UNIVERSIDADE FEDERAL DO CEARÁ

CENTRO DE CIÊNCIAS

DEPARTAMENTO DE BIOLOGIA

PROGRAMA DE PÓS- GRADUAÇÃO EM ECOLOGIA E RECURSOS NATURAIS

JOSÉ MOACIR DE CARVALHO ARAÚJO JÚNIOR

NITROUS OXIDE EMISSIONS AND METAL BIOGEOCHEMISTRY IN COASTAL WETLAND SOILS IN RESPONSE TO BIOTURBATION BY UCIDES CORDATUS

FORTALEZA

2016

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FORTALEZA

2016

Dados Internacionais de Catalogação na Publicação
Universidade Federal do Ceará
Biblioteca Universitária
Gerada automaticamente pelo módulo Catalog, mediante os dados fornecidos pelo autor(a)

A689n Araújo Júnior, José Moacir de Carvalho.
Nitrous oxide emissions and metal biogeochemistry in coastal wetland soils in response to bioturbation by Ucides cordatus / José Moacir de Carvalho Araújo Júnior. – 2016.
96 f. : il. color.

Tese (doutorado) – Universidade Federal do Ceará, Centro de Ciências, Programa de Pós-Graduação em Ecologia e Recursos Naturais , Fortaleza, 2016.
Orientação: Prof. Dr. Tiago Osório Ferreira.
Coorientação: Prof. Dr. Xose Luis Otero.

1. Bioavailability of metals Iron, Zinc, Copper. 2. Bioturbation. 3. Nitrous oxide. 4. Crab. 5. Ucides cordatus. I. Título.

CDD 577

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APROVADA EM: 25 / 08 / 2016

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A todos que me permitiram chegar até aqui, a meus pais pela graça da vida e pela criação maravilhosa que me deram, a minha esposa Alessandra pelo amor, companheirismo e paciência nessa jornada, a meu filho Kal-El por me mostrar o verdadeiro sentido da vida e me fazer uma pessoa melhor a cada dia e, principalmente, a Deus, por todas as conquitas, amizades e aprendizados que me proporcionou.

AGRADECIMENTOS

Antecipadamente gostaria de começar agradecendo a todos que, de alguma forma, da mais simples as mais essenciais, participaram da elaboração deste trabalho e por algum motivo não foram citadas diretamente abaixo. Além desta agradeço de forma muito especial:

A Universidade Federal do Ceará - UFC e a Universidade De Santiago de Compostela – USC, que possibilitaram meus estudos e realização de pesquisas.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior- CAPES e a Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico - FUNCAP, pelo apoio financeiro através da concessão da bolsa de estudo.

Ao Programa de Pós-Graduação em Ecologia e Recursos Naturais da UFC.

Ao Profº. Tiago Osório Ferreira pela orientação, paciência, compreensão, amizade e, principalmente, pelo incentivo a continuar neste trabalho até o fim.

Ao Prof. Dr. Xosé Luiz Otero Perez da Faculdade de Bioloxia do Departamento de Edafologia da Universidade de Santiago de Compostela- España por aceitar me co-orientar, por me receber e possibilitar realizar meus estudos em seu laboratório na USC, pelas valiosas sugestões e pelas análises para o desenvolvimento do presente trabalho.

Aos professores que participaram da Banca examinadora, prof. Dr. Marcos Siqueira Neto, Prof. Dr. Antônio Jeovah de Andrade Meirele e a Profa. Dra. Carla Ferreira Rezende pelas sugestões e contribuições no meu trabalho.

Aos professores José Roberto Feitosa Lima, Adriana Guirado e Célia Maria de Souza Sampaio pelas inúmeras contribuições durante a qualificação e outros momentos do desenvolvimento deste trabalho.

Aos colegas do eterno “mangrupo” Raiana Lira, Adriana, Danielsinho, Camila, Fabi, Hermano, Danilo e, especialmente ao Gabriel Nuto e a Antônia Gislaine, que contribuíram de tantas formas com esse trabalho, desde a coleta em campo, passando pelas análises em laboratório e depois com estatística e trabalho com os dados, por me atender quando preciso e pela amizade e incentivo sempre.

Ao Ronaldo (meu “mateiro” do mangue do Aracati) e toda sua família pela ajuda essecial a conclusão deste trabalho e pela simpatia constante em nos receber.

Aos demais amigos e colegas da UFC (Sâmia Paiva, Rafinha, Izabel, Girão, Leônia, Antônio José, entre outros), UECE (Eugênio, Paiva, Ivo, Francyregis, Anna

Patricya, Argeu, Mírian, Sâmia, Raul, Rommulo, professor Bruno e outros) e CEFET (em especial aos professores Bemvindo e Glória e a meus amigos-irmãos, padrinhos e apadrinhados Heraldo e Elivânia) que me auxiliaram nas diversas fases do projeto.

A todos os colegas guerreiros do curso de Ecologia e Recursos Naturais.

Aos amigos Ricardo e Solange (professor e solzinha) e sua maravilhosa família pelo amizade e carinho tão maravilhoso que nos deram.

Aos amigos e vizinhos espanhóis Saul e Iri, por nos receberem com tanto amor e amizade em seu país e nos mostrarem as belezas da Galícia, tanto as naturais quanto a de seu povo representado por eles mesmo e sua amizade.

Aos meus colegas e amigos da SEUMA (Mansour, Erica, André, Sâmia, Sérgio, Ataciso, Johnnatha, Luciano e tantos outros) pela amizade e companheirismo oferecidos. Em especial a minha eterna amiga, chefe e exemplo vivo de conhecimento e ética, Maria Ester Esmeraldo.

Aos meu “irmãos e irmas do coração” Claver Giovanni, Natália, Júnior (Bakura), Ramon, Fernando Joca, Juliana, Neone, Ivo e aos compadres Tiago e Alana e Marlo Eddy e Denise, que marcaram e estiveram em minha vida.

Ao meu afilhado Matheus, obrigado por sempre me fazer sorrir e me fazer ver que as melhores coisas da vida estão nas coisas e atos mais simples.

A minha família adotiva, meus cunhados Lili e Berg, meus sogros Ivan e Beta, ao Sr. Micias, a Dona Claúdia e ao Lucas (Padawan) e a Dafne, pela acolhida sempre alegre em sua casa, pelas brincadeiras e conversas que me ajudavam.

Aos meus parentes, incluindo aqui tios, tias, primos, primas e tantos outros que me ajudaram de diversas formas e, em especial, a meus avós, a Tia Lidu, tio Tico, e a Monalisa por me propiciarem diversos momentos felizes e inesquecíveis.

E mais do que especialmente a quatro pessoas. Primeiramente aos meus pais Moacir e Luzia pelo apoio e amor incondicional e por me ajudar de todas as maneiras possíveis e imagináveis a concluir este trabalho.

A minha companheira, amiga e amada Alessandra por todo apoio, dedicação e incentivo em todos os aspectos da minha vida.

A meu filho Kal-El por me fazer a cada dia mais feliz, por me fazer persistir na vida e em tudo. Por ser meu super-heroi pessoal. Esse trabalho dedico a você meu filho.

A Deus e ao mestre Jesus, acima de tudo, meus sinceros agradecimentos.

“Eu posso não ter ido para onde eu pretendia ir, mas eu acho que acabei terminando onde eu pretendia estar.” (Douglas Adams)

RESUMO

As zonas úmidas costeiras, dentre elas os manguezais, são ecossistemas com elevada biodiversidade. Nesses ambientes, os caranguejos destacam-se tanto por seu grande número de espécies quanto por sua importância econômica e ecológica e econômica, principalmente devido ao processo de formação de tocas (bioturbação). Neste trabalho foram analisados os efeitos da bioturbação realizada por caranguejos *Ucides cordatus* de manguezais do Rio Jaguaribe (Ceará, Brasil) sob as concentrações das diferentes formas biogeoquímicas dos metais ferro, zinco e mangânes no solo próximo e no tecido desses animais, além das variações no fluxo de óxido nitroso (N_2O) em áreas com e sem esses crustáceos, comparando os valores encontrados entre os períodos chuvoso e seco. Os solos foram coletados no período de maré baixa em duas 2 áreas de coleta, uma com caranguejos e outra sem. Foram realizadas medições de parâmetros bioecológicos dos caranguejos, de parâmetros físico-químicos do solo e as concentrações dos metais associados às diferentes frações do solo (trocável, carbonato, ferridrita, *lepidocrocita, goethita e pirita)* e nos tecidos do caranguejo *Ucides cordatus*, além da determinação do fluxo de N_2O. Os resultados demonstraram claramente uma variação significativamente maior de atividade bioturbadora no período seco, com consequente aumento na oxidação e acidificção do solo nas áreas com caranguejo. As formas mais oxidadas dos metais foram predominantemente maiores na área com tocas de caranguejos em relação a área control, enquanto as de pirita foram menores. Entretanto, a emissão de fluxos de óxido nitroso foi maior na área controle em ambos os períodos climáticos estudados, o que indica que a atividade bioturbadora do caranguejo promove redução das emissçoes desse gás. Os resultados obtidos permitiram compreender o papel da bioturbação na emissão de GEE e na dinâmica dos processos biogeoquímicos nos solos de zonas úmidas costeiras, além de identificar possíveis variações sazonais nesses valores e a determinação das emissões de GEE e da contaminação dos solos e caranguejos dessas áreas por metais traços, de forma a melhorar o monitoramento ambiental.

Palavras-chave: Biodisponibilidade de metais. Ferro. Zinco. Cobre. Óxido Nitroso. Bioturbação. Caranguejo. *Ucides cordatus*.

ABSTRACT

Coastal wetlands, among them the mangroves, are ecosystems with high biodiversity. In these environments, the crabs stand out both for its large number of species as by its economic and ecological importance, mainly due to the dens formation process (bioturbation. In this work, the effects of bioturbation by *Ucides cordatus* crabs from the Jaguaribe River mangrove (Ceará, Brazil) were analyzed under the concentrations of the different biogeochemical forms of the iron, zinc and manganese metals in the nearby soil and in the tissues of these animals, besides the variations in the (N*2*O) in areas with and without these crustaceans, comparing the values found between the rainy and dry periods. Soil samples were collected at low tide period in the demarcated two collection areas, one with and one without crabs. Measurements of bioecological parameters of crabs, soil physical and chemical parameters and concentrations of the metals associated with the different soil fractions (exchangeable, carbonate, ferridrite, lepidocrocite, goethite and pyrite) and *Ucides cordatus* crab Determination of the N_2O flow. The results clearly showed a significantly greater variation of bioturbation activity in the dry period, with consequent increase in oxidation and acidification of the soil in the areas with crab. The more oxidized forms of the metals were predominantly larger in the area with crab burrows in relation to the control area, while those of pyrite were smaller. However, the emission of nitrous oxide fluxes was higher in the control area in both climatic periods, which indicates that the bioturbation activity of the crab promotes reduction of the emissions of this gas. The results allowed us to understand the role of bioturbation in GHG emissions and dynamics of biogeochemical processes in coastal wetlands soils, and identify possible seasonal variations in these values and the determination of GHG emissions and contamination of soil and crabs in these areas by trace metals, to improve environmental monitoring.

Keywords: Bioavailability of metals. Iron. Zinc. Copper. Bioturbation. Nitrous oxide. Crab. *Ucides cordatus*.

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1 INTRODUCTION

Coastal wetlands are transitional ecosystems between the aquatic and terrestrial environments, (i.e. mangroves, hypersaline tidal flats, coral reefs, beaches, cliffs, coastal lagoons, estuaries and salt marshes) that can be found in areas with hydromorphic soils (ALBUQUERQUE et al., 2014) and which cover about 9,2 millions km² in a global scale (BEUEL et al., 2016). This corresponds to only 6% of the earth's surface, but, contrastingly, these ecosystems support approximately 20% of all living organisms on Earth and have an annual production value of 45–160 times higher (product value in terms of dollar value) than of farmland ecosystems (CHEN; WONG, 2016).

Despite this environmental importance, these ecosystems constitute one of the most threatened environments of the world (MITSCH et al., 2010), since its geographical position facilitates the uncontrolled exploitation, and contamination especially originated from human activities. The pollution by domestic and industrial effluents in these ecosystems causes negative effects (i.e biota contamination, biodiversity loss, changes in physicochemical and biological patterns) (LI et al., 2016).

Due to their biological richness,the coastal wetlands play an ecological key role, serving as natural "nurseries", both for the species characteristic of these environments, but also for other animals that migrate to coastal areas during at least one stage of their life cycle (DRAYER; RICHTER, 2016; HOGARTH, 2015) . These ecosystems also, perform several other essential functions , such as the stabilization of the coastal geomorphology, maintenance of large fish stocks, production and export of nutrients to the sea (WINGARD; LORENZ, 2014).

Among the different coastal wetlands, mangroves stand out as the most functionally complex tropical coastal ecosystems that develop in estuary areas, being located in the intersection zone between freshwater rivers and saltwater seas (FAO, 2005). Mangrove forests are distributed in four continents and in Brazil occupy about 92% of the coastline (ANTONIO; DIEGUES, 1970; WALTERS et al., 2008). Mangrove have high biodiversity and are composed of trees and shrubs with great tolerance to salt or brackish water and typically resident animals in these regions, which are important food source for coastal communities (POLIDORO et al., 2010).

Despite its important ecological value, mangroves have suffered excessive human pressure in recent decades (VALIELA; BOWEN; YORK, 2001). The shrimp production industry stands out as one of the main degrading activities. , causing deforestation, migration and loss of local biodiversity, and especially water and soil pollution (HUITRIC; FOLKE; KAUTSKY, 2002; THORNTON; SHANAHAN; WILLIAMS, 2003; TROTT; ALONGI, 2000).

Additionally, mangroves are threatened by the discharge of effluents rich in metals. Many authors studied the forms of release, concentration and effects of trace metals in estuarine environments, in water (Ex: GUHATHAKURTA; KAVIRAJ, 2000; OTERO; MACÍAS, 2002; SUZUKI et al., 2014; TROTT; ALONGI, 2000), in sediments (ANDRADE et al., 2012; DEFEW; MAIR; GUZMAN, 2005; MARCHAND et al., 2006), in the plants (AGORAMOORTHY; CHEN; HSU, 2008; FERREIRA et al., 2007; GRIBSHOLT; KRISTENSEN, 2002), in animals (FERNANDES et al., 2006; FIRAT et al., 2008; JULSHAMN et al., 2015) and in microorganisms (HOLGUIN; VAZQUEZ; BASHAN, 2001; KRISTENSEN et al., 2008).

Understanding the geochemical behavior of trace elements in the soil is essential for the choice of management practices and public policies focused in soil quality and water resources. In this context the bioavailability and toxicity of trace metals have become one of the most frequent topics of studies associated with ecosystems, mainly due to the fact that these metals can be adsorbed to the soil, accumulate in benthic organisms to toxic levels (FAO, 2005; LACERDA, 2002; LACERDA; ITTEKKOT; PATCHINEELAM, 1995).

Under anoxic conditions most metal sulfides are poorly soluble and stable (KRAUSKOPF, 1956) since pyrite and other minerals may control trace metal bioavailability (HOWARTH, 1979, 1984).

The bioavailability of trace metals consists in its release to living beings. However bioavailable forms not always correlate directly with the total contents, since the different soil and sediment components may interact differently with the trace metals. Thus, the study of the cycling of trace elements in the soil requires knowledge of its main forms, combinations and the possible changes in the physicho-chemical environment (BEUEL et al., 2016; CIDU; BIDDAU, 2007)

For coastal wetland soils, bioturbation by crabs is a very important process that may cause the remobilization of sediments and soils, enhance aeration, an thus, the whole geochemical condition of these hydromorphic substrates. Kristensen

(2008) describes the increase in bioavailable forms of metals in mangrove environments due to burrows opening by crabs, as well as remobilization of reduced metals usually found in greater depths to the upper layers. This phenomenon, along with the removal of large particulate organic matter through the ingestion of sediments found in these environments, made Kristensen denominated crabs as "ecosystem engineers".

Due to its impacts in the geochemical environment, bioturbation may also considerably alter the degradation of organic compounds. Due to the anaerobic condition, the accumulation of organic matter and, by consequence, the organic carbon stocks in coastal wetland soils are much higher than those found in terrestrial soils (KRISTENSEN et al., 2008; LORENZ; LAL; JIMÉNEZ, 2010; NÓBREGA et al., 2014). The forms of nitrogen present in the environment are originated from the slower mineralization, predominance of ammonification, decreased of nitrification and increased denitrification. During the process of decomposition of organic matter, some greenhouse gases (GHG) are released into the atmosphere, like CO_2, N_2O e CH_4 (CHEN; TAM; YE, 2010; CHEN et al., 2011; KONNERUP et al., 2014).

GHG emissions are increasingly studied, from its formation to its possible impacts at a local and global level (CHEN; TAM; YE, 2010; FORD et al., 2012). Some of these studies have highlighted the high potential for gases release in coastal wetland soils, such as mangrove forests, seagrass meadows or intertidal saltmarshes (BEAUMONT et al., 2014; NÓBREGA et al., 2016). Among these areas, mangroves are recognized the ecosystem that contribute most to GHG emissions (ALLEN et al., 2007; CHEN; TAM; YE, 2012), mainly due to the intense microbiological activity during the degradation of organic matter and root respiration (CHEN et al., 2014; KREUZWIESER; BUCHHOLZ; RENNENBERG, 2003).

Several edaphic and climatic factors can affect the emission of GHGs into the atmosphere, such as redox potential; salinity; temperature and others (LIVESLEY; ANDRUSIAK, 2012; NÓBREGA et al., 2016). According to Yan et al. (1996), the addition of organic sewage to the soil can cause a rise in pH, by two processes: decarboxylation of organic anions, consuming H^+ and releasing CO_2, and deamination of amino acids. Thus, it is assumed that the addition of effluents from shrimp farming and urban origin in mangrove areas can affect the dynamics of nitrogen and carbon in the soil (HOLGUIN; VAZQUEZ; BASHAN, 2001) and enhance the emission of gases into the atmosphere (SIGNOR; EDUARDO; CERRI, 2013; SUÁREZ-ABELENDA et al., 2014).

This work, divided into three chapters, aimed to: 1) evaluate the role of bioturbation by crabs in coastal wetlands soils, both in the biogeochemical cycle of metals such as the carbon cycle, release of greenhouse gases (GHGs) and in physical-chemical patterns of soil in order to improve understanding about the role of these animals in the dynamics of coastal ecosystems, as well as provide new elements to create more efficient strategies for management and conservation of these ecosystems; 2) assessing the different forms of associated metals in the soil bioturbation by crabs and their concentrations in different soil depths and tissues of crabs themselves; 3) to evaluate the emission of nitrous oxide (N_2O) in the Brazilian semi-arid mangrove soils under the influence of bioturbation by crabs seeking a better understanding of the role of these areas threatened in the issue and sequestration of these greenhouse gases.

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CHAPTER 1

Environmental effects of bioturbation by crabs in soils of coastal environments: A review

Theoretical framework

1 INTRODUCTION

The humid coastal zones (wetlands) are functionally complex, resistant and high-resilience ecosystems, defined as zones of interface between terrestrial and aquatic environments, which may be continental or coastal, natural or artificial and temporarily or permanently flooded by moderately or highly saline waters, serving as shelter for various specific plants and animals adapted to their hydrological dynamics (JUNK et al., 2014).

Among them, the salt marsh, hypersaline tidal flats (apicum ecosystems) and mangrove ecosystems are generally associated with the banks of bays, coves, bars, mouths of rivers, lagoons and coastal indentations, or other sites where river and sea waters meet, or directly exposed to the coastal line (OTERO et al., 2009; SCARLATE ROVAI et al., 2012)

Salt marshs regions are mainly dominated by herbaceous vegetation, perennial or “annual”, and may also have some shrubs, contrasting with the mangrove, which is dominated by arboreal plant species. The plant species of the salt marsh dominate the intertidal coastal zone of temperate regions, while in the tropics and subtropics they tend to behave as pioneers, colonizing recently deposited and poorly consolidated terrains, or where the evapotranspiration rates are too high for mangrove plants, but being able to coexist with them at tropical latitudes, in both natural and man-modified environments (FAGHERAZZI et al., 2012).

Apicuns (term originated from the Tupi-Guarani language, which means a sandbank made by the sea or a swamp of salty water close to the sea) are other relevant coastal humid zones, constituting a transitional environment (ecotone) between the mangrove and the coastal plains, with generally sandy soil, without vegetal cover or occupied by herbaceous vegetation. These environments are situated in the peripheral portion and at higher levels in relation to the mangroves, standing out for an apparent absence of fauna, despite being surrounded by rich ecosystems from the biological point of view, playing an important ecological role as reservoirs of various mangrove species and other animals (ALBUQUERQUE et al., 2014a).

Mangroves cover approximately 137,760 km² of coasts worldwide (GIRI et al., 2011) and accumulate about 26.1 Tg of organic carbon per year (BREITHAUPT et al., 2012). Mangrove soils are originated from poorly consolidated and semifluid marine and fluvial sediments, can be several meters deep and are generally located in regions with flat topography on the coastline (ALBUQUERQUE et al., 2014b). The decomposition of the litter in mangrove regions associated with the saturation by water explain the colors of mangrove soils, which range between grey and black (FERREIRA et al., 2007a). These authors analyzed the properties of mangrove soils and concluded that the pedogenic processes of addition, translocation and transformation that occur in these environments classify the substrate of the mangroves as soil, instead of sediments.

In this context, the soils of mangroves, marismas and apicuns suffer influences from the fauna in their formation, since the animals have significant influence on the biogeochemical conditions of the soil. Activities such as mobilization of soil particles, either through ingestion, construction of channels and burrows (phenomenon known as bioturbation) or through the movement of undecomposed organic matter to subsurface layers and the transfer of reduced compounds to oxidizing regions during the search for food and/or reproduction, contribute to the processes that act on the formation of the soil (ARAÚJO JÚNIOR et al., 2012; FERREIRA et al., 2007b).

In addition, bioturbation also affects the physicochemical patterns of the soils, since it causes the vertical zonation of biogeochemical processes to disappear (BEAUCHARD et al., 2012; WILSON; HUGHES; FITZGERALD, 2012), leading to the formation of oxidized portions and/or oxidized microsites arranged in subsurface due to the disposition of the biological channels. As a consequence of this process, various elements that are sensitive to the changes in the reducing conditions of the environment (iron and sulfur, for instance), when oxidized or reduced, have their dynamics altered as a result of the biological activity, with possible increments or decreases in their concentrations in the interstitial water and in the various fractions of the solid phase of the soil (FANJUL et al., 2011; LA CROIX et al., 2015; PASCAL et al., 2016; SANTOS; EYRE; HUETTEL, 2012).

Some reviews about the processes and effects of bioturbation have been published in the 1980 decade (ANDERSSON; GRANELI; STENSON, 1988; KRANTZBERG, 1985), which mainly focused on the effects of invertebrates on the content of oxygen, redox potential, distribution of metals, cycling of nutrients and microbial activities (ADÁMEK; MARŠÁLEK, 2013). Since then, many other studies have been conducted on bioturbation and its effects (bioirrigation and the reworking of particles) by different animals (CANFIELD; FARQUHAR, 2009; JOHNSON, 2002; KUTSCHERA; ELLIOTT, 2010; NKEM et al., 2000). Among bioturbating animals, a considerable attention has been payed to the impact of bioturbation by crabs, given their wide distribution and intense effect of burrowing (KRISTENSEN, 2008; PENHA-LOPES et al., 2009).

Therefore, this study aimed to describe and evaluate recent knowledge on the processes associated with the bioturbation by crabs in coastal humid zones, with emphasis on their different effects on the water-soil interface of these environments, as well as on the dynamics of gases and substrates reworked by them.

2 BIOTURBATION: CONCEPTS AND CHARACTERISTICS

Kristensen et al. (2012) point out that, although the term bioturbation originates from ichnology (study of traces and footprints of animals), it currently has a diversified use, going from its original meaning, which refers to only redistribution of particles and formation of biogenic structures by burrowing animals, until wider concepts, also encompassing all physical disturbances caused by animals on the substrate or the microbial processes associated with animal disturbances. In this study, we will use the definition for ecology and aquatic sciences proposed by Kristensen et al. (2012) and based on the study of Meysman et al. (2006), in which bioturbation consists, in general, in the revolving and reformulation of matrices of soils and sediments by living organisms, from microorganisms to burrowing macro-animals and plants with roots, and the physical processes and chemical changes associated with this movement of solid particles (rework) and water (ventilation).

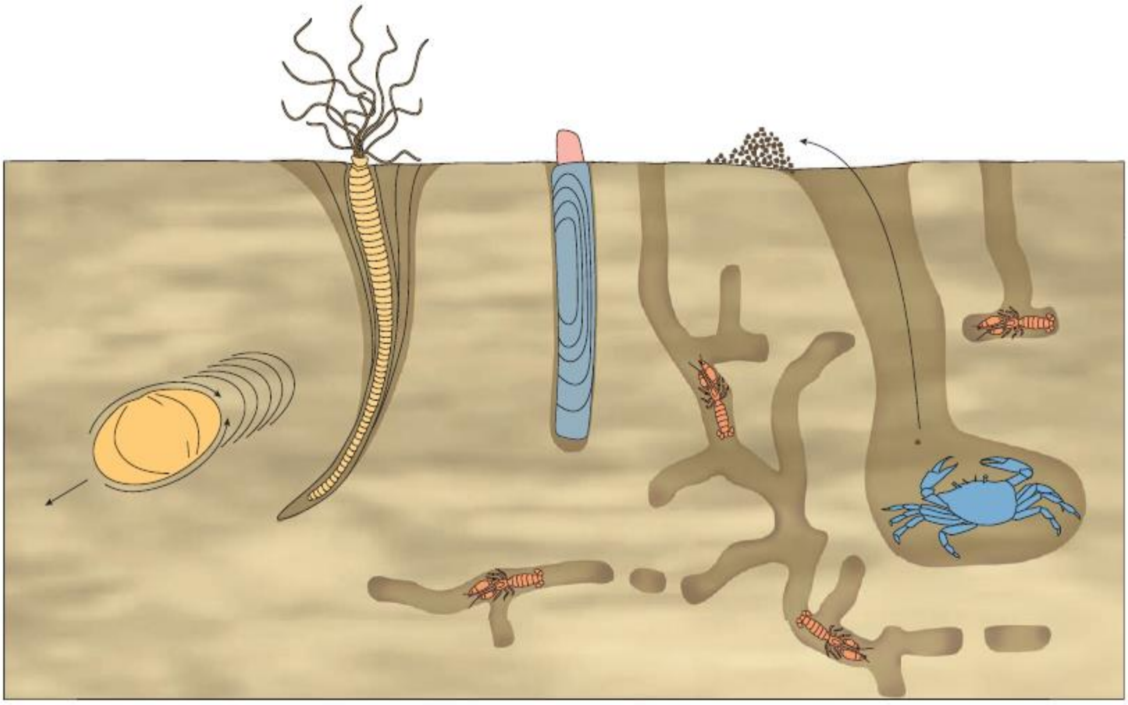
The first record of the term bioturbation occurred in the study of Richter (1952) to describe fossil biogenic structures found in sedimentary rocks. However, the study on bioturbation is older. Various authors (FELLER et al., 2003; JOHNSON, 2002; KRISTENSEN et al., 2012; KUTSCHERA; ELLIOTT, 2010; MEYSMAN;

MIDDELBURG; HEIP, 2006) attribute the first study on bioturbation to Charles Darwin in his last published scientific book, called “On the Formation of Vegetable Mounds through the Action of Worms with Observations on their Habits”, in which, despite not using the term specifically, he describes the effects of worms on the structure of the substrate in which they live (DARWIN, 1881). Kristensen et al (2012) also point out the studies of Davison (1891), who described 10 years after Darwin the dragging of sediments by a worm species to the surface, in the United Kingdom, and the studies of Richter (1927) and Abel (1935) who introduced various concepts used in bioturbation studies, among which the term "Lebensspuren”, which would be a word used to designate the visual traces of burrowing animals.

In the next years, various researchers continued the studies of Darwin (see review by Johnson, 2002) on bioturbation with worms and other benthic macroinvertebrates (Ex: ADÁMEK; MARŠÁLEK, 2013; KRISTENSEN et al., 2013, 1985; QUEIRÓS et al., 2015; RENZ; FORSTER, 2013; VOLKENBORN et al., 2007), and expanded them, including publications about scientific models (DELEFOSSE et al., 2015; LA CROIX et al., 2015; LECROART et al., 2010; MADSEN et al., 2011), flora (FERREIRA et al., 2007b; GRIBSHOLT; KRISTENSEN, 2002; PAWLIK; PHILLIPS; ŠAMONIL, 2016) and various other species of burrowing fauna, such as oysters (ex: Michaud et al., 2006, 2005), insects (LAGAUZÈRE; MOREIRA; KOSCHORRECK, 2011), marine crustaceans (ex: DE BACKER et al., 2011; HOLMER; HEILSKOV, 2008; PASCAL et al., 2016) and even humans (ZALASIEWICZ; WATERS; WILLIAMS, 2014).

Animals that live on the sediment can cause various alterations in it through different forms, sizes and lengths of tunnels. These tunnels can be simple or branched, isolated or interconnected, with only one entry or many, and they can be open or closed in alternate periods, so that the variation of these factors will lead to significant differences between the bioturbation caused by one animal and another, or even between different species of the same animal (GINGRAS; PEMBERTON; SMITH, 2015). (see Figure 1).

Figure 1 – Animals that live on or close to a water-sediment interface build burrows of different sizes, forms and complexities, which cause different effects of bioturbation.



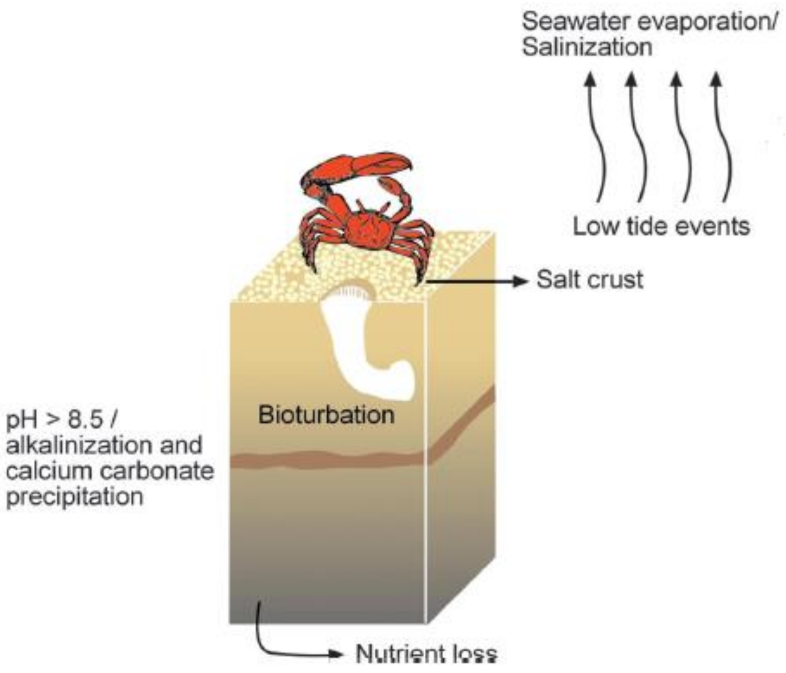
Source: GINGRAS et al. (2015).

Dating and soil morphology are of fundamental importance in the study of strategies developed by macrobenthos for survival and feeding, as well as of bioturbation and its effect on the functioning of soils of ecosystems (ANDREETTA et al., 2014). Koretsky et al. (2002) observed that most of the theoretical models for the study on bioturbation were based on chemical data, instead of ecological data, and then tested stochastic simulation models of 3-D burrow networks to calculate the mean density, volumes and surface area of walls of burrows of crabs and worms as a function of the depth of the sediment. Given the different forms of burrows and excavations, the spatial and temporal patterns of bioturbation are difficult to be analyzed in the generally opaque sediment without altering the natural characteristics of the process. One solution to facilitate these studies was proposed by Delefosse et al. (2015) with a 4-D models of bioturbated environments based on computational and position emission tomography.

In the same way that it has different causing agents, bioturbation has various effects on the environment, such as: physicochemical alteration and alteration in the position of layers of sediments (ESCAPA; PERILLO; IRIBARNE, 2008), increase in turbidity of sediments in suspension (CROEL; KNEITEL, 2011; FLEEGER et al., 2006), alterations in sediment-water interface (WILSON; HUGHES; FITZGERALD, 2012), alteration in the transport of nutrients (HOLMER; HEILSKOV, 2008; MICHAUD et al., 2006), increase in the penetration of oxygen into the sediment (ARAÚJO JÚNIOR et al., 2012; PÜLMANNS et al., 2014), which increases the mineralization processes through which the nutrients (especially phosphate and ammonia) are released to the overlying water (ADAMS; ANDREWS; JICKELLS, 2012; NÓBREGA et al., 2014a), besides altering the ecological and evolutionary dynamics of phytoplankton and zooplankton (ADÁMEK; MARŠÁLEK, 2013).

In the bioturbating fauna associated with coastal humid zones, especially mangrove forests, decapods are among the most researched groups of animals, especially due to their large number of species (about 14,750), great geographic dispersion in aquatic ecosystems, their historic role as biological models and their importance as human food (VOGT, 2012). Among the decapods, crabs (Figure 2) are one of the groups of bioturbators with greatest emphasis in studies, given their wide dispersion, intense burrowing activity and influence on the distribution and density of other animals (ABDULLAH; LEE, 2016; LEE, 2015).

Figure 2 – Example of bioturbation caused by crabs



Source: ALBUQUERQUE et al. (2014a).

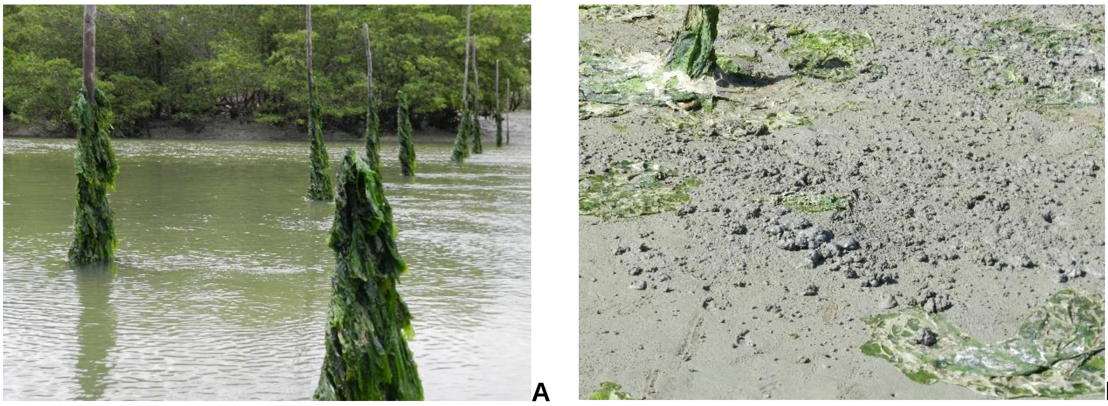
3 EFFECTS OF THE ACTIVITY OF CRABS ON THE SOIL GEOCHEMISTRY OF COASTAL WETLANDS

Kristensen (2008) describes crabs as allogenic engineers of the ecosystem, for transforming, displacing and using biotic and abiotic materials of the environment. These organisms can affect the biogeochemical reactions of an area through alteration of physical conditions, reduction of resources available to microorganisms (ingestion of sediments and organic matter), bioaccumulation of particles and through the formation of burrowed holes that alter the vertical profile of the soil (construction of burrows) (PENHA-LOPES et al., 2009, 2010).

These structures function as a refuge against predators and extreme environmental conditions and as food storage place, besides affecting the topography and biogeochemistry of the sediments, modifying the distribution of particle size, drainage, redox conditions and organic matter, as well as the availability of nutrients in the site (GUTIÉRREZ et al., 2006; MILNER et al., 2010). This last process has great importance in sulfate reduction, because it increases the exposure of the soil to atmospheric oxygen, thus causing its oxidation (ARAÚJO JÚNIOR et al., 2012; CORREIA; GUIMARÃES, 2016; NORDHAUS; DIELE; WOLFF, 2009).

Bioturbation by crabs helps to improve the yield and health of mangrove ecosystems (PENHA-LOPES et al., 2009). Due to the increase in anthropogenic activity close to coastal zones, these areas have suffered with various environmental impacts (DEFEW; MAIR; GUZMAN, 2005; MYHRE et al., 2013), especially deforestation and disposal of domestic effluents and effluents from shrimp farming ponds (HUITRIC; FOLKE; KAUTSKY, 2002; LACERDA; SANTOS; LOPES, 2009; LACERDA et al., 2011; SUÁREZ-ABELENDA et al., 2014; THORNTON; SHANAHAN; WILLIAMS, 2003). The disposal of large amounts of effluents in coastal ecosystems cause alterations in the survival, feeding, burrowing intensity and behavior of various aquatic and semi-terrestrial species (PÁEZ-OSUNA, 2001). One example of a problem resulting from this alteration in the biological patterns of bioturbation through large disposals of effluents is the proliferation of algae mats (Figure 3), which consists in the accelerated growth of microalgae in sediments contaminated by high levels of nutrients from these sewage effluents (KRISTENSEN; ALONGI, 2006), which may lead to extensive anoxia (MARSDEN; BRESSINGTON, 2009). Under normal conditions, the bioturbation activity, with consequent increase of burrowed sediments on the surface by crabs, prevents the development of these algae mats and maintains a high primary benthic productivity (PENHA-LOPES et al., 2009).

Figure 3 – Macroalgae mat (Jaguaribe River mangrove, Northeast Brazil) in trees (A) and soil (B), indicating excess of nutrients and low bioturbation activity in the ecosystem.



A B

Source: The author.

The various effects of bioturbation by crabs on carbon cycling are also very expressive and have been widely studied in the last years (PÜLMANNS et al., 2014; SMITH; WILCOX; LESSMANN, 2009). Andreetta et al. (2014) studied the effect of macrobenthos on soil organic carbon (stock and dynamics) in a mangrove of the Gazi Bay (Kenya) and observed that, despite the low contents of organic carbon (OC) in the studied soils, they were higher than in other ecosystems; therefore, the authors explained that the high spatial heterogeneity in the distribution and amounts of OC in the coastal environments could be explained by the different bioturbating effects from different burrowing animals (ADÁMEK; MARŠÁLEK, 2013; BEAUCHARD et al., 2012; ESCAPA; PERILLO; IRIBARNE, 2008).

Gutiérrez et al. (2006) evaluated the effects of the transfer of sedimentary organic carbon through the excavation of burrows and its exit from more superficial layers to the more buried sediments during this maintenance and cleaning of burrows by the *Chasmagnathus granulatus* crab, observing that the rate of carbon excavated and brought to the surface is higher than that of deposition at the bottom of the burrow, although the excavated sediments showed lower amounts of total and bioavailable carbon in relation to the non-bioturbated sediment, demonstrating an effect of active transposition of sediments, increasing the amount of reduced particles on the surface and oxidized particles on the walls and bottoms of the burrows (Figure 4).

Figure 4 – Mobilization of sediments and organic particles from the bottom of the burrows (more reduced) to the surface (A) and from more superficial layers (more oxidized) to the inside of the burrows (B) during the process of cleaning of burrows.


A


B

Source: The author.

During the maintenance of their burrows, crabs can mix more oxidized sediments from the surface with others reduced, extracted from the bottom of their burrows (residues of organic matter mixed with soil, called pallets) (BARTOLINI et al., 2011; PENHA-LOPES et al., 2009). This increases the potential of reduced soil in the more superficial layers in the distance of a few centimeters from the burrows (GRIBSHOLT; KOSTKA; KRISTENSEN, 2003; GRIBSHOLT; KRISTENSEN, 2002; MOKHTARI et al., 2016; NIELSEN; KRISTENSEN; MACINTOSH, 2003), besides altering the microbial oxidation of soil carbon of mainly anaerobic pathways, such as sulfate reduction and methanogenesis, to partially aerobic pathways (KRISTENSEN, 2008, 2015; KRISTENSEN et al., 2008a).

Organic matter mineralization is also increased by the process of soil bioturbation of coastal humid areas by crabs, due to the greater diffusion of oxygen in the soil by the exchanges (KRISTENSEN, 2008, 2015; NIELSEN; KRISTENSEN; MACINTOSH, 2003; PENHA-LOPES et al., 2010, 2012), which causes the increase in iron (Fe) and manganese (Mn) oxidation in almost the entire soil-water interface (ARAÚJO JÚNIOR et al., 2012; FERREIRA et al., 2007b). Some studies, however, demonstrate that the impact of bioturbation can be spatially limited, with lower sulfate reduction rate in sediments farther from the burrows or more superficial, especially of fiddler crabs such as those of the genus *Uca* (MICHAELS; ZIEMAN, 2013; NIELSEN; KRISTENSEN; MACINTOSH, 2003).

Other environmental factors can alter the impact of bioturbation on carbon cycling and soil biogeochemistry. The presence of roots of bioturbating plants and other vegetal covers directly influence the reduction process in the soil, with consequent oxidation of pyrite and greater precipitation of Fe oxides, increase in redox potential (Eh) and in the fraction of volatile acids (VAS) and consequent decrease in pH (acidification) (FERREIRA et al., 2007b; GRIBSHOLT; KOSTKA; KRISTENSEN, 2003; GRIBSHOLT; KRISTENSEN, 2002; PAWLIK; PHILLIPS; ŠAMONIL, 2016). Climatic variations along the year, such as the increase in rainfalls or temperature in a specific period, influence the bioturbating activity of the crabs, increasing their foraging and reproductive activities in drier and hotter periods, which also increases the excavation of burrows for reproduction and protection against predators and extreme temperatures (ARAÚJO JÚNIOR et al., 2012; GOES et al., 2010; NORDHAUS; DIELE; WOLFF, 2009).

Among the crabs found in the estuaries and other coastal humid zones, the families *Grapsidae, Ucididae* and *Ocypodidae* (Figure 5) are considered as the representatives of bioturbator and bioengineer crabs of ecosystems, due to their high activity of feeding and burrowing (ANDREETTA et al., 2014; KRISTENSEN, 2008; PENHA-LOPES et al., 2009). The crabs of the *Ucididae* family have large size and excavate deep burrows of up to 2 meters, in which they store different foods (CANNICCI et al., 2008; FLEMMING, 2011; KRISTENSEN, 2008).

Figure 5 – *Goniopsis cruentata* (A) of the *Grapsidae* family, *Ucides cordatus* (B) of the Family *Ucididae* and *Uca maracoani* (C) crabs of the *Ocypodidae* family.

A **B** **C**

Source: The author.

Although the crabs of the *Ocypodidae* family may share habitats with those of the *Ucididae* family, they usually have smaller body size and less deep burrows, with higher density (ARAÚJO, M. DE SL; CALADO, 2011; GEIST; NORDHAUS; HINRICHS, 2012; MAUTZ et al., 2011) and different feeding, filtrating food on the surface instead of burying them in the burrow, thus causing higher oxidation of the superficial soil layers (DI VIRGILIO; RIBEIRO, 2013; RIBEIRO; IRIBARNE, 2011).

The differences of bioturbation and feeding between *Ucididae* and *Ocypodidae* families lead to different intensities of bioturbation, with consequent different effects on the biogeochemistry and carbon stock in coastal humid environments (KRISTENSEN, 2008; PENHA-LOPES et al., 2009). Two genera stand out for the wide distribution and intense bioturbating activity: *Ucides* (from *Ucididae* family) and *Uca* (from *Ocypodidae* family) (CRANE, 2015; ERNESTO; BEZERRA, 2012; KRISTENSEN et al., 2008a). The genus *Uca* differs from *Ucides* for having a hairy ventral orifice between the third and fourth pair of pereiopods and about 30% smaller fronto-orbital distance and mean size of body and carapace (SAINT-PAUL; SCHNEIDER, 2010).

The construction of burrows by crabs of the genus *Uca* aims to provide shelter during the periods of high tide, protection against predators, enemies and other crabs, besides serving as a site for mating and incubation of eggs (MILNER et al., 2010; QURESHI; SAHER, 2012). All the material brought from the soil is carried to a short distance from the burrow and left or thrown away. *Uca* burrows are usually simple, unbranched and shallow (approximately 40 cm), individualized or shared (ERNESTO; BEZERRA, 2012; KRISTENSEN, 2008). Qureshi and Saher (2012) evaluated the interspecific differences between three species of the *Uca* genus with respect to the vegetation of the substrate they occupied, composition of sediment and morphology, density, depth, width, volume and opening of their burrows, observing that all morphological characteristics of the burrows varied significantly. In addition, their morphology was correlated with tide height, porosity, percentage of organic matter, vegetal cover and structure of the sediment.

The prominent specie of the genus *Ucides*, *Ucides cordatus,* stands out for its wide distribution and the number of studies about it. *Ucides cordatus* are typical representatives of mangroves of the Atlantic coast of the American continents and are found from south Florida until the state of Santa Catarina in Brazil (ARAÚJO,

M. DE SL; CALADO, 2011; GOES et al., 2010). This crustacean has a bluish-green dorsal color and red legs and is popularly known as “uçá”, “true-crab” or “uçaúna” (MANGUABA et al., 2008).

U. cordatus obtains food during the low tide, close to the burrows (CASTILHO et al., 2007; NORDHAUS; WOLFF; DIELE, 2006), and its diet mainly consists of mangrove leaves (61.2%), unidentified plant material and detritus (28.0%), roots (4.9%), sediment (3.3%), tree barks (2.5%) and material of animal origin, such as crustaceans, polychaetes, insects, bivalves and gastropods (0.1%) (NORDHAUS; WOLFF, 2007). This crab has a territorial behavior in relation to its burrows, building individual galleries that are intensely protected (DOS SANTOS; PINHEIRO; HATTORI, 2009; GOES et al., 2010) The burrows serve as protection against predators and desiccation. Thus, *U. cordatus* plays an important function in the biogeochemistry of the estuaries, promoting degradation of organic matter through its feeding and mobilizing sediments vertically in the soils through the removal and mobilization of detritus from their burrows (NORDHAUS; DIELE; WOLFF, 2009; NORDHAUS; WOLFF; DIELE, 2006).

4 CONTAMINATION OF MANGROVE BIOTA BY TRACE METALS AND THEIR BIOACCUMULATION IN CRABS.

Trace metals can be defined as cationic and oxyanionic metals that are normally present at low concentrations in the environment, usually lower than 1 g kg-1 (ESTEVES, 2011). This term has been used in substitution to the expression “heavy metal”, which is not a consensus and has been modified several times by many authors in scientific studies along the years, because of the density of the elements, which have already varied between 4 and more than 6 g/cm3 (PIAN; ALVES, 2013). These metals can be originated from natural components such as soil, atmosphere, waters or the biota itself (PREDA; COX, 2002), besides effluents from human activities.

The metals found in soils and coastal sediments can present themselves in different geochemical phases, which vary according to the environmental conditions of the sediment and water columns (OTERO; MACIAS, 2003; OTERO et al., 2009). Five of these phases stand out because their behavior is influenced by specific conditions in the environment (OTERO et al., 2014). Among them, the sum of the concentrations of metals of the first four phases is considered as potentially bioavailable and may contain metals of natural or anthropic origin (Nóbrega et al., 2014b):

a) Exchangeable phase – comprehends ions of the metals associated with the exchange surface of clay minerals, Fe and Mn oxides and organic matter, absorbed in the solid/liquid interface as a result of relatively weak intermolecular forces;
b) Oxidizable phase – comprehends metals that are bound to organic matter and sulfides;
c) Carbonate phase – includes metals co-precipitated with carbonates and that are strongly affected by variations and pH;
d) Reducible phase – includes metals bound to Fe and Mn oxides;
e) Residual phase – contains metals of natural origin, in the phase of silicates, bound to the crystalline networks of the minerals.

Besides the contamination of the soils, another great ecological concern refers to the environmental impact caused by the anthropogenic release of metals in the various natural environments and their consequent ingestion or absorption by the local biota, a phenomenon known as bioaccumulation (MOHAPATRA et al., 2009; PENG; HUNG; HWANG, 2011; PERRY et al., 2015).

Some trace metals (e.g. Cu, Fe, Zn and Mn) are essential for the metabolism of plants and animals at low concentrations, participating in respiratory pigments, formation of tissues and carapaces or as activators of enzymatic complexes, etc. At high concentrations, however, these minerals may act as harmful or toxic agents to the organisms, causing metabolic disorders such as reduction in the effect of enzymes, alteration of molecules of proteins or other problems at a biochemical level in aquatic organisms and humans (LAMBAIS; OTERO; CURY, 2008; OTERO et al., 2014; SUÁREZ-ABELENDA et al., 2014).

Various organisms, both animals and plants, can be used as bioindicators or biomonitors of disposal of pollutants in the ecosystem (ÁLVARO et al., 2016; BELTRAME; DE MARCO; MARCOVECCHIO, 2011; PINHEIRO et al., 2012). Aquatic animals, for instance, are good indicators of the quality of the water in which they are, and among them, bivalves and crustaceans stand out for being organisms mostly filtrators or of strong interaction with the sediment of their habitats (NAGELKERKEN et al., 2008; SAHA; SARKAR; BHATTACHARYA, 2006). This relationship can cause accumulation of pollutants in the tissues of these animals and, more importantly, in human populations that feed on them (COLAÇO et al., 2006; LACERDA; SANTOS; LOPES, 2009; VAN DER OOST; BEYER; VERMEULEN, 2003).

Carvalho and Lacerda (1992) performed chemical analysis of various benthonic marine organisms in the Guanabara Bay and did not detect high concentrations of metals, compared with other uncontaminated areas. These authors concluded that domestic sewage disposed in the cited water body created a partially reducing environment, with metals in a form that is unavailable for biological incorporation, due to the high sedimentation rates that maintain the metals strongly bound to the sediment.

Due to the great importance of crabs and swimming crabs in the feeding of coastal populations in Northeast Brazil, the evaluation of the contents of trace metals in these animals is of great relevance (MOHAPATRA et al., 2009; PERRY et al., 2015; PINHEIRO et al., 2012). Among the microorganisms studied with respect to bioaccumulation of metals in mangroves, *Ucides cordatus* crabs stand out for having great importance in the mobilization of sediments and in the flow of nutrients in the mangroves, being responsible for a large part of the reuse of litter in these sites. In addition, they have great economic importance as fishing resource for commercialization and consumption by resident populations (WALTERS et al., 2008).

Crabs of the genus *Ucides* have high sensitivity to various pollutants, which highlights their efficiency as bioindicators of contamination in mangrove areas. Various researchers utilized these animals in studies on environmental biomonitoring (GOES et al., 2010; NORDHAUS; WOLFF, 2007), evaluation of presence of oil in mangroves (NUDI et al., 2007), detection of levels of pollutants and agrochemicals such as DDT (TAVARES; BERETTA; COSTA, 1999), climate changes and increase in solar radiation (DE OLIVEIRA MIGUEL; MEYER-ROCHOW; ALLODI, 2002) and, mainly, as bioindicators of pollutants containing trace metals (PINHEIRO et al., 2012; SÁ; ZANOTTO, 2013). Corréa et al., (2000) reported that the accumulation of zinc in the hepatopancreas of *U. cordatus* caused signs of damage to the hormonal system. Salinity, on the other hand, promotes ideal conditions for larval development when at high concentrations.

However, studies that analyze the effects of bioaccumulation of metals in crabs are still scarce. Some of them are listed in Table 1. Rainbow (1985) observed that decapod crustaceans were sensitive to the increase of non-essential metals in the environment, as well as to high contents of essential metals, although they regulate the internal concentration of essential elements through processes of physiological and biochemical detoxification.

Hence, further studies on the effects of bioaccumulation of trace metals in crabs and their bioavailability for the next trophic levels are necessary due to both environmental and ecological importance and to the social aspect, since it is a species used for human consumption.

Table 1 – Concentrations of various elements in tissues of crabs of different geographic sites (in µg/g)

Reference	Crab	Local	Organ/Tissue	Metals concentration in µg g−1 (ppm)												
				Fe	Zn	Cu	As	Cd	Co	Cr	Hg	Mn	Ni	Pb	Rb	Se
Este trabalho	*Ucides cordatus*	Northeast Brazil - Carciniculture Area	Hepatopancreas	129.2±88.5	137.9±72.6	67.8±43.3	-	-	-	-	-	-	-	-	-	-
Álvaro et al., (2016)	*Pachygrapsus marmoratus*	Lagoon - São Miguel island,	Hepatopancreas	300±0	112,67±2,1	197,67±5	25,93±0,5	0,6±0	0,43±0,05	7,57±1,2	0,07±0	28,67±21,5	6,33±0,1	-	2,6±0,1	2±0,1
		Cais da Sardinha - São Miguel island	Hepatopancreas	450±0	144,25±11,8	32,23±2,4	33,15±2,9	3,48±0,5	0,28±0,04	7,23±3	0,05±0	23±9	5,08±0,5	-	3,18±0,1	2,48±0,1
		Ladeira da Velha - São Miguel island	Hepatopancreas	200±0	159,83±5,8	417,17±21,9	34,73±3,1	0,2±2,8	0,7±0	4,12±0,8	0,09±0,1	28,33±3,9	8,02±1,7	0,63±0	3,87±0,1	2,62±0,4
		Negrito	Hepatopancreas	1683,33±0,1	168±4,7	44,57±2,2	34,53±2,5	0,6±0,1	0,82±0,5	7,02±3,9	0,09±0,1	65,67±9,8	2,82±0,9	0,12±0	5,2±0,1	2,3±0,2
Perry et al., (2015)	*Chaceon quinquedens (red crab)*	Area 1 - Gulf of Mexico	Muscle - males	-	240±36,11	-	402±129	1,14±0,12	4,42±2,49	88,69±106	0,36±0,11	-	-	12,54±3,85	-	-
			Muscle - females	-	226±21,96	-	275±58,39	1,12±0,06	1,12±0,06	6,53±8,25	0,56±0,12	-	-	11,24±0,58	-	-
		Area 2 - Gulf of Mexico	Muscle - males	-	206±26,38	-	372±81,56	1,63±0,53	1,98±0,47	5,44±4,94	0,34±0,21	-	-	12,13±3,70	-	-
			Muscle - females	-	214±20,4	-	291±56,98	1,41±0,54	1,5±0,53	6,85±5,32	0,25±0,22	-	-	17,37±9,35	-	-
		Area 3 - Gulf of Mexico	Muscle - males	-	202±13,64	-	367±90,48	0,54±0,16	3,31±1,98	296±180	0,68±0,42	-	-	16,72±7,70	-	-
			Muscle - females	-	200±13,59	-	354±107	-	2,39±1,72	285±164	0,47±0,24	-	-	6,97±2,68	-	-
Julshamn et al., (2015)	*Paralithodes camtschaticus (red king crabs)*	Barents Sea	Claws and paws	-	-	-	10 ± 5	0.021 ± 0.036	-	-	0.04	-	-	-	-	-
Corrêa et al., (2005)	*Ucides cordatus*	Manguezais da baia de Guanabara, Rio de Janeiro, Brasil	Gills	-	-	-	-	-	-	4,8±0,5	-	5,4±0,2	-	-	-	-
			Hepatopancreas	-	-	-	-	-	-	1,7±0,9	-	10,1±5,5	-	-	-	-
			Muscle	-	-	-	-	-	-	2,5±0,2	-	1,2±0,2	-	-	-	-
Pinheiro et al., (2012)	*Ucides cordatus*	Cubatão, Santos-Sao Vicente estuary, São Paulo, Brasil	Gills	-	-	22,43±2,04	-	0,11±0,01	-	0,37±0,06	<0,001	8,01±0,72	-	<0,05	-	-
			Hepatopancreas	-	-	6,64±0,47	-	0,16±0,02	-	0,52±0,17	<0,001	8,89±1,52	-	<0,05	-	-
			Muscle	-	-	5,31±0,26	-	0,10±0,01	-	0,25±0,07	<0,001	5,09±0,72	-	<0,05	-	-
Peng et al., (2011)	*Xenograpsus testudinatus*	Kueishan Island, Taiwan	Exoskeleton	14,38±8,12	26,12±18,2	1,00±0,14	-	0,13±0,07	0,02±0,02	1,15±0,99	-	19,91±18,68	0,95±0,45	6,99±5,52	-	-
			Gills	158±70	610±170	290±91	-	1,29±0,97	0,67±0,26	2,17±1,11	-	3,31±1,31	4,76±1,71	2,28±1,4	-	-
			Hepatopancreas	175±99	118±66,82	53,83±37,59	-	0,6±0,39	0,2±0,09	1,66±1,14	-	3,95±2,35	1,64±1,5	2,64±2,11	-	-
			Muscle	37,04±21,72	230±41,83	74,63±27,08	-	0,49±0,4	0,1±0,03	0,81±0,3	-	0,69±0,5	0,99±0,64	1,83±0,71	-	-

Firat et al., (2008)	*Charybdis longicollis*	Iskenderun Bay, Turkey	Gills	491,0±161,2	698,1±118,0	827,0±92,61	-	75,50±8,77		677,1±97,66	-	-	-	-	-	-
			Hepatopancreas	684,9±153,2	804,8±103,1	935,1±50,48	-	111,2±22,27		802,3±124,6	-	-	-	-	-	-
			Muscle	32,77±7,31	32,53±7,03	77,55±12,00	-	25,44±7,84		76,55±16,82	-	-	-	-	-	-
Mohapatra et al., (2009)	*Scylla serrata*	Mahanadi estuary, India	Gills - Males	190,4±16,2	42,2±2,9	32,9±2,9	-	-	-	-	-	18,5±1,7	-	0,18±0,009	-	-
			Gills - Females	182,8±18,1	52,8±5,9	31,6±2,4	-	-	-	-	-	17,2±0,9	-	0,13±0,010	-	-
			Carapace - Males	125,9±10,6	63,4±4,9	47,9±3,2	-	-	-	-	-	18,6±2,0	-	0,16±0,01	-	-
			Carapace - Females	112,6±9,7	71,6±6,9	51,6±5,2	-	-	-	-	-	24,8±2,1	-	0,18±0,008	-	-
			Muscle - Males	159,7±13,2	279,3±19,6	114±10,4	-	-	-	-	-	12,8±1,3	-	0,19±0,006	-	-
			Muscle - Females	171,4±12,9	312,6±28,6	132,3±13,0	-	-	-	-	-	11,2±1,3	-	0,23±0,013	-	-
Barrento et al., (2009)	*Cancer pagurus*	Scottish coast	Gonads -Males	4,5±0,6	33±1	14±0	-	-	-	-	-	-	-	-	-	1,4±0,1
			Gonads - Females	19±1	90±4	12,±1	-	-	-	-	-	2,4±0,1	-	-	-	2,6±0,2
			Hepatopancreas - Males	61±20	57±15	53±3	-	-	-	-	-	2,4±1,3	-	-	-	3,5±0,9
			Hepatopancreas - Females	58±18	80±13	166±112	-	-	-	-	-	1,4±0,3	-	-	-	5,3±2,1
			Muscle - Males	3,1±0,4	59±3	7,5±09	-	-	-	-	-	0,24±0,04	-	-	-	0,92±0,19
			Muscle - Females	3,4±0,4	55±4	9,3±2,1	-	-	-	-	-	0,26±0,03	-	-	-	0,98±0,14
		English channel	Gonads -Males	2,4±0,5	16±1	4,8±0,5	-	-	-	-	-	-	-	-	-	1,4±0,1
			Gonads - Females	13±1	72±3	8,1±0,5	-	-	-	-	-	3,2±0,1	-	-	-	2,6±0,2
			Hepatopancreas - Males	38±11	18±2	13±7	-	-	-	-	-	3,4±0,7	-	-	-	2±0,47
			Hepatopancreas - Females	11±3	21±4	58±11	-	-	-	-	-	2,1±0,3	-	-	-	4±1,1
			Muscle - Males	2,2±0,2	52±4	6,7±1,1	-	-	-	-	-	0,32±0,15	-	-	-	0,66±0,12
			Muscle - Females	4,3±0,8	56±5	10±3	-	-	-	-	-	0,28±0,03	-	-	-	1,1±0,3

Source: The author.

5 THE EFFECT OF BIOTURBATION ON THE EMISSION OF GREENHOUSE GASES

The emission of greenhouse gases (GHGs) is nowadays one of the greatest environmental problems of humanity. GHGs are responsible for retaining the heat in the atmosphere so that the temperature remains within a range of values adequate to the survival of living organisms and ecosystems. It is important to point out that GHGs are naturally produced, as a result of volcanic eruptions, decomposition of organic matter and smokes of fires, and that are essential for the existence of life on the planet. Nonetheless, on a global scale, the exaggerated increase of these elements, especially through the anthropic action of burning fossil fuels, leads to global warming and its catastrophic consequences (IPCC, 2014).

The main consequences of the greenhouse effect are the destruction of natural habitats, disappearance of plant and animal species, amplification and multiplication of drought effects, floods, hurricanes and the melting of ice caps and glaciers, with consequent increase in the water level of the oceans and lakes, submerging islands and large densely populated coastal areas. The overheating of tropical and subtropical regions contributes to the intensification of the process of desertification and proliferation of insects that are harmful to human and animal health Among the GHGs, carbon dioxide (CO_2), methane gas (CH_4) and nitrous oxide (N_2O) stand out as the most abundant in the atmosphere and potentially dangerous (CHEN; TAM; YE, 2010). CO_2 contributes with about 55% of the total global emissions of GHGs. The amount of CH_4 emitted is lower, but its warming potential is 23 times higher than that of CO_2. Although N_2O concentrations in the atmosphere are even lower, the power of global warming of these gases is very high, being respectively about 298 higher than that of CO_2 (FORD et al., 2012; YU; FAULKNER; PATRICK, 2006)

It is estimated that the annual global emissions of GHGs of anthropogenic origin increased by 70% in the period from 1970 to 2004, resulting in increment of 0.55 ºC in the mean temperature of the terrestrial surface (IPCC, 2014). Such increment in GHG emission has led the population and competent organs to search for strategies of land use and soil management that can reduce GHG emission to the atmosphere.

CH_4 is one of the main hydrocarbons present in the atmosphere. In anaerobic environments, with low concentrations of sulfate and nitrate, the mineralization of soil organic matter (SOM) occurs through methanogenic fermentation, which releases CH_4 and CO_2. Methanogenesis occurs in anaerobic environment with low redox potential (KRISTENSEN et al., 2008b; KRITHIKA; PURVAJA; RAMESH, 2008; UEDA et al., 2000). CH_4 emission can be affected by diffent factors influencing the processes of oxidation-reduction and transfer of CH_4 to atmosphere, like the nature of clay, type of vegetation, intensity of microbial activity and methanogenesis, particularly the competition with the denitrification and sulfate reduction (ADAMS; ANDREWS; JICKELLS, 2012; KREUZWIESER; BUCHHOLZ; RENNENBERG, 2003).

N_2O formation and emission through microbial processes (nitrification and denitrification) results from complex interactions between various factors, such as temperature, texture, structure, soil pH, availability of nitrogen and degradable organic material and water content in the soil (BLACKWELL; YAMULKI; BOL, 2010; DIVIA et al., 2013). For Dong et al. (2006), N_2O can be formed in various processes (ammonification, nitrification and denitrification); however, in estuaries in the United Kingdom, it was not observed which of these processes was the source of N_2O emission. Bauza et al. (2002) observed, in mangrove sediments in Puerto Rico, satisfactory conditions for the activity of aerobic microorganisms, i.e., for the occurrence of nitrification, and inferred that this process was responsible for the emission of N_2O.

Soil use and management directly affect nutrient cycling, dynamics of SOM and energy flow, negatively affecting soil chemical and physical attributes, as well as biodiversity. During all steps of SOM degradation, GHGs are released to the atmosphere. CO_2 emission through the decomposing action of heterotrophic microorganisms in the soil is mainly dependent on the SOM content and availability of vegetal residues, which constitute the main sources of carbon to the microbiota (PAGE; DALAL, 2011). Hence, SOM degradation is affected by the edaphoclimatic conditions (temperature, humidity, pH, contents of O_2 and nutrients in the soil) and by the quality of the substrates, as soluble fraction, nutrients, lignin and C:N ratio (CHEN; TAM; YE, 2010).

CO_2 emission from the soil to the atmosphere occurs mainly through two biological processes: respiration (organisms and root system of the plants) and decomposition of organic residues. According to Duiker and Lal (2000), the climatic variables directly influence the CO_2 flow to the atmosphere, and its main conditioning factors are temperature (soil and atmosphere) and soil moisture. In soils with high moisture content, SOM mineralization rate decreases, compromising the exchange of gases with the atmosphere.

Bioturbation by crabs stimulates benthic metabolism, mineralization rates of total N and the potential of nitrification and denitrification in flooded environments like mangroves, which could cause increase in the GHG flow, but the effects on the concentration of dissolved organic N and its effects on N_2-fixing organisms are contrasting, increasing the fixation in some flooded sites (saltmarshes), but decreasing in swampy sites (mudflats) (FANJUL et al., 2011, 2014).

Penha-Lopes et al. (2010) evaluated the differences between the benthic metabolism, measured as CO_2 production, and the patterns of carbon oxidation in four mangroves subject to disposal of effluents and bioturbation by roots and crabs, and observed that the biogenic structures (roots and burrows) increased CO_2 emissions during the low tide in the areas contaminated by effluents, since the reduction of Fe was substituted by sulfate reduction and the contribution of microbial aerobic respiration to the total metabolism remained above 50%, thus demonstrating the impact of bioturbation on GHG emission and on the biogeochemistry of the environment.

Pülmanns et al. (2014) analyzed CO_2 flow with and without the bioturbating action of the *Ucides cordatus* crab and observed that climatic and temporal changes in the sediments surrounding the burrows did not influence their CO_2 release (20 to 60% of the CO_2 is released from the burrows through the respiration of the crabs). Thus, these authors demonstrated that the CO_2 released by *U. cordatus* and its burrows can be an important pathway of exportation of this gas from mangrove soils and must be considered in the accounting of carbon estimate of these environments.

While the respiration of *U. cordatus* and the CO_2 release of its burrows increased the carbon dioxide flow rate from 15 to 71%, fiddler crabs (such as those of the *Uca* genus) release 2 to 5 times more CO_2 than non-bioturbated soils, demonstrating a significant increase in the release of GHG in bioturbated areas, and that this increase has interspecific differences (KRISTENSEN et al., 2008b; NIELSEN; KRISTENSEN; MACINTOSH, 2003).

6 CONCLUSIONS

Bioturbation by macrobenthos, especially crabs, causes physical, chemical and biological modifications in coastal humid ecosystems, such as mangroves, marismas and apicuns. Among these effects, the increase in the penetration of oxygen in the various soil layers stands out; it alters the microbiota present in the soil, which starts to mineralize its organic matter more intensely. This causes an increase in the metabolism and benthic flow of the organic matter dissolved in the water column, affecting its distribution and bioavailability, besides increasing the proportion of labile organic carbon in the bioturbated sediments.

These alterations in soil microbiota and organic matter mineralization by the bioturbation of crabs will be directly influenced by the species of crab that is performing the bioturbation, varying according to its size, feeding and behavior, besides density, depth, diameter and structure of its burrow. Many studies about the effects of bioturbation on the biogeochemistry, bioavailability of metals and alterations in carbon cycling have been conducted; however, studies about the effects of bioturbation on the release of the various greenhouse gases, despite their importance for a correct environmental monitoring and, if necessary and possible, the remediation, are still scarce, requiring more publications and deepening.

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CHAPTER 2

The role of bioturbation by *Ucides cordatus* crab in the fractionation and bioavailability of trace metals in tropical semiarid mangroves*

RESUMO

Este estudo avaliou a atividade de construção de tocas por caranguejos *Ucides cordatus* e seus efeitos sobre o fracionamento, biodisponibilidade e bioacumulação de Fe, Cu e Zn em uma área de manguezal semi-árido (estado Ceará, NE-Brasil). Foram analisados o Eh, pH; tamanho de grão, composição da água intersticial. S total, C orgânico e a especiação das fases-sólidas de Fe, Cu e Zn em duas áreas: uma densamente povoada por caranguejos a outra um local de controle. A atividade de construção de tocas e a variação sazonal afetaram as condições biogeoquímicas dos solos de manguezais, aumentando a biodisponibilidade e bioacumulação de metais. As tocas de caranguejo favorecem a entrada de oxigênio no solo, oxidando a pirita e formando minerais Fe pouco cristalinos, aumentando os riscos de biocontaminação. Além disso, os teores de metais no hepatopâncreas são um bom indicador para a avaliação das formas de metal biodisponível e, assim, mais estudos devem ser realizados a fim de avaliar o potencial uso de *U. cordatus* como bioindicador de contaminação traços de metais.

Palavras-chaves: Biogeoquímica de metais traço; bioacumulação; Ferro; Cobre; Zinco.

ABSTRACT

This study evaluated the burrowing activity of *U. cordatus* and its effects on Fe, Cu and Zn fractionation, bioavailability and bioaccumulation in a semiarid mangrove area (Ceará state, NE-Brazil). Were analyzed the Eh; pH; grain size and pore water composition; total S and organic C, and the speciation of Fe, Cu and Zn solid-phases in two areas: a densely populated crab and a control site. The burrowing activity and seasonal variation affect the biogeochemical conditions of mangrove soils increasing metals bioavailability and bioaccumulation. The crab burrows favors the entrance of oxygen into the soil, oxidizing the pyrite and forming poorly-crystalline Fe minerals, increasing the risks of biocontamination. Furthermore, the metals content in the hepatopancreas are a good proxy for the evaluation of bioavailable metal forms and, thus, further studies must be conducted in order to evaluate the potential use of *U. cordatus* as a bioindicator for trace metals contamination.

Keywords: trace metals biogeochemistry; bioaccumulation; Iron; Copper; Zinc

1. INTRODUCTION

Mangrove forests are among the most important coastal ecosystems in the world, covering extensive areas in tropical and subtropical regions (SPALDING et al., 1997). Associated to these ecosystems, brachyuran crabs (mostly fiddler and sesarmid crabs) stand out as the most conspicuous among the Crustaceans (AHYONG et al., 2007), and are key organisms for the mangrove functioning since they influence many biogeochemical processes (KRISTENSEN and ALONGI, 2006; PÜLMANNS et al., 2014). This fact caused the recent recognition of mangrove crabs as true ecosystem engineers (KRISTENSEN, 2008).

Among the wide range of influences that Brachyurans exert in mangrove ecosystems (i.e. through leaf consumption, propagule predation, pre-shredding and macerating leaf-litter), the bioturbation probably stands outwith the greatest number of co-effects. The biological mixing of soil and sediments during the construction and maintenance of burrows for different purposes (refuge, mating, feeding) and its physical and chemical effects have received a great deal of attention in the last few years (WILSON et al., 2012; QUINTANA et al., 2015; REMAILI et al.,2016; MARTINEZ-GARCIA et al., 2015).

In fact, recent studies have evidenced that bioturbation in mangrove substrates may considerably deviate the dominance of anaerobic respiration routes (i.e. sulfate reduction; KRISTENSEN, 2008) to others more energetically favorable (i.e. aerobic and iron reduction; see ALONGI et al., 2001; KRISTENSEN, 2000; QUINTANA et al., 2015). Furthermore, as bioturbation promote the oxidation and dissolution of the sulfidicmaterial (i.e., pyrite; ARAÚJO JÚNIOR et al., 2012), an important trace metal sink (MACHADO et al., 2014), it may also increasing trace metals bioavailability. Thus, crab burrowing activity may not only alter the predominance of anaerobic pathways, but directly affect trace metals behavior with an associated risk of increasing their mobility and environmental impacts.

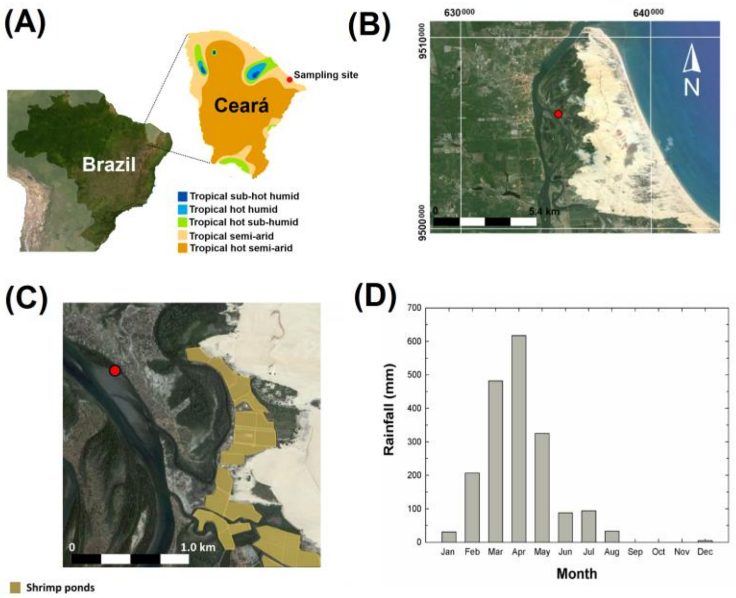
Among these crabs, *Ucides cordatus* stands out as the most extensively distributed along the Brazilian coast (MELO, 1996), and as one of the most dominant burrowing decapod inhabiting mangrove forests in the tropics. Despite the effects of *U. cordatus* burrows in mangroves soil has been studied (PÜLMANNS et al., 2014; ARAÚJO JÚNIOR et al., 2012), any studies have been conducted in order to assess the effects of bioturbation on metals bioavailability and, thus, the effects of crab bioturbation on metal biogeochemistry in mangrove soils still poorly understood. This study aim to evaluate the burrowing activity of *U. cordatus* and its effects on Fe, Cu and Zn fractionation, bioavailability and bioaccumulation rates in a semiarid mangrove area improving the comprehension of these endangered ecosystems and, thereby, promoting better conservation policies and management practices.

2. MATERIAL AND METHODS

2.1. Study site

The sampling site is located at the Jaguaribe river estuary, Ceará State, NE Brazil (Figure 1). The semi-arid climate of the region (ALVARES et al., 2013) presents a rainy period with a mean rainfall of 734 mm (from February to April) and a dry season with an mean rainfall of 189 mm (from June to January) with annual temperatures ranging from 26 °C to 28 °C (IPECE, 2014). Themangrove forest in the Jaguaribe estuary covers 1735 km^2 (MAIA et al., 2006) and is dominated by Rhizophora mangle L., R. racemosa, and Avicennia schaueriana L. species, and the spring-neap tidal cycle ranges between 1.4 and 2.6 m (TANAKA and MAIA, 2006).

Figure 1 - Location of the Jaguaribe estuary (A) and the sampling site (B) in the Ceará State (northeastern Brazil); In detail, shrimp ponds near to the sampling site (C); and precipitation record for the 2009, in mm (D).



Source: The author.

The Jaguaribe estuary stands out as the largest shrimp producer in Brazil (LACERDA et al., 2009; NOGUEIRA et al., 2009) with a total of 12.6km2 of ponds, accounting for 12% of the national shrimp production (NOGUEIRA et al., 2009). Most shrimp ponds are in close contact with mangrove forests that are permanently exposed to heavy metal emissions. In fact, the chronic use of Cu-bearing aquafeeds and fertilizers during shrimp breeding were reported as the main causes for high Cu contents in some Brazilian mangrove ecosystems (LACERDA et al., 2006). Moreover, due to physiological requirements, most shrimp species require a Zn-supplementation (FRIBERG et al., 1974), which frequently results in a large concentration of this metal in shrimp pond effluents.

2.2. Sample collection

Since there are no baseline concentrations for these potentially toxic metals in semiarid mangrove soils affected by shrimp pond effluents and no assessment of the effects of bioturbation on thesemetals dynamics, soil samples were collected in two contrasting sites: one affected by crab burrowing activity ("crab site" - Cb) and another without crab activity ("control site" - Cs). Both sites were closely located (distance <10 m) maintaining identical physiographical position to avoid diferences in flooding frequency, and to minimize vegetation effects (e.g., presence of pneumatophores, roots and plantlets). The sampling sites are vegetated by Rhizophora mangle and directly influenced by the shrimp farming effluents discharge from 28 shrimp ponds (covering 66.5 ha) (Figure 1C). However, all the shrimp farms at the Jaguaribe River are located upstream to the sampling site, which conducts to an intense anthropogenic metal discharge at the study site (LACERDA et al., 2006).

The burrow density was determined and the burrows depths were measured with a soft rubber wire (BRANCO, 1993), whereas the superficial crab burrow diameters were measured with a caliper (CARMONA-SUÁREZ and GUERRA-CASTRO, 2012). In the control site, bioturbation was avoided by the establishment of an exclusion plot by fencing off the soil with a 1 cm mesh nylon net (0.1 m height above the substrate and buried up to 1.5mof depth) during 3months prior to soil sampling. Six soil cores (PVC tubes with 5 cm diameter and 50 cm length) were taken in each site during lowtide,with soils exposed to the atmosphere, during the wet and dry seasons (May and December, respectively) in 1 m^2 plots. The cores were cut into sections of 10 cm depth intervals (0–10, 10–20 and 20–30) for laboratory analysis. The redox potential (Eh) and pH were measured in situ after equilibrating the electrodes for approximately 2 min with the soil samples. The redox potentials Eh) were measured with a platinum electrode and final readings were corrected by adding the potential (+244 mV) of a calomel reference electrode. The pH was measured with a glass electrode (Model MP120; Mettler Toledo) calibratedwith pH 4.0 and 7.0 standards. Additionally to soil samples, 20 adult specimens (10 males and 10 females) of *U. cordatus* were collected during each sampling period in order to access bioaccumulation linking to metals fractionation and bioavailability.

2.3. Analytical procedures

Interstitial water samples were extracted from soil samples by centrifugation at 10,000 rpmfor 30 min at 4 °C in polypropylene bottles (OTERO and MACIAS, 2002a, 2002b) and quantified the concentration of sulfate (precipitation with BaCl2 and quantification by gravimetry; RHOADES, 1982), chloride (titration with 0.05 N AgNO3 in the presence of potassium chromate: RHOADES, 1982) and salinity (hand refractometer).

Total contents of organic carbon (TOC) and S (TS) were determined in 2-mm sieved soil samples by an elemental analyzer (LECO CNS, model 2000). For the TOC quantification, samples were previously pre-treated with 6 N HCl to remove carbonates. The pipette method (GEE and BAUDER, 1986) was used for grain-size analysis.

Partitioning of Fe, Cu and Zn was performed using a sequential extraction procedure derived from the combination of methods proposed by TESSIER et al. (1979); FORTÍN et al. (1993), and HUERTA-DÍAZ and MORSE (1990), commonly used for wetland soils studies (e.g., ARAÚJO JÚNIOR et al., 2012; NÓBREGA et al., 2013; OTERO et al., 2009; and others), and obtaining six operationally distinct fractions, defined as:

a) (F1) Exchangeable metals (Fe_{EX}; Cu_{EX}; Zn_{EX}): extracted with 30 mL of 1 MMgCl2 solution (pH 7.0); samples were shaken for 30 min;

b) (F2) Metals associated to carbonates (Fe_{CA}; Cu_{CA}; Zn_{CA}): extractedwith 30 mL of 1 M NaOAc (pH 5.0); samples were shaken for 5 h;
c) (F3) Metals associated to ferrihydrite (Fe_{FR}; Cu_{FR}; Zn_{FR}): extracted with 30 mL of a solution of 0.04 M hydroxylamine+acetic acid 25% (v/v); samples were shaken for 6 h at 30 °C;
d) (F4) Metals associated to lepidocrocite (Fe_{LP}; Cu_{LP}; Zn_{LP}): extracted with 30 mL of a solution of 0.04 M hydroxylamine + acetic acid 25% (v/v); samples were shaken for 6 h at 96 °C;
e) (F5) Metals associated to crystalline Fe (oxyhydr)oxides (Fe_{OX}; Cu_{OX}; ZnOX): extracted with 20 mL of a solution of 0.25 M sodium citrate + 0.11 M NaHCO3, with 3 g of sodium dithionite; samples were shaken for 30 min at 75 °C;
f) (F6) Metals associated to pyrite (Fe_{PY}; Cu_{PY}; Zn_{PY}): after the elimination of sheet silicates (10MHF extraction) and organicmatter (HsSO4 extraction; see HUERTA-DÍAZ and MORSE, 1990; HUERTA-DIAZ and MORSE, 1992) the pyritic phase was extracted with 10 mL of concentrated HNO3; samples were shaken for 2 h at room temperature and then washed with 15 mL of ultrapure water. Between each step of the extraction procedure, the samples were centrifuged at 10,000 rpm (4 °C) during 30 min and washed with 20 mL of ultrapure water.

The sum of the six fractions (ΣF1 ➔ F6) of each metal were compared as Pseudo-Total contents, since the metals associated to organic matter and to sheet silicates were removed during pre-treatments of the pyritic fraction. The Degree of Iron Pyritization (DOP), which determines the percentage of reactive iron incorporated in the pyritic fraction (BERNER, 1970), was calculated as follows (1):

$$DOP\ (\%)=[(Fe_{PY})/(Fe_{REACTIVE}+Fe_{PY})]\times 100 \quad (1)$$

The $Fe_{REACTIVE}$ was considered to correspond to the sumof F1 to F5 (ΣF1 ➔ F5), which involves the reducible fractions that may react to form pyrite (OTERO and MACIAS, 2002a). Similarly, the DTMP (Degree of Trace Metal Pyritization, see

HUERTA-DÍAZ and MORSE, 1990) was determined based on the percentages of reactive metals incorporated into pyrite and, thus, calculated as (2):

$$DTMP_{METAL}(\%)=[(METAL_{PY})/(METAL_{REACTIVE}+METAL_{PY})]\times 100 \quad (2)$$

The crabs used for metal accumulation analysis were euthanized by chilling in a freezer, than the hepatopancreas was collected and maintained frozen until analysis acid/peroxide digestion (TUROCZY et al., 2001) to determine themetal content accumulated into the crab tissue. The biota-soil accumulation factor (BSAF; GOBAS, 2001; JACOBS, 1998) was assessed dividing the content ofmetal in the hepatopancreas divided by the Pseudo-Total content of the metal in the soil.

The metals concentrations in all the extracts (sequential extraction procedure and crab tissues) were determined by flame atomic absorption spectrophotometry.

2.4. Statistical analysis

Kolmogorov-Smirnov two-sample test (KeS-test) was used to study data homogeneity and normality an alpha-value of 0.05. Two-way ANOVA was performed to assess differences between fixed factors (sites and seasons) for the studied variables. Simple (2-tailed) correlation analyses (Pearson's product-moment; $p<0.05$) among general soil variables, porewater, and partitioning of Fe, Cu and Zn were conducted to investigate whether any relationships existed between variables.

Furthermore, a multivariate discriminant analysis was performed in order to better evaluate the variables related to the distinction among the studied sites. Prior to the discriminant analysis, the variables were divided in two groups: environmental parameters (i.e., pH, Eh, TOC, S, sand, silt, clay, SO_4^{2-} and Cl^-) and metals fractionation (EX, CA, FR, LP, PY, DOP), increasing the number of observation compared to the number of variables and, thus, improving the results.Moreover, the variables that presented highmulticollinearity were removed after a pre-analysis and, thus, were not included in the results.

3. RESULTS AND DISCUSSION

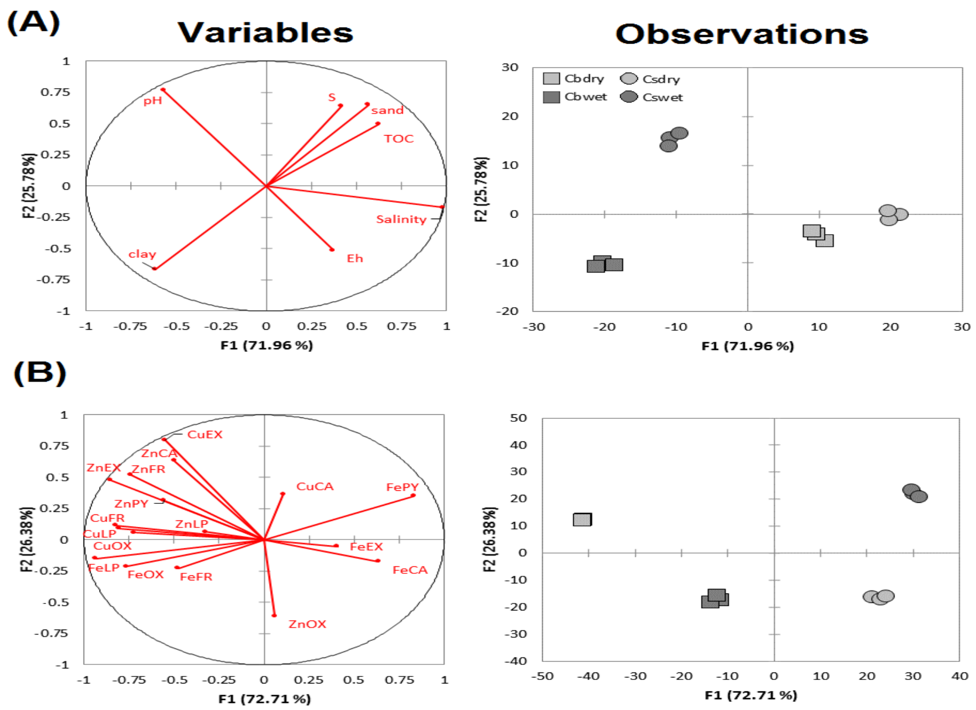
3.1. Effects of bioturbation and seasonal changes on soil properties

The bioturbated area presented burrow densities (12 ± 3 burrows m^{-2} and 38± 12 burrows m^{-2} during the wet and dry season, respectively) higher than those reported in other areas inhabited by *Ucides* in Brazil (1.5–0.9 burrows m^{-2}; WUNDERLICH et al., 2008). The higher number of burrows during the dry season coincides with the greater activity of adults and juveniles after the reproductive stage (from July to September; MANESCHY, 1993). The burrows depth ranged from 50 to 100 cm in both seasons, and the mean diameter of entrances ranged from45 to 75mm, pointing to similar values reported in the literature for other Brazilian mangroves inhabited by *U. cordatus* (e.g.WUNDERLICH et al., 2008; GOES et al., 2010).

The physicochemical characteristics found in the studied soils (Table 1) suggest clear effect of both the burrowing activity and seasons ($p<0.01$). Furthermore, the discriminant analysis allowed a complete distinction among the sites and seasons (Figure 2). Higher pH values were registered in the control site during the wet season (mean value = 8.1 ± 0.1). During the dry season, the pH values dropped to in both studied sites (pH<7.0). On the other hand, the control site presented anoxic conditions during both seasons (Eh from +12 to +156 mV) whereas the crab site showed redox conditions that ranged from oxic during dry season (Eh=+380 ± 11 mV), to suboxic during the wet season (Eh = +117 ± 93).

These results indicate that the soil aeration caused by crab burrows lead to significant soil oxidation and acidification, especially during the dry season (Table 1). The significantly lower pH values in the crab site are a result of the oxidation of reduced compounds (especially e.g. H_2S, FeS, Fe_3S_4, FeS_2) which releases of H^+ (see OTERO et al., 2006; NÓBREGA et al., 2013; FERREIRA et al. 2007; MARCHAND et al., 2011). The negative and highly significant correlation between pH and Eh ($r=-0.65$, $p < 0.05$, $n=12$; Figure 3A), and the inverse vector direction in the discriminant analysis (Figure 2) corroborates this interpretation.

Figure 2 - Discriminant analysis based on environmental (A) and metal fractionation (B) variables.



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Several studies have reported the effect of crab burrows changing the redox and acid-base conditions of mangrove and salt marshes soils (MOUTON and FELDER, 1996; BOTTO and IRIBARNE, 2000; FERREIRA et al. 2007; ARAÚJO JÚNIOR et al., 2012). The burrowing activity promotes the entrance of elements with high oxidant capacity (mostly O_2) and thus may shift the soil redox conditions (KRISTENSEN and ALONGI, 2006; CANNICCI et al., 2008; NORDHAUS et al., 2006).

Moreover, the marked seasonality in the study area with a prolonged period of drought and high evapotranspiration rates (see Figure 1D), caused an intense soil desiccation and, thus, enhanced the oxidizing conditions (ARAÚJO JÚNIOR et al., 2012; NÓBREGA et al., 2013; SUÁREZ-ABELENDA et al., 2014). The significantly higher $SO_4{}^{2-}$ (mean value for all depths: 25.6 ± 3.0 cmol L−1 for both areas), Cl^- (mean value for all depths: 42.4 ± 1.4 cmol L−1 for both areas) and salinity (mean value for all depths: 26.8 ± 3.4‰ for both areas) values during the dry season ($p < 0.05$; Table 1) reinforces the striking physicochemical changes caused by seasonality. Moreover, according to the discriminant analysis, mostly of the distinction among dry and wet seasons in both Cs and Cb areas are based on higher Eh and salinity and lower pH (Figure 2), as a result of the oxidation of sulfides.

Irrespective to seasons, the higher TOC values were found in the control site (mean values for all depths= 1.73%; Table 1) when compared to the crab site (mean values for all depths=1.47%). Although not statistically different, this trend corroborates recent studies that suggest that the oxidizing conditions promoted by burrowing activity of crabs may increase CO_2 emissions and, thus, should be considered in the future carbon budget estimates for in these ecosystems (PÜLMANNs et al., 2014).

One reason for the absent statistical difference would be related to the exclusion time (3 months), which would not be sufficient to promote a relevant change in C dynamic, but sufficient enough to promote changes in iron and trace metals dynamics (ARAÚJO JÚNIOR et al., 2012). On the contrary, despite the highly oxic conditions in the crab site during the dry season, a significant increase in the TOC contents was registered in comparison to the control site. These results seem to indicate an incorporation of organic matter by crabs during the period of higher bioturbation activity, which also would be related to the higher TOC contents in the deeper layers (see Table 1). In fact, according to NASCIMENTO (1995), *U. cordatus* L. can efficiently transport semidecomposed organic matter from the soil surface to deeper soil layers. The higher burrow densities registered at the end of the dry period, when *U. cordatus* is mostly active (MANESCHY, 1993), corroborates this hypothesis. Additionally, it must be highlighted that, despite both bioturbated and control site were set very close, part of the results could be associated to the high spatial variability that occur in mangroves soils (MACHADO et al., 2014; FERREIRA et al. 2010) which may have cause differences among the sites and among the studied seasons.

Total S contents did not vary between seasons ($p > 0.05$) but showed significant differences ($p < 0.05$) between sites. Values were significantly lower in the crab site (0.25 ± 0.12%) when compared to the control site (0.44 ± 0.12%). The significantly lower TS values in the crab site further corroborate the *U. cordatus* ability to promote the oxidation of the metal sulfides (i.e. pyrite) in mangrove soils through bioturbation. The highly significant and positive correlation between total S and TOC ($r= 0.78$, $n = 12$; $p < 0.01$; Figure 3B), also presented by the association among the vector in the discriminant analysis, is another evidence that most S is associated to organic compounds and, thus, not to metallic sulfides.

Table 1 - General chemical parameters for soil samples (Eh, pH, and contents of sand, silt, clay, total organic carbon – TOC, nitrogen – N, and sulfur - S) and porewater (salinity and concentrations of chloride and sulfates) for the studied sites and seasons.

Site and Season		depth (cm)	pH	Eh mV	Sand	Silt	Clay	TOC	N	S	C/N	C/S	Salinity ‰	SO_4^{2-}	Cl^-	SO_4^{2-} / Cl^-
							%							cmol L^{-1}		
Control site	Wet	0-10	8.1	+12	62.8	23.0	14.1	1.23	0.08	0.24	16.0	5.0	2.7	4.0	15.3	0.26
		10-20	8.3	+62	43.8	34.0	22.2	1.92	0.12	0.54	15.9	3.6	5.0	0.6	21.0	0.04
		20-30	8.1	+68	46.0	31.4	22.6	2.08	0.11	0.60	18.7	3.5	7.5	10.4	29.0	0.36
	Dry	0-10	7.2	+156	61.8	21.7	16.5	1.68	0.12	0.40	14.4	4.2	29.7	27.0	43.8	0.62
		10-20	6.6	+116	55.8	27.1	17.1	1.71	0.11	0.46	15.3	3.7	29.5	27.6	43.8	0.64
		20-30	6.8	+123	63.3	19.9	16.8	1.59	0.09	0.41	18.1	3.9	30.0	29.8	40.0	0.74
Crab site	Wet	0-10	7.4	+47	17.2	47.8	35.0	0.91	0.14	0.18	6.5	5.1	2.3	0.4	25.4	0.02
		10-20	7.2	+218	17.7	46.0	36.2	0.83	0.13	0.17	6.3	5.0	4.5	3.0	18.1	0.16
		20-30	7.0	+173	22.5	47.4	30.1	0.70	0.12	0.15	6.0	4.6	3.5	7.8	14.4	0.54
	Dry	0-10	6.6	+375	33.4	39.5	27.1	1.81	0.14	0.32	13.0	5.6	26.3	22.4	41.4	0.54
		10-20	7.0	+389	25.6	47.5	26.9	2.02	0.15	0.23	13.6	8.7	22.0	24.4	43.0	0.56
		20-30	6.8	+391	30.9	44.5	24.6	1.99	0.14	0.48	14.6	4.2	23.5	22.4	42.5	0.52

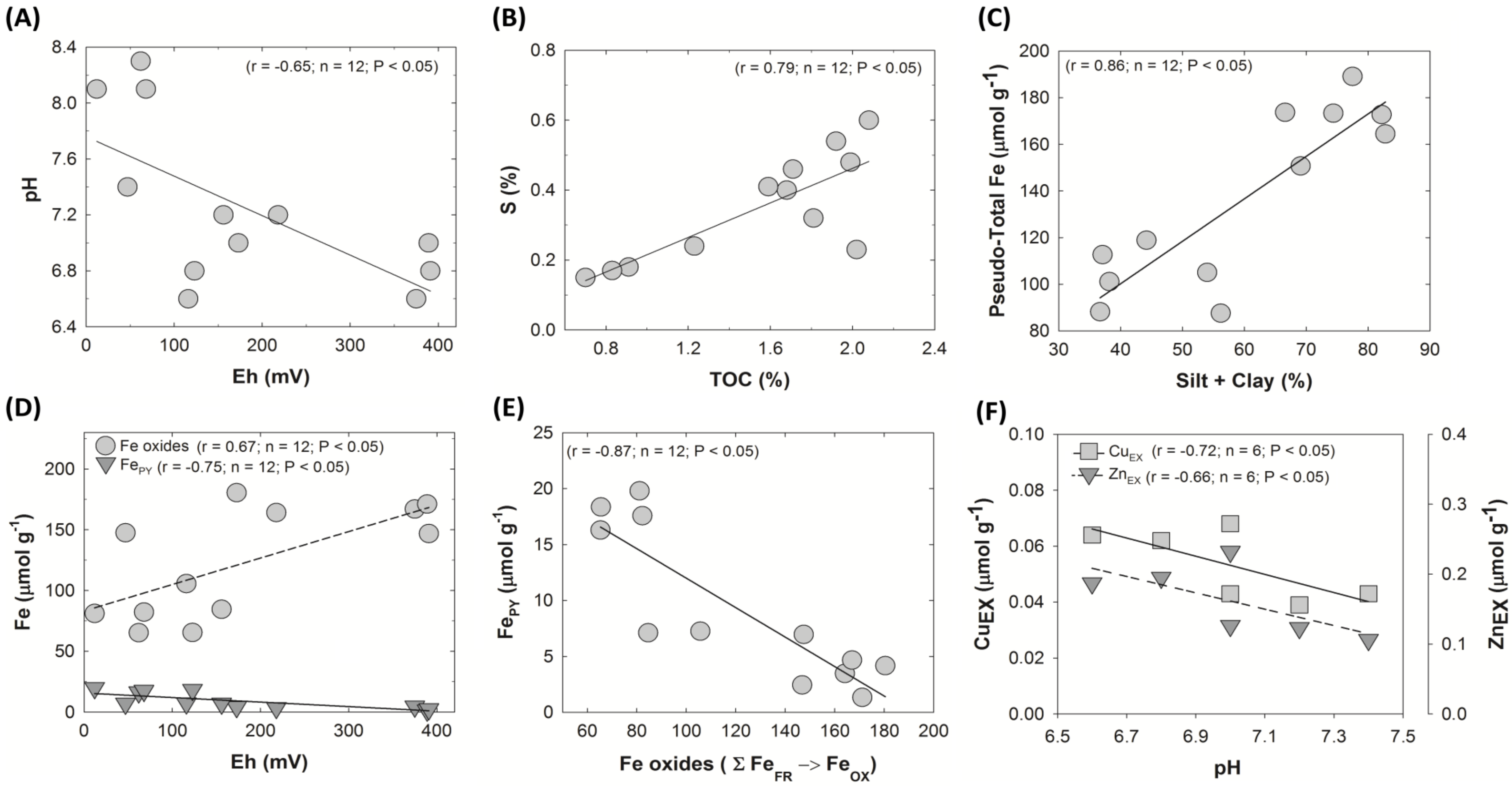
Source: The author.

3.2. Effects of bioturbation and seasonal changes on trace metals bioavailability and bioaccumulation

The new physicochemical environment (e.g.more acidic and oxidizing) caused by the burrowing activity of *U. cordatus* (especially in the dry season) clearly affected the biogeochemical dynamics of metals (Figure 2).

Pseudo-Total Fe contents (accounted as$\Sigma Fe_{EX} \rightarrow Fe_{PY}$) presented significant differences among sites but not between seasons ($p>0.05$). The Pseudo-Total Fe contents showed higher values (approximately 2-fold higher) in the crab site (170 ± 16 μmol g^{-1}; Table 2). This result may be related to the finer soil texture in the crab site (significant correlation positive between finer fractions (clay+silt) and Pseudo-Total Fe contents; $r = 0.86$, $n = 12$, $p < 0.05$; Fig. 3C). Despite this significant textural difference, the participation of iron (oxyhydr) oxides (expressed in percentages; was significantly increased in response to crab activity (Figure 4). The more oxidizing conditions promoted by crabs activity (especially during the dry season) would have favored both Fe_{PY} oxidation and iron oxides (Fe_{FR}, Fe_{LP}, Fe_{OX}) formation, as can be observed by the opposite direction of the vectors Fe_{FR}, Fe_{LP}; Fe_{OX} compared to Fe_{PY} (Figure 2). The positive and highly significant correlation between Eh and iron oxides forms ($r = 0.667$, $p<0.02$, $n = 12$; Figure 3D) and the negative highly significant correlation between Eh and Fe_{PY} ($r= -0.748$, $p<0.01$, $n=12$; Fig. 3D) corroborates this affirmation. Additionally the highly significant and negative ($r=-0.864$, $p<0.001$, $n= 12$; Figure 3E) correlation between iron oxides ($\Sigma FeFr \rightarrow Fe_{OX}$) and pyritic fraction ($Fe_{PY}$) confirms the coupling between pyrite oxidation and iron oxides precipitation. Accordingly, the molar SO_4/Cl ratios higher than the seawater (seawater reference value of 0.05) further corroborate the metal sulfide oxidation (molar rations > 0.05 indicates sulfide oxidation, whereas molar rations lower than 0.05 indicates sulfate reduction. For further details, please see GIBLIN (1988). In fact, in all the studied soils SO_4/Cl values remained above 0.2 (Table 1).

Figure 3 - Relationships between (A) Eh and pH; (B) TOC and S; (C) silt+clay and Pseudo-Total Fe; (D) Fe oxides (ΣFeFR → FeOX), FePY and Eh; (E) Fe oxides (ΣFeFR → FeOX) and. FePY for all sites and seasons; and (F) CuEX, ZnEX and pH for the crab sites.



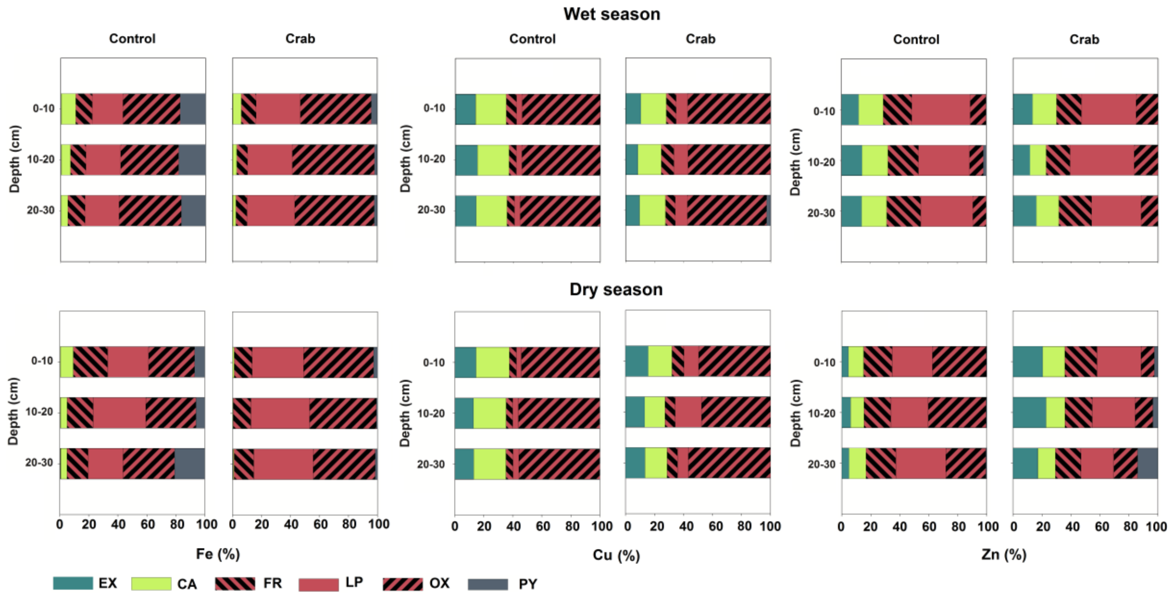
Source: The author.

Table 2 **-** Mean value (± s.d.) for Fe, Cu and Zn speciation for both Cb and Cs site, during both seasons

Site	Season	Depth	EX	CA	FR	LP	OX	PY	DOP
		(cm)	--------------------------------------			μmol g^{-1}	--------------------------------------		%
						Iron			
Cb	wet	0-10	0.3±0.2	9.6±0.4	16.7±3.8	50.4±14.0	80.4±10.6	7.0±0.8	4.2±0.4
		10-20	0.3±0.2	4.8±5.0	12.8±0.7	53.6±14.0	97.7±17.0	3.5±4.2	2.1±2.6
		20-30	0.5±0.3	4.0±4.4	14.5±2.9	62.3±13.2	103.6±36.3	4.2±5.7	2.5±3.4
	dry	0-10	0.2±0.0	1.7±1.5	21.3±11.5	61.8±34.5	83.9±39.8	4.7±4.1	1.8±2.7
		10-20	0.3±0.0	0.6±0.1	20.9±8.3	70.5±12.7	79.8±19.9	1.3±2.2	1.6±1.2
		20-30	0.5±0.1	0.9±0.1	20.7±2.3	61.7±7.3	64.5±6.6	2.5±0.9	0.8±0.5
Cs	wet	0-10	0.5±0.3	11.2±5.0	12.9±2.7	23.9±4.1	44.4±12.7	19.8±8.9	17.7±8.3
		10-20	0.4±0.2	5.5±1.3	9.4±1.0	20.9±4.3	35.1±9.6	16.3±4.3	18.7±5.0
		20-30	0.4±0.2	4.8±2.7	12.4±10.8	24.7±3.1	45.2±2.4	17.6±4.7	16.8±3.2
	dry	0-10	0.3±0.2	9.1±2.5	23.9±7.1	28.5±1.8	32.1±0.4	7.1±6.1	7.2±6.0
		10-20	0.4±0.1	5.4±3.0	21.4±8.3	43.2±21.7	41.2±3.5	7.3±5.7	6.4±5.8
		20-30	0.5±0.2	3.8±1.4	12.8±7.3	21.3±7.6	31.4±8.0	18.4±10.9	24.5±7.7
						Copper			
Cb	wet	0-10	0.04±0.00	0.07±0.00	0.03±0.00	0.03±0.00	0.24±0.00	0.00±0.00	0.00±0.00
		10-20	0.04±0.01	0.08±0.00	0.04±0.02	0.05±0.00	0.26±0.04	0.00±0.00	0.00±0.00
		20-30	0.04±0.00	0.08±0.01	0.03±0.01	0.04±0.00	0.24±0.04	0.01±0.00	0.00±0.00
	dry	0-10	0.06±0.00	0.07±0.00	0.03±0.00	0.04±0.00	0.20±0.00	0.00±0.00	0.00±0.00
		10-20	0.07±0.01	0.07±0.00	0.04±0.01	0.09±0.10	0.25±0.01	0.00±0.00	0.00±0.00
		20-30	0.06±0.00	0.07±0.00	0.03±0.00	0.04±0.01	0.26±0.05	0.00±0.00	0.00±0.00
Cs	wet	0-10	0.05±0.00	0.08±0.01	0.03±0.01	0.02±0.00	0.20±0.06	0.00±0.00	0.00±0.00
		10-20	0.05±0.01	0.07±0.01	0.02±0.00	0.01±0.00	0.18±0.05	0.00±0.00	0.00±0.00
		20-30	0.05±0.01	0.08±0.00	0.02±0.00	0.02±0.00	0.21±0.05	0.00±0.00	0.00±0.00
	dry	0-10	0.04±0.01	0.07±0.01	0.01±0.00	0.01±0.00	0.16±0.01	0.00±0.00	0.00±0.00
		10-20	0.04±0.01	0.07±0.01	0.01±0.00	0.01±0.00	0.17±0.02	0.00±0.00	0.00±0.00
		20-30	0.04±0.01	0.07±0.00	0.01±0.00	0.01±0.00	0.16±0.02	0.00±0.00	0.00±0.00
						Zinc			
Cb	wet	0-10	0.11±0.00	0.13±0.04	0.14±0.00	0.30±0.02	0.12±0.00	0.00±0.00	0.00±0.00
		10-20	0.12±0.00	0.12±0.02	0.17±0.03	0.47±0.22	0.17±0.02	0.00±0.00	0.00±0.00
		20-30	0.13±0.00	0.12±0.01	0.18±0.04	0.27±0.03	0.09±0.08	0.00±0.00	0.00±0.00
	dry	0-10	0.19±0.01	0.14±0.02	0.20±0.05	0.28±0.04	0.08±0.01	0.02±0.01	2.55±1.39
		10-20	0.23±0.06	0.13±0.04	0.19±0.03	0.30±0.11	0.13±0.02	0.04±0.02	3.43±1.46
		20-30	0.20±0.02	0.14±0.01	0.20±0.02	0.26±0.00	0.19±0.12	0.16±0.16	17.42±13.50
Cs	wet	0-10	0.10±0.01	0.15±0.06	0.17±0.05	0.35±0.24	0.09±0.07	0.00±0.00	0.47±0.66
		10-20	0.10±0.00	0.12±0.05	0.15±0.01	0.25±0.05	0.07±0.04	0.01±0.02	1.49±2.11
		20-30	0.10±0.01	0.12±0.05	0.16±0.02	0.25±0.03	0.06±0.02	0.00±0.00	0.00±0.00
	dry	0-10	0.03±0.02	0.07±0.01	0.13±0.03	0.18±0.02	0.25±0.04	0.00±0.00	0.32±0.56
		10-20	0.04±0.03	0.07±0.01	0.13±0.03	0.19±0.01	0.29±0.04	0.00±0.01	0.60±1.05
		20-30	0.03±0.02	0.07±0.01	0.12±0.01	0.20±0.05	0.16±0.12	0.00±0.00	0.49±0.85

Source: The author.

Figure 4 - Percentage of each solid phase fraction (exchangeable, EX; associated to carbonates, CA; associated to ferrihydrite, FR; associated to lepidocrocite, LP; associated to crystalline oxyhydroxides, OX; associated to pyrite, PY) in relation to Pseudo-Total content.



Source: The author.

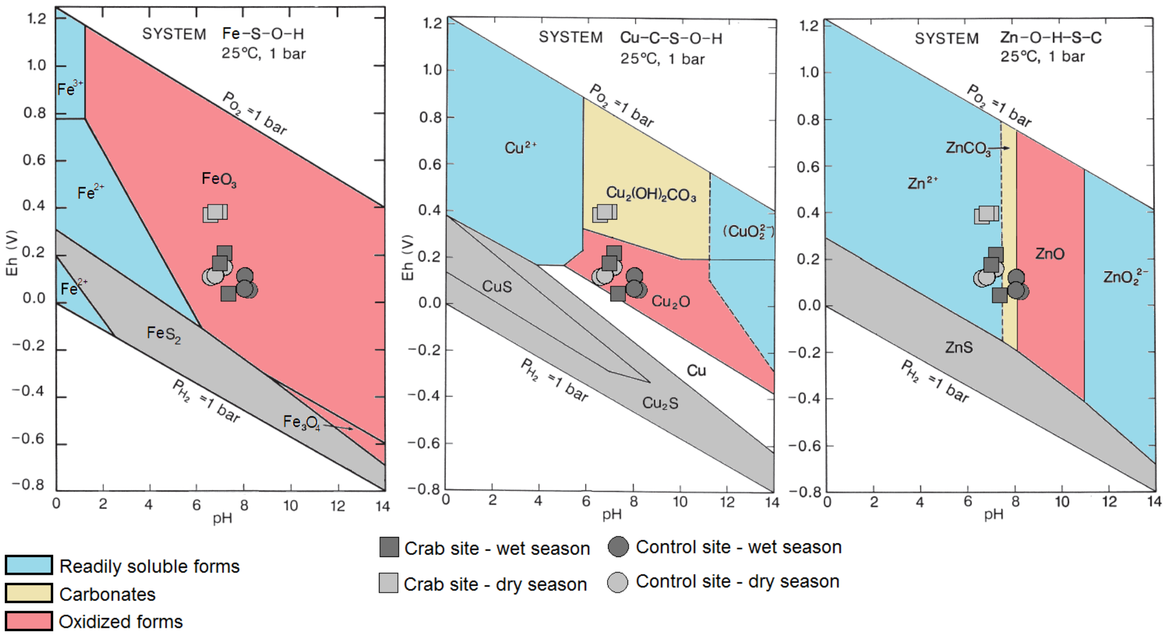
Additionally, in the crab site, the Fe_{PY} values were significantly ($p<0.01$) lower (<5 μmol g^{-1}; especially in the dry season) than those registered in the control site (14±7 μmol g^{-1}; Table 2; Figures 2 and 4). As expected, DOP values (Table 2), showed the same decreasing pattern in response to the more oxidized environment promoted by both crab activity and the drier climate conditions. Irrespective to seasons, DOP values always remained <5% in the crab site, while in the control site the mean value for all depths was 15±7.0% (Figure 4).

The exchangeable Fe fraction (Fe_{EX}) and Fe associated to carbonates (Fe_{CA}) were the least representative of all Fe fractions (mean value: 0.4±0.1 and 5.5±3.9 μmol g^{-1}, respectively; Figure 4) and did not vary either between seasons or sites ($p>0.05$).

In contrast to iron, for both Cu and Zn, the more labile fractions showed the higher percentages in the partitioning results. Cu and Zn showed significantly higher percentages of the exchangeable and carbonate fractions, when compared to the iron partitioning (Figure 4). The exchangeable and carbonate fractions varied with seasons and sites ($p<0.01$), with the highest values of Cu_{EX} and Zn_{EX} occurring in the crab site during the dry season (mean values for all depths: 0.06±0.003 μmol g^{-1} and 0.20±0.02 μmol g^{-1}, respectively).

In agreement with these results, a significant and negative correlation between pH and both Cu_{EX} and Zn_{EX} in the crab site was found ($r = -0.75$ and $r = -0.71$, $p<0.01$, $n = 6$, respectively) (see Figure 3F) evidencing the role of *U. cordatus* in altering the physico-chemical and favoring the bioavailability of both Cu and Zn. In fact, the Eh-pH diagram showed in Figure 5 evidence the prevalence of soluble forms of Cu and Zn under the registered physico-chemical conditions of the studied soils, while most of the Fe would be associated to insoluble forms (i.e.(oxyhydroxides).

Figure 5 - Eh – pH diagrams for the stable phases of (A) Fe; (B) Cu and (C) Zn.



Source: The author.

The fraction Cu_{CA} did not differ either among areas or seasons ($p>0.05$) and values were approximately 0.08 µmol g^{-1}. Contrastingly the contents of Zn_{CA} showed significant differences between both sites and seasons ($p<0.01$), with higher values in the crab and in the dry season (mean values of 0.14±0.01 and 0.12±0.01 µmol g^{-1} dry and wet seasons, respectively). However, for both metals, the partitioning indicated a relative stronger association to carbonates when in comparison to iron ($p<0.05$; Figure 4). In this case, the significantly lower solubility product constants (pK) of both $CuCO_3$ and $ZnCO_3$ (respectively, 9.93 and 10.0; see KRAUSKOPF, 1979) when compared to that of $FeCO_3$ (10.7) may be related to the higher percentages of Cu and Zn carbonates in both sites and seasons.

On the other hand, similarly to Fe, most Cu and Zn were mainly associated to the iron oxides fraction (ΣCu_{FR} ➔ Cu_{OX} and ΣZn_{FR} ➔ Zn_{OX}; Figure 4; Table 2) with significantly higher values ($p < 0.01$) in the crab site (mean values: 0.32±0.03 μmol g^{-1} and 0.62±0.10 μmol g^{-1}, for Cu and Zn respectively) when compared to the control site (mean values: 0.21±0.02 μmol g^{-1} and 0.53±0.07 μmol g^{-1}, for Cu and Zn respectively), especially during the dry season (Figures 2 and 4). Cu was barely present in the pyrite fraction, showing negligible contents (DPTM ~ 0; Figure 4). On the other hand, for Zn, the pyritic fraction was detected, however in significantly lower concentrations than the exchangeable, carbonate and iron oxides fractions (Table 2; Figure 4).

These results evidence that, although several studies have reported the role pyrite as an important sink for metals in intertidal soils (OTERO and MACÍAS, 2002a; OTERO et al., 2000; MACHADO et al., 2014; YE et al., 2010), both the bioturbation and the seasonality in semiarid mangroves shift the biogeochemical conditions away from those favoring sulfate reduction. In this case, the newly established physicochemical environment alters, not only the iron dynamics (e.g. increasing the participation of oxides and reducing the participation of the pyritic fraction), but also enhances the bioavailability of Cu and Zn, which become mostly associated to more labile forms (oxides > carbonates > exchangeable >> pyrite). Although iron oxides are also considered important sinks for metals due to their high reactivity and surface area (PEACOCK and SHERMAN, 2004; ÖHLANDER et al., 2003; HANSEL et al., 2005), their control over the bioavailability of metals in the studied mangrove soils is drastically reduced due to the highly varying redox conditions (owing both to bioturbation and seasonality).

Ultimately, our results show that the concentrations of metals in crab tissue are closely related to its biogeochemical distributions in the different soil fractions. The mean metal concentrations in the crab hepatopancreas followed the following order: Zn > Fe > Cu (mean values: 137.9±72.6; 129.2±88.5 and 67.8±43.3 mg kg^{-1}, respectively). Contrastingly, the biota-soil accumulation factors (BSAFs) calculated for the three metals presented the following order: Zn > Cu >> Fe with mean values of 2.55, 2.35 and 0.01, respectively.

The BSAF results suggest that the concentrations of metals in *U. cordatus* are much more correlated to the contents of more labile fractions (i.e. exchangeable and carbonate fractions) in the soils. Although the Pseudo-Total values of iron in the soils were up to 100-fold higher than those of Cu and Zn, this large difference was not quantified in the crabs hepatopancreas. The higher BSAF values for Zn and Cu are probably related to the significantly higher percentages of more labile fractions, when compared to Fe.

The use of trace metals content in hepatopancreas as a bioindicator have been suggested since it is the major invertebrate organ that sequestre and detoxifies toxic metals circulating in the hemolymph (AHEARN et al., 2004; PINHEIRO et al., 2012). The toxic metals enter the crabs through food consumption (PINHEIRO et al., 2012), but may also enter through the tegument or the gills (AHEARN et al., 2004). In this sense, the direct assimilation of metals from readily dissolved phases or more labile forms in the soil would increase metal contents in crab tissues (see BELTRAME et al., 2011; HEIDARIEH et al., 2013; RAINBOW, 1985).

Another possible interpretation for the higher Zn and Cu BSFAs values regards the oxidation of metal sulfides, mostly pyrite, which would result in, acidification, the release of associated metals and, thus, in an increased bioavailability and bioaccumulation (OTERO et al., 2000). In this case, the crabs themselves may have increased their own exposure to both Zn and Cu, since burrowing activity clearly favored both pyrite oxidation and the more acidic conditions.

4. CONCLUSIONS

The following conclusions were drawn from the present study: (i) the burrowing activity of *U. cordatus* associated to seasonality considerably alter the mangrove soils biogeochemical conditions increasing metals bioavailability and bioaccumulation; (ii) the role of pyrite as a trace metal sink is drastically decreased due to bioturbation, which increases the participation of less stable mineral forms and, thus, the risks of biocontamination; and (iii) the concentrations of metals in the crab tissue are a good proxy for the soil bioavailable fractions, thus future studies should further evaluate the potential use of *U. cordatus* as a bioindicator species for trace metals contamination.

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CHAPTER 3

Seasonal nitrous oxide emission from semi-arid mangrove soils (NE-Brazil) under *Ucides cordatus crab* activity

RESUMO

Conhecido como um dos ecossistemas mais ricos em carbono do planeta, a alta produtividade dos manguezais é comumente associada a condições anóxicas de seus solos, permitindo grande acúmulo de C, embora também seja um local favorável à emissão de gases de efeito estufa (por exemplo, N_2O e CH_4) . Como a macrofauna e as raízes afetam diretamente as condições do solo, é possível que eles também possam afetar as emissões de N_2O. As tocas de caranguejo, por exemplo, mudam os fluxos de ar-água e também modificam as condições biogeoquímicas do solo. Este estudo foi realizado para avaliar o papel da bioturbação por caranguejos na emissão de N_2O em solos de mangue. Assim, amostras de solo e gases foram coletadas em um mangue semi-árido (NE-Brasil), durante as estações chuvosa e seca, em um local bioturbados e em outro local isolado de caranguejos (controle). Carbono Orgânico Total (COT), nitrogênio total (NT), enxofre total (ST), fluxo de N_2O, pH e o potencial redox (Eh) foram analisados . As concentrações de TOC, NT e ST não apresentaram diferenças estatísticas significtivas quando comparados a área bioturbada e a área controle ($p> 0,05$). Em ambas as estações, um maior fluxo de N_2O foi registado para a área controle (47,3 e 8,9 mg de m-2 h-1, para as estações chuvosa e seca, respectivamente), quando comparado com o local bioturbado (36,5 e 4,5 µg m^{-2} h^{-1}, para os períodos chuvoso e seco, respectivamente). Apesar ds diferenças em valores de Eh e pH, o período de isolamento curto não foi suficiente para promover mudanças claras sobre TOC, NT e ST entre as áreas, o que reflete a necessidade de mais estudos com períodos de isolamento mais extensos para melhorar a compreensão do papel dos caranguejos no comportamento biogeoquímico e de nutrients de manguezais. Como nossos resultados indicam, a atividade de caranguejo pode ser responsável por fluxos mais baixos de N_2O, quando comparados com a área controle. Além disso, as variações sazonais são uma importante fonte de variação para o fluxo de óxido nitroso, com fluxos mais elevados emitidos durante o período seco.

Palavras-chaves: Bioturbação. N_2O. Carbono Orgânico. Nitrogênio. Enxofre. Caranguejo.

ABSTRACT

Knows as one of most carbon-rich ecosystems in planet, the mangroves high productivity is commonly associated to anoxic conditions of its soils allowing great accumulation of C, but on the other hand is favorable to emission of greenhouse gases (e.g., N_2O and CH_4). Since macrofauna and roots directly affect soil conditions, it is possible that it would, also, affect N_2O emissions. The crab burrows, for example, changes air-water fluxes and also modify soil biogeochemical conditions. Thus, this study was performed to evaluate the role of crab bioturbation to N_2O emission in mangrove soils. Thus, soil and gases samples were collected at a semiarid mangrove (NE-Brazil), during the rainy and dry seasons, in a bioturbated and a control site (isolation plot). Total organic carbon (TOC), nitrogen (TN) and sulfur (TS), N_2O flux, pH and redox potential (Eh) were analyzed. The TOC, TN and TS content did not presented statistical difference when compared control and bioturbated sites and seasons ($P > 0.05$). In both seasons, a higher N_2O flux was recorded for the control site (47.3 and 8.9 µg m^{-2} h^{-1}, for rainy and dry periods, respectively), when compared to the bioturbated site (36.5 and 4.5 µg m^{-2} h^{-1}, for rainy and dry periods, respectively). Despite the differences on Eh and pH values, the short isolation period was not long enough to promote clear changes on TOC, TN and TS among sites, which reflects the necessity of longer isolation periods studies to improve the understanding of the role of crabs on major nutrients geochemistry. As our results indicates, the crab activity may be responsible for lower N_2O fluxes, when compared to the control site. Moreover, the seasonal variations are an important source of variation for nitrous oxide flux, with higher fluxes emitted during the dry period.

Keywords: Bioturbation; N_2O; Organic carbon; Nitrogen; Sulfur; Crab.

1. INTRODUCTION

Mangroves consists in one of the most carbon-rich forests in the planet due to its high productivity associated to the commonly anoxic conditions of its soils (DONATO et al., 2011). Besides promoting the accumulation of great C stocks, the anoxic soil condition favors the production of nitrous oxide (N_2O) and methane (CH_4) gases by microbial activity pathways (nitrification, denitrification and methanogenesis) (KREUZWIESER; BUCHHOLZ; RENNENBERG, 2003; KRISTENSEN et al., 2008; NÓBREGA et al., 2016).

In mangrove soils, the N_2O emission is governed by denitrification process, consuming NO_3^- and NH_4^+ under anoxic/suboxic conditions (anaerobic pathways), whereas the oxic conditions increase N_2O emission by nitrification process consuming NH_4^+ (ALLEN et al., 2007, CORREDOR et al., 1999, KREUZWIESER; BUCHHOLZ; RENNENBERG, 2003). ALLEN et al., (2007) highlighted the effect of Eh and N source as key factors to N_2O emissions, and the denitrification as the most important pathway to N_2O emissions in mangrove soils. Other factors which affect greenhouse gases fluxes would be related to soil properties (i.e total organic C, total N and P); temperature and tidal conditions, which ultimately affects microbial activity in soil (CHEN et al., 2012).

Mangrove crabs and roots are known drivers of soil geochemical conditions and, thus, can directly N_2O emissions (KRISTENSEN et al., 2008; FERREIRA et al., 2007). Crab burrows, for example, changes air-water fluxes and also modify soil biogeochemical conditions (e.g., Eh, pH, Fe and trace metals fractionation; BOTTO and IRIBARNE, 2000; KRISTENSEN, 2008; ARAÚJO JÚNIOR et al., 2012; 2016). As a result of the crab burrows, the entrance of atmospheric O_2 through the soil forms a centimetric-scale oxidizing environment around the burrows (NIELSEN et al., 2003).

Despite mangrove forest have high potential for N_2O and CH_4 emissions (CHEN et al., 2010), the studies regarding theses emissions are scarce (CORREDOR et al., 1999; KREUZWIESER et al,. 2003; NÓBREGa et al., 2016). Furthermore, the effects of crab bioturbation and greenhouse gases (especially N_2O) emission in mangroves ecosystem are not well understood (PÜLMANS et al., 2014). Only a very few studies (e.g., PÜLMANS et al., 2014) obtained information about the role of crabs bioturbation and CO_2 emissions. Moreover, until present date, it was not found any study regarding the effects of bioturbation to N_2O emission in mangrove soils. Thus, this study was performed with objective to evaluate the role of N_2O emission in mangrove soils under crab bioturbation based on the hypothesis that crab burrows would decrease N_2O emissions since the crab activity increase soil redox potential.

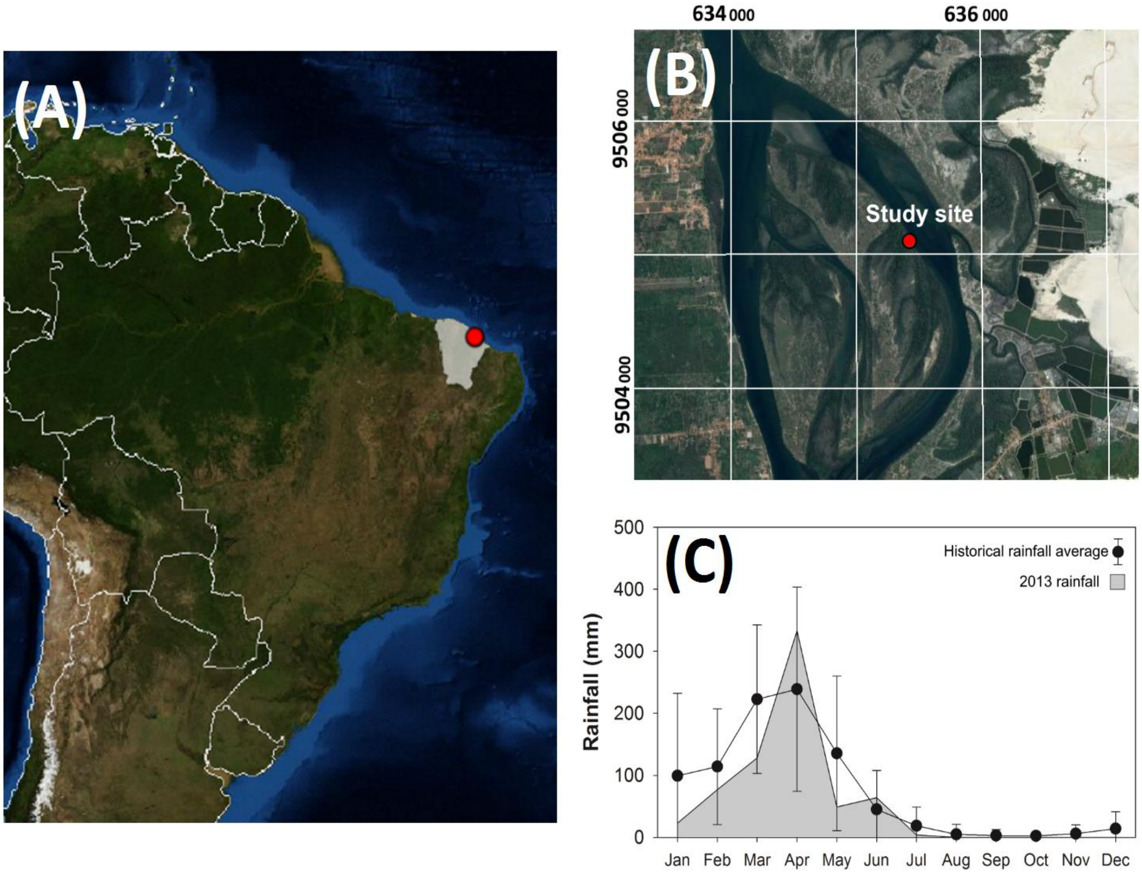
2. MATERIAL AND METHODS

2.1. Site description

The studied mangrove is located at the Brazilian semiarid coast (Aracati, Ceará state), in the Jaguaribe river estuary (Figure 1A and 1B). The climate of the estuary is classified as Aw', according to Köppen climate classification (KOTTEK et al., 2006; PEEL et al., 2007). The average rainfall ranges between 700-800 mm yr^{-1}, occurring mostly between February to April (Figure 1C); and the daily average temperature oscillates between 26 to 28 ºC during the year (IPECE, 2014). Associated to the low precipitation, the high evaporative demand causes high evapotranspiration rates that can reach 5 mm day^{-1} (SALES, 2008). The mangrove forest of the Jaguaribe river cover 11.6 km^2 and is mainly vegetated by *Rhizophora mangle* L., *R. racemosa* and *Avicennia schaueriana* species (TANAKA and MAIA, 2006). However, the water deficit of the studied region increases soil salinity and limit mangrove development compared to the other sections of Brazilian coast (Schaeffer-NOVELLI et al., 1990).

To verify the effects of bioturbation on N_2O emissions, two sampling plots were selected: a site affected by *U. cordatus* crab activity (bioturbated plot) and a control site, without crab activity. The control site consisted in a 1 m^2 exclusion plot, delimited by 1 cm mesh nylon net buried up to 1.5 m depth, which was established 3 months prior to soil and gases sampling (ARAÚJO JÚNIOR. et al., 2012; 2016). Both sites were closely located and mainly vegetated by *Rhizophora mangle*, maintaining identical physiographic position to avoid differences in flooding frequency and to minimize vegetation effects (e.g., presence of pneumatophores; roots and plantlets). In each site, the samples (gas and soil) were collected at the end of the wet (July/2013) and dry (January/2014) seasons, at low tide (OTERO and MACÍAS, 2002; OTERO et al., 2006).

Figure 1 - The studied mangrove located at the Brazilian semiarid coast (Aracati, Ceará state) (A), in the Jaguaribe river estuary (B) and the local average rainfall (C)



Source: A and B - the author. C – FUNCEME (2016), modified by the author.

At the bioturbated plot, were assessed the burrow density (burrow m^{-2}) and the burrows size (diameter of the entrance and burrow depth). The burrow densities were assessed using 3 random measurements with an 1 m^2 frame; whereas the burrow sizes was measured in 10 burrows, using a caliper for diameter measurements and a soft rubber wire for burrow depth assessment (BRANCO, 1993; ARAÚJO JÚNIOR et al., 2016).

2.2. N_2O sampling and analysis

In each sampling site (bioturbated and control), during each season, three static rigid chambers for N_2O collection were installed. The chambers (17.5 cm diameter and 17.5 cm height, inserted 5 cm into the soil) were placed on the soil surface and left opened for the stabilization of the internal pressure (approximately 30 minutes). After stabilization, the chambers were closed and the gas samples were collected using nylon syringes (BD types). The gas samples in each site were collected simultaneously in 4 times within a 45 minutes sampling period (0, 15, 30 and 45 minutes) to avoid significant tidal variation, also measuring air and soil temperature and air humidity (HOWARD et al., 2014; NÓBREGA et al., 2016).

The syringes were taken to the laboratory where the gas concentration was measured by gas chromatography and the N_2O flux (mg m^{-2} h^{-1}) was obtained by the difference on the gas mass inside each chamber. The mass of N_2O in each interval was calculated using the universal gas equation (PV = nRT) and the air temperature, the chamber volume and atmospheric pressure (HOWARD et al., 2014; NÓBREGA et al., 2016), obtaining an equation for mass flux (g h^{-1}) for each chamber. The mass flux was divided by the area inside the chamber to express the emission on a per area basis (mg m^{-2} h^{-1}) (For further details, HOWARD et al., 2014).

2.3. Soil sampling and analysis

To identify soil characteristics that affect N_2O emission, a soil sample was collected in each site, during each season, using PVC tubes attached to a flooded-soil auger. After collection, the PVC tubes were hermetically closed and transported in vertical position (at approximately 4 ºC) to the laboratory, where the samples were cut at 10 cm intervals until a depth of 40 cm, characterizing surface and subsurface layers. At the laboratory, the samples were oven dried (45 ºC) for the determination of total organic carbon (TOC), total nitrogen (TN) and total sulfur (TS). The TOC content were quantified in sub-samples using an elemental analyzer (LECO SE-144DR) after the elimination of inorganic carbon with HCl (HOWARD et al., 2014), whereas the contents of TN and TS were quantified in untreated sub-samples.

Additional soil samples were taken using a semi-opened soil auger used to measure *in situ* the pH and redox potentials (Eh) values. The Eh values were obtained using a platinum electrode after the correction to the reference calomel electrode (adding +244 mV to the readings), whereas the pH were obtained using a glass electrode calibrated with pH 4.0 and 7.0 standard solutions (FERREIRA et al., 2007). The final readings were taken after equilibrating the soil samples and the electrodes (approximately 2 minutes).

2.4. General analytical procedures and statistical analysis

The equipment used during samples collection and laboratory analysis were previously cleaned using HCl 10% overnight and rinsed with distilled water. The contents of the analyzed variables were quantified based in dried weight, which was determined after drying subsamples at 105 ºC to constant weight obtaining a conversion factor.

The mean values of each variable were compared using two-way ANOVA ($p<0.05$) and the correlation among the N_2O fluxes and the other variables were obtained using Pearson's correlation.

3. RESULTS

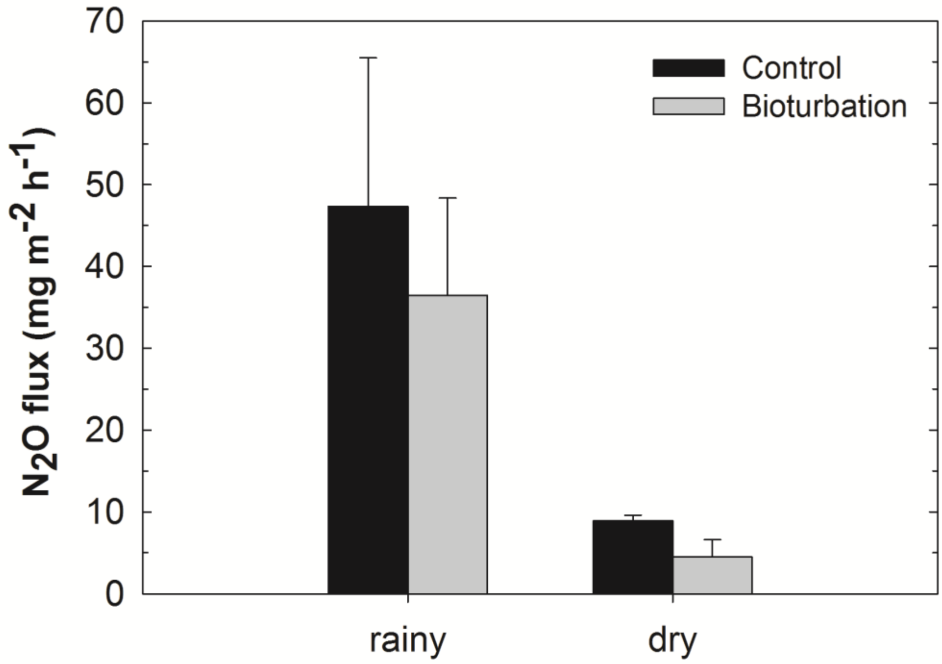
3.1 Crab burrows densities and burrow characteristics

The crab burrow density and sizes did not presented seasonal changes ($P > 0.05$). During the dry season were accounted 3.3±1.2 burrows m^{-2}, measuring 5.7±1.1 cm in diameter and 73±11 cm in depth. During the rainy season, the burrow density was 2.3 ± 0.6 burrows m^{-2}, measuring 5.9±1.0 cm in diameter and 76.2 ± 3.6 cm deep.

3.2 N_2O fluxes

A clear pattern can be observed regarding the N_2O emissions. When compared both seasons, a greater emission was recorded during the rainy period. In fact, the emissions recorded during the rainy season were almost 10 times greater than those recorded for the dry season. Despite the absent statistical difference ($p>0.05$), a higher N_2O flux was recorded for the control site (average: 47.3±9.7 and 8.9±0.5 µg m^{-2} h^{-1}, for rainy and dry periods, respectively; Figure 2), when compared to the bioturbated site (average: 36.5±7.8 and 4.5±2.1 µg m^{-2} h^{-1}, for rainy and dry periods, respectively).

Figure 2 – Values of N_2O flux recorded for the control site (black bar) and the bioturbated site (white bar) on rainy and dru season.

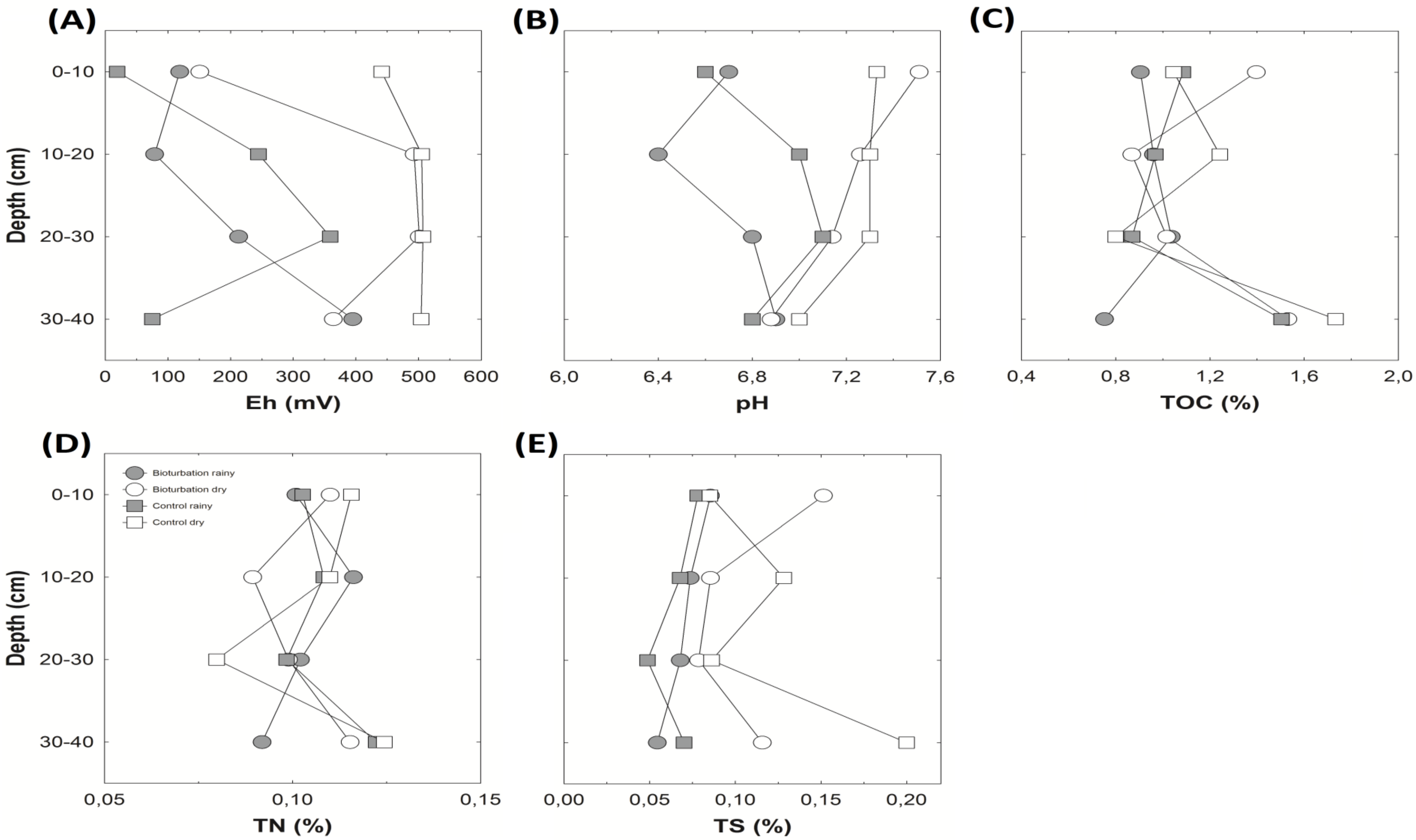


Source: The author

3.3 Soil characteristics

The redox potential (Eh) presented strong seasonal variation in both sites. Higher Eh values were recorded during the dry season, characterizing the predominance of oxic conditions for both sites (Eh>+350 mV, except for the uppermost layer of the bioturbated site; Figure 3A). During the rainy period, the Eh values presented a significant decrease, with values ranging between +79 and + 395 mV, mostly indicating suboxic conditions (Eh: +100 to +350 mV). When evaluated both sites, during the rainy period, higher Eh values were measured at the bioturbated site (site average during rainy season: +202±141 mV) when compared to the control (site average during rainy season: +174±156 mV; Figure 3A). On the other hand, during the dry season, the control site presented higher Eh values (site average during dry period: +489±32 mV; Figure 3A) than the bioturbated site (site average during dry period: +377±163 mV).

Figure 3 – Values of Eh (A), pH (B), % TOC (C), %TN (D) and %TS (E) ranging from diferents depths on each area.



Source: The author

Regarding pH values, as occurred with the redox potential, higher pH were measured during the dry season (values ranging between 6.9 to 7.5; Figure 3B), when compared to the wet season (values ranging between 6.6 to 7.1). During the wet season, higher values were measured at the control site (average of the control site during wet season: 6.9±0.2) when compared to the bioturbated site (average: 6.7±0.2; Figure 3B). During the dry season, on the other hand, the pH average for both areas were similar (average: 7.2±0.2 and 7.2±0.3 for control and bioturbated sites, respectively).

The TOC, TN and TS content did not presented statistical difference when compared both sites and seasons ($P>0.05$). During the dry season, the TOC content was similar in both sites (average: 1.2±0.4% and 1.2±0.3% for control and bioturbated sites, respectively), whereas during the wet season the control site presented a slightly higher TOC (average: 1.1±0.3%) compared to the bioturbated site (average: 0.9±0.1%; Figure 3C).

For the control site, the total nitrogen (TN) content was 0.11±0.02 % and 0.11±0.01% for rainy and dry season, respectively; whereas to the bioturbated site the TN was 0.10±0.01 % for both rainy and dry season (Figure 3D). Similar TS contents (Figure 3E) were quantified for both sites during the rainy season (average: 0.07±0.01%), whereas slight higher TS values were recorded at the bioturbated site (average: 0.11±0.03%) compared to the control (average: 0.09±0.02%) during the dry season.

When compared the C/N ratio among sites, similar values were recorded for both dry (average: 11.5±1.7 and 11.1±2.11, for the bioturbated and control site, respectively), and wet seasons (average: 10.2±1.6 and 8.9±0.9, for control and bioturbated sites, respectively). On the other hand, higher C/S ratios were recorded for the control site (average: 16.9±3.5 and 15.9±11.0, for rainy and dry periods, respectively) compared those recorded for the bioturbated site (average: 13.2±1.9 and 11.4±2.0, for rainy and dry periods, respectively). When compared both seasons, higher C/S ratio were recorded for the rainy period.

4. DISCUSSIONS

The burrow densities at the bioturbated site are in concordance to other burrow densities measured in Brazilian coast (average values ranging between 2 and

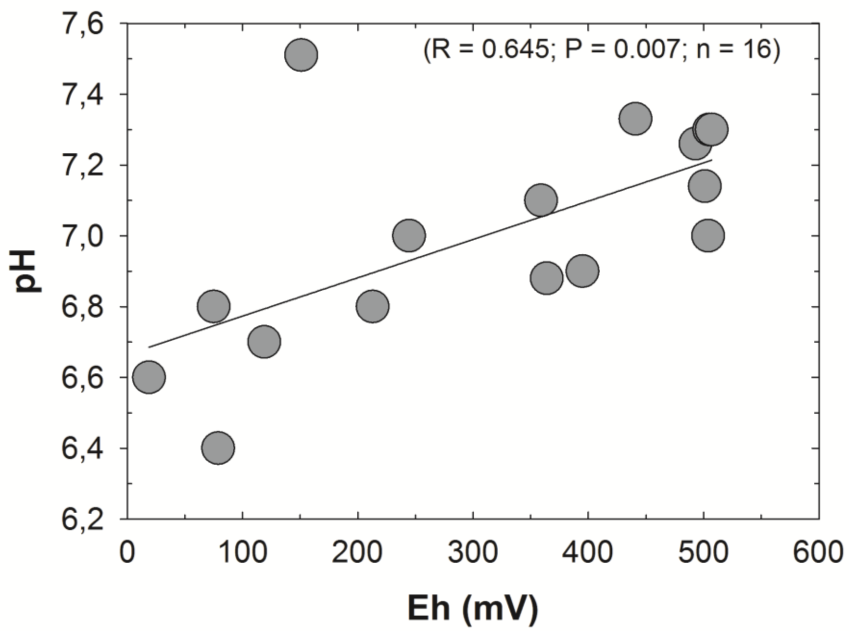
6 burrows m^{-2}; CASTRO et al., 2008; WUNDERLICH et al., 2008; MENDONCA and COSTA PEREIRA, 2009), but lower than the density measured for the same estuary in other studies (e.g., crab burrow density: 12±3 and 38±12 burrows m^{-2} during rainy and dry season, respectively; ARAÚJO JÚNIOR et al., 2012). With regard to crab behavior, the dry period correspond to the period with greater activity of adults and juveniles specimens, after to the reproductive stage (MANESCHY, 1993), which results in a higher burrow density (ARAÚJO JÚNIOR et al., 2012; 2016). In addition, the higher density during the dry season may also indicate an escape of the crabs to the harsh environmental conditions that occur outside the burrows (WARNER, 1969; SALES, 2008). In our study, the absent statistical difference among both seasons may be associated to the unusual lower precipitation that occurred during 2013, as a result of the El Niño Southern Oscillation (Figure 1C), increasing the number of burrows during the rainy season.

Because of the crab activity, the redox potential values varied among sites. In fact, many studies reported that the crab burrowing activity promotes the entrance of highly oxidizing compounds (e.g., O_2) increasing Eh values (ARAÚJO JÚNIOR 2012; 2016). However, during the dry season, the higher burrowing activity seems to promote the entrance of water to the burrows, which would affect the oxygen diffusion through the soil and the establishment of lower redox values compared to the control site, especially at deeper layers (Figure 3A). In fact, the crab burrowing activity is an strategy to prevent the crab desiccation, allowing the crab to reach the water table without being exposed to predation (WARNER, 1969).

As the crab affects the redox potential, the changes on soil oxidation also affected the acid-base conditions. When compared both sites during the wet season, the lower pH values recorded at the bioturbated site (Figure 3B) may be a result of the crab burrows promoting the oxidation of reduced sulfur compounds (e.g., pyrite), which releases protons (MACHADO et al., 2014). This fact could only be recorded during the wet season since occurrence of suboxic conditions, favorable for the production of reduced sulfur (MACHADO et al., 2014; ARAÚJO JÚNIOR et al., 2012), were only recorded during the rain period.

On the other hand, during the dry season, since both sites presented similar redox potential (higher than those recorded during the rainy season) and presumable higher desiccation, the higher pH values would be a result of an increased salinity and the precipitation of calcium carbonate (ALBUQUERQUE et al., 2014a; 2014b). In fact, the circumneutral to alkaline pH conditions found in the soil evidence that the H^+ activity is controlled by calcium carbonate precipitation/dissolution as a result of the concentration of seawater (ALBUQUERQUE et al., 2014a; 2014b). The significant positive correlation between Eh and pH corroborate to this fact (Figure 4).

Figure 4 – Correlation between Eh and pH.



Source: The author.

Despite the differences on soil redox potential and pH, the short isolation period was not long enough to promote clear changes on TOC, TN and TS among sites as occurred to Fe, Zn and Cu fractionation (e.g., ARAÚJO JÚNIOR et al., 2012; 2016). This fact reflects the necessity of longer isolation periods studies to improve the understanding of the role of crabs on major nutrients geochemistry.

As our results indicates, the crab activity may contribute for lower N_2O fluxes, when compared to the control site, especially under highly inhabited area. Moreover, the seasonal variations are an important source of variation for nitrous oxide flux. The Nitrous oxide (N_2O) is the intermediate product of both nitrification and denitrification pathways (KREUZWIESER et al., 2003). The nitrification occurs when ammonium (NH_4^+) is sequentially oxidized to nitrite (NO_2^-) and to nitrate (NO_3^-) under aerobic conditions, and the flux of N_2O occurs by the reduction of the NO_2^-.

The denitrification, on the other hand, consists in the reduction of NO_2^- and NO_3^- to N_2O under anoxic condition by anaerobic processes (MEYER et al., 2008; CHEN et al., 2010; 2012). Lastly, the N_2O emission may also occurs by the anaerobic ammonium oxidation (anammox) pathway, where the N_2O flux results from the partial nitrification (ALLEN et al., 2007; OKABE et al., 2011).

In this sense, the redox potential (Eh) values higher than +200 mV indicates preference for nitrification pathway, and values below +350 mV indicates that denitrification pathway is more favorable (KREUZWIESER et al., 2003). Thus, our data suggest higher nitrification process than denitrification on dry season for both, control and bioturbated sites (+489 mV and +377 mV respectively) and higher denitrification pathway than nitrification on rainy season, also for control and bioturbated sites (+174 and +202 mV).

With regard to seasonal variations, the increment of the redox potential as a result of the soil desiccation and the increase of the pH may be responsible for a lower N_2O emission. According to Dalal et al. (2003), higher N_2O emissions are supposed to be expected under acidic conditions, which is in accordance to our results when compared both seasons. However, the control and disturbed site has been showed similar behaviour for pH values.

In fact, seasonal variations is an important factor that affect N_2O fluxes as showed in many studies with higher N_2O emissions during the dry season (ZHANG et al., 2006; ALLEN et al., 2011; CHEN et al., 2012). On the other hand, our study showed significantly higher N_2O emission during the rainy season, similar to those found in tropical Indians mangroves (CHAUHAN et al., 2015; KRITHIKA et al., 2008). Climatic influences includes temperature variations along seasons, as Chen et al. (2010, 2012) highlight in subtropical mangroves, with higher temperature amplitude than those that occurs with lower amplitude in tropical\equatorial mangroves forests.

5. CONCLUSION

This study reinforce the importance of mangroves as source of N_2O from soil to atmosphere that contributes to climate change. Highlighting the seasonal variations as an important source of variation for nitrous oxide flux, with higher emission on rainy season, suggested by input of N from neighborhood and anthropogenic activities, reflecting on pH and Eh values.

Moreover, our results indicates that the *Ucides cordatus* crab activity, even changing Eh and pH, may not affect N_2O fluxes in rainy or dry seasons, when compared to the area without crabs activities. On the other hand, the short isolation period of this study was not long enough to promote clear changes on TOC, TN and TS between control and bioturbated sites, as occurred to others elements cited in literature (Fe, Zn and Cu). This fact reflects the necessity of longer isolation periods studies to improve the understanding of the role of crabs on major nutrients geochemistry, reducing seasonal, tidal and climate effects.

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CONCLUSIONS

This work presented results that showed the strong influence of biota, in particular crabs, and the process of bioturbation in the biogeochemistry of metals as well as its influence on the decomposition of organic matter and the release of greenhouse gases (GHGs); in specific nitrous oxide (N_2O).

The results evidenced a strong influence of bioturbation in the availability of larger quantities of metal associated with oxidized forms of Fe and less associated with pyrite due to an increased of aeration in burrows. The burrowing activity of *U. cordatus* associated to seasonality considerably increase metals bioavailability and bioaccumulation, altering the mangrove soils biogeochemical conditions, but with a decrease in pyrite.

Besides that, the concentrations of metals in the crab tissue are a good proxy for the soil bioavailable fractions, with the bioavailable amounts of the metals in the hepatopancreas tissue of the crab *Ucides cordatus* differentiating significantly from the animal to the soil in which these animals live, what shows that the concentrations of metals in U. cordatus are much more correlated to the contents of more labile fractions (i.e. exchangeable and carbonate fractions).

Thus, according to data presented in mangroves under the influence of bioturbation by crabs occur significant changes in biogeochemical processes of soil metals, mostly due to increased aeration of soil particles (which causes the increase in oxidized forms of these) but also because of the very soil mobilization by craabs during the burrows building process.

Since mangroves are coastal ecosystems of great importance to the flow of greenhouse gases, thanks to the great carbon storage capacity on their soil, we expected that the physical and chemical changes in the soil caused by bioturbation of crabs also directly influences the rates greenhouse gas emissions, increasing the sites most burrows construction effect. But our results indicates the contrary, with the crab activity may been responsible for lower N_2O fluxes, when compared to the control site. We attribute this result to a possible short isolation period of the control area, that may be not long enough to promote clear changes in the fluxes of GHG. Moreover, the seasonal variations are an important source of variation for nitrous oxide flux, with higher fluxes emitted during the dry period

More studies are necessary to undestand how the bioturbation process can be affected by different species of crabs, as well as environmental changes (such as seasonal climatic changes or increasing organic load or metals in the soil due to the dumping of sewage), both short and long periods of time, in order to better understand the degree and extent of the effects of these changes in burrows building process by crabs, and the consequent change in the dynamics of biogeochemistry of metals and variations in GHG fluxes of these locations.