UNIVERSIDAD NACIONAL
SISTEMA DE ESTUDIOS DE POSGRADO
POSGRADO EN ECOTOXICOLOGÍA TROPICAL

EVALUACIÓN DE LA ACUMULACIÓN DE METALES (Cd, Cu, Cr, Ni, Mn, Pb y Zn) Y MERCURIO TOTAL (THg) EN SEDIMENTOS, MACROALGAS (*Cryptonemia crenulata*) Y ESPONJAS (*Cinachyrella kuekenthali)* DEL ECOSISTEMA CORALINO DE MOÍN (LIMÓN, COSTA RICA): UN ENFOQUE ECOTOXICOLÓGICO

SUSTENTANTE
SUSANA MARÍA MÉNDEZ ALFARO

CAMPUS OMAR DENGO

Heredia, Costa Rica
2018

Tesis sometida a consideración del Tribunal Examinador del Posgrado en Ecotoxicología Tropical, para optar por el grado de Magíster Scientiae en Ecotoxicología Tropical con énfasis Acuático

EVALUACIÓN DE LA ACUMULACIÓN DE METALES (Cd, Cu, Cr, Ni, Mn, Pb y Zn) Y MERCURIO TOTAL (THg) EN SEDIMENTOS, MACROALGAS (*Cryptonemia crenulata*) Y ESPONJAS (*Cinachyrella kuekenthali)* DEL ECOSISTEMA CORALINO DE MOÍN (LIMÓN, COSTA RICA): UN ENFOQUE ECOTOXICOLÓGICO

SUSTENTANTE

SUSANA MARÍA MÉNDEZ ALFARO

Tesis presentada para optar al grado de Magister Scientiae en Ecotoxicología Tropical con énfasis acuático. Cumple con los requisitos establecidos por el Sistema de Estudios de Posgrado de la Universidad Nacional. Heredia, Costa Rica

Miembros del Tribunal Examinador

Ph.D Luis Alfredo Miranda Calderón
Representante del Concejo Central de Posgrado

M.Sc. Margaret Pinnock Branford
Coordinadora Maestría en Ecotoxicología Tropical

Ph.D. Jorge Cortés Núñez
Tutor

M.Sc. Clemens Ruepert
Asesor

M.Sc. Freylan Mena Torres
Asesor

Susana María Méndez Alfaro
Sustentante

IV. Resumen

La contaminación por metales y mercurio en arrecifes de coral, está relacionada principalmente con actividades antropogénicas. Para la región de Moín, con una zona costera catalogada como de uso múltiple y con presencia de arrecifes, el ingreso de estas sustancias al ecosistema coralino puede darse a través de las actividades urbanas, industriales, agropecuarias y portuarias que allí se desarrollan. Los metales, por su alta persistencia y amplia distribución, pueden llegar fácilmente a distintos compartimientos ambientales donde al estar biodisponibles, son acumulados y biomagnificados en los organismos de diferentes niveles tróficos. La presencia de metales en arrecifes representa un riesgo para organismos sensibles como los corales, en los que pueden causar efectos como pérdida de zooxantelas (blanqueamiento), reducción del crecimiento, retracción de pólipos, detrimento de la respiración, fertilización, metamorfosis y el asentamiento larval. Con el fin de conocer el estado actual de contaminación por metales y mercurio en Moín y la bioaccumulación de estos en organismos del arrecife, se evaluó la acumulación de siete metales (Cd, Cu, Cr, Mn, Ni, Pb y Zn) y mercurio total (THg), en tres zonas del ecosistema coralino de Moín ubicadas dentro del área de influencia directa del proyecto “Ampliación de la Terminal Portuaria Petrolera del Atlántico” de la empresa RECOPE, y en tres zonas del Caribe Sur como sitio de referencia. Se analizaron muestras compuestas de sedimentos del fondo marino, de macro-algas (*Cryptonemia crenulata*) y de esponjas (*Cinachyrella kuekenthali*). Los metales se analizaron mediante ICP-MS y el THg mediante DMA. Se hizo una comparación de las concentraciones encontradas con otros estudios para evaluar la contaminación y se calculó el Factor de Bioconcentración (BCF). Los resultados indican que existe una tendencia a que las concentraciones de metales y mercurio en Moín sean mayores que en el Caribe Sur, sin embargo, las diferencias entre las concentraciones de ambos sitios no son significativas, por lo que se sugiere que las condiciones de corrientes marinas del Caribe favorecen la distribución de los metales en toda la región. La relación de concentraciones para algas fue Mn>Cu>Zn>Ni>Cr>Pb>Cd>Hg, para esponjas Mn>Cu>Zn>Ni>Cr>Cd>Pb>Hg y para sedimentos Mn>Cu>Zn>Cr>Ni>Pb>Cd>Hg. Todas las concentraciones en sedimentos se mantuvieron por debajo de los límites de ERL (concentraciones por debajo de las cuales raramente ocurren efectos adversos, por sus siglas en inglés) y ERM (concentraciones en o sobre las cuales ocurren efectos adversos frecuentemente, por sus siglas en inglés) de las guías para la calidad de los sedimentos (SQG, por sus siglas en inglés) y de los ámbitos de concentración encontrados en estudios previos, con excepción del Mn que presentó mayores concentraciones en este trabajo que en estudios previos. Cu presentó ligeramente mayores concentraciones en algas. Cd, Ni y Hg tuvieron mayores concentraciones en esponjas. Cr, Mn y Pb tuvieron mayores concentraciones en sedimentos. Cd, Cu, Ni, Zn y Hg fueron bioacumulados por las algas y esponjas, demostrando su biodisponibilidad en los sedimentos. Las concentraciones de THg encontradas en sedimentos se clasificaron como no contaminados en Moín y en el sitio de referencia, según el índice de geo-acumulación (I_{geo}). Los resultados demuestran que efectivamente, metales y mercurio presentes en el ecosistema coralino de Moín, están siendo bioconcentrados y que, aunque estos se encuentren en bajas concentraciones, podrían darse efectos tóxicos en las especies estudiadas o en organismos de niveles tróficos superiores favorecidos mediante procesos de biomagnificación.

Abstract

Anthropogenic activities are one of the main causes of metal (including mercury) contamination in coral reefs. Moín is a multi-use coastal zone where a variety of activities such as urban settlements, port, industry and agricultural developments can favor the entry of metals to the marine ecosystems. Metals high persistence and distribution capacity makes it easy for them to reach different environmental compartments, were they can become bioavailable and be accumulated and biomagnified through the food chain. Metal presence in coral reefs represent a risk to the ecosystem in general, but specially to the more sensitive organisms like corals to which some metals, even at low concentrations, can cause loss of zooxanthella (bleaching), growth reduction, polyp retraction and detrimental of respiration, fertilization, metamorphosis and larval settlement. With the purpose of knowing which is the current metal and mercury contamination in Moín, and they bioaccumulation in organisms of the coral reef, we evaluate the accumulation of seven metals (Cd, Cu, Cr, Mn, Ni, Pb y Zn) and total mercury (THg) in samples of bottom sediments, macroalgae (*Cryptonemia crenulata*) and sponge (*Cinachyrella kuekenthali*) in three sampling stations of the Moín reef located inside of the direct influence area of the project “Expansion of the Atlantic Oil Port Terminal” (ATPPA, in Spanish), conducted by the Oil Refinery Company of Costa Rica (RECOPE, in Spanish), and in three sampling stations in the South Caribbean as the reference site. Metals and total mercury were analyzed by ICP-MS and DMA methods, respectively. Results were compared to data of previous studies, the bioconcentration factor was calculated and, only for mercury analysis the geo-accumulation index was calculated. Results indicate a tendency where metals and mercury are higher in Moín than in the reference site however, there were no statistical differences found between both sites, which suggest that the Caribbean marine currents are favoring the distribution of contaminants along the entire coastline. The concentration range of metals for algae was Mn>Cu>Zn>Ni>Cr>Pb>Cd>Hg, for sponges Mn>Cu>Zn>Ni>Cr>Cd>Pb>Hg and for sediments Mn>Cu>Zn>Cr>Ni>Pb>Cd>Hg. Sediment concentrations were below the limits ERL (concentrations below which adverse effects rarely occur) and ERM (concentrations at or above which adverse effects frequently occur) of the Sediment Quality Guidelines (SQG), and from the concentrations found in previous studies; only Mn showed higher concentrations compared to previous studies. Cu showed slightly higher concentrations in algae, Cd, Ni and Hg had higher concentrations in sponges and Cr, Mn and Pb in sediments. Cd, Cu, Ni, Zn and Hg were bioconcentrated by algae and sponge, showing possible bioavailability in sediments. Sediment THg concentrations were classified as uncontaminated on both Moín and the reference site, according to the geo-accumulation index (I_{geo}). Results show that all metals and mercury are present in the coral reef ecosystem of Moín and that they are being accumulated by biota. The concentrations found, even if low in some cases, could cause toxicity to the species studied and to other sensitive organism like corals or in higher trophic levels by biomagnification processes.

V. Agradecimientos

Quiero agradecer a mi papá y a mi mamá por ser mi principal apoyo. Gracias por creer en mí, por apoyar mis sueños y nunca soltarme de la mano, por todo el amor, paciencia y dedicación. Agradezco a mi hermana por su inigualable amor, comprensión y apoyo en todo momento. A las diferentes personas en mi familia que me ayudaron de una u otra manera a cumplir esta meta. También a mis amigos y amigas que con su cariño y apoyo me daban las fuerzas para salir adelante. A Claudio por su compañía, amor y apoyo incondicional.

A mi increíble grupo asesor: Jorge Cortés, Clemens Ruepert y Freylan Mena, por todas sus recomendaciones, consejos y por apoyarme cuando quise descubrir otras oportunidades académicas.

Agradezco vehementemente a la Dr. Elba de la Cruz Malavasi, por creer que yo era una estudiante merecedora de una beca, sin su apoyo esto no hubiese sido posible.

Al CEOS y al Dr. Feiyue Wang de la Universidad de Manitoba, por su colaboración con el análisis de las muestras de mercurio. A David Lean por su gran apoyo profesional.

También quiero agradecer a mis compañeros de aventuras subacuáticas: Álvaro Segura, Giovanni Bassey, Carlos Gamboa, Eleazar Ruíz, Jaime Nivia, Rebeca Mallenco y especialmente a Juan Carlos Romero que además de compañero subacuático, fue mi apoyo y contacto para desarrollar este proyecto en Moín. A la empresa Refinadora Costarricense de Petróleo (RECOPE) y al consorcio ICA-MECO por facilitarme los medios para los muestreos.

Al CIMAR y al LAREP por el financiamiento de este proyecto. Al CIMAR por la facilitación de los medios de transporte, colaboradores y equipos para giras. También al CICA por reducir los costos de los análisis y darme asesoría. Al LAREP por las instalaciones para almacenar mis muestras y el desarrollo de ensayos. Al IRET y a la maestría EcoTrop por darme la oportunidad de estudiar este posgrado.

VI. Dedicatoria

Dedico este trabajo a mi papá y a mi mamá. Porque desde pequeña ustedes me enseñaron a ser valiente, a luchar por mis sueños y a nunca rendirme. A mi padre por todos sus consejos y muy especialmente por todas esas horas que dedicó a sus múltiples trabajos para brindarme los medios para seguir estudiando, para que nunca me faltara nada. A mi madre por todas sus atenciones, por su dulzura, por su paciencia y por todo su trabajo para que nuestro hogar siempre fuera un lugar lleno de amor. Para ustedes es este y todos los logros que tenga en mi vida, porque gracias a su esfuerzo esto ha sido posible, los amo.

VII. Índice

VIII. Lista de cuadros

Artículo 1

Artículo 2

IX. Lista de figuras

Artículo 1

Artículo 2

X. Lista de abreviaturas

A: Alga

AID: Área de Influencia Directa

ATPPA: Proyecto de Ampliación de la Terminal Portuaria Petrolera del Atlántico

BFC (por sus siglas en inglés): Factor de Bioconcentración

Cd: Cadmio

CEOS (por sus siglas en inglés): Centro para la Observación de las Ciencias de la Tierra

CICA: Centro de Investigación en Contaminación Ambiental

CIMAR: Centro de Investigación en Ciencias del Mar y Limnología

cm: Centímetros

Cr: Cromo

Cu: Cobre

CS: Caribe Sur

DMA (por sus siglas en inglés): Análisis Directo de Mercurio

DOM (por sus siglas en inglés): Materia Orgánica Disuelta

ERL (por sus siglas en inglés): Efectos de Rango Bajo

ERM (por sus siglas en inglés): Efectos de Rango Medio

HDPE (por sus siglas en inglés): Polietileno de Alta Densidad

Hg: Mercurio

HgMe (por sus siglas en inglés): metil-mercurio

ICP-MS (por sus siglas en inglés): Espectrometría de Masas con Plasma Acoplado Inductivamente

I_{geo} (por sus siglas en inglés): índice de geo-acumulación

IP: Isla Pájaros

IRET: Instituto Regional de Estudios en Sustancias Tóxicas

kg: Kilogramos

km: Kilómetros

L: Litros

LAREP: Laboratorio de Análisis Residuos de Plaguicidas.

LOI (por sus siglas en inglés): Pérdida en ignición

M: Moín

m: Metros

ManZ: Manzanillo

min: Minutos

ml: Mililitros

Mn: Manganeso

NaOH: Hidróxido de Sodio

$Na_2C_2O_4$: Oxalato de Sodio

M/P: Moín/Portete

Ni: Níquel

Pb: Plomo

PM: Punta Mona

PU: Punta Uva

RECOPE: Refinadora Costarricense de Petróleo

RO: Rompeolas

S (por su sigla en inglés): Esponja

SRB (por sus siglas en inglés): Bacterias Reductoras de Sulfato

THg (por sus siglas en inglés): Mercurio Total

TBT (por sus siglas en inglés): Tributiltin

UCR: Universidad de Costa Rica

UNA: Universidad Nacional

WCR (por sus siglas en inglés): Región del Gran Caribe

Zn: Zinc

XI. Descriptores

Accumulación

Metales

Mercurio

Caribe

Macroalga

Esponja

XII. Introducción

Los arrecifes de coral son uno de los ecosistemas más diversos, productivos y ecológicamente complejos del mundo (Pait et al., 2007; Hughes et al., 2017). Miles de especies utilizan los arrecifes de coral como sitios de alimentación, reproducción y refugio (van Damm et al., 2011). Además, funcionan como barreras protectoras ante tormentas y oleajes y millones de personas alrededor del mundo dependen de estos, de forma directa o indirecta, para su subsistencia, obtención de alimentos u otros productos esenciales (Hoegh-Guldberg, 1999; Erftemeijer et al., 2012).

Desde hace varias décadas, la cobertura coralina ha disminuido dramáticamente en muchas regiones del mundo, siendo afectados tanto por perturbaciones naturales como antropogénicas (Alutoin et al., 2001; Elias, 2018; NOAA, 2009). Se cree que los arrecifes de las regiones del Caribe han sido destruidos aproximadamente en un 80% (Elias, 2018). En el Caribe costarricense, estudios han demostrado que las actividades humanas como la agricultura extensiva, la descarga de aguas residuales sin tratamiento, contaminación por residuos sólidos, el transporte marítimo, industrias y las actividades portuarias han provocado un aumento en el ingreso de material sedimentable y sustancias químicas al ambiente marino (Acuña-González et al., 2004; Cortés et al., 2010; Erftemeijer et al., 2012; Guzmán and García, 2002; Guzmán and Jiménez, 1992). Se estima que la contaminación es responsable de impactar negativamente al 25% de los arrecifes de coral en el mundo (Briand et al., 2018).

Entre la gran variedad de contaminantes químicos, los metales representan uno de los problemas ambientales más serios globalmente (El-Metwally et al., 2017). La mayoría de los metales son constituyentes naturales de los ambientes marinos y algunos de estos como el Co, Fe, Cu, Mn and Zn son además elementos esenciales para la vida (Ansari et al., 2004). Sin embargo, en altas concentraciones tienen la capacidad de ser altamente tóxicos e inclusive algunos como el Pb, Hg and Cd son tóxicos a muy bajas concentraciones (Jakimska et al., 2011).

Los metales pueden ser ampliamente distribuidos y transportados por largas distancias por vías atmosféricas o mediante procesos hidrológicos (Berry et al., 2013; Guzmán and Jiménez, 1992). Son persistentes y pueden encontrarse biodisponibles e ingresar a las cadenas tróficas, llegando a potenciar su toxicidad a gran variedad de organismos incluyendo los humanos (Fatoki and Mathabatha, 2001; Peters et al., 1997). Entre los efectos agudos y tóxicos que estos pueden causar en especies de coral se encuentran el detrimento de la respiración, fertilización, metamorfosis y asentamiento larval, también causan estrés fisiológico mediante la pérdida de zooxantelas, reducción del crecimiento, mayor mortalidad y disminución de la biodiversidad (Berry et al., 2013; Heyward, 1988; Negri et al., 2002). En el caso del Hg, su toxicidad aumenta por su capacidad de formar compuestos órgano-metálicos como el metilmercurio (HgMe), que son más fácilmente asimilados por los organismos, favoreciendo su biomagnificación (Ramos et al., 2009; UNEP, 2014).

La costa Caribe de Costa Rica es caracterizada por la presencia de playas de arena de alta energía y lagunas alargadas en la región norte; playas de arena y arrecifes de coral en la región central y sur. Sus ecosistemas principales son los arrecifes de coral, pastos marinos y dos pequeños humedales (Cortés and Wehrtmann, 2009). A pesar de que su línea costera es relativamente corta, 212 km, comparada con la Costa Pacífica, 1 254 km (Cortés and Wehrtmann, 2009), alberga aproximadamente 2 300 especies (Cortés, 2016), lo que representa aproximadamente el 34% del total de especies reportadas para ambas costas (Cortés and Wehrtmann, 2005). Se reconocen tres regiones o zonas con presencia de arrecifes coralinos en el Caribe, una entre Moín y Limón, otra en el Parque Nacional Cahuita y una entre Puerto Viejo y Punta Mona (Cortés, 2016).

Nuestra zona de estudio, Moín, se encuentra en la región coralina ubicada entre la ciudad de Moín y Limón, caracterizada por ser una zona costera de uso múltiple, es decir, donde se desarrolla una amplia variedad de actividades, industriales comerciales y urbanas. En Moín se encuentra el único puerto petrolero del país, la Refinadora Costarricense de Petróleo (RECOPE), un puerto de contenedores por donde se comercia aproximadamente el 75% de los bienes del país y cuenta también con sitios de importancia turística, pesca y transporte marítimo (JAPDEVA, 2008). Asimismo, desde 2015 se construye un mega puerto de contenedores de 78.6 hectáreas de extensión, construidas completamente sobre terreno marino a rellenar, con importantes impactos a la morfología de la costa y los ecosistemas marinos (CCT, 2013; CNC, 2017). También, la región se caracteriza por su proximidad con zonas de producción agrícola extensiva, principalmente de cultivos como el banano, con 41 442 hectáreas cultivadas en el Caribe, y otros productos como la piña, palma, cacao, café, caña, melón, entre otros (MINAE & IMN, 2013). Exactamente la zona de estudio se encuentra ubicada dentro del Área de Influencia Directa (AID) del proyecto de Ampliación de la Terminal Portuaria Petrolera del Atlántico (ATPPA), desarrollado por la Refinadora Costarricense de Petróleo (RECOPE) desde 2012 hasta la actualidad (RECOPE, 2018).

Considerando que las actividades portuarias como el dragado del fondo marino, reparación de embarcaciones, carga y descarga de combustibles y transporte marítimo son una de las principales fuentes de contaminación de metales en zonas costeras (El-Metwally et al., 2017; Guzmán and Jiménez, 1992), añadido a las otras actividades mencionadas que se desarrollas en la zona y a otras fuentes de contaminación como la descarga de aguas residuales sin tratamiento previo o inespecífico para sustancias inorgánicas (Ruiz, 2012), esta región y los arrecifes que se encuentran en ella pueden estar en riesgo por contaminación de metales.

Ante este panorama, el objetivo general del presente trabajo fue: evaluar la acumulación de metales (Cd, Cr, Cu, Ni, Pb, Mn y Zn) y mercurio total (THg) en sedimentos y organismos del arrecife de Moín, mediante un enfoque ecotoxicológico. Para lo que se plantearon tres objetivos específicos: (1) Identificar de la presencia de metales en el arrecife coralino de Moín mediante la evaluación de su acumulación en sedimentos del fondo marino y utilizando las macro algas *Cryptonemia crenulata* y las esponjas *Cinachyrella kuekenthali* como organismos bioindicadores; (2) Comparar la contaminación de metales del sitio de estudio con un sitio de referencia ubicado en un área protegida (Refugio de Vida Silvestre Gandoca-Manzanillo) y con estudios previos; y (3) Evaluar la biodisponibilidad de los metales en las especies estudiadas, su función como bioindicadores de contaminación y los posibles efectos tóxicos que los metales puedan causar en estos y otros organismos del ecosistema coralino. Los objetivos de este trabajo se presentan bajo la modalidad de artículos científicos. Para el primer artículo se desarrollaron los objetivos específicos enfocados en los metales: Cd, Cr, Cu, Pb, Mn y Zn. En el segundo artículo de igual forma se desarrollaron los objetivos específicos, pero enfocados únicamente en el Hg, debido a que las particularidades de este metal en cuanto a fuentes de contaminación, distribución ambiental y toxicidad, así lo requieren.

XIII. Conclusiones generales

Este estudio actualiza la información sobre presencia de metales y mercurio en el Caribe costarricense. Además, provee los primeros datos de concentraciones de metales y mercurio en especies locales de algas y esponjas, y constituye una línea base de información para estudios posteriores.

La homogeneidad en la presencia de metales y mercurio entre Moín y el Caribe Sur sugiere que existe un mecanismo de dispersión costero, posiblemente favorecido por corrientes marinas, que permite el arrastre de sustancias contaminantes como los estudiados a zonas con menor impacto antropogénico directo como lo es el Caribe Sur. Sin embargo, las concentraciones de algunos metales y mercurio ligeramente mayores en Moín, supone que pueden estarse facilitando las condiciones para un problema de contaminación en este sitio.

Las diferencias significativas entre las concentraciones de metales y mercurio según el tipo de muestra analizado, demostraron que los metales y mercurio se acumulan de forma diferente según la matriz que se utilice o inclusive la especie seleccionada. Por lo que se concluye que el uso de diferentes matrices favoreció la evaluación de la acumulación de metales y mercurio en los ecosistemas coralinos estudiados.

Las comparaciones con estudios previos demostraron que en nuestro estudio se obtuvieron en general concentraciones menores de acumulación de metales y mercurio en sedimentos. Sin embargo, el Mn fue el metal que presentó concentraciones mayores, respecto a los estudios previos, posiblemente asociado al uso intensivo de plaguicidas como el mancozeb.

Se estableció que el alga *Cryptonemia crenulata* y la esponja *Cinachyrella kuekenthali* son adecuados bioindicadores de contaminación por metales y mercurio en los arrecifes del Caribe costarricense. Ambas especies permiten evaluar la acumulación y posible biomagnificación de los contaminantes en la cadena trófica, así como representar con mayor eficacia los niveles de contaminación del medio acuático y los posibles riesgos derivados para otras especies.

La esponja *Cinachyrella kuekenthali* presentó en general mejores niveles de acumulación de metales y mercurio, por lo que resulta ser una especie, que con mayor monitoreo, podría funcionar como centinela para evaluar contaminación por metales y mercurio el Caribe.

Existe una necesidad de desarrollo de investigaciones ecotoxicológicas en el ambiente marino del Caribe costarricense, debido a que no existen datos de toxicidad para los contaminantes y las especies estudiadas, lo que impidió evaluar los riesgos asociados a las concentraciones encontradas, especialmente de aquellos metales que fueron bioconcentrados (Cd, Cu, Ni, Zn y Hg).

Las actividades portuarias que se desarrollan actualmente en la región, junto con las demás actividades antropogénicas como crecimiento urbano, industrialización, ausencia de tratamiento de aguas residuales y agricultura intensiva y extensiva, podrían estar contribuyendo a la presencia de metales en el ecosistema coralino Moín. Además, las nuevas construcciones portuarias (ampliación del puerto petrolero y el nuevo megapuerto de contenedores) que, al aumentar el tránsito marino en la región, podrían provocar el ingreso de mayores niveles de metales u otros contaminantes al ecosistema, así como influir en la dinámica marina de la región y cambiar los procesos de dispersión de contaminantes. Por lo que es necesario realizar estudios de monitoreo de acumulación de metales y mercurio en la región, tanto para establecer las principales fuentes de contaminación, así como para identificar riesgos y detectar los posibles cambios en el tiempo.

XIV. Recomendaciones

A partir de la información obtenida con este trabajo, se recomienda continuar con monitoreos periódicos de metales y mercurio en sedimentos, algas (*Cryptonemia crenulata*) y esponjas (*Cinachyrella kuekenthali*) en los sitios estudiados, con el fin de registrar posibles cambios en la acumulación de los metales, identificar fuentes de contaminación y establecer medidas preventivas para evitar el aumento de las concentraciones encontradas.

Se recomienda estudiar los procesos de bioacumulación y biomagnificación de los metales y especialmente del Cd, Cu, Ni, Zn y Hg en esponjas y organismos de niveles tróficos superiores, debido a las altas concentraciones encontradas y su importante participación en el ciclo de la materia orgánica disuelta (DOM) en los arrecifes. Además, se recomienda expandir el monitoreo a otras regiones del Caribe, considerando sitios de menor influencia antropogénica que el Caribe Sur de Costa Rica, con el fin de identificar niveles y zonas de contaminación, utilizando como especie bioindicadora a la esponja *Cinachyrella kuekenthali*.

De igual forma, se recomienda monitorear la presencia de Mn en los ecosistemas estudiados, así como en las afluencias de los ríos y cuencas en general, para determinar si existe relación entre las altas concentraciones encontradas en los ecosistemas coralinos con el uso intensivo del plaguicida mancozeb.

En futuros monitoreos, se recomienda incluir otras especies de algas y esponjas además de las utilizadas en este estudio, debido que existen diferencias en los procesos de acumulación de metales según la especie o tipo de organismo. Además, en el caso de las algas, se recomienda también utilizar diferentes partes del organismo, para detectar posibles diferencias en las concentraciones de metales respecto a la sección estudiada en el alga.

Debido a que se desconocen los posibles riesgos y efectos que la exposición a metales y mercurio pueden estar causando en los ecosistemas estudiados, se recomienda realizar, además de los monitoreos químicos, ensayos ecotoxicológicos en especies bioindicadoras, así como en especies ecológicamente importantes para los arrecifes, con el fin de estableces niveles de toxicidad en especies locales y evaluar los posibles riesgos.

XV. Referencias generales

Acuña-González, J., Vargas-Zamora, J.A., Gómez-Ramírez, E., García-Céspedes, J., 2004. Hidrocarburos de petróleo, disueltos y dispersos, en cuatro ambientes costeros de Costa Rica. Rev. Biol. Trop. 52, 43–50. https://doi.org/https://doi.org/10.15517/rbt.v54i1.26828

Alutoin, S., Boberg, J., Nyström, M., Tedengren, M., 2001. Effects of the multiple stressors copper and reduced salinity on the metabolism of the hermantypic coral Porites lutea. Mar. Environ. Res. 52, 289–299.

Ansari, T.M., Marr, I.L., Tariq, N., 2004. Heavy metals in marine pollution perspective - A mini review. J. Appl. Sci. 4, 1–20. https://doi.org/10.3923/jas.2004.1.20

Berry, K.L.E., Seemann, J., Dellwig, O., Struck, U., Wild, C., Leinfelder, R.R., 2013. Sources and spatial distribution of heavy metals in scleractinian coral tissues and sediments from the Bocas del Toro Archipelago, Panama. Environ. Monit. Assess. 185, 9089–9099. https://doi.org/10.1007/s10661-013-3238-8

Briand, M.J., Bustamante, P., Bonnet, X., Churlaud, C., Letourneur, Y., 2018. Tracking trace elements into complex coral reef trophic networks. Sci. Total Environ. 612, 1091–1104. https://doi.org/10.1016/j.scitotenv.2017.08.257

CCT, 2013. Estudio de Impacto Ambiental Proyecto Terminal de Contenedores Moín: expediente No. 7968-12-SETENA. Centro Científico Tropical. San José, Costa Rica.

CNC, 2017. Terminal de Contenedores de Moín [WWW Document]. Com. -Nacional Concesiones, MOPT. URL http://www.cnc.go.cr/index.php/proyectos/en-desarrollo/tcm (accessed 6.13.18).

Cortés, J., 2016. The Caribbean coastal and marine ecosystems, in: Kappelle, M. (Ed.), Costa Rican Ecosystems. University of Chicago Press, Chicago and London, pp. 591–617.

Cortés, J., Jiménez, C.E., Fonseca, A.C., Alvarado, J.J., 2010. Status and conservation of coral reefs in Costa Rica. Rev. Biol. Trop. 58, 33–50. https://doi.org/10.15517/rbt.v58i1.20022

Cortés, J., Wehrtmann, I.S., 2009. Diversity of marine habitats of the Caribbean and Pacific of Costa Rica, in: Wehrtmann, I.S., Cortés, J. (Eds.), Marine Biodiversity of Costa Rica, Central America. Springer + Business Media B.V., Berlin, pp. 1–45. https://doi.org/10.1007/978-1-4020-8278-8_1

Cortés, J., Wehrtmann, I.S., 2005. Costa Rica, in: Miloslavich, P., Klein, E. (Eds.), Caribbean Marine Biodiversity: The Known and the Unknown. DEStech Publishing Inc., Lancaster, Pennsylvania, pp. 169–179.

El-Metwally, M.E.A., Madkour, A.G., Fouad, R.R., Mohamedein, L.I., Eldine, H.A.N., Dar, M.A., El-Moselhy, K.M., 2017. Assessment the Leachable Heavy Metals and Ecological Risk in the Surface Sediments inside the Red Sea Ports of Egypt. Int. J. Mar. Sci. 7, 214–228. https://doi.org/10.5376/ijms.2017.07.0023

Elias, S.A., 2018. Loss of coral reefs, in: Dellasala, D.A., Goldstein, M.I. (Eds.), Encyclopedia of the Anthropocene. Elsevier, Oxford, pp. 245–258. https://doi.org/10.1016/B978-0-12-809665-9.09917-1

Erftemeijer, P.L.A., Riegl, B., Hoeksema, B.W., Todd, P.A., 2012. Environmental impacts of dredging and other sediment disturbances on corals: A review. Mar. Pollut. Bull. 64, 1737–1765. https://doi.org/10.1016/j.marpolbul.2012.05.008

Fatoki, O.S., Mathabatha, S., 2001. An assessment of heavy metal pollution in the East London and Port Elizabeth harbours. Water SA 27, 233–240. https://doi.org/10.4314/wsa.v27i2.4997

Guzmán, H.M., García, E.M., 2002. Mercury levels in coral reefs along the Caribbean coast of Central America. Mar. Pollut. Bull. 44, 1415–1420.

Guzmán, H.M., Jiménez, C.E., 1992. Contamination of coral reefs by heavy metals along the Caribbean coast of Central America (Costa Rica and Panama). Mar. Pollut. Bull. 24, 554–561. https://doi.org/10.1016/0025-326X(92)90708-E

Heyward, A.J., 1988. Inhibitory effects of copper and zinc sulphates on fertilization in corals, in: Choat, J.H. (Ed.), Proceedings of the 6th International Coral Reef Symposium. 6th International Coral Reef Symposium Executive Committee, Townsville, pp. 299–303.

Hoegh-Guldberg, O., 1999. Climate change, coral bleaching and the future of the world's coral reefs. Mar. Freshw. Res. 50, 839–866. https://doi.org/10.1071/MF99078

Hughes, T.P., Barnes, M.L., Bellwood, D.R., Cinner, J.E., Cumming, G.S., Jackson, J.B.C., Kleypas, J., vn de Leemput, I.A., Lough, J.M., Morrison, T.H., Palumbi, S.R., van Nes, E.H., Scheffer, M., 2017. Coral reefs in the Anthropocene. Nature 546, 82–90. https://doi.org/10.1038/nature22901

Jakimska, A., Konieczka, P., Skóra, K., Namieśnik, J., 2011. Bioaccumulation of metals in tissues of marine animals, part I: the role and impact of heavy metals on organism. Polish J. Environ. Stud. 20, 1117–1125.

JAPDEVA, 2008. Plan Maestro para el complejo portuario Limón - Moín. Junta de Administración Portuaria y de Desarrollo Económico de la Vertiente Atlántica de Costa Rica. Limón, Costa Rica. https://doi.org/9R4672.21 /R/401180/Nijm

MINAE & IMN, 2013. Descripción del Clima Cantón de Limón. Ministerio de Ambiente y Energía & Instituto Meteorológico Nacional. San José, Costa Rica.

Negri, A.P., Smith, L.D., Webster, N.S., Heyward, A.J., 2002. Understanding ship-grounding impacts on a coral reef: Potential effects of anti-foulant paint contamination on coral recruitment. Mar. Pollut. Bull. 44, 111–117. https://doi.org/10.1016/S0025-326X(01)00128-X

NOAA, 2009. Coral Reef Conservation Program: International Strategy 2010-2015. National Oceanic and Atmospheric Administration. Silver Spring, MD.

Pait, A.S., Whitall, D.R., Jeffrey, C.F., Caldow, C., Mason, A.L., Christensen, J.D., Monaco, M., Ramirez, J., 2007. An Assessment of Chemical Contaminants in the Marine Sediments of Southwest Puerto Rico. NOAA/ NOS/Center for Coastal Monitoring and Assessment. Silver Spring, MD.

Peters, E.C., Gassman, N.J., Firman, J.C., Richmond, R.H., Power, E.A., 1997. Ecotoxicology of tropical marine ecosystems. Environ. Toxicol. Chemestry 16, 12–40.

Ramos, R., Cipriani, R., Guzman, H.M., García, E., 2009. Chronology of mercury enrichment factors in reef corals from western Venezuela. Mar. Pollut. Bull. 58, 222–229. https://doi.org/10.1016/j.marpolbul.2008.09.023

RECOPE, 2018. Ampliación de la terminal portuaria petrolera del atlántico [WWW Document]. Ficha Proy. URL https://www.recope.go.cr/proyectos/procesos-industriales-portuarios/ampliacion-de-la-terminal-portuaria-petrolera-del-atlantico/ (accessed 5.14.18).

Ruiz, F., 2012. Gestión de las Excretas y Aguas Residuales en Costa Rica. Instituto Costarricense de Acueductos y Alcantarillados. San José, Costa Rica.

UNEP, 2014. The Minamata Convention on mercury and its implementation in the Latin America and Caribbean region. Montevideo, Uruguay.

van Damm, J.W., Negri, A.P., Uthicke, S., F. Mueller, J., 2011. Chemical Pollution on Coral Reefs: Exposure and Ecological Effects, in: Sánchez-Bayo, F., van den Brink, P.J., Mann, R.M. (Eds.), Ecological Impacts of Toxic Chemicals. Bentham Science Publishers Ltd, Townsville, pp. 187–211. https://doi.org/10.2174/978160805121210187

XVI. Artículo I: Acumulación de metales (Cd, Cr, Cu, Mn, Pb, Ni, Zn) en sedimentos, macroalgas (*Cryptonemia crenulata)* y esponjas (*Cinachyrella kuekenthali*) del ecosistema coralino de Moín, Limón, Costa Rica

Accumulation of metals (Cd, Cr, Cu, Mn, Pb, Ni, Zn) in sediments, macroalgae (*Cryptonemia crenulata*) and sponge (*Cinachyrella kuekenthali*) of a coral reef in Moín, Limón, Costa Rica: An ecotoxicological approach

Sustentante

Susana María Méndez Alfaro

Artículo con el formato de la revista Marine Pollution Bulletin

Accumulation of metals (Cd, Cr, Cu, Mn, Pb, Ni, Zn) in sediments, macroalgae (*Cryptonemia crenulata*) and sponge (*Cinachyrella kuekenthali*) of a coral reef in Moín, Limón, Costa Rica: An ecotoxicological approach

Susana María Méndez Alfaro

Maestría Académica en Ecotoxicología Tropical. Instituto Regional de Estudios en Sustancias Tóxicas. Universidad Nacional, Costa Rica

Abstract Coral reefs are among the most important and yet endangered marine ecosystems in the world. The Costa Rican Caribbean has three coral reefs areas and one of them is located at Moín, were a wide variety of human activities such as urban, commercial and industrial activities are take place. Also Moín is one of the most important commercial ports and the only oil port and oil refinery of the country. Coral reefs of Moín have been affected by both natural and anthropogenic disturbances such as earthquakes, intensive farming runoff, untreated sewage discharge, solid waste pollution, marine transport, oil spills and oil refinery and port activities; all these activities are sources of metal pollution. Some metals can be highly toxic and persistent and can be accumulated and biomagnified through the food webs. In this study, the concentration of seven metals (Cd, Cr, Cu, Mn, Pb, Ni, Zn) were determined in sediments, macroalgae (*Cryptonemia crenulata*) and sponge (*Cinachyrella kuekenthali*), collected between March 2015 and May 2016 at Moín and at a reference site (South Caribbean-SC). The Bioconcentration Factor (BCF) of the metals was calculated for both organisms and sites. Also, we compared our data with previous studies and Sediment Quality Guidelines (SQG) to evaluate sediment pollution and possible ecotoxicological effects. Results indicate that, even if there is a tendency for metal concentrations to be higher in samples of Moín, differences were not significant with the SC, suggesting effective dispersal by currents, throughout the entire coast. Concentration for algae samples was Mn>Cu>Zn>Ni>Cr>Pb>Cd, sponges Mn>Cu>Zn>Ni>Cr>Cd>Pb and sediments Mn>Cu>Zn>Cr>Ni>Pb>Cd. Mn had the highest concentrations in all samples, Cd and Ni had higher concentrations in sponge samples and Cr, Mn and Pb were higher for sediment samples. Metal concentrations in algae were generally lower than in the other samples. Results indicate the presence of metals in the coral reef ecosystem, positive bioconcentration values for Cd, Cu, Ni and Zn and metal concentrations in sediments below the SQG thresholds.

Keywords: Contamination, ecotoxicology**,** metals**,** Caribbean, bioaccumulation

Introduction

Coral reefs are one of the most diverse, productive and ecologically complex ecosystems in the world (Pait et al., 2007; Hughes et al., 2017). This are biogenic structures, formed by calcification process of scleractinian corals and coralline algae, where thousands of species of fish, algae and invertebrates find refuge, food sources and reproduction sites (van Damm et al., 2011). Coral reefs also provide valuable economic, social and cultural services to millions of people around the world through their role in activities like fishing, tourism, coastal protection and medical and biochemical uses (Hoegh-Guldberg, 1999; Elias, 2018)

From several decades now, natural and anthropogenic disturbances had been causing serious decline of coral reefs communities worldwide (Alutoin et al., 2001; Halpern et al., 2008; NOAA, 2009; Elias, 2018). In the Costa Rican Caribbean, studies have shown that human activities, such as intensive and extensive farming, untreated sewage discharge, solid waste pollution, marine transport, oil refineries, oil spills and port activities, have adversely affected coral reefs by increasing the sedimentation rates and causing chemical contamination to seawater, sediments and corals (Guzmán and Jiménez, 1992; Guzmán and García, 2002; Acuña-González et al., 2004; Cortés et al., 2010). However, relationships between pollution and adverse effects in coral reefs have not been studied in the country.

Among the wide variety of chemical contaminants, metals represent one of the most important global environmental problems (OzCoast, 2012; El-Metwally et al., 2017). Metals can be widely distributed and transported long distances via atmospheric and hydrological processes (Guzmán and Jiménez, 1992; Berry et al., 2013), they can be environmentally persistent, become bioavailable and through biomagnification process metals can be potentially toxic to organisms, including humans (Peters et al., 1997; Fatoki and Mathabatha, 2001). Acute and chronic effects of metals in coral organisms can include detriment in respiration, fertilization, metamorphosis and larvae settlement or even cause physiological stress like zooxanthellae loss, reduced growth or biodiversity and enhance mortality (Heyward, 1988; Negri et al., 2002; Berry et al., 2013).

The Caribbean coast of Costa Rica is characterized by high energy sandy beaches and elongates coastal lagoons in the north and sandy beaches and coral reefs, only in the southern half. Its main ecosystems are coral reefs, seagrass beds and two small mangrove forests (Cortés, 2016). The coast line is relatively short, 212 km, compared to the Pacific coast, 1,254 km (Cortés and Wehrtmann, 2009). Nevertheless, the Caribbean has approximately 2,300 species (Cortés, 2016), which represent almost the 34% of the total number of species recorded from both coasts (Cortés and Wehrtmann, 2005). Fishes and benthic gastropods are the most diverse taxonomic groups, followed by macroalgae, bivalves, decapods and benthic opisthobranchs (Cortés and Wehrtmann, 2005), and there are 47 species of reef building corals and 3 hydrocorals (Alvarado et al., 2006; Cortés, 2009). There are three coral reef formations in the Caribbean, one between Moín and Limón (the most populated towns of the region), other at the Cahuita National Park (the most developed coral reef of the Costa Rican Caribbean) and the last one is located from Puerto Viejo to Punta Mona (Cortés and Jiménez, 2003; Cortés, 2016).

Moín, is located in the Central Caribbean coast of Costa Rica, is characterized as a multi-use coastal zone, with a variety of commercial, industrial, and urban activities. The only oil port of the country is located there, and is in constant operation and expansion since 2012 (RECOPE, 2018). The only oil refinery and primary distribution center of oil products in the country, and the commercial port where approximately 75% of the country's goods are moved are in Moín (JAPDEVA, 2008). Also, since 2015 a new commercial port is being built in Moín, with and extension of 78.6 ha of marine land to be filled (CCT, 2013; CNC, 2017). The region is also characterized for its proximity to extensive and intensive farming fields, primarily of pineapple and banana plantations, with 41,442 ha cultivated (INEC, 2015) and an approximately pesticide use of 73.28 kg a.i./ha/year in banana fields (Echeverría-Sáenz et al., 2015). Pesticides possibly drains to the sea because of the high rainfall and deforestation in the region (MINAE & IMN, 2013).

The most significant sources for metal pollution in coastal ecosystems are related with port activities, such as dredging, ships repairing, shipping, loading and effluent oil discharge (Guzmán and Jiménez, 1992; El-Metwally et al., 2017). Also, pesticide use and wastewater, commonly discharged into the sea without previous treatment, makes the coral reefs in the area at risk of metal contamination.

For this reason, the objectives of the present study were to: (1) identify the present level of seven metals (cadmium (Cd), chrome (Cr), copper (Cu), manganese (Mn), lead (Pb), nickel (Ni) and zinc (Zn)) in a coral reef in Moín by assessing their accumulation in bottom sediments and using the macroalgae *Cryptonemia crenulata* and the sponge *Cinachyrella kuekenthali* as bioindicators of contamination; (2) compare the metal concentrations of Moín with a putative less polluted site (reference site) in the southern Caribbean coast of Costa Rica; and (3) evaluate the data with an ecotoxicological approach by analyzing the metals bioaccumulation, the contamination level, the species utility as bioindicators and the possible toxic risks to which the organisms are exposed.

Materials and methods

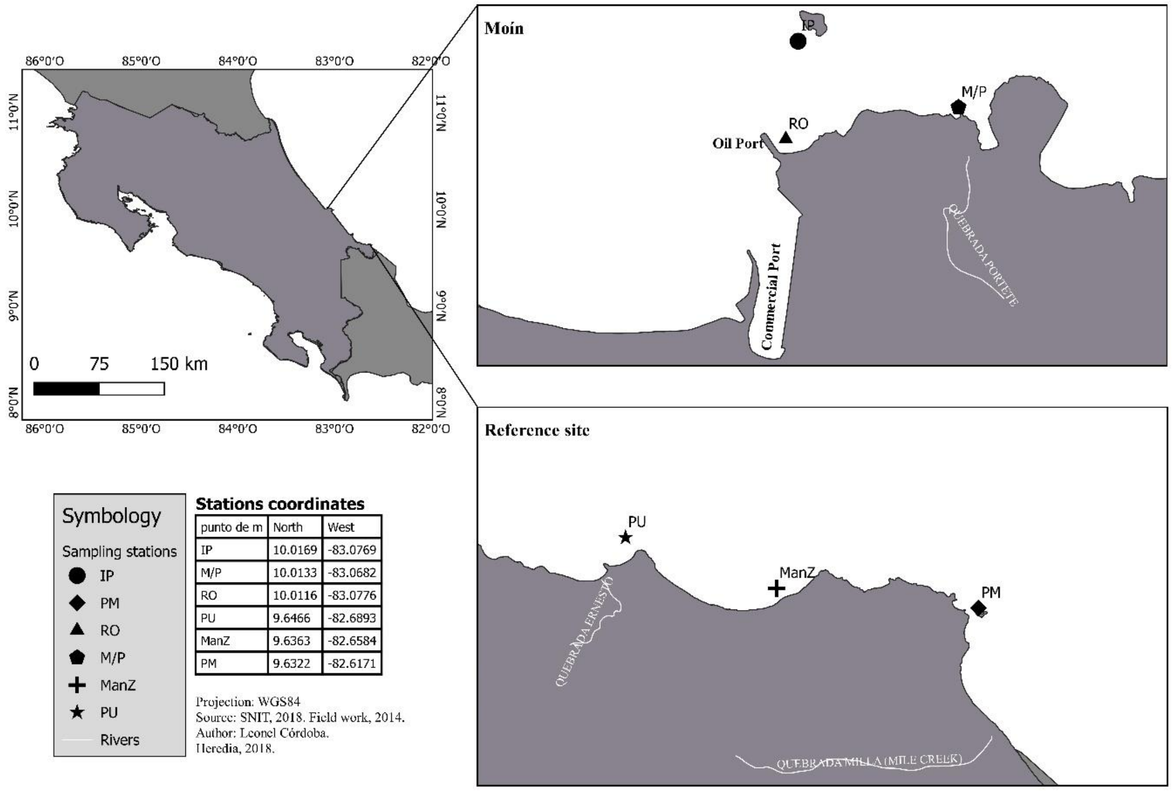
Description of the study site

Moín (M) is located in the central Caribbean region, inside the direct influence area of the project "Expansion of the Atlantic Oil Port Terminal" (ATPPA, in Spanish), conducted by the Oil Refinery Company of Costa Rica (RECOPE, in Spanish). This area comprises 24,489 m^2 of marine zone, and includes one of the three main coral reef formations of the Costa Rica Caribbean, the Moín-Limón reef (Cortés, 2016).

Three sample stations where selected inside the ATPPA, (1) Isla Pájaros (IP), in the south west of the island, where wave action is lower, (2) Moín-Portete (M/P), in the coastal reef band between Moín and Portete, and (3) Rompeolas (RO), wavebraker located in the east side of the oil port (Fig. 1.1).

Description of the reference site (South Caribbean)

The reference site is located in the South Caribbean (SC) region. Unlike Moín, this region has less agricultural and urban development and no mayor industries or port activities. Three sampling stations were selected, all located within the protected area of the Gandoca-Manzanillo National Wildlife Refuge, with presumably very little anthropogenic pressure or pollution. (1) Punta Uva (PU), located in the reef in front of Punta Uva beach (2), Manzanillo (ManZ), located in the reef off Manzanillo beach, and (3) Punta Mona (PM), the farthest site from human activities in the Costa Rican Caribbean (Fig. 1.1). However, the majority of the analyzed samples were from PM sampling point, for ManZ and PU only algae samples were analyzed (Annex 1).



Stations coordinates

punto de m	North	West
IP	10.0169	-83.0769
M/P	10.0133	-83.0682
RO	10.0116	-83.0776
PU	9.6466	-82.6893
ManZ	9.6363	-82.6584
PM	9.6322	-82.6171

Projection: WGS84
Source: SNIT, 2018. Field work, 2014.
Author: Leonel Córdoba.
Heredia, 2018.

Fig. 1.1. Geographical locations of the three sampling stations Moín (study site); IP: Isla Pájaros, M/P: Moín-Portete, RO: Rompeolas; and at the reference site (South Caribbean); PU: Punta Uva, ManZ: Mazanillo, PM: Punta Mona; Costa Rica.

Bioindicators selection

Three recognition field trips were done, two to Moín and one to the reference site, to identify the general conditions of the reefs and to select possible bioindicators. In a review of Fernandez et al., (2007), about trace metal accumulation carried out in the Wider Caribbean Region (WCR), they mention bivalves, sea urchins, echinoderms, oysters and corals as the most commonly used organisms as bioindicators for metal contamination. However, from our recognition field trips we found that these organisms, both in Moín and in the reference site, have low populations and were not the same species at both sites. Also, sampling coral species can cause damage to an already impacted ecosystem. Thus, we selected organisms that were abundant, widely distributed, with sedentary lifestyles, fast growing rates and that can be easily identified, handle and analyzed, such as macroalgae and sponges. Macroalgae and sponge species are recognized as good bioindicators of metal pollution in marine environments (Rao et al., 2009; Padovan et al., 2012; Chakraborty et al., 2014; Davis et al., 2014; García-Seoane et al., 2018).

We selected the macroalgae (also mention as algae) *Cryptonemia crenulata* and the sponge *Cinachyrella kuekenthali* as bioindicator organisms, two abundant and common species in the Costa Rica Caribbean (Bernecker, 2009; Cortés et al., 2009; Araya-Vargas, 2017). *Cryptonemia crenulata* is a species of Rhodophyta, found from the Lesser Antilles to the Western Caribbean; they can grow on rocks, caves, cracks or beneath rock ledges on lower intertidal to 30 m deep (Scullion and Masterton, 2000). *Cinachyrella kuekenthali* is a species of the class Demospongiae, found in the Central American Caribbean, from the Lesser Antilles, the Western Caribbean to the Northeastern Brazil (Van Soest et al., 2018); they are semispherical sponges of a strong yellow color, always covered by sediments and organisms, and can grow on hard substrates and reef rocks to 21 m depth (Gómez, 2003).

Sample collection

Samples were collected between March 2015 and May 2016, by SCUBA diving at depths ranging from 3 m to 12 m. Five field trips were done, three to Moín and two to the reference site. An average of 2 to 3 individual macroalgae, sponge and sediments samples were taken in each sampling station at arbitrarily selected sampling points in a 10-20 m^2 area, to ensure a representative sampling. For May 2015 at MP and RO sampling was precluded due to bad diving conditions. Macroalgae and sponge samples were handpicked, using protective gloves, and stored in high-density polyethylene (HDPE) bags. Sediment samples were collected from the top 2 to 5 cm depth of the bottom sediments using HDPE bags, by dragging the open bag through the marine bottom, avoiding rocks and marine vegetation. All samples were transported to the laboratory in coolers and stored at - 20° C until analyzed.

Sample preparation

Samples were thawed and any foreign matter, such as rock fragments, epiphytes and adhered animals were removed. Macroalgae samples were rinsed with ultrapure water (Milli-Q grade) to remove sediments. For sponge samples, the superficial layer of sediments that accumulates naturally in this sponge species, was removed and then the samples were cut into small pieces. Each individual sample was homogenized and then, accordingly to three conditions (sample type, sampling station and sampling date), composed samples were prepared and homogenized again. Composed samples reduced the analytical effort maintaining the information from all samples taken. We obtained one composed sample of each type for each sample station at each sampling date, and from a total of 114 individual samples, we got 12 macroalgae, 10 sponge and 9 sediment composed samples to be analyzed. All samples were lyophilized at -80° C for 48 h and then pulverized in a ceramic mortar.

Two separate portions of the samples were for grain size and organic matter content analysis. Sediment samples were characterized by grain size using the Bouyocous method (Bouyoucos, 1962), taking 50 g of the sediments in duplicated, in a 600 ml beaker with distillated water and 5 ml of sodium hydroxide (NaOH 1 N) and 5 ml of sodium oxalate ($Na_2C_2O_4$) as dispersants. The solution was transferred to a 1 L graduated cylinder and hydrometer measurements were taken at 40 seconds and 2 hours after mixing. The organic matter content was determined by the Loss on Ignition (LOI) method; biological samples were weighed then heated to 550°C for 4 hours, sediment samples were heated to 350°C for 2 hours then at 550°C for an additional 4 hours (Wood, 2015).

Chemical analysis

For biological samples 0.5 g and for sediment samples 1.0 g were digested in 5 ml of 70% HNO_3 (J.T.BAKER-Trace metal analysis), 1 ml of 80% HCl (SPECTRUM – Trace grade) and 4 ml of ultrapure water in a microwave CEM-MARS*X*. The microwave was programmed on a 20 min ramp, for biological samples 15 min at 180 °C and potency of 1800 W and for sediments 15 min at 180 °C and potency of 1200 W.

After digestion, samples were transferred to a 50 ml volumetric flask and topped with ultrapure water. Then, a portion of the sample was centrifuged and filtered with 0.45 µm syringe filters. Metals concentrations (Cd, Cr, Cu, Mn, Pb, Ni and Zn) were determined by ICP-MS (Agilent – 7500 cx) at the Center for Research on Environmental Pollution (CICA), Universidad de Costa Rica (UCR). Three blanks (5 ml HNO_3 + 1 ml HCl + 4 ml of ultrapure water) were digested and analyzed in the same way as the samples, also three replicates, one of each sample type, were analyzed. Concentrations are reported as dry weight (µg g^{-1} dwt) with a detection limit of 0.004 µg g^{-1} for all samples; concentration limit was 0.001 µg g^{-1}.

Bioconcentration factor

Algae and sponge metal concentrations were compared to those in sediments using the Bioconcentration Factor (BCF), calculated by dividing the mean metal concentration in the organisms by the mean metal concentration in the sediments (Walker et al., 2012). A BCF value of ≥ 1 was set as indicative of bioaccumulation (Mayzel et al., 2014).

Statistical analysis

Descriptive statistics were used to analyzed metal accumulation and contamination in Moín. Comparisons between sites and samples were developed by a linear model were metal concentrations were used as response variable and the sample type and site as explanatory variables. Significance ($p<0.05$) was analyzed by Tukey Multiple Comparison test (software R; version 3.4.2).

Results

Sediment grain size and organic matter content

The sediment grain size and its characterization are presented in Table 1.1. The sediments of Moín were calcareous in IP and RO, and mud and muddy sand in M/P. Probably the sediments found in M/P are influenced by the creek Quebrada Portete (Fig. 1.1) that enters in the small bay next to M/P sampling station. The sediments from the reference site were all sand. Regarding organic matter content, algae had higher values than sponges (74.32 ± 7.52 % and 31.29 ± 5.32 % respectively). When comparing sediment types, the higher values were measured in mud and muddy sand (8.59 ± 1.76 % and 7.25 ± 0.49 % respectively) while calcareous (3.71 ± 0.57 %) and sand (2.81 ± 0.09 %) had the lower scores (Annex 1).

Table 1.1. Sediment sample characterization in regard to grain size (Bouyoucos, 1962) and sediment type at the sampling stations of Moín (M): Isla Pájaros (IP), Moín/Portete (M/P) and Rompeolas (RO), and the reference site (SC): Punta Mona (PM).

Site	Sample Station	Grain size	Sediment type
M	RO	98 % sand, 2 % clay, 0 %silt	Calcareous
M	M/P	35 % sand, 6 % clay, 59 %silt	Mud
M	IP	98 % sand, 2 % clay, 0 %silt	Calcareous
M	IP	98 % sand, 2 % clay, 0 %silt	Calcareous
M	IP	98 % sand, 2 % clay, 0 %silt	Calcareous
M	M/P	76 % sand, 4 % clay, 20 %silt	Muddy Sand
M	RO	98 % sand, 2 % clay, 0 %silt	Calcareous
SC	PM	97 % sand, 3 % clay, 0 %silt	Sand
SC	PM	97 % sand, 3 % clay, 0 %silt	Sand

Moín metal concentrations

Metal concentrations found in algae, sponge and sediment samples at each sampling station in Moín are shown in Figures 1.2A, B (Annex 1). The ranking of concentrations for algae was Mn>Cu>Zn>Ni>Cr>Pb>Cd, sponge Mn>Cu>Zn>Ni>Cr>Cd>Pb and sediments Mn>Cu>Zn>Cr>Ni>Pb>Cd.

Metal concentrations in algae were lower or in the same range as the other samples. Only Cu concentrations were significantly higher ($p<0.05$) in algae than in sediments (Fig. 1.2A). Sponge samples, had higher concentrations of Cd and Ni. Sediment samples had greater mean concentrations of Cr, but the difference was only significant in regard to algae samples (Fig. 1.2A). Mn concentrations were significantly higher in sediment samples, with a mean concentration of 2.6 and 4 times higher than algae and sponge, respectively. The highest Mn concentration found was as high as 766 $\mu g\ g^{-1}$ dwt in sediments of RO sampling station (Fig. 1.2B).

Metal concentration patterns across the sampling stations were similar. However, Cr, Pb and Zn had significantly higher concentrations in sediments at M/P. Cd had significantly higher concentrations in sponge at IP. This suggests that possible variations in metal concentrations in Moín are related to sample rather than location, and that the difference in the concentrations found in sediments of M/P can be related to the higher organic matter content on this samples (Table 1.1). Pb and Zn concentrations patterns were similar for all samples and sampling stations except for previously mentioned high concentrations at M/P in sediment samples.

No significant difference was found between metal concentrations and the sampling dates, which suggest that the concentrations found are stable and does not change during short time periods. However, higher concentrations in individual samples were observed in March 2015, for example Cd in sponges of IP and RO, Cu in sediments of M/P and algae of M/P and RO, Ni in sediments of M/P and sponges of IP and RO, Mn in sediments and algae of M/P and RO, Pb in sediments of M/P and Zn in sediments of M/P and algae of M/P and RO (Fig. 1.2A, B).

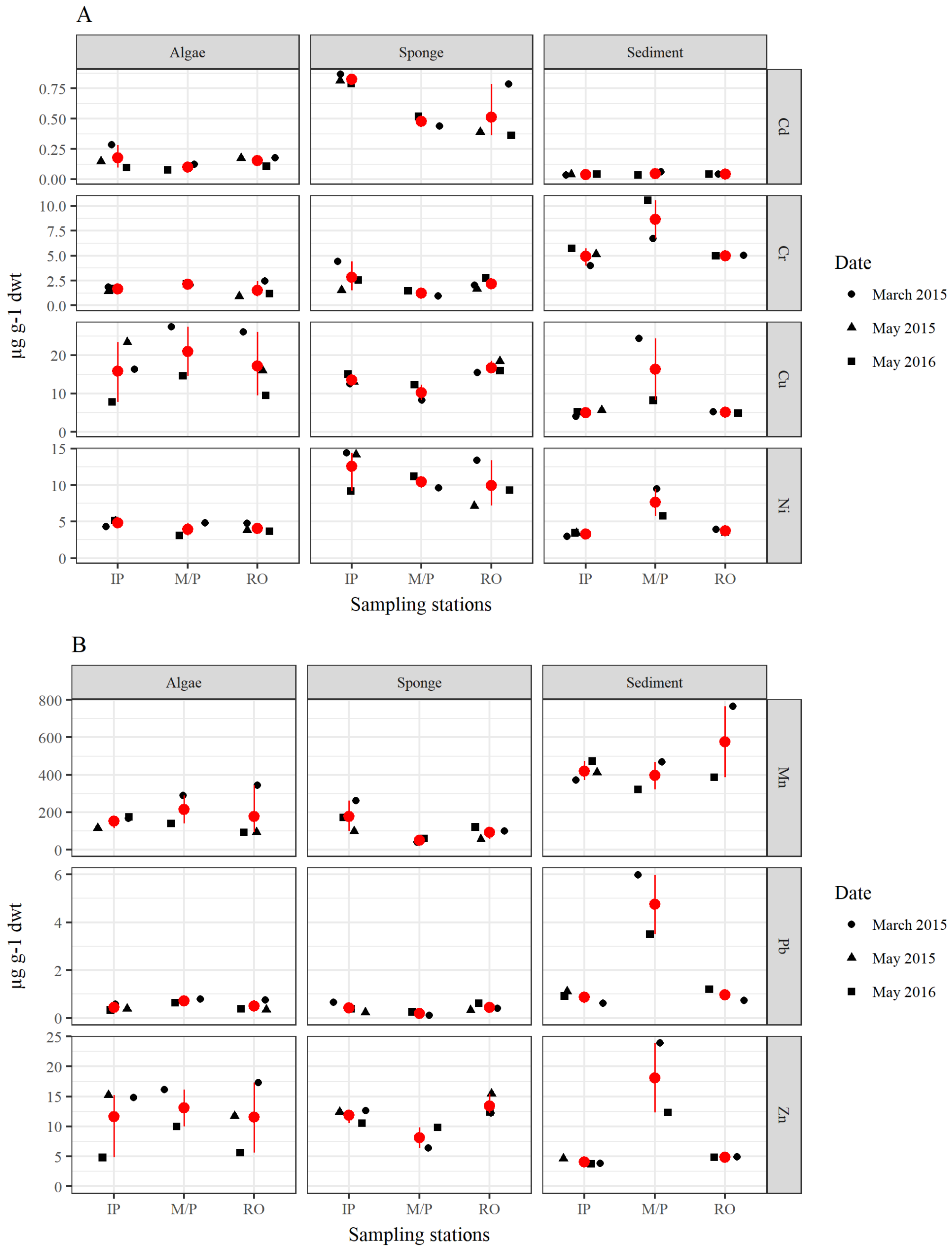


Fig. 1.2. Measured concentrations of (A) Cd, Cr, Cu and Ni and (B) Mn, Pb and Zn, in algae, sponge and sediment samples in the three sampling stations: Isla Pájaros (IP), Moín/Portete (M/P) and Rompeolas (RO) of Moín with the sampling date at which each sample was taken. Samples concentrations are showed in black and the means with standard deviation in red.

Metal concentrations comparison between Moín and the reference site

There were not significant differences ($p < 0.05$) in the metal concentrations between the study and reference site in any of the matrices sampled (Fig. 1.3). Comparisons showed a tendency were the higher mean metal concentrations were found mainly at Moín, with the exception of Cu, Pb and Zn in sediment and algae samples, where the mean concentrations were higher at the reference site (SC).

There was a recognizable concentration pattern of each metal in regard to the sample types, for instance, Cd and Ni mean concentrations were significantly higher ($p < 0.05$) in sponge samples and very similar in algae and sediment, but Cr, Mn and Pb mean concentrations were significantly higher ($p < 0.05$) in sediments samples. Pb mean concentrations were significantly higher ($p < 0.05$) in sediments at the reference site. Cu and Zn concentrations were very similar between samples and sites, no pattern of concentrations was identified as in the other metals and there were no significant differences found (Fig. 1.3). Additionally, the data suggest that the possible variations in metal concentrations are related to the sample type rather than the location were samples are taken.

Bioconcentration Factor (BCF)

The BCF was positive for Cd, Cr, Ni and Zn (BCF > 1) in algae and sponge. Cu and Zn only had BCF > 1 in samples of Moín. The maximum BCF value recorder was for Cd (14.9) in the sponge samples at Moín. The BCF values in algae were Cd>Cu>Zn>Ni and in sponge Cd>Ni>Cu>Zn. Being Cd the metal with higher BCF levels. Cr, Mn and Pb had BCF below 1 in the sampled organisms (Table 1.2).

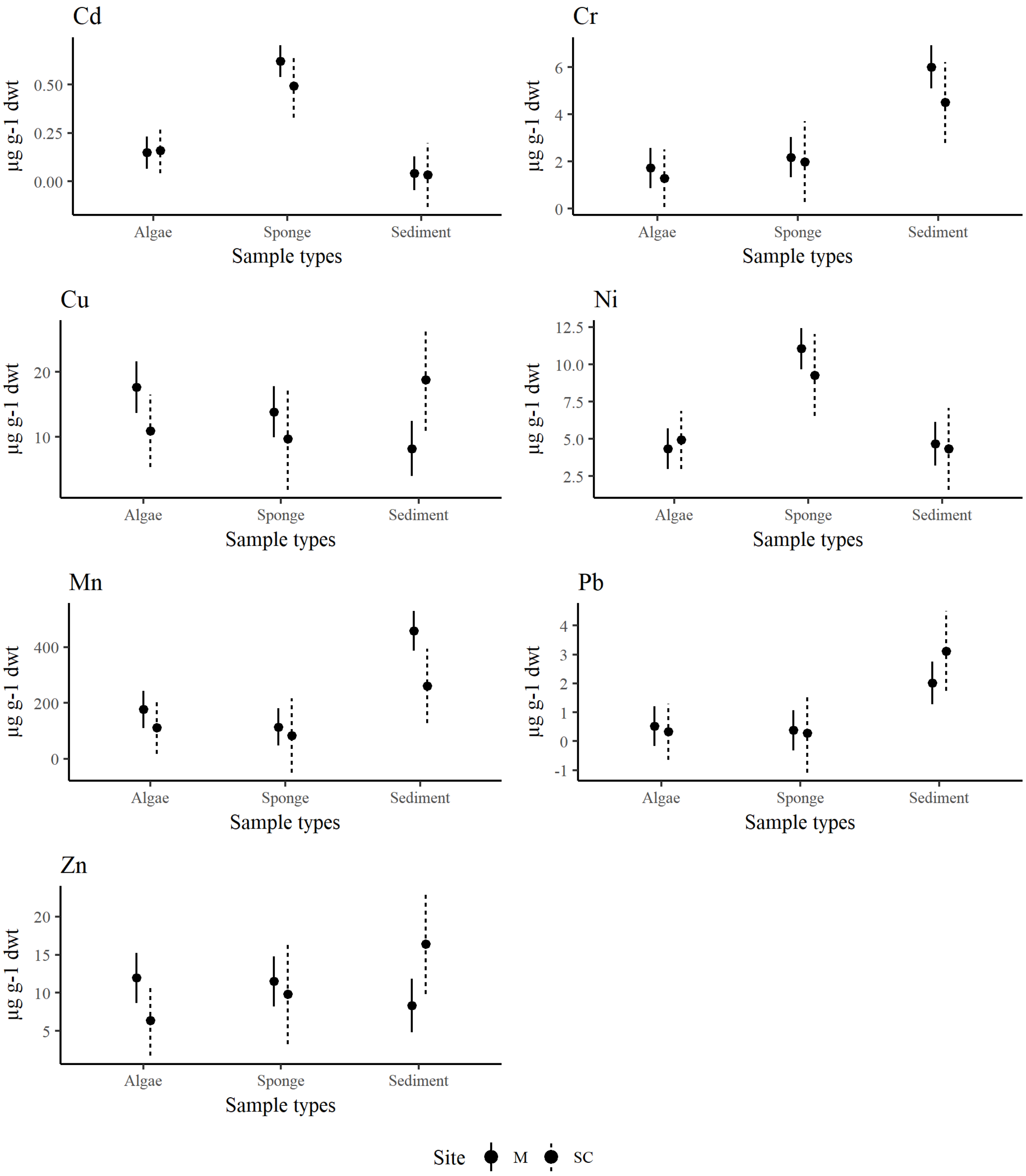


Fig. 1.3. Lineal analysis of mean metal concentrations, with the standard deviations, among the sample types: algae, sponge and sediments samples of Moín (M) and the reference site (SC).

Table 1.2. Bioconcentration Factors (BCFs) of metals in algae (A, *Cryptonemia crenulata*) and sponge (S, *Cinachyrella kuekenthali*) of the study (M) and the reference (SC) sites.

Metal		**Cd**		**Cr**		**Cu**		**Ni**		**Mn**		**Pb**		**Zn**	
Sample type		A	S	A	S	A	S	A	S	A	S	A	S	A	S
Sites	**M**	3.6*	14.9*	0.3	0.4	2.1*	1.7*	0.9*	2.4*	0.4	0.2	0.3	0.2	1.4*	1.4*
	SC	4.9*	15.1*	0.3	0.4	0.6	0.5	1.1*	2.1*	0.4	0.3	0.1	0.1	0.4	0.6

* BCF >1

Sediment metal accumulation analysis

Sediment concentrations were compared to the NOAA Sediment Quality Guidelines (SQG) (Long et al., 1995; NOAA, 1999). Comparisons showed that all concentrations found in our samples were below the limits of the Effect Range-Low (ERL), concentrations at or above which adverse effects rarely occur on marine organisms, and the Effect Range-Media (ERM), concentrations at or above which adverse effects frequently occur (Long et al., 1995) (Table 1.3).

Only one metal contamination study has been carried out in the central and South Caribbean region of Costa Rica. Guzmán and Jiménez (1992) studied metal accumulation in sediments and coral skeletons. Their results show no significant difference in the sediments metal concentrations of both sites, similar to the tendency that we found. However, the present study found lower sediment concentrations of Cd, Cr, Ni, Pb and Zn but higher concentrations for Cu in the South Caribbean at almost double high concentrations of Mn in Moín. (Table 1.3).

Table 1.3. Sediment mean metal concentrations (µg g-1 dwt) at Moín (M) and reference (SC) sites comparison with the mean values of metal concentrations in sediments in Guzmán and Jiménez (1992) and the ERL and ERM values of the SQG. All values are given in µg g-1 dwt.

Metal	**This study**		**Guzmán & Jiménez (1992)**		**SQG***	
	M	SC	Portete*	Manzanillo**	ERL	ERM
Cd	0.04[a]	0.03[a]	7.3	5.7	1.2	9.6
Cr	6.01[a]	4.51[a]	22.6	10.8	81	370
Cu	8.22	18.82[b]	8.4	7.9	34	270
Mn	457[b]	261[a]	268	271.5	-	-
Pb	2.01[a]	3.12[a]	34.5	25.2	46.7	218
Ni	4.67[a]	4.32[a]	102.8	89	20.9	51.6
Zn	8.31[a]	16.38[a]	14.7	26.4	150	410

[a] Lower concentrations than Guzmán & Jiménez (1992)
[b] Higher concentrations than Guzmán & Jiménez (1992)
* Same region than M/P sampling station
**Same region than ManZ sampling station
*** Long et al. (1995)

Discussion

Moín is the most impacted site by human activities on the Costa Rican Caribbean coast (Acuña-González et al., 2004; García-Céspedes et al., 2004). Our results showed that metal concentrations on sediments and biota of this site are no different from the ones in the South Caribbean, regardless of its consideration as a relatively pristine site due to its low population, no ports and category as a protected area. A previous study (Guzmán and Jiménez, 1992) also demonstrated that there were not consistent differences in metal concentrations between the impacted and the pristine areas of the Caribbean, suggesting that there is a wide range of pollution sources and very effective dispersal mechanisms that maintain the metals well distributed in the region. Currents and winds are the primary mechanisms affecting pollutants distribution in Moín coral reefs ecosystems. The currents in the Caribbean coastline move from the northwest to the south-southeast, forming small eddies, favoring sediment removal and transport in some areas, and deposition in others along to the entire coast (Cortés, 2016).

In most cases, the concentrations of metals we measured in this study are lower than those reported previously for this region or other regions considered as polluted areas, except for Mn that is higher compared to other studies (Guzmán and Jiménez, 1992; Fatoki and Mathabatha, 2001; Fernandez et al., 2007). All metals analyzed were also below the effects thresholds of the SQG (Long et al., 1995) which means no biological adverse effects should be expected. Nonetheless, this SQG values are not corrected to the environmental conditions of the study region and do not consider additive stress or other conditions that could increase toxicity (Whitall et al., 2015), and from an ecotoxicological point of view, low concentrations do not mean completely risk free. When a metal becomes bioavailable in the environment, even at low concentrations, it can be taken by different organisms and be biomagnified, increasing the possibility of having adverse biological effects on higher trophic levels (Sijm et al., 2007).

Cd is considered to be an extremely toxic metal, however, unlike in freshwater, the abundance of chloride in seawater forms thermodynamically stable complex that reduces its bioavailability and toxicity (Hudspith et al., 2017). Cd has been proven to have less toxic effects on corals than other metals, but it can cause reduce fertilization success in some corals species (Reichelt-Brushett and Harrison, 2005) and can inhibit sperm mobility and cause ultrastructural damage to sea urchins (Au et al., 2000). In algae, Cd can produce oxidative stress even at sub-letal concentrations (Encina-Montoya et al., 2017), but in sponges Cd is an essential element, however its role in the sponge is yet unknown (Mayzel et al., 2014)

Cu has become a new alternative to antifouling paints for ships and boats. Biocides such as copper oxide (Cu_2O) and copper thiocyanate (CuSCN), are intended to be environmentally less harmful than tributyltin (TBT) biocides (Amara et al., 2018). Despite its lipophilic characteristics, studies have shown that Cu can be very toxic to marine organisms even at low concentrations and specially in locations with high boat traffic (Pait et al., 2007). Cu can cause fertilization inhibition on corals (Heyward, 1988), growth and photosynthetic inhibition in macro algae (Babu et al., 2014; Amara et al., 2018), larvae and embryonic development disruption and survival reduction on invertebrates and fishes (Amara et al., 2018).

Zn has also been proven to cause fertilization inhibition on stone coral species (Heyward, 1988; Reichelt-Brushett and Harrison, 2005), and root anatomy alteration on mangrove seedlings (Bayen, 2012). Ni does not tend to be highly toxic, however in high concentrations it can cause reduce fertilization success and larval settlement of corals (Reichelt-Brushett and Harrison, 2005), and also reduce fertilization, larval and juvenile production, lacerate development and diminution of offspring production in other organisms as anemones, sea urchins, copepods and polychaetes (Reichelt-Brushett and Hudspith, 2016).

Cd and Ni levels in sponge samples were higher than those in sediments or algae, while Cu, Mn and Pb levels were higher in sediments samples than those in algae or sponge. According to our results, Cd, Cu, Zn and Ni are relevant pollutants due to their bioaccumulation, their possible toxicity, but also because a chronic low-level contamination (possibly happening in the region), than can reduce resilience of reef organisms to other environmental stressors such as temperature, sedimentation, acidification and other pollutants (van Damm et al., 2011). Some of these metals become more toxic when combined with other metals or organic compounds, evaluations of mixture effects are need to determine the risk in coral reefs.

Mn was the metal with the highest concentrations. Mn is one of the most abundant and widely distributed metals in nature (Pinsino et al., 2012), and the concentrations found in our study are average Mn concentrations for marine sediments (Howe et al., 2004). However, individual analysis showed Mn concentrations as high as 766 µg g-1 in some sediment samples of Moín, and when compared to other studies, our Mn levels tend to be higher (Guzmán and Jiménez, 1992; Fatoki and Mathabatha, 2001; Pait et al., 2007; Naidu et al., 2012; Whitall et al., 2015; El-Metwally et al., 2017). The high Mn concentrations found could be related to the intense use of mancozeb fungicide on banana plantations in lands near the coast. Mancozeb fungicides contains approximately 20% Mn (van Wendel de Joode et al., 2016), and around 1.3 million kilograms of mancozeb are applied annually in banana plantations in Costa Rica (Mora et al., 2014). van Wendel de Joode et al. (2016) found Mn and derivative Mn compounds in soil and drinking water from villages situated near banana plantations, demonstrating that mancozeb use is an important source for Mn pollution. It has been shown that sediments from banana plantations drain to the Caribbean coast. (Cortés and Risk, 1985; Roder et al., 2009). Also, Mn could enter the marine environment by wastewater discharges (Howe et al., 2004), which could be a pollution source for Moín considering the population growth and the lack of sewage treatment. There is only one local treatment plant in all the Caribbean region and with no treatment for inorganic substances (Ruiz, 2012).

Metals concentrations in sediments and biota showed no significant temporal or local distribution variations, but it had significant differences in metal accumulation in regard to sample type. The BCF results (when BCF > 1) suggest that metal accumulation in biota could be related to the metal presence in the sediments from the study and the reference site (Mayzel et al., 2014). Metal concentrations were higher in sponges compared to algae. However, BCFs were positive for Cd, Cu, Ni and Zn in both organisms. The BCFs for Cd in sponges were the highest observed. Some metals, under certain environmental and chemical conditions, can be oxidized, reduced or complexed, forming chemical species with a different degree of assimilation or toxicity or even making them not bioavailable (García-Céspedes et al., 2004). As well, high concentrations of metals can limit or inhibit the uptake of other metals since the transport channels can become saturated by some metals reducing the accumulation of others (Encina-Montoya et al., 2017).

Accumulation processes are different for algae and sponge. Macroalgae take metals mainly from seawater, by electrostatic attraction or active uptake (Morrison et al., 2015; Fostier et al., 2016; Encina-Montoya et al., 2017). However, metal resuspension from sediments can be an important secondary source to the water column (Covelli et al., 2012) were metals are more likely to be available for algae to accumulate, and thus, correlations between metal concentrations in sediments and algae are possible (Akcali and Kucuksezgin, 2011; Signa et al., 2017). It is also important to consider that algae species can exhibit different affinities towards different metals and that the metal accumulation can vary in different portions of the algae (Astorga et al., 2008; Akcali and Kucuksezgin, 2011). In this case, we analyzed the whole organism to obtain mean concentrations in the algae, but further considerations could be made in the future to study different portions of the algae and other species. On the other hand, sponge filtration feeding enables the metal uptake from a variety of sources like inclusion of sediments and large substrate particles, micro-detritus, dissolve organic matter (DOM) by feeding or by active uptake (Mayzel et al., 2014; Rix et al., 2016). This shows that Cd, Cu, Ni and Zn, are bioavailable in the coral reefs ecosystems, but more information is needed in order to link the bioaccumulation found to a sediment exposure source.

Both algae and sponge are food source for invertebrates and fish (Akcali and Kucuksezgin, 2011), and some sponge species are important food sources for immature hawksbill turtles (*Eretmochelys imbricata*) (van Dam and Diez, 1997). Metal accumulation in sponges represent a risk of biomagnification in this turtle, thus toxic effects could be detrimental for its population and even for humans (Gardner et al., 2006), because many coastal communities, in different Caribbean regions, eat turtle eggs or its meat (Ross et al., 2017). Sponges also play an important role in the organic matter cycling on the Caribbean corals reefs by the “sponge loop” (Rix et al., 2016), where sponges transform DOM, produced mainly by corals and macroalgae (Rix et al., 2018), into particulate organic matter (POM) which is food for benthic detritivores and other organisms (Rix et al., 2016). Pollutants such as metals could be biomagnified through the sponge loop into different trophic levels.

Low metal concentrations in sediments found in this study could be related to analytical method differences, sample types, variations in the sampling seasons or to changes in levels of pollution discharge over time. Regulatory changes like the elimination of lead additives use in fuels since 1996 (RECOPE, 2016) could have had a positive effect on reducing metal pollution. Biogenous sediments, mainly composed of eroded corals and various calcareous remains from marine organisms, as most of our samples, are generally associated with low metal accumulation (Pan et al., 2011). As well, technology improvements, associated with regulatory advances, have resulted in metal contamination decreasing in different marine areas of the world (OSPAR, 2010).

In future studies it is important to consider using other sampling options, like sediment traps or using a broad range of species with different characteristics, to evaluate different metal bioaccumulation processes. Accumulation and toxic effects of metals vary selectively among species and factors such as life span, morphology, contact surface area and growth rate (Batista et al., 2014; Chakraborty et al., 2014; Sánchez-Quiles et al., 2017).

Conclusions

Our study provides the first database of metal concentrations in a macroalgae and a sponge from the Costa Rican Caribbean, and constitutes a baseline information for future studies. Significant differences in metal concentrations and bioacumulation demonstrated that the species studied, *Cryptonemia crenulata* and *Cinachyrella kuekenthali*, are suitable bioindicator organisms to monitor metal contamination in the Caribbean coral reefs. The metal concentrations in biota and sediments, though comparatively low, are indicative of metal bioaccumulation in algae and sponge. Regular monitoring will be necessary to prevent exceeding the safe limits due to increased port activities, which are already occurring in the region. Possible toxic levels of the bioconcentrated metals (Cd, Cu, Ni and Zn) cannot be compared to the environmental levels due to absence of ecotoxicological information about these organisms or other coral reef species and the environmental conditions of the region. Consequently, the possible effects and risks to which the ecosystems are exposed are unknown, creating a need to back up the chemical monitoring with toxicity assays.

References

Acuña-González, J., Vargas-Zamora, J.A., Gómez-Ramírez, E., García-Céspedes, J., 2004. Hidrocarburos de petróleo, disueltos y dispersos, en cuatro ambientes costeros de Costa Rica. Rev. Biol. Trop. 52, 43–50. https://doi.org/https://doi.org/10.15517/rbt.v54i1.26828

Akcali, I., Kucuksezgin, F., 2011. A biomonitoring study: Heavy metals in macroalgae from eastern Aegean coastal areas. Mar. Pollut. Bull. 62, 637–645. https://doi.org/10.1016/j.marpolbul.2010.12.021

Alutoin, S., Boberg, J., Nyström, M., Tedengren, M., 2001. Effects of the multiple stressors copper and reduced salinity on the metabolism of the hermantypic coral Porites lutea. Mar. Environ. Res. 52, 289–299.

Alvarado, J., Fernández, C., Nielsen, V., 2006. Arrecifes y comunidades coralinas, in: Nielsen, V., Quesada, M. (Eds.), Ambientes Marino Costeros de Costa Rica. Comisión Interdisciplinaria Marino Costera de la Zona Económica Exclusiva de Costa Rica, pp. 51–68.

Amara, I., Miled, W., Slama, R. Ben, Ladhari, N., 2018. Antifouling processes and toxicity effects of antifouling paints on marine environment. A review. Environ. Toxicol. Pharmacol. 57, 115–130. https://doi.org/10.1016/j.etap.2017.12.001

Araya-Vargas, A., 2017. Pautas para la conservación y el uso de las comunidades de esponjas en los parches arrecifales del Caribe Sur de Costa Rica. M.Sc. Thesis, Universidad Nacional, Heredia, Costa Rica. https://doi.org/10.13140/RG.2.2.13901.54247

Astorga, M.S., Calisto, N.C., Guerrero, S., 2008. Baseline concentrations of trace metals in macroalgae from the Strait of Magellan, Chile. Bull. Environ. Contam. Toxicol. 80, 97–101. https://doi.org/10.1007/s00128-007-9323-3

Au, D.W.T., Chiang, M.W.L., Wu, R.S.S., 2000. Effects of cadmium and phenol on motility and ultrastructure of sea urchin and mussel spermatozoa. Arch. Environ. Contam. Toxicol. 38, 455–463. https://doi.org/10.1007/s002449910060

Babu, M.Y., Palanikumar, L., Nagarani, N., Devi, V.J., Kumar, S.R., Ramakritinan, C.M., Kumaraguru, A.K., 2014. Cadmium and copper toxicity in three marine macroalgae:

Evaluation of the biochemical responses and DNA damage. Environ. Sci. Pollut. Res. 21, 9604–9616. https://doi.org/10.1007/s11356-014-2999-0

Batista, D., Muricy, G., Rocha, R.C., Miekeley, N.F., 2014. Marine sponges with contrasting life histories can be complementary biomonitors of heavy metal pollution in coastal ecosystems. Environ. Sci. Pollut. Res. 21, 5785–5794. https://doi.org/10.1007/s11356-014-2530-7

Bayen, S., 2012. Occurrence, bioavailability and toxic effects of trace metals and organic contaminants in mangrove ecosystems: A review. Environ. Int. 48, 84–101. https://doi.org/10.1016/j.envint.2012.07.008

Bernecker, A., 2009. Part 2: Marine benthic algae, in: Wehrtmann, I.S., Cortés, J. (Eds.), Marine Biodiversity of Costa Rica, Central America. Springer + Business Media B.V., San José, pp. 109–117.

Berry, K.L.E., Seemann, J., Dellwig, O., Struck, U., Wild, C., Leinfelder, R.R., 2013. Sources and spatial distribution of heavy metals in scleractinian coral tissues and sediments from the Bocas del Toro Archipelago, Panama. Environ. Monit. Assess. 185, 9089–9099. https://doi.org/10.1007/s10661-013-3238-8

Bouyoucos, G.J., 1962. Hydrometer method improved for making particle size analysis of soils. Agron. J. 54, 464–465. https://doi.org/10.2134/agronj1962.00021962005400050028x

CCT, 2013. Estudio de Impacto Ambiental Proyecto Terminal de Contenedores Moín: expediente No. 7968-12-SETENA. Centro Científico Tropical. San José, Costa Rica.

Chakraborty, S., Bhattacharya, T., Singh, G., Maity, J.P., 2014. Benthic macroalgae as biological indicators of heavy metal pollution in the marine environments: A biomonitoring approach for pollution assessment. Ecotoxicol. Environ. Saf. 100, 61–68. https://doi.org/10.1016/j.ecoenv.2013.12.003

CNC, 2017. Terminal de Contenedores de Moín [WWW Document]. Com. -Nacional Concesiones, MOPT. URL http://www.cnc.go.cr/index.php/proyectos/en-desarrollo/tcm (accessed 6.13.18).

Cortés, J., 2016. The Caribbean coastal and marine ecosystems, in: Kappelle, M. (Ed.), Costa Rican Ecosystems. University of Chicago Press, Chicago and London, pp. 591–617.

Cortés, J., 2009. Stony corals, in: Wehrtmann, I.S., Cortés, J. (Eds.), Marine Biodiversity of Costa Rica, Central America. Springer + Business Media B.V., Berlin, pp. 169–173.

Cortés, J., Jiménez, C.E., 2003. Past, present and future of the coral reefs of the Caribbean coast of Costa Rica, in: Cortés, J. (Ed.), Latin American Coral Reefs. Elsevier Science B.V., Amsterdam, pp. 223–239.

Cortés, J., Jiménez, C.E., Fonseca, A.C., Alvarado, J.J., 2010. Status and conservation of coral reefs in Costa Rica. Rev. Biol. Trop. 58, 33–50. https://doi.org/10.15517/rbt.v58i1.20022

Cortés, J., Risk, M.J., 1985. A reef under siltation stress: Cahuita, Costa Rica. Bull. Mar. Sci. 36, 339–356.

Cortés, J., Van Der Hal, N., Van Soest, R.W.M. Van, 2009. Sponges, in: Wehrtmann, I.S., cort (Eds.), Marine Biodiversity of Costa Rica, Central America. Springer + Business Media B.V., San José, pp. 137–142.

Cortés, J., Wehrtmann, I.S., 2009. Diversity of marine habitats of the Caribbean and Pacific of Costa Rica, in: Wehrtmann, I.S., Cortés, J. (Eds.), Marine Biodiversity of Costa Rica, Central America. Springer + Business Media B.V., Berlin, pp. 1–45. https://doi.org/10.1007/978-1-4020-8278-8_1

Cortés, J., Wehrtmann, I.S., 2005. Costa Rica, in: Miloslavich, P., Klein, E. (Eds.), Caribbean Marine Biodiversity: The Known and the Unknown. DEStech Publishing Inc., Lancaster, Pennsylvania, pp. 169–179.

Covelli, S., Protopsalti, I., Acquavita, A., Sperle, M., Bonardi, M., Emili, A., 2012. Spatial variation, speciation and sedimentary records of mercury in the Guanabara Bay (Rio de Janeiro, Brazil). Cont. Shelf Res. 35, 29–42. https://doi.org/10.1016/j.csr.2011.12.003

Davis, A.R., de Mestre, C., Maher, W., Krikowa, F., Broad, A., 2014. Sponges as sentinels: Metal accumulation using transplanted sponges across a metal gradient. Environ. Toxicol. Chem. 33, 2818–2825. https://doi.org/10.1002/etc.2747

Echeverría-Sáenz, S., Pinnock, M., de la Cruz, E., Herrera, G., Ugalde, R., Vargas, S., 2015. Report: Estudio de la contaminación y eco-toxicología en el Río Parismina. Instituto Regional de Estudios en Sustancias Tóxicas, Universidad Nacional, Heredia, Costa Rica.

El-Metwally, M.E.A., Madkour, A.G., Fouad, R.R., Mohamedein, L.I., Eldine, H.A.N., Dar, M.A., El-Moselhy, K.M., 2017. Assessment the Leachable Heavy Metals and Ecological Risk in the Surface Sediments inside the Red Sea Ports of Egypt. Int. J. Mar. Sci. 7, 214–228. https://doi.org/10.5376/ijms.2017.07.0023

Elias, S.A., 2018. Loss of coral reefs, in: Dellasala, D.A., Goldstein, M.I. (Eds.), Encyclopedia of the Anthropocene. Elsevier, Oxford, pp. 245–258. https://doi.org/10.1016/B978-0-12-809665-9.09917-1

Encina-Montoya, F., Vega-Aguayo, R., Díaz, O., Esse, C., Nimptsch, J., Muñoz-Pedreros, A., 2017. Mazzaella laminarioides and Sarcothalia crispata as possible bioindicators of heavy metal contamination in the marine coastal zone of Chile. Environ. Monit. Assess. 189, 584. https://doi.org/10.1007/s10661-017-6297-4

Fatoki, O.S., Mathabatha, S., 2001. An assessment of heavy metal pollution in the East London and Port Elizabeth harbours. Water SA 27, 233–240. https://doi.org/10.4314/wsa.v27i2.4997

Fernandez, A., Singh, A., Jaffé, R., 2007. A literature review on trace metals and organic compounds of anthropogenic origin in the Wider Caribbean Region. Mar. Pollut. Bull. 54, 1681–1691. https://doi.org/10.1016/j.marpolbul.2007.08.007

Fostier, A.H., do N. Costa, F., Korn, M. das G.A., 2016. Assessment of mercury contamination based on mercury distribution in sediment, macroalgae, and seagrass in the Todos os Santos bay, Bahia, Brazil. Environ. Sci. Pollut. Res. 23, 19686–19695. https://doi.org/10.1007/s11356-016-7163-6

García-Céspedes, J., Acuña-González, J., Vargas-Zamora, J.A., 2004. Metales traza en sedimentos costeros de Costa Rica. Rev. Biol. Trop. 52, 51–60.

García-Seoane, R., Fernández, J.A., Villares, R., Aboal, J.R., 2018. Use of macroalgae to biomonitor pollutants in coastal waters : Optimization of the methodology. Ecol. Indic. 84, 710–726. https://doi.org/10.1016/j.ecolind.2017.09.015

Gardner, S.C., Fitzgerald, S.L., Vargas, B.A., Rodríguez, L.M., 2006. Heavy metal accumulation in four species of sea turtles from the Baja California peninsula, Mexico. BioMetals 19, 91–99. https://doi.org/10.1007/s10534-005-8660-0

Gómez, P., 2003. Esponjas marinas del golfo de México y el Caribe, 1st ed. Agt editor S.A, 70-71.

Guzmán, H.M., García, E.M., 2002. Mercury levels in coral reefs along the Caribbean coast of Central America. Mar. Pollut. Bull. 44, 1415–1420. https://doi.org/10.1016/S0025-326X(02)00318-1

Guzmán, H.M., Jiménez, C.E., 1992. Contamination of coral reefs by heavy metals along the Caribbean coast of Central America (Costa Rica and Panama). Mar. Pollut. Bull. 24, 554–561. https://doi.org/10.1016/0025-326X(92)90708-E

Halpern, B.S., Walbridge, S., Selkoe, K.A., Kappel, C.V., Micheli, F., D'Agrosa, C., Bruno, J.F., Casey, K.S., Ebert, C., Fox, H.E., Fujita, R., Heinemann, D., Lenihan, H.S., Madin, E.M.P., Perry, M.T., Selig, E.R., Spalding, M., Steneck, R., Watson, R., 2008. A global map of human impact on marine ecosystems. Science (80-.). 319, 948–952. https://doi.org/10.1126/science.1149345

Heyward, A.J., 1988. Inhibitory effects of copper and zinc sulphates on fertilization in corals, in: Choat, J.H. (Ed.), Proceedings of the 6th International Coral Reef Symposium. 6th International Coral Reef Symposium Executive Committee, Townsville, pp. 299–303.

Hoegh-Guldberg, O., 1999. Climate change, coral bleaching and the future of the world's coral reefs. Mar. Freshw. Res. 50, 839–866. https://doi.org/10.1071/MF99078

Howe, P.D., Malcolm, H.M., Dobson, S., 2004. Manganese and Its Compounds: Environmental Aspect, WHO Concise International Chemical Assessment Document 63. Geneva.

Hudspith, M., Reichelt-Brushett, A., Harrison, P.L., 2017. Factors affecting the toxicity of trace metals to fertilization success in broadcast spawning marine invertebrates: A review. Aquat. Toxicol. 184, 1–13. https://doi.org/10.1016/j.aquatox.2016.12.019

Hughes, T.P., Barnes, M.L., Bellwood, D.R., Cinner, J.E., Cumming, G.S., Jackson, J.B.C., Kleypas, J., vn de Leemput, I.A., Lough, J.M., Morrison, T.H., Palumbi, S.R., van Nes, E.H., Scheffer, M., 2017. Coral reefs in the Anthropocene. Nature 546, 82–90. https://doi.org/10.1038/nature22901

INEC, 2015. VI Censo Nacional Agropecuario: Resultados Generales, 1st ed. Instituto Nacional de Estadísticas y Censos, San José, Costa Rica.

JAPDEVA, 2008. Plan Maestro para el complejo portuario Limón - Moín. Junta de Administración Portuaria y de Desarrollo Económico de la Vertiente Atlántica de Costa Rica. Limón, Costa Rica. https://doi.org/9R4672.21 /R/401180/Nijm

Long, E., MacDonald, D., Smith, S., Calder, F., 1995. Incidence of adverse bilogical effects within ranges of chemical concentrations in marine and estuarine sediments. Environ. Manage. 19, 81–97. https://doi.org/10.1007/BF02472006

Mayzel, B., Aizenberg, J., Ilan, M., 2014. The Elemental Composition of Demospongiae from the Red Sea , Gulf of Aqaba. PLoS One 9, e95775. https://doi.org/10.1371/journal.pone.0095775

MINAE & IMN, 2013. Descripción del Clima Cantón de Limón. Ministerio de Ambiente y Energía & Instituto Meteorológico Nacional. San José, Costa Rica.

Mora, A.M., van Wendel de Joode, B., Mergler, D., Córdoba, L., Cano, C., Quesada, R., Smith, D.R., Menezes-Filho, J.A., Lundh, T., Lindh, C.H., Bradman, A., Eskenazi, B., 2014. Blood and hair manganese concentrations in pregnant women from the Infants' Environmental Health Study (ISA) in Costa Rica. Environ. Sci. Technol. 48, 3467–3476. https://doi.org/10.1021/es404279r

Morrison, R.J., Peshut, P.J., West, R.J., Lasorsa, B.K., 2015. Mercury (Hg) speciation in coral reef systems of remote Oceania: Implications for the artisanal fisheries of Tutuila, Samoa Islands. Mar. Pollut. Bull. 96, 41–56. https://doi.org/10.1016/j.marpolbul.2015.05.049

Naidu, A.S., Blanchard, A.L., Misra, D., Trefry, J., Dasher, D., Kelley, J.J., Venkatesan, M., 2012. Historical changes in trace metals and hydrocarbons in nearshore sediments, Alaskan Beaufort Sea, prior and subsequent to petroleum-related industrial development: Part I. Trace metals. Mar. Pollut. Bull. 64, 2177–2189. https://doi.org/10.1016/j.marpolbul.2012.07.037

Negri, A.P., Smith, L.D., Webster, N.S., Heyward, A.J., 2002. Understanding ship-grounding impacts on a coral reef: Potential effects of anti-foulant paint contamination on coral recruitment. Mar. Pollut. Bull. 44, 111–117. https://doi.org/10.1016/S0025-326X(01)00128-X

NOAA, 2009. Coral Reef Conservation Program: International Strategy 2010-2015. National Oceanic and Atmospheric Administration. Silver Spring, MD.

NOAA, 1999. Sediment Quality Guidelines dveloped for the National Status and Trends Program. National Oceanic and Atmospheric Administration. Silver Spring, MD.

OSPAR, 2010. Hazardous substances, in: Quality Status Report 2010. OSPAR Commision, London, pp. 37–52.

OzCoast, 2012. Coastal indicators [WWW Document]. Met. Contam. URL http://www.ozcoasts.gov.au/indicators/metal_contaminants.jsp# (accessed 5.14.18).

Padovan, A., Munksgaard, N., Alvarez, B., Mcguinness, K., Parry, D., Gibb, K., 2012. Trace metal concentrations in the tropical sponge Spheciospongia vagabunda at a sewage outfall : synchrotron X-ray imaging reveals the micron-scale distribution of accumulated metals. Hydrobiologia 687, 275–288. https://doi.org/10.1007/s10750-011-0916-9

Pait, A.S., Whitall, D.R., Jeffrey, C.F., Caldow, C., Mason, A.L., Christensen, J.D., Monaco, M., Ramirez, J., 2007. An Assessment of Chemical Contaminants in the Marine Sediments of Southwest Puerto Rico. NOAA/ NOS/Center for Coastal Monitoring and Assessment. Silver Spring, MD.

Pan, K., Lee, O.O., Qian, P.Y., Wang, W.X., 2011. Sponges and sediments as monitoring tools of metal contamination in the eastern coast of the Red Sea, Saudi Arabia. Mar. Pollut. Bull. 62, 1140–1146. https://doi.org/10.1016/j.marpolbul.2011.02.043

Peters, E.C., Gassman, N.J., Firman, J.C., Richmond, R.H., Power, E.A., 1997. Ecotoxicology of tropical marine ecosystems. Environ. Toxicol. Chemestry 16, 12–40.

Pinsino, A., Matranga, V., Roccheri, M.C., 2012. Manganese: a new emerging contaminant in the environment, in: Environmental Contamination. Jatin Srivastava, pp. 17–36. https://doi.org/10.5772/31438

Rao, J. V, Srikanth, K., Pallela, R., Rao, T.G., 2009. The use of marine sponge , Haliclona tenuiramosa as bioindicator to monitor heavy metal pollution in the coasts of Gulf of Mannar , India. Environ. Monit. Assess. 156, 451–459. https://doi.org/10.1007/s10661-008-0497-x

RECOPE, 2018. Ampliación de la terminal portuaria petrolera del atlántico [WWW Document]. Ficha Proy. URL https://www.recope.go.cr/proyectos/procesos-industriales-portuarios/ampliacion-de-la-terminal-portuaria-petrolera-del-atlantico/ (accessed 5.14.18).

RECOPE, 2016. Gasolina sin plomo desde hace dos décadas [WWW Document]. URL https://www.recope.go.cr/gasolina-sin-plomo-desde-hace-dos-decadas/ (accessed 5.14.18).

Reichelt-Brushett, A.J., Harrison, P.L., 2005. The effect of selected trace metals on the fertilization success of several scleractinian coral species. Coral Reefs 24, 524–534. https://doi.org/10.1007/s00338-005-0013-5

Reichelt-Brushett, A.J., Hudspith, M., 2016. The effects of metals of emerging concern on the fertilization success of gametes of the tropical scleractinian coral Platygyra daedalea. Chemosphere 150, 398–406. https://doi.org/10.1016/j.chemosphere.2016.02.048

Rix, L., de Goeij, J.M., Mueller, C.E., Struck, U., Middelburg, J.J., Van Duyl, F.C., Al-Horani, F.A., Wild, C., Naumann, M.S., van Oevelen, D., 2016. Coral mucus fuels the sponge loop in warm-and cold-water coral reef ecosystems. Sci. Rep. 6, 1–11. https://doi.org/10.1038/srep18715

Rix, L., de Goeij, J.M., van Oevelen, D., Struck, U., Al-Horani, F.A., Wild, C., Naumann, M.S., 2018. Reef sponges facilitate the transfer of coral-derived organic matter to their associated fauna via the sponge loop. Mar. Ecol. Prog. Ser. 589, 85–96. https://doi.org/10.3354/meps12443

Roder, C., Cortés, J., Jiménez, C., Lara, R., 2009. Riverine input of particulate material and inorganic nutrients to a coastal reef ecosystem at the Caribbean coast of Costa Rica. Mar. Pollut. Bull. 58, 1922–1952. https://doi.org/10.1016/j.marpolbul.2009.08.027

Ross, D.A.N., Guzmán, H.M., Potvin, C., van Hinsberg, V.J., 2017. A review of toxic metal contamination in marine turtle tissues and its implications for human health. Reg. Stud. Mar. Sci. 15, 1–9. https://doi.org/10.1016/j.rsma.2017.06.003

Ruiz, F., 2012. Gestión de las Excretas y Aguas Residuales en Costa Rica. Instituto Costarricense de Acueductos y Alcantarillados. San José, Costa Rica.

Sánchez-Quiles, D., Marbà, N., Tovar-Sánchez, A., 2017. Trace metal accumulation in marine macrophytes: Hotspots of coastal contamination worldwide. Sci. Total Environ. 576, 520–527. https://doi.org/10.1016/j.scitotenv.2016.10.144

Scullion, D., Masterton, M., 2000. Rhodophyta, in: D.S. Littler (Ed.), Caribbean Reef Plants. Off Shore Graphics Inc., Washington, pp. 100–101.

Signa, G., Mazzola, A., Di Leonardo, R., Vizzini, S., 2017. Element-specific behaviour and sediment properties modulate transfer and bioaccumulation of trace elements in a highly-contaminated area (Augusta Bay, Central Mediterranean Sea). Chemosphere 187, 230–239. https://doi.org/10.1016/j.chemosphere.2017.08.099

Sijm, D.T.H.M., Rikken, M.G.J., Rorije, E., Traas, T.P., McLAchlan, M.S., Peijnenburg, W.J.G.M., 2007. Transport, accumulation and transformation processes, in: van Leeuwen, C.J., Vermeire, T.G. (Eds.), Risk Assessment of Chemicals: An Introduction. Springer + Business Media B.V., Dordrecht, pp. 73–158. https://doi.org/10.1007/978-1-4020-6102-8_3

van Dam, J.W., Negri, A.P., Uthicke, S., F. Mueller, J.F., 2011. Chemical Pollution on Coral Reefs: Exposure and Ecological Effects, in: Sánchez-Bayo, F., van den Brink, P.J., Mann, R.M. (Eds.), Ecological Impacts of Toxic Chemicals. Bentham Science Publishers Ltd, Townsville, pp. 187–211. https://doi.org/10.2174/978160805121210187

van Dam, R.P., Diez, C.E., 1997. Diving behaviour of immature hawksbills (Eretmochelys imbricata) in a Caribbean reef habitat. Coral Reefs 16, 133–138. https://doi.org/10.1007/s003380050067

Van Soest, R.W.M., Boury-Esnault, N., Hooper, J.N.A., Rützler, K., de Voogd, N.J., Alvarez, B., Hajdu, E., Pisera, A.B., Manconi, R., Schönberg, C., Klautau, M., Picton, B., Kelly, M., Vacelet, J., Dohrmann, M., Díaz, M.C., Cárdenas, P., Carballo, J.L., Ríos, P., Downey, R., 2018. World Porifera database. Cinachyrella kuekenthali (Uliczka, 1929) [WWW Document]. URL http://www.marinespecies.org/aphia.php?p=taxdetails&id=171308 (accessed 6.15.18).

van Wendel de Joode, B., Barbeau, B., Bouchard, M.F., Mora, A.M., Skytt, Å., Córdoba, L., Quesada, R., Lundh, T., Lindh, C.H., Mergler, D., 2016. Manganese concentrations in drinking water from villages near banana plantations with aerial mancozeb spraying in Costa Rica: Results from the Infants' Environmental Health Study (ISA). Environ. Pollut. 215, 247–257. https://doi.org/10.1016/j.envpol.2016.04.015

Walker, C.H., Sibly, R.M., Hopkin, S.P., Peakall, D.B., 2012. The Fate of Metals and Radioactive Isotopes in Contaminated Ecosystems, in: Principles of Ecotoxicology. CRC Press, Boca Raton, pp. 49–58.

Whitall, D., Pait, A., Hartwell, S.I., 2015. Chemical contaminants in surficial sediment in Coral and Fish Bays, St. John, U.S. Virgin Islands. Mar. Environ. Res. 112, 1–8. https://doi.org/10.1016/j.marenvres.2015.08.001

Wood, J.C., 2015. Determination on moisture content and total organic carbon within basin environments: Loss-on-Ignition, in: Clarke, L.E., Nield, J.M. (Eds.), Geomorphical Techniques. Brithish Society for Geomorphology, London.

XVII. Artículo II: Acumulación de mercurio total (THg) en sedimentos, macroalgas (*Cryptonemia crenulata*) y esponjas (*Cinachyrella kuekenthali*) del ecosistema coralino de Moín, Limón, Costa Rica

Accumulation of total mercury (THg) in sediments, macroalgae (*Cryptonemia crenulata*) and sponge (*Cinachyrella kuekenthali*) of a coral reef in Moín, Costa Rica: An ecotoxicological approach

Sustentante

Susana María Méndez Alfaro

Artículo con el formato de la revista Marine Pollution Bulletin

Accumulation of total mercury (THg) in sediments, macroalgae (*Cryptonemia crenulata*) and sponge (*Cinachyrella kuekenthali*) of a coral reef in Moín, Costa Rica: An ecotoxicological approach

Susana María Méndez Alfaro

Maestría Académica en Ecotoxicología Tropical. Instituto Regional de Estudios en Sustancias Tóxicas. Universidad Nacional, Costa Rica

Abstract Mercury contamination represents a risk to coral reefs because of its high toxicity, persistence and biomagnification capacity. The coral reefs in Moín, Caribbean coast of Costa Rica, are exposed to a variety of anthropogenic activities like increasing urban developments, commercial and industrial activities, and the presence of one of the most important commercial ports and the location of the only oil port and oil refinery of the country. Therefore, total mercury (THg) concentrations were determined in sediments, macroalgae (*Cryptonemia crenulata*) and sponge (*Cinachyrella kuekenthali*), collected from October 2014 and May 2016 at Moín and at a reference site (South Caribbean-SC). The Bioconcentration Factor (BCF) of THg was calculated in both organisms to evaluate accumulation. Also, we compared our data with previous studies, Sediment Quality Guidelines (SQG) and the Geoaccumulation Index (I_{geo}) to evaluate sediment pollution and possible ecotoxicologial effects. THg concentrations in algae were very similar between the study and the reference site (28 ng g^{-1} dwt). Sponge samples had the highest THg accumulation of all samples, ranging from 100 to 192 ng g^{-1} dwt at Moín and from 85 to 211 ng g^{-1} dwt at the reference site. Sediment samples had similar THg concentrations as macroalgae at Moín. Sediment THg concentrations were below SQG and the I_{geo} indicated that sediments at Moín and at the reference site were uncontaminated.

Keywords: Contamination, ecotoxicology, mercury, Caribbean, bioaccumulation

Introduction

Mercury (Hg) is a metal known for its high toxicity on different organisms from a wide variety of ecosystems. It has been recognized as a pollutant of global concern (Ramos et al., 2009; GESAMP, 2013) due to its presence in all environmental compartments, high persistence, capability of long distance transport and its ability to form methylmercury (MeHg) - the most toxic form of Hg (Henriques et al., 2015). This organic-metallic species facilitates its mobility into the trophic chains, becoming bioavailable to organisms and biomagnified in the marine food web (Ramos et al., 2009; UNEP, 2014).

Hg can be released to the environment from either natural or anthropogenic sources. Natural Hg is released by breakdown of minerals and soil through exposure to wind and water, from volcanic sources and from forest fires (UNEP, 2013). The natural release of Hg has remained fairly constant throughout time, but the recent increase found in the environment is commonly attributed to human activities (Lamborg et al., 2014). Most of the Hg released from human activities is related to air emissions by activities such as fossil fuel combustion, coal burning, artisanal gold mining, smelting and production of iron and non-ferrous metals, cement production, Hg compounds pesticides, oil refining and wastes from consumer products including fluorescent lamps, thermometers and others (Ramos et al., 2009; UNEP, 2013). Mercury contamination of marine ecosystems, comes primarily through atmospheric deposition, river runoff, coastal erosion, and domestic effluents (Brown and Depledge, 1998; UNEP, 2014; OSPAR, 2016).

Methylmercury is known for its neurotoxic effects and systemic damage in humans and other organisms (Chand et al., 2008; Morrison et al., 2015). However, in coral reefs the effects are not entirely understood due to the high biodiversity of these ecosystems and the lack of toxicity data on such species. Depending on the coral species and the level of Hg contamination, effects can include bleaching, tissue death, polyp retraction, decreased photosynthetic efficiency and fertilization decline due to inhibition of spawning, gamete production and larval metamorphosis (van Damm et al., 2011; Hudspith et al., 2017). Despite the toxic potential of Hg and the ecological importance of corals as foundation species on coral reefs, the data on Hg accumulation in coral reefs or its toxicity on regionally-relevant tropical species is quite limited, especially for Costa Rica, where only one study on Hg contamination has been done (Guzmán & García, 2002). In that study, they found relatively high levels of THg in sediments and biota in the Costa Rica Caribbean coral reefs, and suggested that Hg is widely distributed in the region and that this contamination may have affected the reef ecosystem.

The Caribbean coast of Costa Rica as very rich marine biodiversity with a variety of ecosystems like coral reefs, mangroves, seagrass beds and others (Cortés and Wehrtmann, 2009). There are three coral reef formations along the 212 km of the Caribbean coast, one between Moín and Limón (the most populated towns of the region), other at the Cahuita National Park (the most developed coral reef of the Costa Rican Caribbean) and the last one is located from Puerto Viejo to Punta Mona (Cortés and Jiménez, 2003; Cortés and Wehrtmann, 2009; Cortés, 2016). Moín, is located in the Central Caribbean of Costa Rica, is characterized as a multi-use coastal zone, with a variety of commercial, industrial, and urban activities. The only oil port of the country is located there is in constant operation, and in expansion since 2012 (RECOPE, 2018). The only oil refinery and primary distribution center of oil products in the country, and the commercial port where approximately 75% of the country’s goods are moved are in Moín (JAPDEVA, 2008). Also, since 2015 a new commercial port is being built in Moín, with and extension of 78.6 ha of marine land to be filled (CCT, 2013; CNC, 2017).

Soil erosion and riverine transport to the ocean is a principal pathway for marine Hg contamination (UNEP, 2015). Many of the rivers of Costa Rica are loaded with suspended sediments (Cortés and Risk, 1985) from soil erosion caused by vegetation loss, cultivation of mono-cultures, and the tropical humid conditions of the country with frequent rainfall (Alvarado and Mata, 2016). It has been found that levels of Hg are elevated in the Costa Rican soils because of volcanic activities (Castillo et al., 2011). Additionally, other possible sources for Hg pollution are related to port activities, such as dredging, ships repairing using Hg based paints, shipping, loading and effluent oil discharge (Guzmán and Jiménez, 1992; El-Metwally et al., 2017). Also to pesticide use, wastewater effluents commonly discharged into the sea without previous treatment (Ruiz, 2012). Moín and the coral reefs there could be considered at risk of Hg contamination.

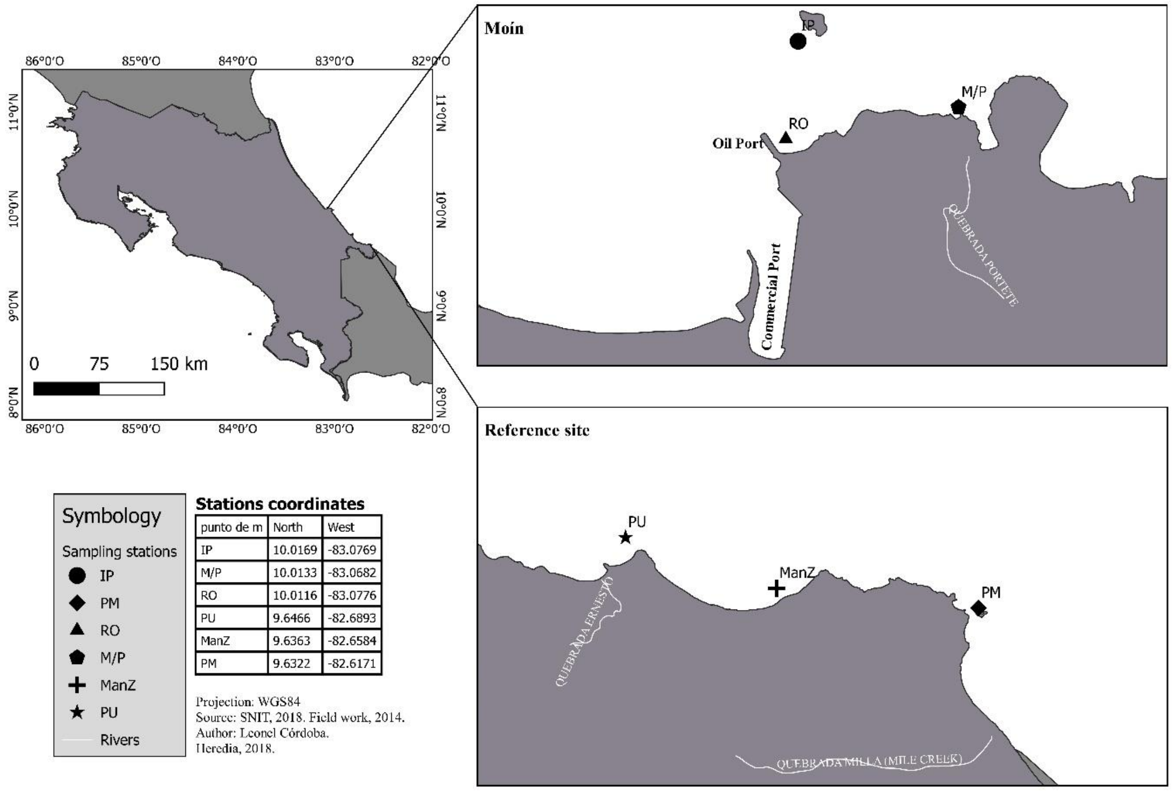
For this reason, the objectives of the present study were: (1) identify the present level of total Hg (THg) in a coral reef area near Moín by assessing the accumulation in bottom sediments and using the macroalgae (*Cryptonemia crenulata*) and the sponge (*Cinachyrella kuekenthali*) as bioindicators of contamination, (2) compare the Hg concentrations of Moín with a putative less polluted site (reference site) in the southern Caribbean coast of Costa Rica, and (3) evaluate the data with an ecotoxicological approach by analyzing the Hg bioaccumulation, the contamination level, the species utility as bioindicators, and the possible toxic risks to which the organisms are exposed.

Materials and methods

This study was developed in the frame of the study “Accumulation of metals (Cd, Cr, Cu, Mn, Pb, Ni, Zn) in sediments, macroalgae (*Cryptonemia crenulata*) and sponge (*Cinachyrella kuekenthali*) of a coral reef in Moín, Limón, Costa Rica: An ecotoxicological approach”. Whence, the detail description of the study and reference site, bioindicator species, sample collection and preparation and results of organic matter analysis are given in the article "Accumulation of metals (Cd, Cr, Cu, Mn, Pb, Ni, Zn) in sediments, macroalgae (*Cryptonemia crenulata*) and sponge (*Cinachyrella kuekenthali*) of a coral reef in Moín, Limón, Costa Rica: An ecotoxicological approach".

Description of Moín and the reference site (South Caribbean)

The study site in Moín (M) is located within the direct influence area (24 489 m^2 of marine zone) of the project “Expansion of the Atlantic Oil Port Terminal” (ATPPA, in Spanish), developed by the Oil Refinery Company of Costa Rica (RECOPE, in Spanish), close to the coral reef of Moín-Limón (Cortés, 2016). Three sample stations where selected inside the ATPPA, (1) Isla Pájaros (IP), south west of the island, where wave action is lower, (2) Moín-Portete (M/P), in the coastal reef band between Moín and Portete and (3) Rompeolas (RO), wavebreaker located in the east side of the oil port. The reference site is located in the South Caribbean (SC) region, where also three sampling stations were selected (all located within the protected area of the Gandoca-Manzanillo National Wildlife Refuge, with presumably lower anthropogenic pressure or pollution than Moín), (1) Punta Uva (PU), located in the reef in front of Punta Uva beach, (2) Manzanillo (ManZ), located in the reef off Manzanillo beach, and (3) Punta Mona (PM), located in the farthest site from human activities in the Costa Rica Caribbean (Fig. 2.1).



Stations coordinates

punto de m	North	West
IP	10.0169	-83.0769
M/P	10.0133	-83.0682
RO	10.0116	-83.0776
PU	9.6466	-82.6893
ManZ	9.6363	-82.6584
PM	9.6322	-82.6171

Projection: WGS84
Source: SNIT, 2018. Field work, 2014.
Author: Leonel Córdoba.
Heredia, 2018.

Fig. 2.1. Geographical locations of the three sampling stations at Moín; IP: Isla Pájaros, M/P: Moín-Portete, RO: Rompeolas; and at the reference site (South Caribbean); PU: Punta Uva, ManZ: Mazanillo, PM: Punta Mona; Costa Rica.

Bioindicators selection

To identify the general conditions of the reefs and to select possible bioindicators, three recognition field trips were done, two in the study site and one in the control site. We selected the macroalgae (also mentioned as algae) *Cryptonemia crenulata* and the sponge *Cinachyrella kuekenthali* as bioindicator organisms, two abundant and common species in the Costa Rica Caribbean (Bernecker, 2009; Cortés et al., 2009; Araya-Vargas, 2017). Macroalgae and sponge species are recognized as good bioindicators of metal pollution in marine environments (Rao et al., 2009; Padovan et al., 2012; Chakraborty et al., 2014; Davis et al., 2014; García-Seoane et al., 2018).

Sample collection and preparation

Samples were collected between October 2014 and May 2016, comprising high and low precipitation seasons, by SCUBA diving at depths ranging from 3 to 12 m. Seven field trips were done, fourth to Moín and three to the reference site. For May 2015 at MP and RO sampling was precluded due to bad diving conditions. From a total of 159 individual samples from the seven field trips, we obtained 20 algae, 17 sponge and 19 sediment composed samples to be analyzed. All samples were lyophilized at -80° C for 48 h and then pulverized in a ceramic mortar. Two separate portions of the samples were used organic matter content analyses. The organic matter content was determined by the Loss on Ignition (LOI) method (Wood, 2015) and sediment samples were characterized by grain size using the Bouyocous method (Bouyoucos, 1962). The samples were sent to the Center for Earth Observation Science (CEOS), University of Manitoba, Canada, to be analyzed for THg. Prior to analysis, the samples were sieved using a standard 300 µm metallic sieve.

Chemical analysis

Analysis was done by the Direct Mercury Analysis (DMA) method using a Teledyne Hydra II Atomic Absorption equipment. Aliquots of 0.1 g of each sample were placed into the trays of the equipment. The program was run and the samples were decomposed at 800 °C. THg was detected and quantified by atomic absorption spectroscopy. Standard reference materials (NIST2709a, PACS-2, NIST2976, MESS-3) were run every 10 samples for quality assurance and quality control. Concentrations are reported in dry weight basis (µg g^{-1} dwt) with a detection limit of 0.25 ng g^{-1} for all samples.

Bioconcentration factor

Algae and sponge THg concentrations were compare to those in sediments by the bioconcentration factor (BCF), calculated by dividing the mean THg concentration in the organisms by the mean THg concentration in the sediments (Walker et al., 2012). A BFC value ≥ 1 was set as indicative of bioaccumulation (Mayzel et al., 2014).

Geoaccumulation index

The Geoaccumulation Index (I_{geo}) is used as a criterion to assess the sediment Hg contamination intensity with a corresponding baseline level as reference (Green-Ruiz et al., 2005; El Zrelli et al., 2015; Fostier et al., 2016). It is calculated using the formula suggested by Müller (1969):

$$I_{geo} = \mathrm{Log}_2\,(C_{Hg}/1.5B_{Hg})$$

Where C_{Hg} is the concentration of Hg in the sediment sample, B_{Hg} is the Hg baseline level, and 1.5 is the established correction factor. Guzmán and García. (2002), proposed a Hg baseline of 71 ng g^{-1} for the marine sediments of the Central America Caribbean, which was used as the B_{Hg} in this study. The I_{geo} classifies seven contamination qualitative classes: uncontaminated ($I_{geo} \leq 0$), uncontaminated to moderately contaminated ($0 < I_{geo} \leq 1$), moderately contaminated ($1 < I_{geo} \leq 2$), moderately to heavily contaminated ($2 < I_{geo} \leq 3$), heavily contaminated ($3 < I_{geo} \leq 4$), heavily to extremely contaminated ($4 < I_{geo} \leq 4$), extremely contaminated ($Igeo > 5$) (Müller, 1969; Fostier et al., 2016).

Statistical analysis

Descriptive statistics were used to analyze metal accumulation and contamination in the study and the reference site. Comparisons between sites and samples were developed by a linear model were THg concentrations were used as response variable and the sample type and site as explanatory variables. Significance ($p < 0.05$) was analyzed by multiple comparison test of Tukey (software R; version 3.4.2).

Results

Sediment grain size and organic matter content

We combined the results of the grain size data and an observational characterization to determined five sediment types: calcareous, fine calcareous, sand, muddy sand and mud (Table 2.1). The sediments of Moín had 4 of the 5 sediment types found. IP sampling station had only calcareous sediments, the RO had sand and calcareous sediments and the M/P had muddy sand and mud sediments. Probably the sediments found in M/P are influenced by the creek Quebrada Portete (Fig. 2.1) that enters in the small bay next to M/P sampling station. In the reference site, PU had calcareous and fine calcareous sediments, ManZ also had calcareous and fine calcareous sediments and PM had only sand sediments. The sampling date had no relation in regard to the sediment type. Algae had higher organic matter content than sponge samples and in the sediment samples the mud and the muddy sand had the higher organic matter content.

Table 2.1. Sediment sample characterization in regard to grain size (Bouyoucos, 1962) and observational description at the sampling stations of Moín (M): Isla Pájaros (IP), Moín-Portete (M/P) and Rompeolas (RO), and the reference site (SC): Punta Uva (PU) Manzanillo (ManZ) and Punta Mona (PM).

Site	Sample Station	Grain size	Sediment type
M	IP	98 % sand, 2 % clay, 0 %silt*	Calcareous
M	M/P	76 % sand, 4 % clay, 20 %silt	Muddy Sand
M	RO	97 % sand, 3 % clay, 0 %silt	Sand
M	RO	98 % sand, 2 % clay, 0 %silt*	Calcareous
M	M/P	35 % sand, 6 % clay, 59 %silt	Mud
M	IP	98 % sand, 2 % clay, 0 %silt*	Calcareous
M	IP	98 % sand, 2 % clay, 0 %silt*	Calcareous
M	IP	98 % sand, 2 % clay, 0 %silt*	Calcareous
M	M/P	76 % sand, 4 % clay, 20 %silt	Muddy Sand
M	RO	98 % sand, 2 % clay, 0 %silt*	Calcareous
SC	PM	97 % sand, 3 % clay, 0 %silt	Sand
SC	ManZ	98 % sand, 2 % clay, 0 %silt*	Calcareous
SC	PU	98 % sand, 2 % clay, 0 %silt*	Calcareous
SC	PM	97 % sand, 3 % clay, 0 %silt	Sand
SC	PU	98 % sand, 2 % clay, 0 %silt**	Fine Calcareous
SC	ManZ	98 % sand, 2 % clay, 0 %silt**	Fine Calcareous
SC	PM	97 % sand, 3 % clay, 0 %silt	Sand
SC	PU	98 % sand, 2 % clay, 0 %silt*	Calcareous
SC	ManZ	98 % sand, 2 % clay, 0 %silt**	Fine Calcareous

*Sediments with big calcareous fractions
**Sediments with fine calcareous fractions

THg concentrations in sediments and organisms at the sampling stations and sites

THg concentrations found in algae, sponge and sediments of Moín and the reference site at each sampling station are shown in Figure 2.2 (Annex 1). The overall concentration range for algae samples was 15.3 – 42.3 ng g $^{-1}$ dwt, 85.5 – 211.0 ng g $^{-1}$ dwt for sponge and 3.8 – 50.5 ng g $^{-1}$

dwt for sediments, showing that sponges had higher THg concentrations than algae and sediments, at both Moín and the reference site. At Moín, sponge samples showed higher THg concentrations for May 2015 and 2016 sampling dates on the M/P and RO, and the lowest on March 2015 in all sampling stations. At the reference site the highest THg sponge concentration was found in PM in October 2014. Sediment samples of M/P had higher THg mean concentrations than in the other sampling stations, probably because of the higher organic matter and small grain size of the samples (Table 2.1). However, there were no significant differences regard to sampling stations and sampling dates in all samples. Overall algae and sediment individual THg concentrations had low variability, especially at the reference site were sediment concentrations varied only between 3.76 and 6.40 ng g^{-1} dwt (Fig. 2.2).

Statistical comparison by sites showed higher THg mean concentration in sponges in the reference site compared to Moín ($p < 0.05$). There was no significant difference between Moín and the reference site in THg content of algae and sediment samples, however, sediments had higher THg mean concentrations at Moín and the lowest THg mean concentration of all samples evaluated at the reference site (Fig. 2.3).

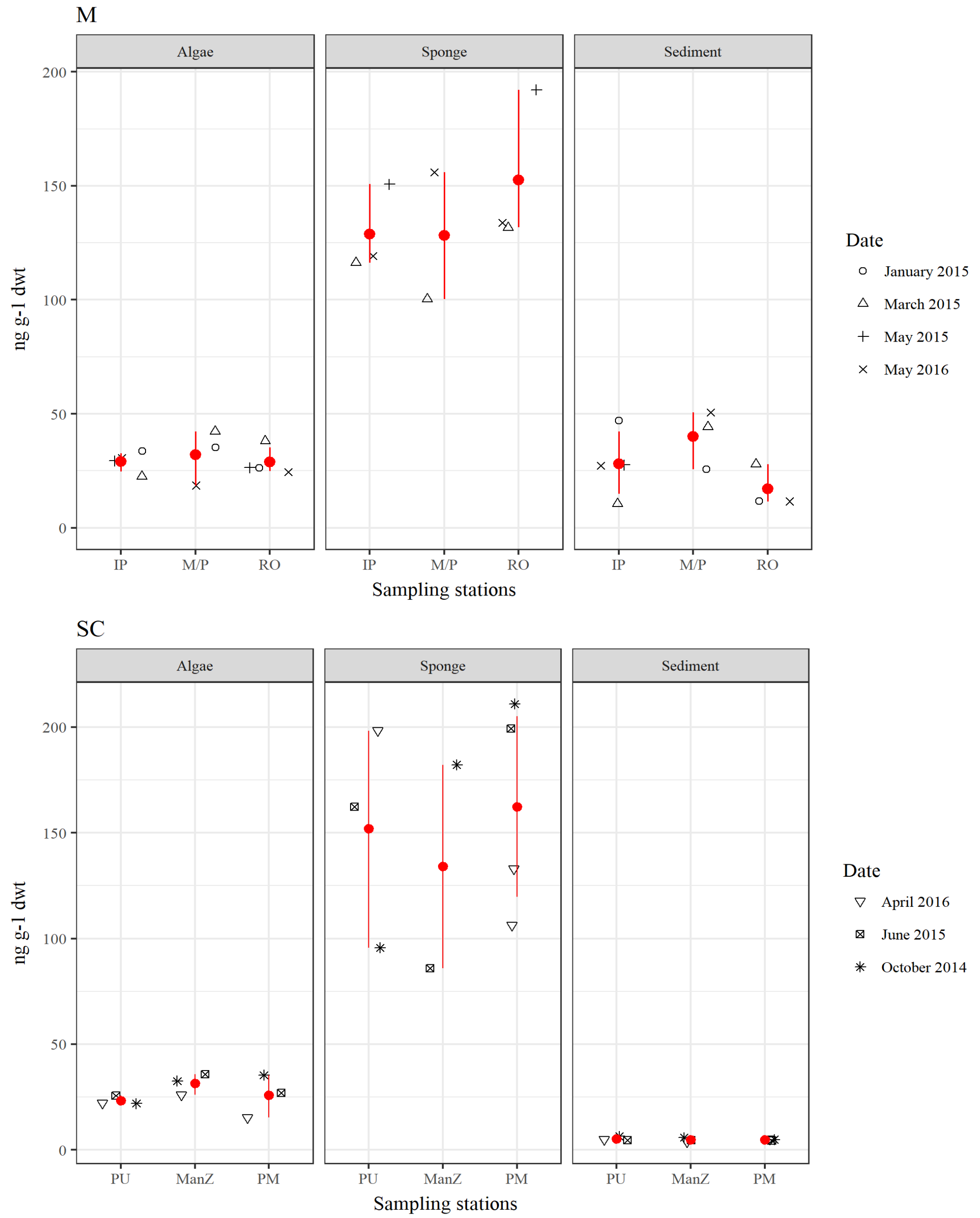


Fig. 2.2. Measured concentrations (ng g^{-1} dwt) of THg on algae, sponge and sediment samples at the sampling stations of Moín (M): Isla Pájaros (IP), Moín/Portete (M/P) and Rompeolas (RO), and of the reference site (SC): Punta Uva (PU) Manzanillo (ManZ) and Punta Mona (PM), with the sampling date at which each sample was taken. Samples concentrations are showed in black and the means with standard deviation in red.

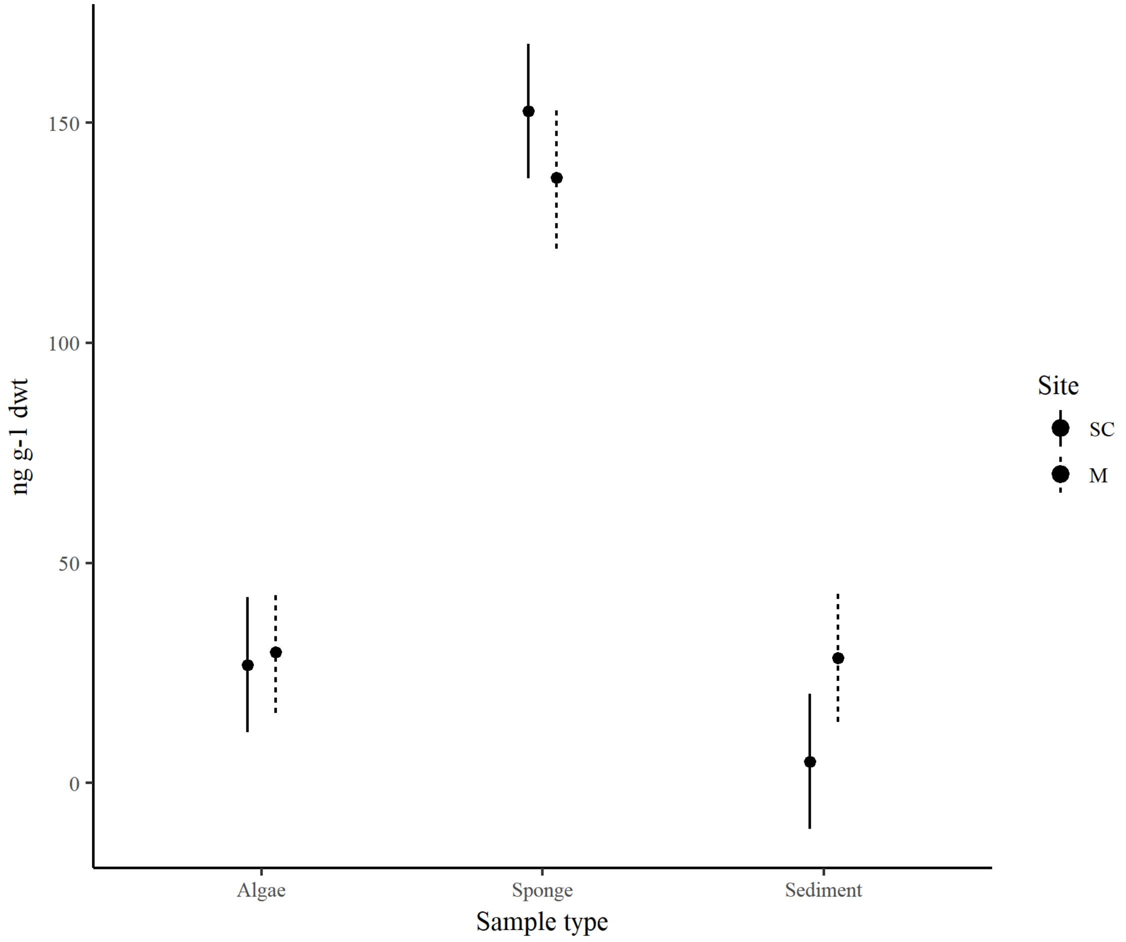


Fig. 2.3. Lineal analysis of THg concentrations among the sample types: algae, sponge and sediments samples of Moín (M) and the reference site (SC).

Bioconcentration Factor

BCFs were more than five times higher in organisms from the reference site compared to organisms from Moín. Among species, BCFs indicate that sponges accumulate six times more Hg than algae (Table 2.2).

Sediment quality guidelines and geoaccumulation index

Sediment concentrations were compared to the NOAA SQG (Long et al., 1995; NOAA, 1999); comparisons showed that all concentrations found in the sediment samples were below the limits of the Effect Range-Low (ERL), that indicates the concentrations below which adverse effects rarely occur to marine organisms, and the Effect Range-Media (ERM), that indicates the concentrations at or above which adverse effects frequently occur to marine organisms (Long et al., 1995) (Table 2.2). I_{geo} calculations classified the sediments of Moín and the reference site as uncontaminated by Hg ($I_{geo} \leq 0$) (Table 2.2).

Table 2.2. THg mean concentrations at Moín (M) and the reference site (SC) in all samples, values of the bioconcentration factors (BCFs) of THg in algae (*Cryptonemia crenulata*) and sponge (*Cinachyrella kuekenthali*) of both sites, values of the NOAA Sediment Quality Guidelines (SQG): ERL: Effect Range-Low, ERM: Effect Range-Median, and the sediments Igeo index results of both sites (M and SC). All concentrations are given in ng g $^{-1}$ dwt.

Sample type	**Average**		**BCF**		**SGQ****		I_{geo}	
	M	**SC**	**M**	**SC**	**ERL**	**ERM**	**M**	**SC**
Algae	29.8	26.9	1.0*	5.5*	-	-	-	-
Sponge	137.5	152.6	4.8*	31.1*	-	-	-	-
Sediment	28.4	4.9	-	-	150	710	-1.3	-3.9

*BCF ≥ 1
** (Long et al., 1995)

Discussion

In the Costa Rica Caribbean there are no Hg based industries or artisanal gold mining, as it happens in the Pacific coast (Reitz, 2007). However, indirect marine exposure through atmospheric deposition and river discharge of erosion materials are possibilities for the Hg presence in the region (Guzmán and García, 2002; Costa et al., 2012). The results showed, for all samples, no statistical difference in the Hg concentrations in regard to location or temporal variance (neither at high or low precipitation periods). Even if sediment samples at Moín had higher THg mean concentrations, the I_{geo} classified them as uncontaminated by Hg, and with no significant differences found between sites, no contamination hot spots could be identified. This might suggest an effective spreading mechanism for the pollutant (Guzmán and Jiménez, 1992; Guzmán and García, 2002), probably favored by currents and winds. Currents in the region move from the northwest to the south-southeast, forming small gyres, in the way, favoring sediment removal and transport, and through this considerations it is likely that Hg is being drag along to the entire coast (Cortés, 2016). However, when used a baseline from another Caribbean region as Brazil (Marins et al., 2004), were the Hg sediment concentrations are similar to the ones we found, the resultant I_{geo} values classified the Moín sediments as moderately contaminated by Hg. This indicates that, even at low concentrations, Hg contamination cannot be discard in Moín and reflects the need to further Hg monitoring.

Guzmán and García (2002) found, in the same region of Moín, higher concentrations of THg in sediments (99.4 ng g^{-1}) and lower concentrations in biota (15.2 ng g^{-1} in coral skeletons) compared to what we report in this assessment. Although methods and analytical resolution could differ among studies, differences in biota could be related to differences in accumulation processes specific to each species (Peters et al., 1997; Hédouin et al., 2011), and differences in sediment concentrations could be related to different sample types. In this regard, the accumulation of Hg is expected to be low in sediments of calcareous origin and low organic matter content as the ones we sampled (Pan et al., 2011).

We found positive Hg bioconcentration in both species sampled, being BCFs higher in sponges of the reference site. Mercury accumulation processes are different among these organisms, on one hand macroalgae take Hg and other metals mainly from the seawater matrix, by absorption through electrostatic attraction to negative sites on the surface of the algae or by active uptake through the cells membrane into the cytoplasm (Morrison et al., 2015; Fostier et al., 2016;

Encina-Montoya et al., 2017). On the other hand, sponge filtration feeding enables the Hg uptake from a variety of sources like inclusion of sediments and large substrate particles, micro-detritus, dissolved organic matter (DOM), by feeding or by active uptake (Mayzel et al., 2014; Rix et al., 2016). Sediments are the primary metal reservoirs on marine environments (El-Metwally et al., 2017; Jahan and Strezov, 2018) which supports the high accumulation observed in sponges. But also, remobilization processes represent an important secondary source of Hg in the water column (Covelli et al., 2012), which can correlate metal concentrations in sediments to bioconcentration in algae (Akcali and Kucuksezgin, 2011; Signa et al., 2017). Results shows that Hg is bioavailable in the coral reefs ecosystems of the Caribbean, and that sponges in the South Caribbean are accumulating more Hg than in Moín. However, the conditions favoring accumulation in this species are yet unknown and more information is needed to link the accumulation found to sediment as exposure source.

Mercury is mostly bioaccumulated when is transformed into methylHg (MeHg) (Costa et al., 2012; Thera and Rumbold, 2014), by methylation processes mediated by microorganisms such as the sulfate reducing bacteria (SRB) (Oliveira et al., 2015). Tropical conditions such as warm temperatures, high organic matter content, elevated bacteria activity and low dissolved oxygen, can favor the MeHg formation (Costa et al., 2012). According to our results, there are no correlations between higher organic matter content in the sediments and higher Hg concentrations in the sampled organisms, which means that methylation processes in sediments is probably not the main bioaccumulation route in these reef ecosystem and other sources need to be considered.

As mentioned, sponges use DOM as an important food source. The DOM in the medium is mostly produced by corals but can also be produced by macroalgae (Rix et al., 2018). It is possible that Hg is being transferred from corals and macroalgae to sponges, which could explain the higher THg concentrations found in sponge samples. Sponges transform a fraction of the DOM into particulate organic matter (POM) which is food for benthic detritivores and other organisms, this process is called the “sponge loop” and is key for the organic matter cycling on

Caribbean reefs (Rix et al., 2016). Pollutants such as Hg could be biomagnified through the sponge loop into different trophic levels. It is possible that the differences found in the THg sponge concentrations of the study and the reference site are related to sponge loop processes at each site. High THg concentrations found in sponge samples, up to 211 ng g^{-1} dwt, represent a risk for toxic effects on other organisms, for example the hawksbill turtles (*Eretmochelys imbricata*), that feeds on sponges (van Dam and Diez, 1997), could have been accumulating Hg in levels 5 times higher than the ones found in the sponge (Thera and Rumbold, 2014). Studies have found possible embryonic development threat from Hg exposure in hawksbill turtles (Dyc et al., 2015).

As sponges, macroalgae also are a food source for invertebrates and fish (Akcali and Kucuksezgin, 2011) and are considered as entrance ways for trace elements into marine food webs (Bonanno and Orlando-Bonaca, 2018) and thus a possible exposure source in the trophic chain. Concentrations detected in primary producers, as macroalgae, are consider as directly proportional to concentrations in the water column (Briand et al., 2018). It is also important to consider that Hg is not an essential element, thus any accumulation in biota is consider as contamination and a potential hazard (Berry et al., 2013). From the results, both organisms are considered good Hg bioindicators in coral reef ecosystems.

Hg concentrations in the sediment samples were below the effects thresholds of the SQG (Long et al., 1995), which means no biological adverse effects should be expected by sediment Hg exposition in the study and the reference site. However, this SQG values are not corrected to the environmental conditions of the study region and do not consider additive stress or other conditions that could increase toxicity (Whitall et al., 2015). As mentioned above, Hg is a priority substance for its high toxicity, persistence and its capacity to move through the food web (GESAMP (IMO/FAO/UNESCO-IOC/WMO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection), 2001; GESAMP, 2013; UNEP, 2013, 2014). Further monitoring, establishment of background Hg levels to the region and toxicity studies on local species are needed to evaluate the ecological risk of Hg in the coral reefs of the Costa Rica Caribbean.

Conclusions

This study provides the first data of Hg concentrations in algae and sponge organisms of the Costa Rica Caribbean, and constitutes a baseline information for future studies. THg concentrations are higher in sponges compared to algae and sediments, and the presence of this contaminant in these organisms makes it bioavailable to other species. The algae *Cryptonemia crenulata* and the sponge *Cinachyrella kuekenthali* are suitable bioindicator organisms to monitor Hg contamination in the Caribbean coral reefs. Higher Hg concentrations in Moín sediments could indicated possible Hg pollution, monitoring is necessary to evaluate possible risks. Special attention is needed to evaluate Hg biomagnification in biota according to high concentrations found in sponges at the study and the reference site.

References

Akcali, I., Kucuksezgin, F., 2011. A biomonitoring study: Heavy metals in macroalgae from eastern Aegean coastal areas. Mar. Pollut. Bull. 62, 637–645. https://doi.org/10.1016/j.marpolbul.2010.12.021

Alvarado, A., Mata, R., 2016. Soils of Costa Rica: An agroecological Approach, in: Kappelle, M. (Ed.), Costa Rican Ecosystems. University of Chicago Press, Chicago and London, pp. 64–93.

Araya-Vargas, A., 2017. Pautas para la conservación y el uso de las comunidades de esponjas en los parches arrecifales del Caribe Sur de Costa Rica. M.Sc. Thesis, Universidad Nacional, Heredia, Costa Rica. https://doi.org/10.13140/RG.2.2.13901.54247

Bernecker, A., 2009. Part 2: Marine benthic algae, in: Wehrtmann, I.S., Cortés, J. (Eds.), Marine Biodiversity of Costa Rica, Central America. Springer + Business Media B.V., San José, pp. 109–117.

Berry, K.L.E., Seemann, J., Dellwig, O., Struck, U., Wild, C., Leinfelder, R.R., 2013. Sources and spatial distribution of heavy metals in scleractinian coral tissues and sediments from the Bocas del Toro Archipelago, Panama. Environ. Monit. Assess. 185, 9089–9099. https://doi.org/10.1007/s10661-013-3238-8

Bonanno, G., Orlando-Bonaca, M., 2018. Trace elements in Mediterranean seagrasses and macroalgae. A review. Sci. Total Environ. 618, 1152–1159. https://doi.org/10.1016/j.scitotenv.2017.09.192

Bouyoucos, G.J., 1962. Hydrometer method improved for making particle size analysis of soils. Agron. J. 54, 464–465. https://doi.org/10.2134/agronj1962.00021962005400050028x

Briand, M.J., Bustamante, P., Bonnet, X., Churlaud, C., Letourneur, Y., 2018. Tracking trace elements into complex coral reef trophic networks. Sci. Total Environ. 612, 1091–1104. https://doi.org/10.1016/j.scitotenv.2017.08.257

Brown, M.T., Depledge, M.H., 1998. Determinants of trace metal concentrations in marine organisms, in: Langston, W.J., Bebianno, M.J. (Eds.), Metal Metabolism in Aquatic Environments. Springer + Business Media B.V., London, pp. 187–217. https://doi.org/10.1007/978-1-4757-2761-6_7

Castillo, A., Valdes, J., Sibaja, J., Vega, I., Alfaro, R., Morales, J., Esquivel, G., Barrantes, E., Black, P., Lean, D., 2011. Seasonal and diel patterns of total gaseous mercury concentration in the atmosphere of the Central Valley of Costa Rica. Appl. Geochemistry 26, 242–248. https://doi.org/10.1016/j.apgeochem.2010.11.024

CCT, 2013. Estudio de Impacto Ambiental Proyecto Terminal de Contenedores Moín: expediente No. 7968-12-SETENA. Centro Científico Tropical. San José, Costa Rica.

Chakraborty, S., Bhattacharya, T., Singh, G., Maity, J.P., 2014. Benthic macroalgae as biological indicators of heavy metal pollution in the marine environments: A biomonitoring approach for pollution assessment. Ecotoxicol. Environ. Saf. 100, 61–68. https://doi.org/10.1016/j.ecoenv.2013.12.003

Chand, D., Jaffe, D., Prestbo, E., Swartzendruber, P.C., Hafner, W., Weiss-Penzias, P., Kato, S., Takami, A., Hatakeyama, S., Kajii, Y., 2008. Reactive and particulate mercury in the Asian marine boundary layer. Atmos. Environ. 42, 7988–7996. https://doi.org/10.1016/j.atmosenv.2008.06.048

CNC, 2017. Terminal de Contenedores de Moín [WWW Document]. Com. -Nacional Concesiones, MOPT. URL http://www.cnc.go.cr/index.php/proyectos/en-desarrollo/tcm (accessed 6.13.18).

Cortés, J., 2016. The Caribbean coastal and marine ecosystems, in: Kappelle, M. (Ed.), Costa Rican Ecosystems. University of Chicago Press, Chicago and London, pp. 591–617.

Cortés, J., Jiménez, C.E., 2003. Past, present and future of the coral reefs of the Caribbean coast of Costa Rica, in: Cortés, J. (Ed.), Latin American Coral Reefs. Elsevier Science B.V., Amsterdam, pp. 223–239.

Cortés, J., Risk, M.J., 1985. A reef under siltation stress: Cahuita, Costa Rica. Bull. Mar. Sci. 36, 339–356.

Cortés, J., Van Der Hal, N., Van Soest, R.W.M. Van, 2009. Sponges, in: Wehrtmann, I.S., cort (Eds.), Marine Biodiversity of Costa Rica, Central America. Springer + Business Media B.V., San José, pp. 137–142.

Cortés, J., Wehrtmann, I.S., 2009. Diversity of marine habitats of the Caribbean and Pacific of Costa Rica, in: Wehrtmann, I.S., Cortés, J. (Eds.), Marine Biodiversity of Costa Rica, Central America. Springer + Business Media B.V., Berlin, pp. 1–45. https://doi.org/10.1007/978-1-4020-8278-8_1

Costa, M.F., Landing, W.M., Kehrig, H.A., Barletta, M., Holmes, C.D., Barrocas, P.R.G., Evers, D.C., Buck, D.G., Claudia Vasconcellos, A., Hacon, S.S., Moreira, J.C., Malm, O., 2012. Mercury in tropical and subtropical coastal environments. Environ. Res. 119, 88–100. https://doi.org/10.1016/j.envres.2012.07.008

Covelli, S., Protopsalti, I., Acquavita, A., Sperle, M., Bonardi, M., Emili, A., 2012. Spatial variation, speciation and sedimentary records of mercury in the Guanabara Bay (Rio de Janeiro, Brazil). Cont. Shelf Res. 35, 29–42. https://doi.org/10.1016/j.csr.2011.12.003

Davis, A.R., de Mestre, C., Maher, W., Krikowa, F., Broad, A., 2014. Sponges as sentinels: Metal accumulation using transplanted sponges across a metal gradient. Environ. Toxicol. Chem. 33, 2818–2825. https://doi.org/10.1002/etc.2747

Dyc, C., Covaci, A., Debier, C., Leroy, C., Delcroix, E., Thomé, J.P., Das, K., 2015. Pollutant exposure in green and hawksbill marine turtles from the Caribbean region. Reg. Stud. Mar. Sci. 2, 158–170. https://doi.org/10.1016/j.rsma.2015.09.004

El-Metwally, M.E.A., Madkour, A.G., Fouad, R.R., Mohamedein, L.I., Eldine, H.A.N., Dar, M.A., El-Moselhy, K.M., 2017. Assessment the Leachable Heavy Metals and Ecological Risk in the Surface Sediments inside the Red Sea Ports of Egypt. Int. J. Mar. Sci. 7, 214–228. https://doi.org/10.5376/ijms.2017.07.0023

El Zrelli, R., Courjault-Radé, P., Rabaoui, L., Castet, S., Michel, S., Bejaoui, N., 2015. Heavy metal contamination and ecological risk assessment in the surface sediments of the coastal area surrounding the industrial complex of Gabes city, Gulf of Gabes, SE Tunisia. Mar. Pollut. Bull. 101, 922–929. https://doi.org/10.1016/j.marpolbul.2015.10.047

Encina-Montoya, F., Vega-Aguayo, R., Díaz, O., Esse, C., Nimptsch, J., Muñoz-Pedreros, A., 2017. Mazzaella laminarioides and Sarcothalia crispata as possible bioindicators of heavy metal contamination in the marine coastal zone of Chile. Environ. Monit. Assess. 189, 584. https://doi.org/10.1007/s10661-017-6297-4

Fostier, A.H., do N. Costa, F., Korn, M. das G.A., 2016. Assessment of mercury contamination based on mercury distribution in sediment, macroalgae, and seagrass in the Todos os Santos bay, Bahia, Brazil. Environ. Sci. Pollut. Res. 23, 19686–19695. https://doi.org/10.1007/s11356-016-7163-6

García-Seoane, R., Fernández, J.A., Villares, R., Aboal, J.R., 2018. Use of macroalgae to biomonitor pollutants in coastal waters : Optimization of the methodology. Ecol. Indic. 84, 710–726. https://doi.org/10.1016/j.ecolind.2017.09.015

GESAMP, 2013. Executive sumary: Mercury in the aquatic environment: sources, releases, transport and monitoring; Working Group 37.

GESAMP (IMO/FAO/UNESCO-IOC/WMO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection), 2001. Protecting the oceans from land-based activities – Land-based sources and activities afecting the quality and uses of the marine, coastal and associated freshwater environment. https://doi.org/71

Green-Ruiz, C., Ruelas-Inzunza, J., Páez-Osuna, F., 2005. Mercury in surface sediments and benthic organisms from Guaymas Bay, east coast of the Gulf of California. Environ. Geochem. Health 27, 321–329. https://doi.org/10.1007/s10653-004-5741-x

Guzmán, H.M., García, E.M., 2002. Mercury levels in coral reefs along the Caribbean coast of Central America. Mar. Pollut. Bull. 44, 1415–1420. https://doi.org/10.1016/S0025-326X(02)00318-1

Guzmán, H.M., Jiménez, C.E., 1992. Contamination of coral reefs by heavy metals along the Caribbean coast of Central America (Costa Rica and Panama). Mar. Pollut. Bull. 24, 554–561. https://doi.org/10.1016/0025-326X(92)90708-E

Hédouin, L., Metian, M., Gates, R.D., 2011. Ecotoxicological approach for assessing the contamination of a Hawaiian coral reef ecosystem (Honolua Bay, Maui) by metals and a metalloid. Mar. Environ. Res. 71, 149–161. https://doi.org/10.1016/j.marenvres.2010.12.006

Henriques, B., Rocha, L.S., Lopes, C.B., Figueira, P., Monteiro, R.J.R., Duarte, A.C., Pardal, M.A., Pereira, E., 2015. Study on bioaccumulation and biosorption of mercury by living marine macroalgae: Prospecting for a new remediation biotechnology applied to saline waters. Chem. Eng. J. 281, 759–770. https://doi.org/10.1016/j.cej.2015.07.013

Hudspith, M., Reichelt-Brushett, A., Harrison, P.L., 2017. Factors affecting the toxicity of trace metals to fertilization success in broadcast spawning marine invertebrates: A review. Aquat. Toxicol. 184, 1–13. https://doi.org/10.1016/j.aquatox.2016.12.019

Jahan, S., Strezov, V., 2018. Comparison of pollution indices for the assessment of heavy metals in the sediments of seaports of NSW, Australia. Mar. Pollut. Bull. 128, 295–306. https://doi.org/10.1016/j.marpolbul.2018.01.036

JAPDEVA, 2008. Plan Maestro para el complejo portuario Limón - Moín. Junta de Administración Portuaria y de Desarrollo Económico de la Vertiente Atlántica de Costa Rica. Limón, Costa Rica. https://doi.org/9R4672.21 /R/401180/Nijm

Lamborg, C.H., Hammerschmidt, C.R., Bowman, K.L., Swarr, G.J., Munson, K.M., Ohnemus, D.C., Lam, P.J., Heimbürger, L.E., Rijkenberg, M.J.A., Saito, M.A., 2014. A global ocean inventory of anthropogenic mercury based on water column measurements. Nature 512, 65–68. https://doi.org/10.1038/nature13563

Long, E., MacDonald, D., Smith, S., Calder, F., 1995. Incidence of adverse bilogical effects within ranges of chemical concentrations in marine and estuarine sediments. Environ. Manage. 19, 81–97. https://doi.org/10.1007/BF02472006

Marins, R. V, Filho, F., Rodrigues, R., Drude de Lacerda, L., Sousa, W., 2004. Total mercury distribution as a proxi of urban and industrial pollution along the Brazilian coast. Quim. Nova 27, 763–770. https://doi.org/10.1590/S0100-40422004000500016

Mayzel, B., Aizenberg, J., Ilan, M., 2014. The Elemental Composition of Demospongiae from the Red Sea , Gulf of Aqaba. PLoS One 9, e95775. https://doi.org/10.1371/journal.pone.0095775

Morrison, R.J., Peshut, P.J., West, R.J., Lasorsa, B.K., 2015. Mercury (Hg) speciation in coral reef systems of remote Oceania: Implications for the artisanal fisheries of Tutuila, Samoa Islands. Mar. Pollut. Bull. 96, 41–56. https://doi.org/10.1016/j.marpolbul.2015.05.049

Müller, G., 1969. Index of geoaccumulation in sediments of the Rhine River. GeoJournal 2, 108–118.

NOAA, 1999. Sediment Quality Guidelines dveloped for the National Status and Trends Program. National Oceanic and Atmospheric Administration. Silver Spring, MD.

Oliveira, D.C.M., Correia, R.R.S., Marinho, C.C., Guimarães, J.R.D., 2015. Mercury methylation in sediments of a Brazilian mangrove under different vegetation covers and salinities. Chemosphere 127, 214–221. https://doi.org/10.1016/j.chemosphere.2015.02.009

OSPAR, 2016. Mercury assessment in the marine environment: Assessment criteria comparison (EAC/EQS) for mercury. OSPAR Commission, London.

Padovan, A., Munksgaard, N., Alvarez, B., Mcguinness, K., Parry, D., Gibb, K., 2012. Trace metal concentrations in the tropical sponge Spheciospongia vagabunda at a sewage outfall : synchrotron X-ray imaging reveals the micron-scale distribution of accumulated metals. Hydrobiologia 687, 275–288. https://doi.org/10.1007/s10750-011-0916-9

Pan, K., Lee, O.O., Qian, P.Y., Wang, W.X., 2011. Sponges and sediments as monitoring tools of metal contamination in the eastern coast of the Red Sea, Saudi Arabia. Mar. Pollut. Bull. 62, 1140–1146. https://doi.org/10.1016/j.marpolbul.2011.02.043

Peters, E.C., Gassman, N.J., Firman, J.C., Richmond, R.H., Power, E.A., 1997. Ecotoxicology of tropical marine ecosystems. Environ. Toxicol. Chemestry 16, 12–40.

Ramos, R., Cipriani, R., Guzman, H.M., García, E., 2009. Chronology of mercury enrichment factors in reef corals from western Venezuela. Mar. Pollut. Bull. 58, 222–229. https://doi.org/10.1016/j.marpolbul.2008.09.023

Rao, J. V, Srikanth, K., Pallela, R., Rao, T.G., 2009. The use of marine sponge , Haliclona tenuiramosa as bioindicator to monitor heavy metal pollution in the coasts of Gulf of Mannar , India. Environ. Monit. Assess. 156, 451–459. https://doi.org/10.1007/s10661-008-0497-x

RECOPE, 2018. Ampliación de la terminal portuaria petrolera del atlántico [WWW Document]. Ficha Proy. URL https://www.recope.go.cr/proyectos/procesos-industriales-portuarios/ampliacion-de-la-terminal-portuaria-petrolera-del-atlantico/ (accessed 5.14.18).

Reitz, M.F., 2007. The Gold Mines of Costa Rica. Rev. Geológica Am. Cent. 36, 125–134.

Rix, L., de Goeij, J.M., Mueller, C.E., Struck, U., Middelburg, J.J., Van Duyl, F.C., Al-Horani, F.A., Wild, C., Naumann, M.S., van Oevelen, D., 2016. Coral mucus fuels the sponge loop in warm-and cold-water coral reef ecosystems. Sci. Rep. 6, 1–11. https://doi.org/10.1038/srep18715

Rix, L., de Goeij, J.M., van Oevelen, D., Struck, U., Al-Horani, F.A., Wild, C., Naumann, M.S., 2018. Reef sponges facilitate the transfer of coral-derived organic matter to their associated fauna via the sponge loop. Mar. Ecol. Prog. Ser. 589, 85–96. https://doi.org/10.3354/meps12443

Ruiz, F., 2012. Gestión de las Excretas y Aguas Residuales en Costa Rica. Instituto Costarricense de Acueductos y Alcantarillados. San José, Costa Rica.

Signa, G., Mazzola, A., Di Leonardo, R., Vizzini, S., 2017. Element-specific behaviour and sediment properties modulate transfer and bioaccumulation of trace elements in a highly-contaminated area (Augusta Bay, Central Mediterranean Sea). Chemosphere 187, 230–239. https://doi.org/10.1016/j.chemosphere.2017.08.099

Thera, J.C., Rumbold, D.G., 2014. Biomagnification of mercury through a subtropical coastal food web off Southwest Florida. Environ. Toxicol. Chem. 33, 65–73. https://doi.org/10.1002/etc.2416

UNEP, 2015. Sedimentation and erosion [WWW Document]. Caribb. Environ. Program. URL http://www.cep.unep.org/publications-and-resources/marine-and-coastal-issues-links/sedimentation-and-erosion (accessed 6.11.18).

UNEP, 2014. The Minamata Convention on mercury and its implementation in the Latin America and Caribbean region. Montevideo, Uruguay.

UNEP, 2013. Global mercury assessment: sources, emissions, realeases and environmental transport, UNEP Chemicals Branch. Geneva. https://doi.org/10.1016/S0300-483X(03)00203-8

van Dam, J.W., Negri, A.P., Uthicke, S., F. Mueller, J.F., 2011. Chemical Pollution on Coral Reefs: Exposure and Ecological Effects, in: Sánchez-Bayo, F., van den Brink, P.J., Mann, R.M. (Eds.), Ecological Impacts of Toxic Chemicals. Bentham Science Publishers Ltd, Townsville, pp. 187–211. https://doi.org/10.2174/978160805121210187

van Dam, R.P., Diez, C.E., 1997. Diving behaviour of immature hawksbills (Eretmochelys imbricata) in a Caribbean reef habitat. Coral Reefs 16, 133–138. https://doi.org/10.1007/s003380050067

Walker, C.H., Sibly, R.M., Hopkin, S.P., Peakall, D.B., 2012. The Fate of Metals and Radioactive Isotopes in Contaminated Ecosystems, in: Principles of Ecotoxicology. CRC Press, Boca Raton, pp. 49–58.

Whitall, D., Pait, A., Hartwell, S.I., 2015. Chemical contaminants in surficial sediment in Coral and Fish Bays, St. John, U.S. Virgin Islands. Mar. Environ. Res. 112, 1–8. https://doi.org/10.1016/j.marenvres.2015.08.001

Wood, J.C., 2015. Determination on moisture content and total organic carbon within basin environments: Loss-on-Ignition, in: Clarke, L.E., Nield, J.M. (Eds.), Geomorphical Techniques. Brithish Society for Geomorphology, London.

Anexo1: Concentraciones de metales y mercurio total (THg) en todas las muestras estudiadas

En estos datos se visualiza el código de identificación de la muestra (año-código numérico), el tipo de muestra (Sedimento, Alga, Esponja), la descripción de la muestra según tipo de sedimento o especie de alga y esponja, la estación de muestreo a la que pertenece la muestra (IP: Isla Pájaros, M/P: Moín/Portete, RO: Rompeolas, PU: Punta Uva, ManZ: Manzanillo y PM: Punta Mona), el sitio de muestreo (SC: Caribe Sur, M: Moín), la fecha en la que se realizó el muestro y finalmente las concentraciones de metales (µg g-1 dwt) y de THg (ng g -1 dwt) en las muestras analizadas.

Código	**Tipo de muestra**	**Descripción**	**Sitio de muestreo**	**Estación de muestreo**	**Fecha de muestreo**	**Metales (µg g-1 dwt)**							**THg (ng g - 1 dwt)**
						Cd	**Cr**	**Cu**	**Mn**	**Pb**	**Ni**	**Zn**	
16-011	Sed.	FineSand	SC	PM	Oct. 2014	n.d	n.d	n.d	n.d	n.d	n.d	n.d	4.8
16-452	Sed.	Calcareous	SC	ManZ	Oct. 2014	n.d	n.d	n.d	n.d	n.d	n.d	n.d	5.8
16-453	Sed.	Calcareous	SC	PU	Oct. 2014	n.d	n.d	n.d	n.d	n.d	n.d	n.d	6.4
16-454	Sed.	Calcareous	M	IP	Ene. 2015	n.d	n.d	n.d	n.d	n.d	n.d	n.d	47.1
16-455	Sed.	Muddy Sand	M	M/P	Ene. 2015	n.d	n.d	n.d	n.d	n.d	n.d	n.d	25.6
16-456	Sed.	Sand	M	RO	Ene. 2015	n.d	n.d	n.d	n.d	n.d	n.d	n.d	11.8
16-012	Sed.	Calcareous	M	RO	Mar. 2015	0.0	5.0	5.3	766	0.7	3.9	4.9	27.8
16-013	Sed.	Mud	M	M/P	Mar. 2015	0.1	6.7	24.3	470	6.0	9.5	23.9	44.3
16-457	Sed.	Calcareous	M	IP	Mar. 2015	0.0	4.0	4.0	371	0.6	3.0	3.8	10.6
16-458	Sed.	Calcareous	M	IP	May. 2015	0.0	5.1	5.6	412	1.1	3.5	4.6	27.6
16-459	Sed.	Sand	SC	PM	Jun. 2015	0.0	4.7	18.3	260	3.1	4.3	16.1	4.3
16-460	Sed.	Fine Calcareous	SC	PU	Jun. 2015	n.d	n.d	n.d	n.d	n.d	n.d	n.d	4.6
16-461	Sed.	Fine Calcareous	SC	ManZ	Jun. 2015	n.d	n.d	n.d	n.d	n.d	n.d	n.d	4.5
16-462	Sed.	Sand	SC	PM	Abr. 2016	0.0	4.3	19.3	262	3.1	4.3	16.6	5.1
16-463	Sed.	Calcareous	SC	PU	Abr. 2016	n.d	n.d	n.d	n.d	n.d	n.d	n.d	4.8
16-464	Sed.	Fine Calcareous	SC	ManZ	Abr. 2016	n.d	n.d	n.d	n.d	n.d	n.d	n.d	3.8
16-465	Sed.	Calcareous	M	IP	May. 2016	0.0	5.7	5.2	473	0.9	3.5	3.8	27.3
16-466	Sed.	Muddy Sand	M	M/P	May. 2016	0.0	10.5	8.2	321	3.5	5.8	12.3	50.5
16-467	Sed.	Calcareous	M	RO	May. 2016	0.0	5.0	4.8	386	1.2	3.6	4.9	11.6
16-492	Alga	*C. crenulata*	SC	ManZ	Oct. 2014	n.d	n.d	n.d	n.d	n.d	n.d	n.d	32.6
16-494	Alga	*C. crenulata*	SC	PM	Oct. 2014	n.d	n.d	n.d	n.d	n.d	n.d	n.d	35.4
16-495	Alga	*C. crenulata*	M	IP	Oct. 2014	n.d	n.d	n.d	n.d	n.d	n.d	n.d	33.6
16-496	Alga	*C. crenulata*	M	M/P	Ene. 2015	n.d	n.d	n.d	n.d	n.d	n.d	n.d	35.2
16-497	Alga	*C. crenulata*	M	RO	Ene. 2015	n.d	n.d	n.d	n.d	n.d	n.d	n.d	26.3
16-501	Alga	*C. crenulata*	M	IP	May. 2015	0.1	1.4	23.4	115	0.4	5.1	15.2	29.4
16-502	Alga	*C. crenulata*	M	RO	May. 2015	0.2	0.9	16.0	94	0.4	3.8	11.7	26.4
16-503	Alga	*C. crenulata*	SC	PM	Jun. 2015	0.2	0.9	16.3	65	0.3	5.6	6.6	26.9
16-504	Alga	*C. crenulata*	SC	PU	Jun. 2015	0.2	1.3	9.0	103	0.3	4.8	6.8	25.7
16-505	Alga	*C. crenulata*	SC	ManZ	Jun. 2015	0.1	1.7	7.6	145	0.3	4.8	6.4	35.8
16-506	Alga	*C. crenulata*	M	IP	May. 2016	0.1	1.7	7.8	174	0.3	5.2	4.8	30.7

16-507	Alga	*C. crenulata*	M	M/P	May. 2016	0.1	2.2	14.6	139	0.6	3.1	10.0	18.6
16-508	Alga	*C. crenulata*	M	RO	May. 2016	0.1	1.2	9.5	92	0.4	3.7	5.6	24.4
16-050	Alga	*C. crenulata*	SC	PM	Abr. 2016	0.1	1.3	11.0	134	0.4	4.5	5.7	15.3
16-054	Alga	*C. crenulata*	SC	PU	Abr. 2016	n.d	n.d	n.d	n.d	n.d	n.d	n.d	22.1
16-058	Alga	*C. crenulata*	SC	ManZ	Abr. 2016	n.d	n.d	n.d	n.d	n.d	n.d	n.d	26.0
16-498	Alga	*C. crenulata*	M	IP	Mar. 2015	0.3	1.8	16.3	166	0.6	4.3	14.9	22.6
16-499	Alga	*C. crenulata*	M	M/P	Mar. 2015	0.1	2.1	27.4	289	0.8	4.8	16.2	42.3
16-500	Alga	*C. crenulata*	M	RO	Mar. 2015	0.2	2.4	26.1	343	0.8	4.7	17.3	38.1
16-509	Esponja	*C. kuekenthali*	SC	ManZ	Oct. 2014	n.d	n.d	n.d	n.d	n.d	n.d	n.d	182.2
16-510	Esponja	*C. kuekenthali*	SC	PU	Oct. 2014	n.d	n.d	n.d	n.d	n.d	n.d	n.d	95.6
16-511	Esponja	*C. kuekenthali*	SC	PM	Oct. 2014	n.d	n.d	n.d	n.d	n.d	n.d	n.d	211.0
16-515	Esponja	*C. kuekenthali*	M	IP	Mar. 2015	0.9	4.4	12.5	262	0.7	14.4	12.7	116.3
16-516	Esponja	*C. kuekenthali*	M	M/P	Mar. 2015	0.4	1.0	8.2	39	0.1	9.7	6.4	100.4
16-517	Esponja	*C. kuekenthali*	M	RO	Mar. 2015	0.8	2.0	15.4	101	0.4	13.4	12.3	131.7
16-518	Esponja	*C. kuekenthali*	M	IP	May. 2015	0.8	1.5	13.0	99	0.2	14.2	12.4	150.7
16-519	Esponja	*C. kuekenthali*	M	RO	May. 2015	0.4	1.7	18.5	57	0.3	7.2	15.5	192.1
16-520	Esponja	*C. kuekenthali*	SC	PM	Jun. 2015	0.5	2.0	11.7	86	0.3	11.8	12.7	199.3
16-521	Esponja	*C. kuekenthali*	SC	PU	Jun. 2015	n.d	n.d	n.d	n.d	n.d	n.d	n.d	162.3
16-522	Esponja	*C. kuekenthali*	SC	ManZ	Jun. 2015	n.d	n.d	n.d	n.d	n.d	n.d	n.d	85.8
16-051	Esponja	*C. kuekenthali*	SC	PM	Abr. 2016	0.5	1.9	7.8	79	0.2	6.8	7.0	106.4
16-055	Esponja	*C. kuekenthali*	SC	PU	Abr. 2016	n.d	n.d	n.d	n.d	n.d	n.d	n.d	198.3
16-059	Esponja	*C. kuekenthali*	SC	PM	Abr. 2016	n.d	n.d	n.d	n.d	n.d	n.d	n.d	132.9
16-523	Esponja	*C. kuekenthali*	M	IP	May. 2016	0.8	2.6	15.0	172	0.4	9.2	10.6	119.3
16-524	Esponja	*C. kuekenthali*	M	M/P	May. 2016	0.5	1.5	12.3	60	0.3	11.2	9.8	156.1
16-525	Esponja	*C. kuekenthali*	M	RO	May. 2016	0.4	2.8	16.0	121	0.6	9.3	12.4	133.7

Anexo2: Guía de autores de la revista Marine Pollution Bulletin