationships

The $\delta^{13}C$ values for mangrove trees ranged from –27.5‰ to –30.6‰, which are typical values for terrestrial C3-plants. Plankton had $\delta^{13}C$ values of approximately –21‰ (Table 1S and Fig. 2A) in according to the carbon signature reported for estuarine phytoplankton (Bouillon et al., 2011). It is

worth to remark that our samples include both phytoplankton and zooplankton.

Considering the $\delta^{15}N$ of the mangrove trees, Table 1S and Figure 2B show that the values of plants from Vitoria Bay are higher than those from Santa Cruz. The range of $\delta^{15}N$ values exhibited by nitrogen compounds, ranging from +5‰ (NO_3) to -3‰ (NH^{+4}) result in a wide natural range of isotopic compositions (Kendall, 1998). Values of $\delta^{15}N$ found in mangrove trees from Santa Cruz have a signature more consistent with the use of ammonium and/or a mixture of NH^{+4} and NO_3 as nitrogen source (Fenech et al., 2012), which indicates marine water as the main nitrogen source for these plants. It is of note that trees from Vitória Bay showed an increase level of $\delta^{15}N$, suggesting the contribution of different nitrogen sources for these plants. According to Fenech et al. (2012), the values for $\delta^{15}N$ in the trees from Vitória Bay can be attributed to fertilizers (+8‰ nitrate to +11‰ ammonia δ15N) (Table 1S; Fig. 2B). However, some contribution from human sewage and industrial effluents at this area cannot be discarded.

Significant differences in $\delta^{15}N$ among different species was observed ($P < 0.05$) when comparing both estuaries (Table 1S and Fig. 2B). Using the $\delta^{15}N$ it is possible to separate, at least, three levels for food web, in both ecosystems. The lowest $\delta^{15}N$ values were found in the plankton (4.7‰ in Santa Cruz and 4.5‰ in Vitória Bay; Table 1S and Fig. 2B), the next upper level is represented by the primary consumer, the shrimp *Macrobranchium sp*. (SH), showing $\delta^{15}N$ of 8.5‰ and 10.2‰ in Santa Cruz and Vitória Bay, respectively (Table 1S and Fig. 2B). As expected, fish (muscle; MU) showed the highest $\delta^{15}N$ values in both estuaries with 12‰ and 12.6‰ for Santa Cruz and Vitória Bay, respectively (Table 1S and Fig. 2B). So far, the range of $\delta^{15}N$ values among the studied species suggests a food web with increasing trophic levels as follows: plankton-shrimp-fish. This is consistent with the 3–5‰ increase in $\delta^{15}N$, commonly proposed per trophic level (Hobson et al., 2002).

Similarly, $\delta^{15}N$ values in plants were significantly higher in the more impacted area (Vitória

Bay) compared to the less impacted one (Santa Cruz) (Table 1S and Fig. 2B). $\delta^{15}N$ in plants (RI, LA and AV) from Santa Cruz ranged -3.1 to -0.5. Conversely, $\delta^{15}N$ in the same plants from Vitória Bay ranged from 7.9 to 11.5 (Table 1S and Fig. 2B). These results show the anthropic influence in the Vitória Bay area (higher $\delta^{15}N$ values), probably associated with sewage, urban run-off and industrial and agricultural activities in this area. The difference of $\delta^{15}N$ between plants (medium value among the tree species studied) and crab (primary consumer) was 3.5‰ in Santa Cruz and 9.4‰ in Vitória Bay, while the difference of crab and fish was 8.5‰ in Santa Cruz and 3.6‰ in Vitória Bay. The Santa Cruz values suggest that shrimp is the principal nutritional source of *C. parallelus,* as previously reported for this species (Cerqueira and Tsuzuki, 2009). However, the Victoria Bay values suggest that crabs may be the greatest nutritional source for *C. parallelus*, as this area has fishing effort reducing the food resource. Thus, it characterized another trophic chain as follows: plants-crab-fish, as already suggested in previous studies (Rabelo et al., 2009; Miranda et al., 2017; Yeager et al., 2016).

It is also possible to assume a third trophic chain formed by plankton and oyster, as the difference between $\delta^{15}N$ values were 1.7 ‰ and 2.9 ‰ in Santa Cruz and Victoria Bay, respectively (Table 1S and Fig. 2B). Probably in Santa Cruz, where the estuary have more influence of the marine waters, the amount of sediment in suspension influences the particles filtered by the oyster, which would explain the small difference between the two trophic levels.

3.2. Strontium Fingerprint

The global general $^{87}Sr/^{86}Sr$ ratio of continental river waters tends to be quite high (approx. 0.711) though, it can vary reflecting the $^{87}Sr/^{86}Sr$ of the underlying geology (Capo et al., 1998). The global marine $^{87}Sr/^{86}Sr$ signature (0.709180; McArthur et al., 2001) is significantly more homogeneous as it has a mixing time in the order of thousands of years. All sites analysed present results more consistent with marine global signature (Table 1S and Fig. 3)

Comparing the studied sites, Tubarão Complex showed the lowest ratio for $^{87/86}Sr$ in both biotic and abiotic samples (Table 1S and Fig. 3) indicating probably higher influence of marine water at this site. Both estuaries showed sediment with higher ratios of Sr than sediment from Tubarão Complex, indicating stronger continental influence in Santa Cruz and Vitória Bay. Biotic samples from both estuaries suggest that all food web is mainly influenced by marine water, as showed the lowest ratios of $^{87/86}Sr$.

For both estuaries, the high marine influence pointed out by $^{87/86}Sr$ ratio suggests that the marine water is the main nutrition source in both mangrove areas. In Santa Cruz estuary, these data are reinforced by the analysis of $\delta^{15}N$, which showed negative values in all mangrove trees from Santa Cruz, in accordance to the absorption of ammonia, related to seawater (Section 3.1). The influence of marine water on mangrove plants is also emphasized by Lin and Sternberg (1994) based on the analysis of oxygen isotopes in *R. mangle*, in which showed that mangrove plants use surface-soil and seawater rather than groundwater as water source, even when the water has high salinity.

3.3. Lead Fingerprint

The isotopic ratios of $^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ (Table 2S) showed large confidence intervals probably due to low ^{204}Pb. Therefore, the discussion was based on $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ ratios (Table 1S and Figure 4). In general, the Pb signature in the sampling sites was compatible with typical metallurgical activities as described by Komárek et al. (2008) and Bollhöfer and Rosman (2000). Since the 1980's no leaded petrol has been used in Brazil (Bollhöfer and Rosman, 2000). Thus, the Brazilian aerosols containing Pb comes from industrial sources, which include mining, smelting, coal burning, crushing, grinding, separation, refining and tailings management. All these processes produce large quantities of dust and aerosols, including the transport of ore by haul trucks and trains (Reed and Westman, 2005). Although most mining operations produce coarse dusts, high temperature processes produce fumes and fine particulates,

potentially laden with metals and metalloids that are present in the ore (Bollhöfer and Rosman, 2000).

In Tubarão Complex, higher Pb ($^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$) isotopic ratios were found in sediments (Table 1S and Fig. 4). In comparing the estuarine sites, the higher Pb radiogenic isotopic ratios found in Tubarão Complex are characteristic of anthropogenic sources. They can be explained by the presence of solid waste from metallurgical activities in this site. There was no Pb isotopic variation between the surface water of the two estuarine sites and the Tubarão Harbour Complex (Table 1S and Fig. 4).

The $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ of biotic and abiotic compartments analysed, show that the values were similar to those of atmospheric contamination (aerosols) in other areas of Brazil and South America (Table 1S and Fig. 4). Iron pelleting and steel smelting process in Tubarão Complex produces and releases steel metallic smoke, another significant part of atmospheric particles in the environment. Solid waste from Tubarão Complex does not correlate with other analysed compartments. Moreover, it is possible to presuppose that the plants are subject to greater contamination through atmospheric particulate matter than through sediment. Similarly, plankton and oyster showed interference from both, sediment and surface water, probably due to suspended PM in water, while fish is probably more influenced by soluble contaminants available in the surface water.

3.4. Integrative stable isotope analysis

The extension of algebra to multi-isotopic data is straightforward estimating the metrics using only bivariate isotopic data (e.g. C and N correlation). The extensive isotope data in terms of ecological niches, community-wide food-web structure, trophic diversity and contamination sources require the development of quantitative metrics to interpret the overall picture (Bouillon et al., 2011). Therefore, an algorithm based on k-means clustering was developed in order to correlate contamination source and trophic level.

Figure 5 shows four groups: Group 1, formed with sediment, plankton, *R. mangle*, *L. racemosa*, *A. schaueriana*, *Aratus* sp. and *C. rhizophorae* from Santa Cruz and Vitória Bay; Group 2 formed with *Macrobrachium* sp. and all tissues of fish, *C. Parallelus*; Group 3, formed with solid waste from Tubarão Harbour Complex and, Group 4 formed with surface water of Santa Cruz, Vitória Bay and Tubarão Harbour Complex and biotic samples of Tubarão Harbour Complex.

The k-means algorithm found a correlation in the Group 1 between the primary producers (mangrove plants and plankton), crabs and oysters from both estuaries, together with sediment explaining the base of the food web. The Group 2, include shrimp, that can be a primary or secondary consumer depending on the nutritional source, and fish that is top of the trophic chain. The Group 3, separates the solid waste from Tubarão Complex indicating that this compartment does not have direct influence in the mangrove ecosystem. The Group 4, formed with particulate matter, water from both estuaries, Santa Cruz and Vitória Bay and Tubarão Complex indicated the correspondence of isotopic signature for marine water and atmospheric particulate matter, even up to 70 km distance, which is the distance between both estuaries. The PM contamination source may be available in the ecosystem by chronic deposition on surface water.

4. Conclusions

The current results indicate an overall influence of marine water in these neotropical mangrove ecosystems. Conversely, contaminated solid waste from Tubarão Harbour Complex does not influence on the other analysed compartments. Mangrove plants from Santa Cruz show the same isotopic signature of marine water, being ammonium their principal source of nitrogen; mangrove plants from Vitória Bay show significant higher levels of $\delta^{15}N$, which can be related to the presence of high anthropic pressure in this area as the releasing sewage and industrial effluents, urban run-off and probably agricultural run-off. Strontium isotope ratios show the higher influence of the marine water to all food web. The Pb isotopic signature matches with metallurgical activities, proper of the

Tubarão Complex, being the atmospheric particulate matter released by this complex the most probable source of contaminants, chronically available to the ecosystem, including estuaries water. The current results showed that efforts to decrease the pollution at the studied area should be firstly concentrated in minimizing the discharge of atmospheric particulate matter (rather than dredging solid waste from the sediment) to decline the anthropogenic impact of the Tubarão Complex on the mangrove ecosystem, and also to the population living nearby to this complex. In conclusion, tool-kit for the evaluation of estuarine mangrove systems affected by anthropic pollution integrate ecological characteristics with contamination sources allowing a feasible model to be used worldwide for the study of pollution in estuaries and other areas affected by similar pollution sources.

Acknowledgements

This study was supported by FAPES, CONICET (National Research Council) and Science and Technology Office from Córdoba National University (Argentina). I.C. Souza acknowledges São Paulo Research Foundation (FAPESP, Proc. 2014/04832-3 and Proc. 2015/05258-1).

References

Arrivabene, H.P., Souza, I.C., Có, W.L.O., Conti, M.M., Wunderlin, D.A., Milanez, C.R.D., 2015. Effect of pollution by particulate iron on the morphoanatomy, histochemistry, and bioaccumulation of three mangrove plant species in Brazil, Chemosphere 127, 27–34 .

Bollhöfer, A., Rosman, K.J.R., 2000. Isotopic source signatures for atmospheric lead: the Southern Hemisphere. Geochim. Cosmochim. Ac. 64 (19), 3251–3262.

Bond, A.L., 2010. Relationships between stable isotopes and metal contaminants in feathers are spurious and biologically uninformative. Environ. Pollut. 158 (5), 1182–1184.

Bouillon, S., Connolly, R.M., Gillikin, D.P., 2011. Use of stable isotopes to understand food webs and ecosystem functioning in estuaries. In: Wolanski E and McLusky DS (eds.) Treatise on Estuarine and Coastal Science 7, 143–173.

Capo, R.C., Stewart, B.W., Chadwick, O.A., 1998. Strontium isotopes as tracers of ecosystem processes: theory and methods. Geoderma 82 (1), 197–225.

Cerqueira, V.R., Tsuzuki, M.Y., 2009. A review of spawning induction, larviculture, and juvenile rearing of the fat snook, *Centropomus parallelus*. Fish Physiol. Biochem. 35 (1), 17–28.

Cheng, H., Hu, Y., 2010. Lead (Pb) isotopic fingerprinting and its applications in lead pollution studies in China: a review. Environ. Pollut. 158 (5), 1134–1146.

Chow, T.J., Earl, J.L., 1970. Lead aerosols in the atmosphere: increasing concentrations. Science 169 (3945), 577–580.

Chow, T.J., Earl, J.L., 1972. Lead isotopes in North American coals. Science 176 (4034), 510–511.

Chow, T.J., Johnstone, M.S., 1965. Lead isotopes in gasoline and aerosols of Los Angeles basin, California. Science 147 (3657), 50–503.

Chow, T.J., Bruland, K.W., Bertine, K., Soutar, A., Koide, M., Goldberg, E.D., 1973. Lead pollution: records in

Southern California coastal sediments. Science 181 (4099), 551–552.

Chow, T.J., Snyder, C.B., Earl, J.L., 1975. Isotope ratios of lead as pollutant source indicators. Univ. of California, San Diego, La Jolla.

Elliott, M., Whitfield, A.K., 2011. Challenging paradigms in estuarine ecology and management. Estuar. Coast. Shelf S. 94 (4), 306–314.

Fenech, C., Rock, L., Nolan, K., Tobin, J., Morrissey, A., 2012. The potential for a suite of isotope and chemical markers to differentiate sources of nitrate contamination: a review. Water res. 46 (7), 2023–2041.

Gazeta, 2016. http://especiais.gazetaonline.com.br/aterro/ [Access: 08/02/2017].

Hobson, K.A., Fisk, A., Karnovsky, N., Holst, M., Gagnon, J.M., Fortier, M., 2002. A stable isotope (δ13C, δ15N) model for the North Water food web: implications for evaluating trophodynamics and the flow of energy and contaminants. Deep Sea Res. II 49, 5131–5150.

Hogarth, P. 1999. The Biology of Mangroves, Ed. Oxford University Press. 228p.

Santos, J.M., Reis Jr N.C., 2011. Caracterização e quantificação de partículas sedimentadas na região da Grande Vitória. Relatório Técnico Instituto Estadual do Meio Ambiente.

Ikemoto, T., Tu, N.P.C., Okuda, N., Iwata, A., Omori, K., Tanabe, S., Tuyen, B.C., Takeuchi,I., 2008. Biomagnification of trace elements in the aquatic food web in the Mekong Delta, South Vietnam using stable carbon and nitrogen isotope analysis.Arch. Environ. Contam. Toxicol. 54, 504–515.

Jiang, C.B., Yeh, C.Y., Lee, H.C., Chen, M.J., Hung, F.Y., Fang, S.S., Chien, L.C., 2010. Mercury concentration in meconium and risk assessment of fish consumption among pregnant women in Taiwan. Sci. Total Environ. 408, 518–523.

Kendall, C., 1998. Tracing nitrogen sources and cycling in catchments. Isotope tracers in catchment hydrology 1, 519–576.

Komárek, M., Ettler, V., Chrastný, V., Mihaljevič, M., 2008. Lead isotopes in environmental sciences: a review. Environment International 34 (4), 562–577.

Lin, G., Sternberg, L.D.S., 1994. Utilization of surface water by red mangrove (*Rhizophora mangle* L.): An isotopic study. B. Mar Sci. 54 (1), 94–102.

McArthur, J.M., Howarth, R.J., Bailey, T.R., 2001. Strontium isotope stratigraphy: LOWESS version 3: best fit to the marine Sr-isotope curve for 0–509 Ma and accompanying look-up table for deriving numerical age. J. Geol. 109 (2), 155–170.

Miranda, J.F., Mendoza-Carranza, M., Sánchez, A.J., Barba, E., 2017. Selective foraging of *Aratus pisonii* (Arthropoda: Sesarmidae) on mangrove leaves in laboratory experiments. J. Exp. Mar. Biol. Ecol. 488, 38–43.

Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. 293–320.

Podio, N.S., Baroni, M.V., Badini, R.G., Inga, C.M., Ostera, H.A., Cagnoni, M., Gautier, E.A., García, P.P., Hoogewerff, J., Wunderlin, D.A., 2013. Elemental and Isotopic Fingerprint of Argentinean Wheat. Matching Soil, Water and Crop Composition to Differentiate Provenance. J. Agric. Food Chem. 6, 3763–3773.

Rabelo, L.B., Muto, E.Y., Soares, L.S.H., 2009. Observações preliminares sobre o hábito alimentar do robalo-flecha *Centropomus undecimalis* (Bloch, 1792) e robalo-peba *Centropomus parallelus* (Poey, 1860) no estuário de Caravelas (Bahia-Brasil). Boletim Técnico Científico do CEPENE, Tamandaré 17 (1), 89–96.

Reed, W.R., Westman, E.C., 2005. A model for predicting the dispersion of dust from a haul truck. International Journal of Surface Mining, Reclamation and Environment 19 (1), 66–74.

Souza, I., Bonomo, M.M., Morozesk, M., Rocha, L.D., Duarte, I.D., Furlan, L.M., Arrivabene, H.P., Monferrán, M.V., Matsumoto, S.T., Milanez, C.R.D., Wunderlin, D.A., Fernandes, M.N., 2014a. Adaptive plasticity of *Laguncularia racemosa* in response to different environmental conditions: integrating chemical and biological data by chemometrics. Ecotoxicology 23, 335–348.

Souza, I.C., Duarte, I.D., Pimentel, N.Q., Rocha, L.D., Morozesk, M., Bonomo, M.M., Azevedo, V.C., Pereira, C.D.S., Monferrán, M.V., Milanez, C.R.D., Matsumoto, S.T., Wunderlin, D.A., Fernandes, M.N., 2013. Matching metal pollution with bioavailability, bioaccumulation and biomarkers response in fish (*Centropomus parallelus*) resident in neotropical estuaries. Environ. Pollut. 180, 136–144.

Souza, I.C., Morozesk, M., Duarte, I.D., Bonomo, M.M., Rocha, L.D., Furlan, L.M., Arrivabene, H.P., Monferrán, M.V., Matsumoto, S.T., Milanez, C.R.D., Wunderlin, D.A., Fernandes, M.N., 2014b. Matching pollution with adaptive changes in mangrove plants by multivariate statistics. A case study, *Rhizophora mangle* from four neotropical mangroves in Brazil. Chemosphere 108, 115–124.

Souza, I.C., Rocha, L.D., Morozesk, M., Bonomo, M.M., Arrivabene, H.P., Duarte, I.D., Furlan, L.M., Monferrán, M.V., Matsumoto, S.T., Milanez, C.R.D., Wunderlin, D.A., Fernandes, M.N., 2015. Changes in bioaccumulation and translocation patterns between root and leaves of *Avicennia schaueriana* as adaptive response to different levels of metals in mangrove system. Marine Pollution Bulletin 94, 176–184.

Usepa, 2009. Risk-based Concentration Table. Philadelphia PA. United States Environmental Protection Agency, Washington.

Wolanski, E., Elliott, M., 2015. Estuarine Ecohydrology: An Introduction. Elsevier, Whatham, 322p.
Yeager, L.A., Stoner, E.W., Peters, J.R., Layman, C.A., 2016. A terrestrial-aquatic food web subsidy is potentially mediated by multiple predator effects on an arboreal crab. J. Exp. Mar. Biol. Ecol. 475, 73–79.

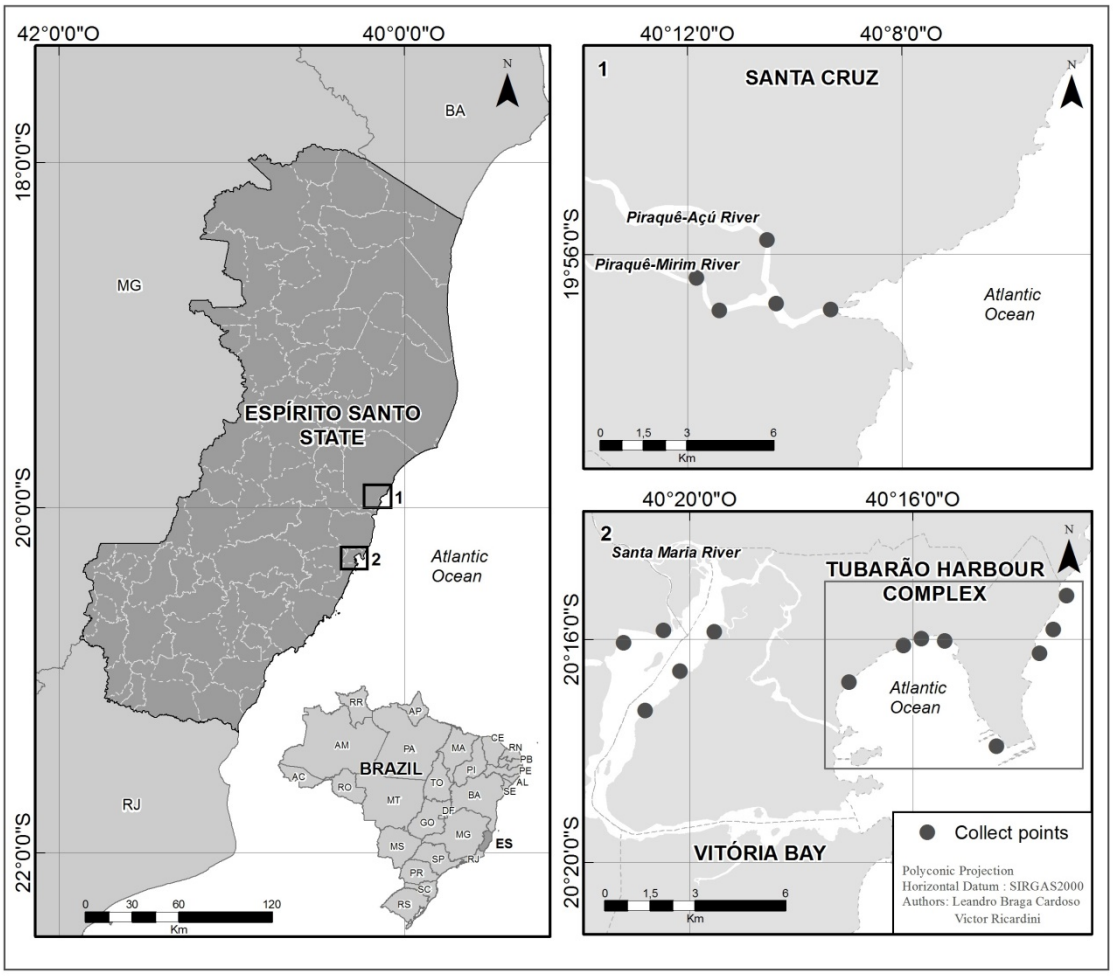


Figure 1. Location of the State of Espírito Santo (South America, Brazil), showing sampling sites. Santa Cruz (S 19º56`26.2``; W 40º 12`87``) and Vitória Bay (S 20º14`31.5``; W 40º19`84.7``) including Tubarão Complex (20º17′03.8″S and 40º14′24.9″W).

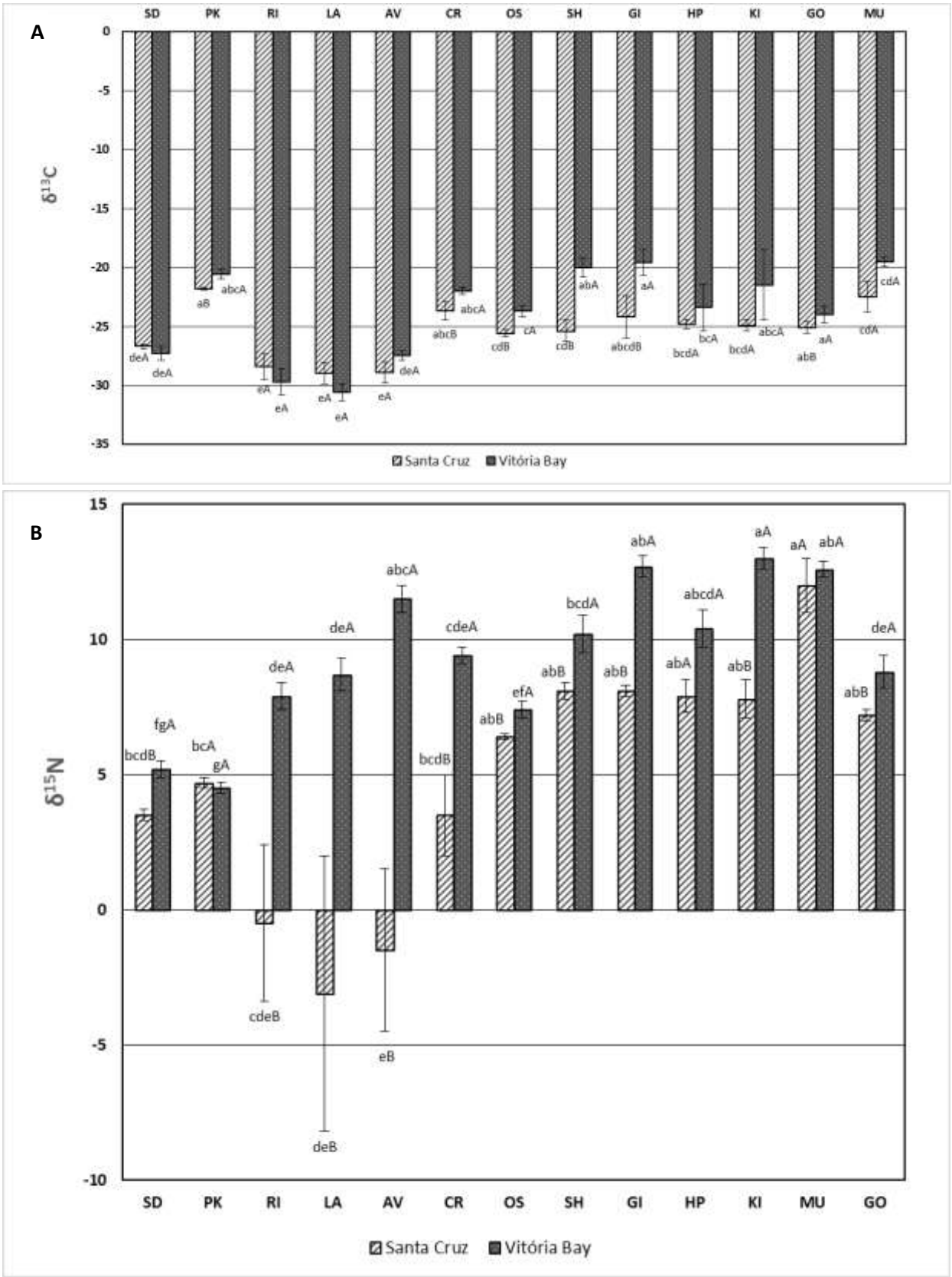


Figure 2. Distribution of (A) carbon and (B) nitrogen stable isotope in abiotic and biotic compartments of the estuaries Santa Cruz and Vitória Bay. SD: sediment, PK: plankton, RI: *Rhizophora mangle*, LA: *Laguncularia racemosa*, AV: *Avicennia schaueriana*, CR: Crab (*Aratus* sp.), OS: oyster (*Crassostrea rhizophorae*), SH: shrimp (*Macrobrachium sp.*), GI: gill of fish (*Centropomus parallelus*), HP: hepatopancreas of fish, KI: kidney of fish, MU: muscle of fish, GO: gonad of fish.

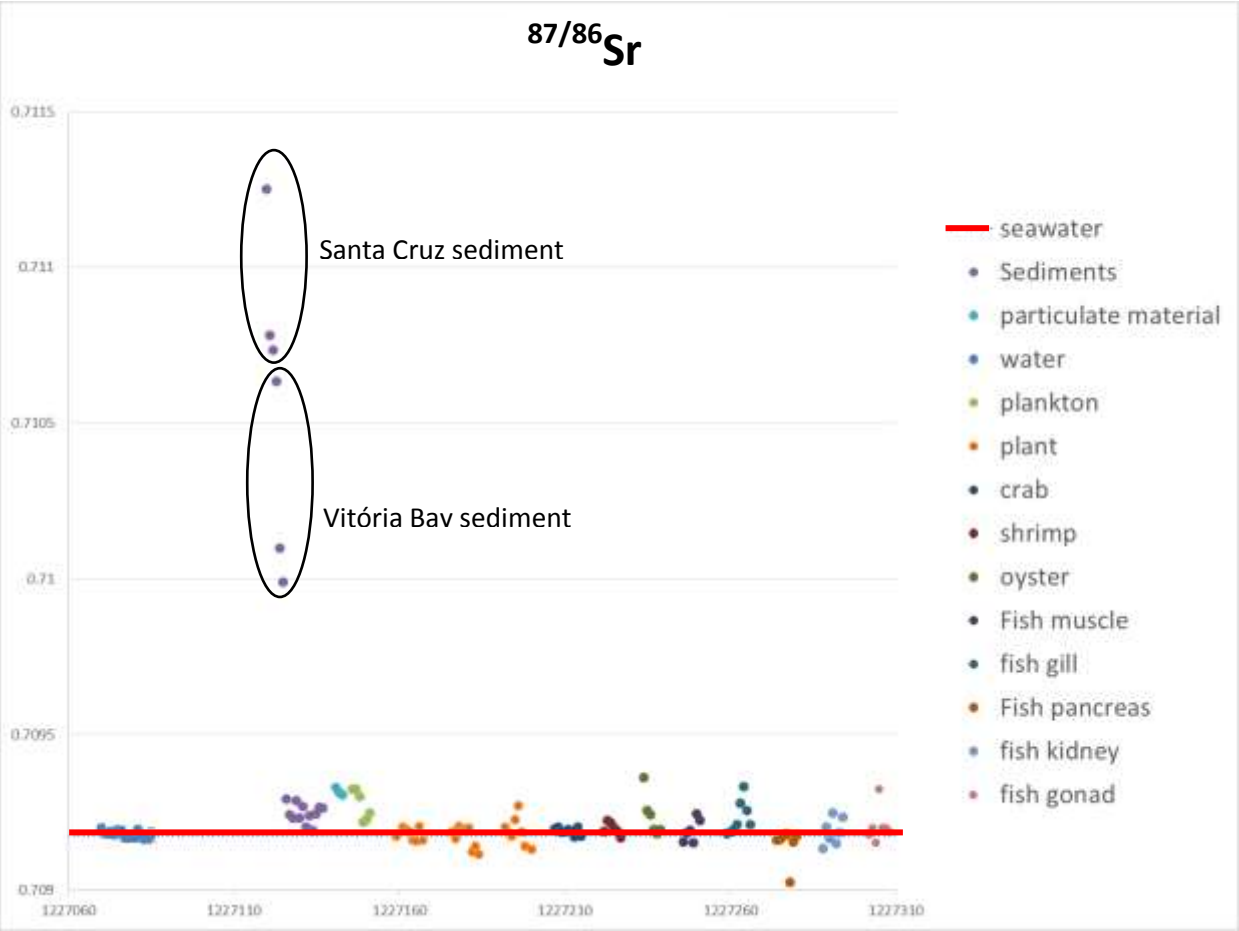


Figure 3. Distribution of strontium ratio ($^{87/86}$Sr) in abiotic and biotic compartments of the estuaries Santa Cruz, Vitória Bay and the Tubarão Complex. The circles differentiate the isotopic ratios found in sediments of both estuaries. There was no difference between the other matrices. SD: sediment, PK: plankton, RI: *Rhizophora mangle*, LA: *Laguncularia racemosa*, AV: *Avicennia schaueriana*, CR: Crab (*Aratus* sp.), OS: oyster (*Crassostrea rhizophorae*), SH: shrimp (*Macrobrachium sp.*), GI: gill of fish (*Centropomus parallelus*), HP: hepatopancreas of fish, KI: kidney of fish, MU: muscle of fish, GO: gonad of fish.

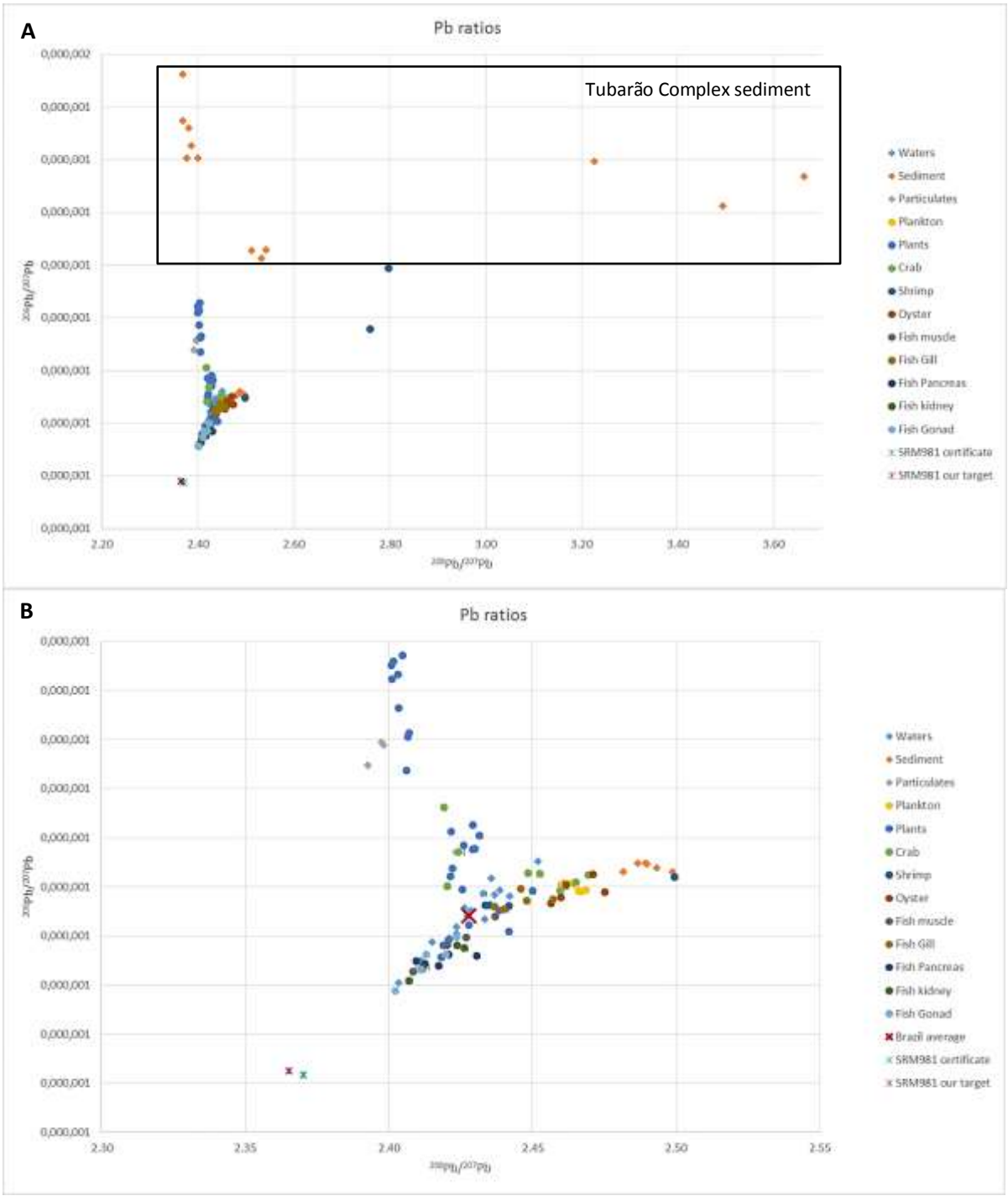


Figure 4. Distribution of lead ratio ($^{206/207}$Pb and $^{208/207}$Pb) isotope in abiotic and biotic compartments of the estuaries Santa Cruz, Vitória Bay and the Tubarão Complex. (A) The rectangle highlights the difference of the isotopic ratios found in Tubarão Complex sediments with the others. (B) Detail of figure A showing all food web.

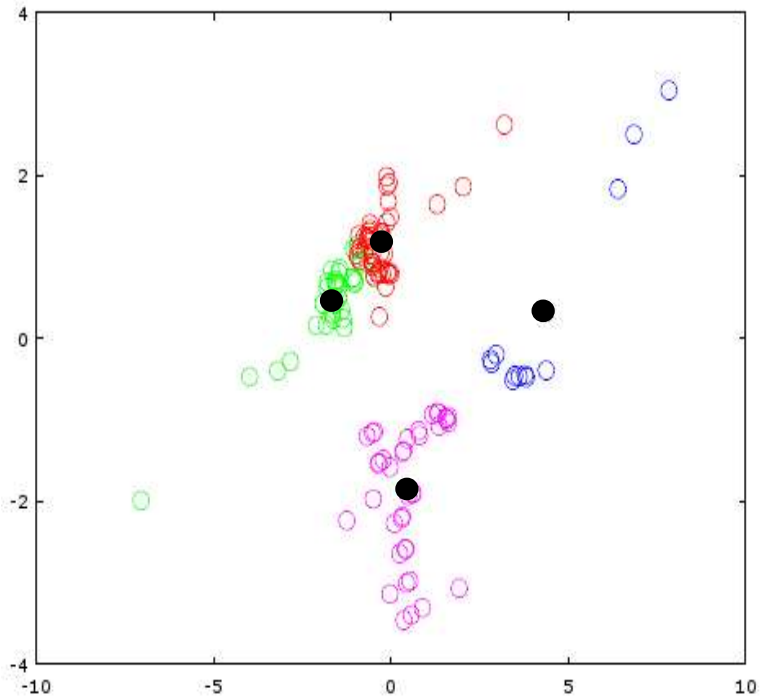


Figure 5. Pixel dataset plotted in 2D of k-means algorithm, using PCA for dimensional reduction. Black circle: centroids of each group. Red (Group 1): sediment, plankton, *R. mangle*, *L. racemosa*, *A. schaueriana*, *Aratus* sp. and *C. rhizophorae* from Santa Cruz and Vitória Bay; Green (Group 2): *Macrobrachium* sp. and all tissues of fish, *C. Parallelus;* Blue (Group 3): sediment of Tubarão Complex; Pink (Group 4): atmospheric particulate matter and surface water of Tubarão Complex, surface water of Santa Cruz and Vitória Bay.

Table 1S. Measurements of the stable isotopes $\delta^{13}C$, $\delta^{15}N$, $^{87/86}Sr$, $^{206/207}Pb$ and $^{208/207}Pb$ in abiotic and biotic compartments of the estuaries Santa Cruz and Vitória Bay and the Tubarão Complex. Values correspond to means ± standard deviation. Lowercase letters indicate significant differences between isotopes in the same estuary; uppercase letters indicate significant differences of same parameter between estuaries (Tukey test, $P < 0.05$). SW: surface water, SD: sediment, PM: particulate matter, PK: plankton, RI: *Rhizophora mangle*, LA: *Laguncularia racemosa*, AV: *Avicennia schaueriana*, CR: Crab (*Aratus* sp.), OS: oyster (*Crassostrea rhizophorae*), SH: shrimp (*Macrobrachium* sp.), GI: gill of fish (*Centropomus parallelus*), HP: hepatopancreas of fish, KI: kidney of fish, MU: muscle of fish, GO: gonad of fish.

			Isotopes				
	Site	Matrix	$\delta^{13}C$	$\delta^{15}N$	$^{87/86}Sr$	$^{206/207}Pb$	$^{208/207}Pb$
Estuaries	Santa Cruz	SW	*NA	*NA	0.70919 ± 0.00001 ^{bA}	1.147 ± 0.007 ^{cdeA}	2.419 ± 0.006 ^{defA}
		SD	-26.7 ± 0.2 ^{deA}	3.5 ± 0.2 ^{bcdB}	0.71092 ± 0.00029 ^{aA}	1.179 ± 0.001 ^{abDE}	2.489 ± 0.002 ^{aB}
		PK	-21.8 ± 0.1 ^{aB}	4.7 ± 0.2 ^{bcA}	0.70932 ± 0.00001 ^{bA}	1.168 ± 0.001 ^{abcdB}	2.467 ± 0.001 ^{bA}
		RI	-28.4 ± 1.1 ^{eA}	-0.5 ± 2.9 ^{cdeB}	0.70919 ± 0.00001 ^{bA}	1.148 ± 0.007 ^{cdeC}	2.422 ± 0.005 ^{cdefA}
		LA	-29.0 ± 0.9 ^{eA}	-3.1 ± 5.1 ^{deB}	0.70919 ± 0.00002 ^{bA}	1.161 ± 0.028 ^{abcdeB}	2.420 ± 0.002 ^{defB}
		AV	-28.9 ± 0.9 ^{eA}	-1.5 ± 3.0 ^{eB}	0.70919 ± 0.00001 ^{bAB}	1.164 ± 0.004 ^{abcdeC}	2.442 ± 0.007 ^{cA}
		CR	-23.7 ± 0.8 ^{abcB}	3.5 ± 1.5 ^{bcdB}	0.70920 ± 0.00001 ^{bA}	1.171 ± 0.003 ^{abcA}	2.465 ± 0.005 ^{bA}
		OS	-25.6 ± 0.3 ^{cdB}	6.4 ± 0.1 ^{abB}	0.70929 ± 0.00007 ^{bA}	1.162 ± 0.001 ^{abcdeA}	2.437 ± 0.004 ^{cdA}
		SH	-25.4 ± 0.9 ^{cdB}	8.1 ± 0.3 ^{abB}	0.70921 ± 0.00002 ^{bA}	1.165 ± 0.002 ^{abcdeA}	2.464 ± 0.010 ^{bA}
		GI	-24.2 ± 1.8 ^{abcdB}	8.1 ± 0.2 ^{abB}	0.70919 ± 0.00001 ^{bA}	1.190 ± 0.021 ^{aA}	2.429 ± 0.017 ^{cdefA}
		HP	-24.8 ± 0.4 ^{bcdA}	7.9 ± 0.6 ^{abA}	0.70916 ± 0.00001 ^{bA}	1.141 ± 0.002 ^{deA}	2.423 ± 0.007 ^{cdefA}
		KI	-24.9 ± 0.5 ^{bcdA}	7.8 ± 0.7 ^{abB}	0.70917 ± 0.00003 ^{bA}	1.139 ± 0.007 ^{deB}	2.416 ± 0.008 ^{efB}
		MU	-22.5 ± 1.3 ^{abB}	12.0 ± 1.0 ^{aA}	0.70918 ± 0.00002 ^{bA}	1.153 ± 0.004 ^{bcdeA}	2.432 ± 0.005 ^{cdeA}
		GO	-25.1 ± 0.5 ^{cdA}	7.2 ± 0.2 ^{abB}	0.70918 ± 0.00002 ^{bA}	1.137 ± 0.005 ^{eA}	2.409 ± 0.004 ^{fA}
	Vitoria Bay	SW	*NA	*NA	0.70919 ± 0.00001 ^{bA}	1.152 ± 0.004 ^{bcA}	2.426 ± 0.006 ^{bA}
		SD	-27.3 ± 0.6 ^{deA}	5.2 ± 0.3 ^{fgA}	0.71024 ± 0.00034 ^{aB}	1.177 ± 0.001 ^{bcE}	2.491 ± 0.009 ^{bB}
		PK	-20.6 ± 0.4 ^{abcA}	4.5 ± 0.2 ^{gA}	0.70923 ± 0.00002 ^{bB}	1.171 ± 0.001 ^{bcA}	2.462 ± 0.002 ^{bA}
		RI	-29.7 ± 1.1 ^{eA}	7.9 ± 0.5 ^{deA}	0.70918 ± 0.00002 ^{bA}	1.173 ± 0.004 ^{bcB}	2.423 ± 0.002 ^{bA}
		LA	-30.6 ± 0.7 ^{eA}	8.7 ± 0.6 ^{deA}	0.70920 ± 0.00001 ^{bA}	1.174 ± 0.020 ^{bcB}	2.433 ± 0.008 ^{bA}
		AV	-27.5 ± 0.4 ^{deA}	11.5 ± 0.5 ^{abcA}	0.70923 ± 0.00004 ^{bA}	1.190 ± 0.005 ^{abB}	2.430 ± 0.001 ^{bB}
		CR	-22.0 ± 0.3 ^{abcA}	9.4 ± 0.3 ^{cdeA}	0.70919 ± 0.00001 ^{bA}	1.172 ± 0.006 ^{bcA}	2.451 ± 0.002 ^{bB}
		OS	-23.7 ± 0.5 ^{cA}	7.4 ± 0.3 ^{efA}	0.70919 ± 0.00001 ^{bA}	1.237 ± 0.062 ^{aA}	2.686 ± 0.162 ^{aA}
		SH	-20.0 ± 0.8 ^{abA}	10.2 ± 0.7 ^{bcdA}	0.70919 ± 0.00002 ^{bA}	1.169 ± 0.007 ^{bcA}	2.459 ± 0.014 ^{bA}
		GI	-19.6 ± 1.1 ^{aA}	12.7 ± 0.4 ^{abA}	0.70927 ± 0.00006 ^{bA}	1.162 ± 0.002 ^{bcA}	2.444 ± 0.004 ^{bA}
		HP	-23.4 ± 2.0 ^{bcA}	10.4 ± 2.7 ^{abcdA}	0.70912 ± 0.00008 ^{bA}	1.136 ± 0.002 ^{cA}	2.413 ± 0.001 ^{bA}
		KI	-21.5 ± 3.0 ^{abcA}	13.0 ± 0.4 ^{aA}	0.70919 ± 0.00005 ^{bA}	1.140 ± 0.004 ^{bcB}	2.416 ± 0.010 ^{bB}
		MU	-19.5 ± 0.4 ^{aA}	12.6 ± 0.3 ^{abA}	0.70921 ± 0.00005 ^{bA}	1.141 ± 0.005 ^{bcA}	2.414 ± 0.006 ^{bA}
		GO	-24.0 ± 0.7 ^{cdA}	8.8 ± 0.6b ^{deA}	0.70924 ± 0.00007 ^{bA}	1.138 ± 0.011 ^{bcA}	2.412 ± 0.011 ^{bA}
	Tubarão Complex	SW1	*NA	*NA	0.70918 ± 0.00001 ^{deA}	1.167 ± 0.006 ^{fA}	2.432 ± 0.005 ^{bA}
		SW2	*NA	*NA	0.70917 ± 0.00001 ^{deA}	1.165 ± 0.005 ^{fA}	2.436 ± 0.008 ^{bA}
		SW3	*NA	*NA	0.70918 ± 0.00002 ^{deA}	1.152 ± 0.019 ^{fA}	2.423 ± 0.017 ^{bA}
		SW4	*NA	*NA	0.70918 ± 0.00002 ^{deA}	1.163 ± 0.017 ^{fA}	2.432 ± 0.019 ^{bA}
		SD1	*NA	*NA	0.70926 ± 0.00003 ^{abcC}	1.411 ± 0.016 ^{abAB}	2.387 ± 0.013 ^{bB}
		SD2	*NA	*NA	0.70926 ± 0.00003 ^{abC}	1.444 ± 0.034 ^{aA}	2.376 ± 0.010 ^{bB}
		SD3	*NA	*NA	0.70921 ± 0.00003 ^{bcdC}	1.311 ± 0.005 ^{cC}	2.530 ± 0.015 ^{bB}
		SD4	*NA	*NA	0.70926 ± 0.00001 ^{abcC}	1.379 ± 0.022 ^{bB}	3.463 ± 0.220 ^{aA}
		PM	*NA	*NA	0.70931 ± 0.00001 ^{aC}	1.225 ± 0.005 ^{deD}	2.396 ± 0.003 ^{bB}
		RI	*NA	*NA	0.70917 ± 0.00003 ^{deA}	1.227 ± 0.008 ^{deA}	2.407± 0.001 ^{bB}
		LA	*NA	*NA	0.70913 ± 0.00001 ^{eB}	1.259 ± 0.004 ^{cdA}	2.401 ± 0.001 ^{bC}
		AV	*NA	*NA	0.70914 ± 0.00001 ^{eB}	1.254 ± 0.011 ^{dA}	2.404 ± 0.001 ^{bC}
		CR	*NA	*NA	0.70918 ± 0.00001 ^{deA}	1.185 ± 0.016 ^{efA}	2.421 ± 0.003 ^{bC}
		GI	*NA	*NA	0.70923 ± 0.00002 ^{bcdA}	1.163 ± 0.002 ^{fA}	2.447 ± 0.014 ^{bA}
		HP	*NA	*NA	0.70917 ± 0.00000 ^{deA}	1.140 ± 0.000 ^{fA}	2.410 ± 0.000 ^{bA}
		KI	*NA	*NA	0.70923 ± 0.00000 ^{bcdA}	1.180 ± 0.000 ^{efA}	2.516 ± 0.000 ^{bA}
		MU	*NA	*NA	*NA	*NA	*NA
		GO	*NA	*NA	0.70919 ± 0.00000 ^{cdeA}	1.142 ± 0.000 ^{fA}	2.420 ± 0.000 ^{bA}

Table 1. Measurements of the stable isotopes $^{206/204}$Pb, $^{207/204}$Pb and $^{208/204}$Pb, in abiotic and biotic compartments of the estuaries Santa Cruz and Vitória Bay and the Tubarão Complex. Values correspond to means ± standard deviation. Lowercase letters indicate significant differences of the same isotope between matrix in the same estuary; uppercase letters indicate significant differences of same parameter between estuaries (Tukey test, $P < 0.05$). SW: surface water, SD: sediment, PM: particulate matter, PK: plankton, RI: *Rhizophora mangle*, LA: *Laguncularia racemosa*, AV: *Avicennia schaueriana*, CR: Crab (*Aratus* sp.), OS: oyster (*Crassostrea rhizophorae*), SH: shrimp (*Macrobrachium* sp.), GI: gill of fish (*Centropomus parallelus*), HP: hepatopancreas of fish, KI: kidney of fish, MU: muscle of fish, GO: gonad of fish. *NA = not analysed.

			Isotopes		
	Site	Matrix	206/204 Pb	207/204 Pb	208/204 Pb
Estuaries	Santa Cruz	SW	17.845 ± 0.121 ^{aA}	15.552 ± 0.013 ^{aA}	37.615 ± 0.132 ^{aA}
		SD	18.477 ± 0.033 ^{aD}	15.666 ± 0.026 ^{aC}	38.988 ± 0.076 ^{aB}
		PK	18.250 ± 0.016 ^{aA}	15.623 ± 0.012 ^{aA}	38.545 ± 0.049 ^{aA}
		RI	17.907 ± 0.065 ^{aC}	15.603 ± 0.032 ^{aB}	37.799 ± 0.045 ^{aA}
		LA	18.069 ± 0.524 ^{aB}	15.567 ± 0.083 ^{aB}	37.637 ± 0.231 ^{aA}
		AV	18.325 ± 0.082 ^{aC}	15.733 ± 0.046 ^{aA}	38.432 ± 0.199 ^{aA}
		CR	18.298 ± 0.069 ^{aA}	15.620 ± 0.022 ^{aA}	38.499 ± 0.123 ^{aA}
		OS	18.120 ± 0.040 ^{aA}	15.592 ± 0.038 ^{aA}	37.987 ± 0.143 ^{aA}
		SH	18.121 ± 0.112 ^{aA}	15.549 ± 0.123 ^{aA}	38.292 ± 0.147 ^{aA}
		GI	14.796 ± 3.543 ^{bA}	12.584 ± 3.101 ^{bA}	30.657 ± 7.697 ^{bA}
		HP	17.647 ± 0.117 ^{abA}	15.472 ± 0.093 ^{aA}	37.490 ± 0.158 ^{aA}
		KI	17.742 ± 0.004 ^{aA}	15.576 ± 0.096 ^{aA}	37.621 ± 0.087 ^{aA}
		MU	17.856 ± 0.185 ^{aA}	15.474 ± 0.221 ^{aA}	37.658 ± 0.437 ^{aA}
		GO	18.349 ± 0.461 ^{aA}	16.135 ± 0.476 ^{aA}	38.859 ± 1.062 ^{aA}
	Vitoria Bay	SW	17.933 ± 0.073 ^{abA}	15.566 ± 0.010 ^{abA}	37.767 ± 0.120 ^{abA}
		SD	18.408 ± 0.034 ^{abD}	15.646 ± 0.021 ^{abC}	38.978 ± 0.187 ^{abB}
		PK	18.288 ± 0.021 ^{abA}	15.615 ± 0.017 ^{abA}	38.443 ± 0.041 ^{abA}
		RI	18.311 ± 0.084 ^{abB}	15.605 ± 0.046 ^{abB}	37.811 ± 0.134 ^{abA}
		LA	18.354 ± 0.324 ^{abB}	15.628 ± 0.047 ^{abAB}	38.012 ± 0.163 ^{abA}
		AV	18.709 ± 0.030 ^{abB}	15.719 ± 0.083 ^{abA}	38.207 ± 0.196 ^{abAB}
		CR	19.947 ± 2.296 ^{aA}	17.034 ± 2.051 ^{aA}	41.757 ± 5.064 ^{aA}
		OS	19.376 ± 0.984 ^{abA}	15.662 ± 0.018 ^{abA}	42.072 ± 2.579 ^{aA}
		SH	17.722 ± 1.448 ^{abA}	15.079 ± 1.292 ^{abA}	37.152 ± 3.235 ^{abA}
		GI	18.143 ± 0.005 ^{abA}	15.613 ± 0.029 ^{abA}	38.171 ± 0.002 ^{abA}
		HP	17.316 ± 0.358 ^{bA}	15.238 ± 0.288 ^{abA}	36.772 ± 0.683 ^{abA}
		KI	17.138 ± 0.890 ^{bA}	14.876 ± 1.024 ^{bA}	36.161 ± 2.145 ^{bA}
		MU	17.652 ± 0.010 ^{abA}	15.467 ± 0.086 ^{abA}	37.433 ± 0.033 ^{abA}
		GO	17.763 ± 0.265 ^{abA}	15.603 ± 0.147 ^{abA}	37.665 ± 0.452 ^{abA}
	Tubarão Complex	SW1	*NA	*NA	*NA
		SW2	18.232 ± 0.140 ^{fghA}	15.624 ± 0.076 ^{cdeA}	37.972 ± 0.202 ^{bcA}
		SW3	18.161 ± 0.096 ^{ghA}	15.595 ± 0.016 ^{deA}	37.991 ± 0.151 ^{bcA}
		SW4	17.948 ± 0.336 ^{ghA}	15.583 ± 0.046 ^{eA}	37.753 ± 0.364 ^{bcA}
		SD1	18.104 ± 0.279 ^{ghA}	15.571 ± 0.019 ^{eA}	37.869 ± 0.332 ^{bcA}
		SD2	22.583 ± 0.298 ^{abAB}	16.007 ± 0.028 ^{aA}	38.205 ± 0.150 ^{bB}
		SD3	23.184 ± 0.626 ^{aA}	16.058 ± 0.051 ^{aA}	38.148 ± 0.131 ^{bB}
		SD4	20.781 ± 0.094 ^{cC}	15.847 ± 0.016 ^{bB}	40.087 ± 0.258 ^{bB}
		PM	21.853 ± 0.390 ^{bB}	15.841 ± 0.033 ^{bB}	54.849 ± 3.445 ^{aA}
		RI	19.214 ± 0.090 ^{defD}	15.681 ± 0.009 ^{cdeC}	37.575 ± 0.066 ^{bcB}
		LA	19.268 ± 0.132 ^{deA}	15.706 ± 0.004 ^{bcdeA}	37.800 ± 0.021 ^{bcA}
		AV	19.818 ± 0.053 ^{cdA}	15.746 ± 0.035 ^{bcdA}	37.810 ± 0.080 ^{bcA}
		CR	19.760 ± 0.196 ^{dA}	15.748 ± 0.016 ^{bcA}	37.858 ± 0.049 ^{bcB}
		GI	18.562 ± 0.276 ^{efgA}	15.659 ± 0.084 ^{cdeA}	37.907 ± 0.250 ^{bcA}
		HP	18.165 ± 0.024 ^{ghA}	15.616 ± 0.054 ^{cdeA}	38.210 ± 0.098 ^{bA}
		KI	17.868 ± 0.000 ^{ghA}	15.676 ± 0.000 ^{cdeA}	37.778 ± 0.000 ^{bcA}
		MU	*NA	*NA	*NA
		GO	16.238 ± 0.000 ^{iA}	13.765 ± 0.000 ^{gA}	34.612 ± 0.000 ^{cA}

CAPÍTULO 4

Metals and metalloids transfer in neotropical mangrove ecosystem

ABSTRACT

Mangrove ecosystem is complex due the wide range of abiotic and biotic variability such as salinity, pH, redox potential, particle size, dissolved organic matter, xenobiotics concentration. This research propose a conceptual framework emphasizing essential and nonessential metal accumulation and transfer in three trophic chains (plankton-shrimp-fish, plankton-crab-fish and plankton-oyster) of a mangrove food web in the Southeast of Brazil: Santa Cruz and Vitória Bay, localized in the Espirito Santo state. In Santa Cruz mangrove ecosystem, the plankton-oyster food chain presented metals biodilution of B, Al, V, Cr, Mn, Fe, Ni, Cu, As, Se, Rb, Sr, Pb, Nb and Hg and biomagnification of Zn, Ag, Cd. Similarly, the plankton-shrimp-fish food chain presented biodilution for most quantified metals such as B, Al, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Si, Rb, Sr, Pb, Nb, while Cd, Hg were biomagnified. The metal transfer data for plant-crab-fish food chain indicated biomagnification of Cr, Zn, As, Rb, Ag, Cd, Pb, Nb, Hg, biodilution of B, Al, Mn, Fe, Sr and no biomagnification or biodilution of V, Ni, Cu. The trophic chain: plant-crab-fish was that has major metal biomagnification potential. Although there is a biodilution in the plankton-shrimp-fish chain and plankton-oyster chain, the great exposure of plankton to metallic contaminants and their corresponding bioaccumulation deserve attention, as it may contribute to decreasing of these organisms that are the base of the marine ecosystem food chain.

Keywords: biodilution, biomagnification, trophic chain, food web, estuaries

1. INTRODUCTION

Estuaries are defined as bodies of semi-enclosed water receiving freshwater runoff water and connected to the sea being exposed to tide or the salt intrusion (Wolanski and Elliot 2015). Among the ecosystems in estuarine areas, mangrove stands out as a coastal ecosystem, predominantly tropical and subtropical regions (Giri et al. 2011), offering a wide range of goods and services around the world, such as protection against coastal storms, sea-level rise, saline intrusion and erosion (Barbier 2016). However, these areas are continuously impacted due to human activities, presenting fastest rate of loss of ecosystems worldwide (Valiela et al., 2001).

Among the impacts suffered by the mangrove ecosystem, metal contamination is a great problem since domestic sewage and industrial residues are carried out by rivers and be adsorbed in sediment which is rich in organic matter and with fine particles (McLuscky and Elliot, 2004). Adsorbed metals have low bioavailability and acid-volatile sulfides primarily reduces they solubility and organic matters as Fe-Mn oxides and fine particles stabilize potentially toxic metals reducing toxicity (Zhang et al. 2014).

Once taken up, the metals behaviour may present differences along the trophic chain; metals can show biodilution or biomagnification through the trophic levels of estuarine ecosystem (Cui et al. 2011, Guo et al. 2016). Biomagnification may be critical for resident organisms and for humans that use the wide variety of fish and crustaceans from thises ecosystems for feeding. The difficulty in assume the toxicity of metals lies in the fact that the tolerance level depends on the organism evaluated (Wood et al., 2012). In addition to the essential metals, some of them are considered non-essential such as As, Cd and Sr; however, they may be necessary in some life forms, so that the essentiality of metals and metalloids depends on the organism being evaluated and, in the case of an entire trophic chain, this definition often becomes difficult (Hartl, 2013; Chapman and Wang 2000).

Studies on the mangrove ecosystem are complex due to difficulties to take the environmental

sampling and the wide range of abiotic and biotic variability (e.g. salinity, pH, redox potential, particle size, dissolved organic matter, xenobiotics concentration). *In situ* studies of metal concentrations through food web may be used as a base for application of conceptual models, which may help to understand the absorption form, transfer and elimination of metals throughout mangrove ecosystem. This chapter propose a conceptual framework emphasized on metal accumulation in three trophic chains (plankton-shrimp-fish, plant-crab-fish and plankton-oyster) of a mangrove food web in the Southeast of Brazil, Santa Cruz and Vitória Bay localized in the Espirito Santo state, evaluating the trophic transfer of essential and nonessential metal data presented in early chapters and finished outlining current limitations and perspectives for future research.

2. MATERIAL AND METHODS

2.1 Study areas:

The study has chosen two sampling areas on the Espírito Santo state, Brazil that extend two mangrove areas, Vitória Bay and Santa Cruz. Sampling collection was made at the end of summer in March 2014 and 2015.

The Santa Cruz mangrove ecosystem (19°56′26.2″S and 40°12′87.0″W) has around 12 km^2 of area coverage and two rivers on its composition. It also presents Piraquê-Açu and Piraquê-Mirim Mangrove Ecological Reserve which represents a great area of preserved mangrove with no introduction of metal contamination industries (Fig. 1).

Vitória Bay (20°14′31.5″S and 40°19′84.7″W) is an estuarine complex formed by five rivers (Fig. 1) after suffers several landfills (made of industrial solid waste of harbour dredging activities or other industrial activities as Tubarão Complex occurred in the 19th and the in final 20th centuries totalling a huge area of 12,000m^2 which half of it was occupied with mangroves. Because of the

landfills, the artificial Camburi Beach located near to Tubarão Complex, presents iron mining bags deposited on its composition and additionally it suffers more impact because has populated area with consequently pollution sources such as sewage sludge, metallurgical and textile activities, steel industry, iron mining and harbours (Gazeta,2016).

2.2 Sampling procedure:

Surface water (SW) and sediment (SD) samples were taken on Santa Cruz, Vitória Bay mangrove ecosystems (Fig. 1). Water samples were collected using acid washed plastic bottles submerged 10-20 cm depth; samples were acidified with ultrapure HNO_3 (sub-boiling grade), stored at 4ºC and before analyses were filtered in 0.45 μm nitrocellulose filter. Sediment samples were collected using a polypropylene spoon among 10-20 cm depth; samples were placed into clean 1L-polypropylene flasks (without headspace), dried at room temperature and sieved through 63 μm nylon meshes.

Samples (n = 9) of *Rhizophora mangle* (RM), *Laguncularia racemosa* (LR), *Avicennia schaueriana* (AS), plankton (PK), shrimp *Macrobranchium sp.* (SH), crab *Aratus sp.* (CR), oyster *Crassostrea rhizophorae* (OS) and organs [muscle (MU), gill (GI), kidney (KI), hepatopancreas (HP) and gonad (GO) from juvenile fish *Centropomus parallelus* were collected in each site (Fig. 1); in 2014 sampling, plants and oysters were not sampled. Plankton (phytoplankton and zooplankton) samples were collected using a 20 um net mesh. Fully expanded leaves from 3rd to 5th node of the mangrove three species were collected and washed with ultrapure water. Crabs were caught from trees by hand and shrimps with pitfall aid. Fish were collected (gills, muscle, hepatopancreas, gonad and kidney of *C. parallelus*), using hook and line and their organs immediately removed and separated for chemical analysis. Each biological sample was freeze-dried until reach constant mass, macerated with mortar and pestle and stored at room temperature until analysis.

2.3 Chemical analysis

Ultrapure water was obtained (<5 µg L−1 TOC) from purification system Arium 61316-RO plus Arium 611 UV (Sartorius, Germany). Multi-element standard solution Merck VI CertiPUR® was obtained from Merck Química Argentina (Buenos Aires, Argentina). Nitric acid (63.7%) sub-boiling grade was prepared from analytical grade acid using a distiller (Figmay Sub-boiling distilled, Córdoba, Argentina). Purity of nitric acid was verified by Mass Spectrometry Inductively Coupled Plasma (ICP-MS), Agilent 7500cx, USA, equipped with an ASX-100 autosampler (CETAC Technologies, Omaha, NE). Filters (0.45 µm, HAWG04756) were obtained from Millipore (São Paulo, Brazil). All glassware and plastic bottles/containers were left overnight with sulfuric/nitric acids solution and posteriorly washed with ultrapure water. ICP probes and pipes were of PTFE previously washed with nitric acid (2% v v^{-1}).

Sediment (0.1 g dry weight) were digested with 4 mL of nitric acid and 1 mL of hydrochloric acid (ultrapure, sub-boiling grade). Biotic samples were ground and homogenized with a mortar and 0.1 g from each sample was digested in 4 mL of nitric acid, 1 ml of hydrochloric acid and 0.5 mL of hydrogen peroxide (ultrapure, sub-boiling grade), filtered in 0.45 µm nitrocellulose filter according to USEPA (2009) and, then metals were analysed. Controls were prepared using only reagents following the same protocol. All digested samples were stored at 4°C until analysis. Pre-cleaned PTFE tubes (Savillex) at constant temperature (90°C) during 24 hours (Chappaz et al. 2012) were used in digestion procedure. After digestion, samples were filtered using 0.45 µm nitrocellulose filter to remove remaining particles according to method described by USEPA (2009) and measured. Controls were prepared using only reagents following the same protocol. All digested samples were stored at 4°C until analysis.

The measurements were performed in triplicate and the repeatability of ICP-MS for metals and metalloids measurements was generally ≥ 97%. Quality assurance (QA) and quality control (QC) were

done using a certified reference material (CRM): estuarine and marine sediment MESS-2, bush branches and leaves NCSDC 73348, peach leaves NIST SRM 1547 and NIST 8414 bovine muscle. Recoveries from CRMs were 91 ± 11%, respectively.

2.7 Statistical analyses

Statistical analyses were conducted depending on each data set. Shapiro-Wilk Test and means for Variances-Levene (ADM) analysis was used to check normal distribution and homogeneity of variance, respectively. Non-parametric Kruskal-Wallis followed by Steel-Dwass test (significance level $p<0.05$) was conducted to detect differences among matrixes and sites. The data was reported in median (q1-q3). To determine metal transfer through the food web (Monferrán et al, 2016), linear regression were made for the three trophic chain (1.plant-crab-fish; 2.plankton-shrimp-fish; 3.plankton-oyster) determined by Souza (2017, Chapter 1) using nitrogen stable isotope data. All statistical analyses were conducted using the software JMP v.12.

3. RESULTS AND DISCUSSION

Machado et al. (2016) concluded that the world's six most heavily polluted aquatic environments by trace metals are estuaries and, according to the European Commission, integrative comprehension of fate and effects of contaminants in different compartments of these transitional environments (estuarine sediment, water, biota) is still required to be established. Although studies regarding metal transfer in estuarine ecosystem exist, no study in mangrove ecosystem was already done. This is the first *in situ* study with metal transfer in mangrove food web proposing a conceptual model for such transfer.

The role of aquatic food chains in transporting contaminants to top-chain fish deserves great attention as the trophic chain organisms may be a contamination source for humans. In this regard, we highlight the bioaccumulation processes, biomagnification and biodilution through food chain.

According to Luoma and Rainbow (2008), bioaccumulation is defined as the accumulation of a metal in an organism tissues, resulting from absorption of multiple sources such as environment and diet. The biomagnification process refers to chemical accumulation to a higher tissue concentration in a predator than occurred in its prey, with trophic transfer being the uptake mechanism. Thus, it is observed that trophic transfer of elements along the food chain can result in either biomagnification or biodilution processes, or even no changes in elemental concentrations (Revenga et al 2012).

These processes are extremely complex as well as the bioaccumulation, considering the absorption and regulation mechanisms of internal concentrations of some metals as well as the nutritional needs of some essential metals (cobalt, chromium, iron, manganese, nickel, molybdenum, selenium, tin and zinc) in metabolism and development of plants and animals (Azevedo, 2003; Azevedo and Chasin, 2004). In addition, the aquatic trophic chain usually presents more trophic levels than the terrestrial ones.

In Santa Cruz and Vitória Bay were possible to identify three metal/metalloid entry routes for resident organisms: (1) atmospheric compartment, derived for example, from air pollution including particulate matter from iron work and steel industry or ground resuspension; (2) aquatic compartment, including dissolved elements and resuspended material from sediment and, (3) sediment, which receive metallic loads from domestic sewage and industrial waste from differ contaminant sources, such as atmospheric, freshwater and coastal water. The source of such metal/metalloid may be both, natural and/or anthropic, due to the industrial contaminants released into these ecosystems.

Isotopic data (Souza, 2017, chapter 1) showed that the contaminants in mangrove areas seems to be more influenced by atmospheric extract as particulate material isotopic signature corresponded to those evaluated in the mangrove biota, in detriment of sediment and surface water. Small size particles contribute for increasing availability of metals as they are chronically released: atmospheric particulate matter deposition in tree tops, and the continuously deposition on the water increased exposure of all

organisms in these ecosystems that can absorb from the water surface or water column.

Among the two estuarine ecosystems evaluated, Santa Cruz mangrove ecosystem did not present anthropogenic nitrogen contamination. Regarding metals quantification performed in these two ecosystems, results from 2014 sampling are shown in Table 1S while results from 2015 sampling are shown in Table 2S. Results from linear regression of all species are shown in Table 3S and 4S.

Considering the nitrogen fertilizer input in Vitória Bay mangrove and its interference in metal transfer analysis in food chain, for the development of transfer model of B, Al, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Rb, Sr, Ag, Cd, Pb, Hg and Nb were used data from metals quantified in Santa Cruz in 2015 sampling. According to Wood et al (2012) classification, the metals Cu, Zn, Fe, Mn, Ni, Se, Cr and V were considered, in this study, as essential metals while, B, Al, As, Se, Rb, Sr, Ag, Cd , Pb, Nb, Ta and Hg as non-essential metals. Tantalum in all matrices was below quantification limit, then transfer model for this metal was not possible.

The three food chains identified by Souza (2017, chapter 1) were considered for development of metal transfer: plankton-oyster, plankton-shrimp-fish and plants-crab-fish. The relationships between the metals, food chains and bioaccumulation, biomagnification and biodilution processes are in Table 3S and 4S. In Santa Cruz mangrove ecosystem (2015), the plankton-oyster food chain presented metals biodilution of B, Al, V, Cr, Mn, Fe, Ni, Cu, As, Se, Rb, Sr, Pb, Nb and Hg and biomagnification of Zn, Ag, Cd (Fig. 2 and Table 3S). Similarly, the plankton-shrimp-fish food chain presented biodilution for most quantified metals such as B, Al, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Si, Rb, Sr, Pb, Nb, while Cd, Hg were biomagnified (Fig.3 and Table 3S). The transfer of Ag was not significant.

Regarding the higher bioaccumulation in phytoplankton, the interaction metal-plankton may occur by two ways favouring bioaccumulation: external adsorption of the metal to the cell wall which provides significant area for adsorption and, uptake through the cell wall into the cell (Broek et al.,

1980). Furthermore, the cell division process and new cells production, metal content increases linearly due to production of new reactive surface material (Fisher et al., 1986).

Isotopic analyses showed that mangrove plants have greater influence of seawater from which directly absorb nutrients and/or contaminants (Souza et al 2017, chapter 1). In general, they have several characteristics that minimizes metal absorption present in the sediment, such as iron plate (Liu et al. 2009; Machado et al. 2005), epidermis and endodermis barriers, at the root (MacFarlane and Burchett 2000) and leaves excretion. However, they may absorb contaminants through leaf due atmospheric particulate matter deposition on it (Arrivabene et al., 2015) and provide substrate for crabs which feed the leaves (McLusky and Elliott, 2004). The metal transfer data for plant-crab-fish food chain indicates biomagnification potential; Cr, Zn, As, Rb, Ag, Cd, Pb, Nb, Hg presented biomagnification while B, Al, Mn, Fe, Sr, biodilution and V, Ni, Cu did not present biomagnification or biodilution (Fig. 4 and Table 3S). Metal translocation by plants could become the metals more bioavailable to next trophic level. Nevertheless, in relation to plankton, due to its habitat in the water column, particles may adsorb to them without internal bioaccumulation leading to decreasing bioavailability. However, further studies are needed to ascertain this hypothesis.

On the other hand, metal bioaccumulation in marine fish depends on physiological process for salt excretion as seawater teleost fishes are hypotonic (Grosell et al., 2007), drink saltwater, produce small amounts of concentrated urine and excrete ions through gills (Wood et al., 2012). Estuarine resident organisms as *C. parallelus* during juvenile stage, metal accumulation may interfere on the enantiostasis process in order to keep homeostasis; they change the properties of interface membranes (transporters, channels, and lipid composition) (Wright, 1995) and also adapt cytosolic enzymes activities (Monserrat et al., 2007) changing the metal availability for the organism (Drexler et al., 2003).

In all trophic chains analysed, Cd exhibited biomagnification in the top-chain consumer, *C.*

parallelus. In the estuaries, Cd remains in Cd-Cl complexes and salinity influencing the bioavailability of this metal; lower salinity lead to free Cd ion formation and consequently higher bioavailability. Cd uptake by estuarine resident organisms occurs by Ca channels facilitated by the similarity of the ionic radius of these metals (Luoma and Rainbow, 2008). Cd affects negatively organs and systems such as the kidney, heart, bones, gonad, brain and central nervous system and development (Castro-González and Méndez-Armenta, 2008). Thus, although the Cd levels in *C. parallelus* muscle were below the maximum intake limit recommendation by the USEPA (2009), monitoring of this metal is necessary since the consumption of this fish is very appreciated. Conversely, B, Al, Mn, Fe and Sr were biodiluted in all analysed trophic chain.

Although mangrove areas occupy almost the entire coastal extension of tropical and subtropical regions, monitoring programs and norms directed specifically to these ecosystems are scarce and usually based on preexisting models for temperate estuaries. Figure 5 shows a conceptual model based in a summarize of metal transfer results and contamination source (Souza, 2017 chapter 1). This research presents novel data for science and for police and government decisions involving these ecosystems highlighting the atmospheric particulate matter as the main source of contaminants input in the trophic chain and the plant-crab-fish chain as the one with the mayor metal biomagnification potential. Although there is a biodilution in the plankton-shrimp-fish chain, the great exposure of plankton to metallic contaminants and their corresponding bioaccumulation deserve attention, as it may contribute to decrease these organisms that are considered the base of the marine ecosystem food chain.

ACKNOWLEDGE

This study was supported by Espírito Santo Research Foundation (FAPES), Brazil and, Science and Technology Office from Córdoba National University (CONICET), Argentina. The authors are thankful to Robert Mcknight and M. Thompson for supporting chemical analyses; I.C. Souza

acknowledges São Paulo Research Foundation (FAPESP, Proc. 2014/04832-3 and Proc. 2015/05258-1) for scholarship support.

REFERENCES

Arrivabene, H.P., Souza, I.C., Có, W.L.O., Conti, M.M., Wunderlin, D.A. & Milanez, C.R.D. 2015. Effect of pollution by particulate iron on the morphoanatomy, histochemistry, and bioaccumulation of three mangrove plant species in Brazil, Chemosphere 127, 27–34.

Azevedo, F.A.D. & Chasin, A.D.M. 2004. As bases toxicológicas da ecotoxicologia. São Carlos, SP: RiMa.

Azevedo, F.A.D. 2003. Toxicologia do mercúrio. In Toxicologia do mercúrio. RIMA/INTERTOX.

Barbier, Edward B. "The protective service of mangrove ecosystems: A review of valuation methods." Marine pollution bulletin 109.2 2016: 676-681.

Castro-González, M.I. & Méndez-Armenta, M. 2008. Heavy metals: Implications associated to fish consumption. Environmental toxicology and pharmacology, 2:3, 263-271.

Chapman, P.M. & Wang, F. 2000. Issues in ecological risk assessment of inorganic metals and metalloids. Human and Ecological Risk Assessment, 6:6, 965-988.

Chappaz, A., Lyons, T.W., Gordon, G.W. & Anbar, A.D. 2012. Isotopic fingerprints of anthropogenic molybdenum in lake sediments. Environmental science & technology, 46:20, 10934-10940.

Cui, B., Zhang, Q., Zhang, K., Liu, X. & Zhang, H. 2011. Analyzing trophic transfer of heavy metals for food webs in the newly-formed wetlands of the Yellow River Delta, China. Environmental Pollution, 159:5, 1297-1306.

Drexler, J., Fisher, N., Henningsen, G., Lanno, R., McGeer, J., Sappington, K. & Beringer, M., 2003, Issue paper on the bioavailability and bioaccumulation of metals. In US Environmental Protection Agency, Risk Assessment Forum, Washington, DC., USA.

Fisher, N.S. 1986. On the reactivity of metals for marine phytoplankton. Limnology and Oceanography, 31:2, 443-449.

Gazeta, 2016. http://especiais.gazetaonline.com.br/aterro/ [Access: 08/02/2017].

Giri, C., Ochieng, E., Tieszen, L.L., Zhu, Z., Singh, A., Loveland, T., Masek, J. & Duke, N., 2011. Status and distribution of mangrove forests of the world using earth observation satellite data. Global Ecology and Biogeography, 20:1, 154-159.

Grosell, M., Blanchard, J., Brix, K. V., & Gerdes, R. 2007. Physiology is pivotal for interactions between salinity and acute copper toxicity to fish and invertebrates. Aquatic toxicology, 84(2), 162-172.

Guo, B., Jiao, D., Wang, J., Lei, K. and Lin, C. 2016. Trophic transfer of toxic elements in the estuarine invertebrate and fish food web of Daliao River, Liaodong Bay, China. Marine Pollution Bulletin, *113*:1, 258-265.

Hartl, M.G. 2013. Homeostasis and Toxicology of non-essential metals- Edited by CM Wood, AP Farrell & CJ Brauner. Journal of Fish Biology, *83*:5, 1476-1477.

Liu, Y., Tam, N.F.Y., Yang, J.X., Pi, N., Wong, M.H., Ye, Z.H. 2009. Mixed heavy metals tolerance and radial oxygen loss in mangrove seedlings. Mar. Pollut. Bull. 58, 1843–1849.

Luoma, S.N., Rainbow, P.S., 2008. Metal contamination in aquatic environments: science and lateral management, first ed. Cambridge University Press, Cambridge.

MacFarlane, G.R., Burchett, M.D. 2000. Cellular distribution of copper, lead and zinc in the grey mangrove, Avicennia marina (Forsk.) Vierh. Aquatic Botany 68, 45-59.

Machado, A.A.S., Spencer, K., Kloas, W., Toffolon, M., Zarfl, C. 2016. Metal fate and effects in estuaries: a review and conceptual model for better understanding of toxicity. Science of the Total Environment 541, 268-281.

Machado, W., Gueiros, B.B., Lisboa-Filho, S.D., Lacerda, L.D. 2005. Trace metals in mangrove seedlings: role of iron plaque formation. Wetlands Ecol. Manage. 13, 199–206.

McLusky, D.S. & Elliott, M., 2004. The estuarine ecosystem: ecology, threats and management. Oxford University

Press on Demand.

Monserrat, J.M., Martínez, P.E., Geracitano, L.A., Amado, L.L., Martins, C.M.G., Pinho, G.L.L., Chaves, I.S., Ferreira-Cravo, M., Ventura-Lima, J. & Bianchini, A. 2007. Pollution biomarkers in estuarine animals: critical review and new perspectives. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 146:1, 221-234.

Revenga, J.E., Campbell, L.M., Arribére, M.A. & Guevara, S.R. 2012. Arsenic, cobalt and chromium food web biodilution in a Patagonia mountain lake. Ecotoxicology and environmental safety, 81, 1-10.

USEPA, 2009. Risk-Based Concentration Table. Philadelphia PA. United States Environmental Protection Agency, Washington, DC.

Valiela, I., Bowen, J.L. & York, J.K. 2001. Mangrove Forests: One of the World's Threatened Major Tropical Environments: At least 35% of the area of mangrove forests has been lost in the past two decades, losses that exceed those for tropical rain forests and coral reefs, two other well-known threatened environments. *Bioscience*, *51*:10, 807-815.

Wolanski, E., Elliott, M., 2015. Estuarine Ecohydrology: An Introduction. Elsevier, Whatham, 322p.

Wood, C.M., Farrell, A.P. & Brauner, C.J. 2012. Homeostasis and toxicology of essential metals. Vol. 31. Academic Press.

Wright, D.A. 1995. Trace metal and major ion interaction in aquatic animals. Mar. Pollut. Bull. 31, 8–18.

Zhang, C., Yu, Z.G., Zeng, G.M., Jiang, M., Yang, Z.Z., Cui, F., Zhu, M.Y., Shen, L.Q. & Hu, L. 2014. Effects of sediment geochemical properties on heavy metal bioavailability. Environment international, 73, 270-281.

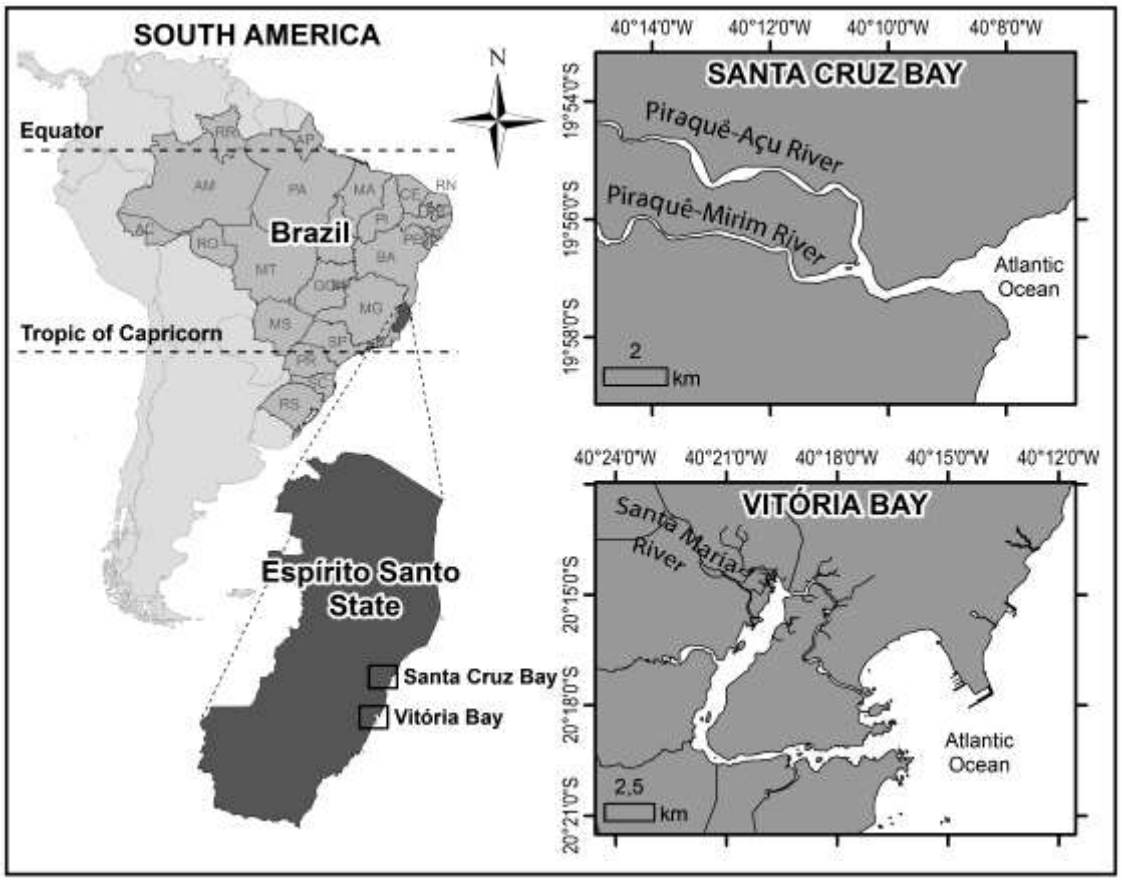


Figure 1. Location of the State of Espírito Santo (South America, Brazil), showing sampling sites. Santa Cruz (S 19º56`26.2``; W 40º 12`87``) and Vitória Bay (S 20º14`31.5``; W 40º19`84.7``).

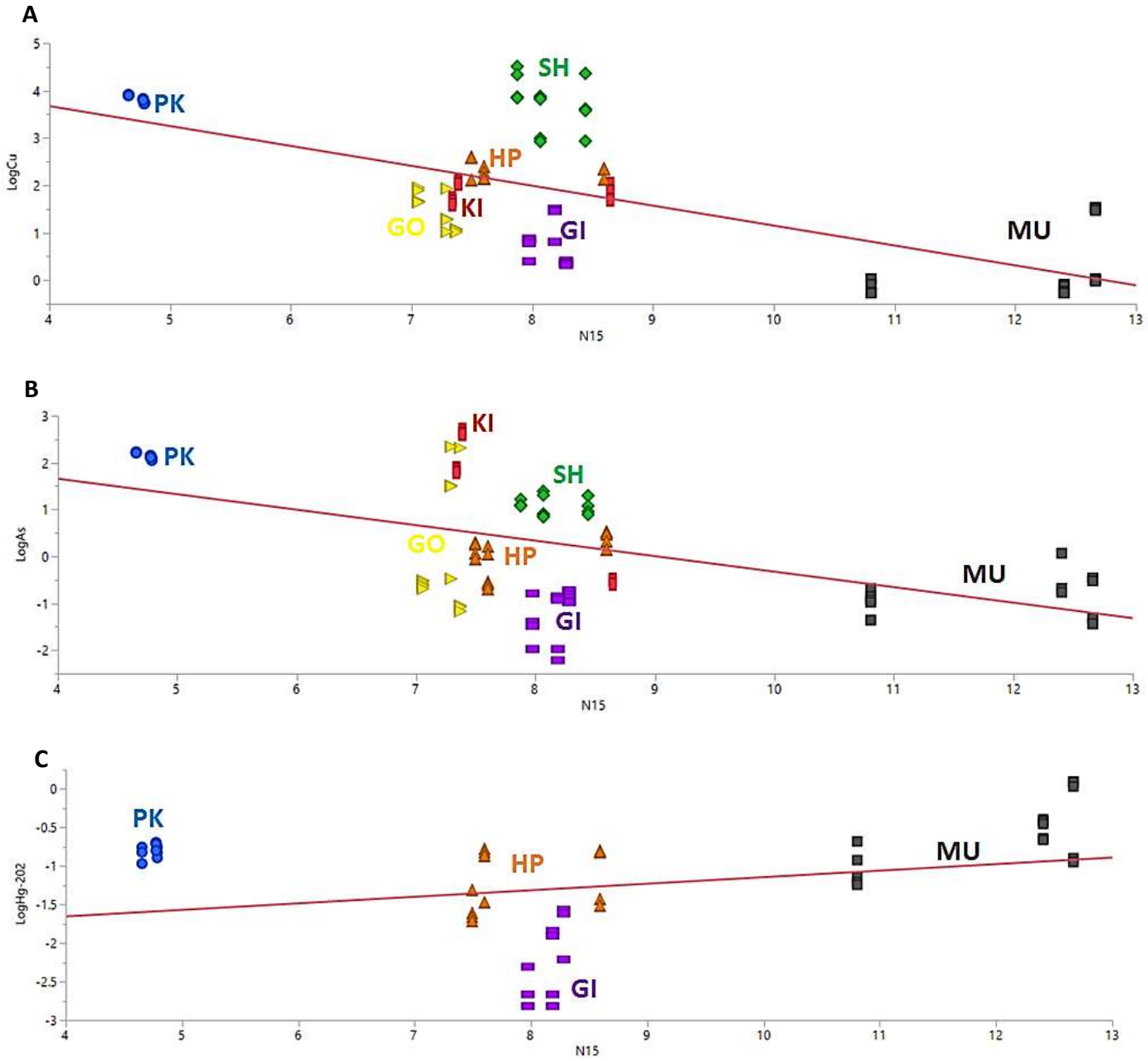


Figure 2. Relationship between Log[µg g−1 dry weight] versus $\delta^{15}N$ for plankton - shrimp (*Macrobranchium sp.*) - fish (*Centropomus parallelus*) trophic chain in Santa Cruz. A) Cu biodilution throughout studied food web; B) As biodilution throughout studied food web; (C-D) Hg-202 biomagnification throughout studied food web.

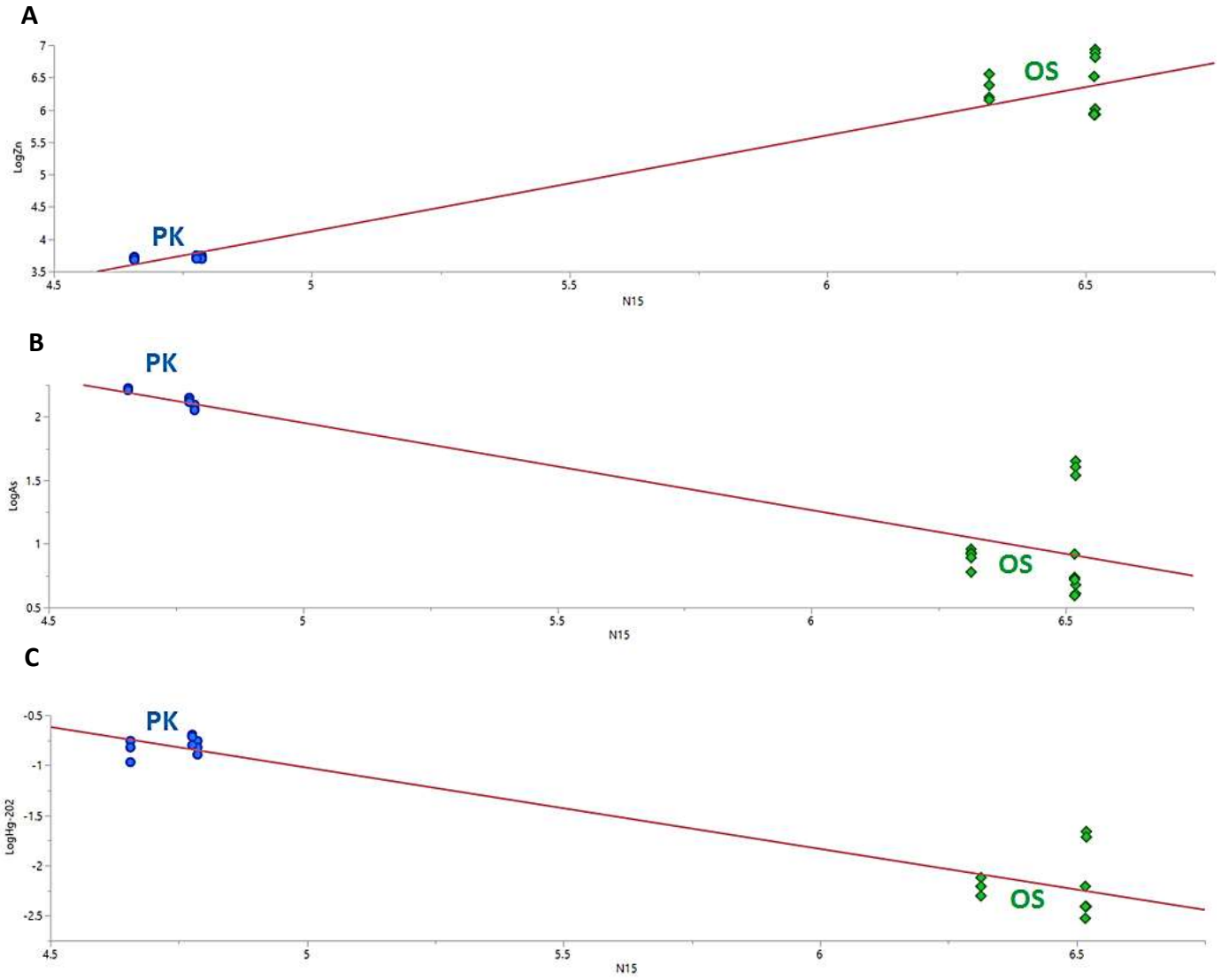


Figure 3. Relationship between Log[µg g−1 dry weight] versus $\delta^{15}N$ for plankton – oyster (*Crassostrea rhizophorae*) trophic chain in Santa Cruz. A) Zn biomagnification throughout studied food web; B) As biodiluition throughout studied food web; C) Hg-202 biodilution throughout studied food web.

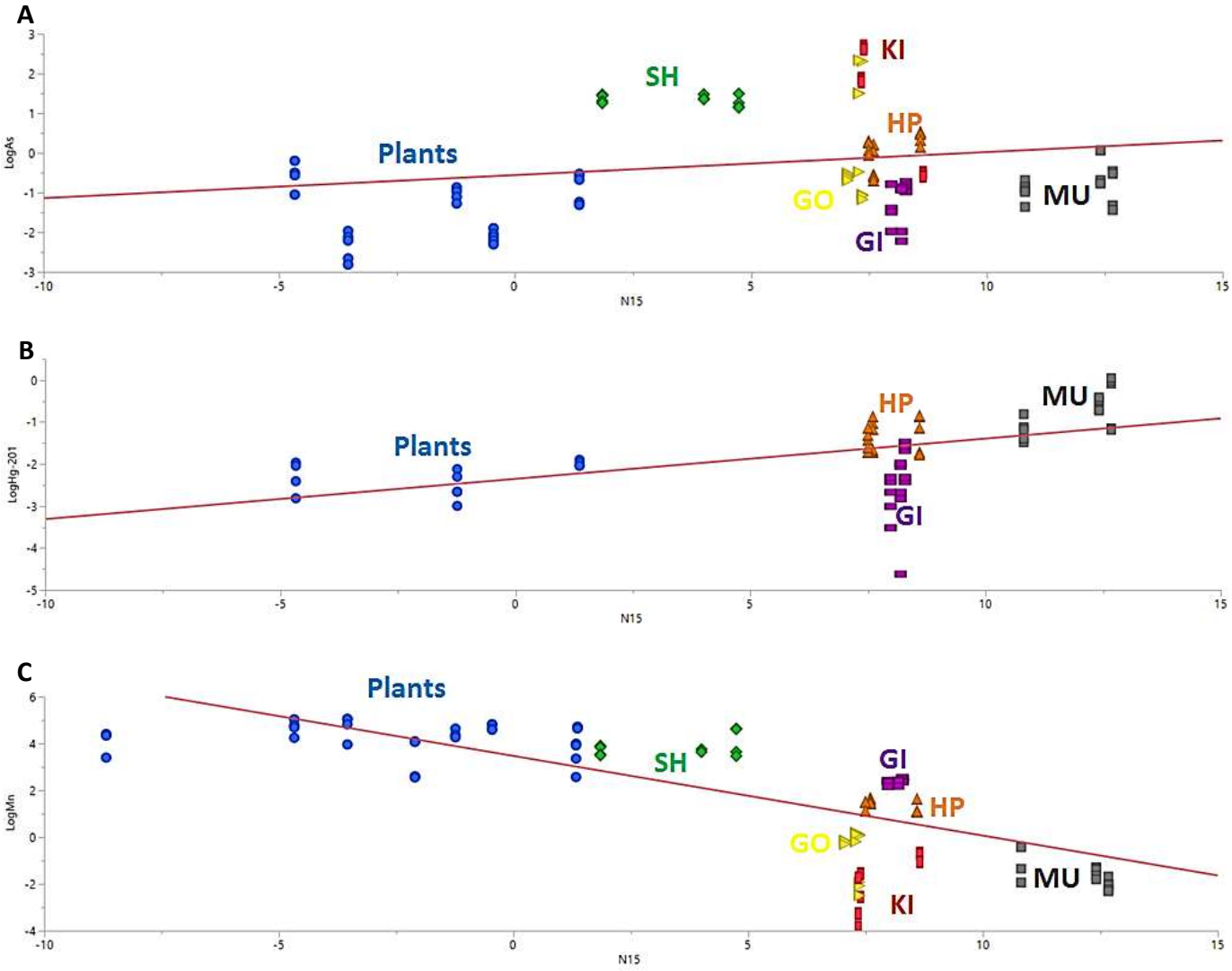


Figure 4. Relationship between Log[µg g−1 dry weight] versus $\delta^{15}N$ for mangrove plants (*Rhizophora mangle*, *Laguncularia racemosa* and *Avicennia shaueriana*) – crab (*Aratus* sp.) – fish (*Centropomus paralllelus*) trophic chain in Santa Cruz. A) As biomagnification throughout studied food web; B) Hg-201 biomagnification throughout studied food web; C) Mn biodilution throughout studied food web.

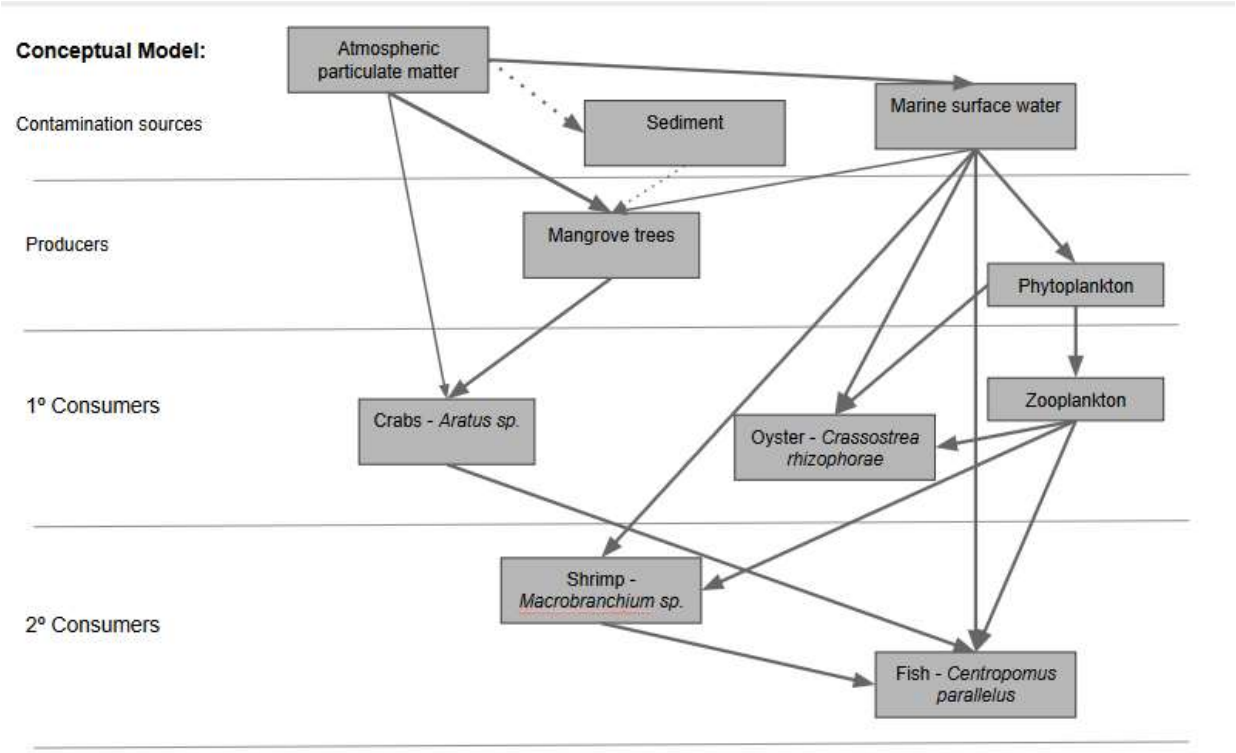


Figure 5. Mangrove food web conceptual model. The arrows indicate the relationship; dotted arrows indicate lower influence than lines.

Table 1S. Measurements of B, Al, V, Cr-52, Cr-53, Mn, Fe-56, Fe-57, Ni, Cu, Zn, As, Se, Rb, Sr, Ag, Cd, Hg-201, Pb and Hg-202 in abiotic and biotic compartments of the estuaries Santa Cruz and Vitoria Bay. Values correspond to median (First-third quartile) µg/g dry weigh. Lowercase letters indicate significant differences between compartments in the same estuary (Kruskal-Wallis test, P < 0.05). * indicate the highest median with significant difference for the same parameter between both estuaries (Student's t-test, P < 0.05). SW: surface water, SD: sediment, PK: plankton, CR: Crab (*Aratus* sp.), SH: shrimp (*Macrobrachium* sp.), GI: gill of fish (*Centropomus parallelus*), HP: hepatopancreas of fish, KI: kidney of fish, MU: muscle of fish, GO: gonad of fish. NA = Not analysed.

			Metals								
			B	Al	V	Cr-52	Cr-53	Mn	Fe-56	Fe-57	Ni	Cu
2014	Santa Cruz	SW	<LOQ	879.80 (647.00-1518.00)de	<LOD	<LOQ	NA	<LOD	<LOD	NA	<LOD	<LOQ
		SD	<LOD	96408.09 (86188.73-98906.50)f	100.35 (97.54-107.37)d	79.98 (77.20-83.51)d	NA	361.24 (304.82-389.19)g*	62264.08 (61837.41-66762.82)f	NA	28.19 (25.15-29.08)c	16.04 (15.25-29.08)de
		PK	<LOD	49367.45 (43211.96-59575.77)ef	66.20 (62.81-68.10)cd	42.01 (34.09-45.03)cd	40.26 (33.89-45.00)d	68.56 (57.07-78.97)fg	31947.92 (25912.33-32952.14)ef	31721.72 (26015.53-32849.26)e	<LOQ	<LOQ
		SH	<LOD	203.96 (131.05-401.41)cd	0.44 (0.34-0.50)a	0.38 (0.17-0.53)b*	0.39 (0.18-0.53)c*	5.61 (5.23-14.81)cd*	262.66 (179.88-356.56)bc	273.58 (203.32-374.18)c	<LOQ	38.01 (36.95-150.07)f*
		CR	<LOD	1825.84 (1470.67-3862.28)de*	2.02 (1.69-3.69)c*	1.50 (1.03-2.63)c*	1,46 (1.02-2.56)d*	45,16(37.80-54.30)ef	1036.96 (853.91-1908.40)cd*	1085.56 (903.70-1940.71)d*	1,39 (1,06-1,66)b	28.68 (27.92-31.56)ef
		MU	<LOD	4.67 (4.17-8.57)a	<LOQ	<LOQ	0.01 (0.01-0.02)a	0.92 (0.88-1.03)ab*	7.32 (4.56-9.17)a	10.22 (6.82-11.24)a	<LOQ	0.10 (0.03-0.22)a
		HP	<LOD	13.50 (12.19-14.19)ab	0.50 (0.38-090)ab*	0.09 (0.02-0.18)a	0.09 (0.02-0.19)b	6.85 (6.44-7.64)d*	2173.33 (1286.59-2816.02)de*	2161.68 (1254.36-2876.34)de*	<LOD	9.87 (9.64-10.87)cd*
		GI	<LOD	85.45 (73.68-96.69)bc	0.39 (0.37-0.43)a*	0.46 (0.22-0.58)b	0.44 (0.22-0.56)c	17.04 (12.73-18.47)de*	117.20 (98.11-136.78)ab*	185.55 (173.44-191.93)bc*	0.50 (0.43-0.59)a	1.08 (0.52-1.29)ab
		KI	<LOD	87.22 (64.25-64.25-96.05)bc*	0.61 (0.50-0.80)b*	0.42 (0.23-0.48)b*	0.42 (0.24-0.48)c*	1.96 (1.78-2.16)bc	1248.94 (1151.11-1282.96)cd*	1242.74 (1150.04-1271.69)d*	<LOQ	5.73 (5.26-6.56)bc*
		GO	<LOD	2.00 (1.29-2.24)a	<LOQ	0.03 (0.01-0.05)a	0.02 (0.01-0.05)ab	0.20 (0.11-0.29)a	12.72 (4.58-38.14)a	13.13 (4.44-36.02)ab	<LOD	0.25 (0.15-1.22)a
	Vitória Bay	SW	<LOQ	815.60 (772.60-1668.00)fg	<LOD	<LOQ	NA	<LOD	<LOQ	NA	<LOD	<LOQ
		SD	<LOD	94813.70 (83277.30-96518.29)h	109.23 (10.6.89-112.11)c*	84.37 (83.02-86.33)d*	NA	30.04 (26.52-30.82)ef	133616.50 (126862.76-134812.27)g*	NA	30.04 (26.52-30.82)c*	28.98 (22.94-60.88)d*
		PK	<LOD	78365.05 (66647.66-98505.51)gh*	82.44 (78.73-85.98)bc*	67.70 (64.97-70.24)cd*	66.06 (62.96-68.65)e*	361.14 (144.46-369.96)f*	60126.73 (57767.82-62273.14)fg*	60569.69 (57896.00-62208.44)e*	16.68 (15.37-20.75)b	31.04 (29.20-33.74)d
		SH	<LOD	269.26 (124.09-309.73)de	0.34 (0.18-0.53)a	0.20 (0.09-0.27)a	0.22 (0.09-0.27)ab	4.31 (3.21-7.20)bc	261.49 (112.22-318.43)c	271.22 (125.17-336.77)b	<LOQ	29.47 (24.20-34.97)d
		CR	<LOD	667.63(605.37-851.63)ef	0.99 (0.92-1.12)b	0.77 (0.55-1.01)bc	0.76 (0.54-0.98)de	61.33 (59.26-62.83)de*	632.52 (541.96-762.15)de	736.69 (637.24-840.81)cd	0.40 (0.32-0.45)a	28.42 (27.79-29.59)d
		MU	<LOD	9.56 (5.84-10.84)ab*	<LOQ	0.17 (0.11-0.19)a	0.16 (0.11-0.18)a*	0.60 (0.53-0.83)a	10.64 (8.40-13.13)a*	11.20 (8.48-16.57)a	<LOQ	0.95 (0.33-1.40)a*
		HP	<LOD	11.12(8.87-17.87)ab	0.30 (0.21-0.33)a	0.33 (0.28-0.35)b*	0.32 (0.13-0.38)bc*	4.27 (4.14-4.62)bc	1178.90 (1160.30-1457.58)ef	1165.14 (1143.92-1450.52)de	<LOD	9.02 (8.57-9.80)c
		GI	<LOD	110.62 (69.46-129.95)cd	0.31 (0.30-0.32)a	0.43 (0.30-0.32)b	0.41 (0.31-0.60)cd	11.24 (10.27-13.68)cd	151.53 (129.54-235.23)bc	206.85(186.30-280.62)b	<LOQ	1.36 (1.26-1.59)ab*
		KI	<LOD	18.11 (17.32-20.06)bc	0.34 (0.25-0.52)a	0.09 (0.04-0.14)a	0.13 (0.06-0.21)a	2.21 (1.84-2.31)ab	549.25 (236.50-691.44)cd	531.01 (228.30-675.80)bc	<LOD	5.06 (3.19-5.36)bc
		GO	<LOD	7.02 (6.02-7.76)a*	<LOQ	0.15 (0.03-0.20)a*	0.15 (0.04-0.19)a*	0.17 (0.10-0.59)a	21.13 (10.62-99.13)ab	20.37 (10.81-98.39)a	<LOD	0.65 (0.52-3.96)ab*

(cont.)

			Metals									
			Zn	As	Se	Rb	Sr	Ag	Cd	Hg-201	Pb	Hg-202
2014	Santa Cruz	SW	71.88 (68.78-72.76)d	<LOD	<LOQ	102.65 (101.47-105.57)f*	7048.76 (6986.76-7356.76)g*	<LOD	<LOD	<LOD	<LOD	<LOD
		SE	61.22 (48.43-68.08)abc	39.23 (36.95-40.29)d	<LOQ	29.60 (27.95-31.66)ef*	136.15 (133.28-156.66)de*	<LOD	<LOD	<LOD	29.05 (28.35-30.25)c	<LOD
		PK	<LOQ	<LOD	<LOQ	16.50 (13.64-17.42)e	68.54 (57.23-71.80)cd	<LOD	<LOD	<LOQ	22.35 (19.69-29.84)c	<LOQ
		SH	73.66 (50.08-86.21)cd	2.45 (1.79-3.48)b	1.97 (1.53-2.31)b	4.10 (3.95-4.24)cd	466.15 (447.74-742.94)ef	0.21 (0.05-0.44)	<LOD	0.76 (0.15-1.14)cd	<LOQ	0.77 (0.15-1.15)cd*
		CR	80.71 (56.76-95.68)d*	5.13 (4.90-5.51)c*	1.43 (1.26-1.65)a	3.41 (3.25-3.49)bc*	1283.53 (1199.19-1314.65)fg	<LOD	<LOD	0.97 (0.43-1.40)d	0.59 (0.46-1.22)b*	0.95 (0.41-1..41)d
		MU	35.65 (32.27-58.50)a*	<LOQ	1.50 (1.21-1.87)ab	4.54 (4.36-5.43)de	13.29 (12.05-18.34)bc	<LOD	<LOD	0.15 (0.12-1.64)bc	<LOD	0.16 (0.13-1.56)bc
		HP	66.61 (57.27-73.15)cd	2.52 (1.30-3.43)ab*	5.79 (5.35-7.64)c	2.56 (2.39-2.65)ab	3.50 (2.75-4.22)ab	<LOD	<LOD	0.74 (0.13-0.98)bcd	<LOD	0.75 (0.12-1.01)bcd
		GI	65.08 (53.84-68.37)bcd	<LOQ	1.26 (1.15-1.42)a	1.07 (1.01-1.13)a	614.59 (485.98-674.13)ef*	<LOD	<LOD	<LOQ	0.27 (0.18-0.34)a	<LOQ
		KI	761.28 (688.47-838.27)e*	8.20 (6.17-12.94)c*	6.49 (5.34-7.29)c	2.64 (2.41-2.78)ab	8.79 (8.28-9.10)ab*	<LOD	<LOD	0.14 (0.06-0.34)a	<LOQ	0.13 (0.05-0.34)a
		GO	10.51 (2.14-73.29)ab	1.39 (0.60-1.70)a	<LOQ	1.14 (0.12-6.43)ab	1.50 (0.74-3.32)a	<LOD	<LOD	0.30 (0.17-0.37)ab	<LOD	0.30 (0.16-0.36)ab
	Vitória Bay	SW	<LOQ	<LOD	<LOQ	79.67 (77.83-83.89)e	5412.76 (5280.76-5488.76)f	<LOD	<LOD	<LOD	<LOD	<LOD
		SE	141.95 (137.47-165.28)de*	54.03 (53.79-55.65)d*	<LOQ	21.77 (20.78-22.61)e	87.08 (80.05-89.87)bc	<LOD	<LOD	<LOD	31.59 (28.80-36.73)b*	<LOD
		PK	<LOQ	19.75 (19.20-21.09)d	<LOQ	22.64 (21.50-24.81)e*	106.21 (98.69-110.49)cd*	<LOQ	<LOD	0.49 (0.39-1.56)bc	123.17 (117.27-130.45)c*	0.42 (0.26-1.46)bc
		SH	65.64 (53.90-76.37)b	3.12 (2.69-3.30)c	2.33 (2.08-2.71)bc*	4.93 (4.81-5.24)d*	434.48 (253.43-503.21)de	0.17 (0.09-0.224)a	<LOD	1.27 (0.57-1.99)cd	<LOQ	<LOQ
		CR	42.98 (39.47-45.97)a	2.93 (2.83-3.04)c	2.54 (2.25-2.85)c*	2.96 (2.88-3.10)ab	1956.12 (1885.87-2014.65)ef*	0.26 (0.25-0.30)b	<LOD	1.30(1.11-1.49)d*	0.28 (0.27-0.33)a	1.29 (1.13-1.48)d*
		MU	29.46 (7.81 37.55)a	<LOQ	1.74 (1.61-2.04)a	5.01 (4.24-5.31)cd	7.00 (3.64-13.60)ab	<LOD	<LOD	0.30 (0.28-0.334)b	<LOD	0.30 (0.29-0.34)b
		HP	73.43 (65.05-76.85)bc*	1.37 (0.97-1.47)a	6.50 (6.13-6.97)d	3.76 (3.68-3.84)bc*	2.58 (2.03-4.01)a	<LOD	<LOD	1.04 (0.87-1.28)cd*	<LOD	1.02 (0.88-1.28)cd*
		GI	88.44 (76.86-101.98)cd*	<LOQ	1.91 (1.78-2.21)ab*	1.78 (1.69-2.67)a*	395.43 (358.91-430.44)de	<LOD	<LOD	0.02 (0.01-0.05)a	0.22 (0.19-0.35)a	0.02 (0.01-0.04)a
		KI	545.30 (317.74-575.63)e	0.94 (0.27-1.49)a	6.41 (3.84-6.99)d	4.46 (3.51-4.64)cd*	3.40 (3.33-4.91)a	<LOD	<LOD	1.02 (0.31-1.99)cd*	<LOD	1.01 (0.31-1.98)cd*
		GO	<LOQ	2.29 (2.25-2.33)b*	<LOQ	0.16 (0.14-0.36)a	3.81 (2.64-7.01)a*	<LOD	<LOD	0.67 (0.58-1.31)cd*	<LOD	0.67 (0.58-1.28)cd*

Table 2S. Measurements of B, Al, V, Cr-52, Cr-53, Mn, Fe-56, Fe-57, Ni, Cu, Zn, As, Se, Rb, Sr, Ag, Cd, Hg-201, Pb and Hg-202 in abiotic and biotic compartments of the estuaries Santa Cruz and Vitoria Bay. Values correspond to median (First-third quartile) µg/g dry weigh. Lowercase letters indicate significant differences between compartments in the same estuary (Kruskal-Wallis test, P < 0.05). * indicate the highest median with significant difference for the same parameter between both estuaries (Student's t-test, P < 0.05). SW: surface water, SD: sediment, PK: plankton, RI: *Rhizophora mangle*, LA: *Laguncularia racemosa*, AV: *Avicennia schaueriana*, CR: Crab (*Aratus* sp.), SH: shrimp (*Macrobrachium* sp.), GI: gill of fish (*Centropomus parallelus*), HP: hepatopancreas of fish, KI: kidney of fish, MU: muscle of fish, GO: gonad of fish.

			Metals									
			B	Al	V	Cr-52	Cr-53	Mn	Fe-56	Fe-57	Ni	Cu
2015	Santa Cruz	SW	4071,74 (4026,66-4169,83)h*	>LOD	>LOD	>LOD	>LOD	>LOD	>LOD	>LOD	>LOD	>LOD
		SD	48,11 (47,93-57,68)gh	49748,92 (47293,28-60996,72)f*	69,11 (68,54-85,42)g*	52,76 (51,76-56,91)d	48,77 (48,06-49,50)d	207,18 (187,60-211,13)e	42523,17 (41292,83-52667,74)h	42270,56 (41889,39-53205,25)e	14,58 (14,07-14,68)d	<LOQ
		PK	719,03 (700,11-859,77)h	23769,45 (22644,63-24318,75)f*	24,88 (24,52-27,50)fg	22,07 (20,54-22,46)cd*	21,82 (20,55-22,39)cd*	164,86 (160,77-182,22)e	14634,26 (14011,99-15521,99)gh	14548,28 (13809,59-15663,08)de	7,95 (7,82-8,08)d*	44,70 (42,64-49,13)f
		RI	44,36 (40,74-52,36)gh*	25,21 (17,97-42,35)bc	0,20 (0,10-0,25)ab	>LOQ	0,16 (0,06-0,32)a	102,09 (52,63-125,29)de	57,73 (40,20-71,94)ab	55,50 (39,51-72,78)a	>LOQ	>LOQ
		LA	21,80 (20,58-25,98)efg	85,98 (27,29-94,18)c	0,20 (0,16-0,24)ab	>LOQ	0,20 (0,11-0,35)ab	50,70 (28,80-60,14)cd	179,78 (161,17-235,80)cd*	180,64 (164,33-233,49)b*	0,07 (0,04-0,08)ab	1,30 (1,12-1,50)a*
		AV	39,94 (38,32-45,04)fgh	130,76 (95,83-216,36)cd	0,27 (0,14-0,47)b	0,03 (0,01-0,14)a	0,35 (0,07-0,40)ab	102,91 (74,29-108,81)de	165,33 (126,41-313,18)cde	168,54 (123,31-313,67)b	0,03 (0,02-0,08)ab*	1,11 (0,95-1,21)a
		OS	9,45 (5,98-10,11)cde	331,54 (189,23-496,90)de	0,53 (0,43-0,76)cd	0,41 (0,19-0,48)b	0,54 (0,32-0,62)b	8,88 (6,10-11,67)b	277,07 (249,74-391,11)def	278,39 (250,23-392,34)bc	0,31 (0,25-0,33)c*	8,16 (6,94-11,95)cde
		SH	5,64 (4,99-6,85)cd	712,15 (490,94-828,03)ef*	1,12 (0,76-1,23)def*	6,04 (1,24-7,84)c*	6,39 (1,64-7,97)c*	6,15 (5,59-7,07)b*	888,73 (567,99-923,41)fg*	892,22 (593,98-942,59)cd*	2,16 (0,57-2,99)d	46,29 (35,75-48,37)f
		CR	16,42 (15,40-16,89)def*	995,73 (566,67-1255,74)ef*	1,22 (0,91-1,72)efg*	1,62 (1,38-1,81)c*	1,89 (1,67-2,06)c*	42,08 (38,03-49,72)cd*	529,58 (335,26-718,54)fg*	576,73 (385,75-757,63)cd*	1,79 (1,64-2,05)d	23.18 (22,41-27,71)ef
		MU	0,83 (0,78-0,99)ab	>LOQ	>LOQ	>LOQ	>LOQ	0,19 (0,14-0,28)a*	7,31 (3,47-7,62)a	7,15 (4,60-7,80)a	>LOQ	0,92 (0,78-1,04)a*
		HP	0,48 (0,47-0,86)a	6,78 (6,38-9,77)ab	0,88 (0,85-0,99)cde*	>LOQ	>LOQ	4,28 (3,08-4,53)ab*	1393,69 (968,97-1646,99)gh	1406,38 (978,65-1640,63)de	0,03 (0,02-0,03)a	10,31 (8,45-11,00)de
		GI	2,74 (2,71-3,08)bc*	134,58 (30,53-165,32)cd	0,40 (0,37-0,45)bc*	0,37 (0,35-0,70)b	0,34 (0,30-0,65)b	11,21 (10,75-11,69)bc*	129,92 (69,05-143,00)bc	185,47 (119,21-197,84)b	0,21 (0,19-0,44)c	2,25 (1,46-2,44)ab
		KI	5,28 (2,74-7,00)cd*	36,15 (9,89-252,37)bc	1,01 (0,56-1,29)def*	>LOQ	>LOQ	0,21 (0,08-0,35)a	1092,20 (149,37-1467,21)efg	1070,85 (140,79-1457,01)cd	>LOQ	6,65 (5,53-7,74)bcd
		GO	0,79 (0,67-1,39)ab	2,14 (1,22-4,51)a*	0,03 (0,01-0,04)a	0,07 (0,06-1,53)ab	0,14 (0,13-1,53)ab	0,86 (0,74-1,13)a	68,40 (55,14-108,89)ab*	64,62 (51,61-105,63)a*	0,14 (0,02-0,66)bc	3,58 (2,77-5,31)bc*
	Vitória Bay	SW	3801,26 (3772,42-3845,64)h	>LOD	>LOD	>LOD	>LOD	>LOD	>LOD	>LOD	>LOD	>LOD
		SD	51,61 (49,58-53,26)fgh	35641,77 (34217,46-36404,69)f	52,36 (50,68-53,04)f	99,41 (74,94-135,42)c*	94,17 (64,27-111,32)e*	179,45 (169,36-207,04)f	96282,74 (76719,74-101266,82)g*	96522,24 (78784,57-101251,49)f*	34,68 (26,16-37,55)g*	>LOQ
		PK	930,45 (868,83-996,8B0)gh	16730,18 (16037,39-17329,74)f	28,00 (27,42-29,13)ef*	14,82 (14,54-15,64)c	14,79 (14,50-15,72)de	202,55 (196,14-210,96)f*	13665,13 (13435,70-14366,27)fg	13579,98 (13418,91-14454,87)f	6,11 (5,99-6,18)f	>LOQ
		RI	40,48 (39,12-41,16)efg	72,94 (51,91-97,32)cd*	0,14 (0,12-0,16)a	>LOQ	0,11 (0,10-0,15)a	184,99 (127,00-197,75)f*	71,11 (69,56-84,16)bc	74,08 (70,48-88,22)ab*	0,10 (0,03-0,50)bc	>LOQ
		LA	29,66 (26,91-30,31)cde*	62,11 (56,76-67,38)cd	0,19 (0,14-0,22)ab	>LOQ	0,15 (0,10-0,17)a	31,91 (30,81-43,87)cde	115,67 (103,93-117,93)cd	120,26 (105,74-124,71)b	0,03 (0,02-0,03)ab	1,01 (0,91-1,24)ab
		AV	44,53 (40,62-53,59)fgh*	177,11 (142,93-202,78)de	0,36 (0,24-0,41)cd	0,03 (0,02-0,10)a	0,38 (0,22-0,58)b	91,01 (81,71-142,26)ef	203,76 (168,43-225,84)de	199,93 (167,49-220,70)cd	0,34 (0,25-0,48)c*	1,99 (1,16-2,49)bc*
		OS	39,03 (26,06-45,17)def*	637,55 (602,43-736,56)ef*	1,33 (1,14-1,68)ef*	0,89 (0,78-0,98)b*	1,21 (1,00-1,28)cd*	80,62 (40,87-81,71)de*	749,75 (622,95-825,12)ef*	766,42 (625,98-839,49)de*	1,92 (1,11-2,03)ef*	28,93 (24,08-40,04)e*
		SH	6,60 (5,97-8,82)bc	102,16 (65,64-115,99)cd	0,21 (0,14-0,35)bc	0,39 (0,33-0,49)b	0,89 (0,60-0,96)bc	2,92 (2,63-3,20)ab	116,86 (75,38-180,90)cd	128,21 (88,81-188,59)bc	0,29 (0,21-0,35)cd	33,08 (26,48-42,64)e
		CR	14,26 (13,92-14,45)bcd	297,04 (214,68-316,32)ef	0,52 (0,48-0,69)de	0,47 (0,44-0,65)b	0,67 (0,58-1,03)bc	33,52 (29,18-38,61)cd	221,22 (161,15-277,49)de	272,46 (210,17-315,75)d	0,70 (0,64-0,90)de	33,55 (29,55-35,20)e*
		MU	0,78 (0,68-0,82)a	>LOQ	>LOQ	0,03 (0,03-0,04)ab	>LOQ	0,13 (0,11-0,17)a	1,44 (1,30-3,63)a*	>LOQ	0,02 (0,01-0,02)ade	0,83 (0,52-0,85)e
		HP	0,87 (0,40-1,04)a	4,68 (3,12-12,54)a	0,24 (0,11-0,27)ab	>LOQ	>LOQ	2,74 (2,53-3,98)ab	1591,10 (1325,04-1739,95)fg	1611,82 (1334,42-1759,42)ef	>LOQ	10,63 (9,01-11,00)d
		GI	2,13 (2,00-2,26)ab	41,82 (30,10-46,91)bc	0,08 (0,05-0,18)a	0,33 (0,26-0,43)b	0,30 (0,22-0,40)bc	8,89 (8,30-9,47)bc	77,51 (74,14-90,08)c	121,44 (111,95-129,85)bc	0,16 (0,16-1,37)cde	1,53 (1,37-3,03)bcd
		KI	1,78 (1,50-2,87)ab	9,97 (6,28-44,32)ab	0,31 (0,19-0,34)bc	>LOQ	>LOQ	0,73 (0,66-2,48)2,48)a*	517,15 (486,65-913,77)efg	501,43 (469,12-900,86)def	>LOQ	4,76 (4,35-9,37)cd
		GO	>LOQ	4,83 (3,15-8,61)a*	>LOQ	>LOQ	>LOQ	>LOD	13,90 (10,33-16,78)ab	12,76 (9,57-17,44)a	>LOQ	0,45 (0,38-1,01)a*

(cont.)

			Metals										
			Zn	As	Se	Rb	Sr	Ag	Cd	Pb	Nb	Hg-201	Hg-202
2015	Santa Cruz	SW	>LOD	>LOD	>LOQ	103.39 (100.62-105.29)h*	7460.44 (7333.13-7511.46)f*	>LOD	6.56 (6.50-6.75)e	>LOD	>LOD	>LOD	>LOD
		SD	>LOQ	26.37 (25.61-33.23)h	>LOD	24.90 (24.08-27.08)gh*	111.24 (106.38-116.41)cd	>LOD	>LOD	22.07 (21.86-26.67)e*	0.69 (0.61-0.99)cd	>LOQ	2.59 (2.11-18.34)c
		PK	40.98 (40.34-41.60)bcd	8.42 (8.14-9.10)gh	>LOQ	11.58 (11.46-11.61)fgh*	900.60 (872.23-965.08)def	0.07 (0.06-0.08)b	0.02 (0.01-0.02)bc	11.96 (11.40-12.49)de*	0.73 (0.70-0.88)d	0.33 (0.30-0.40)b	0.45 (0.44-0.47)bc
		RM	>LOQ	0.11 (0.06-0.12)a	>LOD	1.52 (1.28-1.82)ab	52.66 (46.68-78.27)bc	>LOQ	>LOD	>LOD	0.01 (0.01-0.02)ab*	>LOQ	>LOQ
		LR	9.71 (6.43-10.26)ab	>LOQ	>LOD	1.16 (0.99-1.30)a	84.64 (66.76-107.98)c	>LOD	0.01 (0.01-0.02)b	>LOQ	>LOQ	>LOQ	>LOQ
		AV	8.69 (8.29-10.70)a	0.38 (0.33-0.57)bc*	0.66 (0.59-0.69)a	3.37 (3.20-3.75)cde	111.79 (103.63-120.13)cd*	0.04 (0.03-0.05)a	>LOQ	0.10 (0.07-0.11)a	0.01 (0.01-0.01)a	0.12 (0.07-0.14)a	>LOQ
		OS	491.08 (378.59-701.97)g	2.44 (2.05-2.61)def	1.58 (1.06-2.07)bc	3.18 (1.91-3.50)cd	13.98 (9.34-15.01)ab	0.30 (0.27-0.42)c*	0.15 (0.11-0.16)e	0.22 (0.16-0.34)b	0.02 (0.02-0.04)b	0.11 (0.08-0.13)a	0.11 (0.09-0.12)a
		SH	35.52 (34.11-42.01)cd	2.93 (2.48-3.38)defg	2.57 (1.84-2.74)cd	4.12 (3.95-4.60)def*	363.25 (344.54-421.44)de	0.07 (0.06-0.12)b	0.04 (0.03-0.05)cd	5.76 (5.22-6.30)cd*	0.06 (0.05-0.07)c*	>LOQ	>LOQ
		CR	56.98 (54.54-58.46)de	3.92 (3.51-4.36)fgh	1.30 (1.14-1.40)b	3.25 (2.86-3.44)cd*	1192.08 (1071.70-1465.43)ef	0.04 (0.03-0.04)a	0.02 (0.01-0.02)b	0.50 (0.27-0.53)bc*	0.07 (0.04-0.10)cd*	>LOQ	0.10 (0.06-0.13)a
		MU	12.29 (10.60-14.85)abc	0.51 (0.38-0.64)bc*	0.78 (0.44-0.99)a	3.60 (3.48-4.09)de	5.65 (4.46-15.01)a*	0.11 (0.03-0.53)b	0.01 (0.01-0.01)a	>LOQ	0.01 (0.01-0.02)ab*	0.49 (0.31-0.67)b	0.52 (0.39-0.68)bc
		HP	65.59 (63.40-76.33)ef	1.16 (0.95-1.38)cd	3.98 (3.71-4.25)de	2.73 (2.48-2.76)bc*	8.16 (7.70-10.10)a*	>LOD	0.06 (0.05-0.08)de	>LOQ	>LOQ	0.27 (0.18-0.36)b	0.27 (0.22-0.44)b
		GI	51.63 (45.74-57.07)de	0.39 (0.23-0.43)ab	0.64 (0.59-0.83)a	1.06 (0.98-1.11)a	549.38 (478.77-564.93)de*	>LOQ	0.01 (0.01-0.01)b	0.21 (0.20-0.27)b*	0.02 (0.01-0.02)ab	0.09 (0.06-0.14)a	0.11 (0.07-0.16)a
		KI	329.09 (213.17-693.75)fg	6.51 (0.62-13.72)efgh	10.65 (8.26-11.57)e	3.36 (1.33-6.06)cde	8.47 (3.86-13.07)a	>LOD	>LOD	>LOD	>LOD	>LOQ	>LOQ
		GO	289.00 (60.60-302.79)efg*	0.60 (0.50-4.54)de	1.97 (1.43-3.00)bc	4.47 (3.83-5.46)efg*	20.83 (8.00-23.83)ab*	>LOD	0.04 (0.03-0.04)cd*	>LOQ	>LOQ	>LOD	>LOQ
	Vitória Bay	SW	>LOD	>LOD	>LOQ	94.99 (91.79-100.21)h	6981.20 (6691.35-7100.46)f	>LOD	6.79 (6.79-6.82)e	>LOD	>LOD	>LOD	>LOD
		SD	>LOQ	38.73 (29.13-40.07)f*	>LOD	14.90 (14.74-15.28)h	147.86 (99.56-182.93)cd	>LOD	>LOD	18.53 (18.46-19.01)d	1.76 (1.42-2.07)e*	>LOD	>LOQ
		PK	266.37 (259.91-289.66)fg*	9.52 (8.81-11.90)ef*	>LOQ	6.22 (5.95-7.11)efg	1516.84 (1462.93-1659.79)ef*	0.11 (0.09-0.12)b*	>LOQ	10.11 (9.95-11.58)cd	1.12 (1.02-1.15)de*	>LOQ	0.79 (0.78-0.82)c*
		RM	2.60 (0.82-5.73)a	>LOQ	>LOQ	2.71 (2.39-2.79)cd*	115.19 (92.51-136.73)bc*	>LOQ	>LOD	>LOQ	0.01 (0.01-0.01)b	>LOQ	>LOQ
		LR	9.18 (6.96-10.18)ab	>LOQ	>LOQ	1.60 (1.46-1.66)ab*	118.06 (109.80-131.49)c*	0.04 (0.02-0.07)a*	0.01 (0.01-0.02)abc	0.10 (0.08-0.13)a	>LOQ	>LOQ	>LOQ
		AV	13.24 (8.87-16.67)bc*	0.33 (0.15-0.36)a	0.80 (0.70-1.00)a*	4.31 (3.69-4.76)efg*	96.38 (86.78-111.27)bc	0.07 (0.05-0.12)b	0.01 (0.01-0.01)a	0.19 (0.13-0.24)b*	0.01 (0.01-0.02)bc	>LOQ	0.11 (0.09-0.15)a
		OS	1616.39 (72.44-1901.51)fg*	15.00 (9.51-16.16)ef*	7.91 (4.68-8.58)d*	12.65 (9.23-13.98)gh*	157.12 (149.92-182.21)cd*	0.11 (0.10-0.13)b*	0.52 (0.28-0.56)e*	0.53 (0.44-0.60)bc*	0.05 (0.04-0.05)de*	0.31 (0.25-0.35)ab*	0.38 (0.30-0.43)b*
		SH	38.98 (37.33-42.55)cd	2.63 (2.52-2.74)bc	2.25 (1.88-2.92)bc	3.90 (3.74-3.91)def	393.40 (362.06-437.55)de	0.13 (0.12-0.18)b*	>LOQ	0.07 (0.05-0.11)a	0.01 (0.01-0.02)b	0.20 (0.08-0.75)bc	0.17 (0.08-0.73)b
		CR	61.37 (58.07-66.39)def*	5.20 (4.76-5.44)de*	1.48 (1.19-1.71)b	2.86 (2.60-2.99)cd	1239.54 (1123.75-1317.63)ef	0.39 (0.27-0.41)c	0.01 (0.01-0.02)ab	0.16 (0.12-0.18)b	0.03 (0.02-0.03)cd	>LOQ	>LOQ
		MU	11.44 (10.80-13.29)bc	0.40 (0.33-0.42)a	0.81 (0.59-0.98)a	3.74 (3.65-3.94)def	2.63 (0.72-3.77)a*	0.05 (0.04-0.07)a	0.02 (0.02-0.03)d	>LOQ	0.01 (0.01-0.01)a	0.92 (0.58-1.07)d*	0.95 (0.62-1.07)c*
		HP	72.38 (67.61-76.92)efg	2.65 (2.19-4.10)c*	4.74 (4.24-5.19)cd*	2.03 (1.96-2.14)bc	6.08 (3.15-6.52)a*	>LOQ	0.06 (0.05-0.08)e	>LOD	>LOQ	0.44 (0.36-0.58)cd*	0.48 (0.40-0.67)c*
		GI	55.32 (47.29-60.03)de	0.70 (0.62-0.79)ab*	0.90 (0.84-1.02)a*	1.25 (1.21-1.35)a*	353.57 (345.53-413.52)de*	>LOD	0.02 (0.02-0.02)bcd*	0.07 (0.06-0.11)a	0.01 (0.01-0.01)b	0.18 (0.16-0.19)a*	0.19 (0.17-0.21)ab*
		KI	671.32 (429.48-1066.49)g	2.99 (2.49-4.57)cd	4.80 (4.33-12.72)d	2.66 (1.94-5.22)cde	7.76 (4.17-8.11)ab	>LOD	>LOQ	>LOD	>LOD	0.75 (0.49-0.89)d	0.79 (0.38-1.13)c
		GO	6.88 (4.20-85.56)bc	3.24 (3.05-3.81)cd	>LOQ	0.27 (0.07-0.30)a	1.92 (1.19-3.03)a*	>LOD	0.02 (0.01-0.02)cd	>LOQ	>LOQ	>LOD	>LOQ

Table 3S. Regression parameters and p values for δ 15N vs. trace element concentration (µg g−1 dry weight). PK: plankton, PT: mangrove plants (*Rhizophora mangle*, *Laguncularia racemosa*, *Avicennia schaueriana*), SH: shrimp (*Macrobrachium* sp.), OS: oyster (*Crassostrea rhizophorae*), CR: crab (*Aratus* sp.), FS: fish (*Centropomus parallelus*).-: Element below quantification.

2015							
Element	Sites	Trophic chain	Intercept	Slop	R^2	P	Trophic Transfer
B	Santa Cruz	PK-SH-FS	6.22	-0.62	0.37	<0.0001	Biodilution
		PK-OS	18.97	-2.6	0.98	<0.0001	Biodilution
		PT-CR-FS	2.74	-0.25	0.7	<0.0001	Biodilution
	Vitória Bay	PK-SH-FS	8.56	-0.67	0.72	<0.0001	Biodilution
		PK-OS	11.84	-1.13	0.85	<0.0001	Biodilution
		PT-CR-FS	8.76	-0.64	0.78	<0.0001	Biodilution
Al	Santa Cruz	PK-SH-FS	14.22	-1.34	0.22	<0.0001	Biodilution
		PK-OS	21.96	-2.52	0.97	<0.0001	Biodilution
		PT-CR-FS	4.24	-0.13	0.09	0.0014	Biodilution
	Vitória Bay	PK-SH-FS	9.02	-0.52	0.31	<0.0001	Biodilution
		PK-OS	14.77	-1.15	0.77	<0.0001	Biodilution
		PT-CR-FS	6.05	-0.14	0.03	0.1842	
V	Santa Cruz	PK-SH-FS	5.53	-0.82	0.19	<0.0001	Biodilution
		PK-OS	13.85	-2.24	0.98	<0.0001	Biodilution
		PT-CR-FS	-1.35	0.026	0.009	0.32	
	Vitória Bay	PK-SH-FS	4.96	-0.58	0.71	<0.0001	Biodilution
		PK-OS	8.03	-1.06	0.83	<0.0001	Biodilution
		PT-CR-FS	-1.66	0.09	0.04	0.11	
Cr-52	Santa Cruz	PK-SH-FS	5.5	-0.73	0.18	0.0003	Biodilution
		PK-OS	14.28	-2.37	0.93	<0.0001	Biodilution
		PT-CR-FS	-2.3	0.22	0.25	<0.0001	Biomagnification
	Vitória Bay	PK-SH-FS	4.94	-0.56	0.61	<0.0001	Biodilution
		PK-OS	7.6	-1.13	0.56	<0.0001	Biodilution
		PT-CR-FS	3.84	-0.35	0.57	<0.0001	Biodilution
Cr-53	Santa Cruz	PK-SH-FS	5.7	-0.73	0.23	0.0003	Biodilution
		PK-OS	13.41	-2.19	0.96	<0.0001	Biodilution
		PT-CR-FS	-1.27	0.1	0.14	0.0004	Biomagnification
	Vitória Bay	PK-SH-FS	4.36	-0.42	0.7	<0.0001	Biodilution
		PK-OS	6.9	-0.95	0.73	<0.0001	Biodilution
		PT-CR-FS	-2.08	0.14	0.05	0.1354	
Mn	Santa Cruz	PK-SH-FS	6.55	-0.69	0.4	<0.0001	Biodilution
		PK-OS	13.69	-1.81	0.96	<0.0001	Biodilution
		PT-CR-FS	3.47	-0.34	0.59	<0.0001	Biodilution
	Vitória Bay	PK-SH-FS	6.96	-0.54	0.48	<0.0001	Biodilution
		PK-OS	7.29	-0.45	0.47	0.0002	Biodilution
		PT-CR-FS	12.2	-0.94	0.6	<0.0001	Biodilution
Fe-56	Santa Cruz	PK-SH-FS	13.34	-0.96	0.64	<0.0001	Biodilution
		PK-OS	20.27	-2.26	0.98	<0.0001	Biodilution
		PT-CR-FS	5.32	-0.07	0.06	0.006	Biodilution
	Vitória Bay	PK-SH-FS	9.65	-0.47	0.2	<0.0001	Biodilution

		PK-OS	14.2	-1.06	0.78	<0.0001	Biodilution
		PT-CR-FS	5.32	-0.06	0.004	0.571	
Fe-57	Santa Cruz	PK-SH-FS	13.24	-0.93	0.65	<0.0001	Biodilution
		PK-OS	20.26	-2.26	0.98	<0.0001	Biodilution
		PT-CR-FS	5.35	-0.06	0.05	0.013	Biodilution
	Vitória Bay	PK-SH-FS	7.99	-0.24	0.08	0.0108	Biodilution
		PK-OS	14.16	-1.05	0.8	<0.0001	Biodilution
		PT-CR-FS	5.12	-0.03	0.001	0.768	
Ni	Santa Cruz	PK-SH-FS	5.47	-0.89	0.23	<0.0001	Biodilution
		PK-OS	11.32	-1.96	0.98	<0.0001	Biodilution
		PT-CR-FS	-2.35	0.06	0.03	0.11	
	Vitória Bay	PK-SH-FS	4.26	-0.55	0.53	0.0026	Biodilution
		PK-OS	4.28	-0.58	0.34	0.0026	Biodilution
		PT-CR-FS	7.54	-0.73	0.74	<0.0001	Biodilution
Cu	Santa Cruz	PK-SH-FS	5.35	-0.42	0.37	<0.0001	Biodilution
		PK-OS	8.39	-0.97	0.88	<0.0001	Biodilution
		PT-CR-FS	1.06	0.03	0.03	0.0846	
	Vitória Bay	PK-SH-FS	3.71	-0.21	0.11	0.0011	Biodilution
		PK-OS	3.79	-0.04	0.01	0.6287	
		PT-CR-FS	8.46	-0.58	0.49	<0.0001	Biodilution
Zn	Santa Cruz	PK-SH-FS	6.42	-0.29	0.26	<0.0001	Biodilution
		PK-OS	3.32	1.49	0.94	<0.0001	Biomagnification
		PT-CR-FS	3.19	0.09	0.13	<0.0001	Biomagnification
	Vitória Bay	PK-SH-FS	4.61	-0.07	0.01	0.2426	
		PK-OS	3.57	0.43	0.29	0.0076	Biomagnification
		PT-CR-FS	3.49	0.04	0.003	0.7014	
As	Santa Cruz	PK-SH-FS	2.98	-0.33	0.26	<0.0001	Biodilution
		PK-OS	5.39	-0.69	0.8	<0.0001	Biodilution
		PT-CR-FS	-0.56	0.06	0.04	0.0278	Biomagnification
	Vitória Bay	PK-SH-FS	3.7	-0.29	0.54	<0.0001	Biodilution
		PK-OS	2.1	0.04	0.014	0.5815	
		PT-CR-FS	-2.47	0.18	0.06	0.0294	Biomagnification
Se	Santa Cruz	PK-SH-FS	3.03	-0.28	0.25	<0.0001	Biodilution
		PK-OS	-	-	-	-	
		PT-CR-FS	0.12	0.04	0.03	0.092	
	Vitória Bay	PK-SH-FS	2.86	-0.18	0.12	0.0039	Biodilution
		PK-OS	-	-	-	-	
		PT-CR-FS	-0.99	0.11	0.03	0.2173	
Rb	Santa Cruz	PK-SH-FS	2.07	-0.11	0.09	0.0028	Biodilution
		PK-OS	6.33	-0.82	0.78	<0.0001	Biodilution
		PT-CR-FS	0.75	0.03	0.08	0.0014	Biomagnification
	Vitória Bay	PK-SH-FS	0.55	0.006	0.0002	0.9051	
		PK-OS	1.1	0.17	0.32	0.0042	Biomagnification
		PT-CR-FS	0.24	0.05	0.03	0.1323	
Sr	Santa Cruz	PK-SH-FS	7.77	-0.47	0.19	<0.0001	Biomagnification
		PK-OS	18.22	-2.41	0.96	<0.0001	Biodilution

		PT-CR-FS	4.59	-0.13	0.15	<0.0001	Biodilution
	Vitória Bay	PK-SH-FS	6.7	-0.33	0.1	0.0018	Biodilution
		PK-OS	10.75	-0.75	0.86	<0.0001	Biodilution
		PT-CR-FS	8.83	-0.41	0.15	0.0006	Biodilution
Ag	Santa Cruz	PK-SH-FS	-2.93	0.06	0.03	0.2724	
		PK-OS	-7.12	0.94	0.9	<0.0001	Biomagnification
		PT-CR-FS	-3.37	0.09	0.24	0.0006	Biomagnification
	Vitória Bay	PK-SH-FS	-1.5	-0.11	0.21	0.0036	Biodilution
		PK-OS	-2.38	0.05	0.01	0.6227	
		PT-CR-FS	-7.35	0.42	0.33	0.0009	Biomagnification
Cd	Santa Cruz	PK-SH-FS	-4.65	0.15	0.06	0.0439	Biomagnification
		PK-OS	-10.2	1.29	0.9	<0.0001	Biomagnification
		PT-CR-FS	-4.17	0.07	0.2	<0.0001	Biomagnification
	Vitória Bay	PK-SH-FS	-	-	-	-	
		PK-OS	-	-	-	-	
		PT-CR-FS	-4.96	0.07	0.04	0.2396	
Pb	Santa Cruz	PK-SH-FS	5.84	-0.73	0.34	0.0001	Biodilution
		PK-OS	13.01	-2.23	0.9	<0.0001	Biodilution
		PT-CR-FS	-1.87	0.09	0.29	0.0001	Biomagnification
	Vitória Bay	PK-SH-FS	4.44	-0.6	0.76	<0.0001	Biodilution
		PK-OS	7.08	-1.08	0.76	<0.0001	Biodilution
		PT-CR-FS	1	-0.19	0.34	0.0007	Biodilution
Nb	Santa Cruz	PK-SH-FS	0.58	-0.41	0.43	<0.0001	Biodilution
		PK-OS	9.33	-2.03	0.94	<0.0001	Biodilution
		PT-CR-FS	-4.03	0.05	0.07	0.0261	Biomagnification
	Vitória Bay	PK-SH-FS	2.42	-0.62	0.77	<0.0001	Biodilution
		PK-OS	4.96	-1.1	0.83	<0.0001	Biodilution
		PT-CR-FS	-3.6	-0.01	0.0005	0.8618	
Hg-201	Santa Cruz	PK-SH-FS	-2.35	0.11	0.09	0.0275	Biomagnification
		PK-OS	2	-0.65	0.78	<0.0001	Biodilution
		PT-CR-FS	-2.35	0.1	0.31	<0.0001	Biomagnification
	Vitória Bay	PK-SH-FS	-6.62	0.45	0.29	<0.0001	Biomagnification
		PK-OS	-	-	-	-	
		PT-CR-FS	-	-	-	-	
Hg-202	Santa Cruz	PK-SH-FS	-1.99	0.08	0.08	0.0405	Biomagnification
		PK-OS	3.03	-0.81	0.88	<0.0001	Biodilution
		PT-CR-FS	-3.15	0.19	0.53	<0.0001	Biomagnification
	Vitória Bay	PK-SH-FS	-0.59	-0.03	0.01	0.3276	
		PK-OS	0.98	-0.28	0.49	0.0002	Biomagnification
		PT-CR-FS	-5.25	0.03	0.29	<0.0001	Biomagnification

Table 4S. Regression parameters and p values for δ 15N vs. trace element concentration (µg g−1 dry weight) in the plankton-shrimp (*Macrobrachium* sp.) – fish (*Centropomus parallelus*) trophic chain.

2014						
Element	Sites	Intercept	Slop	R2	p	Trophic Transfer
Al	Santa Cruz	11.91	-0.99	0.34	<0.0001	Biodilution
	Vitória Bay	12.18	-0.76	0.5	<0.0001	Biodilution
V	Santa Cruz	4.8	-0.62	0.48	<0.0001	Biodilution
	Vitória Bay	5.25	-0.56	0.61	<0.0001	Biodilution
Cr-52	Santa Cruz	3.6	-0.59	0.26	<0.0001	Biodilution
	Vitória Bay	5.14	-0.59	0.55	<0.0001	Biodilution
Cr-53	Santa Cruz	5.96	-0.93	0.52	<0.0001	Biodilution
	Vitória Bay	4.71	-0.55	0.46	<0.0001	Biodilution
Mn	Santa Cruz	4.8	-0.42	0.21	<0.0001	Biodilution
	Vitória Bay	5.85	-0.43	0.31	<0.0001	Biodilution
Fe-56	Santa Cruz	13.47	-0.98	0.47	<0.0001	Biodilution
	Vitória Bay	11.59	-0.57	0.36	<0.0001	Biodilution
Fe-57	Santa Cruz	13.28	-0.94	0.47	<0.0001	Biodilution
	Vitória Bay	11.51	-0.56	0.35	<0.0001	Biodilution
Cu	Santa Cruz	6.61	-0.69	0.23	<0.0001	Biodilution
	Vitória Bay	4.56	-0.29	0.31	<0.0001	Biodilution
Zn	Santa Cruz	5.17	-0.11	0.01	0.2303	
	Vitória Bay	2.63	0.16	0.07	0.0139	Biomagnification
As	Santa Cruz	-5.03	0.76	0.15	0.0007	Biomagnification
	Vitória Bay	4.18	-0.34	0.7	<0.0001	Biodilution
Se	Santa Cruz	2.77	-0.2	0.21	<0.0001	Biodilution
	Vitória Bay	1.8	-0.06	0.024	0.1439	
Rb	Santa Cruz	1.56	-0.06	0.01	0.3145	
	Vitória Bay	2.47	-0.12	0.07	0.0027	Biodilution
Sr	Santa Cruz	4	-0.08	0.01	0.4127	
	Vitória Bay	4.7	-0.14	0.04	0.0269	Biodilution
Pb	Santa Cruz	9.35	-1.31	0.99	<0.0001	Biodilution
	Vitória Bay	8.2	-0.75	0.99	<0.0001	Biodilution
Hg-201	Santa Cruz	-0.7	-0.06	0.01	0.3931	
	Vitória Bay	0.61	-0.14	0.07	0.003	Biodilution
Hg-202	Santa Cruz	-0.72	-0.06	0.01	0.3945	
	Vitória Bay	0.38	-0.13	0.06	0.3945	

CAPÍTULO 5

Bismuth occurrence in a food web of neotropical mangrove ecosystem

ABSTRACT

Bismuth (Bi) is an environmentally relevant element that due to its chemical and physical characteristics has been used in numerous industrial processes such as semiconductors, metallurgical additives, cosmetic products, medicines, air cleaning and water purification. Despite its low concentration in nature, there is an increased awareness of the potential exposure arising from industrial release, leading to environmental contamination. The objective of this study was to evaluate the presence of Bi in two neotropical mangrove estuarine ecosystem evaluating abiotic matrix (surface water, sediment, particulate matter) and biota. Six trophic levels were considered to evaluate the Bi potential biomagnification or biodilution through the food web. The results suggested that the main source of Bi in these estuarine ecosystems is the iron and steel industries located in Tubarão Complex. Linear regressions showed biodilution in plankton-fish trophic chain and no influence of trophic level in bismuth bioaccumulation in the others trophic chain analysed. Scanning electron microscope analysis showed most bismuth in oyster shells. In fish from both mangrove sites, the highest Bi concentration was in hepatopancreas followed by the muscle.

Keywords: *Crassostrea rhizophorae, Centropomus parallelus*, biodilution, trophic chain, metal localization.

1. INTRODUCTION

Bismuth (Bi) is an environmentally relevant element that due to its chemical and physical characteristics has been used in numerous industrial processes such as semiconductors, metallurgical additives, cosmetic products (e.g., nail varnish, lipstick, hair dye), medicines (e.g., treatment of ulcers and other gastrointestinal disorders, syphilis, dermatological disorders, radiotherapy), air cleaning and water purification (Das et al, 2006; Fillela 2010; Gao et al 2015). Bismuth is usually obtained as a subproduct from Cu and Pb ores and recovered by the reduction of the oxide by iron or charcoal; Bi (^{209}Bi) is considered as the heaviest stable isotope in nature, with an occurrence of 0.00002% in Earth's crust being its trivalent form the most abundant in geosphere, including native bismuth (Bi), bismuthinite (Bi_2S_3) and bismite (Bi_2O^3) (Kearns and Turner, 2016). Bismuth has even been qualified as "green chemical" since it exhibits low toxicity to humans compared to its periodic neighbours (Pb and Po) and other group 15 elements (e.g. As and Sb). This element is considered as nonessential element with unknowing biological function (Das et al, 2006).

Despite its low concentration in nature, there is an increased awareness of the potential exposure arising from industrial release, leading to environmental contamination. Das et al. (2006) also emphasize the need of studies of environmental concentration of trace bismuth to establish its migration cycle and impact in the environment and showed that bismuth concentrations in soil usually is around 1 µg/g or higher in case of pollution from land soil. In reference materials of sediment, Bi concentrations have been found between 0.07 µg/g (Ivanova et al 2001) and 49.6 µg/g (Hall and Pelchat, 1997). Some coal samples contain more than 1 µg/g Bi, which indicates that, during burning coal, it can be distributed in the ash in the atmosphere suspended matter; Bi levels from 1.05 µg/g to 14.7 µg/g were reported after coal burning

(Aulinger et al., 2002; Moscoso-Perez et al., 2003).

Some studies have reported concentrations of Bi in sea and freshwater. In seawater, Bi was present in concentration of 10 to 30 ng L^{-1} and, most of total Bi (70%) is found in particulate form (Lee 1982; Lee et al 1986). In freshwater systems, Bi concentration varies from 0.7 ng L^{-1} to 40 000 µg L^{-1}, depending on geochemical conditions existing in freshwater systems and/or its proximity of pollution sources (Filella, 2010). In contaminated environments as in Marmara Sea, the Bi value reached 53 000 ng L^{-1} (Tokman and Akman, 2004).

Bismuth is also detected in biota at ng/g (Das et al., 2006). Radiotracer experiments using 207Bi showed significant accumulation by phytoplankton with a factor between about 10^5 and 10^7. Considering that copepods consumed phytoplankton, they were able to assimilate 4% of 207Bi (Fowler et al. 2010). Bismuth concentrations was found in Arctic terns mainly birds not infected with gastrointestinal parasites (Provencher et al., 2014) and, juvenile Bluefin tuna (21 $ng.g^{-1}$ of ^{209}Bi) from the Western Pacific Ocean (Arslan and Paulson, 2002).

Bismuth content in the various environmental media is scarce and often contradictory (Filella et al 2010) likewise, investigation about Bi toxicity is still incipient in the biota of different environmental compartments. At present, the Bi toxicity is considered low compared to others heavy metals as reported in macroalgae (Kears and Turner, 2016). However, numerous toxic effects have been attributed to Bi in humans and other animals such as osteoarthropathy, hepatitis, nephrotoxicity, neuropathology and encephalopathy (Cadore et al 1998).

Therefore, considering the occurrence of bismuth in the aquatic environment and its potential toxicity to biota, the objective of this study was to evaluate the presence of Bi in a neotropical estuarine ecosystem evaluating abiotic matrix (surface water, sediment, particulate matter) and biota. Six trophic levels were taking to evaluate the Bi potential biomagnification or

biodilution through the food web.

This is the first research focusing Bi in different compartments of tropical estuarine ecosystems and its transfer through mangrove food web. Most trophic chain researches emphasise the food web structure, being scarce studies on metal transfer through food web as well as in different environmental compartments. Furthermore, contradictory information is available about bismuth content in the environment (Filella, 2010). This study focuses on Bi in estuarine ecosystems as well as it bioaccumulation in three food chain: (1) plankton - oyster, (2) plankton – shrimp - fish and, (3) mangrove tree - crab - fish.

2. MATERIALS AND METHODS

2.1 Study areas:

Three sampling areas on the Espírito Santo state, Brazil were selected: the Tubarão Complex area and two mangrove areas, Vitória Bay and Santa Cruz, which are located at 10 and 70 km from Tubarão Complex, respectively (Fig. 1). Sampling was taking in March 2015, at the end of summer.

Tubarão Complex (20º17′03.8″S and 40º14′24.9″W) is located in a coastal area between Vitória City and Serra (Fig. 1). It is a steel industrial place, iron ore pelletize, storage and transports. The transportation of these materials is made by open mats to ship and the storage is in open-air courtyard. Moreover, steel smelting process produces steel metallic smoke that is a significant part of particulate material airborne. The industrial discharge from steel industry is also released directly by an outfall in adjacent sea area (Santos and Reis Jr., 2016).

Vitória Bay (20°14′31.5″S and 40°19′84.7″W) is an estuarine complex formed by five rivers. Originally it was formed for 54 islands constituted by mangrove area; between 1830 and

1996, a total of 12,000 km^2 of mangrove area received several landfills, using solid waste from harbour dredging activities or other industrial activities, including industrial solid waste from Tubarão Complex (Gazeta, 2016). Nowadays, it presents only 14 islands. The great Vitoria city located on his place and the artificial Camburi Beach (adjacent to the Tubarão Complex, close to Vitoria city) have iron mining bags deposited on it. Furthermore, this is a very populous area which is impacted by other several pollution sources such as sewage, metallurgical and textile activities, paint industry, steel industry and iron mining (Fig. 1).

The Santa Cruz bay (19°56′26.2″S and 40°12′87.0″W) is a typical mangrove ecosystem covering approximately 12 km^2 formed by two rivers which are part of Piraquê-Açu and Piraquê-Mirim Mangrove Ecological Reservation. It presents a wide preserved mangrove area without occupation (Fig.1).

2.2 Sampling procedure:

Surface water (SW) and sediment (SD) samples were taken simultaneously with biota on Vitória Bay and Santa Cruz mangrove ecosystems (Fig. 1). On the Tubarão Complex, SW was sampled at about 100-200 m far from: 1) Iron effluent (IE) - the discharge of the pelletizing iron industry, 2) Iron port (IP) – port for pelletizing iron industry export, 3) Steel effluent (SE) - the steel industry effluent discharge and, 4) Steel port (SP) – port for steel industry export and (Fig. 1) and SD sampling was collected in four points adjacent to solid discharge from Tubarão Complex (Fig. 1). Atmospheric particulate matter (PM) was collected at about 1 km from from Tubarão Complex was sampled according to Arrivabene et al. (2015).

Water samples were collected in acid washed plastic bottles submerged to 10-20 cm deep. The samples were acidified with ultrapure HNO_3 (sub-boiling grade) and stored at 4ºC. Prior to analyses, water samples were filtered on nitrocellulose filter (0.45 µm). Sediment samples were

collected at 10-20 cm depth using a polypropylene spoon and samples were immediately transferred into clean 1L-polypropylene flasks (without head space). Thereafter, they were dried at room temperature and sieved through 63 μm nylon meshes.

Samples of mangrove biota (n=9): *Rhizophora mangle* (RM), *Laguncularia racemosa* (LR) trees, *Avicennia schaueriana* (AS) - plankton (PK), shrimp *Macrobranchium sp.* (SH), crab *Aratus sp.* (CR), oyster *Crassostrea rhizophorae* (OS) as well as samples of the organs (muscle (MU), gill (GI), kidney (KI), hepatopancreas (HP) and gonad (GO) from juvenile fish *Centropomus parallelus* were collected in each site. Fully expanded leaves from the 3rd to 5th node were collected from each mangrove tree species and immediately washed with ultrapure water. Plankton was collected using plankton net of 20 μm without distinction among phytoplankton or zooplankton and oven-dried until constant mass. Crabs (whole body) and oysters (soft tissue and shell) were collected directly from mangrove trees, by hand, and shrimps (whole body) were collected with pitfall aid. Fish *C. parallelus*; total length = 20 ± 2.5 cm; n=5) were collected with hook and line; their organs were removed and separated. All biological samples for metal analysis were oven-dried at 37ºC until constant mass, macerated with mortar and pestle and, then stored at room temperature until analysis. Oyster shell was stored in room temperature for scanning electron microscope (SEM) analysis and organ samples (gills, muscle, hepatopancreas, gonad and kidney) from *C. parallelus* were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3 solution for transmission electron microscope analysis.

2.3 Bismuth analysis

Ultrapure water (<5 μg L^{-1} TOC) was obtained from a purification system Arium 61316-RO plus Arium 611 UV (Sartorius, Germany). Multi-element standard solution Merck VI

CertiPUR® was obtained from Merck Química Argentina (Buenos Aires, Argentina). Nitric acid with 63.7% was prepared from analytical grade acid using a distiller (Figmay Sub-boiling distilled, Córdoba, Argentina) until sub-boiling grade (70%). Purity of nitric acid was verified by Mass Spectrometry Inductively Coupled Plasma (ICP-MS), Agilent 7500cx, USA, equipped with an ASX-100 autosampler (CETAC Technologies, Omaha, NE). Filters (0.45 μm, HAWG04756) were obtained from Millipore (São Paulo, Brazil). All glassware and plastic bottles/containers were left with sulfuric/nitric acids solution overnight and washed with ultrapure water. ICP probes and pipes were of PTFE previously washed with nitric acid (2% v v^{-1}).

Each sediment and particulate matter sample (0.1 g dry weight each) was digested with 4 mL of nitric acid and 1 mL of hydrochloric acid (ultrapure, sub-boiling grade) for 24hs at 100 ºC. Biotic samples (0.1 g dry weight each) were digested with 4 mL of nitric acid, 1 ml of hydrochloric acid and 0.5 mL of hydrogen peroxide (ultrapure, sub-boiling grade) in pre-cleaned PTFE close-vessels, using a Microwave Accelerated Reaction System (MARS Xpress TM; CEM Corporation, USA) (Souza et al., 2013). Before measurement, all digested samples were filtered using 0.45 μm nitrocellulose filter to remove any remaining particles in according to method described by USEPA (2008). All material used in the digestion procedure were washed out with a sulphuric/nitric acid solution overnight, and further rinsed with ultrapure water.

Concentrations of elements were determined in triplicate; the repeatability of ICP-MS measurements was generally ≥ 97%. Quality assurance (QA) and quality control (QC) were done using a certified reference material (CRM): MESS-2 Marine and estuarine sediment and NCSDC 73348. Recoveries from CRMs were 82 ± 17%.

2.4 Scanning Electron Microscope

Oyster shells were analysed using scanning electron microscope; the shells were fractured

and fixed with araldite on aluminium stub with upwards the inner part and examined at low vacuum condition in a MEV INSPECT S50 with an electron backscatter detector (EBSD, Electron Backscatter Diffraction) lighter regions (LFD) and X-ray Dispersive Energy Spectroscopy (EDS). The composition was analysed and, whenever it detected bismuth and/or other metal, a secondary electron detector determined if the particles were on the surface or inner layer. Ten shells were analysed for each study area.

2.6 Statistical analysis

Data are reported as mean ± standard deviation (data were previously tested for normal distribution). One-way analysis of variance (ANOVA), followed by the Tukey's test or T-test (for two groups) was applied with a significance level $P<0.05$. Metal transfer through the food web was determined according to Monferrán et al. (2016) using linear regression for the three trophic chain (1.plant-crab-fish; 2.plankton-shrimp-fish; 3.plankton-oyster) determined by Souza and collaborators (2017, chapter 1) using nitrogen stable isotope data. All statistical analyses were conducted using the software Infostat.

3. RESULTS AND DISCUSSION

In abiotic samples from Santa Cruz and Vitória Bay, Bi was lower than the limits of detection. However, Bi was quantified in water samples collected in IP, SE and SP in Tubarão Complex from which the highest level were found in IP (ironport, 15.22 ± 8.31 µg Bi/mL seawater) followed by SE (steel industry effluent discharge, 4.44 ± 0.73 µg/mL seawater) and SP (steel industry port, 1.87 ± 0.71µg/mL seawater) (Table 1) suggesting that Tubarão Complex may be the source of this element. During pelletizing iron process and steel production Bi and other rare elements from the primary ore or used for increasing malleability and conduction of

amalgams can be chronic released to environment by the effluent discharge and/or metallic smoke generated during those processes. Although Bi was identify, Bi was not possible to quantified in particulate matter samples.

In the biota, Bi was quantified at ng/g; the highest levels were found in plankton, followed by fish organs and oysters in both, Vitória Bay and Santa Cruz; in shrimp, Bi was quantified only in Santa Cruz. Among mangrove trees, Bi was quantified in *A. schaueriana* from Santa Cruz and Tubarão and *L. racemosa* from Tubarão (Table 1).

Bi values in plankton from Vitória Bay (230 ng/g) and Santa Cruz (130 ng/g) were lower than those found in some marine plankton such as artemia, *Monochysis lutheri* (2000 ng/g) and *microalga Chamydomonas sp.* (7700 ng/g) (Riley and Roth, 1971) cultivated in experimental conditions. Plankton community constituted by phytoplankton and zooplankton living in suspension in water column and subjected to passive movements caused by winds and marine currents (Reynolds 1984), are a key step in carbon and trace elements transfer through marine food web (Fenchel, 2008). They are able to accumulate trace elements dissolved in the aquatic environment or via ingested food playing significant role in nutrient cycling and elemental vertical fluxes (Wang and Fisher, 1998). Besides, nutrients input direct into water, atmospheric nutrient inputs are recognized as an important source of major and trace elements in seawater (Jickells, 1995) contributing to increased phytoplankton biomass (Paytan et al 2009). The plankton Bi levels in the present study suggested possible water and atmospheric Bi input as the smoke released from Tubarão Complex may contain this element although, it was not quantified in PM from this complex.

Metal transferring process from phytoplankton did not imply in decreasing metal bioavailability but, may potentiate metal increment to higher trophic levels by, simultaneously,

altering metal chemistry and/or exposing routes to other organisms (Sunda and Huntsman, 1998). In this sense, in Vitória Bay and Santa Cruz, Bi concentration in oyster (*Crassostrea rhizophorae*) was 22 ± 1.5 ng/g and 24 ± 1 ng/g, respectively. Oysters are an organisms susceptible to the contamination in the water column, as they are filter organisms. Bi concentrations in mussels was reported be positively correlated with the degree of metals contamination in immediate environment, as well as the age, sex and zinc content of these animals; Bi concentration were higher in mussels (*Choromytilus meridionalis*) collected in places with high Bi content in water and higher in larger female mussels having elevated content of zinc (Watling and Watling, 1976). However, in Vitoria Bay and Santa Cruz such correlation was not observed as no Bi was detected in water.

Bi concentration relationship between plankton and mollusks, rather than water, was reported in blue mussel *Mytilus edulis* in Swedish coast, scallops *Pecten maximu*s and *Chlamys opercularis* in the English Channel suggesting a transference through food (Phillips, 1980). The present study corroborates this author as, following nitrogen isotopes, a trophic chain plankton - oyster was identified in Vitoria Bay and Santa Cruz together with the two other trophic chains: plankton-shrimp-fish and trees-crab-fish (Souza, 2017, chapter 1). Metals accumulation via diet is as much as important as metal uptake from aqueous phase and usually dominates metal accumulation in bivalves from marine environments (Dimitriadis et al., 2003).

The trophic chain plankton-oyster may explain the Bi accumulation in it. Bi concentration in oyster soft tissue was lower than in plankton (Table 1). It is well-known that some metals may be deposited together with calcium carbonate in the oyster's shell as animal growth being one form of the detoxification process in order to eliminate metals from metabolic active tissues (Dimitriadis et al., 2003). The analysis in different areas of shell of oysters showed higher metal

concentration, in those areas with continuous contact with oyster soft tissue; some metals were detected and identified in, at least, one sample (Ba, S, Al, Mg, Na, K, Ni, Cl, Si, Cu, W, Ti, Zr, Fe, Mn, Y, P, Zn, La, Ce), while Bi were found in all analysed samples.

Bi element was identified in samples analysed from Vitória Bay and Santa Cruz and Bi particle was identified under the shell surface (Fig. 2). In Brazil, bismuth can originate from Carajás iron mining. According Tallarico et al. (2004), Carajás has a unique formation in which iron and manganese deposits preserves a signature of the underlying primary ore, with a strong enrichment in Cu, Au, W, Mn, Bi, Sn, La and As relative to the host sedimentary rocks and the underlying granite intrusion; significant concentrations of Bi in the Carajás's greisen are mainly due to bismuthinite and native Bi that occur as inclusions in arsenopyrite and chalcopyrite. Considering the Bi levels found in surface water of Tubarão Complex and the rare elements found in oyster shell suggested that the Bi source may be Carajás's greisen, the most important iron and manganese deposits in Brazil, suggesting the industrial activity carried out in Tubarão Complex as the possible source for this emerging contaminant.

Bi concentration in shrimp (*Macrobrachium* sp.) from Santa Cruz was 15 ± 13 ng/g while, in those from Vitória Bay, Bi was always below the quantification limit; such variability may be due to the shrimp diet. There is great variation in the diet of this species that can be herbivorous, detritus or predator. For example, Mantel and Dudgeon (2004) reported that *Macrobrachium sp.* from forest streams has a predaceous nature through its life stages in contrast to other *Macrobrachium* species which have omnivorous and/or detritivorous habit; Xu et al. (2008) using isotope analysis, suggested that benthic food web provides the primary carbon source for *Macrobrachium nipponensis.*

As other base of the mangrove trophic chain, the mangrove trees *R. mangle* (red mangrove),

L. racemosa (white mangrove) and *A. schaueriana* (black mangrove), Bi concentrations in the leaves were quantified in *A. schaueriana* (22 ± 15 ng/g) from Santa Cruz and in *A. schaueriana* (10 ± 2.90 ng/g) and *L. racemosa* (36 ± 6 ng/g) from Tubarão Complex (Table 1) which may indicate low influence of Bi in bioaccumulation through trophic chain plant-crabs-fish identified in these estuarine regions and possible atmospheric input as no Bi was detected in surface water and, isotopic study, showed that these plants have more influence from seawater than sediment (Souza, 2017, chapter 1). These mangrove plants are capable of metal foliar absorption (Arrivabene et al., 2015, 2016); large amount of Fe in leaves from *R. mangle* (131.7 ± 15.6 μg/g DW), *L. racemosa* (180.1 ± 16.2 μg/g DW) and *A. schaueriana* (282.9 ± 15.9 DW) from Santa Cruz was explained by atmospheric input rather water and/or sediment (Arrivabene et al., 2015). Then, despite the distance from the Tubarão Complex, Santa Cruz is potentially subject to atmospheric contamination due to wind direction in this regions. As crab eats leaves from these plants, it was expected found Bi in this organism from Santa Cruz; however, Bi concentration in crabs was lower than the quantification limits (Table 1).

At the top of the two trophic chain (mangrove trees-crabs-fish and plankton-shrimp-fish) of the studied ecosystems is the fat snook *C. parallelus* (Souza, 2017, chapter 1). This species is a protandric predator fish (Taylor et al., 2000) widely distributed through tropical and subtropical coasts in Florida (USA) to Brazilian southern coasts (Rivas, 1986) and has carnivore diet (Gilmore et al., 1983). During juvenile life stage has benthic habits, feeding with crustaceans and shrimps (Volpe, 1959; Cháves, 1963; Gilmore et al., 1983, Souza 2017, Chapter 1) and, when adult, is benthic-pelagic feeding habits mainly with small fish (Carvajal, 1975). Bi was quantified in the gills, hepatopancreas, muscles, gonads and kidney, excepting in the kidney of fish from Santa Cruz and Vitória Bay (Table 1). The Bi concentration differences among the

organs may be explained by fish organ composition, nutritional needs and function (Lorrain, et al., 2002). The highest Bi concentrations were in hepatopancreas followed by the muscle from fish collected in all sites. Hepatopancreas is the main detoxification organ and was expected has higher Bi concentration and, in general, muscle accumulation occurs when the hepatopancreas capacity was exceeded, excepting for some metals. The highest concentrations of Bi in gonads were detected in Santa Cruz (0.045 ± 0.024) and Tubarão Complex (0.043 ± 0.0028). Bi presence in gonads, open an alert as no information is available about Bi effect reproduction processes, and/or on biological processes and cellular structures. Bi in gills are expected due to respiratory and osmoregulatory processes, mainly if the contaminant is dissolved in water.

C. Parallelus are able to detect different substances dissolved in water, influencing capture strategies and selection of food of the species (Hanke et al., 2008). Considering that, according to Souza (2017, chapter 1), two main trophic food chain was identified for this species depending on food availability, a mangrove plant-crab-fish in Vitória Bay and a plankton-shrimp-fish in Santa Cruz, it may explain the differences in Bi concentration in this top chain animal in this both sites. Shrimp eats plankton that showed higher Bi accumulation, and is the major nutritional source for fish in Santa Cruz and crabs eats mangrove leaves that did not show Bi accumulation in Vitoria Bay as well as crabs which is the main nutritional source for fish in this site.

Linear regressions performed using data from each identified trophic chain showed that in plankton-oyster trophic chain, there was a biodilution in both sites (Fig. 3) and in plankton-shrimp-fish and plants-crab-fish trophic chain did not show a pattern, neither biomagnification or biodilution were observed in both sites (Fig. 3). Although when analysed only plankton-fish a biodilution were observed (Fig. 3). Metal deposition in oyster's shell can be the main reasons for

low Bi concentration in oyster in soft tissues and is considered one mechanisms for detoxification in these species (Luoma and Rainbow, 2008).

Ba, Bi, W, Ti, Zr, Y, La, Ce (Fig. 1S, 2S and 3S) are not usually analysed in environmental monitoring and are considered as rare elements with no determined biological role but, nowadays, these elements have been used in electronic components, for corrosion resistance and new alloys by electronic industry and other metallurgical industries. Although, all these rare elements are nonessential to the humans and the analysed biota, special attention should be given to the presence of these elements in the environment in future researches. Most elements detected by SEM analysis coupled with EDS, can be considered as emerging contaminants in the environment and research in the biota are scarce.

In conclusion, the results suggested that the main source of Bi present in these estuarine ecosystems is the iron and steel industries localized in Tubarão Complex. In general, the industries are always a step ahead of government and environmental regulations due to the needs of improving products; the industries are continuously creating new compounds with elements that are not regulated by environmental agencies yet and being considered as emerging contaminants such as Bi. The detection of Bi in the biota evidences its potential for accumulation in it. Therefore, Bi effects on the biological processes need to be investigated in the biota.

ACKNOWLEDGE

This study was supported by Espírito Santo Research Foundation (FAPES), Brazil and, Science and Technology Office from Córdoba National University (CONICET), Argentina. The authors are thankful to M. Thompson for supporting chemical analyses and V.A.S. Mendes acknowledges the Material Engineering Department/Federal University of São Carlos for facilities. I.C. Souza acknowledges São Paulo Research Foundation (FAPESP, Proc.

2014/04832-3 and Proc. 2015/05258-1) fellowships.

REFERENCE

Arrivabene, H.P., Campos, C.Q., Souza, I.C., Wunderlin, D.A., Milanez, C.R.D. & Machado, S. R. 2016. Differential bioaccumulation and translocation patterns in three mangrove plants experimentally exposed to iron. Consequences for environmental sensing. Environmental Pollution, 215, 302-313.

Arrivabene, H.P., Souza, I.C., Có, W.L.O., Conti, M.M., Wunderlin, D.A. & Milanez, C.R.D. 2015. Effect of pollution by particulate iron on the morphoanatomy, histochemistry, and bioaccumulation of three mangrove plant species in Brazil, Chemosphere 127, 27–34.

Arslan, Z., & Paulson, A. 2002. Analysis of biogenic carbonates by inductively coupled plasma–mass spectrometry (ICP–MS). Flow injection on-line solid-phase preconcentration for trace element determination in fish otoliths. Analytical and bioanalytical chemistry, 372:7, 776-785.

Aulinger, A., Prange, A., Niedergesäß, R., Schmolke, S., & Einax, J. W. 2002. Characterization of elemental pollution and its variations in sediments and suspended particulate matter from the River Elbe via multi-element analysis combined with chemometric data evaluation. Journal of environmental monitoring, 4:6, 942-949.

Cadore, S., Dos Anjos, A.P., & Baccan, N. 1998. Determination of bismuth in urine and prescription medicines using atomic absorption with an on-line hydride generation system. Analyst, 123:8, 1717-1719.

Carvajal JR. 1975. Contribucion al conocimiento de la biología de los robalos *Centropomus undecimalis* y *C. Poeyi* en la laguna de terminos, Campeche, México. Instituto Oceanográfico, Universidad de Oriente, Cumaná, Venezuela. Boletín del Institudo Oceanografica 14: 51-70.

Cháves H. 1963. Contribucion al conocimiento de la biología de los robalos, chucumite y constantino (*Centropomus* spp.) del estado de Veracruz. Contribución de la Estación de Biología Marina del Instituto Tecnológico de Veracruz, México. Ciencia 22: 141-161.

Das, A.K., Chakraborty, R., Cervera, M.L., & Guardia, M. 2006. Analytical techniques for the determination of bismuth in solid environmental samples. Trends in Analytical Chemistry, 25:6, 599-608.

Dimitriadis, V.K., Domouhtsidou, G.P., & Raftopoulou, E. 2003. Localization of Hg and Pb in the palps, the digestive gland and the gills in *Mytilus galloprovincialis* (L.) using autometallography and X-ray microanalysis. Environmental Pollution, 125:3, 345-353.

Fenchel, T. 2008. The microbial loop–25 years later. Journal of Experimental Marine Biology and Ecology, 366:1, 99-103.

Filella, M. 2010. How reliable are environmental data on 'orphan'elements? The case of bismuth concentrations in surface waters. Journal of Environmental Monitoring, 12:1, 90-109.

Fowler, S.W., Teyssie, J.L., & Church, T.M. 2010. Scavenging and retention of bismuth by marine plankton and biogenic particles. Limnology and Oceanography, 55:3, 1093-1104.

Gao, X., Wang, Y., Peng, S., Yue, B., Fan, C., Chen, W. & Li, X. 2015. Comparative toxicities of bismuth oxybromide and titanium dioxide exposure on human skin keratinocyte cells. Chemosphere, 135, 83-93.

Gazeta, 2016. http://especiais.gazetaonline.com.br/aterro/ [Access: 08/02/2017].

Gilmore R.G., Donahoe C.J. & Cooke D.W. 1983. Observations on the distribution and biology of the common snook, *Centropomus undecimalis* (Bloch). Florida Scientist 46: 313–336.

Hall, G.E. & Pelchat, J.C. 1997. Analysis of geological materials for bismuth, antimony, selenium and tellurium by continuous flow hydride generation inductively coupled plasma mass spectrometry. Journal of Analytical Atomic Spectrometry, 12:1, 97-102.

Hanke, B.V., Meyer, A.A.N. & Oliveira, E. 2008. Análise histológica de estruturas sensoriais de *Centropomus parallelus* (Poey, 1896) (Centropomidae) relacionadas ao hábito alimentar. RUBS, 1, 16-23.

Ivanova, J., Djingova, R., Korhammer, S. & Markert, B. 2001. On the microwave digestion of soils and sediments for determination of lanthanides and some toxic and essential elements by inductively coupled plasma

source mass spectrometry. Talanta, 54:4, 567-574.

Jickells, T. 1995. Atmospheric inputs of metals and nutrients to the oceans: their magnitude and effects. Marine Chemistry, 48:3-4, 199-214.

Kearns, J. & Turner, A. 2016. An evaluation of the toxicity and bioaccumulation of bismuth in the coastal environment using three species of macroalga. Environmental Pollution, 208, 435-441.

Lee, D.S. 1982. Determination of bismuth in environmental samples by flameless atomic absorption spectrometry with hydride generation. Analytical Chemistry, 54:11, 1682-1686.

Lee, D.S., Edmond, J.M. & Bruland, K.W. 1986. Bismuth in the Atlantic and North Pacific: a natural analogue to plutonium and lead?. Earth and planetary science letters, 76:3-4, 254-262.

Lorrain, A., Paulet, Y.M., Chauvaud, L., Savoye, N., Donval, A. & Saout, C. 2002. Differential δ13 C and δ15 N signatures among scallop tissues: implications for ecology and physiology. Journal of experimental marine biology and ecology, 275:1, 47-61.

Luoma, S.N., Rainbow, P.S., 2008. Metal contamination in aquatic environments: science and lateral management, first ed. Cambridge University Press, Cambridge.

Mantel, S. K. & Dudgeon, D. 2004. Dietary variation in a predatory shrimp Macrobrachium hainanense (Palaemonidae) in Hong Kong forest streams. Archiv für Hydrobiologie, 160:3, 305-328.

Monferrán, M.V., Garnero, P., Bistoni, M.A., Anbar, A.A., Gordon, G.W. & Wunderlin, D.A. 2016. From water to edible fish. Transfer of metals and metalloids in the San Roque Reservoir (Córdoba, Argentina). Implications associated with fish consumption. Ecological Indicators, 63, 48-60.

Moscoso-Pérez, C., Moreda-Piñeiro, J., López-Mahía, P., Muniategui-Lorenzo, S., Fernández-Fernández, E. & Prada-Rodríguez, D. 2003. Bismuth determination in environmental samples by hydride generation–electrothermal atomic absorption spectrometry. Talanta, 61:5, 633-642.

Paytan, A., Mackey, K.R., Chen, Y., Lima, I.D., Doney, S.C., Mahowald, N., Labiosa, R. & Post, A. F. 2009. Toxicity of atmospheric aerosols on marine phytoplankton. Proceedings of the National Academy of Sciences, 106:12, 4601-4605.

Phillips, D.J.H. 1980. Quantitative Aquatic Biological Indicators. Applied Science Publishers, London.

Provencher, J.F., Braune, B.M., Gilchrist, H.G., Forbes, M.R. & Mallory, M.L. 2014. Trace element concentrations and gastrointestinal parasites of Arctic terns breeding in the Canadian High Arctic. Science of the Total Environment, 476, 308-316.

Reynolds, C.S. 1984. The ecology of freshwater phytoplankton. Cambridge University Press.

Riley, J.P. & Roth, I. 1971. The distribution of trace elements in some species of phytoplankton grown in culture. Journal of the Marine Biological Association of the United Kingdom, 51:01, 63-72.

Rivas LR. 1986. Sistematic Review of the Perciform fishes of the genus Centropomus. American Society of Ichthyologist and Herpetologist. Copeia 3: 579-611.

Santos, J.M., Reis Jr N.C., 2011. Caracterização e quantificação de partículas sedimentadas na região da Grande Vitória. Relatório Técnico Instituto Estadual do Meio Ambiente.

Souza I.C., Duarte I.D., Pimentel N.Q., Rocha L.D., Morozesk M., Bonomo M.M., Azevedo V.C., Pereira C.D.S., Monferrán M.V., Milanez C.R.D., Matsumoto S.T., Wunderlin D.A., Fernandes M.N. 2013. Matching metal pollution with bioavailability, bioaccumulation and biomarkers response in fish (*Centropomus parallelus*) resident in neotropical estuaries. Environmental Pollution 180: 136-144.

Sunda, W.G. & Huntsman, S.A. 1998. Processes regulating cellular metal accumulation and physiological effects: phytoplankton as model systems. Science of the Total Environment, 219:2, 165-181.

Tallarico, F.H.B., McNaughton, N.J., Groves, D.I., Fletcher, I.R., Figueiredo, B.R., Carvalho, J.B., Rego, J.L. & Nunes, A.R. 2004. Geological and SHRIMP II U-Pb constraints on the age and origin of the Breves Cu-Au-(W-Bi-Sn) deposit, Carajás, Brazil. Mineralium Deposita, 39:1, 68-86.

Taylor R.G., Whittington J.A., Grier H.J., Crabtree R.E. 2000. Age, growth, maturation, and protandric sex reversal in the common snook, *Centropomus undecimalis*, from South Florida waters. Fishery Bulletin 98: 612–624.

Tokman, N. & Akman, S. 2004. Determination of bismuth and cadmium after solid-phase extraction with

chromosorb-107 in a syringe. Analytica chimica acta, 519:1, 87-91.

Volpe A.V. 1959. Aspects of the biology of the common snook, *Centropomus undecimalis* of southwest Florida. In: MITTS, Ernest, State Board of Conservation. Technical Series. The Marine Laboratory. Florida: University of Miami 31: 37.

Wang, W.X. & Fisher, N.S. 1998. Accumulation of trace elements in a marine copepod. Limnology and Oceanography, 43:2, 273-283.

Watling, H.R. & Watling, R.J. 1976. Trace metals in *Choromytilus meridionalis*. Marine Pollution Bulletin, 7:5, 91-94.

Xu, J., Zhang, M. & Xie, P. 2008. Stable isotope changes in freshwater shrimps (*Exopalaemon modestus* and *Macrobrachium nipponensis*): trophic pattern implications. Hydrobiologia, 605:1, 45-54.

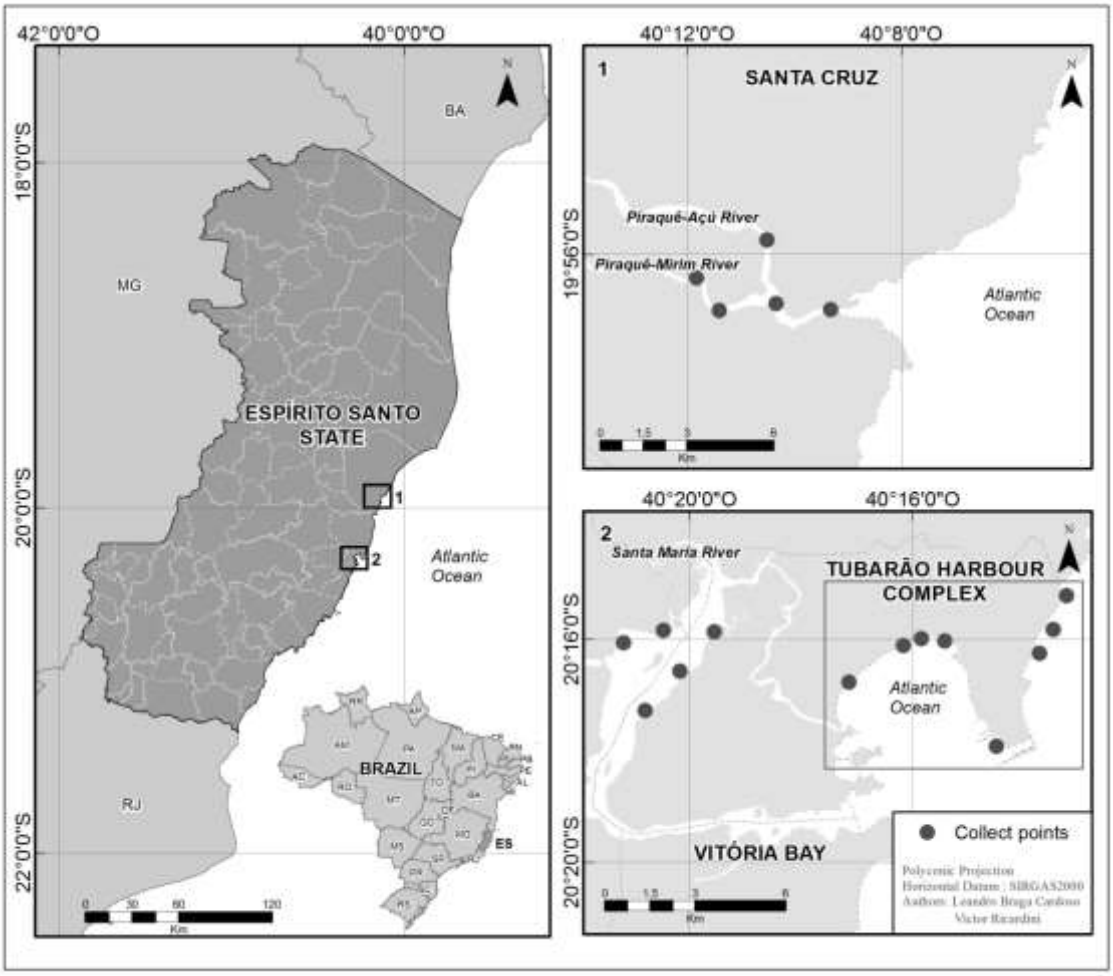


Figure 1. Location of the State of Espírito Santo (South America, Brazil), showing sampling sites. Santa Cruz (S 19º56`26.2``; W 40º 12`87``), Vitória Bay (S 20º14`31.5``; W 40º19`84.7``) and Tubarão Harbour Complex (20º17′03.8″S and 40º14′24.9″W).

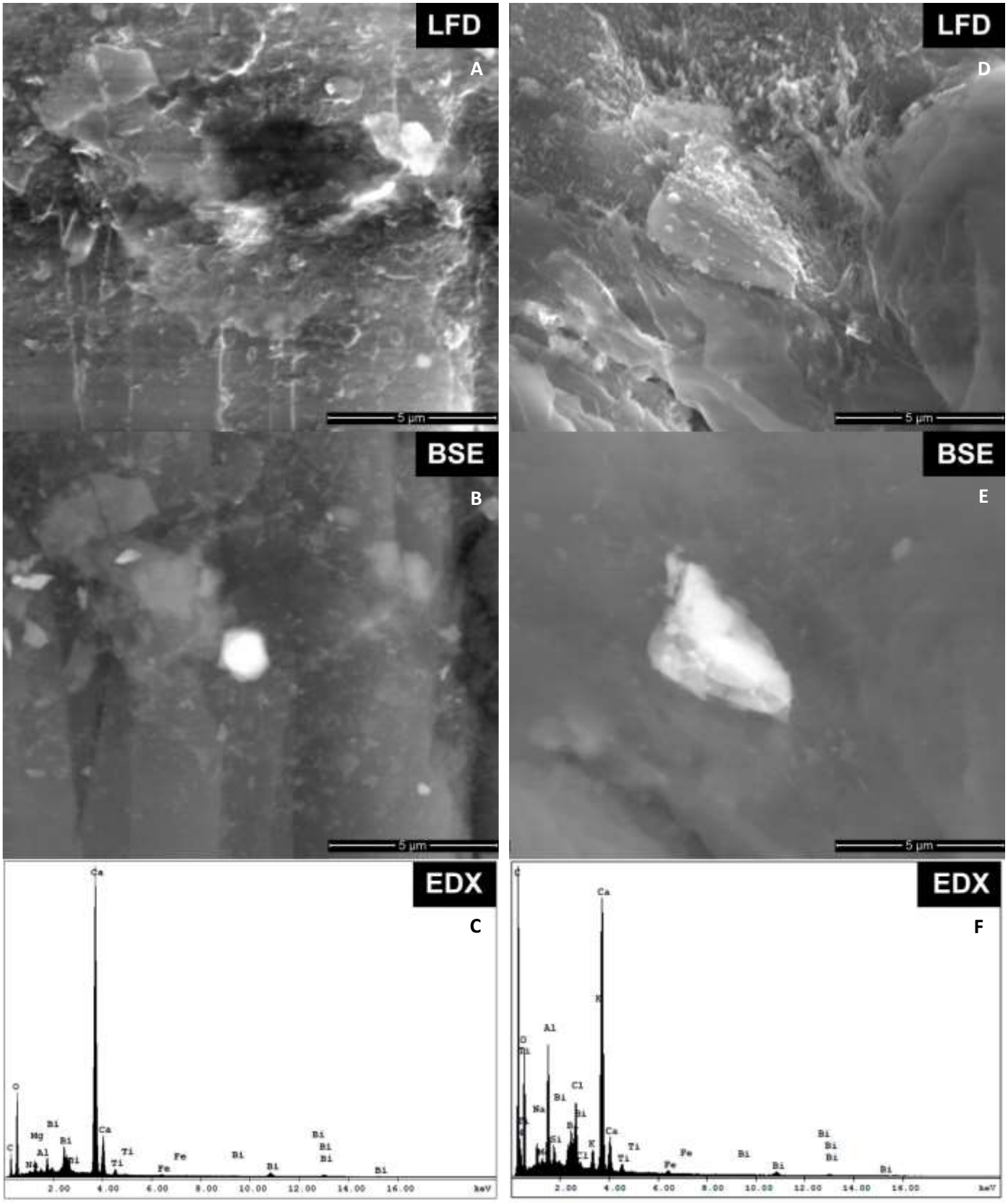


Figure 2. Scanning microscope images showing bismuth particles inside shell oyster of *Crassostrea rhizophorae*. LFD: Large Field Detector for topographic, BSD: Electron Backscatter Diffraction for chemical contrast, EDX: energy dispersive X-ray spectroscopy for chemical identification. A-C: Imagens from Santa Cruz, D-F: Images from Vitória Bay.

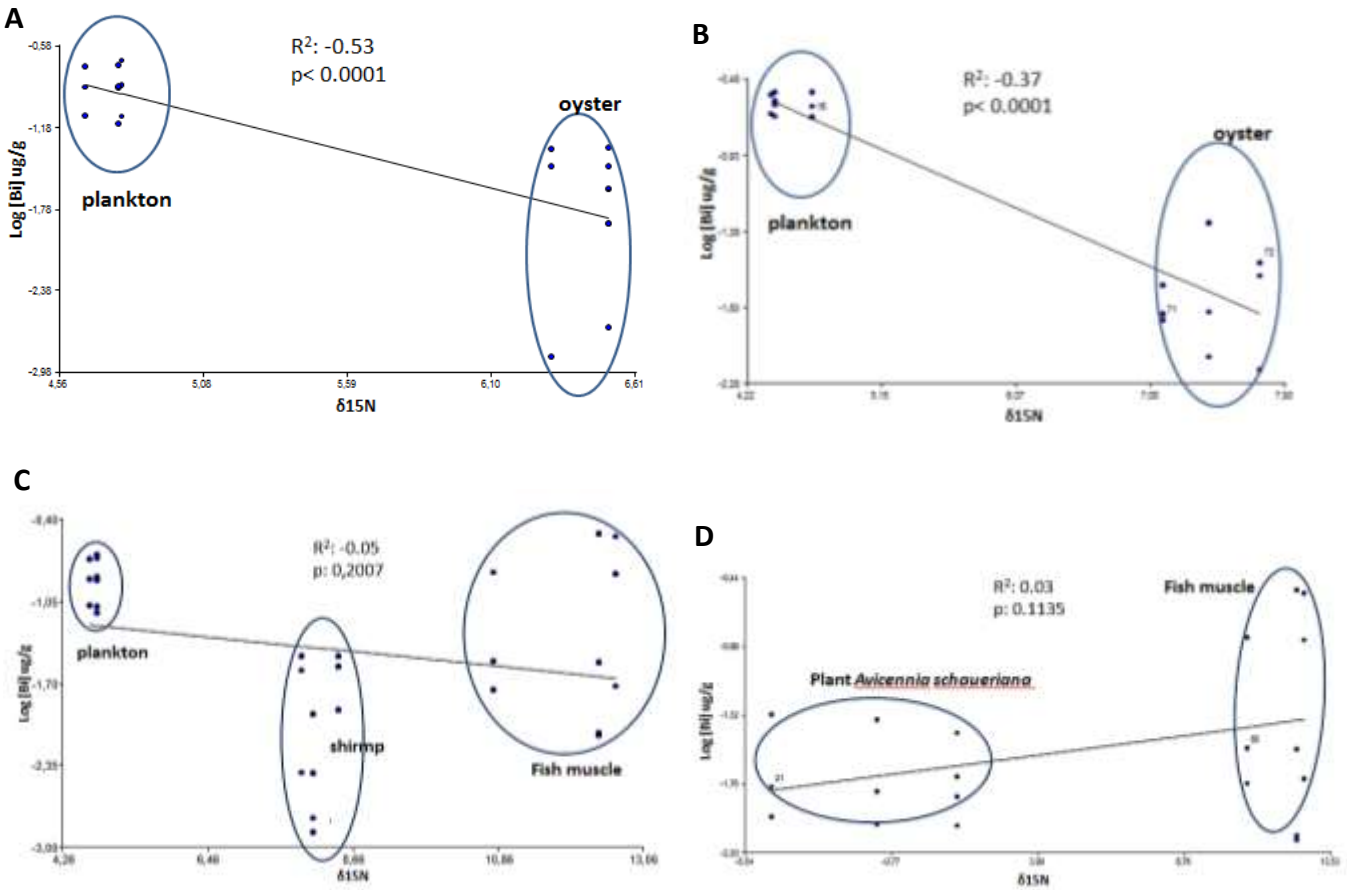


Figure 3. Relationship between Log[Bi, µg g−1 dry weight] versus $\delta^{15}N$ for bismuth. A) Bi biodiluted throughout Santa Cruz studied food web; B) Bi biodiluted throughout the Vitória Bay studied food web; (C-D) Bi without significant change throughout Santa Cruz studied food web.

Table 1. Measurements of bismuth in abiotic and biotic compartments of the estuaries Santa Cruz and Vitoria Bay and Tubarão Complex. Values correspond to means ± standard deviation ng/g dry weigh. Lowercase letters indicate significant differences between sites (Tukey test, $P < 0.05$). SW: surface water, SD: sediment, PM: atmospheric particulate matter, IF: Iron Effluent, IP: Iron Port, SE: Steel effluent, SP: Steel Port, RI: *Rhizophora mangle*, LA: *Laguncularia racemosa*, AV: *Avicennia schaueriana*, PK: plankton, CR: Crab (*Aratus* sp.), OS: oyster (*Crassostrea rhizophorae*), SH: shrimp (*Macrobrachium* sp.), GI: gill of fish (*Centropomus parallelus*), HP: hepatopancreas of fish, KI: kidney of fish, MU: muscle of fish, GO: gonad of fish. *NA = Not analysed. *NC = Not analysed.

		Sites		
		Santa Cruz [ng/g]	**Vitoria Bay [ng/g]**	**Tubarão Complex [ng/g]**
Abiotic	**SW**	<LOD	<LOD	**IF** <LOD **IP** 15224.55 ± 8306.2 **SE** 4442.41 ± 727.95 **SP** 1868.61 ± 705.16
	SD	<LOQ	<LOQ	<LOQ
	PM	*NA	*NA	<LOQ
Primary Producers	**RI**	<LOQ	<LOQ	<LOQ
	LA	<LOQ	<LOQ	36.10 ± 5.97
	AV	22.45 ± 14.94^{b}	<LOQ	10.47 ± 2.89^{a}
	PK	134.33 ± 48.28^{a}	233.07 ± 31.16^{b}	*NC
Primary Consumers	**CR**	<LOQ	<LOQ	*NC
	SH	14.91 ± 12.62	<LOQ	*NC
	OS	23.51 ± 16.34^{a}	16.95 ± 13.02^{a}	*NC
Top of food web	**GI**	33.00 ± 45.25^{a}	32.37 ± 71.11^{a}	12.02 ± 6.36^{a}
	KI	<LOQ	<LOQ	43.31 ± 7.95
	HP	184.80 ± 371.88^{a}	53.39 ± 30.67^{a}	77.80 ± 6.13^{a}
	MU	98.83 ± 115.44^{a}	44.00 ± 30.68^{a}	34.03 ± 1.13^{a}
	GO	45.19 ± 23.76^{b}	15.83 ± 5.97^{a}	43.23 ± 2.85^{b}

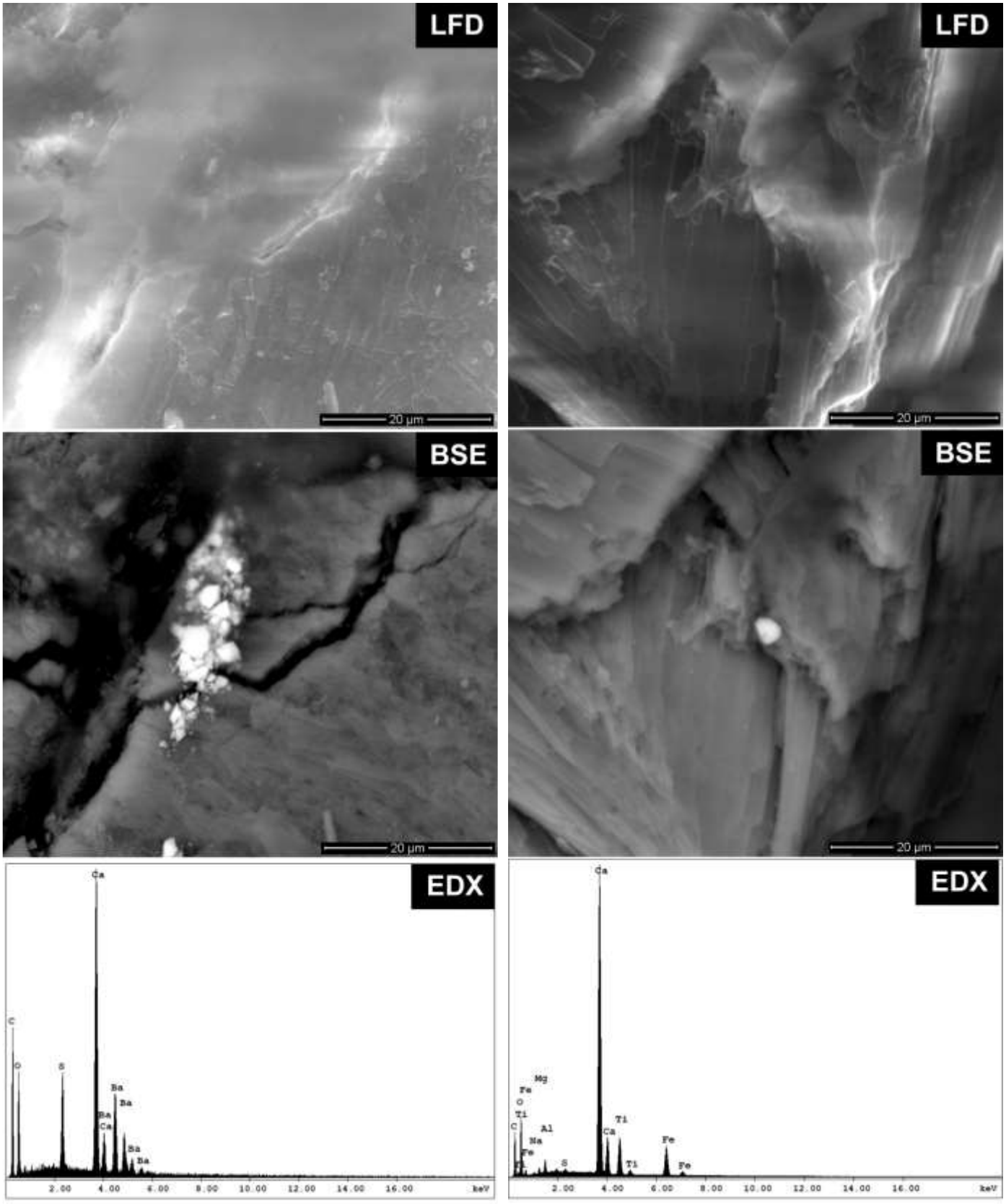


Figure 1S. Scanning microscope images showing metallic particles inside shell oyster of *Crassostrea rhizophorae*. Large Field Detector for topographic, BSD: Electron Backscatter Diffraction for chemical contrast, EDX: energy dispersive X-ray spectroscopy for chemical identification.

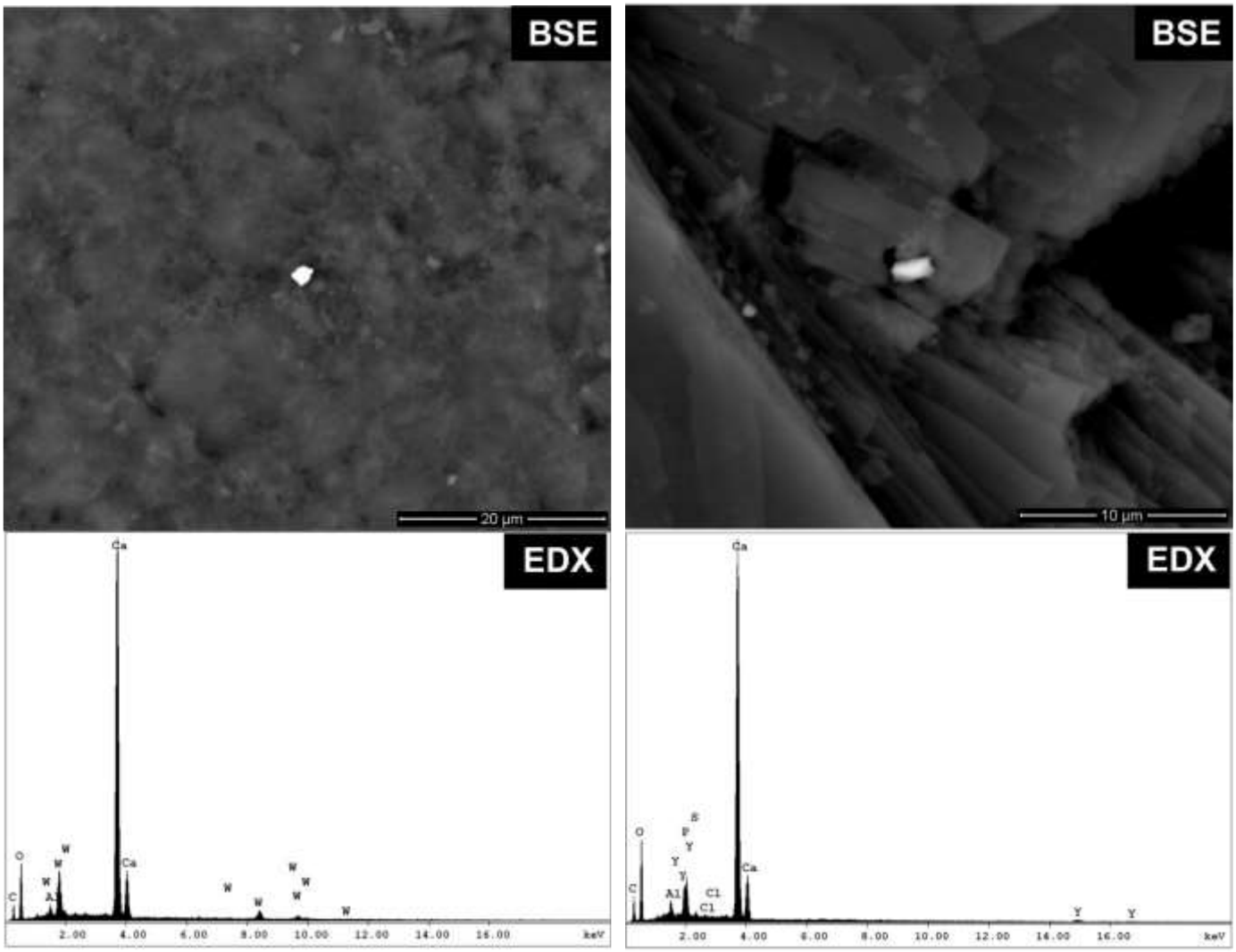


Figure 2S. Scanning microscope images showing metallic particles inside shell oyster of *Crassostrea rhizophorae* Large Field Detector for topographic, BSD: Electron Backscatter Diffraction for chemical contrast, EDX: energy dispersive X-ray spectroscopy for chemical identification.

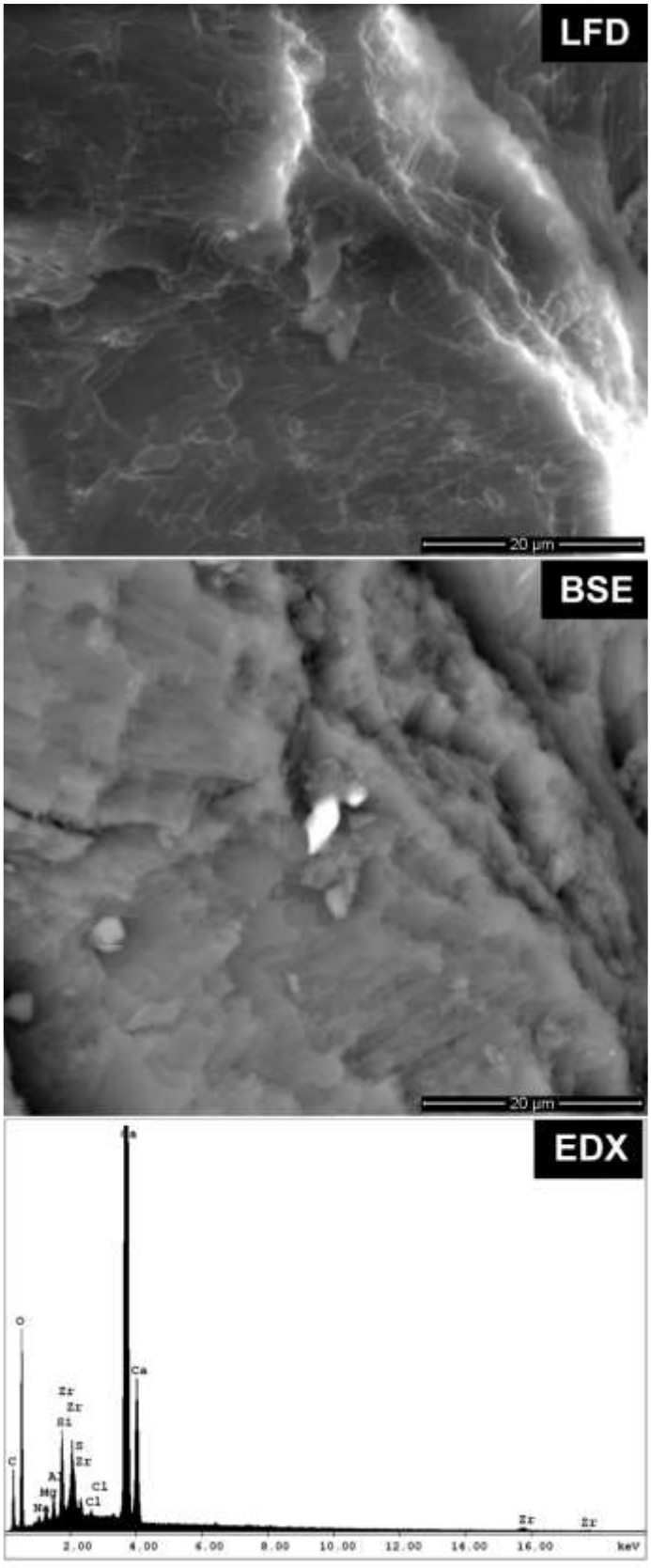


Figure 3S. Scanning microscope images showing metallic particles inside shell oyster of *Crassostrea rhizophorae*. Large Field Detector for topographic, BSD: Electron Backscatter Diffraction for chemical contrast, EDX: energy dispersive X-ray spectroscopy for chemical identification.

CAPÍTULO 6

Titanium dioxide nanoparticles identification in neotropical mangrove ecosystem: transfer through food web and subcellular internalization in top trophic chain fish

ABSTRACT

Among the varieties of nanoparticles (NP) produced, metal oxides have been increasingly applied due to their photocatalytic nature, particularly, the titanium dioxide NP (TiO_2-NP). Considering the presence of TiO_2-NP in the environment due to industrial and sewage releasing, especially in aquatic ecosystems, *in-situ* studies are required to evaluate the behaviour of theses NP in the environmental compartments and biota. Therefore, the purpose of this study was to quantify if Ti is present in abiotic samples (surface water, sediment, atmospheric particulate matter) and in six trophic levels (plankton, plants, oyster, crab, shrimp, fish) of neotropical estuarine mangrove ecosystems and evaluate its potential for biomagnification or biodilution in the food web. Moreover, the nanocrystallographic structure of Ti in abiotic matrixes and in fish organ/tissues was analysed to identify the TiO_2-NP oxidation state. Ti was identified and quantified in almost all matrix analysed, excepting for surface water in all sites. Linear regression showed biodilution through trophic chain based in plankton as primary producer and biomagnification when plants are the primary producers. Nanocrystallographic analyses showed that TiO_2-NP in atmospheric particulate matter samples were similar to those found in each fish organ, the top trophic chain organism, independent of their localization: inside cytoplasm and in the cell nucleus suggesting that atmospheric particulate matter may be the source of TiO_2-NP in these mangrove ecosystems.

Keywords: nanocrystallographic, oxidation state, atmospheric particulate matter, rutile

1. INTRODUCTION

Nanotechnology has been considered one the greatest industrial innovations since the beginning of industry having numerous possibilities for applications on products available to industries and private consumers (Som et al., 2010) being the nanoparticles (NP) are characterized as particles in which, at least, one of its external dimension ranges from 1 to 100 nm (ASTM, 2006). Several consumer products are currently listed containing NP, including sunscreens and cosmetics as well as drug delivery systems, paints, surface coatings, semiconductors and electronics (Johnson et al., 2015). Among the varieties of NP produced, metal oxides have been increasingly applied due to their photocatalytic nature, particularly, the titanium dioxide NP (TiO_2-NP). They are one of the most synthesized metal oxide NP (Klaine et al 2008) and have been estimated as 165,050,000 metric tonnes of TiO_2 produced worldwide between 1916 and 2011 (Jovanovic 2015).

TiO_2 occurs naturally and is obtained from minerals. Four TiO_2 polymorphs are commonly known and found in nature: anatase (tetragonal), rutile (tetragonal), brookite (orthorhombic) and TiO_2 (B) (monoclinic) (Carp et al 2004). Additional high-pressure forms have been synthesized such as TiO_2 (II) with a PbO_2 structure and TiO_2 (H) with a hollandite structure (Simons and Dachilleet, 1967; Latroche et al 1989). The crystallization forms influence its photocatalytic activity; so that, anatase has higher photocatalytic activity compared to rutile (Linsebigler et al 1995; Thompson and Yates 2006). Furthermore, most TiO_2-NP have a zero charge point in the range of neutral pH values (Kosmulski 2009; Loosli et al 2013) which allows its rapid aggregation in natural aquatic systems.

Nowadays, TiO_2-NP are used many personal care products such as sunscreens and cosmetics due to their transparency and solar UV radiation absorption (Melquiades et al 2008).

In addition, it is worth highlighting in manufacturing of textiles and electronics, production of medicines, water purification processes and photodegradation of pollutants in water (Theron et al 2008; Paschoalino et al 2010). In steel industry, TiO_2-NP addition on steel matrix improved its mechanical properties (Amondarain et al 2013) and the application of a thin film of TiO_2-NP on steel act as photoprotection against corrosion (Ohko et al 2014). Titanium alloys are widely used due to its corrosion resistant which permit compose aircraft's structure and aircraft turbine parts, chemical processing equipment, marine hardware and medical application (Ngajib et al 2013). Then, due to its intense application in several industrial branches, the TiO_2-NP in the environment needs attention as they may be potentially toxic for living systems (Xiong et al 2011); TiO_2-NP may accumulate in the environment via waterway releasing from industrial and urban sewage (Robichaud et al 2009; Menard et al 2011; Gondikas et al 2014).

Regardless of its release source, TiO_2-NPs eventually will end up into the sea in which it may represent a risk to marine organism health (Matranga and Corsi 2012). Even at low concentrations, these NP and their aggregates can affect microorganisms in natural aquatic systems (Battin et al 2009). Laboratory studies have reported TiO_2-NP toxicity on biota such as algae, zooplankton, bacteria and fish (Handy et al 2008; Sharma 2009). The TiO_2-NP effects on green alga, *Desmodesmus subspicatus* (Sharma 2009), zooplankton, *Daphnia magna* (Lovern and Kepler 2006), oyster *Crassostrea virginica* (Johnson et al 2015), estuarine polychaete *Laeonereis acuta* (Nunes et al 2016), rainbow trout *Oncorhynchus mykiss* (Frederici et al 2007), plants (Demir et al 2014) and mammalian cells (Freyre-Fonseca et al 2011; Park et al 2008) were reported in studies under controlled experimental conditions. In summary, TiO_2-NP present presumed low risk to the ecosystem, being classified as non-harmful material (Kahru and Dubourguier, 2010). However, despite low or moderate toxicity in short-term evaluations,

the toxicity after long-term TiO_2-NP exposure may be greater (Kim et al, 2010).

Considering the presence of TiO_2-NP in the environment due industrial and sewage releasing, especially in aquatic ecosystems, *in-situ* studies are required to evaluate the behaviour of theses NP in the environmental compartments and biota. Therefore, the purpose of this study was to quantify if Ti is present in abiotic samples (surface water, sediment, particulate matter) and in six trophic levels (plankton, plants, oyster, crab, shrimp, fish) of neotropical estuarine mangrove ecosystems and evaluate its potential for biomagnification or biodilution in the food web. Moreover, the nanocrystallographic structure of Ti in abiotic matrixes and in fish organ/tissues was analysed to identify the TiO_2-NP oxidation state.

2. MATERIAL AND METHODS

2.1 Study areas:

Three sampling areas in the Espírito Santo state, southeastern Brazil were studied: the Tubarão Complex and two mangrove areas, Vitória Bay and Santa Cruz (situated at 10 and 70 km from Tubarão Complex, respectively) (Fig. 1). Sampling collection was done at the end of summer (March 2014 and 2015). Tubarão Complex (20º17′03.8″S and 40º14′24.9″W) is located in a coastal area between Vitória City and Serra (Fig. 1) and is a metal industrial area which include iron pelletization, iron ore storage and transportation in an open-air courtyards mechanism to cargo ships and steel industry which produces steel metallic smoke during steel smelting process in addition to solid discharge waste adjacent to sea (Camburi Beach) and the liquid effluent by outfall (Santos & Reis Jr, 2011).

Santa Cruz mangrove ecosystem (19°56′26.2″S and 40°12′87.0″W) is constituted by two rivers and coverage an area of about 12 km^2. It includes the Piraquê-Açu and Piraquê-Mirim Mangrove Ecological Reserve which represents a preserved mangrove area (Fig. 1).

Vitória Bay (20°14′31.5″S and 40°19′84.7″W) is an estuarine complex formed by five rivers which received several landfills [industrial solid waste from harbour dredging activities or other industrial activities from Tubarão Complex (Gazeta, 2016)] since 19th to the end of 20th centuries, totalling 12,000 m^2; at least a half of this area was originally occupied with mangroves (Fig. 1).

2.2 Sampling procedure:

Surface water (SW) and sediment (SD) samples were taken in Tubarão Complex, Santa Cruz and Vitória Bay mangrove ecosystems (Fig. 1). In the Tubarão Complex were 4 SW and 4 SD sampling points. SW sampling were at about 100-200 m far from: 1) Iron effluent (IF) - the discharge of the pelletizing iron industry, 2) Iron port (IP) – port for pelletizing iron industry export, 3) Steel effluent (SE) - the steel industry effluent discharge and, 4) Steel port (SP) – port for steel industry export (Fig. 1). SD sampling was collected in four points adjacent to solid discharge from Tubarão complex. Atmospheric particulate matter (PM) was collected at about 1 km from Tubarão Complex according to Arrivabene et al. (2015). Water samples were collected using acid washed plastic bottles submerged 10-20 cm depth; samples were acidified with ultrapure HNO_3 (sub-boiling grade), stored at 4ºC and before analyses were filtered in 0.45 µm nitrocellulose filter. Sediment samples were collected using a polypropylene spoon among 10-20 cm depth; samples were placed into clean 1L-polypropylene flasks (without headspace), dried at room temperature and sieved through 63 µm nylon meshes.

Samples (n = 9) of *Rhizophora mangle* (RM) (leaves), *Laguncularia racemosa* (LR) (leaves), *Avicennia schaueriana* (AS) (leaves), plankton (PK), shrimp *Macrobranchium sp.* (SH) (whole body), crab *Aratus sp.* (CR) (whole body), oyster *Crassostrea rhizophorae* (OS) (soft tissue) and organs [muscle (MU), gill (GI), kidney (KI), hepatopancreas (HP) and gonad

(GO) from juvenile fish *Centropomus parallelus* were collected in each site, excepting plankton, shrimp, crab and oyster in Tubarão Complex because they differ from mangrove community. Plankton (phytoplankton and zooplankton) samples were collected using a 20 μm net mesh. Fully expanded leaves from 3rd to 5th node of the mangrove three species were collected and washed with ultrapure water. Crabs were caught from trees by hand and shrimps with pitfall aid. Fish were collected using hook and line and their organs immediately removed and separated for chemical and ultrastructural analysis. For chemical analysis each biological sample was freeze-dried until reach constant mass, macerated with mortar and pestle and stored at room temperature until analysis. For ultrastructural analysis by transmission electron microscope, fish samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3 solution.

2.3 Titanium analysis and transfer through food web

Ultrapure water was obtained (<5 μg L^{-1} TOC) from purification system Arium 61316-RO plus Arium 611 UV (Sartorius, Germany). Multi-element standard solution Merck VI CertiPUR® was obtained from Merck Química Argentina (Buenos Aires, Argentina). Nitric acid (63.7%) sub-boiling grade was prepared from analytical grade acid using a distiller (Figmay Sub-boiling distilled, Córdoba, Argentina). Purity of nitric acid was verified by Mass Spectrometry Inductively Coupled Plasma (ICP-MS), Agilent 7500cx, USA, equipped with an ASX-100 autosampler (CETAC Technologies, Omaha, NE). Filters (0.45 μm, HAWG04756) were obtained from Millipore (São Paulo, Brazil). All glassware and plastic bottles/containers were left overnight with sulfuric/nitric acids solution and posteriorly washed with ultrapure water. ICP probes and pipes were of PTFE previously washed with nitric acid (2% v v^{-1}).

Sediment and particulate matter (0.1 g dry weight each) were digested with 4 mL of nitric acid and 1 mL of hydrochloric acid (ultrapure, sub-boiling grade). Biotic samples were ground and homogenized with a mortar and 0.1 g from each sample was digested in 4 mL of nitric acid, 1 ml of hydrochloric acid and 0.5 mL of hydrogen peroxide (ultrapure, sub-boiling grade), filtered in 0.45 µm nitrocellulose filter according to USEPA (2009) and, then Ti was measured. Controls were prepared using only reagents following the same protocol. All digested samples were stored at 4°C until analysis. Pre-cleaned PTFE tubes (Savillex) at constant temperature (90°C) during 24 hours (Chappaz et al. 2012) were used in digestion procedure. After digestion, samples were filtered using 0.45 µm nitrocellulose filter to remove remaining particles according to method described by USEPA (2009) and measured. Controls were prepared using only reagents following the same protocol. All digested samples were stored at 4°C until analysis.

The measurements were performed in triplicate and the repeatability of ICP-MS measurements was generally ≥ 97%. Quality assurance (QA) and quality control (QC) were done using a certified reference material (CRM): estuarine and marine sediment MESS-2, bush branches and leaves NCSDC 73348, peach leaves NIST SRM 1547 and NIST 8414 bovine muscle. Recoveries from CRMs were 88 ± 16%, respectively.

Thereafter Ti transfer in each trophic chain (1.plant-crab-fish; 2.plankton-shrimp-fish; 3.plankton-oyster) determined by Souza (2017) in Santa Cruz and Vitória Bay using linear regression analysis between Ti concentration and $\delta^{15}N$ data reported by Souza (2017, chapter 1).

2.4 X-ray and Scanning Electron Microscope (SEM)

X-ray diffraction was used to determine the general phase of sediment and atmospheric particulate matter; 1 cm^2 of sediment or particulate matter was fixed in a glass slide and analysed with 5º to 90º scanning at 2º/min using a Rigaku Geiger-Flex with target copper at 30kV and 40 mA. For SEM, sediment and atmospheric particulate matter were direct fixed on carbon tape glued on aluminium stub. Scanning MEV MAGELLAN electron backscatter detector (BSE) were used to identify chemical contrast, secondary electrons (SE) were used to identify morphology and X-ray Dispersive Energy Spectroscopy (EDS) were used to identify chemical elements lower than 5% of total composition.

2.5 High Resolution Transmission Electron Microscopy and Crystallographic Analyses

Atmospheric particulate matter were sonicated in a 100% solution of isopropyl alcohol for 24 minutes and 20 µl of the supernatant were removed and put on carbon covered gold grids for analysis of particles below 200 nm. Water (20 µl) was put on a carbon covered gold grid, left to dry and to verify the presence of metallic particles. All fish samples were post-fixed and contrasted in 1% osmium tetroxide. The samples were dehydrated in alcohol crescent series and propylene oxide, embedded in Spurr's resin (EMS − Low Viscosity Embedding Media, U.S.) and ultrathin sections (90 nm in thickness) were obtained with an ultramicrotome. From each fish organ 5 fields per sections (n = 5) were analysed per site.

Analyses were carried out in a FEI TECNAI G^2 F20 equipped with STEM-HAADF detector and chemical contrast difference in which the lighter particles have a larger atomic radius and have high angle diffraction in an annular detector with a higher atomic weight than the darkest. EDS detector was used in order to confirm particle composition. Nanodiffraction was used for particles size close to 100 nm and lower to identify the crystallographic structure (oxidation

state and particle morphology). Electron nanodiffraction was performed to find axes of high symmetry zone in nanoparticles containing Ti and O using the holder TEM double tilt. Nanodiffraction pattern were indexed (comparison between the obtained data and the theoretical pattern) using Jems software (Electron Microscopy Software, P.Stade Imann, Switzeland) and theoretical crystallography of the ICSD FIZ Karlsruhe, Germany. At least 2 high symmetry zone axes of the same nanoparticle were indexed.

2.6 Statistical analysis

Statistical analyses were conducted depending on each data set. Shapiro-Wilk Test and means for Variances-Levene (ADM) analysis was used to check normal distribution and homogeneity of variance, respectively. Two way ANOVA followed by Tukey test (significance level $p<0.05$) was conducted in 2014 and 2015 Victoria Bay and Santa Cruz data set, after logarithmic data transformation, to detect differences of the same matrix between sites and differences among matrixes within the same site. The data was reported in mean (95% confidence interval). Non-parametric Kruskal-Wallis followed by Steel-Dwass test (significance level $p<0.05$) was conducted in 2015 Tubarão complex data set, to detect differences among matrixes. The data was reported in median (q1-q3). One Way ANOVA followed by Tukey test (significance level $p<0.05$) was conducted in 2014 matrixes of Tubarão complex data set, to detect differences among matrixes. The data was reported in mean (95% confidence interval). Linear regression between Ti concentration determined in the present study and $\delta^{15}N$ data determined by Souza (2017, chapter 1) determined Ti transfer through the food web. All statistical analyses were conducted using the software JMP v.12.

3. RESULTS AND DISCUSSION

This is the first study, in field, that identified significant amounts of Ti in abiotic (sediment and atmospheric particulate matter) and biotic (plankton, mangrove trees, oysters, shrimps, crabs and fish) matrixes in neotropical mangrove ecosystems and evaluated its transfer through food web. Nanocrystallographic analyses in sediment, atmospheric particulate matter and each fish organ, the top trophic chain organism, characterized TiO_2-NP in abiotic samples and its localization inside cytoplasm and cell nucleus of different organ/tissue of fish.

3.1 Ti in abiotic and biotic samples

Ti concentration in abiotic and biotic matrix in Santa Cruz, Vitória Bay and Tubarão Complex are shown in Tables 1S and 2S. Ti highest levels in the sediment were found in abiotic matrixes; the quantification carried out, in 2014, showed Ti levels ranging from 569.65.66 μg/g in Tubarão Complex to 1252.53 μg/g in Vitória Bay while, in 2015, the variation was between 39.83 μg/g in the first cited site and 991.68 μg/g in Santa Cruz; Ti was not detected in the surface water from all sites (Table 1S and 2S). The higher levels of Ti in the sediment may be related to physical-chemical processes, in which metals may be adsorbed to oxides or organic matter reducing their mobility and availability (Ducrotoy et al, 2013). On the other hand, in water, the behaviour of metals is influenced by the O_2 concentration and the pH which is determinant on the speediness of chemical and biochemical transformation processes increasing or reducing the availability of metals (Luoma and Rainbow, 2008). The concentration of dissolved Ti in interstitial water (water removed from sediment) may reach up to 50 times higher than in water column, even closely to the sediment being significant importance in transport and bioavailability of them (Luoma and Rainbow, 2008). Ti in atmospheric particulate material (Table 1S and 2S) collected in Tubarão Complex was 1519.73 μg/g (2014) and 386.18

μg/g (2015). In the mangrove sites, Vitória Bay and Santa Cruz, low atmospheric particulate matter was collected probably due to canopy of trees which prevents it to be deposited in water/sediment.

Among biotic samples, plankton showed the highest Ti values (Table 1S and 2S), followed by crabs and shrimp. Plankton, that is the base of aquatic food web (Anandraj et al., 2008), is able to accumulate trace elements present in the aquatic environment (Wang and Fisher, 1998) being an important route of metal transfer through the marine food chain (Fenchel, 2008).

Ti levels of other primary producers varied among Vitória Bay, Santa Cruz and Tubarão Complex; Ti levels in the leaves of *A. schaueriana* and *R. mangle* were higher in Vitória Bay and Tubarão Complex while in *L. racemosa* ti was higher in Tubarão Complex (Table 1S and 2S). The highest Ti levels in *A. schaueriana* from all sites may be related to salt-exclusion mechanism in the leaves of this species which results in a differentiated leaf composition in relation to the others mangrove plant species and favour higher accumulation of Ti (Table 1S). Similar finds was reported for accumulation of other metals detected in these mangrove ecosystems (Souza et al, 2014a, 2014b, 2015).

In general, Ti quantification in crab (CR), shrimp (SH) and fish from both sites, Santa Cruz and Vitória bay, was lower than plankton (Table 1S and 2S). Ti levels in crabs from Santa Cruz were higher than in shrimp and oysters and it levels in all matrixes from Santa Cruz was higher than those from Vitória Bay excepting, the oysters, which levels were higher in Vitória Bay (Table 1S). In fish, Ti was detected in all analysed organs and the highest Ti concentration was in the gills.

Bioaccumulation implies in contaminant absorption faster than excretion depending on toxicokinetic processes (Menard et al 2011; Hartmann et al 2012); aquatic species can uptake

metals from the surrounding water and/or feeding contaminated preys however, some metals are efficiently excreted reducing the possibility of accumulation and trophic transfer throughout food chains (Monferrán et al, 2016). Conversely, biodilution or biomagnification, which implies in decreasing or increasing concentration, respectively of an element correlated with increasing trophic level in food chain, depend on contaminant behaviour of such element into biota (Azevedo and Chasin, 2004; Azevedo, 2003). The linear regressions obtained between Ti concentration (present study) and $\delta^{15}N$ data (Souza, 2017, chapter 1) in each food chain in Santa Cruz and Vitória Bay were presented in Table 3S, only significant results were discussed. According to Monferrán et al. (2016), positive slope indicates increasing concentration through the food chain due to element persistence, while negative slope indicate decreasing concentration due to possible elimination of an element from the food chain or an interrupted trophic transfer. In the present study, the relationships between Ti concentration and $\delta^{15}N$ showed a dependence of the trophic level in the accumulation in a given organism in the studied food web (Table 3S).

Considering the trophic chain plankton-shrimp-fish, linear regression showed that Ti was biodiluted throughout this food chain in Santa Cruz and Vitória Bay (Fig. 2 and Table 3S); plankton presented the highest Ti levels, shrimp the intermediate one and, the fat snook, the highest trophic level in this food chain, presented lowest concentration of Ti in the muscle. Similar biodilution was also identified in food chain involving plankton-oysters; oysters take plankton during the filtration processes and, consequently, feed them (Fig. 3 and Table 3S).

In the plant-crab-fish trophic chain, linear regression showed a biomagnification of Ti (Fig. 4 and Table 3S). These crabs living most of time on trees and feed leaves from the studied mangrove plant species (Rabelo et al., 2009; Miranda et al., 2017; Yeager et al., 2016) which

suggested that they may be more susceptible to contamination by Ti considering the possible foliar atmospheric deposition of atmospheric particulate matter but the element was not available to following trophic level. The elevated levels of Ti in gills of *C. parallelus* compared to other organ-tissues may be related to the fact that the gills in these animals are responsible for excretion. Biomagnification of Ti in trophic chain was reported by Zhu and collaborators (2010) from *Daphnia* to zebrafish and Chen et al. (2015) between the single-celled alga *Scenedesmus obliquus* and aquatic flea *Daphnia magna* in laboratory studies.

3.2. Ti structure and subcellular internalization

Ti analyses in sediment from Santa Cruz and Vitória Bay and sediment and atmospheric particulate matter from Tubarão Complex, using SEM, revealed that they are mainly constituted by hematite (Fig. 5), a ore from which iron is extracted from Brazilian iron mining and, in the atmospheric particulate matter, besides Fe, other metal particles were also identified such as Ti, Cl, Si, Al, Si, Ca, Na, S, K and Mg (Fig. 6). TEM analysis of surface water did not show metallic particles possible due the little amount of it in water samples. Although Santa Cruz is approximately 70 km north far from Tubarão Complex, the high Ti level in this site may be related to the northeast and east wind predominant directions in these regions (Santos and Reis Jr., 2011) favouring the dispersion of particulate material to long distances until its deposition, especially when it is composed of nanoparticles.

SEM and TEM analyses of particulate matter showed that Ti particles are titanium dioxide NP (TiO_2- NP) (Fig. 6 and 7). Nanoparticles reach the environment (land or water) by different routes during industrial production: solid and liquid effluents and/or solid and gases atmospheric emissions and during transportation of products (Gottschalk et al., 2009). Considering potential risk to human and environment health, transportation by smoke

emissions, which may include atmospheric particulate matter, is perhaps most important due to faster dispersion depending on wind speed and direction may reaching long distance (Csavina et al., 2012). In general, large particles are transported by surface creep (>2000 μm) and saltation (60 – 2000 μm), being responsible for the majority of mass movement at local scale (Stout and Zobeck 1996; Ravi et al 2011); smaller particles from the soil (<60 μm) are transported by atmospheric suspension and are available for long-range transport at regional, continental, and global scales (Chadwick et al 1999; Prospero et al 2002). The TiO_2-NP, identified in the atmospheric particulate matter from Tubarão Complex, may reach long distances before deposition which may explain it in Santa Cruz.

Nanocrystallographic structure analysis of TiO_2-NP in the atmospheric particulate matter, using nano diffraction, showed that this NP has rutile structure, tetragonal form in the crystalline system of space group P4/mnm (tetragonal/tetrahedron configuration) (Fig. 7). Rutile is the most stable form of TiO_2-NP being the most common form in nature (EPA, 2009). Anatase and brookite forms are stable at normal temperatures however, when heated at temperatures between 550 and 750 °C, during the cooling process, below 500ºC, they are slowly converted to rutile form; a process usually occurring in industrial furnace and other iron and steel manufacturing (Carp et al., 2004). Rutile can contain up to 10% iron and other impurities such as tantalum, niobium, chromium, vanadium and tin; furthermore, rutile may be associated with hematite, quartz, tourmaline, barite and silicates, minerals occurring mainly in Brazilian, North American, African and Swiss Alps regions (Carp et al., 2004).

The ultrastructural analyses in fish organs and tissue identified TiO_2-NP in all organs of *C. parallelus* from Santa Cruz and Vitória Bay and, in general, they were associated with iron nanoparticles (Fig. 8 and 9). The nanocrystallographic structure of TiO_2-NP in organs and

tissue of *C. parallelus* showed that titanium oxide was rutile, tetragonal form in the crystalline system of space group P4/mnm (tetragonal/tetrahedron configuration) (Fig. 9); the same nanocrystallographic structure identified in the atmospheric particulate matter collected in Tubarão Complex. These findings suggested that the possible source of TiO_2-NPs in *C. parallelus* is the atmospheric particulate matter. TiO_2-NP in rutile phase, which present Ti oxidation state +4 [the most Ti stable form (Britannica, 2016) may interfere in biochemical and metabolic process through chemical characteristics.

In the muscle, TiO_2-NP was localized in the sarcomeres, encapsulated in a like-cyst structure (Fig. 8A) which may suggest a defence mechanism of the organism against foreign bodies. However, as this species is very appreciate by humans, these NP localized in this edible tissue of fish (Ti concentration from 1.21 to 2.71 µg/g, respectively 2014 and 2015) may be transferred to humans consumers at high amount than those recommended by the National Institute for Occupational Safety and Health (NIOSH, 2016) which indicates the permissible exposure level (PEL) as 1.5 µg/g (mL) and he recommended exposure level (REL) of 0.1 µg/g (mL) for TiO_2-NP based materials.

In the gonad (Fig. 8B), kidney (Fig 8C), gill (Fig. 8D) and liver (Fig 9A, 9B) the majority of TiO_2-NP and Fe-NP were usually found into cytoplasmic vesicles and cell nucleus, being Fe-NPs mostly found in the cellular nucleus of kidney and hepatopancreas. NP cellular internalization occurs mainly by endocytosis process, mediated by membrane proteins such as clathrin and caveolin; Ti-NPs founded in this study for atmospheric particulate matter and in fish organs/tissue had at least one dimension with approximately 50nm, and as described by Rejman et al. (2004) and Chithrani et al. (2006), NPs having 50 nm may be internalized faster than those having 14 nm and/or the larger ones, up to 500 nm. Metal accumulation induces

cellular detoxification processes from which metal lysosomal and peroxisome compartmentalisation are among such processes (Lawrence and Hemingway, 2003); thereafter, the presence of TiO_2-NP in cytoplasmic vesicles suggest that these NP may be biologically unavailable. Neverless,, it is important at individual level, such processes did not prevent the NPs from reaching the next trophic levels, represented by birds and humans, for example. However, in bivalve mollusc *Mytilus sp.*, lysosomal disorders and changes in the expression of antioxidant and immune-related genes were induced by TiO_2-NP agglomerates present in the seawater e absorbed by the gills and digestive glands during the feeding process (Canesi et al., 2015). The action of TiO_2-NP and Fe-NP in the nucleus of cells are unknown but, it is possible to cause mutagenic and/or genotoxic effects as reported in *Allium cepa* tests, in which TiO_2-NP suspensions at 12.5, 25, 50, 100 mg/mL caused oxidative stress and cytotoxic effect by decreasing mitotic cell index, genotoxic effects by increasing DNA fragmentation and chromosomal aberrations which were attributed to the uptake of TiO_2 in particulate form (Pakrashi et al., 2014). Then, studies are needed on the action of such NP in the gill, the main organ for respiratory gas exchange and having an important role in the osmoregulatory process, kidney which also participate of ionic and osmoregulation, hepatopancreas, the main detoxification organ, and gonads to evaluate the interference of TiO_2-NP on the organ function and on the sex-changed processes of juvenile *C. parallelus* as well on the adult reproductive processes to assure the species continuity.

In conclusion, considering the increasing commercial applications and use of TiO_2-NP in industrial production, the impact on the biota may be relevant in the future. The high levels of Ti in the plankton from both mangrove sites may be related to deposition of atmospheric matter in the marine environment, although Ti was not quantified in surface water; water is

susceptible to atmospheric contamination was already emphasised by Jickells (1995) and Paytan et al. (2009). The similar characterization of TiO_2-NP in atmospheric particulate matter and fish organs suggested that Tubarão Complex industries may be the source of the TiO_2-NP contamination in such environment. Furthermore, the localization of TiO_2-NP in cytoplasmic vesicles and cell nucleus become mandatory more studies to understand the real TiO_2-NP availability to the cells.

ACKNOWLEDGE

This study was supported by Espírito Santo Research Foundation (FAPES), Brazil and, Science and Technology Office from Córdoba National University (CONICET), Argentina. The authors are thankful to Robert Mcknight and M. Thompson for supporting chemical analyses; A. Lowry for her instruction in microscope sample preparation and V.A.S. Mendes acknowledges the Material Engineering Department/Federal University of São Carlos for facilities. I.C. Souza acknowledges São Paulo Research Foundation (FAPESP, Proc. 2014/04832-3 and Proc. 2015/05258-1) for scholarship support, I.D. Duarte acknowledges Coordination of Improvement of Higher Education Personal (CAPES); L.D. Rocha acknowledges FAPES fellowship and V.C. Azevedo acknowledges CNPQ fellowship, from Brazil.

REFERENCE

American Society for Testing and Materials (ASTM), 2006. Standard terminology relating to nanotechnology. ASTM E 2456-06. Philadelphia, Pennsylvania. 5 p.

Amondarain, Z., Arribas, M., Arana, J.L. & Lopez, G. A. 2013. Mechanical Properties and Phases Derived from TiO2 Nanopowder Inoculation in Low Carbon Steel Matrix. Materials Transactions, 54:10, 1867-1876.

Anandraj, A., Perissinotto, R., Nozais, C. & Stretch, D. 2008. The recovery of microalgal production and biomass in a South African temporarily open/closed estuary, following mouth breaching. Estuarine, Coastal and Shelf Science, 79:4, 599-606.

Arrivabene, H.P., Souza, I.C., Có, W.L.O., Conti, M.M., Wunderlin, D.A. & Milanez, C.R.D. 2015. Effect of pollution by particulate iron on the morphoanatomy, histochemistry, and bioaccumulation of three mangrove plant species in Brazil, Chemosphere 127, 27–34.

Azevedo, F.A.D. & Chasin, A.D.M. 2004. As bases toxicológicas da ecotoxicologia. São Carlos, SP: RiMa.

Azevedo, F.A.D. 2003. Toxicologia do mercúrio. In Toxicologia do mercúrio. RIMA/INTERTOX.

Battin, T.J., Kammer, F.V., Weilhartner, A., Ottofuelling, S. & Hofmann, T. 2009. Nanostructured TiO2: transport behavior and effects on aquatic microbial communities under environmental conditions. Environmental science & technology, 43:21, 8098-8104.

Britannica, 2016. https://global.britannica.com/science/titanium [Access: 28/02/2017].

Canesi, L., Ciacci, C. & Balbi, T. 2015. Interactive effects of nanoparticles with other contaminants in aquatic organisms: Friend or foe?. Marine environmental research, 111, 128-134.

Carp, O., Huisman, C.L. & Reller, A. 2004. Photoinduced reactivity of titanium dioxide. Progress in solid state chemistry, 32(1), 33-177.

Chadwick, O.A., Derry, L.A., Vitousek, P.M., Huebert, B.J. & Hedin, L.O. 1999. Changing sources of nutrients during four million years of ecosystem development. Nature, 397:6719, 491-497.

Chappaz, A., Lyons, T.W., Gordon, G.W. & Anbar, A.D. 2012. Isotopic fingerprints of anthropogenic molybdenum in lake sediments. Environmental science & technology, 46:20, 10934-10940.

Chen, J., Li, H., Han, X. & Wei, X. 2015. Transmission and Accumulation of Nano-TiO2. Bulletin of environmental contamination and toxicology, 95:2, 145-149.

Chithrani, B.D., Ghazani, A.A. & Chan, W. C. 2006. Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. Nano letters, 6:4, 662-668.

Csavina, J., Field, J., Taylor, M.P., Gao, S., Landázuri, A., Betterton, E.A. & Sáez, A.E. 2012. A review on the importance of metals and metalloids in atmospheric dust and aerosol from mining operations. Science of the Total Environment, 433, 58-73.

Demir, E., Kaya, N. & Kaya, B., 2014. Genotoxic effects of zinc oxide and titanium dioxide nanoparticles on root meristem cells of *Allium cepa* by comet assay. Turkish Journal of Biology, 38:1, 31-39.

Ducrotoy, J.P., Mazik, K. and Elliott, M. 2013. Bio-sedimentary indicators for estuaries: A critical reviews. Journal of Oceanography, Research and Data, 5:1.

Federici, G., Shaw, B.J. & Handy, R.D. 2007. Toxicity of titanium dioxide nanoparticles to rainbow trout (*Oncorhynchus mykiss*): gill injury, oxidative stress, and other physiological effects. Aquatic Toxicology, 84:4, 415-430.

Fenchel, T. 2008. The microbial loop–25 years later. Journal of Experimental Marine Biology and Ecology, 366:1, 99-103.

Freyre-Fonseca, V., Delgado-Buenrostro, N.L., Gutiérrez-Cirlos, E.B., Calderón-Torres, C.M., Cabellos-Avelar, T., Sánchez-Pérez, Y., Pinzón, E., Torres, I., Molina-Jijón, E., Zazueta, C. & Pedraza-Chaverri, J. 2011. Titanium dioxide nanoparticles impair lung mitochondrial function. Toxicology letters, 202:2, 111-119.

Gondikas, A.P., Kammer, F.V.D., Reed, R.B., Wagner, S., Ranville, J.F. & Hofmann, T. 2014. Release of TiO2 nanoparticles from sunscreens into surface waters: a one-year survey at the old Danube recreational Lake. Environmental science & technology, 48:10, 5415-5422.

Gottschalk, F., Sonderer, T., Scholz, R.W. & Nowack, B. 2009. Modeled environmental concentrations of engineered nanomaterials (TiO2, ZnO, Ag, CNT, fullerenes) for different regions. Environmental science & technology, 43:24, 9216-9222.

Handy, R.D., Owen, R. & Valsami-Jones, E. 2008. The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. Ecotoxicology, 17:5, 315-325.

Hartmann, N.B., Engelbrekt, C., Zhang, J., Ulstrup, J., Kusk, K.O. & Baun, A. 2012. The challenges of testing metal and metal oxide nanoparticles in algal bioassays: titanium dioxide and gold nanoparticles as case studies. Nanotoxicology, 7:6, 1082-1094.

Jickells, T. 1995. Atmospheric inputs of metals and nutrients to the oceans: their magnitude and effects. Marine Chemistry, 48:3-4, 199-214.

Johnson, B.D., Gilbert, S.L., Khan, B., Carroll, D.L. & Ringwood, A.H. 2015. Cellular responses of eastern oysters, Crassostrea virginica, to titanium dioxide nanoparticles. Marine environmental research, 111, 135-143.

Jovanović, B., Bezirci, G., Çağan, A.S., Coppens, J., Levi, E.E., Oluz, Z., Tuncel, E., Duran, H. & Beklioğlu, M. 2016. Food web effects of titanium dioxide nanoparticles in an outdoor freshwater mesocosm experiment. Nanotoxicology, 10:7, 902-912.

Kahru, A. & Dubourguier, H.C. 2010. From ecotoxicology to nanoecotoxicology. Toxicology, 269:2, 105-119.

Kim, K.T., Klaine, S.J., Cho, J., Kim, S.H. & Kim, S.D. 2010. Oxidative stress responses of Daphnia magna exposed to TiO 2 nanoparticles according to size fraction. Science of the Total Environment, 408:10, 2268-2272.

Klaine, S.J., Alvarez, P.J., Batley, G.E., Fernandes, T.F., Handy, R.D., Lyon, D.Y., Mahendra, S., McLaughlin, M.J. & Lead, J.R. 2008. Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environmental Toxicology and Chemistry, 27:9, 1825-1851.

Kosmulski, Marek. Surface charging and points of zero charge. Vol. 145. CRC Press, 2009.

Latroche, M., Brohan, L., Marchand, R. & Tournoux, M. 1989. New hollandite oxides: TiO2 (H) and K0. 06TiO2. Journal of Solid State Chemistry, 81:1, 78-82.

Lawrence, A., Hemingway, K., 2003. Effects of Pollution on Fish: molecular effects and population responses. Blackwell Science, Oxford.

Linsebigler, A.L., Lu, G. & Yates, J.T. 1995. Photocatalysis on TiO2 surfaces: principles, mechanisms, and selected results. Chemical reviews, 95:3, 735-758.

Loosli, F., Le Coustumer, P. & Stoll, S. 2013. TiO 2 nanoparticles aggregation and disaggregation in presence of alginate and Suwannee River humic acids. pH and concentration effects on nanoparticle stability. Water research, 47:16, 6052-6063.

Lovern, S.B. & Klaper, R. 2006. Daphnia magna mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles. Environmental toxicology and chemistry, 25:4, 1132-1137.

Luoma, S.N., Rainbow, P.S., 2008. Metal contamination in aquatic environments: science and lateral management, first ed. Cambridge University Press, Cambridge.

Matranga, V. & Corsi, I. 2012. Toxic effects of engineered nanoparticles in the marine environment: model organisms and molecular approaches. Marine Environmental Research, 76, 32-40.

Melquiades, F.L., Ferreira, D.D., Appoloni, C.R., Lopes, F., Lonni, A.G., Oliveira, F.M., Duarte, J.C. 2008. Titanium dioxide determination in sunscreen by energy dispersive X-ray fluorescence methodology. Anal Chim. Acta 613, 135-143.

Menard, A., Drobne, D. & Jemec, A. 2011. Ecotoxicity of nanosized TiO 2. Review of in vivo data. Environmental Pollution, 159:3, 677-684.

Miranda, J.F., Mendoza-Carranza, M., Sánchez, A.J., Barba, E. 2017. Selective foraging of Aratus pisonii (Arthropoda: Sesarmidae) on mangrove leaves in laboratory experiments. J. Exp. Mar. Biol. Ecol. 488, 38–43.

Monferrán, M.V., Garnero, P., Bistoni, M.A., Anbar, A.A., Gordon, G.W. & Wunderlin, D.A. 2016. From water to edible fish. Transfer of metals and metalloids in the San Roque Reservoir (Córdoba, Argentina). Implications associated with fish consumption. Ecological Indicators, 63, 48-60.

Ngajib, M., Jannah, N., Liman, M.A., Mohid, Z. & Wahab, M. S. 2013. Melted Zone Characteristics of Laser Welded Titanium Alloy (Ti-6Al-4V) under Different Process Parameters. In Applied Mechanics and Materials, 315, pp. 304-308. Trans Tech Publications.

NIOSH, 2016. https://www.cdc.gov/niosh/docs/2011-160/pdfs/2011-160.pdf [Access: 28/02/2017].

Nunes, S.M., Josende, M.E., Ruas, C.P., Gelesky, M.A., da Silva Júnior, F.M.R., Fattorini, D., Regoli, F., Monserrat, J.M. & Ventura-Lima, J. 2017. Biochemical responses induced by co-exposition to arsenic and titanium dioxide nanoparticles in the estuarine polychaete *Laeonereis acuta*. Toxicology, 376, 51-58.

Ohko, Y., Saitoh, S., Tatsuma, T. & Fujishima, A. 2001. Photoelectrochemical anticorrosion and self-cleaning effects of a TiO2 coating for type 304 stainless steel. Journal of the Electrochemical Society, 148:1, B24-B28.

Pakrashi, S., Jain, N., Dalai, S., Jayakumar, J., Chandrasekaran, P.T., Raichur, A.M., Chandrasekaran, N. &

Mukherjee, A. 2014. In vivo genotoxicity assessment of titanium dioxide nanoparticles by Allium cepa root tip assay at high exposure concentrations. PLoS One, 9:2, e87789.

Park, E.J., Choi, J., Park, Y.K. & Park, K. 2008. Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells. Toxicology, 245:1, 90-100.

Paschoalino, M.P., Marcone, G.P. & Jardim, W.F. 201). Nanomaterials and the environment. Quimica Nova, 33:2, 421-430.

Paytan, A., Mackey, K.R., Chen, Y., Lima, I.D., Doney, S.C., Mahowald, N., Labiosa, R. & Post, A. F. 2009. Toxicity of atmospheric aerosols on marine phytoplankton. Proceedings of the National Academy of Sciences, 106:12, 4601-4605.

Prospero, J.M., Ginoux, P., Torres, O., Nicholson, S.E. & Gill, T.E. 2002. Environmental characterization of global sources of atmospheric soil dust identified with the Nimbus 7 Total Ozone Mapping Spectrometer (TOMS) absorbing aerosol product. Reviews of geophysics, 40:1.

Rabelo, L.B., Muto, E.Y., Soares, L.S.H. 2009. Observações preliminares sobre o hábito alimentar do robalo-flecha *Centropomus undecimalis* (Bloch, 1792) e robalo-peba *Centropomus parallelus* (Poey, 1860) no estuário de Caravelas (Bahia-Brasil). Boletim Técnico Científico do CEPENE, Tamandaré 17:1, 89–96.

Ravi, S., D'Odorico, P., Breshears, D.D., Field, J.P., Goudie, A.S., Huxman, T.E., Li, J., Okin, G.S., Swap, R.J., Thomas, A.D. & Van Pelt, S. 2011. Aeolian processes and the biosphere. Reviews of Geophysics, 4:3.

Rejman, J., Oberle, V., Zuhorn, I.S. & Hoekstra, D. 2004. Size-dependent internalization of particles via the pathways of clathrin-and caveolae-mediated endocytosis. Biochemical Journal, 377:1, 159-169.

Robichaud, C.O., Uyar, A.E., Darby, M.R., Zucker, L.G., & Wiesner, M.R. 2009. Estimates of upper bounds and trends in nano-TiO2 production as a basis for exposure assessment.

Santos, J.M., Reis Jr N.C., 2011. Caracterização e quantificação de partículas sedimentadas na região da Grande Vitória. Relatório Técnico Instituto Estadual do Meio Ambiente.

Sharma, V.K. 2009. Aggregation and toxicity of titanium dioxide nanoparticles in aquatic environment—a review. Journal of Environmental Science and Health Part A, 44:14, 1485-1495.

Simons, P.Y. & Dachille, F. 1967. The structure of TiO2II, a high-pressure phase of TiO2. Acta Crystallographica, 23:2, 334-336.

Som, C., Berges, M., Chaudhry, Q., Dusinska, M., Fernandes, T.F., Olsen, S.I. & Nowack, B. 2010. The importance of life cycle concepts for the development of safe nanoproducts. Toxicology, 269:2, 160-169.

Souza, I., Bonomo, M.M., Morozesk, M., Rocha, L.D., Duarte, I.D., Furlan, L.M., Arrivabene, H.P., Monferrán, M.V., Matsumoto, S.T., Milanez, C.R.D., Wunderlin, D.A., Fernandes, M.N., 2014a. Adaptive plasticity of *Laguncularia racemosa* in response to different environmental conditions: integrating chemical and biological data by chemometrics. Ecotoxicology 23, 335–348.

Souza, I.C., Morozesk, M., Duarte, I.D., Bonomo, M.M., Rocha, L.D., Furlan, L.M., Arrivabene, H.P., Monferrán, M.V., Matsumoto, S.T., Milanez, C.R.D., Wunderlin, D.A., Fernandes, M.N., 2014b. Matching pollution with adaptive changes in mangrove plants by multivariate statistics. A case study, *Rhizophora mangle* from four neotropical mangroves in Brazil. Chemosphere 108, 115–124.

Souza, I.C., Rocha, L.D., Morozesk, M., Bonomo, M.M., Arrivabene, H.P., Duarte, I.D., Furlan, L.M., Monferrán, M.V., Matsumoto, S.T., Milanez, C.R.D., Wunderlin, D.A., Fernandes, M.N., 2015. Changes in bioaccumulation and translocation patterns between root and leaves of *Avicennia schaueriana* as adaptive response to different levels of metals in mangrove system. Marine Pollution Bulletin 94, 176–184.

Stout, J.E. & Zobeck, T.M. 1996. The Wolfforth field experiment: a wind erosion study. Soil Science, 161:9, 616-632.

Theron, J., Walker, J.A. & Cloete, T.E. 2008. Nanotechnology and water treatment: applications and emerging opportunities. Critical reviews in microbiology, 34:1, 43-69.

Thompson, T.L. & Yates, J.T. 2006. Surface science studies of the photoactivation of TiO2 new photochemical processes. Chemical Reviews, 106:10, 4428-4453.

Usepa, 2009. Risk-based Concentration Table. Philadelphia PA. United States Environmental Protection Agency, Washington.

Wang, W.X. & Fisher, N.S. 1998. Accumulation of trace elements in a marine copepod. Limnology and Oceanography, 43:2, 273-283.

Xiong, D.W., Fang, T., Yu, L.P., Sima, X.F., Zhu, W.T. 2011. Effects of nano-scale TiO2, ZnO and their bulk counterparts on zebrafish: Acute toxicity, oxidative stress and oxidative damage. Sci. Total. Environ. 409, 1444-1452.

Yeager, L.A., Stoner, E.W., Peters, J.R., Layman, C.A. 2016. A terrestrial-aquatic food web subsidy is potentially mediated by multiple predator effects on an arboreal crab. J. Exp. Mar. Biol. Ecol. 475, 73–79.

Zhu, X., Wang, J., Zhang, X., Chang, Y. & Chen, Y. 2010. Trophic transfer of TiO 2 nanoparticles from daphnia to zebrafish in a simplified freshwater food chain. Chemosphere, 79:9, 928-933.

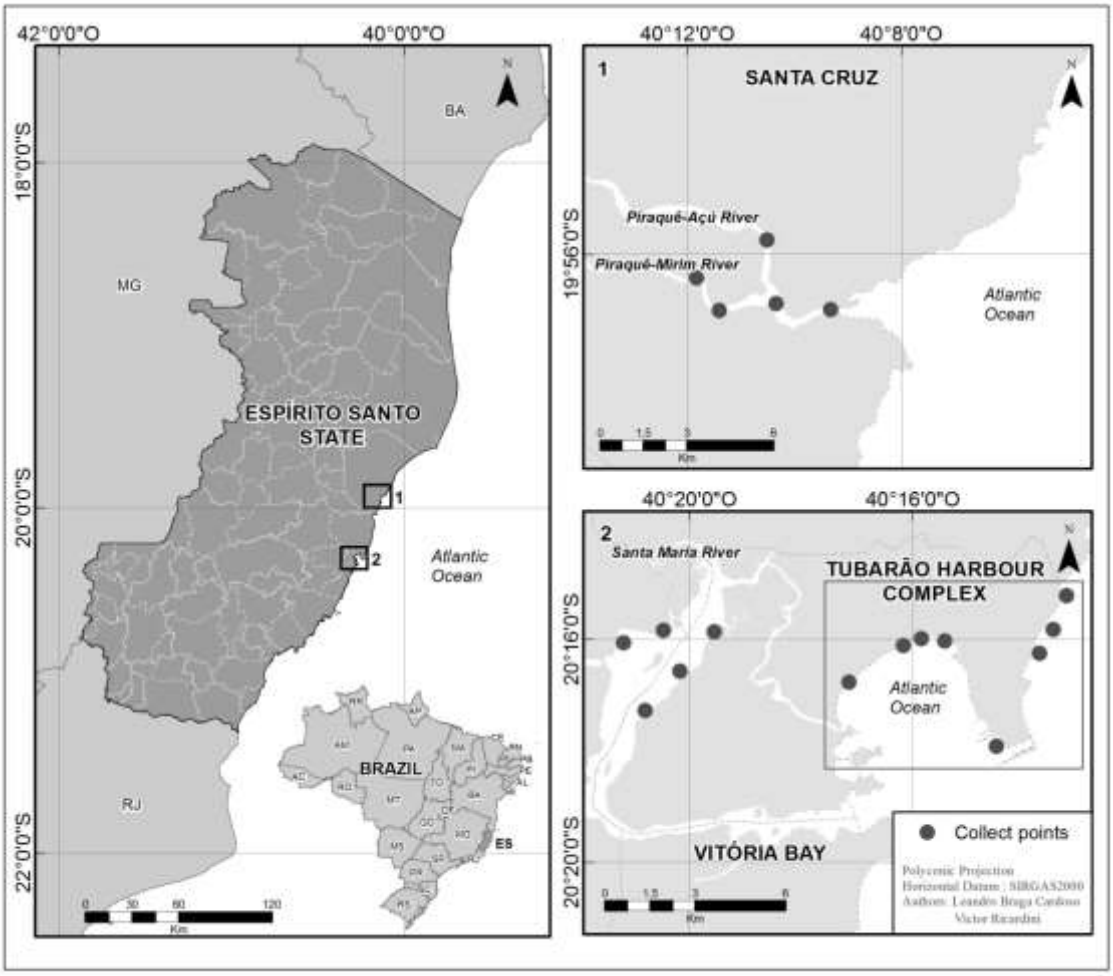


Figure 1. Location of the State of Espírito Santo (South America, Brazil), showing sampling sites. Santa Cruz (S 19º56`26.2``; W 40º 12`87``), Vitória Bay (S 20º14`31.5``; W 40º19`84.7``) and Tubarão Harbour Complex (20º17′03.8″S and 40º14′24.9″W).

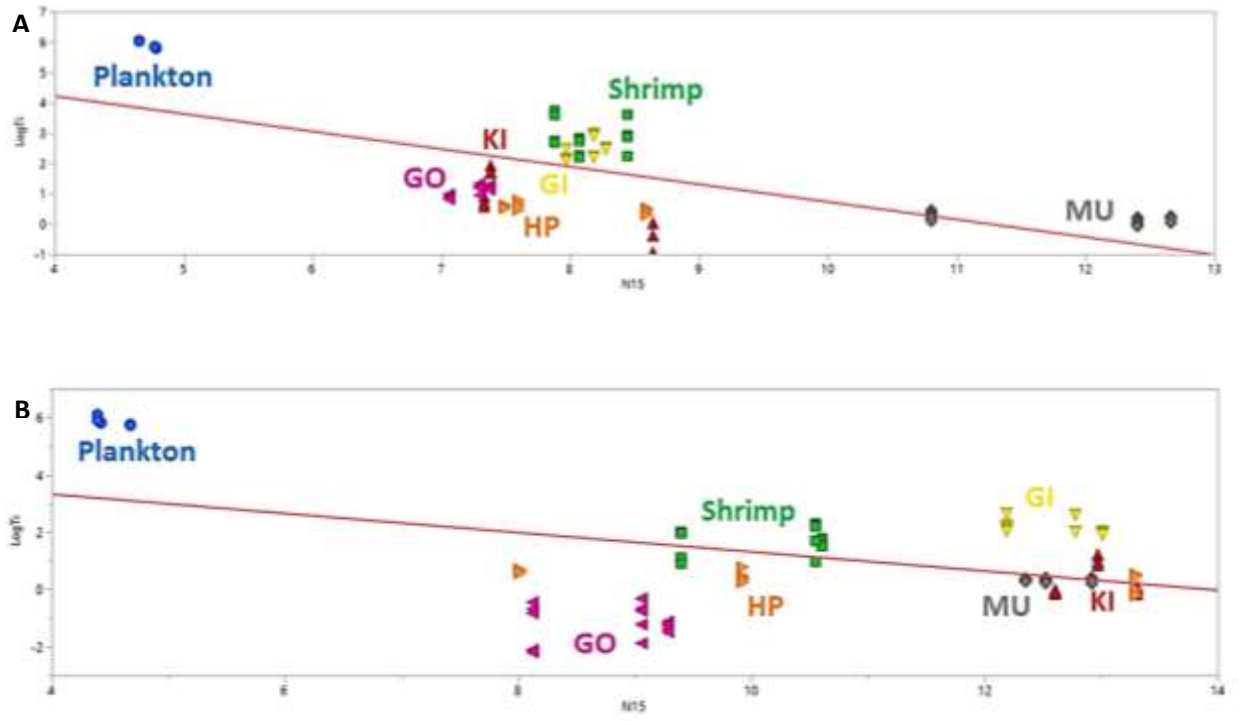


Figure 2. Relationship between Log[Ti, µg g−1 dry weight] versus $\delta^{15}N$ for titanium. A) T i biodiluted throughout Santa Cruz studied food web; B) Ti biodiluted throughout the Vitória Bay studied food web. GI: gill of fish (*Centropomus parallelus*), HP: hepatopancreas of fish, KI: kidney of fish, MU: muscle of fish, GO: gonad of fish.

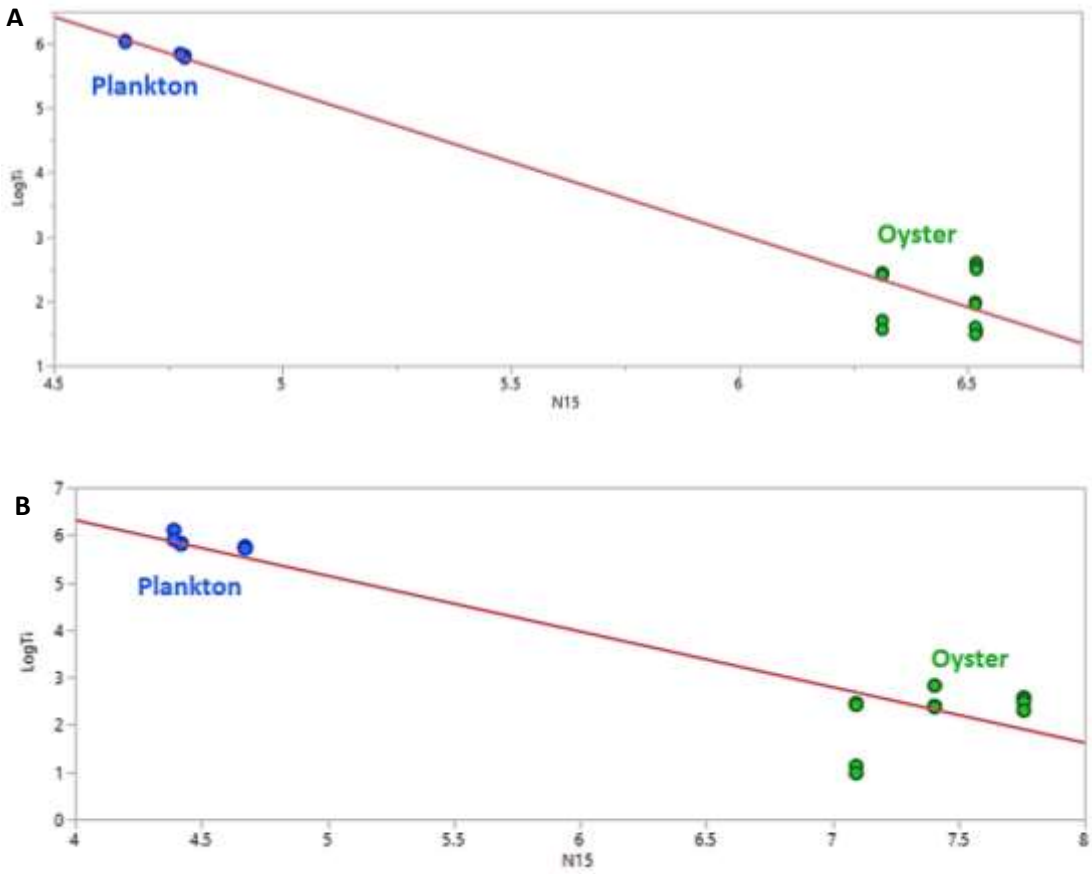


Figure 3. Relationship between Log[Ti, µg g−1 dry weight] versus $\delta^{15}N$ for titanium. A) Ti biodiluted throughout Santa Cruz studied food web; B) Ti biodiluted throughout the Vitória Bay studied food web.

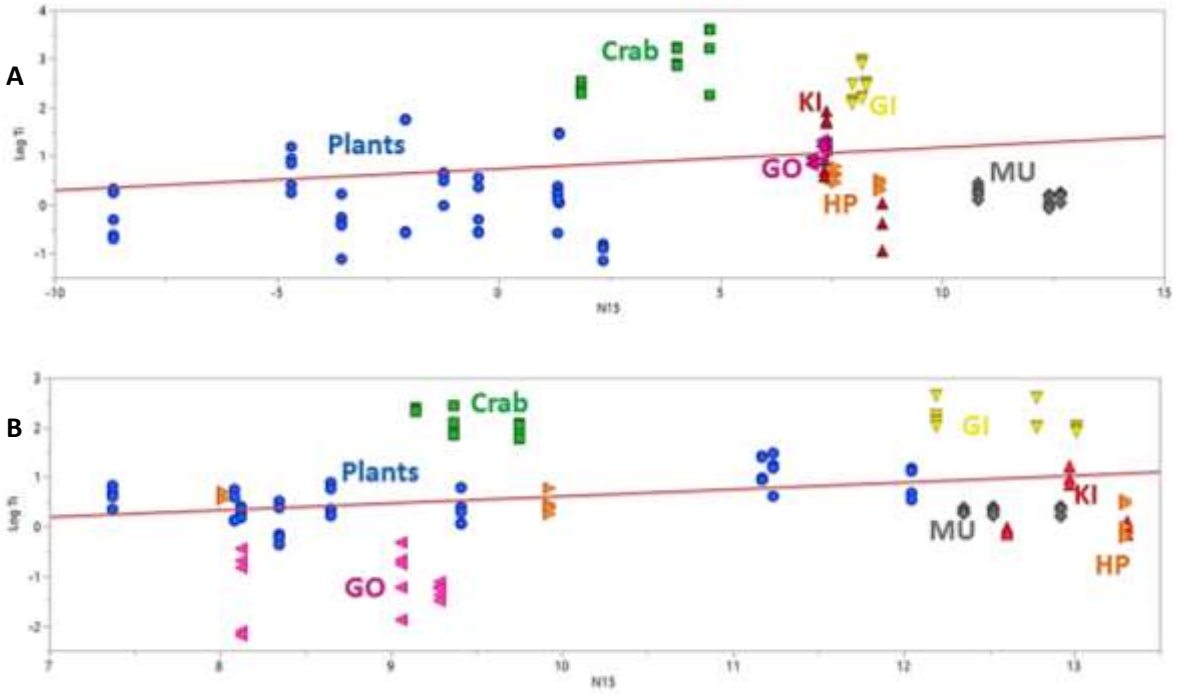


Figure 4. Relationship between Log[Ti, µg g−1 dry weight] versus $\delta^{15}N$ for titanium. A) Ti biomagnification throughout Santa Cruz studied food web; B) Ti biomagnification throughout the Vitória Bay studied food web. GI: gill of fish (*Centropomus parallelus*), HP: hepatopancreas of fish, KI: kidney of fish, MU: muscle of fish, GO: gonad of fish.

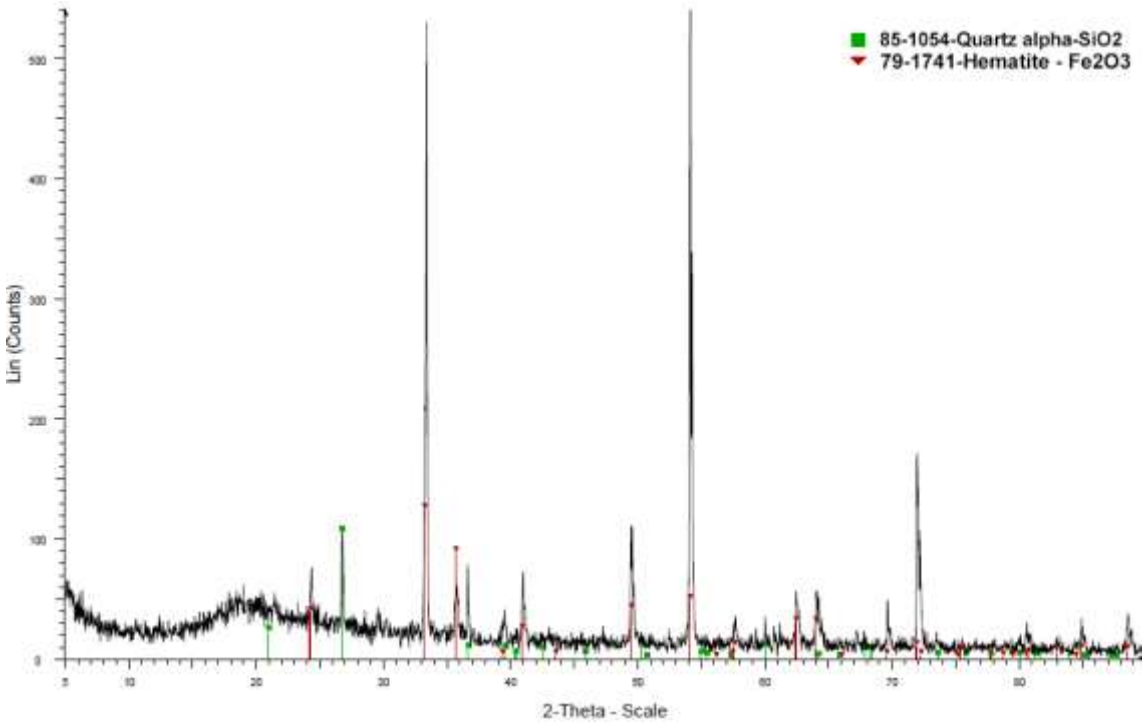


Figure 2. X-Ray analysis of atmospheric particulate matter from Tubarão Complex.

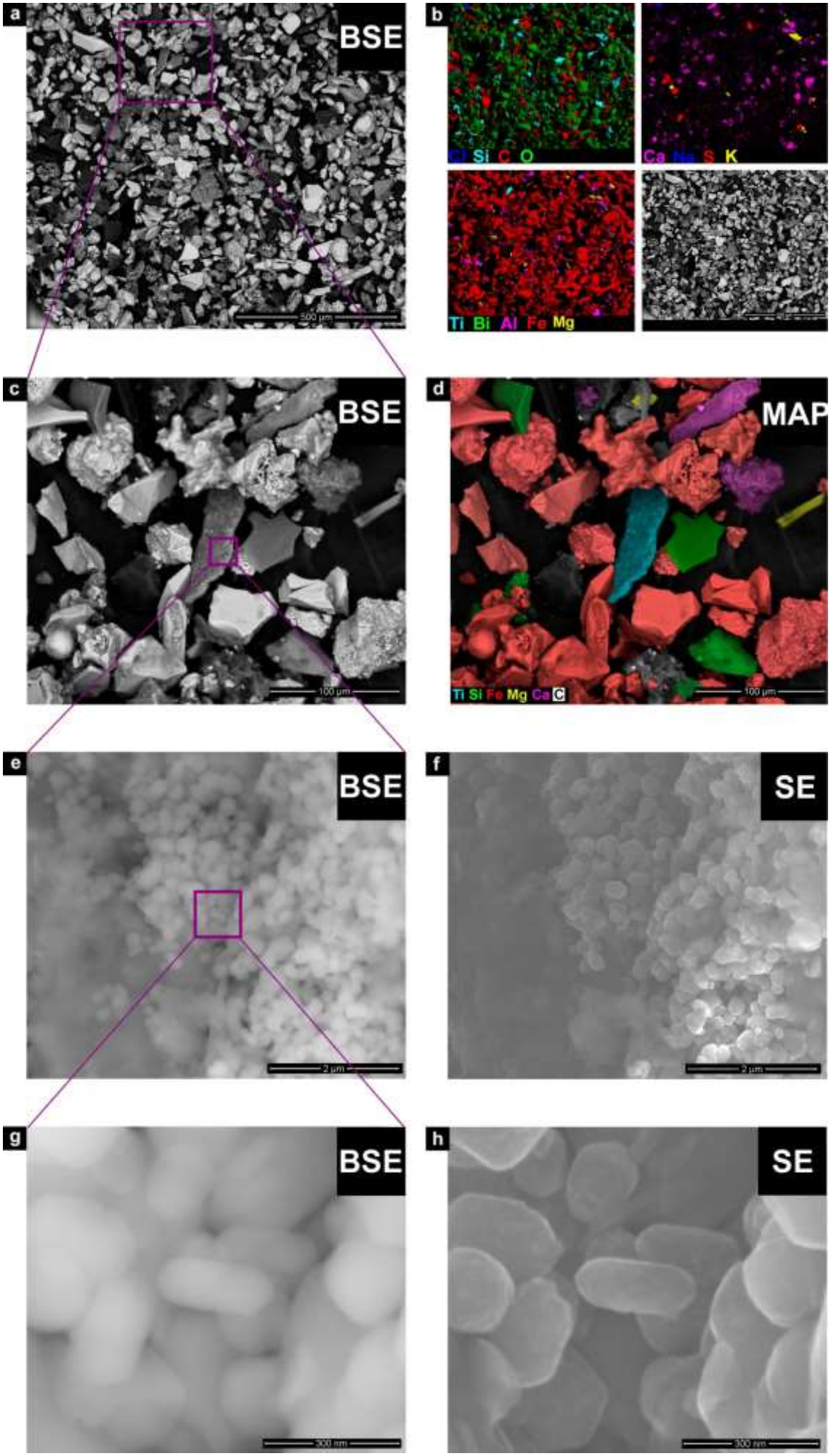


Figure 3. SEM analysis of atmospheric particulate matter from Tubarão Complex.

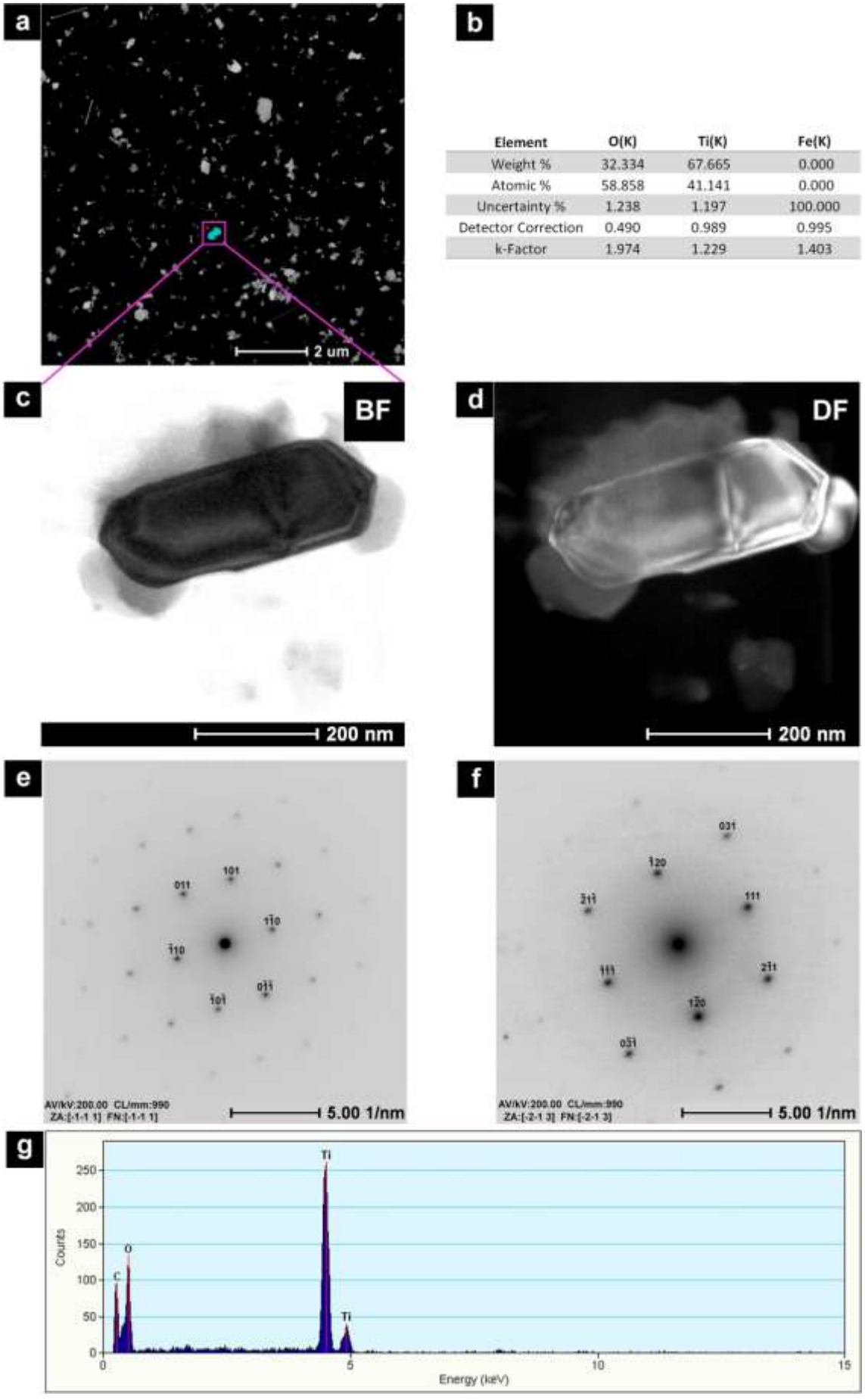


Figure 4. TEM analysis of atmospheric particulate matter from Tubarão Complex.

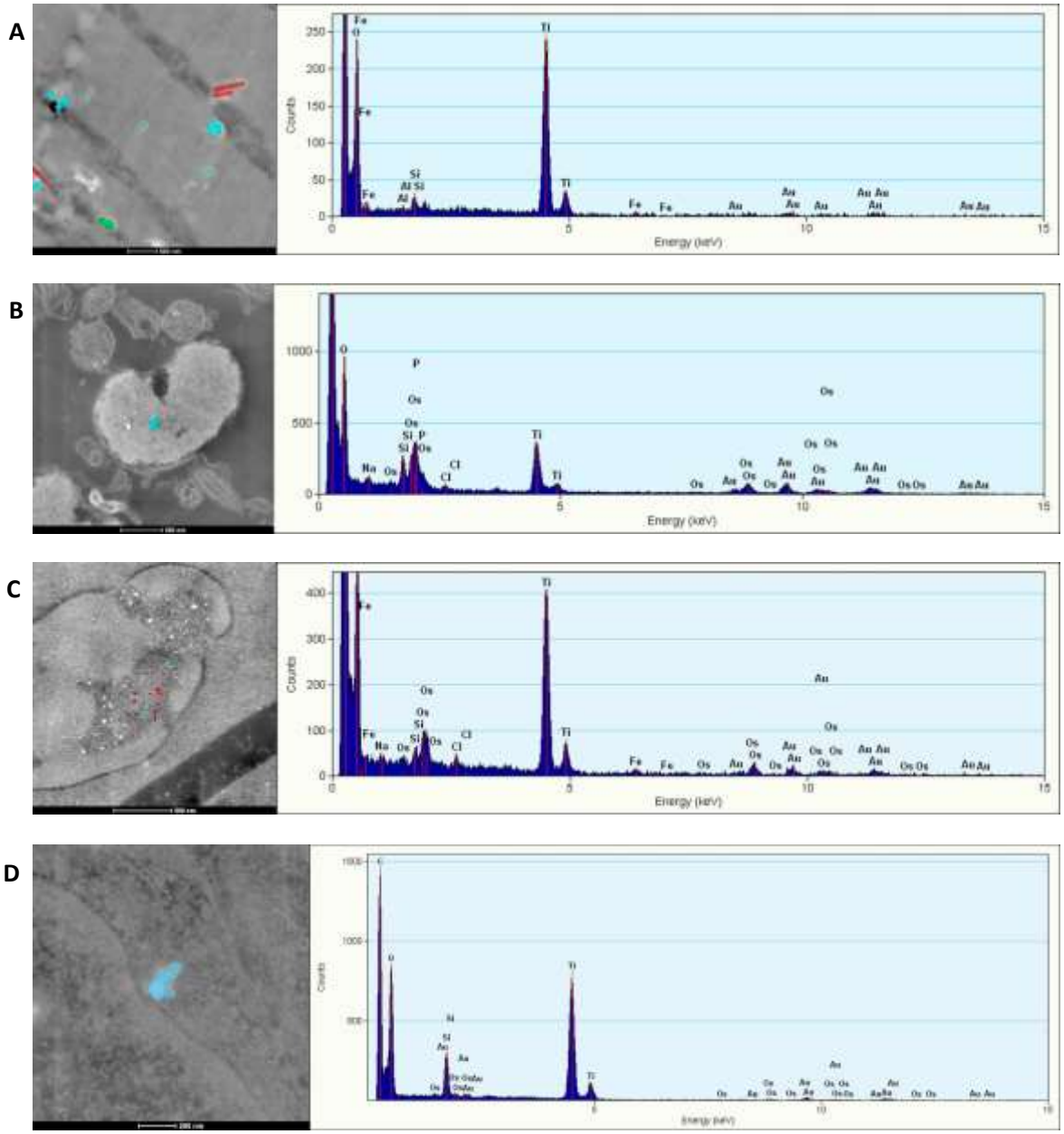


Figure 8. TEM analysis of *Centropomus parallelus*. A: muscle, B: Gonade, C: Kidney, D: Gill.

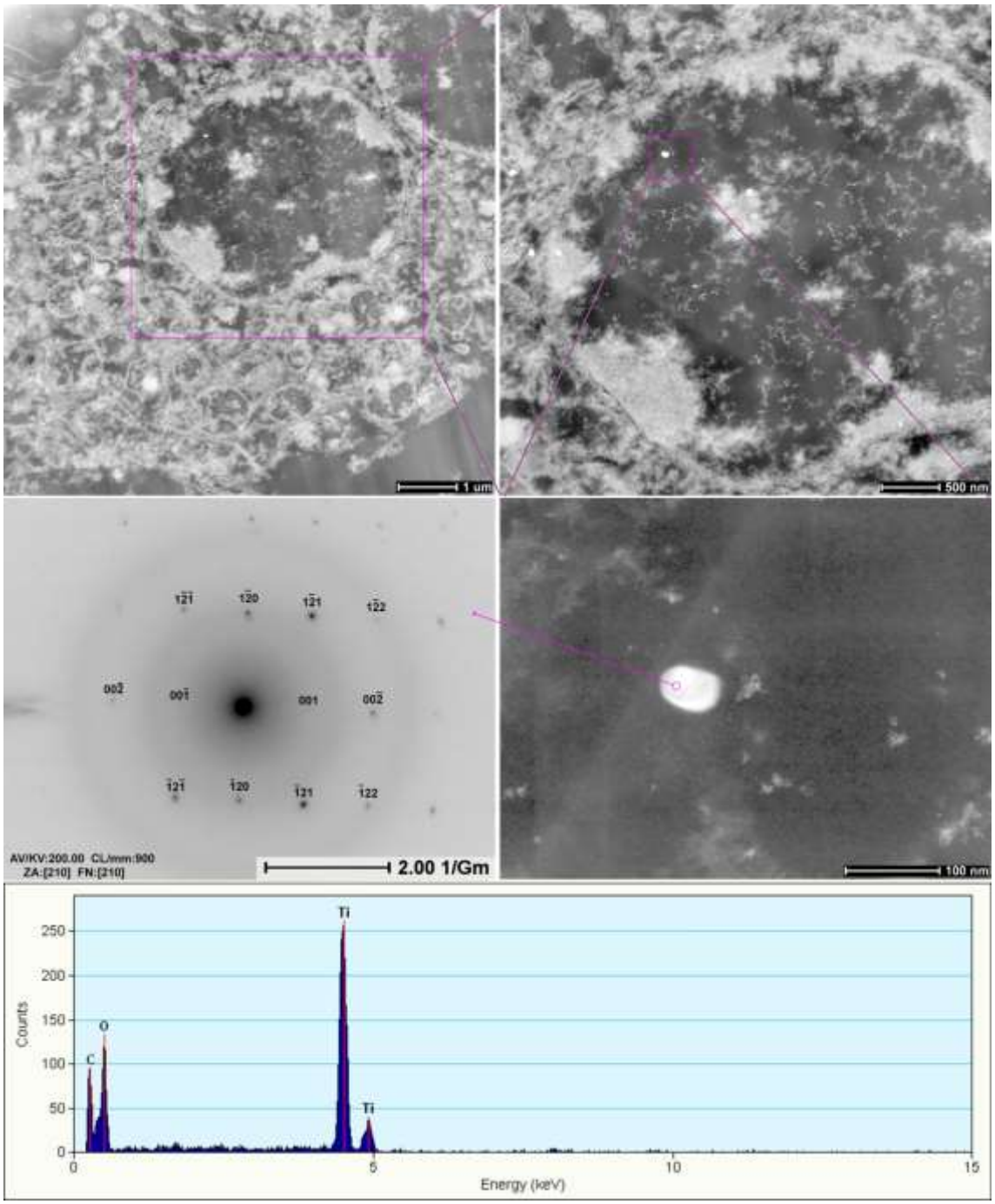


Figure 9. TEM analysis of *Centropomus parallelus* hepatopancreas.

Table 1S. Measurements of Titanium in abiotic and biotic compartments of the estuaries Santa Cruz and Vitória Bay and the Tubarão Complex in 2014. Values correspond to means (confidence interval). Lowercase letters indicate significant differences between matrix in the same estuary; Uppercase letters indicate significant differences between Santa Cruz and Vitória Bay (Tukey test, $P < 0.05$). SW: surface water, SD: sediment, PM: particulate matter, PK: plankton, CR: Crab (*Aratus* sp.), SH: shrimp (*Macrobrachium* sp.), GI: gill of fish (*Centropomus parallelus*), HP: hepatopancreas of fish, KI: kidney of fish, MU: muscle of fish, GO: gonad of fish. –: without sampling. <LOD: Below detection limit.

	Santa Cruz	Vitória Bay	Tubarão Complex
SW	<LOD	<LOD	**IF** <LOD **IP** <LOD **SE** <LOD **SP** <LOD
SD	1056.85 (770.87 - 1448.85)^{aA}	1252.53 (913.84 - 1717.55)^{aA}	**SD1** 569.65 (433.00 – 706.02)^{d} **SD2** 1231.08 (1095.00 – 1367.60)^{b} **SD3** 925.41 (789.00 – 1062.00)^{c} **SD4** 1755.44 (1619.00 – 1892.00)^{a}
PM	-	-	1519.73 (1383.00 - 1656.30)^{ab}
PK	672.28 (537.64 - 840.66)^{aA}	240.39 (192.40 - 300.53)^{bB}	-
CR	56.95 (44.97 - 72.17)^{bA}	14.62 (11.69 - 18.28)^{cB}	39.43 (-97.00 - 176.00)^{e}
SH	29.73 (23.79 - 37.16)^{bA}	18.47 (14.34 - 23.78)^{cA}	-
GI	12.19 (9.75 - 15.23)^{cA}	16.02 (12.82 - 20.03)^{dA}	-
KI	3.06 (2.23 - 4.19)^{dA}	2.70 (1.97 - 3.70)^{eA}	-
LI	2.50 (1.96 - 3.20)^{dA}	2.64 (2.07 - 3.37)^{eA}	-
MU	2.38 (1.90 - 2.97)^{dA}	2.71 (2.13 - 3.46)^{eA}	-
GO	0.44 (0.33 - 0.58)^{eA}	0.59 (0.46 - 0.76)^{fA}	-

Table 2S. Measurements of Titanium in abiotic and biotic compartments of the estuaries Santa Cruz and Vitória Bay and the Tubarão Complex in 2015. Values correspond to means (confidence interval). Lowercase letters indicate significant differences between matrix in the same estuary; Uppercase letters indicate significant differences between Santa Cruz and Vitória Bay (Tukey test, $P < 0.05$). SW: surface water, SD: sediment, PM: particulate matter, PK: plankton, CR: Crab (*Aratus* sp.), SH: shrimp (*Macrobrachium* sp.), GI: gill of fish (*Centropomus parallelus*), HP: hepatopancreas of fish, KI: kidney of fish, MU: muscle of fish, GO: gonad of fish. –: without sampling. <LOD: Below detection limit.

	Santa Cruz	Vitória Bay	Tubarão Complex
SW	<LOD	<LOD	**IF** <LOD **IP** <LOD **SE** <LOD **SP** <LOD
SD	991.68 (771.84-1273.83)^{aA}	910.22 (708.54-1169.35)^{aA}	**SD1** 39.83 (34.66 - 44.38)^{c} **SD2** 47.12 (30.18 - 50.66)^{c} **SD3** 64.57 (56.37 - 66.48)^{b} **SD4** 265.82 (127.87 - 467.62)^{a}
PM	-	-	386.18 (370.03 - 399.63)^{a}
PK	365.74 (284.84 - 469.62)^{bA}	346.65 (269.91- 445.01)^{bA}	-
AV	2.30 (1.90 - 2.80)^{efA}	3.06 (2.52 - 3.71)^{eA}	2.98 (2.66 - 3.33)^{e}
LA	0.95 (0.76 - 1.18)^{ghB}	1.50 (1.24 - 1.82)^{*fA}	1.85 (1.66 - 2.07)^{f}
RI	0.72 (0.59 - 0.87)^{hA}	1.51 (1.24 - 1.83)^{*fB}	1.08 (0.96 - 1.20)^{g}
CR	20.72 (17.07 - 25.16)^{cA}	8.49 (6.99 - 10.31)^{cB}	-
SH	19.79 (16.19 - 24.18)^{cA}	6.11 (5.03 - 7.42)^{dB}	-
OS	8.25 (6.79- 10.01)^{dA}	11.58 (9.54 - 14.06)^{cA}	-
GI	11.94 (9.84 - 14.49)^{cA}	8.84 (7.28 - 10.74)^{cB}	7.59 (6.61 - 8.71)^{d}
KI	2.63 (1.88 - 3.68)^{efA}	1.58 (1.23 - 2.03)^{fB}	1.17 (0.96 -1.42)^{fgh}
GO	3.01 (2.48 - 3.66)^{eA}	0.37 (0.30 - 0.44)^{gB}	0.56 (0.46 - 0.68)^{h}
HP	1.73 (1.43 - 2.10)^{fA}	1.55 (1.28 - 1.89) ^{fA}	1.71 (1.41 - 2.08)^{fh}
MU	1.21 (1.00 - 1.47)^{gB}	1.39 (1.13 -1.69)^{*fA}	1.33 (1.10 - 1.62)^{gh}

Table 3S. Regression parameters and p values for δ 15N vs. trace Ti (µg g−1 dry weight). PK: plankton, PT: mangrove plants (*Rhizophora mangle*, *Laguncularia racemosa*, *Avicennia schaueriana*), SH: shrimp (*Macrobrachium* sp.), OS: oyster (*Crassostrea rhizophorae*), CR: crab (*Aratus* sp.), FS: fish (*Centropomus parallelus*).

			2015				
Year	Sites	Trophic chain	Intercept	Slop	R^2	P	Trophic Transfer
2015	Santa Cruz	PK-SH-FS	6.54	-0.58	0.45	<0.0001	Biodilution
		PK-OS	16.56	-2.25	0.97	<0.0001	Biodilution
		PT-CR-FS	0.74	0.04	0.04	0.0204	Biomagnification
	Vitória Bay	PK-SH-FS	4.66	-0.33	0.21	<0.0001	Biodilution
		PK-OS	11	-1.17	0.86	<0.0001	Biodilution
		PT-CR-FS	-0.78	0.14	0.07	0.0024	Biomagnification
2014	Santa Cruz	PK-SH-FS	7.08	-0.6	0.32	<0.0001	Biodilution
		CR-FS	3.79	-0.29	0.26	<0.0001	Biodilution
	Vitória Bay	PK-SH-FS	5.5	-0.36	0.27	<0.0001	Biodilution
		CR-FS	-0.66	0.17	0.06	0.0226	Biomagnification

CAPÍTULO 7

Differential biochemical responses to metals/metalloids accumulation in organs of an edible fish (*Centropomus parallelus*) from Neotropical estuaries

ABSTRACT

Metals/metalloids accumulation in fish organs elicits biochemical responses that indicate the overall fish and environmental health status. This study evaluated the bioaccumulation of metal/metalloids (B, Al, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Ag, Cd, Hg and Pb) and biochemical biomarkers in different organs of fish, *Centropomus parallelus*, in two Neotropical estuaries, Vitória Bay and Santa Cruz (State of Espírito Santo, Brazil), having different levels of contamination. Chemometric evaluation between metal/metalloid accumulation and the biomarkers superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione (GSH) in all organs, Na^+/K^+-ATPase, H^+-ATPase in gills and kidneys, metallothionein (MT), lipid peroxidation (LPO) and oxidazed protein (OP) in hepatopancreas and muscles and, acetylcholinesterase (AChE) in muscle were done using principal components analysis (PCA), Pearson correlation and generalized procrustes analysis (GPA) to evaluate spatial and temporal changes in different organ/tissues. Metal/metalloid concentrations differ in each organ and were significantly higher in winter. GSH in gills, CAT and OP in hepatopancreas, GST in kidney and, CAT and MT in muscles were higher in Vitória Bay than Santa Cruz; SOD and GST in gills and GST, GSH and LPO in hepatopancreas were higher in Vitória Bay (winter) and Santa Cruz (summer). Biochemical biomarkers did not differ between seasons in muscles and kidney and AChe activity did not change between sites and seasons. The responses and/or absence of them in a given organ show organ/tissue-specific sensibility to metals accumulation. Metal levels in gills indicate water contamination and lower sensitivity of this organ to most metals and muscle was the less reactive tissue. Biochemical responses suggested that the metal elimination pathway is through the gills and kidney. Hepatopancreas and kidneys are important detoxification organs. *C. parallelus* was able to support environmental conditions

however, it probably implicates in energy expenditure that may influence the growth rate and reproduction processes.

Keywords: gills, hepatopancreas, kidney, muscle, antioxidant system, osmoregulatory enzymes.

1. INTRODUCTION

Estuaries are characterized by high environment physical and chemical variability as they constitute an interface zone between continent and sea and, most of them are under the influence of anthropogenic activities which are the main contamination sources of these areas (Borja et al., 2012, Wolanski and Elliot, 2015). Metal/metalloids inputs in estuarine areas and their transfer through the trophic web may disrupt biological processes resulting in toxicity which may affect the structure of population and community, even in those organisms well-adapted to tolerate such stressors (Elliott and Quintino, 2007). Essential metals play important roles in biological systems but, they become toxic at high levels; non-essential metals are toxic disturbing biological process, even at trace amounts (Mazon et al., 2002; Hartl et al, 2013; Rosabal et al, 2015).

Metal/metalloid bioaccumulation differs among organs/tissues depends on mode of exposure (dietary and/or water), uptake, regulation and excretion mechanisms as well as they roles in these processes (Jaric et al., 2011). Metals may interfere in cellular enzymatic pathways by generating reactive oxygen species (ROS) which promote oxidative stress and degenerative processes in the cells (Oliveira et al., 2010; Carvalho et al., 2012; Sakuragui et al., 2013; Barbee et al., 2014). ROS can be detoxified by enzymatic and non-enzymatic cell defence systems that include the enzymes superoxide

dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST) and, the levels of glutathione (GSH) and metallothionein (MT) among others enzymes and compounds (Storey, 1996). The activation and/or inhibition of these systems reflect the exposure to metals and/or their toxicity (Oliveira et al., 2010; Souza et al., 2013). Numerous studies on wild fish emphasize the metal accumulation in the muscles, the edible part of fish consumed by humans (Bosch et al, 2016, Cerveny et al, 2014) but, it is important to analyze other organs/tissues to evaluate metal distribution and their effects in fish organism.

Among fish organs, the gills have many important functions such as gas exchange, ions transport and nitrogen excretion and are the first organs exposed to waterborne chemicals; contaminants uptake as metals is facilitated by the gills large surface area and thin water-blood diffusion distance (Fernandes and Mazon, 2003). Furthermore, the high osmoregulatory activity in estuarine fish becomes the gills and, at less extension, the kidney, which also participate of this process, susceptible to contaminants in water (Monserrat et al, 2007). The liver, the main detoxification organ in all vertebrates, effectively take up metals/metalloids from blood stream and its dysfunction are early indicator of toxicant exposure/effects providing information about the environment and/or food contamination (Fonseca et al., 2011; Paul et al., 2014).

In Brazil, the coast of Espírito Santo state (ES) has been impacted by metal/metalloids coming from metallurgy industries and harbours for iron export. The estuaries, Santa Cruz and Vitória Bay, located in this state, have different levels of metal/metalloids contamination and, despite the lower levels of metal contamination in Santa Cruz estuary; this area presents higher bioavailability of some metals (Souza et al 2013). Numerous biological changes in the biota from these areas were associated to metal contamination (Souza et al., 2014a, b; 2015; Arrivabene et al., 2014; 2015).

Regarding to fish, previous study showed that the fat snook fish, *Centropomus parallelus*, inhabiting theses areas, presented erythrocyte genotoxicity, absence of gill histopathology, changes in some antioxidant enzymes and moderate damage in liver as well as metal bioaccumulation in muscles (Souza et al., 2013). These results opened de following questions: 1) Does metal distribution and accumulation differ among organs? 2) How each organ responds to metal accumulation? 3) Are there differences between season in metal/metalloid accumulation and fish responses?

In this context, the metals/metalloids accumulation in gills, hepatopancreas, kidneys and muscle were determined and enzymatic and non-enzymatic biochemical biomarkers were analysed in juvenile fat snook, *Centropomus parallelus* Poey 1860 (Centropomidae) from Vitoria Bay and Santa Cruz estuaries, ES, Brazil. Thereafter, all data were integrated in order to associate metal/metaloid accumulation and organ response. The activity of phase II enzyme, the glutathione-S-tranferase (GSH), the antioxidant enzymes as superoxide dismutase (SOD), catalase (CAT) and the levels of glutathione (GSH) in gills, hepatopancreas, kidney and muscle; Na+/K+-ATPase and H+-ATPase in gills and kidney; metallothionein, lipid peroxidation and oxidized protein in hepatopancreas and muscles; and acetylcholinesterase (AChE) in muscles. *C. parallelus* is a protandric top-predator fish which does not undergo migratory cycles during its juvenile stage (Volpe, 1959; Taylor et al, 2000) being resident in estuarine areas and has been considered as a possible bioindicator organism in Brazilian coastal regions (Rocha et al., 2007; Souza et al., 2013).

2. MATERIALS AND METHODS

2.1 Fish sampling

Samples from gills, kidney, hepatopancreas and muscles from the same juvenile *C. parallelus* of previous study (Souza et al., 2013) were used in this study. Briefly, in the previous study, *C. parallelus* (n = 40; body mass = 150 ± 30 g; total length = 15 ± 5 cm) were collected in two seasons (winter/2009 and summer/2010) in Vitoria Bay (20° 19′S and 40° 20′W) and Santa Cruz estuary (19° 58′S and 40° 07′W), ES, Brazil (Figure 1). Victoria Bay is an estuarine complex formed by five rivers showing environmental degradation caused by harbour and industrial activities, including air pollution by smoke metallic particles and, Santa Cruz estuary is formed by two rivers with large mangrove. The gills, hepatopancreas, kidneys (posterior region) and muscle were removed using non-metal instruments to avoid adding metals from tools on the organs, immediately after fish were killed by medullar section. Organ/tissues samples were stored at −80°C.

2.2 Multi-elemental analyses

Ultra pure water (<5 μg L^{-1} TOC) was obtained from a purification system Arium 61316-RO plus Arium 611 UV (Sartorius, Germany). Multi-element standard solution Merck VI CertiPUR® was obtained from Merck Química Argentina (Buenos Aires, Argentina). Nitric acid (63.7%) sub-boiling grade was prepared from analytical grade acid using a distiller (Figmay Sub-boiling distilled, Córdoba, Argentina). Purity of nitric acid was verified by Mass Spectrometry Inductively Coupled Plasma (ICP-MS), Agilent 7500cx, USA, equipped with an ASX-100 autosampler (CETAC Technologies, Omaha, NE). Filters (0.45 μm, HAWG04756) were obtained from Millipore (São Paulo, Brazil). All glassware and plastic bottles/containers were left with sulfuric/nitric acids solution overnight and washed with ultrapure water. ICP probes and pipes were of PTFE previously washed with nitric acid (2% v v^{-1}).

For multi-elemental analysis organ/tissue samples (n = 5 animals per season per site) were dried at 37°C until constant weight and stored at room temperature. Samples in triplicate were ground and homogenized with a mortar and digested (0.1 g from each organ) according to Chappaz et al. (2012), using 4 mL nitric acid (ultra pure, sub boiling grade) and 1 mL hydrogen peroxide (30%, Merck), in pre-cleaned PTFE tubes (Savillex) at constant temperature (90°C) during 24 hours. Controls were prepared using the same protocol without sample (only reagents). Digested samples were stored at 4°C until analysis for B, Al, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Ag, Cd, Hg and Pb.

Concentrations of elements were determined in triplicate, the repeatability of ICP-MS measurements was generally ≥ 97%. Quality assurance (QA) and quality control (QC) were done using a certified reference material (CRM): typical diet NIST1548a) and bovine muscle (NIST 8414). Recoveries from CRMs were 94 ± 17%, respectively. Spiked samples were also prepared for gills, kidney, hepatopancreas and muscle samples. Variable amounts of mix standard solutions, containing all the elements analyzed in the samples, were added to 0.1 g of dried tissue, doubling the starting concentration for each element. The rest of the procedure was the same as used for non-spiked samples. The average recovery of these assays was 87 ± 16%.

2.3 Biochemical Analyses

Individual samples of gills, hepatopancreas, kidney and muscle from each animal was homogenated (n = 5 animals per season per site). The total protein in each sample was determined according to Bradford method (Bradford, 1976), in a microplate reader (SpectraMax M5, Molecular Devices, USA) using bovine serum albumin as standard. Four biomarkers were measured in all sampled organ/tissues: the phase II biotransformation enzyme activity glutathione-S-transferase (GST) was measured using

1-chloro-2,4-dinitrobenzene (CDNB) as substrate and following the change in absorbance at 340 nm (Habig and Jakoby, 1981); the activity of antioxidant enzymes superoxide dismutase (SOD) was measured following the inhibition of the cytochrome c reduction by the superoxide radicals at 550 nm (McCord and Fridovich, 1969) and catalase (CAT)was measured by monitoring H_2O_2 decomposition using the decrease in absorbance at 240 nm (Beutler, 1982); the levels of glutathione (GSH) were determined using Ellman's reagent (5,50-dithiobis-2-nitrobenzoic acid, DTNB) and measuring the thiolate anion formation at 412 nm (White et al., 2003). The metallothionein (MT) concentration, other antioxidant compound, was quantified as described by Viarengo et al (1997) in hepatopancreas and muscle. The oxidative stress biomarkers were measured in hepatopancreas and muscle; lipid peroxidation (LPO) was determined as described by Jiang et al. (1991, 1992) and oxidized protein (OP) as described by Levine et al. (1994). Acetylcholinesterase activity (AChE) was assessed only in muscle as described by Ellman et al. (1961). Na^+/K^+-ATPase (NKA) and H^+-ATPase activities (HAT) (Gibbs and Somero, 1989) were measured in gills and kidneys, considering the osmoregulatory and excretory functions of these organs in fish. All measurements were performed in triplicate.

2.4 Statistical Analysis

Data are reported as mean ± standard deviation and analysed using the statistical packages, STATISTICA 7.1 from StatSoft Inc. (2005), and Infostat. All data were tested for normal distribution using Shapiro-Wilk test and homogeneity of variance using Levene's test and Brown & Forsythe's test. The one-way analysis of variance (ANOVA) was applied followed by Tukey's post-hoc test to compare data from each organ between sites and seasons (significance $p < 0.05$). Principal Component Analysis

(PCA) was performed crossing all metal concentrations and biological effects data to identify potential relationships between these variables in each organ and studied areas; Pearson correlation coefficient determined the significant correlations ($p < 0.05$). The results were present as an array with the following characteristics: 1) The number of rows is equal to the number of columns and equal to the number of variables selected; 2) The elements of the diagonal are all equal to 1 since they represent the correlation of a variable with itself; 3) Below the main diagonal and in position it is the coefficient of correlation between the variables of the list; 4) Above the diagonal and in position it is the probability associated with the test of hypothesis of null correlation between the variables in the list.

In addition, Generalized Procrustes Analysis (GPA) (Di Paola-Naranjo et al., 2011) was performed to evaluate the correspondence between biomarkers and metals/metalloids content in organs/tissues of fish with metal data in sediment and water samples from the studied estuaries reported by Souza et al (2013) as the fish samples were the same used in the previous study and it could be ecologically relevant. GPA is based on PCA results by transforming data in a consensus configuration of data sets groups. Grower algorithm was used to minimize within-samples variance by applying translation, scaling and rotation and generate a dimensional average configuration Yc. A q-dimensional group average space ($q \leq p$) was constructed from Yc by PCA (Wunderlin et al., 2001).

3. RESULTS

3.1 Metal accumulation and biochemical biomarker responses

There were significant differences between metal/metalloid concentrations in gills, hepatopancreas, kidneys and muscle of *C. parallelus* from Vitoria Bay and Santa

Cruz estuaries (Table 1). In Vitória Bay, the concentration of B and Cr was higher in gills, Fe in hepatopancreas and Cu, As and Se in both, hepatopancreas and kidneys. In contrast, fish from Santa Cruz had higher levels of Al, Mn in gills, Cr, in gills and muscle, Cu in both, hepatopancreas and kidneys, and V, Fe and Zn in kidneys. In Vitoria Bay was detected Hg in kidneys and Pb in gills and kidney (winter); in Santa Cruz was detected Hg and Pb in gills (winter and summer) and, Pb in kidney (summer). In general, concentrations of metals/metalloids in fish in winter were significantly higher than in summer in both estuaries (Table 1).

Concerning to antioxidant defences, the highest values in these biochemical biomarkers were found in hepatopancreas of fish from both sites (Table 2). In the gills, the activity of CAT and GSH (winter and summer) and GST (winter) were higher in fish from Vitória Bay; GSH (summer) and SOD, CAT and GST (winter) activities were higher in those from Santa Cruz. In hepatopancreas, SOD, CAT, MT and OP were higher in fish from Vitória Bay (winter and summer) and, GST and GSH (winter), SOD and CAT (summer) and LPO (winter and summer) in those from Santa Cruz. In kidney, CAT (winter) and GST (winter and summer) were higher in Vitória Bay than in Santa Cruz. In muscle, CAT, GSH, MT and OP were higher in fish from Vitoria Bay (winter and summer) and MT (winter) and OP (summer) were higher in Santa Cruz. AChE in muscle did not differ between sites and seasons.

The H^+-ATPase activity did not change in gills of fish from both sites but, in kidney, the activity of this enzyme was higher (winter) in both estuaries; Na^+/K^+-ATPase activity in kidney was higher than gills in fish from both sites; and the highest values were in winter.

3.2 Integrated analysis between metals/metalloids and biochemical biomarkers

Metal accumulation differs in each organ between winter and summer in the two estuaries as well as the biomarkers responses. The integrated view of entire data set in each site/season and a general view in each fish organ, independent of sites were given by PCA complemented by Pearson analyses. The first and second principal components of PCA analyses explained more than 80% of total data variability for all tissues and showed the main associations between metal/metalloid concentrations and biomarkers in each organ highlighting clear differentiation between sampling sites and season (Fig. 2, Tables 1S, 2S, 3S, 4S). Comparing Vitória Bay and Santa Cruz, most correlations were found in Vitória Bay, in winter, independent of fish organ (Fig. 2).

In the gills, significant correlations were detected between B (r = 0.48), As (r = 0.56), Se (r = 0.69), Al (r = -0.59), Mn (r = - 0.66) and GSH (winter) and, Cu and CAT (r = 0.42) (summer) in Vitoria Bay. In Santa Cruz there was no correlation between metal/metalloid and enzyme activity during winter; in summer, Mn and Fe were correlated with SOD (r = 0.68, r = 0.42, respectively) and Mn with HAT (r = 0.51) (Fig. 2A, Table 1S).

In the hepatopancreas, positive correlation occurred between Fe (r = 0.60), Se (r = 0.56), Zn (r = 0.52), Mn (r = 0.53) and As (r = 0.47) and MT concentration; Fe (r = 0.70), Se (r = 0.68), Zn (r = 0.46), Mn (r = 0.55), As (r = 0.66) and SOD; Fe (r = 0.85), Se (r = 0.89), Zn (r = 0.65), Mn (r = 0.65), As (r = 0.81) and CAT, in Vitória Bay (winter); summer samples showed the highest activity of most of enzymes and correlations were significant between B-CAT (r = 0.53) and enzymatic correlations between SOD-CAT (r = 0.80); SOD-OP (r = 0.55), SOD-LPO (r = -0.68) and CAT-LPO (r = -0.87) were observed (Fig. 2B, Table 2S). In contrast, no significant correlation occurred in Santa Cruz between metals and biomarkers.

In the kidneys, correlations between metals/metalloids and biomarkers occurred in the winter in Vitória Bay between Cr (r = 0.67), As (r = 0.49), Se (r = 0.48) and GST; Cr (r = 0.57), Se (r = 0.53), Cu (r = - 0.64) and NKA and, Se (r = 0.56), Cr (r = 0.42), B (r = -0.42), Cu (r = -0.59) and HAT (Fig. 2C, Table 3S). Enzymatic correlations were found between HAT-NKA (r = 0.96), NKA-GST (r = 0.75) and HAT-GST (r =0.65). In Santa Cruz, the levels of Fe (r = 0.43), V (r = 0.56) and Mn (r = 0.51) were correlated with SOD and, V (r = 0.65) and Mn (r = 0.59) were also correlated with GSH.

In muscle, the correlations occurred only in Vitoria Bay (Fig. 2D). In winter the correlation was between Hg-AChe (r = 0.44) although the absolute values of Hg and AChe did not differ significantly between sites. In summer, correlations were between Se-CAT (r =0.45), Se-MT (r = 0.60), Cu-MT (r = 0.60), Hg-GST (r = 0.47) and Se-GST (r = -0.41) (Fig. 2D, Table 4S).

3.3 Integrated analysis between metals/metalloids in the environment, accumulation and biomarkers in fish organs

Considering the environmental metal/metalloid data in water and sediment reported by Souza et al. (2013) and those accumulated in the organs of fish in the present study, B and V was not analyzed in the environment but detected in fish organs. Al, Mn, Fe and Pb were quantified in water and sediment, Ag in water and Cr and As in the sediment in both sites and seasons; Ni and Cu were quantified in the water and sediment in Santa Cruz and in water in Vitória Bay. Zn was quantified in the water in Vitoria Bay and was lower than quantification limit in Santa Cruz, although detected in this site. Cd and Hg were detected but were lower than the quantification limit in both sites and seasons.

GPA analyses using metal accumulation and biomarkers in each fish organ in this study and the environmental metal data (water and sediment) reported by Souza et al. (2013) showed a configuration consensual of four data sets discriminating the seasons and sites. This configuration, defined by its first (CP1- 54.9%, indicate the site segregation) and second (CP2- 27.3%, indicates to seasonal differences) principal axes, explained >80% variability of data, indicating that the difference of metal concentrations and they bioavailability between sites were more important than season (Fig. 3) .

4. DISCUSSION

Metals/metalloids accumulation, the activity of enzymes (SOD, CAT, GST) GSH and MT content related to antioxidant defences in gills, hepatopancreas, kidneys and muscles and those related to osmoregulation processes (NKA and HAT) in gills and kidney of *C. parallelus* revealed complex scenario in which the enzyme responses depend on metal accumulation and organ sensitivity. In general, metal availability in estuarine water is highly variable, depends on tide and season as well as water chemical and physical characteristics (Souza et al., 2013, Wolanski and Elliot, 2015). Therefore, it is expected that bioaccumulation of metals in the fish reflect such bioavailability in the environment. The general higher accumulation in winter season are probably related to higher metal availability in winter (Souza et al., 2013) combined to lower fish metabolism during this season.

The liver and kidney have been considered the organs that accumulate the highest levels of essential and non-essential metals as they are the main detoxification and excretion organs (Lawrence and Hemingway, 2003). However, in juvenile *C. Parallelus*, although most metals/metalloids were distributed in all organs; in general,

some metals were higher in a particular organ such as B, Al, Cr, Mn and Ni in gills, V and Zn in kidney, Cu and As in hepatopancreas and Fe in hepatopancreas and kidney evidencing different capability of each organ for storage/detoxification and excretion of such elements as already emphasized by Cazenave et al. (2006). Chronic field exposure and the exposure route (gills or gut) alter tissue-specific accumulation patterns as the results of different blood perfusion rate among organs; metal bioaccumulation in gills is expected to be higher in waterborne exposures while higher bioaccumulation in liver is followed by gut metal uptake (Wood, 2012).

The respiratory process involves large amount of water moving throughout the lamellar epithelium and the high blood perfusion rate in these organs and the tide dynamic in estuarine areas implies in more intense osmoregulatory activity in resident fish favouring metal uptake by gills (Bianchini et al., 2008) which, at least in part, may explain the presence of all metals in these organs. Gills are also potential excretory organs; for example, Al accumulated in the gills at higher levels than other organs (Jarić et al., 2011; Wood, 2002), but it may return to water as aluminum hydroxide and discarded as mucus-bound metal (Playle and Wood, 1991) reducing the entrance in fish body and having higher accumulation in gills. On the other hand, the thin diffusion distance between water-blood in gills favour metals diffusion into blood and them, they distribution to other organs, via blood stream, evidencing metals burden clearance into blood such as occurs with Cu (Grosell et al., 1997; Wood et al., 2002).

Despite the high metal bioaccumulation in gills, these organs showed low sensitivity to most of them; the negative correlations between Al and Mn with GSH may explain the lower content of GSH in the gills of fish from Santa Cruz in which were found the higher Al levels. These metals have strong affinity for reduced GSH producing oxidized glutathione (GSSG) and formation of glutathione-aluminum (GS-

Al) conjugate (Khan et al., 2012) and kept them inactive in lysosomes (Luoma and Rainbow, 2008; Nikinmaa et al, 2014). Conversely, B and As positive correlation with GSH may induce GSH production related to their strong interaction with sulfhydryl groups of molecule favouring the formation of metal complex and excretion. Concerning to enzymatic detoxification processes, SOD is a first-line metalloenzyme defence that uses Mn as cofactor and acts against superoxide radical (O_2^-) converting them in hydrogen peroxide (H_2O_2) and, CAT which cofactor is Fe, neutralize H_2O_2 to O_2 and H_2O (Pereira et al, 2010). Copper even at low concentration in the gill may induce ROS production, including H_2O_2, through Fenton reactions which may explain the positive correlation with CAT in the gills (Collén et al., 2003, Carvalho et al 2015). The activation of such antioxidant systems by Mn and Fe as well as Cu positive correlation with SOD and CAT, respectively, represents an important biochemical defence response to metals avoiding oxidative damage of lipids, proteins and nucleic acids (Collén et al., 2003). The morphological integrity of gills in this species living in these estuarine areas (Souza et al., 2013) reinforces the gill tolerance to metals.

The kidneys are important organs for metal excretion although in estuarine or marine fish the urine excretion is lower than freshwater fish. The great accumulation of Cu in the kidney of fish from Vitoria Bay and Fe and Zn in fish from Santa Cruz is probably associated to the bioavailability of these metals in these estuarine areas. Such high accumulation in this organ was unexpected however, some seawater fish such as *Thunnus albacares*, also accumulate very high levels of Zn in the kidney (Kojadinovic et al., 2007). MT is the main Zn binding protein in the cells but, small molecules such as glutathione, cysteine, and histidine may be Zn ligands increasing Zn accumulation in the kidney (Hogstrand, 2012) that was not correlated with any antioxidant defense system in this organ. Concerning to Fe bioaccumulation in the kidney, it may bind

intracellular proteins such as ferritin that keeps Fe in a soluble bioavailable non-toxic form in the cytoplasm (Arozio and Levy, 2010) until excretion. Fe are mainly eliminated via the liver (biliary routes) (LeSage et al., 1986) and kidney also participate of Fe excretion (Ferguson et al., 2003). Nevertheless, V and Fe are required for production of hydroxyl radical (.OH) production from H_2O_2, via Fenton reaction, and by conversion of O_2- to .OH, via catalysis of Haber-Weiss reaction (Stohs and Bagchi, 1995). The positive correlation between Fe, V and Mn with SOD activity and GSH levels may reflect an attempt to contain .OH; Mn can also catalyse these reactions.

Another important positive correlations are those found between Mn and HAT in the gills in fish from Santa Cruz (summer) and Cr and Se with NKA and negative correlation of Cu and NKA in the kidneys in fish from Vitória Bay (winter) These enzymes are directly related to ion and acid base regulation in fish in these organs but, little is known about the direct action of these metals on these enzymes, excepting for Cu that inhibit NKA activity (Dang et al., 2000; Atli and Canli, 2007; Li et al., 1996; Li et al, 2009). However, such correlations may be an indirect effect of Mn, Cr and Se on these enzymes. For example, high Cu concentration inhibits NKA activity but, increases plasma cortisol which, in turn, increases NKA activity (Dang et al., 2000; McCormick, 1995) being the total NKA activity the result of Cu-inhibition action and cortisol up-regulation. The correlation between NKA and HAT activity of *C. parallelus* evidenced the role of gills and kidney in keeping an electrical gradient and ion regulation in the organism.

The hepatopancreas (liver region) is usually the main detoxification organ for contaminant biotransformation or storage reducing biological damages (Pacheco and Santos, 2002). The positive correlation between SOD-CAT in hepatopancreas is expected when high level of H_2O_2 is produced and evidenced the activation of this first-

line antioxidant system as occurred in fish from Vitória Bay (winter). Furthermore, the positive correlation of SOD and CAT with its metals cofactors (Se, Mn, Fe and Zn) and Al and As levels in hepatopancreas reinforced this hypothesis and evidence an effective protector role against non-essential metals given by the negative correlation with lipid peroxidation (LPO) levels in *C. parallelus*. The possible peroxisome proliferation that contains high levels of CAT has an important role in peroxide degradation (Lawrence and Hemingway, 2003). Furthermore, MT positively correlation with Fe, Se, Zn, Mn and As evidenced a non-enzymatic antioxidant response to maintain metal homeostasis in Victoria Bay although, OP was higher than in Santa Cruz. Metallothioneins regulate the free concentrations of essential metals and sequester non-essential ones protect cells, at least until certain level, against their toxic effect (Luoma and Rainbow, 2008).

Muscle is the ultimate soft tissue for metal accumulation and, in general, indicates that the liver and other excretion organ exceeded the capacity to remove metals, excepting Hg which preferentially accumulated in muscle. The positive correlation between Se with CAT and MT, Cu with MT and Hg with GST suggest that even at low levels these metals stimulate the activity of these antioxidant defences protecting the cells.

The differences between metal bioaccumulation and the biological responses in both sites highlight the importance to evaluate different locations instead of season to evaluate the responses in estuarine fish. Overall, biological responses to metal accumulation was observed in both sites, however such responses were more evident in Vitória Bay in which metal concentration in the surface water were higher. Although, the physicochemical properties of the water in each site are different, it was possible to observe metal bioaccumulation in both areas.

In conclusion, the evaluation of metals/metalloids accumulation in different organs of *C. parallelus* evidenced that the responses and/or absence of them in a given organ show organ/tissue-specific sensibility suggesting that, at least in some extension, *C. parallelus* is able to support such environmental conditions. The metal levels in gills indicate water contamination and low sensitivity of this organ to most metals. Moreover, biochemical responses suggested that the metal elimination pathway of *C. paralellus* is through the gills and kidney. Hepatopancreas and kidneys revealed to be important detoxification organs while muscle was the less reactive tissue. It is important emphasize that it implicates in energy expenditure that may influence the growth rate and reproduction processes. Considering the ecological and economic importance of *C. parallelus*, the level of toxic metals/metalloids in juvenile fish is an important alert for maintenance, preservation and commercialization of this species.

ACKNOWLEDGMENTS

This study was supported by National Council of Technological and Scientific Development (CNPq)/National Institute of Science and Technology in Aquatic Toxicology (INCT in Aquatic Toxicology, Proc. 573949/2008-5)(Brazil), Scientific and Technological Research Fund (FONCyT/PICT-1597), CONICET (National Research Council) and Science and Technology Office from Córdoba National University (Argentina). I.C. Souza acknowledges São Paulo Research Foundation (FAPESP, Proc. 2014/04832-3).

REFERENCES

Arrivabene, H.P., Souza, I.C., Có, W.L.O., Conti, M.M., Wunderlin, D.A., Milanez, C.R.D., 2015. Effect of pollution by particulate iron on the morphoanatomy, histochemistry, and bioaccumulation of three mangrove plant species in Brazil. Chemosphere 127, 27–34. doi: 10.1016/j.chemosphere.2015.01.011

Arrivabene, H.P., Souza, I.C., Có, W.L.O., Rodella, R.A., Wunderlin, D.A., Milanez, C.R., 2014. Functional traits of selected mangrove species in Brazil as biological indicators of different

environmental conditions. Science of the Total Environment 476–477, 496–504. doi: 10.1016/j.scitotenv.2014.01.032

Atli, G., Canli, M., 2007. Enzymatic responses to metal exposures in a freshwater fish *Oreochromis niloticus*. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 145.2, 282-287. doi: 10.1016/j.cbpc.2006.12.012

Barbee, N.C., Ganio, K., Swearer, S.E., 2014. Integrating multiple bioassays to detect and assess impacts of sublethal exposure to metal mixtures in an estuarine fish. Aquatic Toxicology 152, 244-255. doi: 10.1016/j.aquatox.2014.04.012

Beutler, E., 1982. Catalase. In: Beutler, E. Red cell metabolism, a manual of biochemical methods. Grune and Stratton, New York, pp. 105-106.

Bianchini, A., Lauer, M.M., Nery, L.E.M., Colares, E.P., Monserrat, J.M., Santos Filho, E.A., 2008. Biochemical and physiological adaptations in the estuarine crab *Neohelice granulata* during salinity acclimation. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 151, 423-436. doi: 10.1016/j.cbpa.2007.12.001

Borja, A., Basset, A., Bricker, S., Dauvin, J., Elliot, M., Harrison, T., Marques, J.C., Weisberg, S., West, R., 2012. Classifying ecological quality and integrity of estuaries. In: Wolanski, E., McLusky, D. (Eds.), Treatise on Estuarine and Coastal Science. Academic Press, Waltham, pp. 125-162.

Bosch, A.C., O'Neill, B., Sigge, G.O., Kerwath, S.E., Hoffman, L.C., 2016. Mercury accumulation in Yellowfin tuna (*Thunnus albacares*) with regards to muscle type, muscle position and fish size. Food chemistry 190, 351-356. doi: 10.1016/j.foodchem.2015.05.109

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254. doi: 10.1016/0003-2697(76)90527-3

Carvalho, C.S., Bernusso, V.A., Araújo, H.S.S., Espíndola, E.L.G., Fernandes, M.N., 2012. Biomarker responses as indication of contaminant effects in *Oreochromis niloticus*. Chemosphere 89, 60-69. doi: 10.1016/j.chemosphere.2012.04.013. doi: 10.1016/j.aquatox.2015.08.003.

Carvalho, C.S., Bernusso, V.A., Fernandes, M.N., 2015. Copper levels and changes in pH induce oxidative stress in the tissue of curimbata (*Prochilodus lineatus*). Aquatic Toxicology 167, 220–227.

Cazenave, J., Bistoni M.A., Pesce, S.F., Wunderlin, D.A., 2006. Differential Detoxification and Antioxidant Response in Organs of Corydoras paleatus Exposed to Microcystin-RR. Aquatic Toxicology 76,1-12. doi: 10.1016/j.aquatox.2005.08.011

Cerveny D., Zlabek V., Velisek J., Turek J., Grabic R., Grabicova K., Fedorova G., Rosmus J., Lepic P., Randak T., 2014. Contamination of fish in important fishing grounds of the Czech Republic. Ecotoxicology and Environmental Safety 109, 101-109. doi: 10.1016/j.ecoenv.2014.07.034.

Chappaz, A., Lyons, T.W., Gordon, G.W., Anbar, A.D., 2012. Isotopic fingerprints of anthropogenic molybdenum in lake sediments. Environmental science & technology, 46:20, 10934-10940.

Collén, J., Pinto, E., Pedersen, M., Colepicolo, P., 2003. Induction of oxidative stress in the red macroalga *Gracilaria tenuistipitata* by pollutant metals. Archives of Environmental Contamination and Toxicology 45.3, 337-342.

Dang, Z., Lock, R.A.C., Flick, G., Bonga, S.W, 2000. Na/K-ATPase immunoreactivity in branchial chloride cells of Oreochromis mossambicus exposed to copper. Journal of Experimental Biology 203.2, 379-387.

Di Paola-Naranjo, R.D., Baroni, M.V., Podio, N.S., Rubinstein, H.R., Fabani, M.P., Badini, R.G., Inga, M., Ostera, H.A., Cagnoni, M., Gallego, E., Gautier, E., Peral-García, P., Hoogewerff, J., Wunderlin, D.A., 2011. Fingerprints for main varieties of Argentinean Wines: terroir differentiation by inorganic, organic and stable isotopic analyses coupled to chemometrics. Journal of Agricultural and Food Chemistry 59, 7854-7865. doi: 10.1021/jf2007419

Elliott, M., Quintino V., 2007. The Estuarine Quality Paradox, Environmental Homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. Marine Pollution Bulletin 54, 640-645. doi: 10.1016/j.marpolbul.2007.02.003

Elliott, M., Whitfield, A., 2011. Challenging paradigms in estuarine ecology and management. Estuarine, Coastal & Shelf Science 94, 306-314. doi: 10.1016/j.ecss.2011.06.016

Ellman, G.L., Courtney, K.O., Andres, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 7, 88-95. doi: 10.1016/0006-2952(61)90145-9.

Ferguson, C.J., Wareing, M., Delannoy, M., Fenton, R., McLarnon, S.J., Ashton, N., Cox, A.G., McMahon, R.F., Garrick, L.M., Green, R., Smith, C.P., and Riccardi, D., 2003. Iron handling and gene expression of the divalent metal transporter, DMT1, in the kidney of the anemic Belgrade (b) rat. Kidney International 64, 1755-1764.

Fernandes, M.N., Mazon, A.F., 2003. Environmental pollution and fish gill morphology. In: Val, A.L., Kapoor, B.G. (Eds.). Fish Adaptation. Science Publishers, Enfield, pp. 203-231.

Fonseca, V.F., França, S., Serafim, A., Company, R., Lopes, B., Bebianno, M.J., Cabral, H.N., 2011. Multi-biomarker responses to estuarine habitat contamination in three fish species: Dicentrarchus labrax, Solea senegalensis and Pomatoschistus microps. Aquatic Toxicology 102, 216-227. doi: 10.1016/j.aquatox.2011.01.018

Gibbs, A., Somero, G.N., 1989. Pressure Adaptation of NA+/K+-ATPase in gills of marine teleosts. Journal of Experimental Biology 143, 475-492.

Grosell et al., 1997; -livro Wood

Habig, W.H., Jakoby, W.B., 1981. Assays for differentiation of glutathione S-Transferases. Methods Enzymology 77, 398-405.

Hartl, M.G.J., 2013. Homeostasis and Toxicology of Non-essential metals. Journal of Fish Biology 83: 1476–1477. doi: 10.1111/jfb.12243

Hogstrand, C., 2012. Zinc. In: Homeostasis and Toxicology of Essential Metals (Wood, C.M., Farrell, A.P., Brauner, C.J., eds.). Fish Physiology series, v. 31A, p. 136-200.

Jarić, I., Višnjić-Jeftić, Ž., Cvijanović, G., Gačić, Z., Jovanović, L., Skorić, S., & Lenhardt, M., 2011. Determination of differential heavy metal and trace element accumulation in liver, gills, intestine and muscle of sterlet (Acipenser ruthenus) from the Danube River in Serbia by ICP-OES. Microchemical Journal 98.1, 77-81. doi: 10.1016/j.microc.2010.11.008

Jiang, Z-Y., Hunt, J.V., Wolff, S.P., 1992. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Analytical Biochemistry 202, 384-389. doi: 10.1016/0003-2697(92)90122-N

Jiang, Z-Y., Woollard, A.C.S., Wolff, S.P., 1991. Lipid hydroperoxides measurement by oxidation of Fe2+ in the presence of xylenol orange. Comparison with the TBA assay and an iodometric method. Lipids 26, 853-856. doi: 10.1007/BF02536169.

Khan, H., Khan, M.F., Khan, B.A., Razaque, G., Haque, N., Akhter, B., Zareen, B., 2012. Evaluation of the interaction of aluminium metal with glutathione in human blood components. Biomedical Research 23, 237-240.

Kennish, M.J., Elliott, M., 2011. Volume 8. Human-induced problems (uses and abuses) in Estuaries and Coasts. In: Wolanski, E., McLusky, D.S. (Eds). Treatise on Estuarine & Coastal Science. Elsevier, Amsterdam, 315 p.

Kojadinovic, J., Potier, M., Le Corre, M., Cosson, R. P., and Bustamante, P. (2007).

Bioaccumulation of trace elements in pelagic fish from the Western Indian Ocean. Environ. Pollut. 146, 548–566.

Lawrence, A., Hemingway, K., 2003. Effects of Pollution on Fish: molecular effects and population responses. Blackwell Science, Oxford.

LeSage, G. D., Kost, L. J., Barham, S. S., & LaRusso, N. F. 1986. Biliary excretion of iron from hepatocyte lysosomes in the rat. A major excretory pathway in experimental iron overload. Journal of Clinical Investigation, 77(1), 90.doi: 10.1172/JCI112307.

Levine, R.L., Williams, J.A., Stadtman, E.R., Shacter, E., 1994. Carbonyl assays for determination of oxidatively modified proteins. Methods in Enzymology 233, 346-357. doi: 10.1016/S0076-6879(94)33040-9

Li, J., Lock, R.A., Klaren, P.H., Swarts, H.G., Stekhoven, F.M.S., Bonga, S.E.W., Flik, G., 1996. Kinetics of Cu 2+ inhibition of Na+ K+-ATPase. Toxicology Letters 87(1), 31-38. doi: 10.1016/0378-4274(96)03696-X.

Li, X.B., Hou, X.L., Mao, Q., Zhao, Y.L., Cheng, Y.X., Wang, Q., 2009. Toxic effects of copper on antioxidative and metabolic enzymes of the marine gastropod, *Onchidium struma*. Archives of Environmental Contamination and Toxicology 56(4), 776-784. doi: 10.1007/s00244-009-9290-2.

Luoma, S.N., Rainbow, P.S., 2008. Metal contamination in aquatic environments: science and lateral management. Cambridge University Press, Cambridge.

Mazon, A.F., Cerqueira, C.C.C., Fernandes, M.N., 2002. Gill cellular changes induced by copper exposure in the South American tropical freshwater fish *Prochilodus scrofa*. Environmental Research Section A 88, 52-63. doi: 10.1006/enrs.2001.4315.

McCord, J.M., Fridovich, I., 1969. Superoxide dismutase an enzymic function for erythrocuprein (hemocuprein). Journal of Biological Chemistry 244, 6049-6055.

McCormick, S.D., 1995. Hormonal control of gill Na^+, K^+-ATPase and chloride cell function. Fish Physiology 14, 285-315. doi: 10.1016/S1546-5098(08)60250-2.

Monserrat, J.M., Martínez, P.E., Geracitano, L.A., Amado, L.L., Martins, C.M.G., Pinho, G.L.L., Chaves, I.S., Ferreira-Cravo, M., Ventura-Lima, J., Bianchini, A., 2007. Pollution biomarkers in estuarine animals: Critical review and new perspectives. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 146(1–2), 221-234. doi: 10.1016/j.cbpc.2006.08.012.

Nikinmaa, M., 2014. An Introduction to aquatic toxicology. Elsevier, Whaltam, 253p.

Oliveira, M., Ahmad, I., Maria, V.L., Serafim, A., Bebianno, J., Pacheco, M., Santos, M.A., 2010. Hepatic metallothionein concentrations in the golden grey mullet (*Liza aurata*) – Relationship with environmental metal concentrations in a metal-contaminated coastal system in Portugal. Marine Environmental Research 69, 227–233. doi: 10.1016/j.marenvres.2009.10.012

Pacheco, M., Santos, M.A., 2002. Biotransformation, genotoxic, and histopathological effects of environmental contaminants in European eel (*Anguilla anguilla* L.). Ecotoxicology and Environmental Safety 53(3), 331-347. doi: 10.1016/S0147-6513(02)00017-9.

Paul, N., Chakraborty, S., Sengupta, M., 2014. Lead toxicity on non-specific immune mechanisms of freshwater fish *Channa punctatus*. Aquatic Toxicology 152, 105-112. doi: 10.1016/j.aquatox.2014.03.017.

Playle, R.C., Wood, C.M., 1991. Mechanisms of aluminium extraction and accumulation at the gills of rainbow trout, *Oncorhynchus mykiss* (Walbaum), in acidic soft water. Journal of Fish Biology 38, 791–805.

Pereira, P., Pablo, H., Vale, C., Pacheco, M., 2010. Combined use of environmental data and biomarkers in fish (*Liza aurata*) inhabiting a eutrophic and metal-contaminated coastal system – Gills reflect environmental contamination. Marine Environmental Research 69, 53-62. doi: 10.1016/j.marenvres.2009.08.003.

Rocha, A.J.S., Gomes, V., Ngan, P.V., Passos, M.J.A.C.R., Furia, R.R., 2007. Effects of anionic surfactant and salinity on the bioenergetics of juveniles of *Centropomus parallelus* (Poey). Ecotoxicology and Environmental Safety 68, 397-404. doi: 10.1016/j.ecoenv.2006.10.007.

Rosabal, M., Pierron, F., Couture, P., Baudrimont, M., Hare, L., Campbell, P.G.C., 2015. Subcellular partitioning of non-essential trace metals (Ag, As, Cd, Ni, Pb, and Tl) in livers of American (*Anguilla rostrata*) and European (*Anguilla anguilla*) yellow eels. Aquatic Toxicology 160, 128-141. doi: 10.1016/j.aquatox.2015.01.011.

Sakuragui, M.M., Paulino, M.G., Pereira, C.D.S., Carvalho, C.S., Sadauskas-Henrique, H., Fernandes, M.N., 2013. Integrated use of antioxidant enzymes and oxidative damage in two fish species to assess pollution in man-made hydroelectric reservoirs. Environmental pollution 178, 41-51. Doi: 10.1016/j.envpol.2013.02.032.

Souza, I., Bonomo, M.M., Morozesk, M., Rocha, L.D., Duarte, I.D., Furlan, L.M., Arrivabene, H.P., Monferrán, M.V., Matsumoto, S.T., Milanez, C.R.D., Wunderlin, D.A., Fernandes, M.N., 2014a. Adaptive plasticity of Laguncularia racemosa in response to different environmental conditions: integrating chemical and biological data by chemometrics. Ecotoxicology 23, 335–348. doi: 10.1007/s10646-014-1191-0.

Souza, I.C., Duarte, I.D., Pimentel, N.Q., Rocha, L.D., Morozesk, M., Bonomo, M.M., Azevedo, V.C., Pereira, C.D.S., Monferrán, M.V., Milanez, C.R.D., Matsumoto, S.T., Wunderlin, D.A., Fernandes, M.N., 2013. Matching metal pollution with bioavailability, bioaccumulation and biomarkers response

in fish (*Centropomus parallelus*) resident in neotropical estuaries. Environmental Pollution 180, 136-144. doi: 10.1016/j.envpol.2013.05.017.

Souza, I.C., Morozesk, M., Duarte, I.D., Bonomo, M.M., Rocha, L.D., Furlan, L.M., Arrivabene, H.P., Monferrán, M.V., Matsumoto, S.T., Milanez, C.R.D., Wunderlin, D.A., Fernandes, M.N., 2014b. Matching pollution with adaptive changes in mangrove plants by multivariate statistics. A case study, *Rhizophora mangle* from four neotropical mangroves in Brazil. Chemosphere 108, 115–124. doi: 10.1016/j.chemosphere.2014.02.066.

Souza, I.C., Rocha, L.D., Morozesk, M., Bonomo, M.M., Arrivabene, H.P., Duarte, I.D., Furlan, L.M., Monferrán, M.V., Matsumoto, S.T., Milanez, C.R.D., Wunderlin, D.A., Fernandes, M.N., 2015. Changes in bioaccumulation and translocation patterns between root and leaves of *Avicennia schaueriana* as adaptive response to different levels of metals in mangrove system. Marine Pollution Bulletin 94, 176–184. doi: 10.1016/j.marpolbul.2015.02.032.

Stohs, S.J., Bagchi, D., 1995. Oxidative mechanisms in the toxicity of metal ions. Free Radical Biology and Medicine 18(2), 321-336. doi: 10.1016/0891-5849(94)00159-H

Storey, K.B., 1996. Oxidative stress: animal adaptations in nature. Brazilian Journal of Medical and Biological Research 29, 1715-1733.

Taylor, R.G., Whittington, J.A., Grier, H.J., Crabtree, R.E., 2000. Age, growth, maturation, and protandric sex reversal in the common snook, *Centropomus undecimalis*, from South Florida waters. Fishery Bulletin 98, 612–624.

Viarengo, A., Ponzano, E., Dondero, F., Fabbri, R., 1997. A simple spectrophotometric method for metallothionein evaluation in marine organisms: an application to Mediterranean and Antarctic molluscs. Marine Environmental Research 44, 69-84. doi: 10.1016/S0141-1136(96)00103-1.

Volpe, A.V., 1959. Aspects of the Biology of the Common Snook, *Centropomus undecimalis* (Bloch) of Southwest Florida. In: Technical Series No 31. University of Miami, Florida, p. 37.

White, C.C., Viernes, H., Krejsa, C.M., Botta, D., Kavanagh, T.J., 2003. Fluorescence-based microtiter plate assay for glutamate–cysteine ligase activity. Analytical Biochemistry 318, 175-180. doi: 10.1016/S0003-2697(03)00143-X.

Wolanski, E., Elliott, M., 2015. Estuarine Ecohydrology: An Introduction. Elsevier, Whaltam, 322p.

Wood, C.M., 2012. An introduction to metals in fish physiology and toxicology: basic principles. In: Homeostasis and Toxicology of Essential Metals (Wood, C.M., Farrell, A.P., Brauner, C.J., eds). Fish Physiology series, Vol 31A, p.1-51.

Wood, C. M., Grosell, M., Hogstrand, C., Hansen, H., 2002. Kinetics of radiolabeled silver uptake and depuration in the gills of rainbow trout (*Oncorhynchus mykiss*) and European eel (*Anguilla anguilla*): the influence of silver speciation. Aquatic Toxicology 56, 197–213.

Wunderlin, D.A., Díaz, M.P., Amé, M.V., Pesce, S.F., Hued, A.C., Bistoni, M.A., 2001. Pattern recognition techniques for the evaluation of spatial and temporal variations in water quality. A case study: Suquía River Basin (Córdoba, Argentina). Water Research 35, 2881–2894. doi: 10.1016/S0043-1354(00)00592-3.

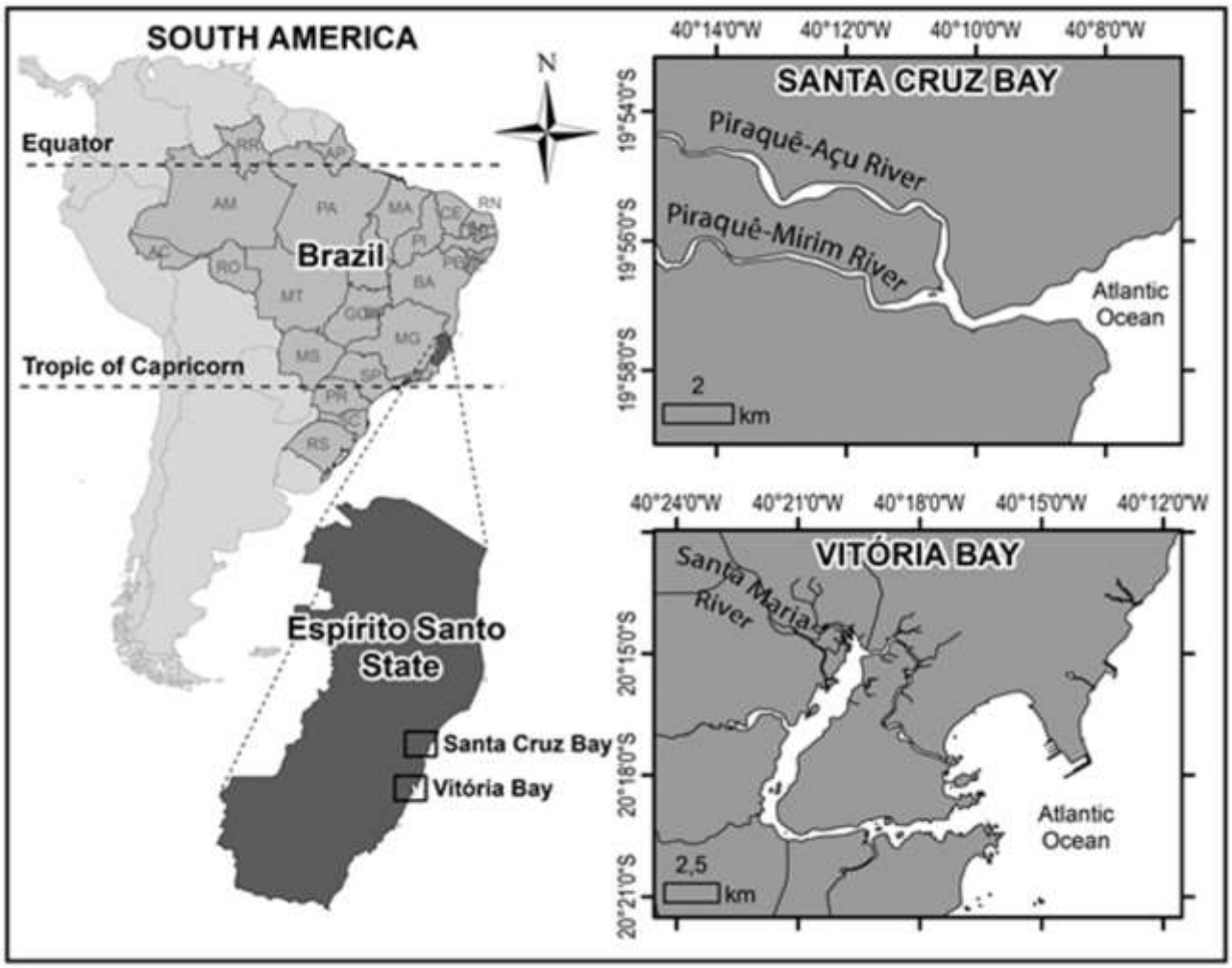
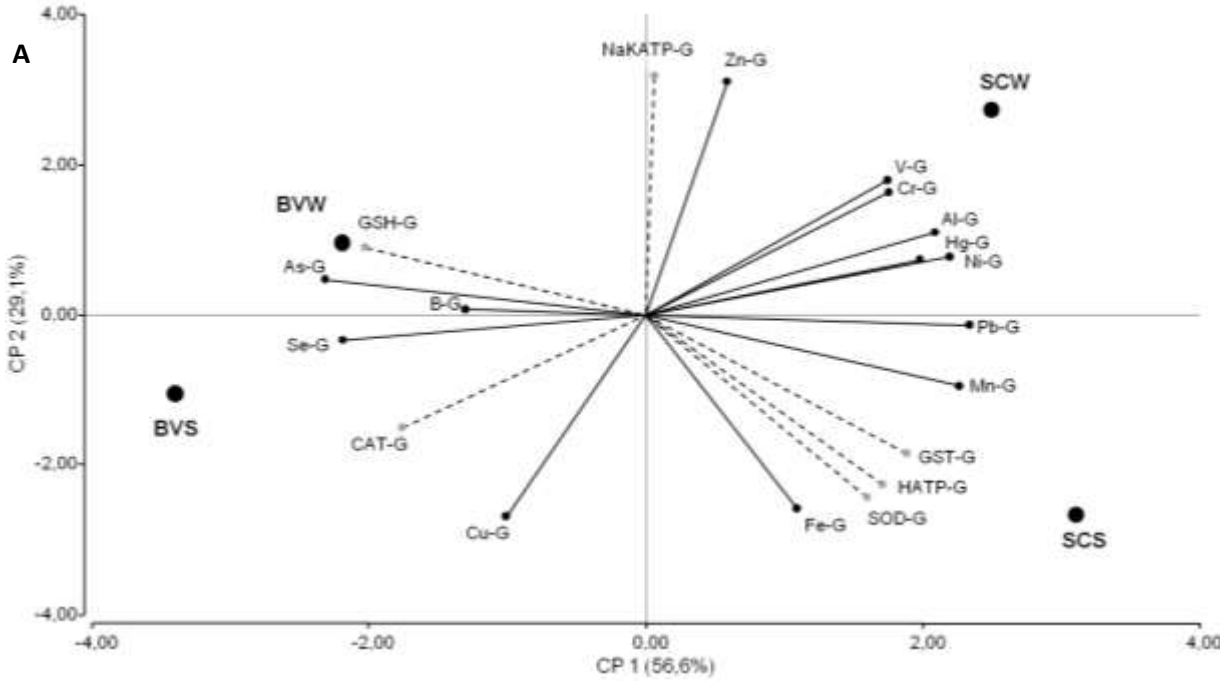
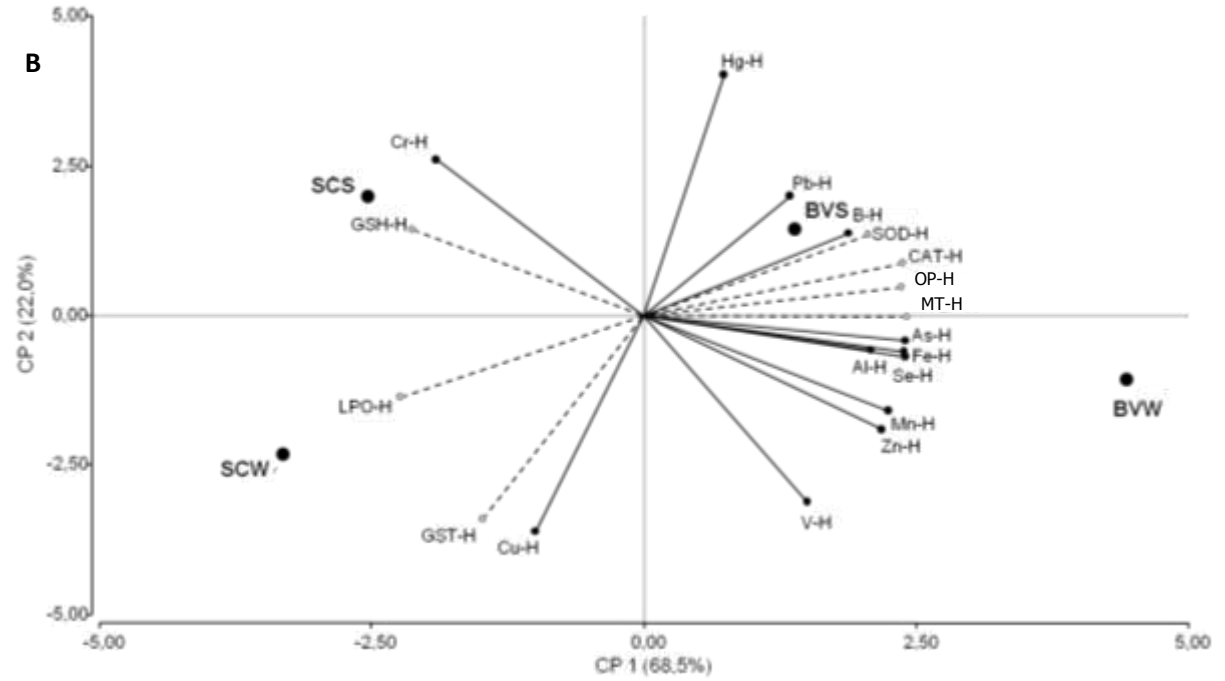


Figure 1. Map of the State of Espírito Santo (Brazil, South America) showing sampling sites. Santa Cruz (S 19º56`26.2``; W 40º 12`87``) and Vitória Bay (S 20º14`31.5``; W 40º19`84.7``).


A
NaKATP-G
Zn-G
SCW
V-G
Cr-G
BVW
GSH-G
Al-G
Hg-G
Ni-G
As-G
B-G
Pb-G
Se-G
Mn-G
BVS
CAT-G
GST-G
HATP-G
SOD-G
Cu-G
Fe-G
SCS
CP 2 (29,1%)
CP 1 (56,6%)
4,00
2,00
0,00
-2,00
-4,00


B
Hg-H
Cr-H
Pb-H
SCS
BVS
B-H
GSH-H
SOD-H
CAT-H
OP-H
MT-H
As-H
Fe-H
Al-H
Se-H
BVW
LPO-H
Mn-H
Zn-H
SCW
V-H
GST-H
Cu-H
CP 2 (22,0%)
CP 1 (68,5%)
5,00
2,50
0,00
-2,50
-5,00

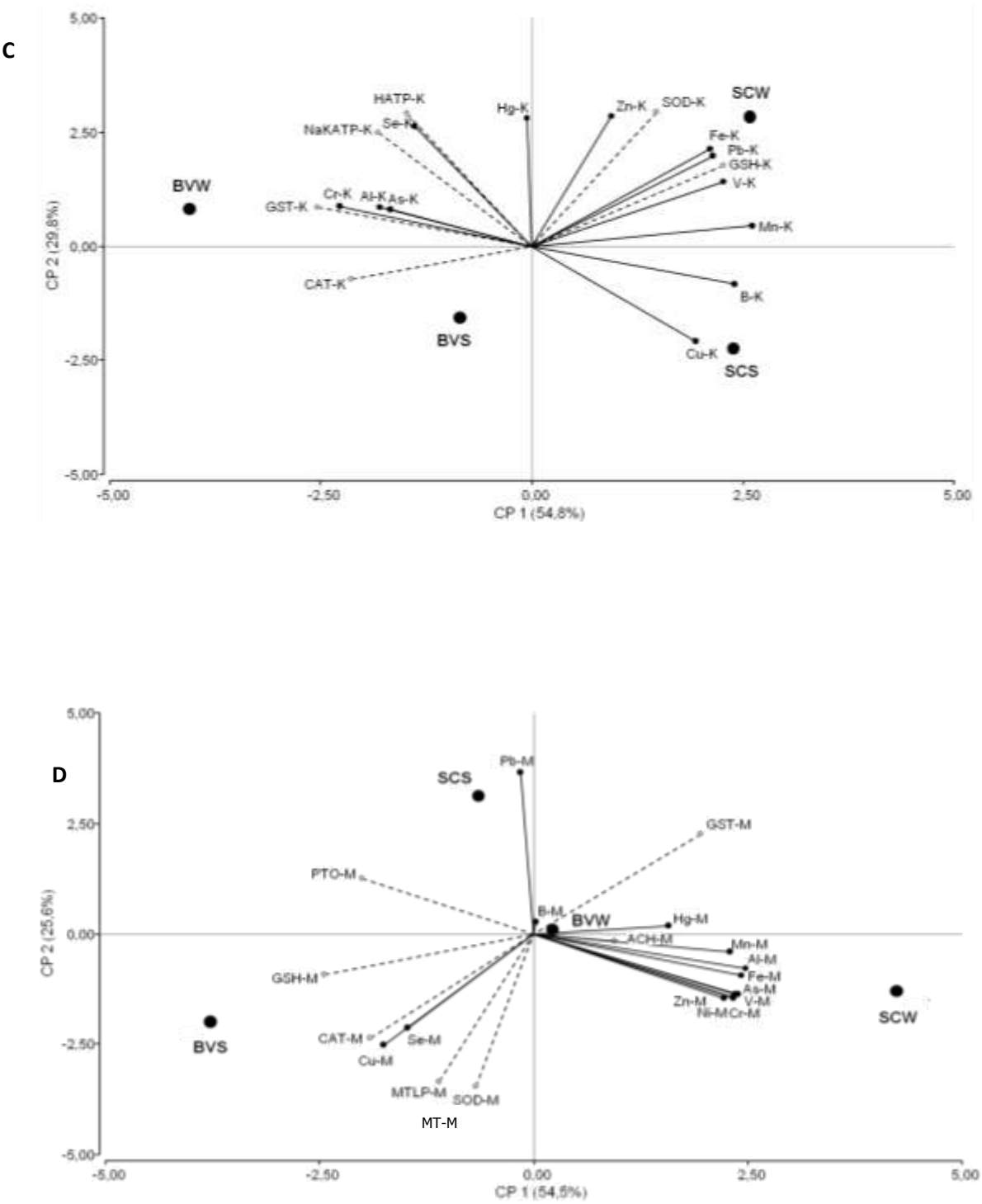


Figure 2. Graphical representation of Principal Component Analysis (PCA). A: G – Gill; B: H – Hepatopancreas; C: K – Kidney; M - Muscle. BVS: Baía de Vitória Summer; BVW: Baía de Vitória Winter; SCS: Santa Cruz Summer; SCW: Santa Cruz Winter.

Metais ——; Biomarkers - - - - -

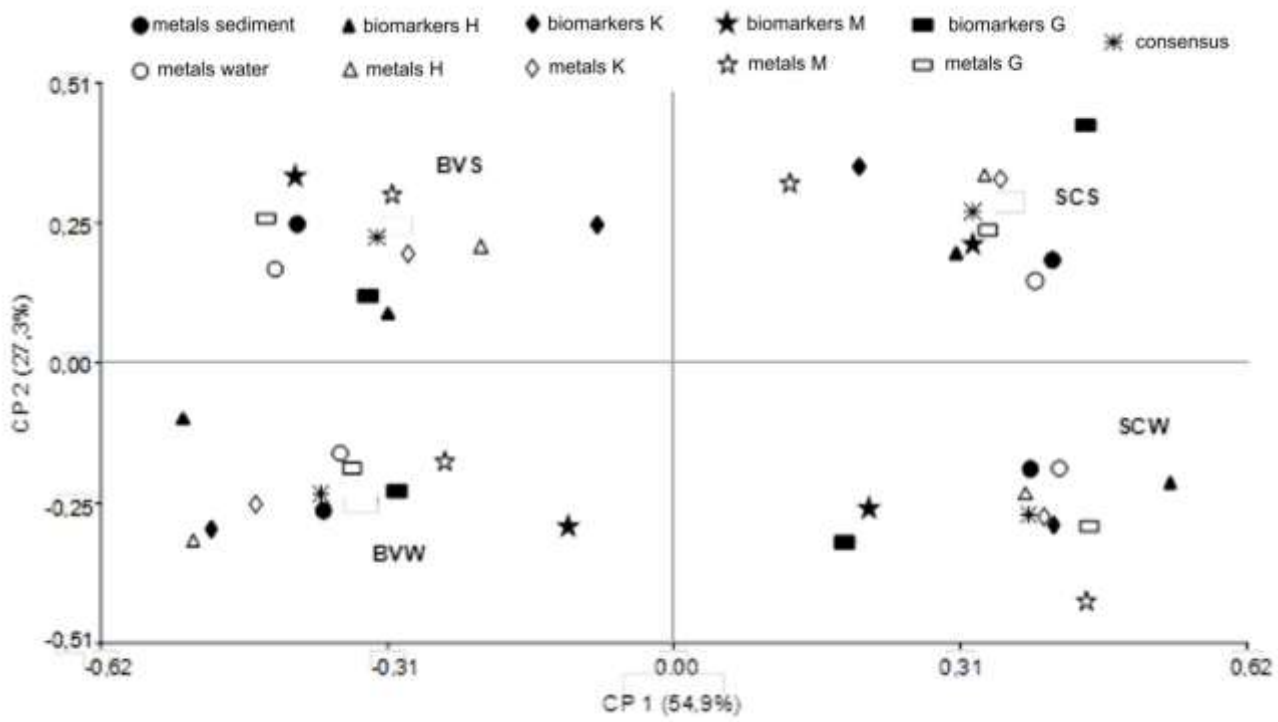


Figure 3. Graphical representation of Generalized Procrustes Analysis (GPA). BVS: Vitória Bay Summer; BVW: Vitória Bay Winter; SCS: Santa Cruz Summer; SCW: Santa Cruz Winter. H: Hepatopancreas; K: Kidney; M: Muscle; G: Gill.

Table 1. Metal concentrations (μg g^{-1} dry mass) in different organs/tissues of *C. parallelus* (n = 5 in each site) from Vitória Bay and Santa Cruz estuaries. Values are mean ± SD. VBW: Vitória bay winter; VBS: Vitória bay summer; SCW: Santa Cruz winter; SCS: Santa Cruz summer.

	Metals														
	B	**Al**	**V**	**Cr**	**Mn**	**Fe**	**Ni**	**Cu**	**Zn**	**As**	**Se**	**Ag**	**Cd**	**Hg**	**Pb**
Gill															
VBW	4.66 ± 3.77^{b}	55.6 ± 6.4^{a}	0.20 ± 0.03^{a}	9.42 ± 6.59^{a}	11.5 ± 2.3^{a}	128 ± 28^{a}	<LOQ^{a}	0.52 ± 0.22^{a,b}	92 ± 12^{a}	0.50 ± 0.22^{b,c}	1.46 ± 0.16^{b}	<LOD	<LOD	<LOQ^{a}	0.31 ± 0.12^{a}
VBS	1.26 ± 0.52^{a,b}	74 .3 ± 10.9^{a}	0.20 ± 0.03^{a}	7.02 ± 2.02^{a}	11.1 ± 1.6^{a}	121 ± 13^{a}	3.03 ± 0.99^{b}	0.84 ± 0.14^{b}	82 ± 3^{a}	0.64 ± 0.17^{c}	1.29 ± 0.08^{b}	<LOD	<LOD	<LOD^{a}	<LOQ^{a}
SCW	<LOD^{a}	279.5 ± 72.5^{c}	0.25 ± 0.06^{a}	10.23 ± 3.83^{a}	28.9 ± 10.1^{b}	117 ± 32^{a}	4.53 ± 1.74^{b}	0.43 ± 0.04^{a}	96 ± 47^{a}	0.26 ± 0.15^{a,b}	0.60 ± 0.19^{a}	<LOD	<LOD	0.81 ± 0.26^{a}	0.38 ± 0.12^{a}
SCS	1.17 ± 0.15^{a}	187.8 ± 69.3^{b}	0.21 ± 0.03^{a}	9.38 ± 0.27^{a}	40.6 ± 9.8^{b}	151 ± 4^{a}	4.05 ± 0.14^{b}	0.70 ± 0.31^{a,b}	83 ± 16^{a}	<LOQ^{a}	0.76 ± 0.01^{a}	<LOD	<LOD	0.83 ± 0.46^{a}	0.41 ± 0.04^{a}
Hepatopancreas															
VBW	<LOD	7.7 ± 2.2^{b}	0.25 ± 0.14^{b}	<LOQ^{a}	7.4 ± 2.2^{b}	1571 ± 414^{c}	<LOD	10.28 ± 3.02^{a}	88 ± 21^{b}	4.34 ± 1.99^{b}	5.90 ± 1.07^{c}	<LOD	<LOD	<LOD	0.45 ± 0.35^{a}
VBS	<LOD	3.7 ± 0.8^{a,b}	0.11 ± 0.05^{a,b}	<LOQ^{a}	5.0 ± 0.6^{a,b}	777 ± 383^{b}	<LOD	8.47 ± 1.85^{a}	55 ± 10^{a,b}	2.84 ± 1.27^{b}	4.10 ± 0.35^{b}	<LOD	<LOD	<LOQ	<LOD^{a}
SCW	<LOD	2.8 ± 2.8^{a}	0.17 ± 0.01^{a,b}	<LOQ^{a}	4.3 ± 0.9^{a}	98 ± 68^{a}	<LOD	11.63 ± 6.10^{a}	46 ± 27^{a}	0.33 ± 0.14^{a}	1.50 ± 0.31^{a}	<LOD	<LOD	<LOD	<LOD^{a}
SCS	<LOD	4.0 ± 2.9^{a,b}	0.10 ± 0.04^{a}	0.33 ± 0.27^{a}	3.5 ± 2.3^{a}	43 ± 11^{a}	<LOD	9.78 ± 6.97^{a}	28 ± 14^{a}	<LOD^{a}	0.98 ± 0.21^{a}	<LOD	<LOD	<LOD	<LOD^{a}
Kidney															
VBW	<LOD^{a}	5.9 ± 0.8^{a}	0.29 ± 0.05^{a}	4.44 ± 3.74^{b}	<LOQ^{a}	378 ± 75^{a}	<LOD	3.74 ± 1.20^{a}	569 ± 98^{a}	2.49 ± 0.86^{b,c}	5.74 ± 1.18^{b}	<LOD	<LOD	<LOD	<LOD^{a}
VBS	<LOD^{a}	13.2 ± 8.6^{a}	0.19 ± 0.04^{a}	<LOD^{a}	3.3 ± 0.6^{a,b}	405 ± 29^{a}	<LOD	5.57 ± 0.50^{a}	787 ± 155^{a}	3.11 ± 0.51^{c}	5.29 ± 0.67^{b}	<LOD	<LOD	<LOD	<LOD^{a}
SCW	<LOD^{a}	31.6 ± 6.8^{b}	1.39 ± 0.45^{b}	<LOD^{a}	4.5 ± 0.9^{b}	1095 ± 309^{b}	<LOD	5.72 ± 1.38^{a}	1398 ± 559^{b}	1.95 ± 0.53^{b}	5.65 ± 0.60^{b}	<LOD	<LOD	<LOD	<LOQ^{b}
SCS	2.05 ± 0.27^{a}	43.4 ± 11.8^{b}	1.06 ± 0.12^{b}	<LOD^{a}	4.3 ± 0.7^{b}	670 ± 279^{a}	<LOD	9.94 ± 3.29^{b}	381 ± 111^{a}	<LOQ^{a}	3.59 ± 0.13^{a}	<LOD	<LOD	<LOD	<LOQ^{b}
Muscle															
VBW	<LOD	37.4 ± 15.0^{a}	0.07 ± 0.02^{a,b}	4.58 ± 3.31^{a,b}	0.8 ± 0.1^{a}	34 ± 16^{a}	1.87 ± 1.74^{a}	0.71 ± 0.41^{a}	37 ± 5^{a}	1.22 ± 0.52^{a,b}	1.16 ± 0.18^{b}	<LOD	<LOD	0.68 ± 0.34^{a}	1.28 ± 1.20^{a}
VBS	<LOD	15.8 ± 6.2^{a}	0.04 ± 0.01^{a}	2.26 ± 0.89^{a}	0.7 ± 0.1^{a}	20 ± 6^{a}	0.78 ± 0.51^{a}	<LOD^{a}	30 ± 2^{a}	0.67 ± 0.11^{a}	1.18 ± 0.13^{b}	<LOD	<LOD	<LOQ^{a}	<LOQ^{a}
SCW	<LOD	53.2 ± 39.5^{a}	0.12 ± 0.06^{b}	13.21 ± 9.21^{b}	2.7 ± 1.4^{b}	84 ± 38^{b}	6.52 ± 5.11^{a}	<LOD^{a}	235 ± 196^{b}	1.75 ± 0.48^{b}	0.61 ± 0.12^{a}	<LOD	<LOD	<LOQ^{a}	<LOQ^{a}
SCS	<LOD	22.7 ± 10.9^{a}	0.04 ± 0.01^{a}	2.60 ± 0.49^{a}	1.3 ± 0.3^{a}	30 ± 7^{a}	0.84 ± 0.18^{a}	<LOD^{a}	29 ± 20^{a}	0.66 ± 0.43^{a}	0.52 ± 0.03^{a}	<LOD	<LOD	<LOQ^{a}	1.93 ± 1.83^{a}

<LOD (below detection limit); <LOQ (below quantification limit). LOQs: B (2.84 μg g^{-1}); Al, Cr, Ni, Cu, Zn, As, Ag, Cd, Pb (0.46 μg g^{-1}); V (0.03 μg g^{-1}); Mn (0.86 μg g^{-1}); Fe (4.42 μg g^{-1}); Se (1.60 μg g^{-1}) and Hg (0.81 μg g^{-1}). Different letters indicate significant difference between site/seasons for each organ ($p < 0.05$).

Table 2. Activity of superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and level of glutathione (GSH) in gills, liver, kidney and muscle; activity of H^+-ATPase, Na^+/ K^+-ATPase in gills and kidneys; level of metallothionein (MT), lipid peroxidation (LPO) and oxidized protein (OP) in liver and muscle and, activity of acetylcholinesterase (AChe) in muscle of *Centropomus parallelus* (n=5 in each site) from Vitória Bay and Santa Cruz estuaries. Values are mean ± SD. VBW: Vitória bay winter; VBS: Vitória bay summer; SCW: Santa Cruz winter; SCS: Santa Cruz summer; ND: not detected; -: not analyzed. Different letters indicate significant difference between site/seasons for each organ ($p < 0.05$).

	SOD (U mg Pt^{-1})	CAT (µmol g Pt^{-1} min^{-1})	GST (nmol mg Pt^{-1} min^{-1})	GSH (nmol mg Pt^{-1})	H^+-ATPase (µmol Pi mg Pt^{-1} h^{-1})	Na^+/K^+-ATPase (µmol Pi mg Pt^{-1} h^{-1})
Gills						
VBW	16.05 ± 1.64^{a}	11.48 ± 2.28^{a,b}	8,76 ± 2,40^{b}	92.76 ± 28.37^{c}	0.12 ± 0.08^{a}	0.05 ± 0.03^{a,b}
VBS	26.95 ± 7.73^{a}	14.65 ± 2.53^{b}	4,58 ± 0,90^{a}	68.24 ± 22.64^{b,c}	0.12 ± 0.10^{a}	0.05 ± 0.01^{a,b}
SCW	27.46 ± 7.49^{a}	10.57 ± 1.47^{a}	11,69 ± 1,54^{b}	44.03 ± 10.61^{a,b}	0.14 ± 0.06^{a}	0.08 ± 0.03^{b}
SCS	71.61 ± 18.01^{b}	11.48 ± 2.72^{a,b}	30,00 ± 3,32^{c}	33.29 ± 6.74^{a}	0.24 ± 0.08^{a}	0.03 ± 0.01^{a}
Hepatopancreas						
VBW	70.63 ± 5.66^{b}	225.72 ± 21.17^{d}	49.63 ± 4.09^{a}	57.27 ± 25.29^{a}	-	-
VBS	47.41 ± 6.23^{b}	186.78 ± 28.97^{c}	35.18 ± 6.67^{a}	71.70 ± 17.87^{a}	-	-
SCW	22.08 ± 9.44^{a}	31.08 ± 14.52^{a}	141.30 ± 29.92^{b}	100.74 ± 12.20^{a,b}	-	-
SCS	49.37 ± 5.78^{b}	80.48 ± 14.56^{b}	48.27 ± 19.08^{a}	132.67 ± 51.40^{b}	-	-
Kidney						
VBW	1.12 ± 0.59^{a}	12.36 ± 2.99^{b}	33.21 ± 4.47^{c}	30.11 ± 2.20^{a}	1.00 ± 0.07^{c}	1.27 ± 0.04^{c}
VBS	1.07 ± 1.14^{a}	9.79 ± 1.05^{a,b}	18.34 ± 5.57^{b}	31.93 ± 3.56^{a}	0.56 ± 0.36^{a,b}	0.69 ± 0.39^{a,b}
SCW	2.77 ± 1.80^{a}	8.78 ± 1.80^{a}	13.69 ± 4.18^{a,b}	56.60 ± 39.50^{a}	0.84 ± 0.10^{b,c}	0.94 ± 0.09^{b,c}
SCS	1.16 ± 0.74^{a}	10.44 ± 1.90^{a,b}	10.56 ± 1.56^{a}	44.15 ± 7.89^{a}	0.41 ± 0.21^{a}	0.54 ± 0.14^{a}
Muscle						
VBW	5.81 ± 2.22^{a}	0.79 ± 0.07^{a,b}	9.47 ± 2.94^{a}	409.66 ± 94.85^{a,b}	-	-
VBS	9.29 ± 3.95^{a}	0.90 ± 0.22^{b}	5.97 ± 1.95^{a}	814.73 ± 579.10^{b}	-	-
SCW	7.50 ± 3.94^{a}	0.67 ± 0.13^{a}	9.59 ± 2.30^{a}	274.81 ± 124.38^{a}	-	-
SCS	4.68 ± 0.63^{a}	0.66 ± 0.08^{a}	9.68 ± 2.48^{a}	486.85 ± 90.52^{a,b}	-	-

	MT (µmol-SH mg Pt^{-1})	LPO (nmol mg Pt^{-1})	OP (nmol mg Pt^{-1})	AChE (µmol mg Pt^{-1} min^{-1})
Hepatopancreas				
VBW	0.30 ± 0.14^{b}	0.43 ± 0.86^{a}	7.55 ± 0.67^{b}	-
VBS	0.21 ± 0.02^{a,b}	0.18 ± 0.36^{a}	6.92 ± 2.27^{b}	-
SCW	0.11 ± 0.07^{a}	4.15 ± 1.20^{b}	2.90 ± 1.53^{a}	-
SCS	0.14 ± 0.12^{a,b}	2.81 ± 1.14^{b}	3.30 ± 1.00^{a}	-
Muscle				
VBW	0.16 ± 0.01^{b,c}	ND	1.86 ± 0.23^{a,b}	29.84 ± 2.07^{a}
VBS	0.26 ± 0.10^{c}	ND	2.76 ± 0.47^{b,c}	26.92 ± 1.38^{a}
SCW	0.13 ± 0.12^{a,b}	ND	1.81 ± 0.54^{a}	27.95 ± 1.71^{a}
SCS	0.03 ± 0.01^{a}	ND	2.80 ± 0.90^{c}	27.12 ± 2.78^{a}

Table 1S. Pearson values (correlation between metal accumulation and biochemical analyses) of *C. parallelus* gills.

	SOD	GST	CAT	GSH	HA	NKA	B	Al	V	Cr	Mn	Fe	Ni	Cu	Zn	As	Se	Ag	Cd	Hg	Pb
SOD	1.00	0.00	0.97	0.00	0.01	0.07	0.21	0.17	0.96	0.96	0.00	0.04	0.55	0.63	0.59	0.00	0.02	1.00	1.00	0.31	0.08
GST	0.84	1.00	0.20	0.00	0.01	0.09	0.58	0.10	0.81	0.76	0.00	0.03	0.56	0.85	0.90	0.00	0.01	1.00	1.00	0.14	0.02
CAT	-0.01	-0.27	1.00	0.99	0.81	0.46	0.76	0.10	0.27	0.59	0.23	0.96	0.77	0.04	0.44	0.15	0.14	1.00	1.00	0.16	0.32
GSH	-0.62	-0.60	0.00	1.00	0.08	0.93	0.02	0.00	0.27	0.79	0.00	0.34	0.11	0.98	0.87	0.00	0.00	1.00	1.00	0.24	0.04
HAT	0.55	0.53	-0.05	-0.37	1.00	0.87	0.61	0.29	0.24	0.60	0.01	0.13	0.72	0.39	0.91	0.12	0.19	1.00	1.00	0.60	0.18
NKA	-0.38	-0.35	-0.16	0.02	-0.04	1.00	0.14	0.39	0.64	0.38	0.46	0.17	0.19	0.40	0.48	0.89	0.36	1.00	1.00	0.50	0.86
B	-0.27	-0.12	-0.06	0.48	-0.11	-0.31	1.00	0.01	0.43	0.01	0.10	0.19	0.00	0.15	0.56	0.02	0.00	1.00	1.00	0.42	0.04
Al	0.29	0.35	-0.34	-0.59	0.23	0.19	-0.52	1.00	0.00	0.18	0.00	0.51	0.08	0.11	0.63	0.00	0.00	1.00	1.00	0.02	0.02
V	0.01	0.05	-0.24	-0.23	0.25	0.10	-0.17	0.66	1.00	0.05	0.05	0.20	0.08	0.49	0.01	0.96	0.15	1.00	1.00	0.02	0.01
Cr	0.01	0.07	-0.11	-0.06	0.11	0.19	-0.50	0.29	0.40	1.00	0.17	0.00	0.00	0.59	0.10	0.16	0.27	1.00	1.00	0.00	0.00
Mn	0.68	0.83	-0.26	-0.66	0.51	-0.16	-0.35	0.64	0.41	0.29	1.00	0.01	0.11	0.96	0.36	0.00	0.00	1.00	1.00	0.12	0.00
Fe	0.42	0.45	0.01	-0.20	0.32	-0.29	-0.28	0.14	0.27	0.71	0.50	1.00	0.00	0.20	0.12	0.14	0.81	1.00	1.00	0.01	0.00
Ni	0.13	0.13	0.06	-0.33	0.08	0.28	-0.67	0.37	0.37	0.87	0.34	0.61	1.00	0.33	0.29	0.10	0.06	1.00	1.00	0.01	0.00
Cu	0.10	-0.04	0.42	0.01	0.18	-0.18	-0.30	-0.33	-0.15	0.11	-0.01	0.27	0.21	1.00	0.06	0.66	0.54	1.00	1.00	0.15	0.94
Zn	-0.12	-0.03	-0.16	0.03	0.02	0.15	0.12	0.10	0.53	0.34	0.19	0.33	0.22	-0.39	1.00	0.23	0.62	1.00	1.00	0.24	0.01
As	-0.58	-0.71	0.30	0.56	-0.33	0.03	0.46	-0.59	0.01	-0.29	-0.69	-0.31	-0.34	0.09	0.25	1.00	0.00	1.00	1.00	0.03	0.02
Se	-0.49	-0.53	0.31	0.69	-0.27	-0.20	0.70	-0.79	-0.30	-0.24	-0.68	-0.05	-0.39	0.13	0.11	0.73	1.00	1.00	1.00	0.08	0.02
Ag	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00
Cd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00
Hg	0.22	0.31	-0.30	-0.25	0.11	-0.14	-0.17	0.47	0.46	0.62	0.32	0.51	0.50	-0.30	0.25	-0.44	-0.36	0.00	0.00	1.00	0.00
Pb	0.36	0.49	-0.21	-0.42	0.29	-0.04	-0.42	0.47	0.52	0.79	0.72	0.76	0.71	-0.02	0.51	-0.47	-0.48	0.00	0.00	0.70	1.00

Table 2S. Pearson values (correlation between metal accumulation and biochemical analyses) of *C. parallelus* hepatopancreas.

	SOD	GST	CAT	GSH	LPO	MT	OP	B	Al	V	Cr	Mn	Fe	Ni	Cu	Zn	As	Se	Ag	Cd	Hg	Pb
SOD	1.00	0.00	0.00	0.13	0.00	0.01	0.01	0.08	0.00	0.14	0.31	0.01	0.00	1.00	0.50	0.02	0.00	0.00	1.00	1.00	0.62	0.08
GST	-0.73	1.00	0.00	0.67	0.00	0.06	0.01	0.09	0.35	0.59	0.92	0.37	0.05	1.00	0.23	0.80	0.06	0.04	1.00	1.00	0.79	0.34
CAT	0.80	-0.66	1.00	0.01	0.00	0.00	0.00	0.01	0.00	0.14	0.21	0.00	0.00	1.00	0.56	0.00	0.00	0.00	1.00	1.00	0.75	0.25
GSH	-0.32	0.09	-0.55	1.00	0.03	0.36	0.00	0.22	0.14	0.28	0.67	0.01	0.00	1.00	0.54	0.00	0.00	0.00	1.00	1.00	0.33	0.43
LPO	-0.68	0.72	-0.87	0.44	1.00	0.00	0.00	0.01	0.02	0.30	0.23	0.01	0.00	1.00	0.71	0.02	0.00	0.00	1.00	1.00	0.29	0.81
MT	0.52	-0.39	0.60	-0.20	-0.69	1.00	0.00	0.07	0.09	0.36	0.19	0.01	0.00	1.00	0.13	0.01	0.02	0.00	1.00	1.00	0.31	0.96
OP	0.55	-0.51	0.80	-0.58	-0.79	0.64	1.00	0.11	0.08	0.36	0.18	0.01	0.00	1.00	0.78	0.00	0.00	0.00	1.00	1.00	0.62	0.97
B	0.36	-0.36	0.53	-0.26	-0.55	0.38	0.33	1.00	0.59	0.82	0.88	0.32	0.13	1.00	0.38	0.41	0.03	0.05	1.00	1.00	0.61	0.53
Al	0.62	-0.20	0.56	-0.31	-0.46	0.35	0.37	0.12	1.00	0.00	0.92	0.02	0.00	1.00	0.86	0.01	0.01	0.00	1.00	1.00	0.15	0.22
V	0.31	0.12	0.31	-0.23	-0.22	0.20	0.20	0.05	0.59	1.00	0.11	0.00	0.00	1.00	0.42	0.00	0.20	0.02	1.00	1.00	0.33	0.49
Cr	-0.22	0.02	-0.26	0.09	0.25	-0.28	-0.28	-0.03	-0.02	-0.34	1.00	0.03	0.03	1.00	0.41	0.14	0.24	0.07	1.00	1.00	0.30	0.90
Mn	0.55	-0.19	0.65	-0.51	-0.49	0.53	0.52	0.21	0.47	0.64	-0.45	1.00	0.00	1.00	0.15	0.00	0.00	0.00	1.00	1.00	0.83	0.02
Fe	0.70	-0.41	0.85	-0.61	-0.74	0.60	0.69	0.32	0.59	0.57	-0.44	0.75	1.00	1.00	0.81	0.00	0.00	0.00	1.00	1.00	0.93	0.58
Ni	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cu	-0.14	0.25	-0.13	0.13	0.08	0.32	0.06	-0.19	0.04	0.17	-0.17	0.30	-0.05	0.00	1.00	0.06	0.94	0.95	1.00	1.00	0.03	0.46
Zn	0.46	-0.05	0.65	-0.58	-0.49	0.52	0.63	0.18	0.54	0.64	-0.31	0.80	0.78	0.00	0.39	1.00	0.00	0.00	1.00	1.00	0.86	0.18
As	0.66	-0.39	0.81	-0.67	-0.70	0.47	0.74	0.44	0.50	0.27	-0.25	0.58	0.75	0.00	0.02	0.69	1.00	0.00	1.00	1.00	0.61	0.81
Se	0.68	-0.42	0.89	-0.68	-0.79	0.56	0.78	0.40	0.56	0.47	-0.37	0.71	0.92	0.00	0.01	0.79	0.94	1.00	1.00	1.00	0.70	0.63
Ag	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00
Cd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00
Hg	0.11	-0.06	0.07	0.21	-0.22	0.21	0.11	0.11	0.31	0.21	-0.22	0.05	-0.02	0.00	0.44	0.04	0.11	0.08	0.00	0.00	1.00	0.67
Pb	0.36	-0.20	0.25	-0.17	-0.05	0.01	0.01	-0.13	0.26	0.15	-0.03	0.48	0.12	0.00	0.16	0.28	0.05	0.10	0.00	0.00	-0.09	1.00

Table 3S. Pearson values (correlation between metal accumulation and biochemical analyses) of *C. parallelus* kidneys.

	B	Al	V	Cr	Mn	Fe	Ni	Cu	Zn	As	Se	Ag	Cd	Hg	Pb	SOD	GST	CAT	GSH	HAT	NKA
B	1.00	0.57	0.00	0.07	0.00	0.24	0.34	0.00	0.62	0.00	0.11	1.00	1.00	0.43	0.16	0.03	0.01	0.44	0.00	0.04	0.02
Al	-0.12	1.00	0.63	0.00	0.18	0.54	0.00	0.28	0.31	0.23	0.16	1.00	1.00	0.00	0.38	0.66	0.11	0.43	0.67	0.33	0.11
V	0.65	-0.10	1.00	0.10	0.00	0.00	0.38	0.09	0.33	0.00	0.43	1.00	1.00	0.57	0.00	0.00	0.00	0.12	0.00	0.67	0.37
Cr	-0.37	0.90	-0.35	1.00	0.02	0.12	0.00	0.02	0.24	0.78	0.95	1.00	1.00	0.01	0.07	0.42	0.00	0.10	0.26	0.04	0.00
Mn	0.62	-0.28	0.79	-0.49	1.00	0.00	0.07	0.01	0.44	0.06	0.49	1.00	1.00	0.54	0.00	0.01	0.00	0.07	0.00	0.25	0.10
Fe	0.25	-0.13	0.81	-0.33	0.68	1.00	0.50	0.90	0.07	0.18	0.83	1.00	1.00	0.54	0.00	0.03	0.01	0.02	0.10	0.99	0.79
Ni	-0.20	0.88	-0.19	0.80	-0.37	-0.14	1.00	0.10	0.41	0.56	0.33	1.00	1.00	0.02	0.29	0.77	0.21	0.97	0.52	0.37	0.13
Cu	0.65	-0.23	0.35	-0.46	0.51	0.03	-0.34	1.00	0.47	0.00	0.00	1.00	1.00	0.98	0.56	0.80	0.00	0.84	0.26	0.00	0.00
Zn	-0.11	-0.22	0.21	-0.25	0.16	0.38	-0.18	-0.15	1.00	0.20	0.03	1.00	1.00	0.06	0.03	0.21	0.39	0.10	0.50	0.22	0.51
As	-0.69	-0.25	-0.60	0.06	-0.40	-0.28	-0.13	-0.63	0.27	1.00	0.00	1.00	1.00	0.19	0.09	0.55	0.02	0.90	0.08	0.11	0.10
Se	-0.34	-0.29	-0.17	0.01	-0.15	-0.05	-0.21	-0.59	0.43	0.74	1.00	1.00	1.00	0.54	0.82	0.19	0.02	0.89	0.49	0.00	0.01
Ag	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Hg	-0.17	0.60	0.12	0.52	-0.13	0.13	0.48	0.01	0.39	-0.28	-0.13	0.00	0.00	1.00	0.33	1.00	0.43	0.63	0.54	0.09	0.08
Pb	0.30	-0.19	0.89	-0.37	0.69	0.93	-0.22	0.12	0.46	-0.35	-0.05	0.00	0.00	0.21	1.00	0.05	0.01	0.04	0.07	0.84	0.78
SOD	0.45	-0.09	0.56	-0.17	0.51	0.43	-0.06	0.06	0.26	-0.13	0.28	0.00	0.00	0.00	0.41	1.00	0.18	0.09	0.00	0.95	0.72
GST	-0.54	0.33	-0.58	0.67	-0.58	-0.49	0.27	-0.57	-0.18	0.49	0.48	0.00	0.00	0.17	-0.55	-0.28	1.00	0.00	0.17	0.00	0.00
CAT	-0.16	0.17	-0.33	0.34	-0.37	-0.46	0.01	-0.04	-0.34	0.03	0.03	0.00	0.00	0.10	-0.42	-0.35	0.61	1.00	0.16	0.43	0.37
GSH	0.76	-0.09	0.65	-0.24	0.59	0.34	-0.14	0.24	0.14	-0.36	0.15	0.00	0.00	-0.13	0.38	0.69	-0.29	-0.30	1.00	0.88	0.63
HAT	-0.42	0.21	-0.09	0.42	-0.25	0.00	0.19	-0.59	0.26	0.33	0.56	0.00	0.00	0.36	0.04	-0.01	0.65	0.17	-0.03	1.00	0.00
NKA	-0.48	0.34	-0.19	0.57	-0.34	-0.06	0.32	-0.64	0.14	0.35	0.53	0.00	0.00	0.37	-0.06	-0.08	0.75	0.19	-0.10	0.96	1.00

Table 4S. Pearson values (correlation between metal and biochemical analyses) of *C. parallelus* muscle.

	SOD	GST	CAT	GSH	MT	OP	ACH	B	Al	V	Cr	Mn	Fe	Ni	Cu	Zn	As	Se	Ag	Cd	Hg	Pb
SOD	1.00	0.02	0.28	0.03	0.02	0.78	0.65	0.26	0.77	0.57	0.45	0.37	0.48	0.44	0.75	0.13	0.54	0.17	1.00	1.00	0.21	0.43
GST	-0.46	1.00	0.36	0.01	0.04	0.17	0.91	0.72	0.21	0.37	0.41	0.61	0.21	0.42	0.60	0.85	0.37	0.04	1.00	1.00	0.02	0.50
CAT	0.23	-0.19	1.00	0.55	0.03	0.55	0.75	0.76	0.35	0.44	0.50	0.02	0.29	0.56	0.18	0.10	0.49	0.03	1.00	1.00	0.27	0.54
GSH	0.44	-0.54	0.13	1.00	0.04	0.07	0.51	0.93	0.07	0.06	0.06	0.06	0.03	0.06	0.62	0.11	0.05	0.07	1.00	1.00	0.08	0.58
MT	0.49	-0.43	0.43	0.43	1.00	0.99	0.56	0.70	0.21	0.90	0.93	0.31	0.60	0.88	0.00	0.84	0.83	0.00	1.00	1.00	0.29	0.13
OP	0.06	-0.29	0.13	0.38	0.00	1.00	0.22	0.31	0.14	0.16	0.19	0.07	0.14	0.20	0.80	0.05	0.06	0.95	1.00	1.00	0.39	0.75
ACH	0.10	0.02	-0.07	-0.14	0.13	-0.26	1.00	0.72	0.82	0.88	0.98	0.47	0.87	0.92	0.58	0.25	0.42	0.20	1.00	1.00	0.03	0.35
B	-0.24	0.08	0.07	-0.02	0.08	-0.22	0.08	1.00	0.64	0.79	0.54	0.46	0.48	0.54	0.91	0.59	0.77	0.28	1.00	1.00	0.46	0.35
Al	-0.06	0.26	-0.20	-0.37	-0.26	-0.31	0.05	-0.10	1.00	0.00	0.01	0.00	0.00	0.01	0.39	0.02	0.13	0.48	1.00	1.00	0.02	0.80
V	-0.12	0.19	-0.17	-0.39	-0.03	-0.30	0.03	-0.06	0.76	1.00	0.00	0.00	0.00	0.00	0.58	0.00	0.00	0.22	1.00	1.00	0.20	0.71
Cr	-0.16	0.18	-0.15	-0.39	0.02	-0.28	-0.01	-0.13	0.55	0.94	1.00	0.01	0.00	0.00	0.67	0.01	0.00	0.08	1.00	1.00	0.68	0.29
Mn	0.19	0.11	-0.46	-0.39	-0.22	-0.38	0.16	-0.16	0.58	0.59	0.52	1.00	0.00	0.01	0.25	0.00	0.01	0.01	1.00	1.00	0.43	0.84
Fe	-0.15	0.26	-0.23	-0.45	-0.11	-0.31	-0.03	-0.15	0.70	0.97	0.97	0.64	1.00	0.00	0.43	0.00	0.00	0.03	1.00	1.00	0.38	0.56
Ni	-0.17	0.17	-0.13	-0.39	0.03	-0.27	-0.02	-0.13	0.53	0.94	1.00	0.52	0.96	1.00	0.71	0.01	0.00	0.09	1.00	1.00	0.73	0.30
Cu	0.07	-0.11	0.28	0.11	0.60	-0.05	0.12	-0.02	-0.18	-0.12	-0.09	-0.24	-0.17	-0.08	1.00	0.61	0.53	0.01	1.00	1.00	0.84	0.56
Zn	0.32	-0.04	-0.34	-0.33	0.04	-0.40	0.24	-0.12	0.48	0.56	0.52	0.94	0.59	0.52	-0.11	1.00	0.00	0.12	1.00	1.00	0.53	0.51
As	0.13	0.19	-0.15	-0.40	0.05	-0.40	0.17	-0.06	0.32	0.57	0.64	0.51	0.61	0.64	-0.14	0.58	1.00	0.20	1.00	1.00	0.72	0.22
Se	0.29	-0.41	0.45	0.38	0.60	-0.01	0.27	0.23	-0.15	-0.26	-0.36	-0.52	-0.44	-0.36	0.51	-0.33	-0.27	1.00	1.00	1.00	0.47	0.37
Ag	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00
Cd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00
Hg	-0.27	0.47	-0.23	-0.37	-0.23	-0.18	0.44	0.16	0.46	0.27	0.09	0.17	0.19	0.07	-0.04	0.13	0.08	0.15	0.00	0.00	1.00	0.52
Pb	-0.17	0.14	-0.13	-0.12	-0.32	0.07	-0.20	0.20	0.06	-0.08	-0.22	-0.04	-0.12	-0.22	-0.13	-0.14	-0.26	-0.19	0.00	0.00	0.14	1.00

CAPÍTULO 8 – CONSIDERAÇÕES FINAIS E PERSPECTIVAS FUTURAS

Na costa sudeste do Brasil, o ambiente tem sido impactado negativamente pela metalurgia, incluindo complexos de mineração, siderurgia e indústrias de pelotização. Este estudo estabeleceu a fonte de poluição e a sua provável via de poluição e transporte na teia alimentar de dois ecossistemas estuarinos de manguezal: Baía de Vitória e Santa Cruz, localizadas no Estado do Espírito Santo.

Metais e isótopos estáveis ($\delta^{13}C$, $\delta^{15}N$, $^{87}Sr/^{86}Sr$ e assinatura de Pb) analisados em água, sedimento, árvores do manguezal (*Rhizophora mangle*, *Laguncularia racemosa*, *Avicennia schaueriana*), plâncton, camarão (*Macrobranchium sp.*), caranguejo (*Aratus sp.*), ostra (*Crassostrea rhizophorae*) e peixe (*Centropomus parallelus*) da Baía Victoria e Santa Cruz, bem como metais em água, sedimentos e material particulado atmosférico oriundos do Complexo de Tubarão foram identificados, constando-se a diferença entre Santa Cruz e Baía de Vitória, causada pela contaminação de fertilizantes; a influência quase única da água marinha neste ecossistema estuarino; a identificação de três cadeias tróficas neste ecossistema: 1 plâncton-camarão-peixe, 2) Plantas-caranguejo-peixe e, 3) plâncton-ostra; e por último a influência do material particulado atmosférico, com caraterística associada a atividade metalo-siderúrgica em todas as amostras bióticas analisadas.

A análise de contaminantes metálicos na biota (plâncton, ostra, caranguejo, camarão e peixe) e no material particulado atmosférico constatou a presença de contaminantes metálicos emergentes, ainda não avaliados em programas de monitoramentos e sem limites pré-estabelecidos na legislação, como o bismuto, tungstênio, titânio, zircônio, ítrio, lantânio, nióbio, tântalo e cério que são utilizados em

material e também fazem parte da cadeia alimentar. Desta forma estudos futuros deverão focar em avaliar a internalização de NP metálicas de contaminantes emergentes presentes no material particulado atmosférico em células de pulmão humano, utilizando metodologias inovadoras, como a nano difração para determinação de estruturas cristalográficas de nano partículas em células humanas, para a avaliação de biodisponibilidade a nível subcelular; estudo necessário para a criação de normas e regimentos governamentais, de segurança para o trabalhador e sociedade exposta a estes contaminantes.

9. REFERÊNCIAS

AGER, L.; HAMMOND, D. E.; E WARE, F.. Artificial spawning of snook. Florida Game and Fresh Water Fish Commission. **Proceedings of the Thirtieth annual conference southeastern association of fish and wildlife agencies**. Jackson, Mississippi: 9 p. 1976.

AKAISHI, F.M., SILVA DE ASSIS, H.C., JAKOBI, S.C.G., STJEAN, S., COUTERNAY, S.C., LIMA, E., WAGNER, A.L.R., SCOFIELD, A., OLIVEIRA RIBEIRO, C.A. Morphological and neurotoxicological findings in tropical freshwater fish (Astyanax sp.) after waterborne and acute exposure to water soluble fraction (WSF) of crude oil. Arch**. Environ. Contam. Toxicol**. v. 46, p. 244–253, 2004.

ALLEN, H.E.; FU, G. & DENG, B. 1993. Analysis of acid – volatile sulfide (AVS) and simultaneously extracted metals (SEM) for the estimation of potential toxicity in aquatic sediments. **Environ. Toxicol. Chem**., 12: 1441 – 1453.

ARAÚJO,L.F.P., SILVA, F.J.A., ALMEIDA, M.M.M. 2000. Caracterização e avaliação sanitária das águas do Rio Maranguapinho. *In*: IX SILUBESA-Simpósio Luso-Brasileiro de Engenharia Sanitária e Ambiental. ABES. Porto Seguro p 21-26.

AUSTIN H.; E AUSTIN S. The feeding habitats of some juvenile marine fishes from the mangroves in western Puerto Rico. **Caribean Journal of Science**. v. 11, n.3, p.171-178, 1971.

AYYLON F AND GARCIA-VAZQUEZ G (2000) Induction of micronuclei and other nuclear abnormalities in European minnow Phoxinus phoxinus and mollie Paecilia latipinna:An assessment of the fish micronucleus test. **Mutat Res** 467:177-186.

BARROSO, G. F. 2004. Development of an evaluation framework for sustainable bivalve aquaculture: a strategic plan approach in Espirito Santo, Brazil: Unpublished PhD Thesis (Geography), University of Victoria, Canada, 229 pp.

BEVILACQUA, J. E. 1996. Estudo sobre a estabilidade de amostras de sedimento do rio Tietê, SP. (Tese de doutorado). IQ, USP, São Paulo, 171 p.

BOMBAIL V, GORDON E AND BATTY J (2001) Application of the comet and micronucleus assays to butterfish (Pholis gunnelus) erythrocytes from the Firth of Forth, Scotland. **Chemosphere** 44:283-392.

BORGES-OSÓRIO, M. R., ROBINSON, W. M. Genética Humana. Porto Alegre: Artmed, 2001.

BÖTTCHER, M.; HESPENHEIDE, B. & LLOBET-BROSSA, E. The biogeochemistry, stable isotope geochemistry, and microbial community structure of a temperate intertidal mudflat: an integrated study. **Cont. Shelf Res**., 20:1749-1769, 2000.

BOWEN, J.J.M. 1979. **Environmental chemistry of the elements**. London. Academic Press. 333p.

BRADFORD, M.N. 1976. A rapid amd sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, 72 248-254.

BUCHELI, T. B., FENT, K. (1995), Induction of cytochrome P450 as a biomarker for environmental contamination in aquatic ecosystems. **Crit. Rev. Environm. Sci. Technol**., 25, 201-268.

CARRASCO KR, TILBURY KL AND MAYERS MS (1990) Assessment of the piscine micronuclei test as an in situ biological indicator of chemical contaminants effects. **Can J Fish Aquat Sci** 47:2123-2136.

CARREIRA, R.; WAGENER, A.L.R.; FILEMAN, T. & READMAN, J. 2001. Distribuição de coprostanol (5ß(H)-COLESTAN-3ß-OL) em sedimentos superficiais da Baía de Guanabara: indicador da poluição recente por esgotos domésticos. **Química Nova** 24:37-42.

CARTER, M. R.; BURNS, L. A.; CAVINDER, T. R.; DUGGER, K. R.; FORE, P. L.; HICKS, D. B.; REVELLS, H. L.; SCHMIDT, T. W.; FARLEY, R. Ecossystems analysis of the big cypress swamp and estuaries. **United States Environmental Protection Agency**. South Florida Ecological Study. Florida: 20 p.,1973.

CARVAJAL, J. R. Contribucion al conocimiento de la biología de los robalos *Centropomus undecimalis* y C. Poeyi en la laguna de terminos, Campeche, México. Instituto Oceanográfico, Universidad de Oriente, Cumaná, Venezuela: **Boletín del Instituto Oceanografica**, v.14, n.1, p. 51-70, 1975.

CERQUEIRA, C.C.C., & FERNANDES, M.N. 2002 Gill tissue recovery after copper exposure and blood parameter responses in the tropical fish, Prochilodus scrofa. **Ecotoxicol. Environ. Safety**, 52: 83-91

CHAPMAN, P. M.; M. D. PAINE; A.D. ARTHUR; L.A. TAYLOR. 1996. A Triad study of sediment quality associated with a major, relatively untreated marine sewage discharge. **Marine pollution Bulletin**, v.32, n.1, p.47-64.

CHÁVES, H. Estudio de una nueva especie de robalo del Golfo de México y redescripción de *Centropomus undecimalis* (BLOCH). **Contribución de la Estación de Biología Marina del Instituto Tecnológico de Veracruz. México: Ciencia**, v.21, n.1, p. 75-83. 1961.

CHÁVES, H. Contribucion al conocimiento de la biología de los robalos, chucumite y constantino (*Centropomus spp*.) del estado de Veracruz. **Contribución de la Estación de Biología Marina del Instituto Tecnológico de Veracruz. México: Ciencia**, v.22, n.3, p. 141-161. 1963.

CLARCK, R.B. **Marine Pollution**. Clarendon Press. Oxford. 1997

COIMBRA, A. G. 2003. Distribuição de metais pesados em moluscos e sedimentos nos manguezais de Coroa Grande e das Enseadas das Garças, Baía de Sepetiba, RJ. 2003. 72 f. Dissertação Mestrado em Geociências - Universidade Federal Fluminense. Niterói.

CONSELHO NACIONAL DO MEIO AMBIENTE - CONAMA. 2005. Resolução Conama nº 357. www.mma.conama.gov.br/conama (acesso em 08/11/2010).

COSTA, R.M.A.; MENK, C.F.M. Biomonitoramento de mutagênese ambiental. **Biotecnologia: ciência e desenvolvimento**. v.3, n.12, p.24-26, 2000.

COUILLARD, C. M., WILLLIAMS, P. J., COURTENAY, S. C., RAWN, G. P. Histopatological evaluation of Atlantic Tomcod (*Microgadus tomcod*) collected at estuarine sites receiving pulp and paper mill effluent. **Aquatic Toxicology**. v. 44, p. 263-278. 1999

DANG, Z. R. A. C., LOCK, G. FLIK AND S. E. WENDELAAR BONGA. Na+/K+ - ATPase Immunoreactivity in branchial chloride cells of Oreochromis mossambicusn exposed to copper. **J. of Exp. Biol**. 203: 379-387, 2000.

DE ZWART, D., 1995. Monitoring water quality in the future, Volume 3:Biomonitoring. National Institute of Public Health and Environmental Protection (RIVM), Bilthoven, The Netherlands.

DEPLEDGE, M.H., FOSSI, M.C., 1994. The role of biomarkers in environmental assessment (2). **Ecotoxicology** 3, 161_/172.

ECETOC, 1993. Environmental hazard assessment of substances. European Centre for Ecotoxicology and Toxicology of Chemicals: Technical Report No. 51. Brussels, Belgium.

EVANS, D. H., PIERMARINI, P. M., CHOE, K. P. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid – base regulation, and excretion of nitrogenous waste. **Physiol. Ver**. 85: 97 - 177, 2005.

CHASIN, A.A.M. & AZEVEDO, F.A. Intoxicação e avaliação da toxicidade. In: Azevedo, F.A. & Chasin, A.A.M. (Org.). As bases toxicológicas da ecotoxicologia. 2003. p. 127-65.

FENECH, M. 2000. The in vitro micronucleus technique. Mutation Research. nº 455. p.81-95.

FERNANDES, M. N., MAZON, A. F. Environmental pollution and fish gill morphology. In: Val, A. L., Kapoor, B. G. (Eds.). **Fish Adaptation**., 418 p., 2003.

FORE, P. L.; E SCHMIDT, T. W. Biology of juvenil and adult snook, *Centropomus undecimalis*, in the Ten Thousand Islands. Ecosystems analysis of the Big Cypress Swamp and estuaries. U.S. Environmental Protection AGENCY Region IV, Atlanta, Georgia Publication Number EPA 904/9-74-002. 18 p. 1973.

FORSTNER, U. G.; WITTMANN, G. T. W. 1981. Metal Pollution in the Aquatic Environmental, Springer-Verlag: Berlin.

GILMORE, R.G., DONOHOE, J., COOKE, D.W., 1983. Observations on the distribuition and biology of east-central Florida populations of the common snook, Centropomus undecimalis Bloch. **Florida Sci**. 46 (3), 313–336.

GRENWOOD, P. H. 1976 A review of the family Centropomidae (Pisces,Perciformes). Bulletin British Museum of Natural History Zoology. 29 (1): p. 1-81. (1976 apud RIVAS, 1986, p.579)

GÜL, S., BELGE-KURUTAS, E., YILDIZ, E., SAHAN, A., DORAN, F. Pollution correlated modifications of liver antioxidant systems and histopathology of fish (Cyprinidae) living in Seyhan Dam Lake. **Turkey Environment International**. v. 30, n. 5, p. 605-609. 2004.

HARBINSON, P. 1986. Mangrove muds-a sink and a source for trace metals. Marine Polution Bulletin, 17 (6), p. 246-250, Great Britain.

HINTON, D.E., 1994. Cells, cellular responses, and their markers in chronic toxicity of fishes. In: Malins, D.C., Ostrander, G.K. (Eds.), Aquatic Toxicology; Molecular, Biochemical and Cellular Perspectives. Lewis Publishers CRC press, pp. 207_/240.

HINTON, D.E., BAUMANN, P.C., GARDNER, G.C., HAWKINS, W.E., HENDRICKS, J.D., MURCHELANO, R.A., OKIHIRO, M.S., 1992. Histopathologic biomarkers. In: Huggett, R.J., Kimerly, R.A., Mehrle, P.M., Jr, Bergman, H.L. (Eds.), Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress. Lewis Publishers, Chelsea, MI, USA, pp. 155_/210.

JESUS, HONÉRIO COUTINHO DE . Distribuição de metais pesados em sedimentos do sistema estuarino da Ilha de Vitória-ES. **Quím. Nova**, São Paulo, v. 27, n. 3, June 2004 .

JOHNSON, L.L., CASILLAS, E., SOL, S., COLLIER, T., STEIN, J., VARANASI, U. Contaminant effects on reproductive success in selected benthic fish. **Mar. Environ. Res.**, 35, 165–170, 1993.

KING, M. Fisheries biology assessment and management. Fishing New Books. Osney Mead, Oxford, England. 321p. 1995.

KRUGER, N. J. The Bradford method for protein quantification. In: Methods in Molecular Biology. V- 32. Basic Protein and Peptide Protocols. University of Hertfordshire, Hatfiel, UK, Humana Press, New Jersey,1994

LACERDA, LD.; SANTOS, JA.; LOPES, DV.. Fate of copper in intensive shrimp farms: bioaccumulation and deposition in pond sediments. **Braz. J. Biol**., São Carlos, v. 69, n. 3, Aug. 2009 .

LYONS, B. P., STENTIFORD, G. D., GREEN, M., BIGNELL, J., BATEMAN, K., FEIST, S. W., GOODSIR,F., REYNOLDS, W. J., THAIN, J. E. DNA adduct analysis and histopathological biomarkers in european flounder (*Platichthys flesus*) sampled from UK estuaries. **Mutation Research**. v. 552, p. 177-186. 2004.

MARSHALL, A. R. A survey of the snook fishery of Florida with Studies of the Biology of the principal species *Centropomus undecimalis* (Bloch). State Board of Conservation, Techinical series. The Marine Laboratory. University of Miami. Florida. n. 22, 37 p., 1958.

MARTIN, J. H.; E KING T. T. Occurrence of fat snook (*Centropomus parallelus*) in Texas: evidence for a range extension. **Contributions in Marine Science**. v. 32, p. 123-126, 1991.

MATSUMOTO, S. T. ; FONSECA, I. ; MARIN-MORALES, ; MANTOVANI, M. S. ; MALAGUTTI, M. I. ; DIAS, A. L. . Genotoxicity and mutagenicity of water contaminated with tannery effluents, as evaluated by the micronucleus test and comet assay using the fish Oreochromis niloticus and chromosome aberrations in onion root-tips. **Genetics and molecular biology**, v. 29, n. 1, p. 148-158, 2006.

MAZON, A. F., MONTEIRO, E. A. S., PINHEIRO, G. H. D., FERNANDES, M. N. Hematological and physiological changes induced by short – term exposure to copper in the freshwater fish, Prochilodus scrofa. **Braz. J. Biol**. 62: 1 – 7, 2002.

MCCARTHY, J.F., SHUGART, L.R., 1990. Biological markers of environmental contamination. In: McCarthy, J.F., Shugart, L.R. (Eds.), Biomarkers of Environmental Contamination. Lewis Publishers, Boca Raton, FL, USA, pp. 3_/16.

MCCARTHY, J.F., HALBROOK, R.S., SHUGART, L.R., 1991. Conceptual strategy for design, implementation, and validation of a biomarkerbased biomonitoring capability. Publication no. 3072, ORNL/TM- 11783. Environmental Sciences Division, Oak Ridge National aboratory, Tennessee, USA.

MCMICHAEL, R. H., JR.; PETERS, K. M.; PARSONS, G. R.. Early life history of the snook, *Centropomus undecimalis* in Tampa Bay, Florida. Northeast Gulf Science. v. 10, n. 2, p.113–125, 1989.

MEZZARI, G. M.; ANTONELLI, P. Análise de atividade mutagênica. Criciúma, SC: UNESC, 2005. 20 f.

MULLER, R. G. The 2000 stock assessment update of common snook, *Centropomus undecimalis*. Fish and Wildlife Conservation Commission. Florida Marine Research Institute. St. Petersburg, Florida, 22 p. 2000.

NELSON, D.W. & SOMMERS, L.E. Total carbon, organic carbon and organic matter. In: PAGE, A.L.; MILLER, R.H. & KEENEY, D.R., eds. Methods of soil analysis: Chemical and microbiological properties. Part 2. Madison, Soil Science Society of America, 1982. p.539-579.

NOREÑA-BARROSO, E., SIMÁ-ÁLVAREZ, R., GOLD – BOUCHOT, G. E ZAPATA-PÉREZ, O. Persistent organic pollutants and histological lesions in Mayan catfish *Ariopsis assimilis* from the the Estuary of Chetumal, Mexico. **Marine Pollution Bulletin**. v. 48, p. 263- 269. 2004.

OLIVEIRA RIBEIRO, C.A., BELGER, L., PELLETIER, É., ROULEAU, C. Histopathological evidence of inorganic mercury and methyl mercury toxicity in the arctic charr (Salvelinus alpinus). USA: Environ. Res., 90, 217 – 225, 2002b.

OLIVEIRA RIBEIRO, C.A., SCHATZMANN, M., SILVA DE ASSIS, H.C., SILVA, P.H., PELLETIER, E., AKAISHI, F.M.. Evaluation of tributyltin subchronic effects in 37 tropical freshwater fish (Astyanax bimaculatus, Linnaeus, 1758). **Ecotoxicol. Environ. Saf**., 51, 161–167, 2002a.

OLIVEIRA RIBEIRO, C.A., VOLLAIRE, Y., SANCHEZ-CHARDI, A., ROCHE, H.. Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the Eel (Anguilla anguilla) at the Camargue Nature Reserve, France. **Aquat. Toxicol**., 74, 53–69, 2005.

ORGANIZAÇÃO MUNDIAL DE SAÚDE (1993). The health of young people: A challenge and a promise. Geneva: WHO.

ONOFRE, C.R.E, CELINO, J.J., NANO, R.M.W., QUEIROZ, A.F.S. 2007. Biodisponibilidade de metais traços nos sedimentos de manguezais da porção norte da Baía de Todos os Santos, Bahia, Brasil. **Revista de Biologia e Ciências da Terra** 7:65-82.

PACHECCO, M E SANTOS MA Induction of liver EIROD and erythrocytic nuclear abnormalities by cyclophosphamide and PAHs in Anguilla anguilla L. **Ecotoxicol Environ Saf** 40:71-76, 1998.

PEAKALL, D.W., 1994. Biomarkers: the way forward in environmental assessment. **Toxicol. Ecotoxicol.** News 1, 55_/60.

PEAKALL, D.W., WALKER, C.H., 1994. The role of biomarkers in environmental assessment (3). Ecotoxicology 3, 173_/179.

PEREIRA, C. D. S. ; ABESSA, DENIS MOLEDO DE SOUZA ; ZARONI, LETICIA PIRES ; GASPARRO, MARCIA REGINA ; BAINY, A. C. D. ; BICEGO, M. C. ; TANIGUCHI, S. ; FURLEY, T. H. ; SOUZA, EDUINETTY CECI PEREIRA MOREIRA DE . Integrated assessment of multi-level biomarker responses and chemical analysis in mussels from São Sebastião - Brazil. **Environmental Toxicology and Chemistry**, USA, v. 26, p. 462-469, 2007.

PÉREZ-PINZÓN, M. A.; LUTZ, P. L. Activity related cost of osmoregulation in the juvenile snook (*Centropomus undecimalis*). Florida: **Bulletin of Marine Science**. v.48, n.1, p. 58-66, 1991.

PETERSON, M. S.; GILMORE, R. G. JR. Eco-physiology of juvenile snook *Centropomus undecimalis* (Bloc): life-history implications. **Bulletin of Marine Science**. v. 48, n.1, p. 46–57, 1991.

PHILLIPS, D.J.H. 1977. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments – a review. ***Environ. Pollut***., 13, 281-317.

PHILLIPS, D. J. (1991). selected trace elements and the use of biomonitors in subtropical and tropical marine ecosystems**. *Environ. Contam. Toxicol*.,** 120, 105-129.

QUABIUS, E.S.; BALM, P.H.M.; WENDELAAR BONGA, S.E. Interrenal stress responsiveness of tilapia (Oreochromis mossambicus) is impaired by dietary exposure to PCB 126. **Gen. Comp. Endocrinol**., v. 108, p. 472-482, 1997.

VAN DER OOST, R.; BEYER, J.; VERMEULEN, N.P.E. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. **Environmental Toxicology and Pharmacology**. v. 13, p. 57-149. 2003.

RIBEIRO, L.R.; SALVADORI, D.M.F.; MARQUES, E.K. Mutagênese Ambiental. Canoas-RS. Editora da ULBRA. 2003. 356p.

RIVAS, L. R. The Florida fishes of the genus *Centropomus*, commonly known as snook. Quarterly Journal of the Florida Academy of Science. Ichthyological Laboratory and Museum, Departament of Zoology, University of Miami. Contribution n. 42. Florida: **Florida Academy of Sciences**, v. 25, n.1, p. 53-64, 1962.

RIVAS, L. R. Sistematic Review of the Perciform fishes of the genus *Centropomus*. American Society of Ichthyologist and Herpetologist. Copeia, v.3, p. 579-611, 1986.

RODRIGUES, P.P. 2005 Aspectos reprodutivos do robalo peva, *Centropomus parallelus*, na foz do Rio Doce, Linhares-ES. Vitória. 51p. (Monografia apresentada ao Curso de Graduação em Oceanografia da Universidade Federal do Espírito Santo).

RUSSELL, R.W., GOBAS, F.A.P.C., HAFFNER, G.D., 1999. Role of chemical and ecological factors in trophic transfer of organic chemicals in aquatic food webs. **Environ. Toxicol. Chem.** 18, 1250_/1257.

SALOMONS, W.; FÖRSTNER, U. (1984) Metals in the Hydrocycle, Ed. Springer-Verlag, Berlin.

SCHMID, W. 1976. The micronucleus test for cytogenetics analysis. *In:* Principles and Methods for Their Detection (ed. Hollaender,A.). Plenum Press, New York, Vol.4, p.31-53.

SCHMALZ, W.F., HERNANDEZ, A.D., WEIS, P. Hepatic histopathology in two populations of the mummichog, Fundulus heteroclitus. USA: **Mar. Environ. Res**., 54, 539–542, 2002.

SCHAEFFER-NOVELLI, Y. 1995. Manguezal: ecossistema entre a terra e o mar. São Paulo: Caribbean Ecological Research, p 64.

SHAFLAND, P. L.; E K. J. FOOTE. A lower lethal temperature for fingerling snook, *Centropomus undecimalis*. Florida Game and Fresh Water Fish Commission. Florida. **Northeast Gulf Science**. v.6. n.2, p.175–178. 1983.

SHUGART, L.R., 1996. Molecoular markers to toxic agents. In: Newman, M.C., Jagoe, C.H. (Eds.), Ecotoxicology: a Hierarchial Treatment. CRC Press, Boca Raton, USA, pp. 133_/161.

SHUGART, L.R., BICKHAM, J., JACKIM, G., MCMAHON, G., RIDLEY, W., STEIN, J., STEINERT, S., 1992. DNA alterations. In: Huggett, R.J., Kimerly, R.A., Mehrle, P.M., Jr, Bergman, H.L. (Eds.), Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress. Lewis Publishers, Chelsea, MI, USA, pp. 155_/210.

SILVERIO, P.F. 2003. Bases Técnico-cientificas para derivação de valores - guias de qualidade de sedimentos para metais: experimentos de campo e laboratório. Tese de Doutorado-Escola de engenharia São Carlos, Universidade de São Paulo, São Carlos.

SUTER, G.W., II, 1990. Use of biomarkers in ecological risk assessment. In: McCarthy, J.F., Shugart, L.R. (Eds.), Biomarkers of Environmental Contamination. Lewis Publishers, Boca Raton, FL, USA, pp. 419_/428.

TABACHINICK, B. G. & FIDELL, L. S. *Using multivariate statistics*. New York: HarperCollins, 1996.

TAYLOR, R. G.; WHITTINGTON J. A.; GRIER H. J.; E CRABTREE R. E. Age, growth, maturation, and protandric sex reversal in the common snook, *Centropomus undecimalis*, from South Florida waters. **Fishery Bulletin**. v. 98, n.3, p. 612–624, 2000.

TEIXEIRA, R. L. Distribution and feeding habits of the young common snook,*Centropomus undecimalis* (Pisces: Centropomidae), in the shallow waters of a tropical brazilian estuary. Boletim do Museu de Biologia Mello Leitão, n. 6, p. 35-46, 1997.

TUCKER, J. W. JR.; CAMPBELL, S. W. Spawning season of common snook along the east central Florida coast. Quarterly Journal of the Florida Academy. Biological Sciences. Florida Scientist, v. 51. n.1, p.1-6, 1988.

USEPA (200.8). Determination of trace elements in waters and wastes by inductively coupled plasma - mass spectrometry. United States Environmental Protection Agency.

VALE, C.C. & FERREIRA, R.D. 1998. Os manguezais do litoral do Estado do Espírito Santo. Pp. 88-94. In: Anais do Simpósio de Ecossistemas da Costa Brasileira. São Paulo, ACIESP, v. I.

VAN GESTEL, C.A.M., VAN BRUMMELEN, T.C., 1996. Incorporation of the biomarker concept in ecotoxicology calls for a redefinition of terms. Ecotoxicology 5, 217_/225.

VARJABEDIAN, E. Impactos sobre os manguezais, In: Yara Schaeffer-Novelli (Org.). Manguezal: Ecossistema entre a terra e o mar, São Paulo: **Caribbean Ecological Research**, 1995, cap. 11, p. 49-52.

VANNUCCI, M. Os manguezais e nós, São Paulo: Edusp, 233 p. 1999.

VAZZOLER, A. E. A. M. Biologia da reprodução de peixes teleósteos: teoria e prática. Eduem, Nupelia.1995.

VETHAAK AD, RHEINALLT T. (1992). Fish diseases as a monitor for marine pollution: The case of the North Sea. Rev. **Fish Biology an Fisheries**. **2**: 1–32.

VETHAAK, A.D., AP RHEINALLT, T., 1992. Fish disease as a monitor for marine pollution: the case of the North Sea. Rev. **Fish Biol. Fish**. 2,1 _/32.

VOLPE, A. V. Aspects of the biology of the common snook, *Centropomus undecimalis* (BLOCH) of southwest Florida. In: MITTS, Ernest, State Board of Conservation. Technical series. The Marine Laboratory. Florida: University of Miami, n. 31, 37 p., 1959.

WALKLEY, A. AND I.A. BLACK. An examination of the Degtjareff method for determining soil organic matter and proposed modification of the chromic acid titration method. **Soil Sci**. 1934. 37:29-38.

WENDELAAR BONGA, S. E., FLIK, G., BALM, P. H. M., VAN DER MEIJ, J. C. A. the ultrastructure of chloride cells in the gills of the Teleost Oreachromis mossambicus during exposure to fresh water. **Cell Tissue Res**., 259: 575 – 585, 1997.

WESTER, P. W., CANTON, J. H. The usefulness of histophatology in aquatic toxicity studies. **Comparative Biochemistry and Physiology**. v. 100C, p. 115-117. 1991.

WESTER, P. W., VAN DER VEN, L. T. M., VETHAAK, A. D., GRINWIS, G. C. M., VOS, J. G., Aquatic toxicology: opportunities for enhancement through histopathology. **Environmental Toxicology and Pharmacology**. v. 11, p. 289-295. 2002.

WHO INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY (IPCS), 1993. Biomarkers and risk assessment: concepts and principles. **Environmental Health Criteria** 155, World Health Organization, Geneva.