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MASTER THESIS PROJECT

(IZENBURUA/TITULO/TITLE)

Spatial and Temporal Changes of Surface Sediment Organic Matter Characteristics in a Mangrove subjected to Shrimp Farm Effluents, New Caledonia.

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Dr .....
as teaching staff of the MER Master of the University of .....
.....

CERTIFIES:
That the research work entitled. Dispersion and evolution of shrimp farm effluents in a mangrove of New Caledonia (South West Pacific) .Spatial and Temporal changes of Surface Sediment Organic Matter Charcteristics in a Mangrove receiving Shrimp Farm Effluent, New Caledonia.

has been carried out by Adélaïde Aschenbroich
in Muséum National d'Histoire Naturelle, Département des Peuplements et Milieux Aquatiques

under the supervision of Tarik MEZIANE
in order to achieve 30 ECTS as a part of the MER Master program.

In ……., September 2011

Signed: Tutor (……..)...Supervisor ( )

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ABSTRACT

Mangroves, organic matter, fatty acid, isotope, shrimp farming

MANGROVES are intertidal ecosystems, which colonies tropical and subtropical coasts and have a fundamental role in recycling and exportation of terrestrial organic matter (OM). This habitat is among the world's most productive ecosystem and one of the most threatened. Shrimp farming development is one of the main anthropogenic pressure acting on this ecosystem. In New Caledonia, shrimp farm activities begun in the 1970's and have increasingly developed in the last decade. Farms are open systems, which diffuse their effluents into adjacent mangroves that are commonly considered natural biofilters by the local population. Characterization of the effects of the effluent discharges on these singular ecosystems is primordial with this blooming farm activity. Therefore the present work aims to characterize the sources of OM of surface sediments within a mangrove receiving the discharge shrimp farm effluent.

TO THAT END, surface sediments of a mangrove located in the Saint Vincent Bay (21°56'S 166°04'E, New Caledonia) submitted to shrimp farm effluent discharge were sampled (51 locations) during two distinct periods: during a non active period (NAP) and an active period (AP; i.e. of the shrimp farm) in which farm discharges its wastewaters into the mangrove. Chlorophyll-a and phaeopigment concentration, fatty acid (FA) characterization and concentrations as well as the natural isotopic signatures ($\delta^{13}C$ $\delta^{15}N$) of the surface sediment were obtained following analytical methods after extraction and statistical treatments were performed.

THE RELEASE OF EFFLUENTS rich in particulate OM and nutrients, as well as the food used in ponds, into the mangrove induced change in the benthic OM nature and spatial distribution. During the farm activity, the OM was fresher (more unsaturated FAs) than during NAP, and the litter (from mangrove tree litter fall such as leaves, fruit and wood fall) was highly degraded, associated by an increase in fungal and anaerobic bacteria markers (e.g. 18:ω7 and branched 15:0). Additionally during AP, sediment was more $\delta^{15}N$ depleted and the phaeopigment concentrations decreased inducating a more reduced sediment and degraded bacterial. In parallel, during AP, FA marker of some micro-phytobenthic communities (18:3ω6, 20:3ω6) declined whereas the diatom bloom (16:1ω7 and 20:5ω3) is outlined contrarily to the seasonal tendency (summer bloom). However the primary production (chlorophyll-a) at both periods was significantly higher than those found in literature which suggests long-standing consequences of the nutrient-rich effluent releases. The monitoring of two FA from typical 'anthropogenic' sources in this habitat (18:2ω6 and 18:1ω9) allowed pointing up the exportation of the OM originated from ponds to the seafront. However, OM from the *Rhizophor*a stand seem to exhibit less FA composition changes, and exhibited low isotopic signature of the effluents, which could traduce its high assimilation capacity. Finally, he bacterial activity stimulated by farm OM inputs could induce a strong degradation activity notably of the additional OM.

RESUME

Mangrove, matière organique, acide gras, isotopes, crevetticulture

LA MANGROVE est un écosystème d'interface clé entre les domaines terrestres et marins des littoraux tropicaux et subtropicaux et ayant un rôle fondamental dans le recyclage et l'exportation de la matière organique (MO) d'origine terrestre. Cet habitat est l'un des écosystèmes les plus productifs (et les plus menacés) au monde. Parmi les pressions anthropiques exercées sur cet écosystème, le développement de la crevetticulture est l'un de ceux qui l'affecte le plus. En Nouvelle-Calédonie, les fermes de crevettes mises en place depuis les années 70 font l'objet d'un réel essor depuis une dizaine d'années. Elles ne fonctionnent pas en vase clos et rejettent leurs effluents de manière diffuse dans les mangroves adjacentes qui sont considérées comme des biofiltres naturels par les populations locales. Face à la croissance de la crevetticulture, la caractérisation des effets de ses rejets sur ces écosystèmes si singuliers est primordiale. Ainsi, ce travail a pour objectif de caractériser la MO du sédiment de surface au sein d'une mangrove soumise à des rejets d'effluents d'une ferme de crevette.

DANS CE BUT, les sédiments de surface d'une mangrove située dans la Baie de St Vincent (21°56'S 166°04'E, Nouvelle Calédonie) recevant les effluents de la ferme dite « FAO » (Ferme Aquacole de la Ouenghi) ont été collecté (51 localisations) à deux périodes : lorsque la ferme est à l'arrêt (NAP) et lorsque la ferme est active (AP) et rejette donc ses eaux usées dans la mangrove. Les concentrations en chlorophylle-a et pheopigments, la caractérisation et les concentrations en acides gras ainsi que les valeurs isotopiques des sédiments ont été mesurées, après extraction, selon des techniques d'analyses spécifiques, et furent soumise à des traitements statistiques.

LE REJET D'EFFLUENTS chargée en MO particulaire et en nutriments, ainsi que de la nourriture utilisée dans les bassins, sont à l'origine d'un changement de la nature et de la distribution de la MO. Pendant l'activité de la ferme, la MO est plus fraiche (plus d'AG insaturés) sur le sédiment de la mangrove et la litière apportée par les palétuviers est hautement dégradée, associée à une augmentation des marqueurs de fungi et de bactéries anaérobiques (ex. 18:1ω7 et les branchés du 15:0). A cette période le $\delta^{15}N$ du sédiment est appauvri et les concentrations en phéopigments diminuées indiquant ainsi un sédiment plus réduit et, dégradé par les bactéries. En parallèle, les AG marqueurs de certaines communautés micro-phytobenthiques (18:3ω6, 20:3ω6) déclinent alors que la croissance des diatomées (16:1ω7 et 20:5ω3) est mise en avant à l'encontre de la tendance saisonnière (bloom estival). Cependant, la chlorophylle-*a* mesurée aux deux périodes est nettement supérieures à celles rencontrées dans la littérature et suggère que le rejet d'effluent aurait des conséquences à long terme sur la production primaire. Le suivi de deux AG typiquement 'anthropiques' dans cet habitat (18:2ω6 et 18:1ω9) a mis en avant l'exportation de la MO provenant des bassins jusqu'au front de mer. Cependant, la MO de la zone de *Rhizophora* semble subir moins de changement d'une période à l'autre. Finalement, l'activité bactérienne stimulée par les apports en MO de la ferme semble aboutir sur une plus forte activité de dégradation notamment de la MO additionnelle.

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INTRODUCTION

Mangrove forests are among the world's most productive ecosystems (Kathiseran, 2001; Kristensen et al., 2008) and host a large biodiversity. They are important ecosystems to maintain biodiversity and for Humans as it provide an important commercial fishery resource (Barbier, 2000), a shoreline protection from erosion and cyclonic condition frequent in tropical area (Giri et al., 2010), and is a major carbon sink (see Barr et al., 2004) reliable in the present global context.

Today, this singular environment is about the most threatened ecosystems (Alongi, 2002) because of coastal development (urban and economic activities). Mangroves are largely used as natural biofilters and receive effluents of aquaculture farms and notably of shrimp farms which activity is strongly blooming since few decades. Indeed, it has increased of more than 600% between 1984 and 2004 and is expected to continue this pattern through 2030 (FAO 2006).

In New Caledonia, research for shrimp farm development begun in the 1970s managed by IFREMER[1], and commercial exportation begun ten years later. Nowadays, New Caledonia accounts for 19 shrimp farms corresponding to 620 hectares of ponds (Thomas et al., 2006). In 2005, the annual production was about 2,004 tons making the shrimp production the second largest economic exporting activity after the nickel industry and a doubling of the production is expected by 10 years (Della Patrona, 2009). Waters from lagoon, river or from mangrove are imported to ponds and none filtered waters from shrimp pond, richer in nutrient and particulate organic matter (Martin et al., 1998; Riviera-Monroy et al., 1999; Lemonnier et al., 2003; Thomas et al., 2010) than supply water are released into the mangrove swamps. These daily discharges can represent until 30 % of pond volumes (Della Patrona, 2009).

Mangroves have been reported to be efficient for removing suspended solids and nutrients (Twilley et al., 1993; Robertson and Phillips, 1995) and for cleaning discharge waters before entering the lagoon. But the lack of information about the direct and indirect impacts of effluents on mangrove ecosystems and their reversibility have led the ZoNéCo[2] program to create a specific research branch in New Caledonia since 2004. Improve the knowledge about the shrimp farm effluent effects on the receiving mangrove ecosystem is needed in order to optimize the management practices respectful of the environment.

In this context, Tarik Meziane (MNHN[3], Paris, France) and Cyril Marchand (IRD[4], Nouméa, New Caledonia), biogeochemists and mangrove-lovers have shaped a PhD project carried out by Nathalie Molnar. Her 3-years thesis aims to estimate the impacts of the FAO (Ferme Aquacole de la Ouenghi) shrimp farm effluents on a receiving mangrove ecosystem in New Caledonia (St Vincent bay), by the characterization of its ecological changes at several levels involved by shrimp farm

[1] *Institut francais de le Recherche pour l'Exploitation de la Mer*
[2] *Zone economique de Nouvelle Caledonie, which aims to study marine resource in New Caledonia for 15 years.*
[3] *Museum National d'Histoire Naturelle*
[4] *Institut de Recherche pour le Développement*

activities. Included in this plan the present Msc work mainly deals with the surface sediment analysis to qualify and quantify the organic matter of the FAO mangrove by using three main tracers: chlorophyll-*a*, stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and fatty acid signatures.

Chlorophyll-a (chl-*a*) is used to quantify the primary production in sediment and to informs about the ecological status of an environment as an algal bloom with an increase of organic matter production, in response to changing environmental condition results in low levels of dissolved oxygen which is a limiting factor for many marine organisms (Pelley, 1998). Fatty acids (FAs) are hydrophobic carbon chain constituting lipids in living tissues, which can be used as biomarker[5] since several FAs are referred to be specific of some organisms (Parrish, 1991). Therefore they inform about the OM sources, the health of an ecosystem or the degree to which it has been influenced by anthropogenic inputs (Boon and Duineveld, 1996; Parrish et al., 2000). Potential sources of FAs are largely discussed in literature and their biomarker powers strongly depend on the environment (wetland, lake, Open Ocean; see review in Napolitano, 1998). Similarly, analysis of $\delta^{15}N/\delta^{14}N$ and $\delta^{13}C/\delta^{12}C$ stable isotopic ratios are regularly used in ecology to assess relative contributions of multiple sources to bulk OM pool, and to follow the flow of OM in marshes and estuaries (Peterson et al., 1985). The stable isotopes ^{14}N and ^{15}N occur overall on earth and their ratios differ among specific N pools in the environment (Peterson and Fry 1987). Therefore, N-isotopic signature from distinct sources can be identified and traced (MClelland et al., 1997). The heavy form of the atom carbon, ^{13}C, is always found in small proportion in photosynthetic organisms since the latest preferably use the ^{12}C from the atmosphere, which is lighter for photosynthesis. Measurement of the $\delta^{13}C$ marks the deviation of isotopic concentration in any sample with respect to a standard measurement (PDB marine fossil shell, Rodelli et al., 1984) and is characteristic of specific sources. Both ratios have been largely used to trace wastewater plume in coastal sediment (Sweeney et al 1978a; Rogers 1999; Ramírez-Álvarez et al., 2007).

On this basis, the present work aims to (1) characterize, identify and quantify the source of the surface sediment organic matter (SOM) in a mangrove receiving shrimp farm effluent and (2) compare the temporal SOM composition and dispersion between a non active period and an active period (i.e. of the shrimp farm). Sampling was performed within a unique mangrove receiving shrimp farm effluent waters half of the year, in order to assess within an unique environment the direct influences of shrimp farm discharge.

[5] *Compounds or groups of compounds that can be used as signatures of individual organisms or groups of organisms, or of certain environmental processes*

CHAPTER I: CONTEXT AND REVIEW

This first chapter aims to describe mangrove ecosystems, their particularities and adaptations, their ecological, socio-cultural and economic interests, and their threats. In parallel knowledge about shrimp farming effluent impacts on receiving adjacent mangroves is developed, focusing on the case of New Caledonia (NC).

I.1 ECOLOGY OF MANGROVE ECOSYSTEMS

I.1.1. Presentation and Zonation of New Caledonian mangroves

Mangroves are intertidal ecosystems characterized by a halophyte (i.e. adapted to saline environment) vegetations, which take place in sheltered tropical and subtropical coasts, lagoon, bays, estuaries or deltas (i.e. small topographic gradient; Kathiseran, web source). This singular ecosystem covers 75% of the world tropical and subtropical coastline (Spalding et al., 1997) and is present in 112 countries between 30°N and 30°S (Appendix A). In New Caledonia (South West Pacific), they represent 35,100 hectares of the island and 88% are situated on the flat landscape at the western part sheltered from the easterlies winds (Figure I.1; Virly, 2005).

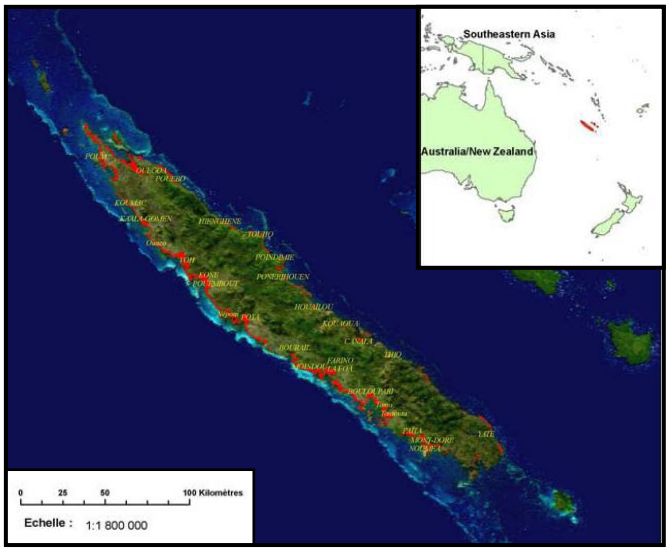


Figure I.1: Location of mangrove forests in New-Caledonia (Pacific Ocean; from Virly, 2008)

A specific vegetal zonation can be attributed to mangroves conditioned by tidal flooding, land elevation, slope and salinity (Martin, 2005). In NC three zones, generally distributed parallel from the shoreline, can be differentiated (shown on Figure I.2 or on Appendix B): a fringe along the seafront where *Rhizophora stylosa* trees dominate, behind which a second fringe is dominated by *Avicennia marina* trees and behind, the saltpan is dominated by *Salicornia australis* (Appendix B). Within the third fringe a sailing algal expense (Cyanophycae; Appendix B) or zones where the sediment does not

present any vegetal cover (because of coarse sediment) can also be found (Della Patrona and Brun, 2009). The *Rhizophora* and *Avicennia* stands constitute an "active swamp" submitted to a regular tidal immersion whereas the salt flat is more occasionally immerged, making this area more saline. Mangrove zonation is generally parallel to the coastline however some conditions can make it different (e.g. Mangrove of Voh; Appendix C).

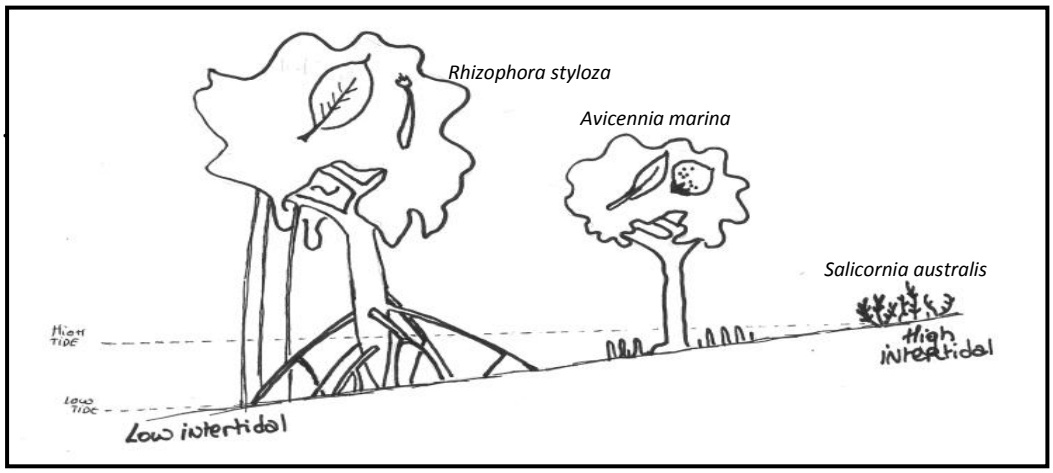


Figure I.2: Scheme of the typical zonation of the three vegetation stands of the mangroves in New Caledonia, usually distributed parallel to the shoreline.

I.1.2. Intertidal Adaptations and their Limits

Mangroves are intertidal forests which have a high adaptation capacity to extreme environmental condition changes as they are frequently submitted to saline (variations from 0‰ to 90 ‰; Clough, 1992), waterlogging and contrasting redox conditions variations (Marchand et al., 2005). However additional changes in hydrological properties (i.e. immersion time and frequency, nature and amount of the freshwater inputs, wave exposure, current, salinity, temperature and oxygen levels) affect directly or indirectly the mangrove (Virly et al., 2005) influencing the recruitment, the survival and the growth of mangrove vegetations. All these factors combined determined the ecological limits of mangrove ecosystem and can be modified by climate or by anthropogenic activity.

Mangrove tree adaptations include roots going down from branches and trunk, which provide a stable support to confront high current intensity of intertidal zones, and unstable soft sediment. Aerial roots (i.e. pneumatophores) are additionally built to adapt hypoxic to anoxic soils characteristic of mangrove area; such adaptations appear only after 4-8months thus plantlets generally suffer oxygen lack. Indeed, anoxia has been reported to lowers nutrient uptake capacity of *Avicennia marina* plantlets leading to their death (Boto et al., 1985). However, bottom oxygenation is usefully enhanced with crab holes which improve sea water evacuation and bring oxygen to underground roots (Nielsen et al., 2003). But anthropogenic activity (i.e. nutrient inputs from wastewater discharges) and eutrophication can prevent oxygen access to roots and lead to mangrove trees death with the formation of Fe plaque on roots (Pi et al., 2010). Mangrove trees withstand hypersaline environments thanks to the presence of salt exclusion (through filtration membrane preventing salt entering) and excretion

(through salt gland) adaptations in roots and leaves respectively. But great changes in water salinity affect water pumping (through the roots) efficiency of mangrove trees. The more salty the water is, the more difficult the pumping results, because the external osmotic pressure is enhanced compare to the internal in roots (Passioura et al., 1992) and mangrove trees metabolism can be affected irreversibly (Flowers et al., 1977). Finally mangrove ecosystems are adapted to specific air temperatures averaging 24°C, and any rise of temperature may lead to the spreading of some species in to higher latitudes (Kathiresan, web source) and to a decrease of the leaf photosynthesis capacity of mangrove trees (which is optimum at 28-32°C; Clough et al 1982). Water temperature also determine their environmental limit as their global distribution is believed to be delimited by major ocean currents and the 20° C isotherm of seawater in winter (Alongi, 2009).

Mangroves are therefore ecosystems well adapted to extreme environmental condition but they remain sensitive to any changes. Environmental variations could be induced by climate change or anthropogenic activities which more and more put in jeopardy mangrove forests.

I.2. **FUNCTIONS OF MANGROVE ECOSYSTEMS**

I.2.1. Ecological and socio-economical functions

Worldwide mangroves comprise about 80 species from 20 families of vascular trees (Tomlinson, 1986). In NC, 24 mangrove species have been repertoried (Rateau and Marchand, 2007). This involves that mangroves are highly productive areas providing food and organic carbon for the local and adjacent environment food webs (such as the lagoon in NC; Odum and Heald, 1975). Some species are exclusive to mangrove forest such as the *Periophtalmus sp* (Appendix D). In NC 64 families of fish have been listed in the mangroves of the southwestern lagoon (Erwan Roussel, conservatoire du littoral). Some species are permanent mangrove inhabitants such as the fiddler crab (Appendix D), the mud crab (Appendix D) and other inhabit temporary mangroves to feed or spawn as mangroves are valuable nursery site for numerous of crustacean and reef fish species (Robertson and Duke, 1987; Robertson and Duke, 1990) because of physical protection (from predation) and food sources these ecosystems provide. It has been reported in Moreton Bay (Australia) that mangrove estuaries hosted 4-10 times more fish than the adjacent sea grass bed habitats (Laegdsgaard et al., 1995). Such environments provide a major food source for local communities and adjacent coastal foodweb (Bouillon et al., 2008; Kristensen et al., 2008) notably for the lagoon in NC (Marchand et al., 2011).

In a lot of country many human activities depend on mangrove forests since they are used for medicine and wood collect (Marius, 1989) and provide an important commercial fishery resource (Barbier, 2000; Diele et al., 2005). Moreover mangrove forests help stabilizing the shorelines from erosion and lessen the devastating impact of natural disasters such as hurricanes, cyclone and storm quite frequent in tropical areas (Giri et al., 2010).

I.2.2. Role of mangroves in the carbon cycle

Additionally mangrove forests have been recently considered to have a significant impact on global carbon cycling (Bouillon et al., 2008). Their intertidal position and the potential exchanges with coastal water suggest that they have a unique contribution to carbon biogeochemistry in coastal ocean (Odum and Heald, 1975; Twilley et al., 1992). Barr et al (Florida, United States; 2004) estimated the amount of dioxide carbon of the atmosphere assimilated by mangrove to 7-9 tC y^{-1} ha^{-1}. Other studies found an assimilation rate in the litter and in the sediment about 7-18 tC y^{-1} ha^{-1} (Matang mangrove, Malaysia; Eong 1993). At this rate mangrove forest outperform other leaf forests assimilating about 0-6 tons of carbon per hectare per year (Baldocchi et al., 2001) and is a valuable and interesting carbon sink in the present global context. However global mangrove carbon budget and notably outwelling of carbon are not well known as more than the half of inputted carbon into mangrove sediment was not detected and quantified in exports considered (i.e. burial, CO2 efflux, POC and DOC export; see Bouillon et al., 2008).

I.3. **MANGROVE THREATS AND SHRIMP FARMING**

I.3.1. Deforestation of mangroves: some numbers

For 30 years, mangrove loss ranged from 35 to 86 % (Wilkie and Fortuna 2003) and this ecosystem is currently disappearing at 1-2 % per year (Valiela et al., 2001; Wilkie and Fortuna 2003). The activity most responsible for mangroves deforestation is the aquaculture pond construction and especially for shrimp farming (Barbier and Sathitaria, 2004). Some countries such as Philippines or Mexico have lost 50% of their mangroves over few decades to aquaculture ponds constructions. In Equador, 14% of the mangrove surface has been used for aquaculture construction only in 1987 (Virly et al., 2005). This deforestation put in jeopardy the mangrove ecosystem itself but also its ecological, physical and economical functions affecting coastal fishery, clean water supplies, salinization of coastal soils, erosion of land or release of carbon dioxide into the atmosphere. (Barbier et Sathirathai 2004 and references therein). However the extend of the deforestation will depend on the farm intensification type chosen (Kongkeo 1997) as an extensive farming needs more space than intensive farming.

I.3.2. Shrimp farm functioning in New Caledonia

In New Caledonia, shrimp farm construction do not deforest as ponds are built on the saltpan, upstream of mangrove forest. Nevertheless, to build aquaculture ponds it is necessary to dig, divert water and matter inputs from watershed, drain or flood some zones and construct artificial channels, which all ends by therefore modifying the mangrove topography and hydrology (Virly et al., 2005).

Farming is based on a “semi-intensive intensified” model, with pond sizes averaging 8.1 ha in which are cultivated about 20-30 *Litopenaeus stylirostris* (or Blue shrimp) per m². Shrimps produced are introduced species originated from the East Pacific coast (SOPAC©). Farms are open systems

composed of several ponds (from 2 to 12) around a main channel which allows the water (taking from lagoon, river or directly from mangrove) distribution into them. Dissolved oxygen is the first limiting factor of shrimp growth in ponds, is exclusively brought by the water incoming and oxygenation is enhanced by the activity of phytoplankton which grows in ponds (Chien 1992). Non filtrated water from ponds is released across railings adapted to shrimp size, and drained back into the mangrove swamps. In average shrimp farms discharge from 5 to 30 % of pond volumes to renewal it daily (Della Patrona, 2009). Shrimp farming activity takes place during the fresh season (i.e. from December to July) in order to prevent diseases and bacterial growth. Fishermen proceed to a "partial fishery" i.e. from 2 to 12 harvests by year are done to obtain different shrimp sizes according to the demand (the diminution of the shrimp density in ponds going along harvests allows the increase of shrimp size).

I.3.3. Review about the evolution of shrimp farm effluents in receiving mangroves

Water discharges of shrimp farm are rich in nutrients, organic matter, phytoplankton, chlorophyll a, bacteria and other suspended solids and are even richer than supply water (Riviera-Monroy et al., 1999; Lemonnier et al., 2003; Thomas et al., 2010) due to physical and chemical cycling taking place in ponds. Nutrients, such as nitrogen, incorporating in ponds through feed pellet (6.5 % of feed pellets; Thomas, 2006), are taken up by animal targets or transformed through shrimp excretion (into ammonia) which can either settle down or be assimilated by phytoplankton (as nitrite and nitrate; Rataud and Marchand, 2007). The amount of nutrient found in effluent water of shrimp farm varies from one study to another; it probably depends on the intensity of inorganic fertilization (Gautier et al., 2001) used to stimulate microalgae growth which in turn serves as food for shrimp. Boyd (1995) observed that all nutrients increase in ponds except nitrite (NO_2^-) because of denitrification[6] process due to anaerobic condition. In the study of Molnar[7] (in prep) a net increase of particulate organic nitrogen and phosphorus was detected in wastewater of the FAO shrimp farm. However, nitrogen and phosphorus fluxes and magnitudes depend on the intensification degree of farms (Virly et al., 2005). Indeed, total suspended solid in effluent water increases with intensification of shrimp farming system since high shrimp density enhances the aeration by bioturbation i.e. the erosion of pond bottom (Lemonnier et al., 2004) and increases organic matter inputs in ponds and generated waste (Martin et al., 1998).

As shrimp ponds are phytoplankton producers representing the main part of discharged elements (Paez-Osuna et al, 1997, Martin et al, 1998)**,** effluents dispersion have been used to be traced by chlorophyll-a which concentration is high close to discharge points, and decreases going downstream (Martin et al., 1998). Furthermore, primary production, phytoplankton growth and bacterial activity of the receptor environment, are reported enhanced by the increase of nutrient available and might lead to the eutrophication (McKinnon et al., 2002) becoming an important

[6] *The denitrification process is the transformation of the nitrates into nitrites then into ammonia by specific bacteria in reduced environments:* $NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$.

[7] *The present work is included in the PhD study of N.Molnar, and therefore deals with the same study site, described below.*

additional food and nutrient source. Indeed growth of microbial organisms enhances the degradation of organic matter accumulated in the sediment (unconsumed feed pellets, feces) producing ammonia, nitrite and nitrate (Blackburn et al., 1988**),** which in turn improves the microbial activity. Eutrophication could lead to a pH and a dissolved oxygen decreases in mangrove receiving shrimp ponds effluent (Powell end Martens 2005) and can therefore lead to asphyxia of mangrove trees (see above) limiting oxygen access to roots. On the long run, constant water inputs in mangroves can prevent evaporation process, changing hypersaline condition of the bottom, making the environment more favorable for *Rhizophora stylosa* (Virly et al., 2005) which takes precedence over *Avicennia marina*, the canopy become thicker and the light access to sediment is reduced.

Previous studies on shrimp farming in NC reported that the high nutrients and suspended solids in aquaculture effluents could be (partially) removed within mangrove ecosystem, acting as an efficient biofilter (Twilley et al. 1992; Robertson and Phillips 1995). Indeed, this ecosystem has a great capacity of matter (particulate/dissolved, organic/mineral) retention and treatment, being able to eliminate suspended solids discharged by effluents. However mangrove as phosphorus and nitrogen sink is a phenomenon poorly understood (Massaut, 1999) and its biofilter efficiency is less predictable than expected (Gautier et al., 2001). Processes involved in suspended solid and nutrient removal are various and those reported in literature are: sedimentation on the bottom or dissolution in the water column, decomposition of organic matter (bacteria, fungi, macrozoobenthic feeding), uptake of nutrient by plants (for mangrove tree growth) and bacteria, nitrification[8]/denitrification and absorption of ions by soils (Boyd and Tucker, 1998; Rivera-Monroy et al., 1999; among others). Considering these processes Rivera-Monroy et al (1999) estimated that 0.04-0.12 ha of mangrove was necessary to removed nutrients of 1ha shrimp pond waste.

Nevertheless mangrove suspended matter and nutrient removal/assimilation depends on many factors such as the composition of wastewater, the daily quantity of inputs, and the physico-chemical properties of the receptor environment.

[8] *The nitrification is the transformation by aerobic micro-organism of the ammonia into nitrite and nitrate.*

CHAPTER II: MATERIAL AND METHODS

II.1. STUDY SITE DESCRIPTION

Study was conducted in a mangrove area located on the west coast of New-Caledonia (21°56'S 166°04'E, Saint Vincent Bay) (figure II.1) which receives shrimp farm effluents from the "Ferme Aquacole de la Ouenghi" (FAO).

FAO farm, opened in 1989, is made of two 1 m deep rearing ponds (L and K, 10.5 and 7.5 ha, respectively), extend over 28.9 ha over a former mangrove saltpan (figure II.1). Ponds are stocked with the blue shrimp and farm operates a semi intensive rearing system with an average of 17 individuals per m². Activity is launched in December/January for ~8months after which farm proceeds a ~4 months break (August-November) in order to drain and dry the ponds (Della Patrona and Brun, 2009). Shrimps are fed with locally produced feed pellets (35-40% protein, SICA ©, NC) which are added daily throughout the rearing period, with inputs increasing over the rearing cycle as the shrimp grow, from ~0.25 to ~3.5 kg ha^{-1} d^{-1} (Farm manager, Pers. Comm). Ponds are continuously irrigated with water pumped directly from the lagoon to maintain water column oxygenation, at a rate of 5 to 25% of the ponds volume over the course of the rearing cycle (Farm manager, Pers.Comm.). Excess water from ponds is released at multiple points into the adjacent mangrove (figure II.1). Effluents flow on sediment mangrove forest surface and are eventually collected by short channels which penetrate the mangrove fringe. The main discharge outflow from the dyke after collecting effluents from both ponds diverted around the saltpan area through little sandy-dykes from which frequent overflows occur.

The vegetation developed in the study area is represented by *Salicornia australis* on the saltpan and by *Avicennia marina* and *Rhizophora stylosa* (figure II.1) successively distributed from ponds to the sea front. More rarely, few *Bruguiera sp* trees have been noted.

Saltpan is either covered by *Salicornia australis* or by cyanophycae (Appendix B). Downstream, mangrove is occupied by *Avicennia marina* shrubs forming a zone submerged at each high tide, and which covers ~15% of the mangrove area. Shrubs are densely present and are about 2 m height; however they reach higher sizes along the K pond, in front of the effluent discharges. Along both ponds, shrubs account for algae and/or moss presence on their pneumatophores. Additionally some zones have higher pneumatophores density than other ones notably at *Avicennia-Rhizophora* interphase in front of discharges.

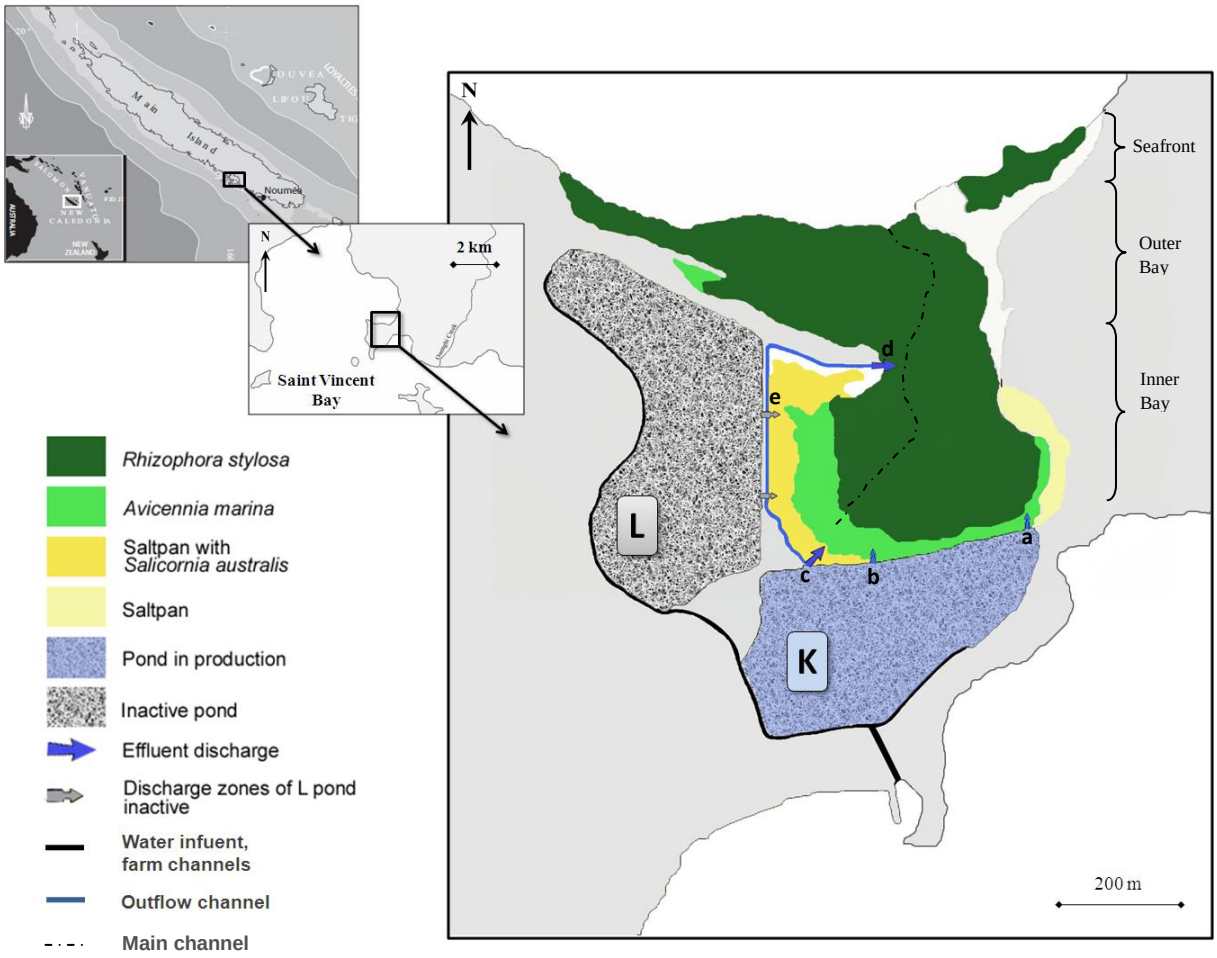


Figure II.1: The FAO constituted of two ponds L and K, located in the St Vincent Bay, New Caledonia. During the study period only the pond K was in production. Wastewaters are released in the adjacent mangrove composed of three vegetation stands: the Rhizosphora stand, the Avicennia stand and the saltpan. Lowercases name the discharge zones. 'e' refers to the frequent overflows which occur from the channel (sandy-made).

Rhizophora stylosa is the dominant vegetation of the mangrove and forms a stand which represents about 85% of forest extending to the seaward edge. It is a quite heterogeneous group since it includes a large range of tree sizes. In a general pattern, trees are smaller on stand borders and higher at the West Sea front. Besides, there is a notable and exceptional trees fringe reaching up to 5 m of height along the main channel, close to the output zone'd'(Appendix F).

Within the mangrove area several crab species are present such as *Uca spp*, particularly in *Salicornia* and *Avicennia* stands with a large number of visible burrows. Density was estimated to be 20 burrows m^{-2} in *Salicornia* stand, and 30 burrows m^{-2} in *Avicennia* stand. *Uca spp* were not present in *Rhizophora* stand, in where *Grapsidae sp* are dominant, with an estimated density of 40 burrows m^{-2} (Molnar, in prep).

Surface sediment of the FAO mangrove was collected during two contrasting periods: during a non active period (NAP) and during an active period (AP) of the shrimp farm. The NAP corresponds to the early hot and wet summer (December 2009). Rainy and cyclonic season only starts in February and generally last 2 months. The AP corresponds to the dryer and colder winter and sampling was

done during July 2010. Winter sampling period was however characterized by higher daily precipitation than summer (Figure II.2). Due to a shortage of shrimp larvae, only the smaller 7.5ha pond K was in production at sampling time.

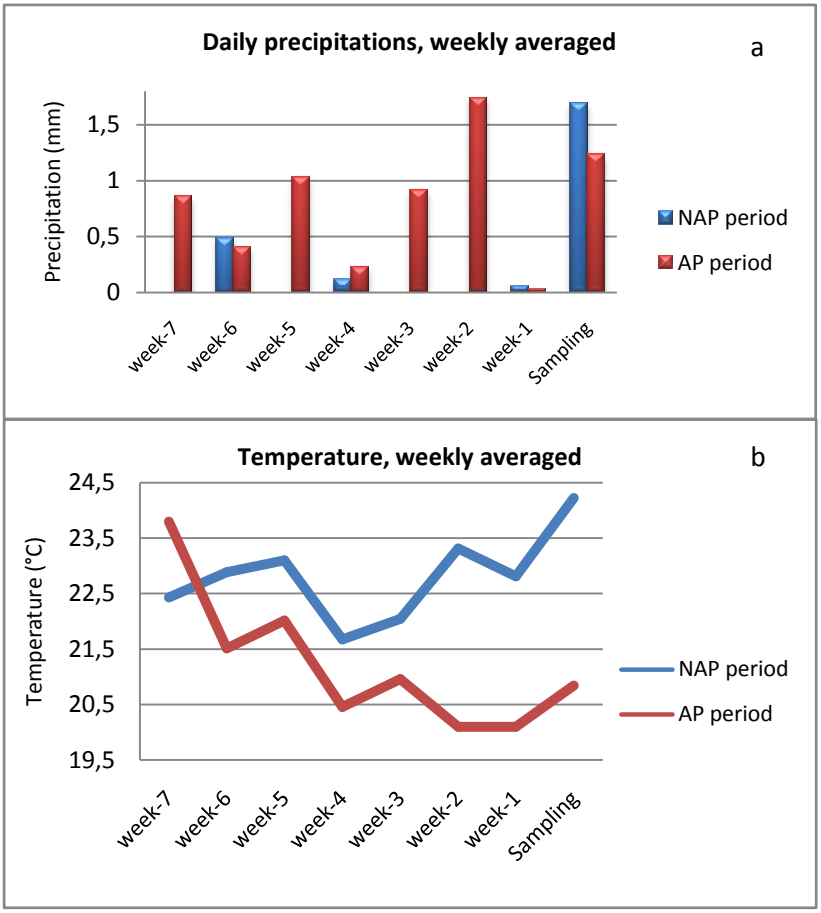


Figure II.2: Data of (a) precipitations and (b) temperatures from "Meteo France" for the sampling weeks and 7 weeks before. Data are daily values weekly averaged.

II.2. FIELD SAMPLING

Surface sediment was collected randomly throughout the FAO mangrove at low tide during NAP (i.e. no effluent releases) and at the end of a rearing period corresponding to the maximum effluent discharge within the mangrove (AP). Because of the high density of trees and aerial roots, several sites were not accessible and the use of a systematic sampling approach (Caeiro et al, 2003) was therefore not always possible. Thus, sampling was optimized to consider a maximum of the mangrove area. To that end, mangrove forest was sub-divided into 51 sites (considered as a minimum for a good statistical treatment; as shown on figure II.3) using a hand-held GPS receiver (Colorado 300, Garmin) for geographical coordinate's registrations.

Sediment samples for fatty acid and stable isotopic analysis were collected in triplicates during AP whereas only one replicate in each site was sampled during NAP. One replicate corresponded in fact to the pooling of five 1cm depth x 2cm Ø syringes contents. For chlorophyll-a samples one replicate corresponded to one syringe core. Samples were swiftly transported to the laboratory (IRD

Noumea Center, New Caledonia), freeze-dried and stored at -20°C until analysed.

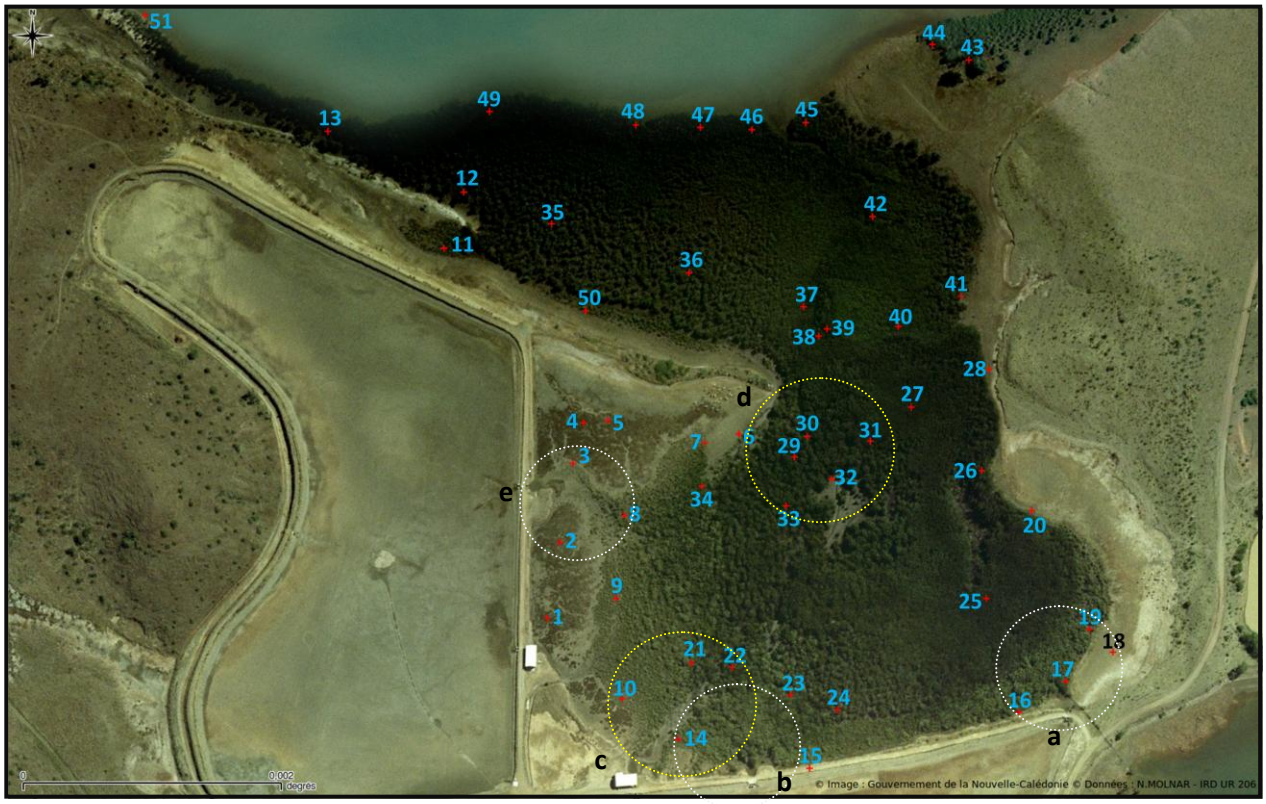


Figure II.3: The FAO mangrove with the sample locations. The circles indicate the output zones of the effluents. The yellow circles refer to the main discharge zones and the white circles refer to the secondary discharge zones.

In order to identify isotopically the sources which could influence the sediment, mangrove leaves (from *Salicornia australis*, *Avicennia marina* and *Rhizosphora stylosa*) were randomly collected (pool of 5 leaves x 3 trees), as well as cyanophycae presented within the surface sediment in the *Salicornia* stand. Additionally, suspended particulate organic matter (POM) of effluents was collected by filtration through Glass-Fibre Filters (GF/F) and FAO farm food (granule) using for shrimp development was also sampled and grounded to assess their fatty acid and isotopic compositions. All samples were freeze-dried and stored at -25°C until analyze. To measure stable isotopic signatures of the microphytobentos, an additional sampling was conducted in February 2011 (corresponding to an active effluent release). Surface sediment was collected (in triplicates) within the three vegetation stands of FAO and benthic microalgae able to migrate, were extracted from sediment following the Riera and Richard (1996) method slightly modified from Couch (1989). Sediment was collected by scraping the upper 5 mm part and spread on 50x30 cm flat tray, a nylon screen (60 µm) was laid upon the sediment surface, then covered by a thin combusted silica powder (60 to 200 µm grain size) layer kept wet by spraying filtered seawater from adjacent lagoon. Silica allows recovering microphytobenthos which use to migrate at low tide to reach light. Trays were held under light during 3 hours and silica powder into which microalgae had migrated was collected and sieved through a 60 µm nylon screen to separate diatoms from the greater part of the silica powder. Filtrates were then filtered on GF/F previously combusted at 450°C. Filters containing microalgae were freeze-dried and

stored at -20°C until analysed.

Finally, in order to realize microscopic observation of the microphytobenthic composition, complementary samples were collected (in triplicate within the three mangrove stands) by scraping the upper 5mm sediment layer. Samples were stored in the dark at 4°C in a 50% filtered lagoon water 50% formalin solution in pillboxes. On the other hand, microalgae able to migrate were extracted from sediment following the method of Easton and Moss (1966). A double layer (1x1 cm on 15x10 cm) of Whatman's No 105 lens tissue was placed on sediment during 30 minutes. Lens tissues saved were stored in 50% formalin and 50% filtered lagoon water solution in the dark at 4°C before microscopic observation.

II.3. SAMPLES ANALYSIS

II.3.1. Chlorophyll a and Phaeopigment

Chl-a and Phaeopigments were measured fluorometrically according to the slightly modified method of Yentsch et *al*. (1963) in the Chemistry laboratory of the IRD center of Noumea. Fluorimeter used was a Turner Designs TD700 equipped with an optical kit n°7000-961 including an excitation filter of 340-500 nm wavelength, and an emission filter up to 665 nm wavelength.

Between 0.4 g and 0.5 g of freeze-dried and ground sediment were extracted with 8 ml methanol 93% and shacked during 30 minutes (sheltered from light), allowing pigments transfer in methanol, improved by centrifugation (3000 rpm, 5 min). The supernatant methanol was x10 diluted in small vials.

Pigments in methanol were then excited in the fluorometer with a 450 nm wavelength beam of light and fluorescence emitted at 664 nm. Fluorescence was measured twice, before and after acidification with HCl (20 µl; 0.3 mol/l and 20s shaking). Before acidification both chl-a and phaeopigments were measured, and acidification converts all chl-a contents into phaeopigments which also emitted fluorescence but less intensively. Therefore fluorescence after acidification (Fa) decreased compare to fluorescence before acidification (Fo) due to the chl-*a* degradation by HCl.

Finally chl-a and phaeopigments were calculated using the following equations (from Chifflet et al., 2004):

$$\text{Chl a} = (Fa^{\lambda} - Fo^{\lambda})/(Ka - Ko)$$

$$\text{Phaeo} = (Ko.Fa^{\lambda} - Ka.Fo^{\lambda})/[ka.(Ka - Ko)]$$

Where Ko is the fluorometer calibration factor for pure Chl-a, and Ka is the fluorometer calibration factor after acidification.

II.3.2. Stable isotopic analysis

Samples for stable isotopic analysis ($\delta^{13}C$ and $\delta^{15}N$) were firstly freeze-dried in the IRD center of Noumea. Carbonates were removed from sediment previously grounded in the BOREA laboratory (Paris, France) using a 1N HCl solution. Plant and sediment samples as well as filters POM of

effluent waters and including migratory microalgae were weighted (20±1 mg and 30±1 mg respectively) in tin caps.

Isotopic analyses were carried out by the UC Davis Stable Isotope Facility laboratory, using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). These samples were burnt at 1000°C in reactor packed with chromium oxide and silvered cobalts oxide. Thereafter oxides were removed from samples in a reduction reactor (reduced copper at 650°C). Helium gas carried flow through a water trap (magnesium perchlorate) and through an optimal CO_2 trap (for N analysis only). Finally, N_2 and CO_2 were separated on a Carbosieve GC column (65°C, 65 mL/min) before entering the IRMS (Isotope Ratio Mass Spectrometry). The analytical precision (standard deviation for repeated measurement of the internal standards) for the measurement was 0.06‰ and 0.13‰ for δ13C and δ15N respectively.

δ13C and δ15N are calculated according to a standard value. Stable nitrogen isotope data are normalized relative to $^{15}N/^{14}N$ of atmospheric N_2 as $\delta^{15}N$ (‰) = $[(Ratio_{sample}/Ratio_{atm})-1]x10^3$ (Peterson and Fry, 1987) and the $\delta^{13}C$ marks the deviation of isotopic concentration in any sample with respect to a standard measurement (PDB marine fossil shell, Rodelli 1984) as $\delta^{13}C(‰) = [(Ratio_{sample}/Ratio_{PDB})-1]x10^3$ and.

II.3.3. Fatty acids methyl ester analysis

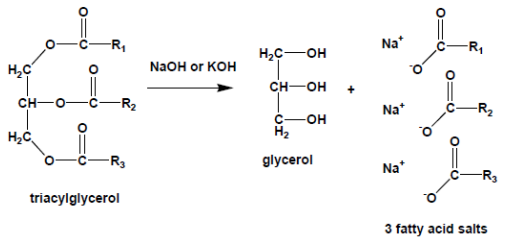
Fatty acids were extracted following the method of Bligh and Dyer (1959) slightly modified as in Meziane et al. (2007) from 0.4 to 0.5 g of sediment and from 0.12 to 0.18 g of filter (including migratory microphytobenthos and POM from effluent waters). A standard fatty acid (23:0; 4µl) was added to samples before extracting to allow concentration calculations further. 23:0 (i.e. chain of 23 carbons) is a useful internal standard since it cannot be found naturally in the environment.

Lipids were extracted ultrasonically for 20 min with a mixture of distilled water:methanol:chloroform (1:2:1; v:v:v). Addition of distilled water:chloroform mixture (1:1; v:v) formed an aqueous-organic two-layers system . Lipids were transferred into the lower chloroform phase improved by centrifugation (3000 rpm, 5 min). Some of the chloroform phase was kept aside, and chloroform mixture was re-added to samples, ultra-sonicated and centrifuged once more to make sure fatty acid were totally transferred into chloroform.

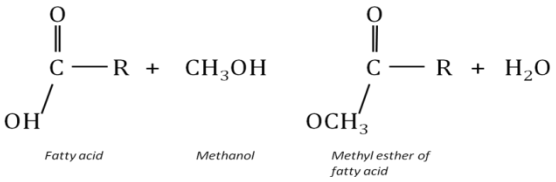
Chloroform was evaporated under a rotary evaporator and the remaining extract (i.e Fatty acids) was once again diluted in chloroform. To separate structural Fatty Acids (FAs) from other organic compounds, the extract was saponified after final evaporation under nitrogen. The saponification (reaction [1]) hydrolyses esterified molecules such as Tryacylglycerols (TAGs) by addition a mixture of NaOH (2N) solution in methanol and distilled water (2:1; v: v) under reflux (1h30, 90°C).

After acidification with ultra pure HCl solution (35%), 1.5 mL of chloroform were added successively to recover the fatty acids which transfer was improved by centrifugation (3000 rpm, 5 min) and partially transferred into another tube. Once more 1.5 mL of chloroform was added and after

centrifugation all chloroform with lipids was transferred in a third tube. The third tube content was evaporated under a N_2 stream and the fatty acids of the total lipids were then converted to methyl esters under reflux with 1 mL BF3-Methanol (14% Borontrifluoride and 86% Methanol) for 10 min at 90°C. Methylation (reaction [2]) consists in add a methyl group to fatty acids by using methanol, producing a fatty acid methyl ester (FAME) and a H_2O molecule and making molecules volatiles and available for Gas Chromatography.



[1] Reaction of Saponification, Rs are carbon chain,



[2] Reaction of Methylation, R is a carbon chain

Total fatty acids (methyled) were washed with chloroform (in two times). After evaporation under a N_2 stream, the extracts were transferred in hexane (200µl).

Fatty acids were then separated and quantified using Gas Chromatography (GC; Varian CP-3800 with flame ionization detector) equipped with a Supelco OMEGAWAX 320 column (30 m x 0.32 mm i.d., 0.25 µm film thickness) using Hydrogen as carrier gas.

Extracts (1µl) in hexane were injected in GC at 60°C. GC oven raised 150°C at 40°Cmin-1, and then 240°C at 3°C min-1. GC gives chromatograms with peaks successions; each refers to a specific fatty acid, given that lighter carbonated chains appear earlier than heavier ones (Figure II.4). Therefore most of fatty acid peaks were identified according to their retention times and comparing with those of standards. Identification were completed or confirmed by Gas Chromatography-Mass Spectrometry (GC-MS; Varian 450-GC).

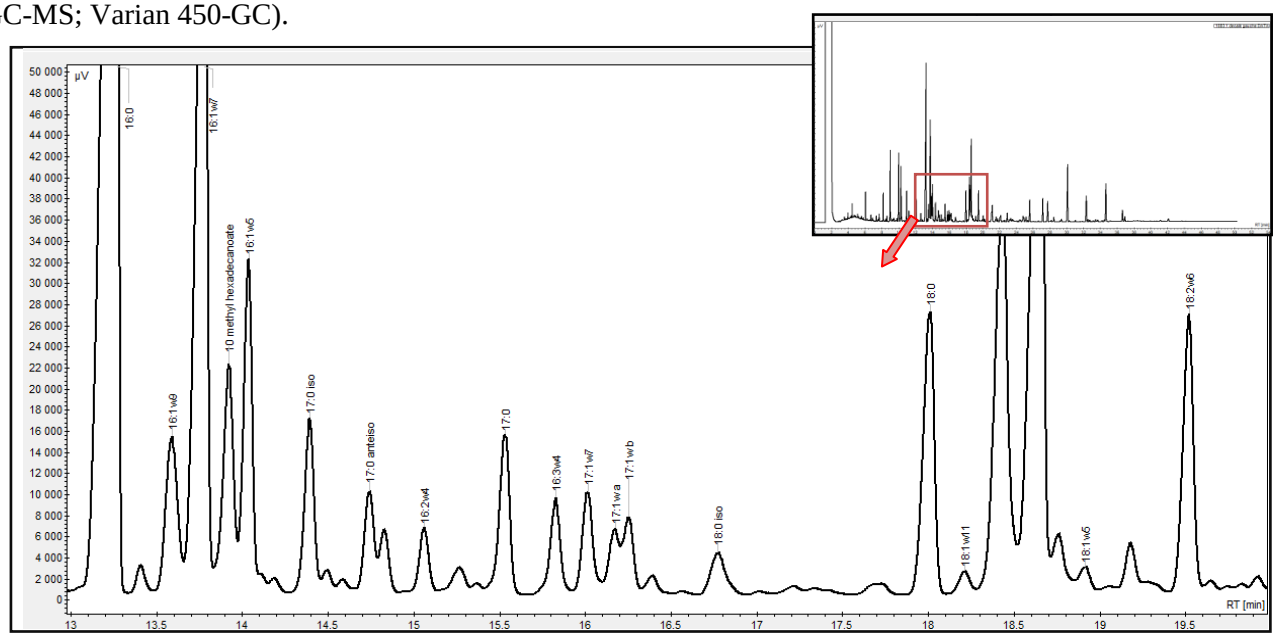


Figure II.4: Example of FA profile for a given sample: lighter FAs are detected firstly by the GC column. Peak intensity of a given FA traduces the quantity of this FA in the sample when comparing with the 23:0 standard (4µl).

Finally, calculation of fatty acid concentration was performed using Schomburg equation (1987) slightly modified.

$C_{FA} = A_S / A_{IS} \times C_{IS} / W_{S,}$ where A_S is the peak area of the FA, A_{IS} the peak area of the internal standard, C_{IS} the concentration of the internal standard (mg) and W_S the dry weight of sample (g). Result is given in $mg.g^{-1}$.

FAs which potentially found can be either saturated or unstaturated when they account for at least one double bond carbon=carbon. Nomenclature of FAs is variable, but in this study the ω-z nomenclature system has been chosen. FAs are designed by the form X:YωZ, where X is the number of carbons, Y the number of double bonds and Z the position of the ultimate double bond from the terminal methyl.

II.3.4. Micro-phythobenthos observations

Micro-phytobenthos was observed and identified using inverted microscopy (Leica DM500). Observation was performed at a magnification of x400. Sediment was diluted x2 and observed on slide. Lens tissues were cleaned through 60µm nylon screen, after products settled down 24h long micro-phytobenthos was observed on decantation slide. Inverted microscopy often does not allow smallest specimen identification (Jacquet et al, 2006). Observation was done by a novice observer (the writer) resulting probably in some misidentifications. In this study, phytobenthos was identified at the branch level, whereas tentative identification at genus and species levels was made.

II.4 STATISTICAL TREATMENTS

Chlorophyll a and phaeopigment concentrations, percentage of total FAs sequences, concentration of selected individual FAs as well as $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures, were statistically compared according to several factors summarized in table II.1 with their diminutives used in this report.

Factors	Levels	Diminu
Period of activity	*1. Non active period*	*NAP*
	2. Active period	*AP*
Vegetation cover	*1. Salptan*	*Sz*
(figure II.1)	*2. Avicennia*	*Az*
	3. Rhizophora inner bay	*Rhi*
	4. Rhizophora outer bay	*Rho*
	5. Rhizophora at the sea front	*RhSF*
	6. Avicennia and Rhizophora mixed	*Av/Rh*
	7.Saltpan and Avicennia and/or	*SVeg*
Distance from effluent	*1. Output zone*	*Oz*
(figure II.3)	*2. Farther (None Output zone)*	*NOz*

Table II.1: Factor and their different levels used for statistical treatments and diminutives associated

Differences in concentrations of chlorophyll-a were tested using a three-way crossed analysis of variance (ANOVA) (Period x Vegetation cover x Distance from effluent discharge). Prior to ANOVA, all data were log (x+1) transformed and tested for homoscedasticity (Bartlett test) and normal distribution of residuals (Shapiro-Wilk). Tukey's HSD Post-hoc tests were then used to determine the differences between groups (as the factor Vegetation included six groups, this test allowed to compare them one another). For phaeopigment data homoscedasticity and normal distribution of residuals condition were not fulfilled. Therefore differences in phaeopigment concentrations were tested using non parametric test (Kruskal-Wallis test), each of the three factors was test one after the other. Wilcoxon Post-hoc tests (pairwise test) were used for multiple comparisons to determine differences between groups. Pearson correlation were used to explore data and identify relationship between chlorophyll-a and pheaopigment concentrations. All these tests were performed using the R version 2.9.0 2009 software and for all tests the probability α was set at 0.05. The initial hypothesis Ho (means of the groups are equals one another) is rejected if the p-values $< \alpha$ i.e at least one group is different from other ones.

The PRIMER 6 software was used for multivariate analysis (Clarke, 1993). The data matrices (% of total FAs sequences) were used to create triangular similarity matrices, based on Bray–Curtis similarity coefficient. All FAs were used in the analyses and no transformation was performed on the data. Differences in FAs composition among factors were tested using separate one-way, and two-way crossed analysis of similarity (ANOSIM) and the statistic test was computed after 5,000 permutations. Where differences in FAs compositions were detected, similarity of percentage (SIMPER) tests, a module of PRIMER 6, were used to determine which FAs drive the observed differences between two sets of data. Here was report FAs which contributed at 60% to difference between groups (cumulated contribution) for sediment samples and at 80 % for migratory micro phytobenthos. Temporal and vegetation variation in FAs composition of sediment sample was displayed using multi-dimensional scaling (MDS) plots based also on Bray-Curtis similarity measures.

Differences in concentrations of selected individual FAs as well as $\delta^{13}C$ and $\delta^{15}N$ stable isotopic signatures vs. factors were tested using one-way analysis of variance (ANOVA) or Kruskal wallis test of variance, and data were compared and explored using Pearson correlation tests. The FAs selected for analysis of variance included the ones which contributed at 60% (80% for migratory phytobenthos) to dissimilarities between groups in term of concentration, identified by way of SIMPER.

To estimate the influence of some isotopic sources to sediment Stable Isotope Analysis in R (SIAR; Parnell et al., 2010) was performed.

II.5. CONTOUR MAP REPRESENTATIONS

Surface maps were used to illustrate spatial variation in the data sets (chlorophyll-a, phaeopigments, selected FAs and $\delta^{13}C$ and $\delta^{15}N$ stable isotope), using Surfer for Windows (Golden Softwer Inc.2002, version 8). The krigging algorithm was employed as the interpolation method with a linear variogram model. The spatial interpolation aims to estimate the value of a function F(X) at a

point Xp (x,y), when knowing some F values at some surrounding point Xi (x,y), and calculate a coefficient (weight which link the position initial and the position interpolated). To that end the method uses the covariance between points Xi in term of the distance between points. To apply the Krigeage method, means and variance of F do not have to be dependent to point positions, but to the distance between points.The Krigeage method only use a semi-variogram for the points Xi and Yi separated by a distance h. The semi-variance according to the distance between points can be plot (P). The method consist in the determination of the combination of weights which guarantees that semi-variance calculated with the target point Xp, will be on the curb given by (P). The weights can be assessed and the search values at Xp are then calculated by using known values of F in (1) (Gratton et al 2002).

CHAPTER III: RESULTS

III.1. PIGMENTS

III.1.1. Chlorophyll a

Chlorophyll a (chl-*a*) concentrations in surface sediments ranged from 1.72 ± 0.2 µg.g^{-1}*dw* to 42.25 ± 9.3 µg.g^{-1}*dw* during the non active period (NAP), and from 2.92 ± 1.62 µg.g^{-1} to 41.56 ± 9.71 µg.g^{-1} during the active period (AP; i.e. of the shrimp farm). Chlorophyll a (chl-*a*) concentrations demonstrated patchiness at sediment surface for both periods (figure III.1a and III.1b).

Chl-*a* concentrations were significantly lower (*F=32.25, p<0.001*) in mangrove sediments during AP (12.06 ± 9.5 µg.g^{-1} in average) than during NAP (16.15 ± 9.15 µg.g^{-1} in average). The decrease mainly occurred within Rhi (*Tuckey's HSD, p<0.001*), whilst within other vegetation stands, no differences were observed between both sampling periods. ANOVA analysis found that at least one vegetation stand significantly differed from others (*F= 16.48, p<0.001*) in term of sediment chl-*a* content at both periods. Seasonal and Vegetation factors interacted (*two-way crossed ANOVA; period activity x vegetation*; *F=2.73, p<0.05*) i.e. differences between vegetation stands were influenced by seasonality differences (i.e. change in farm activity). Chl-*a* concentrations of surface sediments of the output zones (figure II.3) did not differ significantly from those of other sites during NAP as well as during AP.

During NAP highest chl-a concentrations were measured in the sediment at the *Avicennia-Rhizophora* interphase (station (stn) 24; figure III.1a). Another zone of high concentration was measured within *Rhizophora stylosa* sediments (stn 36; 34.01 ± 9.3 µg.g^{-1}*dw*) outer bay from the dyke. Sediment samples collected at the sea front and on saltpan where characterized by lower chl-*a* concentrations (table III.1). Concentrations were significantly higher in the Az, Av/Rh, Rhi and Rho sediments than those measured at the saltpan stations (*Tuckey's HSD, p<0.01* for all) and those situated in the Sea front *(Tuckey's HS; p<0.01* for all).

During AP, maximum chl-*a* concentrations were measured along the K pond in the *Avicennia* stand at the station 16 (see figure III.1b), other high concentrations were found outer bay (station 35 and 36; 25.70 ± 4.55 µg.g^{-1} *dw* in average). Lowest concentrations appeared at the saltpan along the L pond, at the seafront and where the mangrove forest narrows (stn 26, 27 and 38, 39; 4.36 ± 1.42 µg.g^{-1} *dw* in average). However, sediments within the *Avicennia* stand did not have statistically higher concentrations than at other parts of the mangrove. Sediment chl-*a* concentrations were the highest at the *Avicennia -Rhizophora* interphase (*Tukey's HSD, p<0.05* for all comparison with others groups). Secondly, values at Rho were significantly higher than those at the Saltpan (*Tukey's HSD; p<0.01*), Rhi (*Tukey's HSD; p< 0.001*) and RhSF (*Tukey's HSD; p<0.01*).

III.1.2. Phaeopigments

During NAP, phaeopigment (phaeo) ranged from 0.88 ± 0.14 µg.g^{-1} to 63.3 ± 17.19 µg.g^{-1} *dw* and from 0.76 ± 0.39 µg.g^{-1} *dw* to 16.80 ± 2.29 µg.g^{-1} *dw* during AP. Distribution of phaeo concentrations demonstrated a gradient from ponds to lagoon especially during AP (see figure III.1 c and d). At both periods, chl-*a* and phaeopigments were significantly correlated (*Pearson correlation*; *r=0.783, p<0.001, n=179 for NAP; r=0.537, p<0.001, n=182 for AP*).

Phaeo concentrations significantly decreased (*Kruskal-Wallis; χ^2=101.12, p<0.001*) from NAP (14.83 ± 9.47 µg.g^{-1} *dw* in average) to AP (6.18 ± 4.69 µg.g^{-1} *dw* in average). In fact, they decreased in the surface sediment of the Az (χ^2=21.9925, p<0.001), Rhi (χ^2=71.9781, p<0.001), Rho (χ^2=26.8538, p<0.001), RhSF (χ^2=20.9875, p<0.001) and Av/Rh (χ^2=15.6355, p<0.001) but not in the Sz sediment.

During NAP, maximum of phaeo concentrations were measured in the sediment of Az and Av/Rh (stn 15, 23 and 24; figure III.1c) and in the *Rhizosphora* stand (stn 34; 37.04 ± 9.97 µg.g^{-1}*dw*). The lowest concentrations were found in saltpan and RhSF sediment (table III.1; figure III.1c). Similarly, Wilcox analysis found that Az sediments had significantly higher phaeo concentrations than the other groups (i.e.saltpan, Rhi, Rho, and RhSF; *p<0.001 for all*). Sediment phaeo concentrations at the limit between *Avicennia* and *Rhizophora* stands (Av/Rh) were not significantly differentiable either from those of Rhi sediments or from those of Az. Additionally, phaeo concentrations in Rhi sediments were significantly higher (*p<0.01*) than those of Rho which exhibited high concentrations as well.

During AP, maximum concentrations were measured in the sediment along the K pond in the *Avicennia* and *Rhizophora* stands (stn 16, 19 and 34; 15.39 ± 3.90 µg.g^{-1} *dw* in average) and the Sz and the RhSF showed the lowest phaeo concentration (table III.1). Indeed, Wilcox analysis indicates that phaeo concentrations of the Sz were not significantly different than those of the seafront (and of the Rho sediment). Both were significantly lower than values measured in *Avicennia* and *Rhizophora* stand sediments (*p<0.01 for both*) which were not significantly different one another.

Additionally, Kruskal analysis showed that during both NAP and AP, phaeo concentrations in surface sediments of the output zones (Oz) were significantly higher than those measured in sediments of the rest of the studied mangrove (NOz; *χ^2=15.66, p<0.001 for NAP; χ^2=32.66, p<0.001 for AP*).

Chl-a (µg.g^{-1} dw)	NAP	AP	Phaeo (µg.g^{-1} dw)	NAP	AP
Sz	*7.15 ± 3.15*	*8.09+3.77*	**Sz**	*3.30± 2.02*	*3.62±3.83*
Az	*20.48±10.37*	*17.45+15.96*	**Az**	*23.42±11.81*	*7.37±5.66*
Av/Rh	*20.62±9.24*	*17.53+12.27*	**Av/Rh**	*22.63±11.71*	*8.91±5.64*
Rhi	*15.58±7.13*	*8.79+5.87*	**Rhi**	*18.72±6.29*	*7.72±4.09*
Rho	*24.93±7.04*	*17.60+8.58*	**Rho**	*13.99±3.82*	*4.22±2.23*
SVeg	*11.22±5.41*	*14.59+9.74*	**Sveg**	*8.78±4.39*	*4.86±5.57*
RhSF	*11.57±6.28*	*8.09+3.93*	**RhSF**	*7.32±3.53*	*2.74±1.53*
			Oz	*18.36 ± 11.69*	*8.41 ± 5.37*
			NOz	*12.45 ± 6.68*	*4. 44 ± 3.13*

Table III.1: Chl-a and phaeo average concentrations according to sample locations (Vegetation stands or distance from effluent discharges)

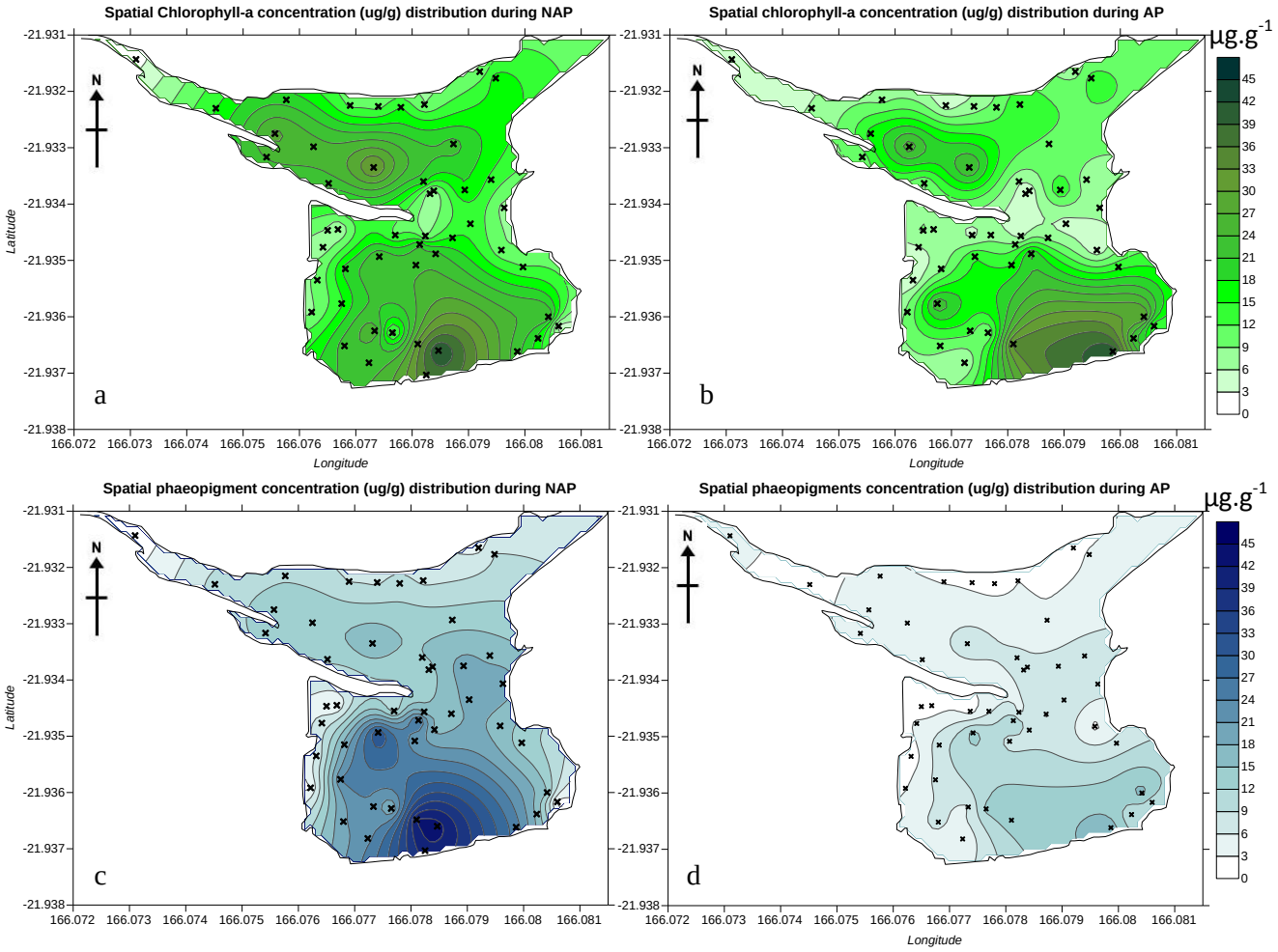


Figure III.1: Maps of benthic chl-a concentrations distribution during NAP (a), AP (b), and of phaeo concentrations during NAP (c) and AP (d).

III.2. FATTY ACIDS

Concentrations of FAs in sediment samples and the contribution of selected (or summed) FAs according to the sampling zones and the studied periods are given in table III.2 and III.3 (following page). Figures III.2a and III.2b presented respectively the contribution and the concentrations of the FA species detected in samples, averaged by period. From 30 to 62 FAs were identified in sediment samples. Identified FAs included long chain fatty acids (LCFAS; ≥24:0), saturated fatty acids (SAFAs; ≥ 11:0-30:0), polyunsaturated fatty acids (PUFAs; e.g. 18:2ω6 and 20:5ω3), monounstaturated fatty acids (MUFAs; e.g. 16:1ω7 and 18:1ω9) and branched fatty acids (BFAs; e.g. *iso*-15:0 and *anteiso*-15:0). Figures III.6 to III.14 illustrate the absolute concentrations distribution of selected (or summed) FAs in the surface sediment of the FAO mangrove.

ug.g^{-1}	NAP																					
	Sz			Az			Av/Rh			Rhi			Rho			RhSF			SVeg		
FAs names	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**
11:0	0,0	±	0,1	0,0	±	0,1	0,1	±	0,2	0,1	±	0,2	0,0	±	0,0	0,2	±	0,2	0,1	±	0,1
12:0	2,2	±	1,0	3,0	±	0,6	4,3	±	1,0	6,2	±	1,8	4,9	±	1,7	2,3	±	1,1	2,8	±	1,0
13:0	0,5	±	0,1	1,0	±	0,2	1,5	±	0,4	1,9	±	0,6	1,6	±	0,5	0,6	±	0,3	1,0	±	0,3
14:0	6,9	±	3,7	17,7	±	5,8	24,0	±	9,6	22,4	±	12,7	20,2	±	6,8	8,9	±	4,2	13,1	±	5,6
15:0	4,8	±	4,5	18,6	±	5,6	19,6	±	10,5	13,4	±	10,5	26,1	±	5,7	5,2	±	2,6	18,5	±	11,7
16:0	43,1	±	21,1	82,5	±	29,1	113,8	±	50,3	83,4	±	42,2	86,5	±	28,0	36,9	±	16,3	57,1	±	25,2
17:0	2,4	±	0,9	5,8	±	1,4	8,0	±	2,4	7,2	±	2,4	7,4	±	2,2	2,3	±	1,1	5,1	±	2,7
18:0	5,7	±	1,6	10,3	±	2,8	14,3	±	4,3	14,4	±	4,5	11,8	±	4,5	6,0	±	2,5	7,9	±	3,6
19:0	0,1	±	0,2	0,2	±	0,2	0,2	±	0,3	0,2	±	0,3	0,2	±	0,3	0,2	±	0,2	0,2	±	0,2
20:0	1,6	±	0,8	3,7	±	1,2	4,7	±	1,2	4,6	±	1,2	3,4	±	1,2	1,4	±	0,7	2,7	±	1,3
21:0	0,0	±	0,0	0,0	±	0,0	0,1	±	0,2	0,1	±	0,4	0,1	±	0,3	0,4	±	0,3	0,3	±	0,5
22:0	3,7	±	2,3	8,0	±	2,9	12,2	±	3,8	16,3	±	5,7	9,8	±	5,3	3,3	±	2,0	6,3	±	3,2
Σ SAFA	**70,8**	**±**	**34,0**	**150,8**	**±**	**44,5**	**202,9**	**±**	**74,2**	**170,2**	**±**	**78,5**	**172,1**	**±**	**53,4**	**67,6**	**±**	**29,4**	**115,0**	**±**	**53,0**
24:0	5,2	±	2,3	18,0	±	5,8	23,8	±	6,8	25,6	±	8,2	19,9	±	7,8	7,8	±	4,9	13,3	±	6,4
25:0	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0
26:0	5,4	±	3,6	9,3	±	2,0	11,2	±	2,2	20,5	±	8,0	17,7	±	7,3	8,0	±	5,7	8,7	±	4,5
28:0	7,4	±	5,9	5,0	±	1,2	5,2	±	0,8	12,1	±	5,3	9,2	±	5,2	4,8	±	3,8	5,9	±	4,1
30:0	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0
Σ LCFA	**18,1**	**±**	**11,7**	**32,3**	**±**	**8,0**	**40,1**	**±**	**8,7**	**58,2**	**±**	**20,4**	**46,8**	**±**	**19,5**	**20,6**	**±**	**14,1**	**27,9**	**±**	**12,7**
12:0iso	0,1	±	0,2	0,1	±	0,2	0,2	±	0,2	0,3	±	0,3	0,0	±	0,0	0,1	±	0,1	0,0	±	0,0
13:0iso	0,1	±	0,2	0,5	±	0,1	0,8	±	0,3	1,0	±	0,2	0,6	±	0,3	0,3	±	0,2	0,4	±	0,2
13:0anteiso	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0	0,2	±	0,3	0,0	±	0,0	0,0	±	0,1	0,0	±	0,0
14:0iso	1,0	±	0,6	3,0	±	1,1	5,1	±	1,9	7,5	±	2,4	4,1	±	1,8	1,7	±	1,0	2,6	±	1,1
15:0iso	6,2	±	3,2	11,9	±	4,6	17,7	±	5,9	21,9	±	5,7	13,1	±	6,2	5,8	±	3,0	8,7	±	4,4
15:0anteiso	2,8	±	2,4	10,1	±	4,5	14,1	±	4,2	19,0	±	4,8	10,2	±	4,6	4,3	±	2,3	7,1	±	3,8
10Me	2,4	±	1,6	3,7	±	1,3	6,7	±	1,6	8,8	±	1,8	7,0	±	2,2	4,0	±	2,3	3,3	±	1,7
16:0iso	2,4	±	1,1	4,5	±	1,4	6,3	±	1,4	7,6	±	1,5	4,9	±	1,8	2,3	±	1,2	3,5	±	1,6
17:0iso	2,1	±	0,8	3,3	±	1,4	5,8	±	1,9	5,7	±	1,8	4,0	±	1,6	2,1	±	1,3	2,7	±	1,3
17:0anteiso	1,5	±	0,5	2,5	±	0,9	3,2	±	0,8	3,8	±	0,8	2,3	±	0,8	1,0	±	0,5	1,7	±	0,9
18:0iso	0,4	±	0,1	1,1	±	0,8	2,5	±	1,7	2,3	±	1,3	1,2	±	0,9	0,7	±	0,4	0,9	±	0,6
Σ BFA	**19,1**	**±**	**10,4**	**40,6**	**±**	**15,9**	**62,6**	**±**	**19,0**	**78,0**	**±**	**18,6**	**47,5**	**±**	**19,8**	**22,4**	**±**	**12,2**	**30,8**	**±**	**15,1**
12:1ω7	0,2	±	0,2	0,3	±	0,2	0,7	±	0,3	0,9	±	0,3	0,6	±	0,4	0,2	±	0,2	0,3	±	0,2
14:1ω3	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0
14:1ω5	0,4	±	0,2	0,6	±	0,2	0,8	±	0,3	0,9	±	0,3	0,7	±	0,3	0,2	±	0,3	0,4	±	0,2
15:1ω1	0,0	±	0,0	0,1	±	0,2	0,7	±	0,8	0,8	±	0,7	1,0	±	0,5	0,1	±	0,2	0,1	±	0,3
16:1ω5	0,2	±	0,2	0,3	±	0,7	3,4	±	4,3	5,6	±	3,3	1,8	±	2,3	0,3	±	0,5	0,1	±	0,3
16:1ω7	0,2	±	0,1	4,0	±	4,9	24,8	±	28,0	30,9	±	15,2	15,4	±	16,5	2,4	±	2,8	3,2	±	4,1
16:1ω9	0,1	±	0,2	0,5	±	0,3	4,2	±	5,2	5,0	±	3,8	2,0	±	2,6	0,6	±	0,9	0,7	±	0,5
17:1ω7	0,0	±	0,0	0,6	±	0,6	3,3	±	3,1	3,8	±	3,4	2,6	±	2,7	0,2	±	0,2	0,9	±	1,2
17:1ωa	1,0	±	0,8	1,7	±	0,7	3,6	±	3,2	3,6	±	1,1	2,1	±	1,3	1,1	±	0,9	1,0	±	0,6
17:1ωb	0,0	±	0,0	0,0	±	0,0	0,9	±	1,0	1,8	±	0,8	0,8	±	1,0	0,1	±	0,1	0,2	±	0,2
18:1ω11	0,0	±	0,0	0,1	±	0,2	0,5	±	0,6	0,9	±	0,3	0,5	±	0,5	0,0	±	0,1	0,1	±	0,2
18:1ω5	0,0	±	0,1	0,0	±	0,0	0,4	±	0,6	0,7	±	0,4	0,3	±	0,3	0,0	±	0,0	0,1	±	0,2
18:1ω7	0,7	±	0,3	2,4	±	2,5	14,1	±	15,1	34,8	±	15,9	11,2	±	13,3	2,7	±	3,5	2,0	±	1,7
18:1ω9	0,0	±	0,0	1,4	±	1,8	9,0	±	10,1	14,4	±	7,3	6,1	±	6,9	1,3	±	1,6	1,3	±	1,6
19:1ω9	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0	0,1	±	0,2	0,2	±	0,4	0,0	±	0,0	0,0	±	0,0
20:1ω11	0,0	±	0,0	0,1	±	0,2	0,9	±	0,5	1,7	±	0,5	0,9	±	0,6	0,2	±	0,3	0,2	±	0,2
20:1ω7	0,3	±	0,1	0,1	±	0,2	0,2	±	0,4	0,7	±	0,8	0,1	±	0,2	0,1	±	0,2	0,1	±	0,1
20:1ω9	0,0	±	0,0	0,0	±	0,0	0,6	±	0,8	1,0	±	0,7	0,3	±	0,5	0,1	±	0,2	0,0	±	0,0
22:1ω9	1,1	±	1,0	0,4	±	0,2	0,9	±	0,5	0,6	±	0,4	0,8	±	0,5	0,0	±	0,0	0,4	±	0,2
24:1ω9	0,2	±	0,2	0,7	±	0,4	0,6	±	0,6	0,5	±	0,8	0,6	±	0,7	0,2	±	0,3	0,5	±	0,5
Σ MUFA	**4,4**	**±**	**2,0**	**13,2**	**±**	**11,5**	**69,7**	**±**	**72,4**	**108,6**	**±**	**52,7**	**48,2**	**±**	**49,1**	**9,8**	**±**	**11,1**	**11,6**	**±**	**10,4**
16:2ω4	0,1	±	0,1	0,1	±	0,2	1,0	±	1,3	1,3	±	0,8	0,6	±	0,7	0,0	±	0,1	0,0	±	0,0
16:2ω6	0,0	±	0,0	0,1	±	0,2	1,4	±	2,2	1,9	±	6,6	0,4	±	0,7	0,3	±	0,6	0,0	±	0,0
16:3ω3	0,1	±	0,1	0,0	±	0,0	0,0	±	0,0	0,3	±	0,9	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0
16:3ω4	0,0	±	0,1	0,1	±	0,2	1,7	±	2,1	1,9	±	1,3	1,0	±	1,5	0,2	±	0,3	0,1	±	0,2
17:2ω5	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0	0,2	±	0,4	0,0	±	0,0	0,0	±	0,0	0,0	±	0,0
18:2ω6	0,0	±	0,0	0,2	±	0,5	4,8	±	6,6	5,3	±	4,1	1,9	±	2,7	0,3	±	0,6	0,1	±	0,3
18:3ω3	4,6	±	2,6	3,7	±	1,4	8,7	±	7,0	6,5	±	4,2	4,4	±	2,8	1,6	±	1,0	2,3	±	1,5
18:3ω6	2,1	±	3,6	6,2	±	4,2	5,7	±	3,2	3,8	±	5,0	6,5	±	2,0	3,3	±	2,3	4,5	±	4,0
20:3ω6	0,6	±	1,0	1,6	±	0,7	1,8	±	0,7	2,1	±	0,8	2,1	±	0,9	1,9	±	1,1	1,4	±	1,0
20:4ω3	0,7	±	0,2	0,9	±	0,6	1,4	±	0,5	0,2	±	0,5	0,5	±	0,5	0,2	±	0,2	0,6	±	0,3
20:4ω6	0,0	±	0,0	0,5	±	0,6	6,2	±	8,3	9,0	±	15,3	3,4	±	5,0	0,8	±	1,0	0,5	±	0,5
20:5ω3	0,0	±	0,0	0,4	±	0,7	7,3	±	9,5	6,8	±	6,0	3,4	±	5,3	0,3	±	0,5	0,2	±	0,4
22:6ω3	0,3	±	0,2	0,3	±	0,2	0,7	±	0,8	1,3	±	0,8	0,4	±	0,9	0,0	±	0,0	0,0	±	0,1
Σ PUFA	**8,5**	**±**	**7,3**	**14,0**	**±**	**6,2**	**40,7**	**±**	**37,6**	**40,5**	**±**	**44,1**	**24,5**	**±**	**19,1**	**9,0**	**±**	**5,5**	**9,7**	**±**	**6,5**
MTAD	1,0	±	0,8	1,8	±	1,5	2,1	±	1,0	1,7	±	0,8	1,3	±	0,6	0,4	±	0,2	1,0	±	0,6
TOTAL Fas	**121,7**	**±**	**62,2**	**252,7**	**±**	**79,8**	**418,1**	**±**	**204,1**	**457,2**	**±**	**200,2**	**340,5**	**±**	**144,7**	**129,7**	**±**	**65,2**	**196,0**	**±**	**94,6**
16:1ω7/16:0	0,0	±	0,0	0,0	±	0,1	0,2	±	0,2	0,4	±	0,1	0,2	±	0,2	0,1	±	0,1	0,0	±	0,0
Σ odd-branched	20,4	±	11,9	54,0	±	15,2	71,1	±	20,3	74,5	±	23,7	65,6	±	20,0	22,0	±	10,2	45,5	±	24,6
Σ 18:3ω6+20:3ω6	2,7	±	4,6	7,8	±	4,9	7,4	±	3,9	5,9	±	5,6	8,6	±	2,6	5,2	±	3,1	5,9	±	5,0
%SAFA	58,8	±	3,9	60,2	±	4,0	50,9	±	7,6	37,6	±	5,7	53,3	±	9,2	57,1	±	13,0	60,2	±	4,9
%LCFA	13,9	±	3,8	13,2	±	2,7	11,0	±	3,6	13,2	±	3,4	14,2	±	3,0	14,7	±	4,7	14,5	±	1,9
%BFA	16,5	±	6,1	15,8	±	2,2	16,5	±	4,4	18,0	±	2,8	14,0	±	0,4	15,2	±	6,2	15,2	±	3,2
%MUFA	3,7	±	0,4	4,6	±	3,1	13,0	±	10,3	23,1	±	5,5	11,5	±	8,7	6,4	±	4,6	5,1	±	2,5
%PUFA	6,4	±	2,6	5,5	±	2,2	8,0	±	4,3	7,7	±	3,3	6,5	±	2,7	6,0	±	3,3	4,5	±	1,9
%16:0	35,6	±	5,0	32,3	±	4,0	27,8	±	4,1	18,4	±	3,4	26,8	±	5,1	32,0	±	9,2	30,3	±	3,7
%16:1ω7	0,2	±	0,1	1,3	±	1,5	4,5	±	4,2	6,5	±	1,8	3,6	±	3,1	1,7	±	1,3	1,3	±	1,2
%16:1ω7/16:0	0,0	±	0,0	0,0	±	0,1	0,2	±	0,2	0,4	±	0,1	0,2	±	0,2	0,1	±	0,1	0,0	±	0,0
%18:1ω7	0,6	±	0,2	0,8	±	0,7	2,6	±	2,2	7,4	±	2,0	2,6	±	2,4	1,5	±	1,6	0,8	±	0,5
%18:1ω9	0,0	±	0,0	0,4	±	0,6	1,6	±	1,5	3,1	±	0,9	1,4	±	1,3	0,8	±	0,8	0,5	±	0,5
%18:2ω6	0,0	±	0,0	0,1	±	0,2	0,8	±	1,0	1,1	±	0,4	0,4	±	0,5	0,2	±	0,3	0,0	±	0,1
%20:5ω3	0,0	±	0,0	0,1	±	0,2	1,2	±	1,4	1,3	±	0,7	0,7	±	1,0	0,1	±	0,2	0,1	±	0,1
% Σ (18:3ω6+20:3ω6)	1,5	±	2,4	3,3	±	2,2	1,9	±	0,9	1,2	±	0,4	2,8	±	0,9	3,8	±	2,8	2,7	±	2,0
% Σ odd-Branched	17,1	±	5,5	21,8	±	3,0	18,6	±	4,5	16,9	±	2,2	20,4	±	3,3	16,1	±	3,9	22,7	±	2,6

Table III.2 : Fatty acid (FA) concentrations for the zone defined, and proportion of selected or added FAs, for the Non Active Period of the shrimp farm

ug.g^{-1}	AP																							
	Sz			Az			Av/Rh			Rhi			Rho			RhSF			SVeg					
FAs names	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**			
11:0	1,2	±	1,0	1,2	±	1,1	1,3	±	0,1	0,9	±	0,5	0,8	±	0,3	0,5	±	0,1	1,3	±	1,9			
12:0	4,6	±	1,6	8,6	±	4,5	11,3	±	1,8	13,0	±	7,4	7,6	±	1,5	5,7	±	1,8	9,0	±	5,2			
13:0	1,5	±	0,7	3,2	±	2,6	3,6	±	0,6	4,1	±	1,8	2,7	±	0,5	1,5	±	0,6	2,8	±	2,4			
14:0	21,8	±	10,8	37,5	±	19,6	46,8	±	7,2	44,2	±	21,4	32,8	±	8,0	22,0	±	7,2	35,8	±	25,6			
15:0	25,2	±	16,7	43,3	±	34,3	44,8	±	1,9	17,9	±	11,3	30,4	±	8,7	9,1	±	1,9	30,2	±	32,2			
16:0	97,3	±	54,0	163,9	±	102,1	175,4	±	29,5	151,0	±	66,1	134,0	±	38,6	88,2	±	29,5	142,4	±	120,2			
17:0	8,8	±	5,8	14,3	±	10,1	14,2	±	1,8	12,6	±	5,9	11,6	±	3,5	5,4	±	1,8	12,1	±	10,1			
18:0	13,7	±	6,2	26,4	±	15,0	24,6	±	3,8	28,5	±	11,5	19,8	±	4,7	14,8	±	3,8	22,4	±	16,8			
19:0	0,9	±	0,3	1,4	±	0,5	1,4	±	0,3	1,6	±	0,5	1,2	±	0,3	1,0	±	0,3	1,4	±	0,7			
20:0	3,5	±	2,0	7,4	±	4,7	6,8	±	1,3	8,5	±	3,7	5,0	±	1,6	3,0	±	1,3	6,3	±	5,3			
21:0	0,6	±	0,5	1,2	±	0,7	1,3	±	0,3	1,7	±	1,0	1,0	±	0,4	0,6	±	0,3	1,2	±	1,0			
22:0	8,1	±	5,9	15,0	±	10,6	15,6	±	3,9	22,3	±	9,5	14,1	±	6,4	7,0	±	3,9	17,3	±	16,1			
Σ SAFA	**187,2**	**±**	**101,9**	**323,4**	**±**	**199,1**	**347,1**	**±**	**48,0**	**306,1**	**±**	**133,1**	**260,9**	**±**	**70,4**	**158,6**	**±**	**48,0**	**282,3**	**±**	**233,1**			
24:0	12,0	±	12,5	24,8	±	18,3	24,9	±	6,0	28,4	±	8,4	25,5	±	10,8	11,5	±	6,0	23,7	±	21,0			
25:0	1,5	±	0,9	2,1	±	1,3	2,1	±	0,6	2,4	±	0,7	2,1	±	0,9	1,0	±	0,6	1,9	±	1,7			
26:0	7,2	±	5,5	11,1	±	8,8	13,2	±	6,0	20,7	±	9,6	23,2	±	11,5	9,7	±	6,0	12,4	±	10,3			
28:0	5,7	±	3,3	6,5	±	6,9	6,2	±	3,3	9,7	±	7,1	10,5	±	5,0	4,4	±	3,3	6,4	±	6,7			
30:0	0,0	±	0,2	0,1	±	0,1	0,0	±	0,1	0,1	±	0,2	0,2	±	0,3	0,0	±	0,1	0,1	±	0,3			
Σ LCFA	**26,4**	**±**	**21,9**	**44,6**	**±**	**34,4**	**46,3**	**±**	**15,7**	**61,3**	**±**	**22,9**	**61,5**	**±**	**27,4**	**26,7**	**±**	**15,7**	**44,6**	**±**	**38,9**			
12:0iso	0,4	±	0,4	1,1	±	0,9	1,7	±	0,3	2,1	±	1,4	1,0	±	0,3	0,6	±	0,3	1,2	±	1,1			
13:0iso	0,9	±	0,4	1,6	±	0,9	2,3	±	0,4	2,7	±	1,2	1,4	±	0,5	0,9	±	0,4	1,6	±	1,2			
13:0anteiso	0,2	±	0,3	0,6	±	0,5	0,8	±	0,2	1,0	±	0,6	0,5	±	0,2	0,4	±	0,2	0,4	±	0,5			
14:0iso	5,5	±	3,1	11,5	±	6,5	14,9	±	2,7	17,6	±	7,6	9,6	±	2,7	6,6	±	2,7	11,2	±	8,7			
15:0iso	15,7	±	7,5	28,7	±	18,1	38,2	±	9,1	50,3	±	23,0	25,2	±	7,4	18,2	±	9,1	29,5	±	24,1			
15:0anteiso	12,2	±	8,1	28,5	±	20,1	36,4	±	8,3	49,3	±	23,8	23,0	±	7,1	15,7	±	8,3	29,5	±	25,2			
10Me	6,3	±	2,0	10,7	±	5,9	17,2	±	4,9	22,4	±	7,4	14,1	±	3,7	11,7	±	4,9	11,7	±	6,7			
16:0iso	6,4	±	2,8	11,7	±	6,8	13,9	±	2,9	17,5	±	7,5	9,9	±	2,7	6,8	±	2,9	11,3	±	8,5			
17:0iso	5,2	±	2,2	8,3	±	5,1	10,7	±	2,0	11,7	±	4,9	7,6	±	2,1	5,1	±	2,0	8,5	±	6,5			
17:0anteiso	3,8	±	1,5	6,5	±	4,1	7,4	±	1,6	9,7	±	4,0	5,0	±	1,5	3,5	±	1,6	6,3	±	5,2			
18:0iso	2,2	±	1,3	4,6	±	3,2	4,8	±	0,6	4,1	±	1,8	3,3	±	0,9	2,0	±	0,6	4,0	±	3,4			
Σ BFA	**58,7**	**±**	**28,7**	**114,0**	**±**	**70,9**	**148,3**	**±**	**32,1**	**188,5**	**±**	**80,3**	**100,5**	**±**	**28,4**	**71,6**	**±**	**32,1**	**115,1**	**±**	**90,1**			
12:1ω7	0,3	±	0,3	0,6	±	0,4	0,7	±	0,6	1,0	±	0,6	0,5	±	0,2	0,4	±	0,6	0,5	±	0,5			
14:1ω3	0,5	±	0,5	0,8	±	0,5	1,2	±	0,3	1,1	±	0,3	1,0	±	0,4	0,5	±	0,3	0,9	±	0,7			
14:1ω5	1,2	±	0,6	1,9	±	0,8	2,3	±	0,5	3,0	±	1,3	1,7	±	0,5	1,1	±	0,5	1,9	±	1,3			
15:1ω1	3,0	±	1,8	5,2	±	3,6	5,7	±	0,3	2,8	±	1,4	4,1	±	1,4	1,1	±	0,3	3,6	±	3,8			
16:1ω5	6,9	±	2,6	11,5	±	7,1	19,5	±	3,0	20,0	±	9,8	11,5	±	3,6	6,9	±	3,0	13,4	±	10,4			
16:1ω7	60,0	±	37,4	87,5	±	56,5	93,5	±	14,4	79,5	±	33,7	81,4	±	31,9	42,4	±	14,4	71,9	±	47,3			
16:1ω9	8,3	±	5,3	16,6	±	13,3	19,9	±	2,4	13,2	±	6,8	11,1	±	2,4	6,9	±	2,4	14,4	±	16,3			
17:1ω7	11,3	±	8,0	22,3	±	22,1	21,3	±	0,9	8,5	±	5,3	14,3	±	4,5	3,5	±	0,9	14,9	±	17,8			
17:1ωa	2,2	±	0,9	4,7	±	3,3	5,3	±	1,6	8,0	±	3,1	3,8	±	1,5	2,5	±	1,6	3,9	±	3,2			
17:1ωb	3,3	±	2,5	5,7	±	4,1	6,8	±	1,1	6,6	±	2,8	5,6	±	1,7	2,5	±	1,1	5,4	±	4,5			
18:1ω11	0,9	±	0,4	1,4	±	0,8	1,7	±	0,2	1,6	±	0,6	1,4	±	0,3	0,8	±	0,2	1,4	±	0,9			
18:1ω5	0,9	±	0,5	1,5	±	1,2	1,8	±	0,3	2,2	±	0,9	1,6	±	0,5	0,9	±	0,3	1,6	±	1,5			
18:1ω7	26,1	±	14,7	56,0	±	43,4	71,1	±	16,6	92,6	±	39,3	50,8	±	15,9	35,5	±	16,6	56,5	±	48,1			
18:1ω9	20,3	±	8,2	34,3	±	20,0	36,3	±	6,0	36,5	±	16,0	27,6	±	7,8	22,2	±	6,0	29,9	±	20,6			
19:1ω9	8,3	±	3,1	10,6	±	6,1	12,2	±	1,6	9,4	±	4,2	7,3	±	2,6	3,1	±	1,6	9,3	±	7,5			
20:1ω11	1,2	±	0,6	1,5	±	0,9	2,1	±	0,8	3,8	±	1,8	2,4	±	0,6	1,6	±	0,8	1,6	±	1,1			
20:1ω7	1,1	±	0,5	2,1	±	1,4	1,8	±	0,4	2,9	±	2,0	1,6	±	0,4	1,1	±	0,4	1,9	±	1,5			
20:1ω9	1,3	±	0,5	2,9	±	2,2	2,6	±	0,5	3,2	±	1,0	2,4	±	0,8	1,9	±	0,5	3,0	±	3,4			
22:1ω9	0,3	±	0,6	1,1	±	0,8	0,9	±	0,1	0,4	±	0,4	0,3	±	0,1	0,2	±	0,1	0,7	±	1,1			
24:1ω9	0,6	±	1,2	2,4	±	2,2	2,0	±	0,2	0,7	±	0,6	0,6	±	0,2	0,3	±	0,2	1,2	±	1,7			
Σ MUFA	**158,3**	**±**	**86,2**	**270,6**	**±**	**182,4**	**308,7**	**±**	**43,5**	**297,0**	**±**	**119,7**	**230,9**	**±**	**67,8**	**135,4**	**±**	**43,5**	**238,0**	**±**	**188,9**			
16:2ω4	2,5	±	1,1	3,5	±	1,9	4,0	±	0,6	2,8	±	1,3	3,4	±	0,9	1,9	±	0,6	3,6	±	2,8			
16:2ω6	1,2	±	0,8	1,9	±	1,5	4,3	±	1,6	1,2	±	0,9	3,1	±	1,1	2,3	±	1,6	2,0	±	1,3			
16:3ω3	0,0	±	0,0	0,8	±	2,6	2,6	±	2,3	0,2	±	1,1	0,1	±	0,4	2,9	±	2,3	0,1	±	0,2			
16:3ω4	3,4	±	1,8	4,8	±	2,6	6,2	±	0,8	3,6	±	2,5	6,2	±	1,7	3,0	±	0,8	5,8	±	3,2			
17:2ω5	0,9	±	0,7	2,2	±	2,5	1,4	±	0,2	0,7	±	1,0	1,1	±	0,3	0,5	±	0,2	1,2	±	1,8			
18:2ω6	5,1	±	4,6	12,6	±	9,8	16,8	±	6,7	18,0	±	14,2	15,8	±	9,0	16,1	±	6,7	13,9	±	12,1			
18:3ω3	2,1	±	1,4	4,4	±	3,6	5,7	±	3,6	7,7	±	6,5	5,1	±	2,6	6,6	±	3,6	5,5	±	6,3			
18:3ω6	1,5	±	1,0	2,5	±	1,7	3,1	±	1,5	1,3	±	0,8	2,3	±	1,1	2,1	±	1,5	4,0	±	8,8			
20:3ω6	0,4	±	0,8	1,1	±	0,8	1,3	±	0,2	1,1	±	0,6	1,2	±	0,4	0,7	±	0,2	1,1	±	0,9			
20:4ω3	0,2	±	0,4	0,9	±	0,9	0,8	±	0,8	3,4	±	3,1	1,1	±	0,5	1,0	±	0,8	1,2	±	1,4			
20:4ω6	7,3	±	5,8	14,1	±	10,2	14,4	±	4,0	10,5	±	4,3	18,5	±	6,6	10,4	±	4,0	14,8	±	9,1			
20:5ω3	13,1	±	7,7	19,1	±	11,8	20,8	±	4,0	9,9	±	7,3	21,2	±	6,4	11,9	±	4,0	19,1	±	13,3			
22:6ω3	1,6	±	0,9	2,2	±	1,6	2,1	±	0,5	1,8	±	1,1	2,1	±	0,7	1,6	±	0,5	2,1	±	1,6			
Σ PUFA	**39,1**	**±**	**26,2**	**70,0**	**±**	**47,1**	**83,5**	**±**	**17,3**	**62,3**	**±**	**34,0**	**81,4**	**±**	**24,5**	**61,0**	**±**	**17,3**	**74,4**	**±**	**59,7**			
MTAD	2,5	±	1,0	4,2	±	3,2	3,3	±	0,8	3,7	±	1,6	3,0	±	1,0	1,5	±	0,8	2,5	±	2,5			
TOTAL FAs	**472,3**	**±**	**262,0**	**826,8**	**±**	**526,4**	**937,2**	**±**	**137,9**	**918,9**	**±**	**356,9**	**738,1**	**±**	**206,2**	**454,7**	**±**	**137,9**	**756,9**	**±**	**605,4**			
16:1ω7/16:0	0,6	±	0,1	0,5	±	0,1	0,5	±	0,1	0,5	±	0,1	0,6	±	0,1	0,5	±	0,1	0,6	±	0,1			
Σ odd-branched	75,4	±	42,0	137,5	±	93,7	161,1	±	24,9	161,7	±	72,4	109,3	±	27,4	61,2	±	24,9	123,6	±	107,2			
Σ 18:3ω6+20:3ω6	1,9	±	1,7	3,6	±	2,5	4,5	±	1,6	2,4	±	1,2	3,5	±	1,5	2,8	±	1,6	5,1	±	9,4			
%SAFA	39,8	±	1,4	39,9	±	1,9	35,5	±	2,7	32,9	±	1,8	35,6	±	2,0	35,0	±	2,7	37,6	±	3,0			
%LCFA	5,3	±	1,9	5,4	±	2,1	6,2	±	2,5	7,7	±	4,3	8,1	±	2,4	5,8	±	2,5	5,6	±	1,4			
%BFA	12,8	±	1,3	14,0	±	1,4	16,3	±	3,1	20,4	±	2,0	13,7	±	1,6	15,1	±	3,1	14,7	±	2,6			
%MUFA	33,6	±	1,6	32,0	±	2,2	32,9	±	1,9	32,1	±	1,8	31,3	±	1,5	29,7	±	1,9	31,9	±	1,2			
%PUFA	7,9	±	1,0	8,2	±	0,8	8,7	±	3,8	6,6	±	1,8	11,0	±	1,4	14,1	±	3,8	10,0	±	1,2			
%16:0	20,6	±	1,3	20,2	±	1,2	17,6	±	2,5	16,2	±	1,2	18,2	±	1,3	19,4	±	2,3	19,1	±	2,1			
%16:1ω7	12,2	±	1,7	10,8	±	1,5	9,1	±	2,4	8,6	±	1,5	11,0	±	2,0	9,4	±	1,7	10,7	±	2,7			
%16:1ω7/16:0	0,6	±	0,1	0,5	±	0,1	0,5	±	0,1	0,5	±	0,1	0,6	±	0,1	0,5	±	0,1	0,6	±	0,1			
%18:1ω7	5,5	±	0,6	6,0	±	1,4	7,9	±	2,3	10,0	±	0,9	6,8	±	0,8	7,5	±	1,8	7,0	±	1,2			
%18:1ω9	4,7	±	0,8	4,4	±	0,8	4,1	±	0,7	4,0	±	0,5	3,8	±	0,6	5,0	±	0,7	4,2	±	0,5			
%18:2ω6	0,9	±	0,3	1,3	±	0,4	1,8	±	0,7	1,8	±	0,8	2,1	±	1,0	3,6	±	1,1	1,7	±	0,5			
%20:5ω3	2,7	±	0,3	2,4	±	0,3	2,1	±	0,8	1,1	±	0,6	2,9	±	0,6	2,9	±	1,4	2,8	±	0,6			
% Σ (18:3ω6+20:3ω6)	0,3	±	0,1	0,4	±	0,1	0,4	±	0,2	0,3	±	0,1	0,5	±	0,1	0,6	±	0,2	0,5	±	0,3			
% Σ odd-branched	15,8	±	2,1	16,1	±	1,5	16,9	±	3,2	17,3	±	1,3	14,9	±	0,8	13,1	±	2,0	15,4	±	1,9			

Table III.3 : Fatty acid (FA) concentrations for the zone defined, and proportion of selected or added FAs, for the Active Period of the shrimp farm

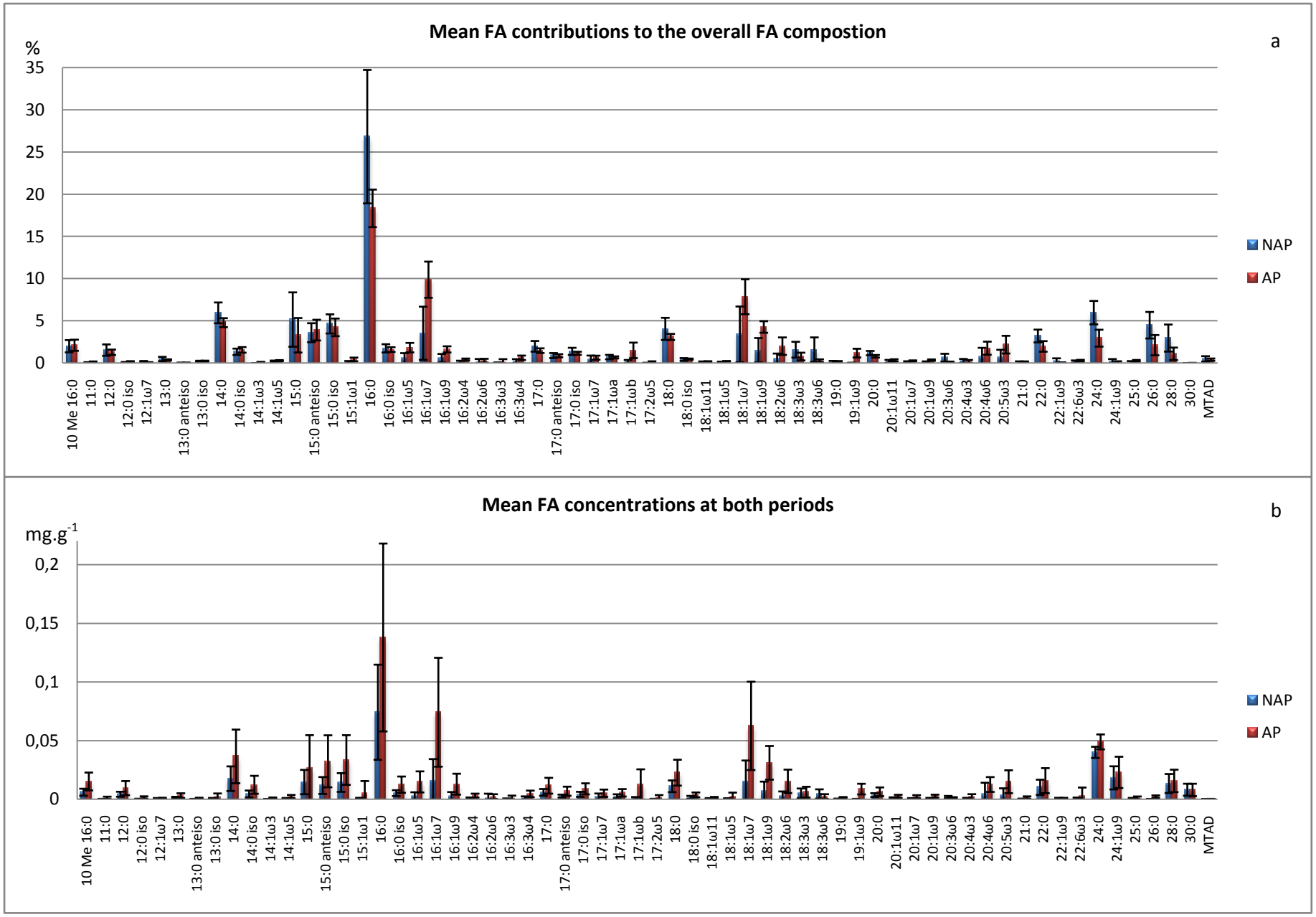


Figure III.2: Profiles of (a) the mean contributions of the FAs detected to the total FA composition of the surface sediment and of (b) their mean concentrations. These values are an average of the 51 stations.

III.2.1. Effluent and food FA composition

Completes FA composition of the granule given to shrimp in pond and of the effluent are given in Appendix F. Food was mainly composed of the FA 18:2ω6 (42.08 ± 1.3 %), 18:1ω9 (10.50 ± 4.0 %) and 16:0 (18.62 ± 1.0%). Effluent waters were mostly composed of the FAs 16:0 (22.1 ± 1.2%), 18:1ω9 (15.3 ± 5.4%), 16:1ω7 (10.0 ± 5.0%), and notably of the 18:2ω6 (3.8 ± 1.3%).

III.2.2. Temporal variation of mangrove sediment FAs composition

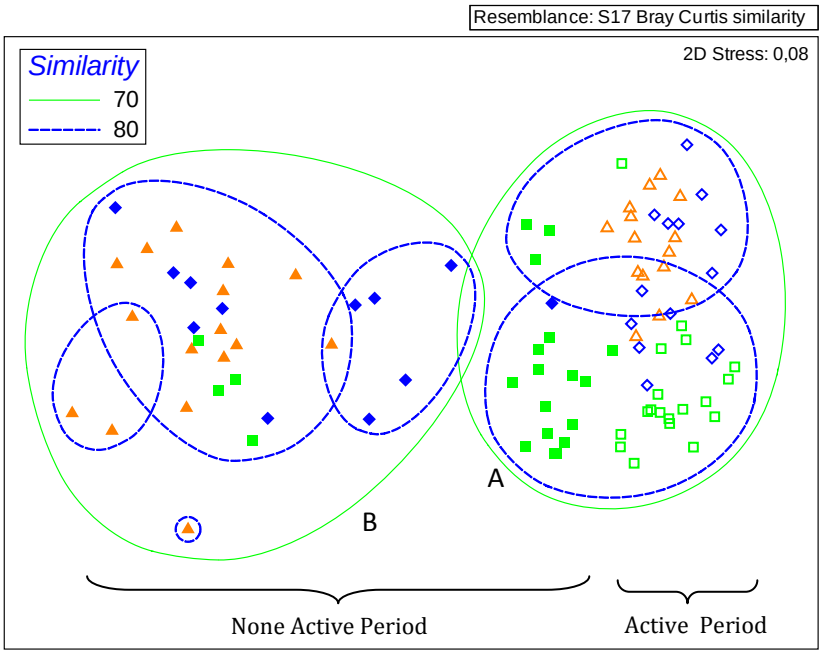
ANOSIM analysis showed significant differences between FA compositions of surface sediments (FASS) of NAP and AP (*R=0.694, p=0.02* %). An average dissimilarity (AD) of ca. 30% (*SIMPER analysis*) was found between both periods due to a greater contribution of the FAs 16:1ω7, 18:1ω7, 18:1ω9, 20:5ω3 and 18:2ω6, and a lower contribution of 16:0, 15:0 and LCFAs (24:0, 26:0, 28:0) to the FASS of the AP than to the FASS of NAP. From NAP to AP there was, in concentration, a significant increase of 16:1ω7 (*one-way ANOVA; F=70.88, p<0.001*), 18:1ω9 (*F=107.6, p<0.001*), 18:1ω7 (*F=65.05, p<0.001*), 20:5ω3 (*F=53.90, p<0.001*), 18:2ω6 (*F=71.32, p<0.001*), 16:0 (*F=27.07, p<0.001*), 15:0 (*F=7.85, p<0.001*), *iso*-15:0 (*F=37.64, p<0.001*), *anteiso*-15:0 (*F=38.82, p<0.001*), 20:4ω3 (*F=14.39, p<0.001*), and a significant decrease of 18:3ω6 (*F=12.17, p<0.001*) and 20:3ω6 (*F=21.07, p<0.001*).

At the both periods, the sum of 18:3ω6 + 20:3ω6 and the chl-*a* concentration were significantly correlated (*Pearson test; r=0.737, p<0.001, n=49 and r=0.489, p<0.001, n=49, respectively*).

As a general trend, there was a significant increase of unsaturated FAs (UFAs e.g. 16:1ω7, 20:5ω3; *one-way ANOVA; F=147.18, p<0.001*) in the sediment from NAP to AP. Relative LCFA contributions to sediments decreased but the figures III.6 show a slight increase of concentrations from NAP to AP, however not significant (*one-way ANOVA; F=29.253, p>0.05*).

The MDS (Multi Dimensional Scale) analysis is given in figure III.3; it regroups samples according to their similarities. In other words, the closer samples are, the more similar are their FA profiles. As shown on figure III.3, both sampling periods are well distinct in term of sediment FA profile compositions, and all profiles of the AP are 70% similar to each other, whereas the FASS highly varied from a sampled station to another during NAP.

Circles A and B regroup samples similarity at the 70% level. The group A includes all samples of the AP and some of the NAP, and the group B includes the rest of NAP samples. ANOSIM showed that these two groups were significantly different (*R=0.956, p=0.02%*) with an AD of ca.36% (*SIMPER analysis*). This difference was mainly due to higher contribution of 16:1ω7, 18:1ω7, 18:1ω9, 20:5ω3 and 18:2ω6, and to a lower contribution of 16:0, 15:0, LCFAs and 18:3ω3 to the group A than in the group B. FAs 16:1ω7, 18:1ω7, 18:1ω9, 18:2ω6, 20:5ω3, *iso*-*anteiso*-15:0 concentrations were significantly higher in the group A than in the group B (*one-way ANOVA; p<0.01 for all*).



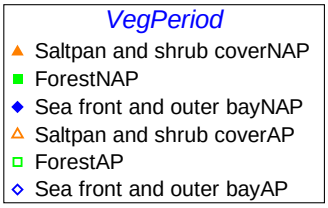


Figure III.3: MDS plot: FA profiles of sediment samples are grouped at 70 % (green circle) and 80% (blue circle) similarity. Samples of NAP are represented by filled forms whereas those of AP are unfilled forms. "Saltpan and shrub cover" refers to sediment samples within the *Salicornia* and the Avicennia, "Forest" refers to sediment sampled in the in the mixed *Avicennia-Rhizosphora* and in the *Rhizosphora* at the inner bay, and "Seafront and outer bay" refers to sediment sample in the mixed *Avicennia-Rhizosphora* at the sea front and in the *Rhizosphora* outer bay and at the seafront.

III.2.3. Spatial changes of surface sediment FA composition

a) Spatial differences during NAP.

ANOSIM indicates a significance difference between FA compositions according to the collected sites during NAP (*R=0.660 and p=0.02%*). Only sediments of the Az, Rho and RhSF zones could be regrouped as their sediment FA profiles were not different (*ANOSIM analysis; p>5%*). The Sz and RhSF sediments were weakly, but significantly different (*R=0.246, p=4.4%*). All other zones defined were significantly different one another (e.g. the Rhi from all other; *R>0.600, p<1%*) with an AD between areas that were up to 20% (*SIMPER analysis*).

Relative contribution of FAs 18:1ω7, 16:1ω7 and 18:1ω9 were significantly higher in the Rhi sediment than in the other parts of the mangrove (*SIMPER analysis*, table III.2), and the SAFAs contributed lower to Rhi than to the rest of the mangrove (14:0, 15:0, 16:0 and/or LCFAs, according to the zones that are compared). The BFAs *iso- anteiso*-15:0 contributed more to Sz, Az and RhSF sediments than to the *Rhizosphora* forest, however, according to the SIMPER analysis, BFAs were not among the main contributors of spatial differences in FASS.

In term of absolute FAs concentrations, it appeared that Rhi clearly differed from other zones by hosting the maximum FA concentrations of the FAO mangrove. Concentrations of the 16:1ω7 ranged from 0 to 74.3 $\mu g.g^{-1}$dw (at the station 25, figure III.7a) and were significantly higher in the Rhi

sediment than in the other parts of the mangrove (*Tukey's HSD; p<0.05*, Table III.2). The 20:5ω3 had the highest concentration at the station 25 (25.1 μg.g^{-1}dw; figure III.9a) and presented significant higher concentrations in Rhi and Az/Rh than in the rest of the mangrove (*Tukey's HSD; p<0.05*). The FA 18:1ω7 ranged from 0 to 78.8 μg.g-1dw (at the station 25, figure III.12a) and was also significantly higher in the Rhi sediment (*Tukey's HSD; p<0.01*) than in the rest of the mangrove. BFAs *iso and anteiso*-15:0 ranged respectively from 0.1 to 38.2 μg.g-1*dw* and from 0 to 29.1 μg.g-1*dw*, and are the main constitutors to the odd-branched FAs, which like other, presented the highest concentrations in the *Rhizosphora* stand (*Tukey's HSD; p<0.01*, figure III.11a) and were detected in all sediment samples. FAs 18:1ω9 and 18:2ω6 which contributed in lower amount to spatial differences in FASS, were, nevertheless, mostly present in the Rhi sediment (*Tukey's HSD; p<0.01 for all*) and ranged from 0 to 35.0 μg.g-1*dw*, and from 0 to 18.6 μg.g-1dw respectively (Figure III.13a and 14a). LCFAs were well represented all over the mangrove area. Theirs concentrations were significantly higher in Rhi sediments than in Sz and RhSF sediments (*Tukey's HSD; p<0.05 for all*), with the highest concentrations found at the stations 25 and 32 (94.1 and 92.0 μg.g-1*dw*, respectively; Figure III.6a).

b) FA composition change of each vegetation stand, from NAP to AP

Surface sediment FA (FASS) composition of defined zones in the FAO mangrove, significantly change from NAP to AP (*ANOSIM; R≤1, p<0.8%)* with an AD up to 15% (*SIMPER*). The highest AD has been found between Sz during NAP and Sz during AP (43.23%) and the lowest between Rhi during NAP and Rhi during AP (17.44%). Differences in vegetation stands between both periods were mainly due to the higher contributions of the FAs 16:1ω7, 18:1ω7 and 18:1ω9, and to the lower contribution of 16:0 during AP than during NAP (*SIMPER*). The concentrations of these MUFAs significantly increased from NAP to AP in all vegetation stand (*one-way ANOVA; p<0.01 for all*). Additionally the FA 20:5ω3 concentration increased (*one-way ANOVA; p<0.01 for all*) from NAP to AP in each vegetation stand except in the Rhi. Relative contributions to FASS of the 16:1ω5 highly increased in Rhi sediments from NAP to AP as well as its concentrations (*one-way ANOVA; p<0.001 both*). BFA iso-anteiso 15:0 concentrations increased (*one-way ANOVA; p<0.05 for all*) everywhere.

From NAP to AP, FA 17:1ω7 concentrations significantly increased in saltpan (*one-way ANOVA; p<0.05*), Az (*p<0.05*) and in Rho (*p<0.001*) sediments. The FA 18:2ω6 concentrations significantly increased from NAP to AP in the Rhi (*p<0.05*), Rho (*p<0.05*) and RhSF (*p<0.001*) contributing more to the sediment of these zones during AP than during NAP (*SIMPER analysis*). Additionally the FA 20:4ω6 significantly increased from NAP to AP in the Rho sediment (p<0.01).

c) Spatial differences of FASS composition during AP

During AP, defined zones were significantly different in term of FASS, (*R=0.324, p=0.02; ANOSIM*). Sz and Az were the less different zones (*ANOSIM; R=0.141, p=1.7%)*, and all other zones defined were significantly different from one to another (*R>0.300; p=0.02% for all*). ADs between

groups during AP were lower than during NAP, ranging from ca.11% (between Sz and Az) to ca. 19% (between Az and Rhi).

FAs16:1ω7 contributed more to the Sz sediment than to other defined zones and showed the lowest contribution in the Rhi sediment, as shown on figure III.4. In the same manner, 20:5ω3 contributed more to the Sz sediment, as well as to the Rho and RhSF sediments (*SIMPER*), and contributed less to the Rhi sediment than to the rest of the mangrove. In contrast, FAs 18:1ω7 (figure III.4) and *iso-anteiso*-15:0 more contributed to FASS of Rhi sediment than to sediment of other zones (*SIMPER*). The FA 18:1ω9 contributed more to the Sz (the highest contribution) and to the RhSF sediments than to Rhi and Rho (*SIMPER*, Figure III.4) but its contribution to the mangrove sediment was quite homogeneous. The FA 17:1ω7 contributed more to the Sz, Az and Av/Rh zones than to the rest of the mangrove FASS composition. Additionally, the FA 10 Me 16:0 contributed more to the Rhi and RhSF zones than to the rest of the mangrove. The FAs 20:4ω6 and 18:2ω6 contributed more to the Rho and RhSF (low intertidal sediments) than to the Rhi (mid-intertidal sediment), Sz and Az (high intertidal sediments) i.e. theirs contributions decreased from the lagoon to the ponds. Finally, LCFAs contributed more to the Rhi, Rho and RhSF sediments than to the Az and Sz.

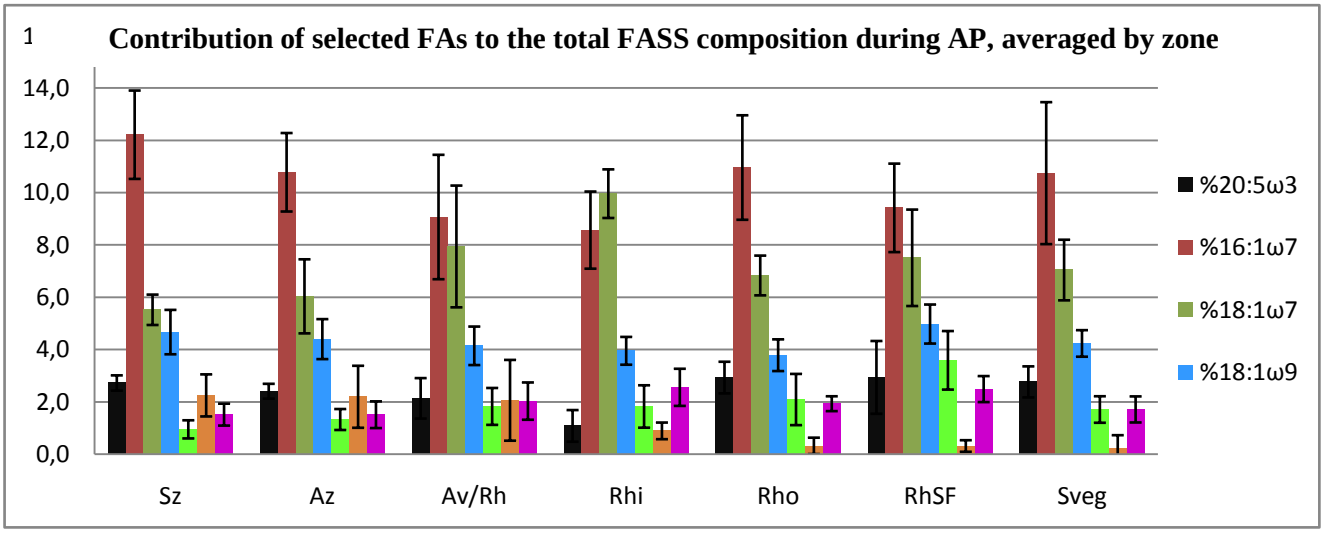


Figure III.4: Mean contribution of selected FAs to the FASS composition according to the vegetation stands during AP.

d) Spatial difference FA concentration during AP.

Concentration of 16:1ω7 and 20:5ω3 during AP ranged from 25.8 ±10.8 to 290.3 ± 145.1µ.g.g^{-1}*dw* and from 3.5 ± 0.5 to 49.3 ± 9.9 µg.g^{-1}*dw*, respectively. For these both FA, higher concentrations were found at stn 17 (figure III.7b and III.9b). Distribution of these FA concentrations in the mangrove sediment did not exhibit any significant spatial differences (*Tukey's HSD; p>0.05*).

Concentration of the FA 18:1ω7 ranged from 8.8 ± 2.6 to 160.5 ±3.1 µg.g^{-1}dw and its highest concentration was found at stn 34 (figure III.12b). Concentrations of this FA were significantly lower at the saltpan and at the seafront than at the rest of the mangrove (*Tukey'HSD; p<0.01 for all*). FAs iso-anteiso15:0 ranged from 6.5 ± 1.3 to 102.8 ± 4.5 µg.g^{-1}dw and from 5.0 ± 1.7 to 108.6 ± 7.0 µg.g^{-}

[1]dw, respectively (the highest concentration were found at the station 34). Concentrations of these both BFAs were significantly higher in Rhi sediment than in Sz and RhSF sediments (*Tukey's HSD; p<0.01 for all*). The odd-branched sum, shown on figure III.11b exhibited its highest concentrations at the stns 17 and 34. The FA 10Me 16:0 (ranged from 3.6 ± 1.1 to 31.0 ± 2.3 $\mu g.g^{-1}$*dw*, reached at stn 32) exhibited the same trend that of the odd-branched and the highest concentrations in the *Rhizophora* stand.

Concentrations of the FA 18:1w9 ranged from 9.9 ± 3.4 to 76.9 ± 3.9 $\mu g.g^{-1}$*dw* and the highest concentration was found at the station 34 (figureIII.13b). High concentrations were also detected at the stn 17 and 10. Concentrations of the FA 18:1w9 did not present significant spatial differences (*one-way ANOVA; p>0.05*) as well as the FA 18:2ω6, which ranged from 1.7 ± 0.4 to 54.3 ± 11.5 $\mu g.g^{-1}$*dw*. The highest concentration of the 18:2ω6 was also detected at stn 34 (figure III.14b).

The FA 20:4ω6, ranged from 2.7 ± 0.8 to 28.4 ± 3.1 $\mu g.g^{-1}$*dw*, with the highest concentration detected outer bay at stn 35. No significant differences in concentrations were found between most of the vegetation stands, however this FA was significantly higher in Rho sediment than in Sz (*Tukey's HSDp<0.001*). The concentration of FAs 18:3ω6 and 20:3ω6 (relatively low) did not involve spatial difference in the mangrove sediment during AP. However, when looking at the map of their sum (figure III.10b), it appears that the highest concentrations were located at the output zones (stn 17 and 29) and that saltpan sediments were particularly poor in these two FAs.

Sum of LCFA concentrations were homogeneously distributed over the mangrove sediment and it did not exhibit significant differences between vegetation stands excepted between Rhi and RhSF sediments in where LCFA concentrations were lower (*Tukey'sHSD; p<0.05*).

III.2.4. Difference in FA compositions between output and other zones.

During NAP, output zones (Oz) of saltpan, *Avicennia* and *Rhizophora* stands did not differ from the non output zones (NOz) of the same vegetation stands (*ANOSIM; p>5%*; output zones are shown on the figure II.3).

However during AP, FASS composition of Oz and NOz on the saltpan were significantly different (*ANOSIM; R=0.296 and p=1.8%)* with an AD of ca. 11% (*SIMPER).* This difference was mainly due to higher contributions of 16:0, 16:1ω7, 18:2ω6, and 20:4ω6, and lower contributions of 18:1ω9, 17:1ω7, 18:1ω7, 10Me 16:0 and iso-15:0 in Oz than in NOz of the Sz sediment. However, no differences in term of FA concentration were found between these two zones (*one-way ANOVA; p>0.05*). Nevertheless, their ranges of concentration were larger in Oz of Sz than in NOz (Figure III.5).

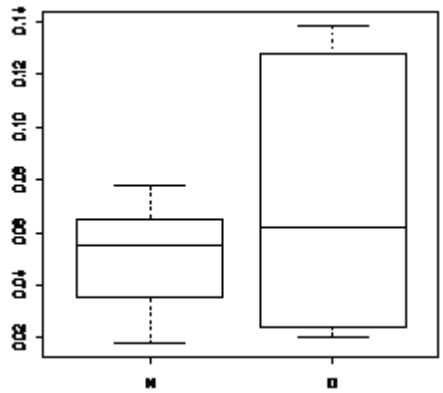


Figure III.5: Boxplot of the 16:1ω7 concentrations (mg.g^{-1}dw) at the output zone ad in the rest of the mangrove sediment. The same pattern of change in concentrations appeared for : 16:0, 18:2ω6, 20:4ω6, 18:1ω9, 17:1ω7, 18:1ω7, 10Me 16:0 and anteiso-iso-15:0.

During AP, Oz and NOz sediments significantly differed within Rhi (*ANOSIM; R=0.319, p=0.02%*) with an AD of ca. 12% (SIMPER). This difference is mainly due to the higher contributions of 16:1ω7, 18:1ω7, 18:2ω6 and 20:5ω3, and to the lower contribution of iso-anteiso-15:0 to Oz than to the rest of the Rhi zone (NOz). FAs 18:1ω9 (*one-way ANOVA; F=4.59 p<0.05*), 16:1ω7 (*F=7.84; p<0.01*) and 20:5ω3 (*F=11.97; p<0.01*) were significantly higher close to effluent discharges than farther. No significant differences in term of FA contribution to the sediment were found between Oz and NOz in the *Avicennia*. Moreover, the FA composition of the saltpan Oz sediment were not different from the FA composition of the *Avicennia* NOz (*ANOSIM; p=15.5%*) and of the *Avicennia* Oz (*ANOSIM; p=35.9%*).

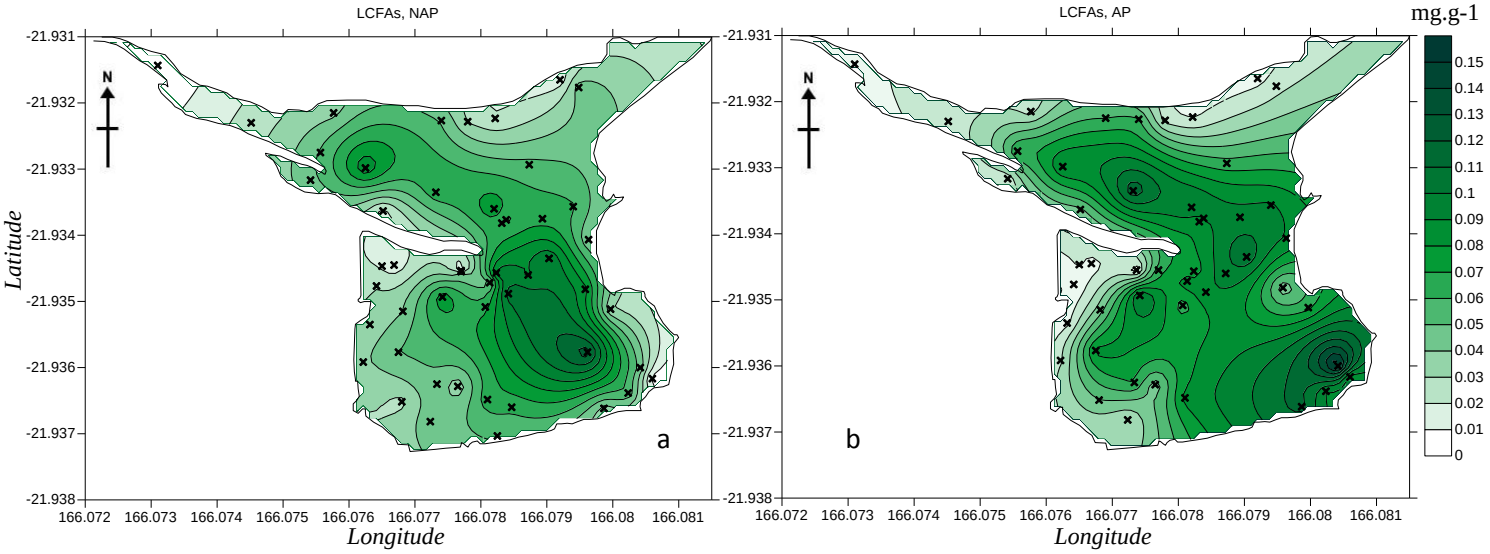


Figure III.6: Distribution of the LCFAs (≥24:0) concentrations in the mangrove surface sediment during NAP (a) and during AP (b).

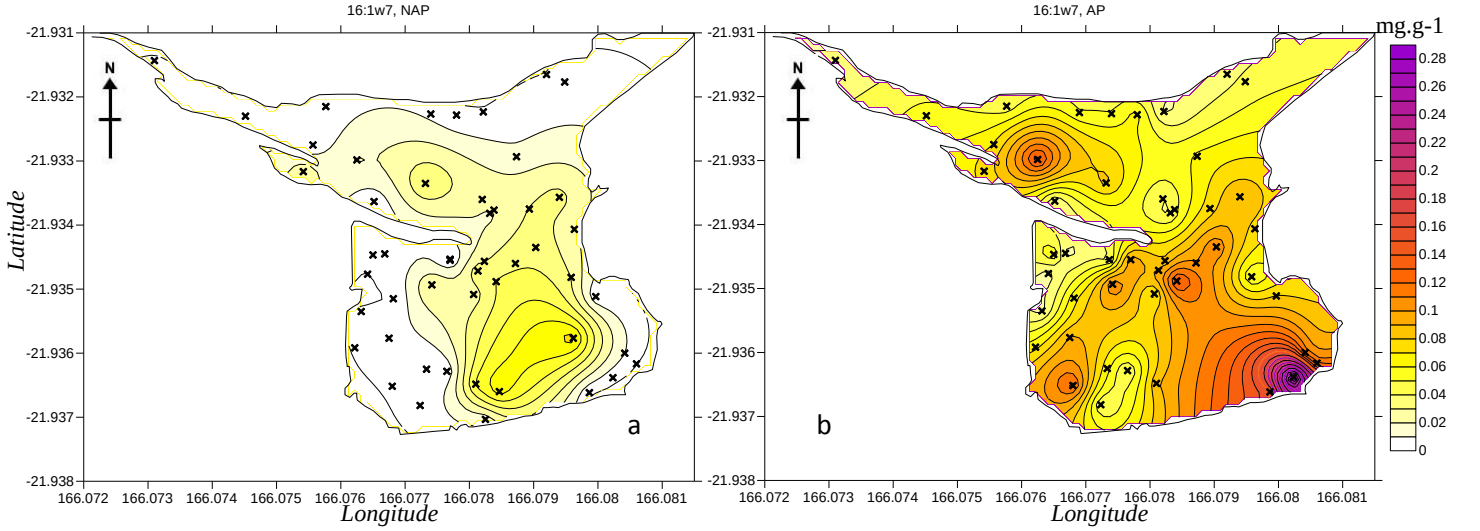


Figure III.7: Distribution of the 16:1ω7 concentrationsin the mangrove surface sediment during NAP (a) and during AP (b)

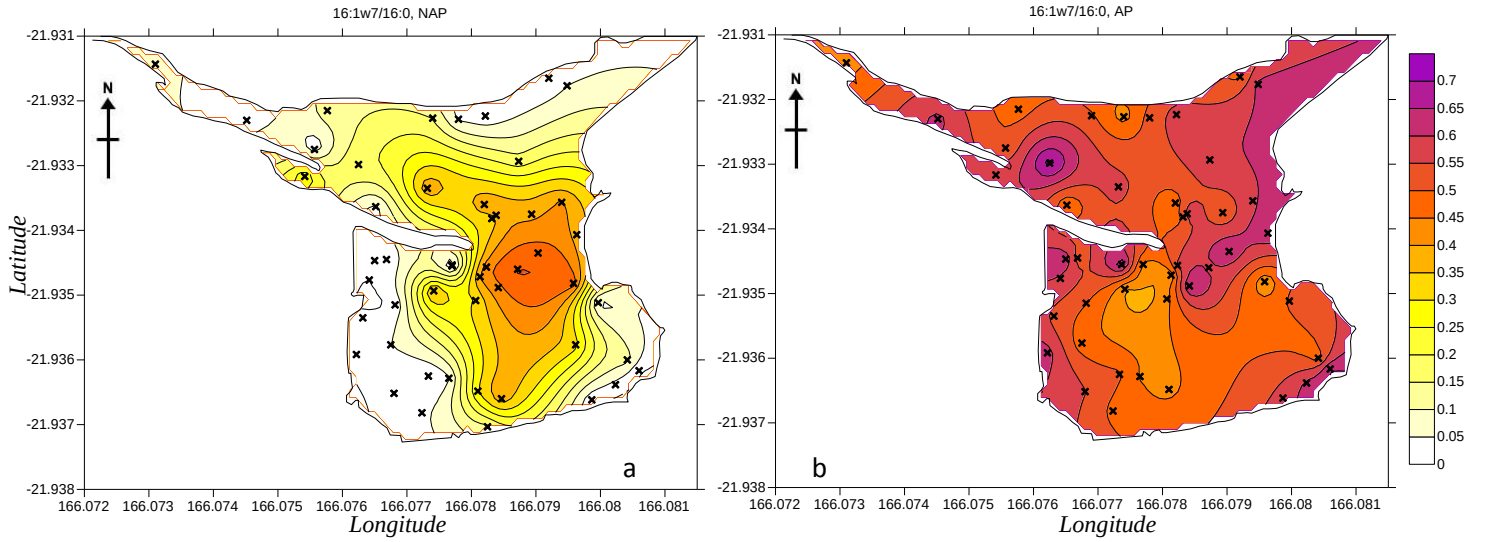


Figure III.8: Distribution of the 16:1ω7/16:0 ratio in the mangrove surface sediment during NAP (a) and during AP (b)

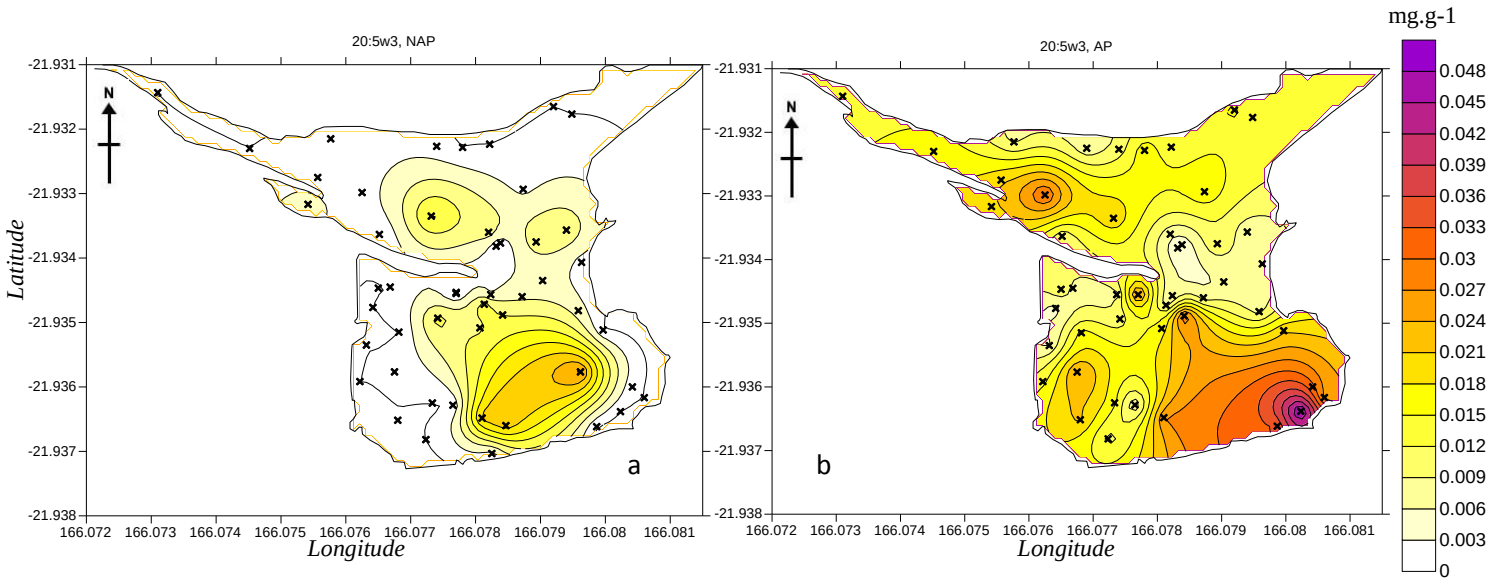


Figure III.9: Distribution of the 20:5ω3 concentrations in the mangrove surface sediment during NAP (a) and during AP (b)

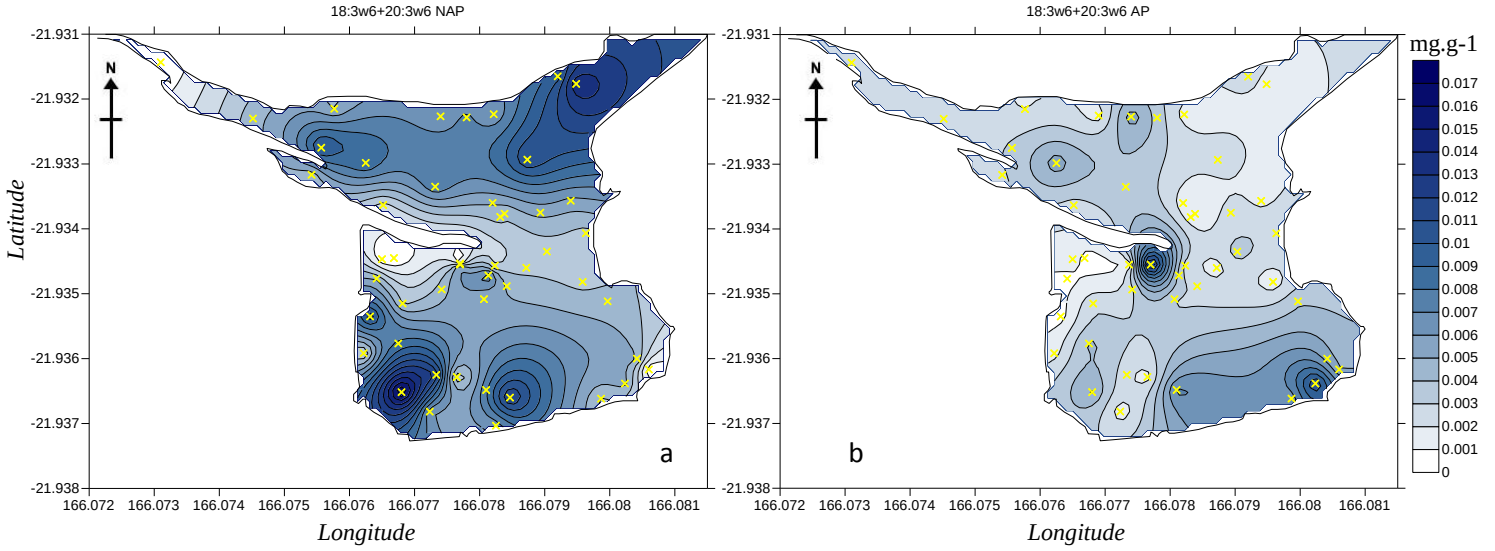


Figure III.10: Distribution of the 18:3ω6 + 20:3ω6 concentrations in the mangrove surface sediment during NAP (a) and during AP (b)

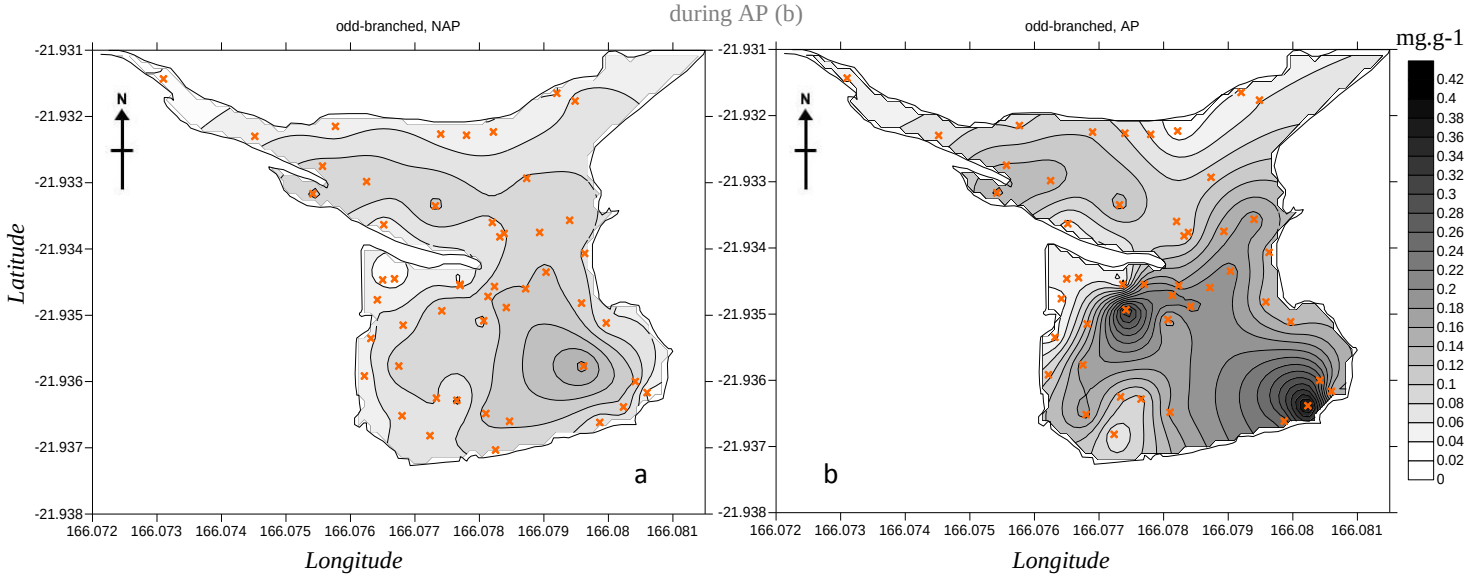


Figure III.11: Distribution of the sum of the odd-branched (11:0+ iso-anteiso 13:0 +iso-anteiso-15:0+iso-anteiso-17:0+19:0) concentrations in the mangrove surface sediment during NAP (a) and during AP (b)

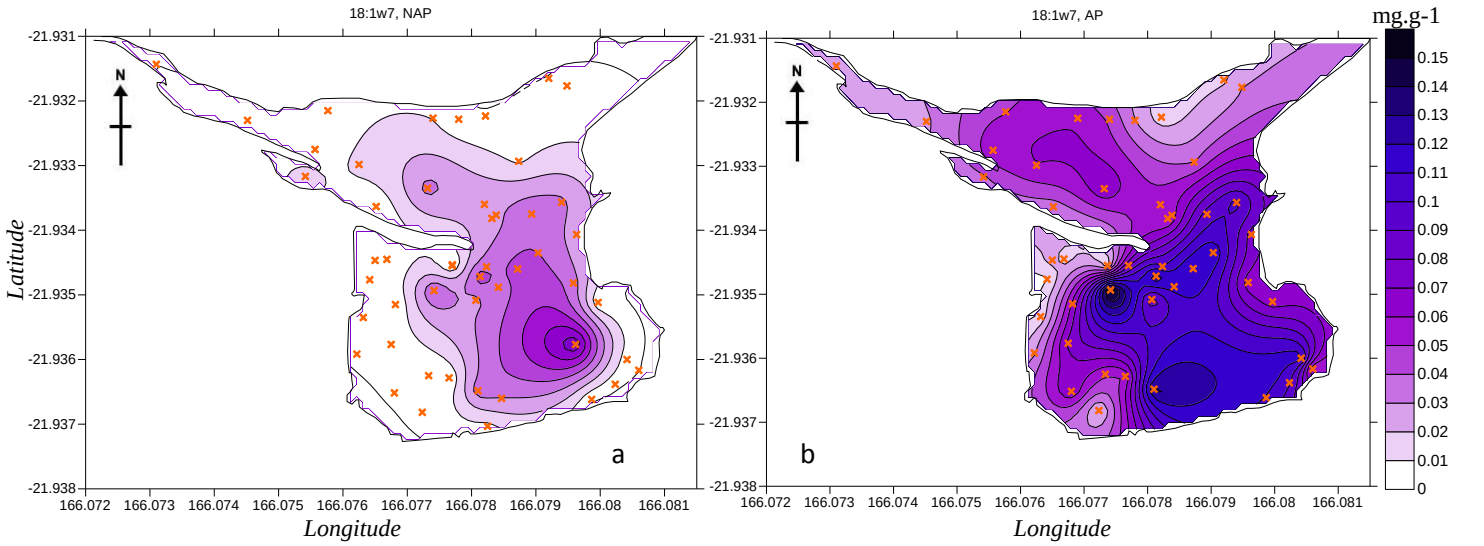


Figure III.12: Distribution of the 18:1ω7 concentrations in the mangrove surface sediment during NAP (a) and during AP (b)

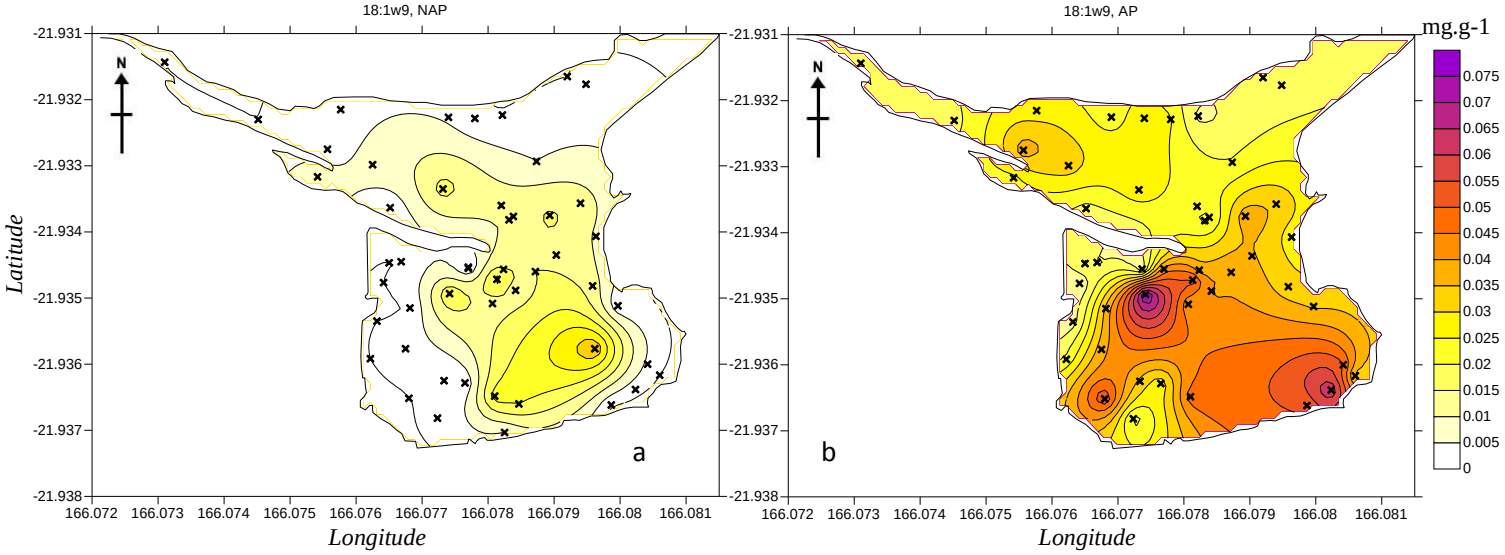


Figure III.13: Distribution of the 18:1ω9 concentrations in the mangrove surface sediment during NAP (a) and during AP (b)

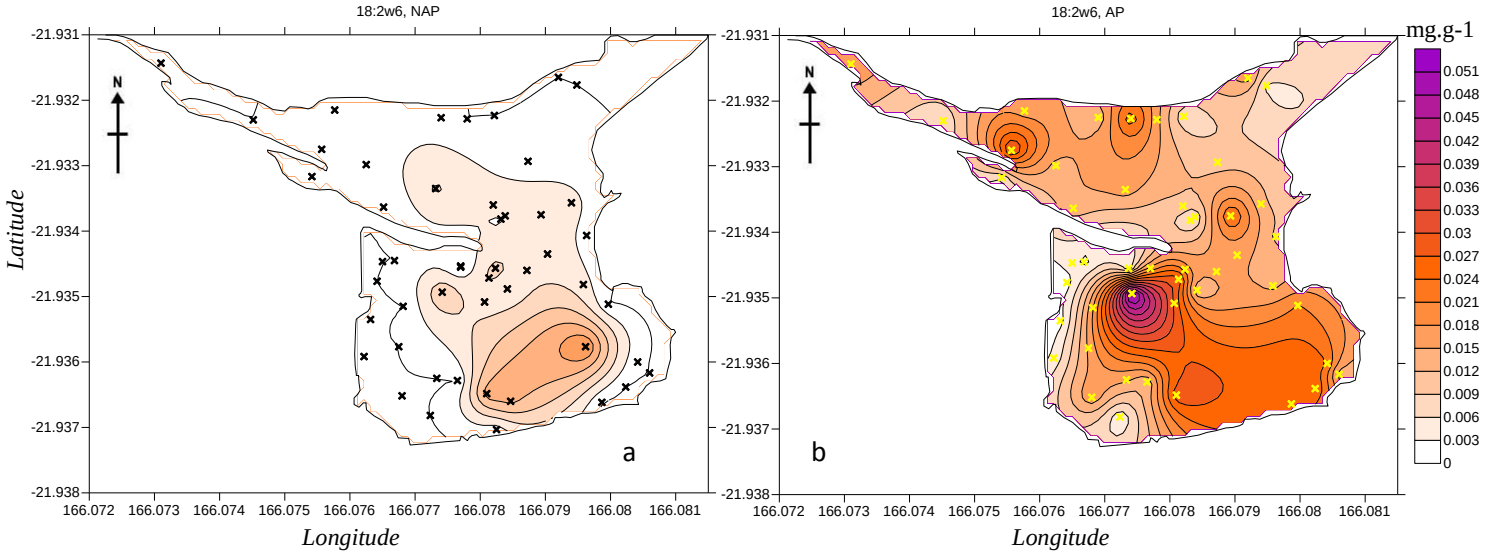


Figure III.14: Distribution of the 18:2ω6 concentrations in the mangrove surface sediment during NAP (a) and during AP (b)

III.3. ISOTOPES

III.3.1. Stable isotope spatial and temporal distribution

Kruskal wallis test found that both $\delta^{13}C$ and $\delta^{15}N$ values were significantly lower during AP than during NAP ($\chi^2=4.99$, $n=2$, $p<0.05$; *and* $\chi^2=0.064$, $n=2$, $p<0.05$ *respectively*). During NAP there were no significant differences ($p>0.05$) between output zones (Oz) and the rest of the mangrove (NOz) for both stable isotopes. However, Oz had significant enrichment of the $\delta\ ^{15}N$ during AP ($\chi^2=9.31$, $n=2$, $p<0.01$) and lower $\delta^{13}C$ values ($\chi^2=13.17$, $n=2$, $p<0.001$) than the rest of the mangrove.

The C:N ratio (Redfield ratio) did not significantly change between NAP and AP (Kruskal Wallis; $p>0.05$).

As shown on the figure III.15 a and b, changes in $\delta^{13}C$ values occurred mainly within the saltpan and *Avicennia* stands where values significantly decreased from NAP to AP (*Wilcoxon test;* $p<0.05$), No significant change occurred within the *Rhizophora* sediment (either at the inner bay, at the outer bay or at the seafront; $p>0.05$). During NAP $\delta^{13}C$ values detected in the Sz and Az sediments were significantly higher than those found in the forest sediment (Rhi; $p<0.01$), as well as during AP (all other vegetation stands; $p<0.01$). Wilcoxon analysis also shown that $\delta^{13}C$ values from the forest (Rhi) significantly decreased from NAP to AP ($p<0.05$).

Maps of figures III.16 a and b indicated that the highest values of the $\delta^{15}N$ were found at the output zones (stns 10, 18 and 30) during NAP. At both periods, it appeared a gradient of the $\delta^{15}N$ from ponds to the lagoon. Indeed, sediments collected in the saltpan, *Avicennia* stand, and at the *Avicennia-Rhizophora* interphase formed a group, which $\delta^{15}N$ values were significantly higher than those collected in the forest sediments (Ri, Rho and RhSF; *Wilcoxon*, $p<0.05$). During AP, this group (Sz, Az, Av/Rh) is also composed of the Rhi zone and had higher values than in the rest of the mangrove sediment (*Wilcoxon;* $p<0.05$). Zones mainly affected by the changes in $\delta^{15}N$ were the saltpan (*Wilcoxon;* $p<0.05$) and *Avicennia* stands ($p<0.01$), which sediments $\delta^{15}N$ significantly decreased from NAP to AP. No significant changes were recorded in forest sediments (Rhi, Rho and RhSF; $p>0.05$).

The C:N ratio did not significantly change from NAP to AP (*one-way ANOVA;* $p>0.05$), and presented almost the same spatial distribution pattern during the both periods (figures III.17 a and b). The Rhi, Rho and RhSF sediments formed a distinct group of higher C:N values than all other zones during the NAP (*Tukey's HSD;* $p<0.001$), and than in the Az and Sz sediments during the AP (*Tukey's HSD,* $p<0.05$ *for all*).

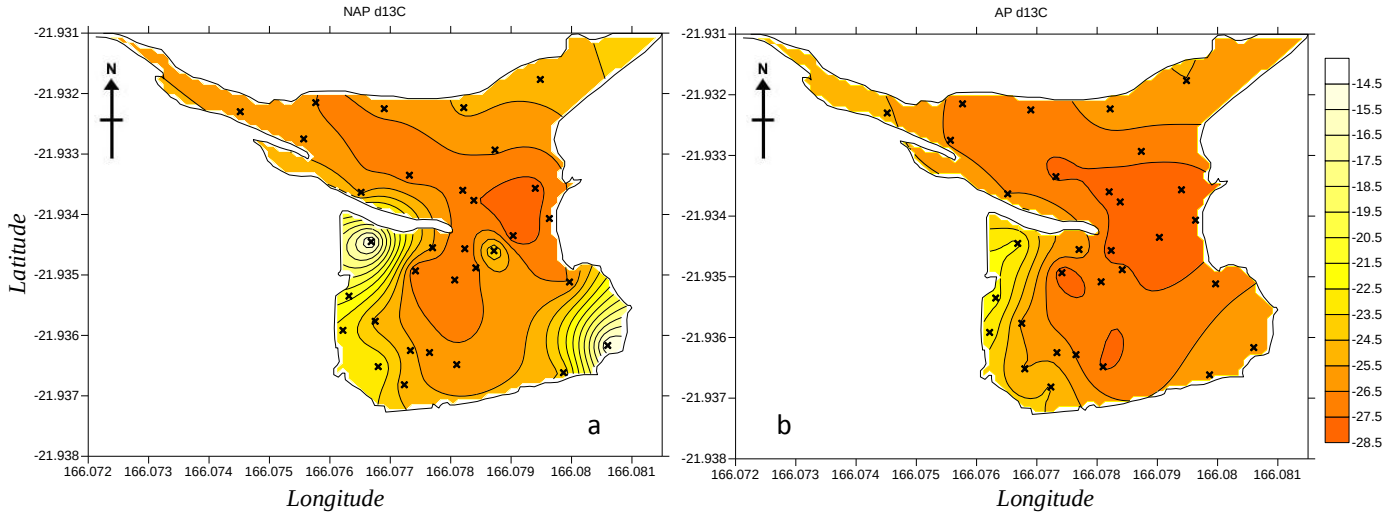


Figure III.15: Distribution of the $\delta^{13}C$ values in the mangrove surface sediment during NAP (a) and during AP (b)

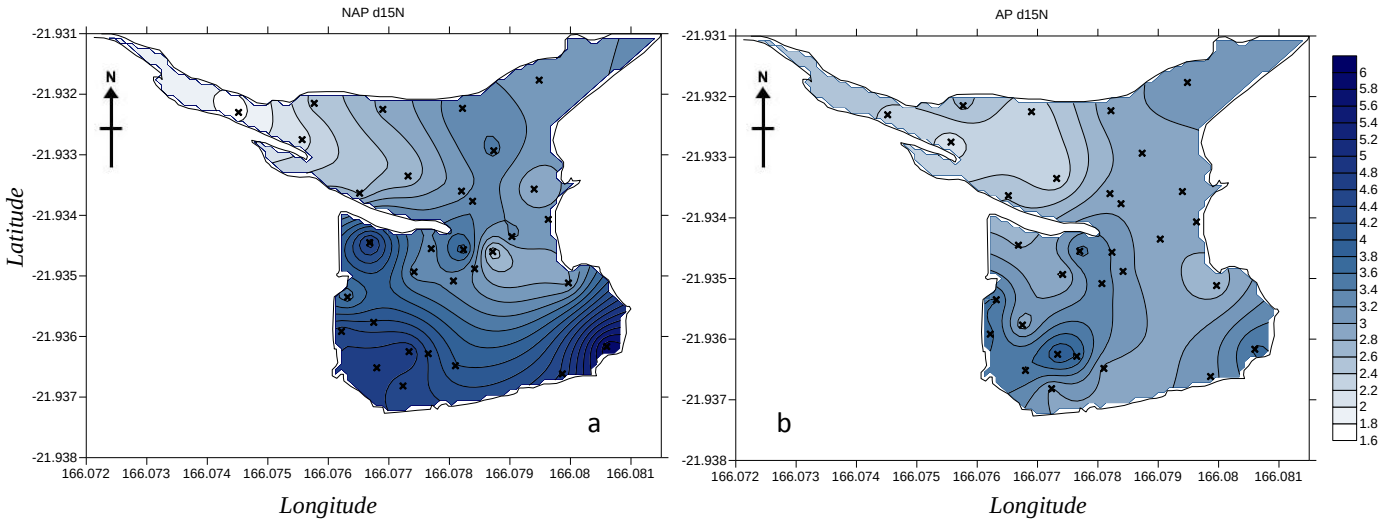


Figure III.16: Distribution of the $\delta^{15}N$ values in the mangrove surface sediment during NAP (a) and during AP (b)

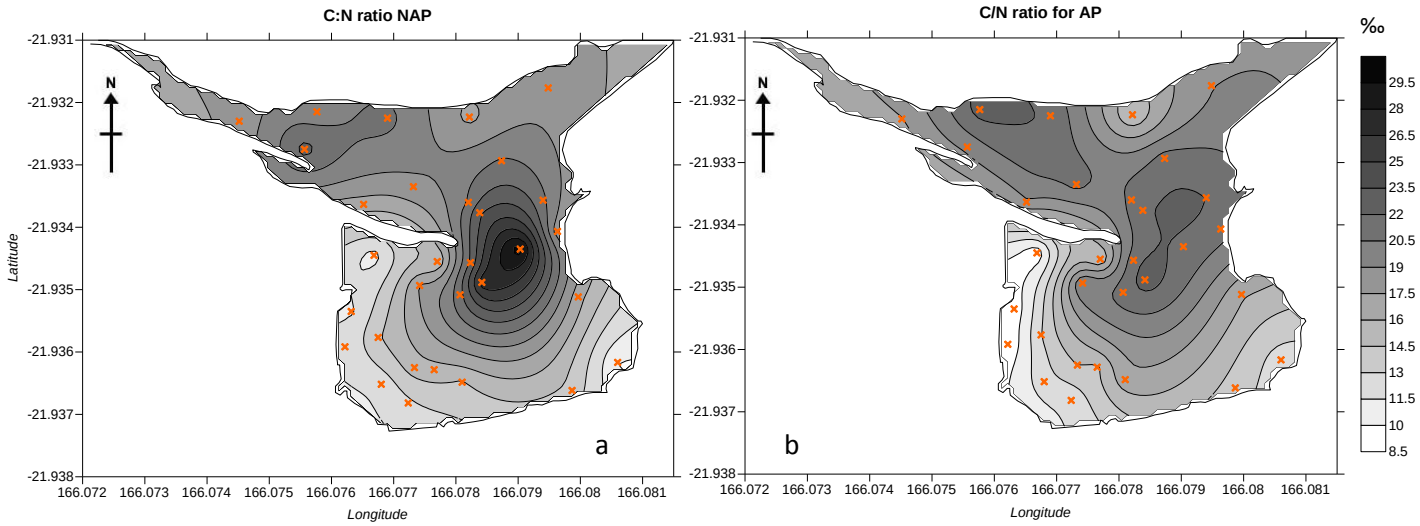


Figure III.17: Distribution in the mangrove surface sediment of the C:N ratio during NAP (a) and AP (b)

III.3.2.Isotopic sources in the sediment

Mean values of $\delta^{13}C$, $\delta^{15}N$ and C:N sources considered in this works are given in table III.4 and theirs contributions (%) to the mangrove sediment are given in Appendix I.

During NAP, all considered sources of $\delta^{13}C$ and $\delta^{15}N$ collected (i.e. litter of *Salicornia australis, Avicennia marina, Rhizophora stylosa*, cyanophycae and diatoms) almost equally contributed to the sediment isotopic signature. Contribution of *Salicornia sp* was maximum (27.2 ± 10.3%) to the saltpan sediment. *Avicennia marina* contributed about 20% of stable isotopic signature of sediments at each stand (figure III.18b), and *Rhizophora stylosa* contribution ranged from 15.5 ± 10.1%, to 25.2 ± 9.3% everywhere with the highest contribution in the forest (figure III.18c). Isotopic signature of cyanophycae collected on saltpan contributed to the entire mangrove sediment with higher level at Sz, Az, Av/Rh (sediment collected inner bay close to ponds; boxplot *d*, figure III.18). Diatoms contribution was higher outer bay and at the seafront (22.8 ± 9.9 and 25.8 ± 10.8% respectively) than inner bay (all vegetations considered, below 20%).

During AP, *S.australis*, *A.marina* and *R.stylosa* litters contributed more to the sediments of forest zones (Rhi, Rho and RhSF) than to the Sz, Az and Av/Rh zones (figure III.19 a, b and c). During AP, isotopic signature of diatoms was (almost) equally detected everywhere (boxplot, figure III.19 g) and cyanophycae was especially found on saltpan (figure III.19 f). Additional source of stable isotope were taken in consideration during AP, i.e. effluents and shrimp food distributed in ponds. Shrimp food and effluent isotopic signatures highly contributed to the saltpan sediment (19.6 ± 7.0% and 15.3 ± 7.9% respectively) as well as to the sediment of the *Avicennia* stand (9.9 ± 6.0% and 16.7 ± 7.3%, respectively). Going seawards, effluent signature was still highly detected (about 15%) but was minimum in the Rhi (boxplot *d*, figure III.19). Shrimp food signature in sediment dropped (about 3% decreased) going seawards and had as well the lowest contribution to the *Rhizophora* stand sediment located inner bay (boxplot *e*, figure III.19).

Sources	**$\delta^{13}C$ ‰**			**$\delta^{15}N$ ‰**			**C/N**		
Salicornia australis	*-28,5*	±	*0,5*	*6,5*	±	*1,1*	*24,5*	±	*5,6*
Avicennia marina	*-27,2*	±	*0,8*	*4,0*	±	*1,7*	*22,9*	±	*2,7*
Rhizophora stylosa	*-28,8*	±	*2,4*	*2,4*	±	*2,0*	*54,1*	±	*11,3*
Diatoms	*-25,1*	±	*0,8*	*1,3*	±	*1,6*	*8,2*	±	*2,1*
Effluents	*-23,6*	±	*0,3*	*1,1*	±	*1,7*	*5,9*	±	*3,0*
Shrimp food	*-22,6*	±	*0,5*	*6,5*	±	*0,7*	*6,8*	±	*0,7*
cyanophycae	*-17,9*	±	*2,1*	*2,4*	±	*1,9*	*9,5*	±	*0,2*

Table III.4: values of isotopic signatures of the sources considered.

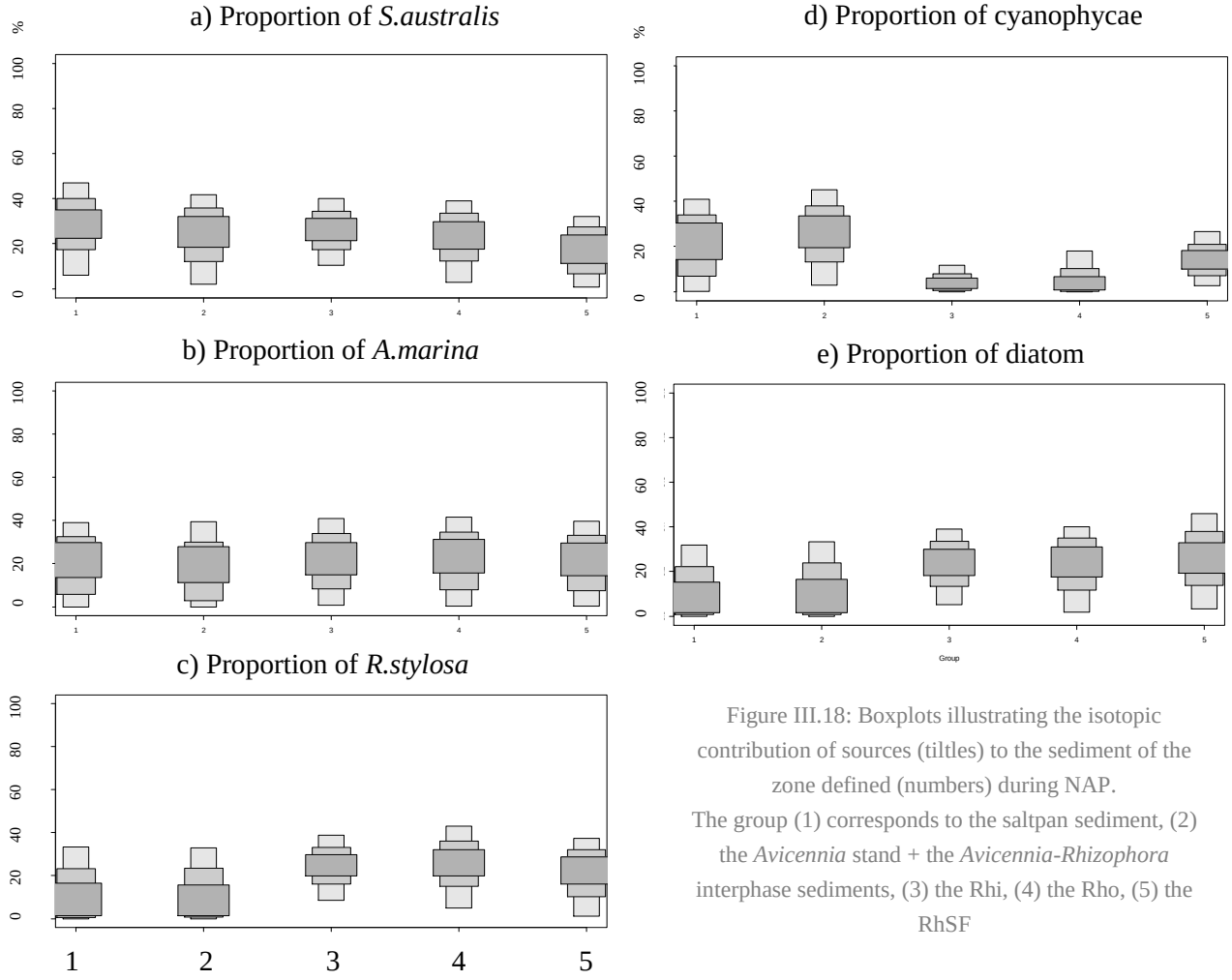


Figure III.18: Boxplots illustrating the isotopic contribution of sources (tiltles) to the sediment of the zone defined (numbers) during NAP.
The group (1) corresponds to the saltpan sediment, (2) the *Avicennia* stand + the *Avicennia-Rhizophora* interphase sediments, (3) the Rhi, (4) the Rho, (5) the RhSF

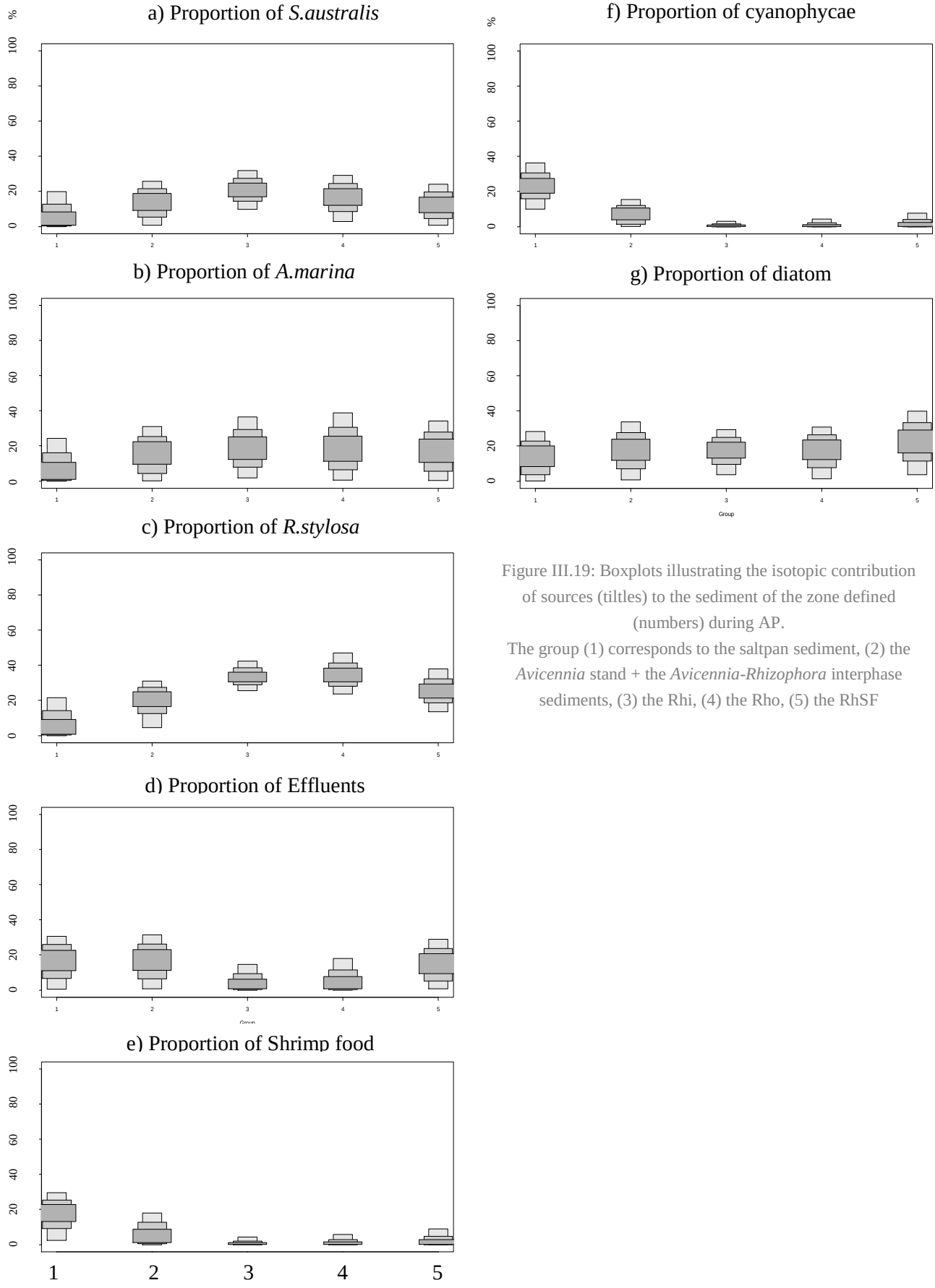


Figure III.19: Boxplots illustrating the isotopic contribution of sources (tiltles) to the sediment of the zone defined (numbers) during AP.
The group (1) corresponds to the saltpan sediment, (2) the *Avicennia* stand + the *Avicennia-Rhizophora* interphase sediments, (3) the Rhi, (4) the Rho, (5) the RhSF

III.4. MICROSCOPIC OBSERVATIONS OF THE MICRO-PHYTOBENTHOS

Microscopic observations allowed a branch-level identification. Indeed, at least three branches were identifiable: Rhodophyta (or red algae), Cyanophyta (or blue algae) and Heterokontophyta (Fam. Diatomphyceae). Additionally, some foraminifers, fungal spores and mangrove detritus were observed.

Some genus have been identified such as Gyrosigma (Branch: Diatomophyceae, Order: Naviculales; figure III.20), Surirella (Branch: Diatomophyceae, Order: Surirellaceae; figure III.20), and Nitzschia (Branch: Diatomophyceae, Order Naviculales; figure III.20) were the dominant organisms present in the sediment of both FAO and pristine mangrove. Additionally, some Nematodes, chains Coscinodicus (Branch: Diatomophyceae, Order: Coscinodiscales), and genus *Spirullina* (Branch Cyanophyta, Order: Oscillatoriales) were found in both mangrove sediments. Moreover, some Dinophyceae were counted in the sediment of the FAO and some Chroococus (Branch: Cyanophyta, Order: Chroococcales , figure III.20) in the sediment of the pristine mangrove.

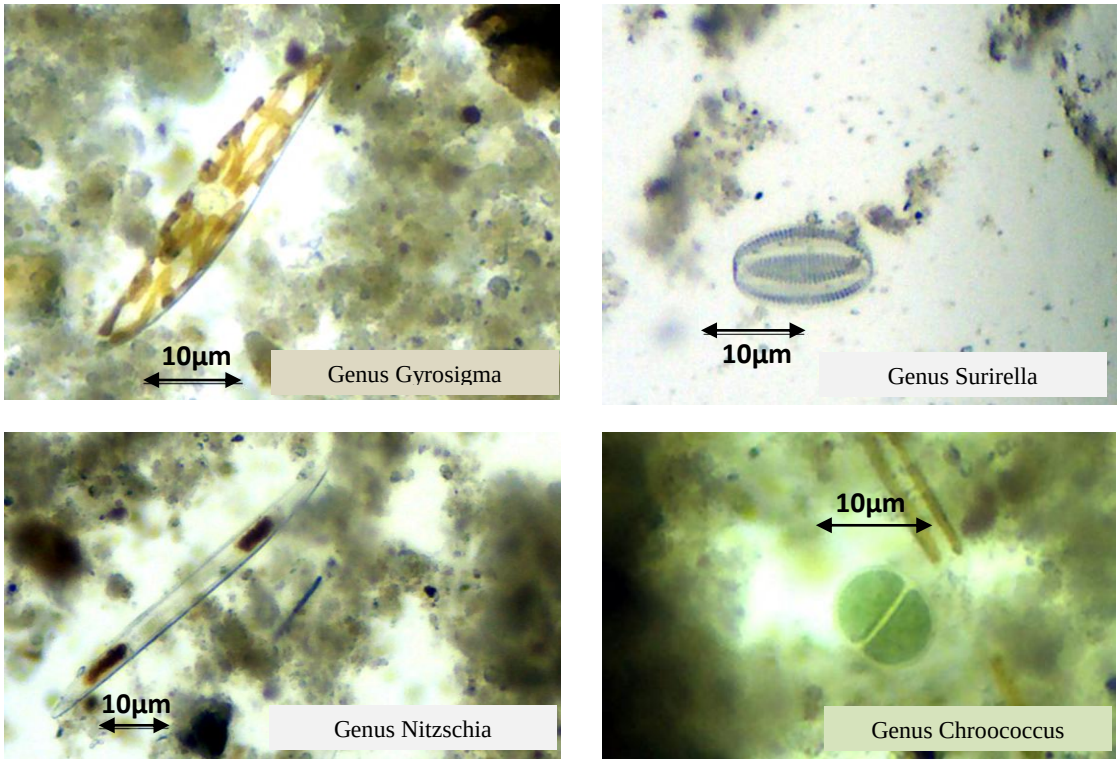


Figure III.20: Microscopic pictures of the FAO mangrove surface sediment

CHAPTER IV: DISCUSSION

The contributions and the concentrations of various specific, coupled and grouped FAs known as markers of several organic matter (OM) sources were used in order to assess the inputs of potential sources to the OM of surface sediments in the FAO mangrove receiving shrimp farm effluents. FA markers also allowed evaluate the role of the FAO shrimp farm discharges in the fate of these inputs. Additionally, the results of stable isotopic signatures of sources and sediments are discussed in a separated chapter to emphasize hypothesis drawn with FAs interpretation.

From non active period (NAP), corresponding to the early hot summer, to active period (AP; i.e. of the shrimp farm), related to the early dryer and colder winter, the differences between FA and stable isotopic compositions in the FAO mangrove surface sediment indicated changes in the contribution of organic matter sources. Additionally, FA and isotopic compositions of the mangrove sediments exhibited spatial marked variations along a gradient from the *Salicornia* area to the lagoon, which reveals that some mangrove areas were more prone than other to OM input, degradation, biosynthesis and exportation.

IV. 1. TEMPORAL CHANGES IN THE ORGANIC MATTER QUALITY

The ratio of unsaturated fatty acid (UFAs) to saturated fatty acid (SAFAs) was largely used to qualify the degradation state of the OM within the sediment (Canuel and Martens, 1993; Carrie et al., 1998; Volkman et al., 2008). Indeed, the dominance of UFAs (figure III.2) characterizes a fresh OM (i.e. undegraded or at the earliest degradation stage). Thus, as the OM was "fresher" during AP than during NAP in the surface sediment, it seemed that recent OM inputs occurred, which could only originate either from a local mangrove production or from the shrimp farm effluents. In fact, it has been reported in previous studies that shrimp farm discharges significantly enhance primary and bacterial production in mangrove ecosystems (Trott and Alongi 2000; Costanzo et al 2004), which seems to be the case at this season in the FAO mangrove. During NAP, FA compositions of sediments were dominated by SAFAs (contributed at >50% to total FAs of sediment; table III.2), which emphasize the abundance of a more degraded OM at the surface sediment.

IV.2. MANGROVE-DERIVED ORGANIC MATTER INPUTS AND DEGRADATION

OM derived from vascular plants can be revealed by long chain fatty acids (LCFAs; from 24:0 to 30:0; Volkman et al 1980). Indeed in mangroves trees, contribution of LCFAs to *Avicennia marina* leaves was up to 5% and about 1-8% in *Rhizophora stylosa* leaves in the West Pacific Ocean mangrove forests (Meziane et al 2007). The high relative contribution of these FAs in the sediment organic matter (SOM) during NAP confirms that the litter originating from mangrove production was the major source of OM at this season. This is in agreement with what was measured elsewhere in a pristine mangrove (Okinawa, Japan; Meziane et Tsuchia, 2000; Mfilinge et al., 2003). The secondary

OM sources were bacteria (source of 18:1ω7 and of *iso-anteiso*-15:0 and *iso-anteiso*-17:0; Kharlamenko et al., 1995) and diatoms (source of 16:1ω7 and 20:5ω3; Parrish et al., 2000 and Meziane and Tsychiya, 2000, respectively). Conversely, during AP these living OM sources (i.e. bacteria and diatoms) bloomed and thus moderated the relative contribution of mangrove litter markers.

However, LCFAs in surface sediment during AP were, in quantity, equally abundant (or slightly more abundant) than during NAP (figure III.6). High LCFAs content could offhand be due to a high litter fall, as both parameters (i.e. LCFAs and litter fall) have been shown to be positively correlated (Mfilinge et al., 2005). Since winter (AP) is the reproductive season (fruit fall) and the maximum litter fall for *Avicennia marina* (Mackey and Smail, 1995), the hypothesis of litter exportation from *Avicennia marina* to the entire mangrove could be considered but seems negligible. Indeed, considering that its litter production is most probably weak on account of the small *Avicennia* extend (10% of the FAO mangrove vegetal cover), and that the litter derived from these trees is highly degradable (Lacerdata et al., 1995), one can only think that a large organic matter content of litter fall would be due to *Rhizophora stylosa* production itself. However, it has been reported in previous studies that winter was the season of the minimum *Rhizophora styloza* litter fall (considering both fruits and leaves fall; Mfilinge et al., 2005; Sharma et al., 2010). The unexpected high winter rainfalls that affected the Saint Vincent Bay could possibly result in a higher litter fall from mangrove trees as shown by Meziane et al. (2006; figure II.2). However precipitations and resulting litter falls were probably not strong enough to explain alone such an abundance of LCFAs in the sediment. Therefore, instead of indicating litter abundance, it is thought that high LCFA concentrations in sediments during AP could be the result of strong leaf degradation (i.e. no recent litter fall). In other words, as the superficial sediment receives the products and detritus of the litter degradation (such as free LCFAs or LCFAs associated with lignin), high LCFA content in sediment could traduce high detritus releases as a result of enhanced litter degradation in relation to a higher bacterial activity. In agreement, previous studies pointed out that litter from *Rhizophora stylosa* is hardly degradable (Lacerdata et al., 1995), mostly because of the presence of lignin (frequently observed in microscopic sediment observations), which decomposes very slowly (Mfilinge et al., 2003) and is degraded only by few bacteria (Bhat et al, 1998), releasing even more LCFAs (long to degrade) able to remain for very long time (from weeks to years; Mfilinge et al., 2003) in sediment.

Litter in the *Rhizophora* stand sediment was most probably *in situ* formed (i.e. from local trees) and is most likely weakly exported because this area (especially at stations 30, 32, 37, 38 and 39) exhibits a dense web of roots that entraps the litter. This contributes to spatial differences in term of OM quality within the FAO mangrove, especially during NAP. The presence of litter markers (LCFAs) on saltpan and at the seafront results probably partly to its exportation as proposed by Meziane and Tsuchiya (2000) who observed that the mangrove OM is exported towards the tidal flat up to hundreds meters away. This exportation from the *Rhizophora* stand is most probably driven by

tidal flow. Thus, particulate OM might be accumulated at the seafront and in upstream parts where lagoon water remains stationary at low and high tide, respectively. The relative decrease of LCFAs contribution on saltpan and at the seafront from NAP to AP suggests an enhanced colonization of the litter by microorganisms (source of other FAs), such as bacteria or fungi. Indeed, organically rich farm effluents could enhance their growths and activities and therefore improve litter degradation (Ziemann et al., 1992; Jones et al., 2001).

IV.3. ENHANCEMENT OF FUNGAL ACTIVITY IN DECAYING LEAVES AND ADDITIONAL ANTHROPOGENIC ORGANIC MATTER INPUT

The polyunsaturated fatty acids (PUFAs) 18:2ω6 and 18:3ω3 are very abundant (about 8-17 % and 17-30% respectively) in mangrove leaves of *Avicennia marina*, *Rhizophora stylosa* (Meziane et al., 2007) and in *Salicornia sp* (about 30% in *Saliornia europa*; Meziane et al 1997). These FAs usually indicates the terrestrial material origin in marine sediments (Budge and Parrish, 1998). The monounsaturated fatty acid (MUFA) 18:1ω9 is also highly detected in mangrove leaves (Meziane et al 2007). It has been recently discovered that these 3 FAs were also abundant in thraustochytrid (fungal strains) that colonizes mangrove leaves (Chen et al 2001, Fan et al., 2001). In addition, FAs 18:2ω6 and 18:1ω9 are the two main FAs found in food pellets used in the shrimp ponds. Consequently, these FAs were highly detected in the water discharges (Appendix F).

During NAP (i.e. summer) FAs 18:2ω6 and 18:1ω9 were most probably originated from mangroves leaves and fungal activity associated in decaying litter, as they were detected mostly at the surface sediment of *Rhizophora* stands where litter fall was maximum (figures III.13a and III.14a). The comparatively lower contributions of the 18:2ω6 on saltpan and *Avicennia* sediments were probably due to the rapid degradation affecting *Avicennia marina* litter (Lacerda et al., 1995). Additionally, crab activity also contributes to this degradation by feeding on the litter (Meziane et al., 2006). Indeed, *Uca sp* has been densely reported in FAO mangrove particularly in saltpan and *Avicennia* zones with burrow density reaching up to 20 and 60 burrows per m² respectively.

During AP, the high contributions of the FAs 18:2ω6 and 18:1ω9 to total FASS (Fatty Acid of the Surface Sediment) composition and their high concentrations are probably partly due to an exceptional fungal growth, as nutrients (from effluent discharges) are more available and thus enhances fungal activity and sporulation on decaying leaves (Suberkropp 1995; Gulis and Suberkropp 2003). Moreover, the FA 16:1ω5, usually detected in Arbuscular mucorrhizal fungi, which inhabits saline and anaerobic environment such as *Rhizophora stylosa* roots (Miller and Bever 1999), is largely detected in most of mangrove trees (Wang et al., 2010). In FAO mangrove, relative contribution of this FA as well as its concentration significantly increased from NAP to AP in the *Rhizophora* sediment at the inner bay, and especially in the zone close to the effluent discharge (dyke effluent d

shown on figure II.1 and II.3). This increase is another argument that confirms the enhancement of the fungal biomass during the release of nutrient-rich effluents into the litter layer.

FAs 18:2ω6 and 18:1ω9 did not exhibit the same pattern of distribution than LCFAs during AP since they highly contributed to total FAs of surface sediment everywhere in the mangrove. Additionally, given that the decomposition rate of UFAs (i.e 18:2ω6 and 18:1ω9) is higher than SAFAs (i.e. LCFAs; Mfilinge et al 2003) it appears that the presence of such a large amount of UFAs cannot only be due to the litter degradation (and to microbial activity associated), but also to additional sources (i.e. pond effluents) that significantly enrich mangrove sediments. Several authors have also reported an anthropogenic origin of 18:2ω6 such as Napolitano et al. (1997) in Bahia Blanca (Argentina) and Meziane and Tsuchiya (2002) in Okinawa (Japan). In the present case, effluent discharges on saltpan (and overflows from channel) could easily explain that both FAs were highly detected on saltpan and that the 18:1ω9 exhibited high concentration at the main output zone 'c' (South West, figure III.13b) of the K pond during AP. To resume, two sources could result in the presence of the 18:2ω6 and 18:1ω9 in the surface sediment: effluent themselves and microbes, which activity and growth have been enhanced by nutrient-rich effluents.

Given that the 18:2ω6 is the biosynthesis product of the 18:1ω9 in plants and in some animals (Dewik et al., 2009), effluents could enhance the activity of benthic (micro)organisms and therefore their ability to synthesis 18:2ω6, adding another potential source for this FA in the sediment OM. Conversely, high levels 18:1ω9 could be due in turn to the microbial degradation in sediment of the 18:2ω6. The contribution of 18:1ω9 to sediments diminished from saltpan to the forest during AP compared to other FAs (such as the 18:1ω7 or the 18:2ω6, figureIII.4). This pattern suggests that OM released from effluents is deposited on saltpan and mostly contributes to OM pool at the output zones, and that in the forest organisms use this OM. Among these organisms, *Uca sp* crabs are able to biosynthesize the 18:2ω6 (Hall et al., 2006).

The tide current within the mangrove can also influence the spatial distributions of the FAs 18:2ω6 and 18:1ω9. The low tide current in relation with the high retention time of water lagoon within the mangrove could permit wastewater particulate OM settlement close to the discharge zones, as both FAs released from effluent remained in high concentration in these areas. Maximum concentrations were actually detected at the west sides of the output zones suggesting a clockwise flow in the bay. On the figures III.13b and III.14b it appears that effluent POM is particularly deposed around the station 34 (south west of the dyke outflow d). This accumulation is thought to be the result of the tide current deceleration (when the sea rises) after flowing through the narrow formed by the dyke (the narrow passage induce a current acceleration; Fieux, 2010) coupled to the presence of high pneumatophore density (favoring particulate retention). However, part of the effluent OM is re-distributed over the mangrove area by tide balancing as they are detected everywhere during AP (in addition to biosynthesis and degradation processes) especially at the seafront. The exportation to the

seafront occurred quickly, probably over a tide cycle, since PUFAs rapidly degrade (Mfilinge et al., 2003). Presence of these UFAs at the seafront clearly suggests that effluent OM is exported to the lagoon.

IV.4. CHANGE IN PHYTOPLANKTON COMMUNITIES AND GROWTH OF MACROALGAE

The PUFAs 18:3ω6, 20:3ω6 and 20:4ω3 are biosynthesized in both macroalgae (Kharlamenko et al, 1995, Meziane et Tsuchiya 2000) and microalgal (Napolitano et al., 1990; Khozin et al., 1997). However, the absence of macroalgae on the mangrove sediment (only few phanerogams were observed at the seafront; Pers.Obs.) suggests that these FAs have a phytoplanktonic origin.

Contribution and concentration of FAs 18:3ω6 and 20:3ω6 halved from NAP to AP, as they were about 6.17 ± 4.63 $\mu g.g^{-1}$ *dw* during NAP and about 3.14 ± 3.33 $\mu g.g^{-1}$*dw* during AP (in average; se figure III.10). The correlation found between these FAs and chl-*a* concentration in the sediment over the mangrove area confirms that 18:3ω6 and 20:3ω6 are originated by primary producers. Thus, it seems that farm activity inhibit the growth of some phytoplanktonic communities outcompeted by other organisms.

Despite its sharp decrease from NAP to AP, which is in agreement with the seasonal pattern of primary production previously documented in the intertidal superficial sediment of the same Bay (St Vincent, New Caledonia; Baron et al., 1993), primary production remains high at both periods. Indeed, chl-*a* concentrations in the FAO mangrove sediment were higher (16.87 ± 11.40 $\mu g.g^{-1}$ *dw* and 12.07 ± 9.57 $\mu g.g^{-1}$*dw* in average during NAP and AP respectively) than those previously found in other estuarine mangroves (see review in Alongi et al., 1992) where they were <5$\mu g.g^{-1}$ *dw*. This clearly confirms that the primary production in a mangrove receiving shrimp farm effluents is enhanced all over the year as previously observed by Trott and Alongi (2000), and McKinnon (2002). The lower primary production detected at both periods within the *Rhizophora* compared to others stands (figure III.1 a and b), where trees exhibit the highest sizes and create a thick canopy, is most probably linked to the difficult light-accessibility for the phyto-organisms at the surface sediment.

During AP the maximal primary production was mostly detected inner bay as shown by markers (i.e. chl-*a* and FAs). Spatial distributions of 18:3ω6 and 20:3ω6 (figure III.10b), with maximum mainly surrounding output zones during AP, suggest that nutrient availability originated from the shrimp farm release is useful for phytobenthos. Thus, the space and grazing pressure are more probably the limiting parameters that could explain the diminution of the primary production observed from the NAP to AP (Martin, 1968). The decrease (ca.50% decrease) of PUFAs markers from NAP to AP of these phytobenthic communities did not equal the decrease of chl-*a* concentrations in the sediment (ca.28% decrease). It appears therefore that a bloom of other photosynthetic phytobenthos communities may have occurred, which could maintain a high chl-*a* contents in the

sediment. This could explain the weak chl-*a* seasonal difference in spite of the strong decrease in 18:3ω6 and 20:3ω6. Additionally, high concentration of chl-*a* could partly traduce the presence of fresh leaves on the sediment, and the increase of litter degradation outlined above, might corresponds to the decrease of fresh leaves and therefore of chl-*a* from NAP to AP.

Diatoms can be assessed in aquatic environment with the presence of the PUFA 20:5ω3 (Meziane and Tsuchiya 2000) and/or the ratio 16:1ω7/16:0 (Napolitano et al, 1990; Mudge et al 1998; Dalsgaard et al, 2003). The higher the value of the ratio is, the higher diatoms contribute to SOM. Another essential FA, 22:6ω3, indicates preferably dinoflagellates in the environment (Napolitano et al, 1997; Dalsgaard et al, 2003).

From NAP to AP, the general rising of relative contributions and concentrations of 16:1ω7 in contrast to 16:0 which dropped (figure III.8) and 20:5ω3 (figure III.9) indicate an increasing biomass of benthic diatoms in the mangrove sediment and could easily explain the high chl-*a* content in the sediment during AP. Indeed, diatoms were found in all vegetation stands collected during an active period of the shrimp farm (figure III.20). This bloom, usually observed in summer within comparable biotopes (Meziane and Tsuchyia, 2000; Meziane et al, 2006), appeared therefore stimulated by nutrient inputs (i.e. released by effluents), and confirms the competitive behavior of diatoms in a nutrient-rich environment (Bennion, 1995). Diatoms were also directly exported by the shrimp ponds to the mangrove as suggested by the presence of the 16:1ω7 (and in lesser proportion of the 20:5ω3) in the effluents leaving their footprint in the surface sediment (figure III.7b and III.9b).

During NAP, proportions of both FA were higher in the wet *Rhizophora* stand sediment in comparison with the other defined zones whereas during AP, diatoms largely spread over the mangrove sediment. Indeed during AP diatom markers highly contribute to saltpan FASS compositions and particularly to its output zones, where 16:1ω7 was the most abundant FA. Diatoms less comparatively contribute the *Rhizophora* stand OM composition, which is probably more appropriate for bacterial growth than for algae because of its high canopy cover resulting in a lower light availability, more anoxic sediments and denser roots web.

The spatial repartition (and spreading from NAP to AP) of diatoms could be explained by the pattern outlined in a laboratory setting by Patterson (1990) and in field by Trites et al (2004) who stated that the wetter the sediment is, the highest diatoms density is. Initially the *Rhizophora* stand is a wet area and saltpan is formed of dry sediments (cracks are observable at low tide), but effluent discharges clearly (observable on field) load the saltpan sediment of water. In addition, the non limiting nutrient and light factors at this zone contribute to diatoms growth.

The FA 20:4ω6 presents in a large range of phytoplankton (included in diatoms; Napolitano, 1998) increased from NAP to AP also accounting for the diatom bloom hypothesis. Note that crabs could be involved in the 20:4ω3 increase as grapsidae crabs (found in the *Rhizophora* stand) synthesize this FA (found in feces) from the 18:2ω6 and 18:3ω3 (Ruess et al 2002) highly available

during AP. The ratio 20:5ω3/22:6ω3 is an interesting tool to observe the dominance of diatoms in sediment compared to dinoflagelattes proposed by Budge and Parrish (1998). During both periods, diatoms dominate mangrove sediments, however during AP, dinoflagellates were more detectable in the surface sediment than during NAP.

All these observations confirm that anthropogenic activity favors the diatom growth. Indeed, diatoms are largely used as a marker of environmental quality disturbance (among others: Patrick, 1973; Kelly et al., 1998; Harding et al 2005).

IV.5. BACTERIAL DEVELOPMENT

The FA 18:1ω7 and the odd-branched (especially the *iso-anteiso*-15:0 and 17:0) are classically used to assess the bacterial contribution to the surface sediment (Carrie et al, 1998; Meziane and Tsuchiya, 2002, Dalsgaard, 2003; Dunn et al., 2008). The monounsaturated 18:1ω7 indicates bacteria living in anaerobic as well as aerobic conditions (Edlund et al 1986; Pinturier-Geiss et al. 2002), whereas the odd branched have been specifically ascribed sulfate reducing bacteria (SRB; Canuel 2001; Pinturier-Geiss et al., 2002). Some other FAs, detected in minor proportions in the FAO mangrove sediments, are present in bacterial composition such as the branched the 17:1ω7 (Dowling et al., 1988) and 10Me 16:0 in SRB (Findlay et al 1998). Bacterial markers (i.e. 18:1ω7, odd-branched and 10Me 16:0) did not follow the same patterns of spatial distribution in concentrations and in relative contributions, which confirms that they are not synthesized by the same type of bacteria. Bacteria source of 18:1ω7 will be qualify by the acronym AB and those source of odd-branched and 10 Me 16:0 will be qualified by SRB. In the FAO mangrove sediment, all these FAs most probably indicate the presence of anaerobic bacteria.

Indeed, mangroves are known to be mainly characterized by two organic matter decomposition pathways, aerobic respiration only present in a few mm of the surface sediment, and by anaerobic sulfate reduction (Alongi, 1988; Kristensen and Alongi, 2006). The sediment of the forest is reduced already at the surface and exhibits a black colour whereas in saltpan the first millimeters are oxidized giving a brown color to the surface sediment after which sediment reduces. Thus, this environment could sustain shallow sulfate reduction processes, above the first cm sampled. In summer, high temperatures enhance litter degradation, which in turn contributes to reduce the sediment. In autumn, OM accumulation favors anaerobic metabolisms as well as sediment reduction (Fenchel et al. 1998). In the present study, winter corresponding to the AP that exhibits high degradation (see above) processes favoring sediment reduction. In a general way, AB were more detected at both periods in the *Rhizophora* stand (higher 18:1ω7 contributions), which is known to have a more reducing sediment and favored sulfato reducing processes (Alongi et al., 2000).

Throughout the year, sediment colonization by bacteria is improved by crab activities. Indeed, crab consumption reduces litter into small particulates (~200µm) increasing surface area to volume

ratio of the litter and enhancing penetration into detritus (Werry and Lee, 2005). Bacteria growth is also encouraged because crab feeding incorporates some nutritionally important PUFAs (as 18:2ω6) in the litter reliable for bacteria (hall et al., 2006). During NAP as well as during AP, *iso* and *anteiso*-15:0 distribution (tables III.2 and III.3) and concentrations (figure III.11) indicate that SRB always contribute significantly to the OM pool whereas AB contribute more greatly to the pool of organic matter during AP. High bacterial number are usually found in tropical mangrove sediments during winter (Alongi, 1988), but such a high increase of AB (figure III.12b) from NAP to AP could easily be the consequence of shrimp farm wastewater outputs as well. Indeed, a previous study reported that shrimp feeding and activity induces bacteria growth in pond (Avnimelech and Rityo 2003), which are probably exported to the environment through effluents. In agreement, FAO effluent waters were rich in 18:1ω7 (about 10% of water FA composition). Additionally, the higher relative contributions of AB and SRB markers to output zones (especially in the *Rhizophora* stand sediment) during AP support this hypothesis. This could also reveal that bacteria proliferate from the output zone and that their growth is directly enhanced by nutrient discharge.

IV.6. DEGRADATION OF ORGANIC MATTER AND RECOVERY

During AP, more FA species were detected than during NAP. However, the FA combinations of sediment samples collected during AP were more similar one another than those collected during NAP (MDS, figure III.3). Indeed, dissimilarities in FASS composition between stands defined (i.e. saltpan, *Avicennia* stand …) was higher (20%) during NAP than during AP (11%). This could indicate that effluent released from shrimp farm indirectly homogenizes the sediment FA composition in addition to enrich it. Indeed it has been found in this study that FA composition of the sediment collected on saltpan at the effluent output zones during NAP could not be differentiated from the sediment sampled in the *Avicennia* stand (which is initially a more productive area), whether it is closer or farther from effluents.

The FA compositions of sediments collected within the forest located at the inner bay (i.e. *Rhizophora* plus the *Avicennia-Rhizophora* interphase, inner bay) during NAP are comparable (70% similar) to the FA compositions of the entire mangrove sediment during AP (figure III.3). Indeed the *Rhizophora* stand (inner bay) during NAP is characterized by a high contribution of diatoms, bacterial FA markers as well as the anthropogenic 18:2ω6 and 18:1ω9 FA markers. The *Rhizophora* stand was the one which FASS composition less change from NAP to AP (SIMPER dissimilarity: 17%) contrarily to others (such as saltpan: 47%). This could be interpreted in two ways. Firstly, the high productivity of the *Rhizophora* area (Clough, 1992) is reached by the other zones (i.e. saltpan, *Avicennia* stand…) because of nutrient supply by shrimp farm during AP. This means that the forest at the inner bay could be lesser affected by the farm activity; or secondly that the forest at the inner bay is irreversibly affected by shrimp farm activity whereas the productivity of other zones are lowers during the NAP. The assimilation capacity attributed to mangrove forests (Tam et al., 1993; Gautier et

al., 2001) tips the scales for the former hypothesis. Therefore the detection of the 18:1ω9 and of 18:2ω6 in the *Rhizophora* stand during NAP could be attributed to leaf detritus and fungi associated (Meziane et al., 2006; Dewick, 2009) as proposed above. The hypothesis of a rapid (1-2 months) recovery after an enhanced primary production of mangrove ecosystem receiving shrimp farm effluent is indeed reported in the litterature (Thomas et al., 2010). However, in the present study, the high chl-*a* and pheo content during both winter and summer (compared to previous studies) suggests that shrimp farm wastewater releases have a long-term effect on sediment primary production.

Bacterial growth plays an important role in the degradation of litter leading to a bloom decline of microalgae (Fukami et al., 1983). OM consumption by bacteria throughout the active period of the shrimp farm could be the reason of the decrease of most algal FAs (18:3ω6 and 20:3ω6) from NAP to AP and of diatoms from AP to NAP. Once the OM is degraded, bacterial communities decline. It is the case for the saltpan area were either diatoms or bacteria were detected during NAP. Consumption of OM by bacteria could explain the degradation of chl-*a* (which results in phaeopigments) as both bacterial FA markers and phaeopigments were significantly higher at the output zones. However, high phaeopigment concentrations on saltpan (figure III.1 c and d) at both periods suggest, given that bacteria were not so much detected during NAP, that degradation could also be performed by zooplankton. Phaeo measured in the present study were in the same order of concentration than those found in literature (Alongi, 1988; Rajesh et al., 2001). The gradient from pond to lagoon of phaeopigment concentration suggests that chl-*a* degradation was enhanced close to pond (K) at both periods and the low concentration of phaeo outer bay, (where chl-*a* concentration was high) could be the consequence of out leaching and exportation processes (Alongi 1988). The decrease of the phaeo concentrations from NAP to AP in the study context can hardly be attributed to an increase in degradation activities as phaeo are finally degraded with the dead matter by bacteria and fungi (GIP Loire estuaire) that are highly detected in the FAO sediment during AP.

IV.7. ISOTOPES INDICATIVE OF A MANGROVE-DERIVED ORGANIC MATTER AND EFFLUENT DISPERSION

Organic carbon to nitrogen ratios (C:N) have been reported useful in differentiating between organic matter sources in estuaries (Jaffé et al., 2001) and in mangrove sediments (Meziane and Tsuchiya, 2000; Bosire et al., 2005). Indeed, sedimentary OM characterized by low C:N suggests microalgae or bacteria inputs (C:N=4-10), and high C:N indicates terrestrial plant origin (C:N> 20; Meyers, 1994). FAO mangrove sediment C:N ratios were in the order of magnitude of those measured in mangrove sediment or in receiving mangrove-derived OM (i.e. mangrove leaf, fruit and wood) environment (Meziane and Tsuchiya, 2000; Jaffé et al., 2001).However its range was narrower (from 8 to 30 against 5 to 280). Typical values for higher plants (C:N> 20) were observed within the forest sediment at both periods, whereas the lowest values were observed on saltpan and at the seafront at both periods (figure III.17). Lowest C:N (C:N=8-10) within the mangrove sediment did not

correspond to region of high chl-*a* and phaeo concentrations, and are maybe too high to indicate algal communities. It could traduce the presence of cyanophycae (C:N = 9.5 ± 0.2) or indicated diatoms (C:N = 8.2 ± 2.1; not correlated with chl-a) and bacterial communities at least during AP, but this organisms were scarce on saltpan during NAP. Therefore low C:N ratio detected on saltpan during NAP could principally indicate a highly degraded mangrove-derived litter as it have been shown that degradation of OM in marine sediment tends to lower C:N (Müller, 1977), and that during the degradation of vascular plant detritus C:N decreases asymptotically with time, to approach C:N about ≈10 (Meyers, 1994). Values measured in the forest (C:N=25-30) probably indicate a (early) degraded mangrove OM, since C:N in freshly exported mangrove OM (to intertidal flat) has been measured in previous study to be about 40-83 and can reach up to 200 (Boto et Bunt, 1981; Meziane et Tsuchiya, 2000). Low C:N at both periods, particularly at the seafront, could also be the result of a mixing between marine-derived and terrestrial OM as suggested by Jaffé et al. (2001). Relatively low C:N (<30) in parallel to high LCFAs content in sediments detected at both periods confirms the presence of mangrove-derived OM (partially) degraded.

The stable carbon isotope ($\delta^{13}C$) composition of coastal wetland and marine plant has been also used for the differentiation of OM sources (Kenicutt et al., 1987; Fleming et al., 1990; Jaffé et al., 2001). However contrarily to C:N, it is not an indicator of the anaerobic degradation state as it remains relatively unchanged during bacterial colonization and leave decomposition (Kennedy and al., 2004; Werry and Lee, 2005) even after a long-time microbial (anaerobic) decomposition (Haine, 1976). $\delta^{13}C$ values of mangrove detritus have been estimated to be about -26‰ by Fleming et al (South Florida mangrove estuary; 1990) and from -30‰ to -24‰ in coastal sediment ecosystem receiving mangrove plant OM (Hemminga et al., 1994; Lee, 2000). The large content of depleted $\delta^{13}C$ (from -20‰ to -28‰; figure III.15) in the FAO mangrove sediment at both periods of the present study confirms the high contribution of mangrove-derived OM to the sediment. $\delta^{13}C$ becomes significantly depleted from NAP to AP which traduces an increase in mangrove litter inputs (degraded or not). The combination between a lower $\delta^{13}C$ content during AP than during NAP, the high LCFAs, C:N ratio and FAs markers of bacteria during AP could still agree the previously outlined hypothesis, which specifies that sediment was characterized by a highly degraded mangrove-derived litter during AP. Saltpan and *Avicennia* stand sediments, were more $\delta^{13}C$-enriched than in the other mangrove areas, confirming the lower influence of plant litter accumulation there.

In the present work, the $\delta^{13}C$ for diatoms has been estimated at -25.1±0.8 ‰, which is lower than values found by Rodelli et al. (1984) for *Navicula sp.* and *Nitzschia sp.* (about -14.8 ‰ and -19.8 ‰ respectively; observed on slide: figure III.20). Diatoms are therefore mainly present in the forest during NAP and spread during AP (as shown before). Lower values of the $\delta^{13}C$ probably indicate the presence of cyanophycae ($\delta^{13}C$= 17.9 ± 2.1‰), or other phytoplankton (when $\delta^{13}C \geq$-20‰) notably on saltpan during NAP where chl-*a* concentrations were high.

Stable isotope $\delta^{15}N$ has been largely used to identify OM sources, and especially from shrimp farm inputs (Primavera 1993; Preston et al., 2000; Costanzo et al. 2004). This marker not only indicates the OM origin but also the degree of diagenetic alteration (Thornton and McManus, 1994). In the forest sediment and at the seafront, $\delta^{15}N$ values measured at both periods are in the same order of magnitude than those reported for typical mangrove-derived OM (about 2‰; Boutton, 1991; Coffin et al., 1994). During NAP, the $\delta^{15}N$ gradient observed from pond to lagoon (figure III.16a) is the result of the difference in isotopic sources contribution to the sediment. Indeed, the highest values were detected on saltpan because of the presence of *S.australis* ($\delta^{15}N$=6.5 ± 1.1‰) followed by the influences of *A.marina* ($\delta^{15}N$=4.0 ± 1.7‰) to the *Avicennia* stand sediment and of the *R.stylosa* ($\delta^{15}N$=2.4 ± 2.0‰) to the *Rhizosphora* stand sediment. The significant stable isotopic changes from NAP to AP are mainly drive by the $\delta^{15}N$ and occurred inner bay from the dyke. Negative shift in $\delta^{15}N$ could indicate reinforcement of anaerobic sediment condition (Terane et al., 2000) and consequently of high anaerobic bacteria colonization previously outlined by FA markers. Additionally, as diatoms bloomed during AP, lower $\delta\ ^{15}N$ value in sediments could easily be due to high N_2-fixation by phytoplankton (and cyanobacteria) which produces a more depleted $\delta\ ^{15}N$ OM, or to a recycling of ammonium ($NH4^+$; Montoya, 2007). It appears, in agreement with previous authors quoted (Primavera 1993; Preston et al., 2000; Costanzo et al. 2004), that $\delta^{15}N$ is largely imported from pond as the concentration in the sediment located at the output zone were higher than to the rest of the mangrove sediment and the figure III.16b well shows the trace left by effluents in the surface sediment.

The mixing of sources (marine and/or terrigenous) of organic matter in sediments can be traced by a linear correlation between $\delta\ ^{13}C$ to $\delta\ ^{15}N$ (Peters et al., 1978). When comparing the $\delta^{13}C$ *vs* $\delta^{15}N$ in sources analyzed it appeared first that all the mangrove area is influenced by plant litter fall (figures III.18a,b,c and III.19a,b,c) pointing out an efficient export of the OM. Secondly, the presence of diatoms influence more forest sediment isotopic signature during NAP and they bloom everywhere during AP as outlined by FA markers. During AP, effluent isotopic signature was detected everywhere showing therefore their dispersion and the redistribution by tide, as well as their exportation to the seafront. They were, however, more assimilated in the *Rhizophora* stand (figure III.19 d) in agreement with the assimilation capacity attributed to mangrove forests in literature (Tam et al., 1993; Gautier et al., 2001). Food shrimp is not totally consumed in ponds (figure III.19e) and is probably used the organisms blooming when moving away from pond as its influence becomes lower.

CONCLUSIONS

The present work highlights firstly several tendencies about the organic matter nature and distribution in the FAO mangrove sediment and their changes when receiving effluents from the adjacent shrimp farm. Secondly, the evolution of the benthic OM in mangrove ecosystem is characterized by comparing our present data with previous research projects. Major conclusions reached throughout this study could be sum up as following:

1. When there is no release of shrimp farm wastewater (NAP) FAO mangrove sediments are characterized by a relatively degraded OM, and mangrove tree litter falls are a major source of OM in the sediment. During shrimp farm activities (AP) food dispatched in pond (not totally consumed by shrimps), and microorganisms and from the pond are also discharged in the mangrove through effluents, leading to a renewal of the mangrove benthic OM during AP.

2. The fate of the "anthropogenic FAs" allowed us to conclude that the current direction influence the exportation of the discharged OM which settles and remains in a great proportion close to the output zones but is also partly exported to seafront over a tide cycle.

3. Discharge of nutrient-rich effluents induces a bloom of competitive diatoms, and a slight increase of phytoflagellates. Conversely, some other microphytobenthic communities decline, either inhibited by diatoms growth or because of the natural algal seasonal variations (as reported in the literature).

4. Fungal and especially bacterial activities are highly enhanced because of the nutrient supply from effluents. Part of the huge bacterial bloom is to be ascribed to a seasonal variation but in any case it has to be related to a higher degradation rate of the litter (of mangrove trees) and to the additional OM during farm activity. In consequence, the OM is potentiality remineralized and therefore available for other organisms.

5. Primary production is enhanced during both AP and NAP by shrimp farm activities, in response to a direct environmental enrichment during AP, and to *a postiori* impact of this enrichment during NAP.

6. Farm activity tends to homogenize the nature of the OM in the mangrove sediment towards a *Rhizophora*-like productive area and the OM nature of the *Rhizophora* stand sediment is the less modified by effluent discharges. Because of the high bacterial content there, this zone (the only one in which isotopic effluent signature was low) has most likely a higher assimilation capacity than the rest of the mangrove.

To conclude, shrimp farm effluent discharge induces the growth of various micro-bentic organisms, which participate to the degradation of the additional OM inputs. However remineralization, could easily favors, in cascade, the growth of some organisms (which could inhibit the growth of others) even far after the farm activity stops. In any case, the relative assimilation efficiency of OM by the *Rhizophora* does not avoid additional inputs (i.e. from shrimp farm) reaching the seafront. Thus, the retention/filtration power ascribed to mangrove ecosystem has to be reconsidered.

Comparing the present results with the OM sources and distributions in pristine mangrove sediments, during the same periods would have lifted the veil on uncertainties, such as the potential existence of a bacterial growth in winter or the recovery to primary production ambient levels.

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ABBREVIATIONS

AD: average dissimilarity between two groups, given by the SIMPER analysis.

Av/Rh: *Avicennia-Rhizophora* interphase

Az: *Avicennia* zone

BFA: Branched Fatty acid (e.g *iso*-15:0)

Chl-*a*: Chlorophyll-a

FA: Fatty Acid

FAO: Ferme Aquacole de la Ouenghi (Name of the shrimp farm of the present study)

FASS: Fatty Acid of the Surface sediment

LCFA: Long-Chain Fatty Acid (≥24:0)

MUFA: Monounsaturated Fatty Acid (one double-bond)

NC: New Caledonia

NOz : Non Output zone

OM: Organic Matter

Oz: Output zone (i.e. effluent discharge zone)

Phaeo: Phaeopigments

POM: Particulate Organic Matter

PUFA: Polyunsaturated Fatty Acid (at least two double-bonds)

Rhi: *Rhizophora* stand, located at the inner part of the bay

Rho: *Rhizophora* stand, located at the outer part of the bay

RhSF: *Rhizophora* stand, located at the seafront

SAFA: Saturated Fatty Acid (without any double bond)

SOM: Sediment Organic Matter

Stn: station

SVeg: Mixed zone of saltapan and vegetation (i.e. *Avicennia*, *Rhizophora* stands)

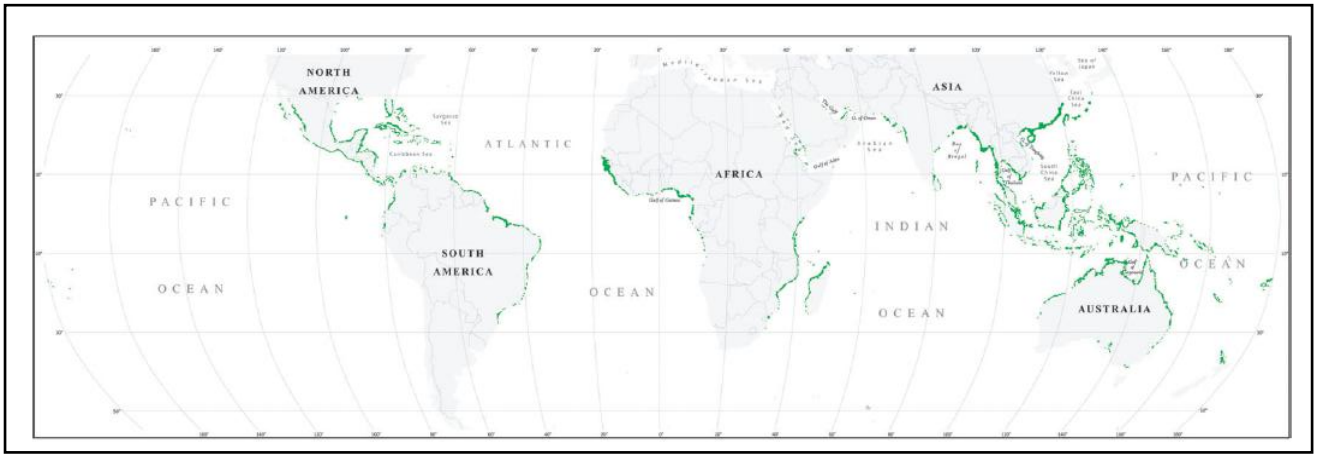
Sz: Saltpan zone

UFA: Unsaturated Fatty Acid (at leat one double bond)

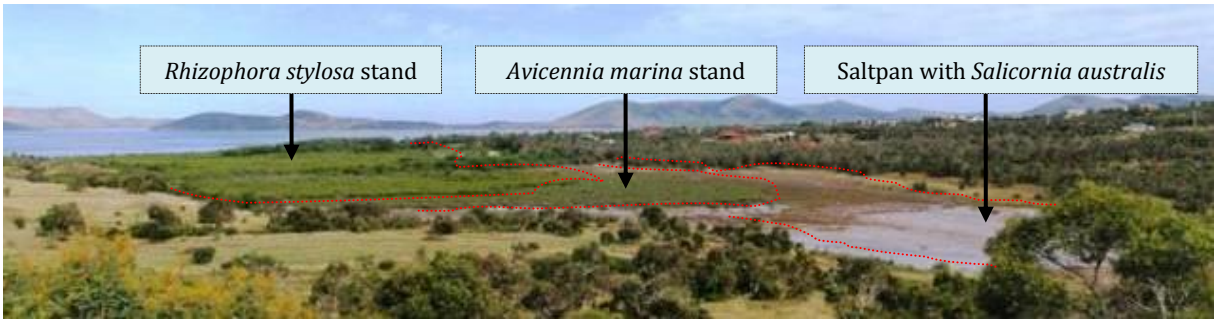
X:YwZ: Nomination of the fatty acids, X refers to the number of carbon, Y to the number of double bonds, and Z to Z the position of the ultimate double bond from the terminal methyl.

APPENDICES

Appendix A: Mangrove worldwide distribution in 2000 (Giri et al., 2010)

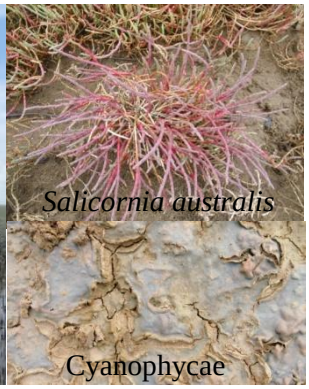


Appendix B: Zonation of the mangrove ecosystem



Rhizophora stylosa

Avicennia marina



Appendix C: The mangrove of Voh (picture of Yann Arthus Bertrand)

The mangrove of Voh is characterized by a particular topography. An *Avicennia* and a saltpan stands elevate at the midst of a *Rizophora stylosa* area where the height is higher. This change in topography and therefore in salinity favors the growth of saline-adapted species in spite of *Rhizophora stylosa.*

Appendix D: Fauna of Mangrove ecosystems

Periophtalmus sp

Fiddler crab

Mud crab

Appendix E: Picture of the FAO farm discharging its effluent in the mangrove which trees exhibit different heights (picture from the IFREMER web page)

AppendixF: FA composition of granule given to shrimps and to effluent waters.

Shrimp food				Effluents		
FA names	Mean %	*Std D*		**FA names**	**Mean**	**St D**
12:0	*0,03*	*0,004*		12:0	*0,6*	*0,4*
13:0	*0,01*	*0,008*		14:0iso	*0,8*	*0,4*
14:0iso	*0,01*	*0,007*		14:0	*12,9*	*3,7*
14:0	*1,17*	*0,063*		14:1ω3	*0,3*	*0,2*
15:0iso	*0,02*	*0,007*		15:0iso	*1,5*	*0,3*
15:0anteiso	*0,02*	*0,013*		15:0anteiso	*0,9*	*0,2*
15:0	*0,16*	*0,015*		15:0	*1,0*	*0,2*
16:0	***18,62***	*1,087*		**16:0**	***22,1***	*1,2*
16:1w9	*0,46*	*0,695*		16:1ω9	*0,7*	*0,3*
16:1w7	*0,79*	*0,652*		**16:1ω7**	***10,0***	*5,0*
16:1w5	*0,05*	*0,028*		16:1ω5	*0,8*	*0,4*
17:0iso	*0,12*	*0,038*		17:0iso	*0,3*	*0,1*
16:2w4	*0,19*	*0,066*		16:2ω6	*0,3*	*0,1*
17:0	*0,34*	*0,088*		16:2ω4	*0,6*	*0,4*
16:3w6	*0,15*	*0,058*		17:0	*0,4*	*0,1*
17:1w7	*0,19*	*0,124*		17:1ω7	*0,4*	*0,1*
16:4	*0,59*	*0,495*		18:0iso	*1,5*	*1,4*
18:0	*6,93*	*3,177*		18:0	*4,9*	*2,7*
18:1w9	***10,50***	*3,913*		**18:1ω9**	***15,3***	*5,4*
18:1w7	*0,97*	*0,314*		**18:1ω5**	***5,5***	*0,6*
18:2w6	***42,08***	*1,358*		18:1ω7	*2,9*	*1,6*
18:3w6	*5,55*	*0,358*		**18:2ω6**	***3,8***	*1,3*
FA 1 unidentified	*0,24*	*0,013*		18:3ω3	*4,7*	*3,6*
20:0	*0,22*	*0,055*		18:4ω3	*1,1*	*0,7*
20:1w9	*1,26*	*0,133*		20:0	*0,6*	*0,3*
FA 2 unidentified	*0,12*	*0,022*		20:1ω9	*0,9*	*0,4*
20:3w6	*0,16*	*0,134*		20:1ω7	*0,3*	*0,1*
21:0iso	*0,02*	*0,001*		20:3ω6	*0,2*	*0,2*
20:4w6	*0,43*	*0,023*		20:4ω6	*0,3*	*0,2*
20:0	*0,34*	*0,188*		20:4ω3	*0,1*	*0,0*
20:4w3	*0,09*	*0,011*		20:5ω3	*1,1*	*0,4*
20:5w3	*3,45*	*0,256*		22:6ω3	*1,9*	*1,5*
22:0	*0,29*	*0,086*		A unidentified	*0,7*	*1,5*
22:5	*0,36*	*0,047*		B unidentified	*0,5*	*1,5*
22:6w3	*4,10*	*0,601*		Total	*100,0*	
Total	*100,00*					

Appendix I: Relative contribution of some sources to the sediment of the FAO mangrove.

NAP %	*Salicornia australis*			*Avicennia marina*			*Rhizosphora stylosa*			Diatom			cyanophycea		
Saltpan	*27,7*	*±*	*10,3*	*20,4*	*±*	*11,3*	*15,5*	*±*	*10,1*	*14,8*	*±*	*9,7*	*21,6*	*±*	*11,4*
***Avicennia* stand**	*18,0*	*±*	*9,7*	*19,2*	*±*	*11,0*	*19,5*	*±*	*10,7*	*19,7*	*±*	*10,9*	*23,6*	*±*	*8,4*
***Rhizosphora* stand (inner bay)**	*24,7*	*±*	*8,2*	*20,8*	*±*	*11,2*	*18,4*	*±*	*10,3*	*17,1*	*±*	*9,9*	*19,0*	*±*	*9,5*
***Rhizosphora* stand (outer bay)**	*22,4*	*±*	*9,0*	*22,3*	*±*	*11,4*	*25,2*	*±*	*9,3*	*22,8*	*±*	*9,9*	*7,2*	*±*	*5,5*
***Rhizosphora* stand (seafront)**	*17,4*	*±*	*8,6*	*21,1*	*±*	*10,8*	*21,0*	*±*	*9,3*	*25,8*	*±*	*10,8*	*14,7*	*±*	*6,0*
Avicennia- Rhizosphora	*18,4*	*±*	*10,3*	*19,6*	*±*	*11,5*	*19,0*	*±*	*11,2*	*20,0*	*±*	*11,2*	*23,1*	*±*	*11,4*
Saltpan-*Avicennia* and/or *Rizosphora*	*25,3*	*±*	*9,9*	*21,8*	*±*	*11,2*	*18,1*	*±*	*9,9*	*18,2*	*±*	*10,7*	*16,6*	*±*	*6,8*

AP %	*S.australis*			cyanophycea			*A.marina*			*R.stylosa*			Diatoms			Effluent			Shrimp food		
Salicorne	*9,3*	*±*	*6,2*	*23,2*	*±*	*6,5*	*10,7*	*±*	*7,2*	*8,6*	*±*	*6,2*	*13,4*	*±*	*7,8*	*15,3*	*±*	*7,9*	*19,6*	*±*	*7,0*
Avicenne	*14,0*	*±*	*6,6*	*9,3*	*±*	*4,8*	*15,0*	*±*	*7,7*	*17,6*	*±*	*6,3*	*17,5*	*±*	*7,5*	*16,7*	*±*	*7,3*	*9,9*	*±*	*6,0*
Avicenne-Rhizosphere	*11,7*	*±*	*6,6*	*5,2*	*±*	*3,7*	*16,9*	*±*	*9,3*	*20,6*	*±*	*7,0*	*21,3*	*±*	*9,7*	*19,0*	*±*	*8,8*	*5,4*	*±*	*4,1*
Rhizosphere (inner bay)	*20,3*	*±*	*5,4*	*0,9*	*±*	*0,8*	*20,0*	*±*	*8,5*	*34,7*	*±*	*4,1*	*17,0*	*±*	*6,2*	*5,8*	*±*	*4,1*	*1,2*	*±*	*1,1*
Rhizosphere (outr bay)	*9,6*	*±*	*5,5*	*2,8*	*±*	*2,4*	*15,6*	*±*	*8,9*	*33,7*	*±*	*7,7*	*21,6*	*±*	*9,5*	*13,6*	*±*	*7,7*	*3,1*	*±*	*2,6*
Rhizosphere (seafront)	*12,7*	*±*	*6,2*	*2,9*	*±*	*2,3*	*18,1*	*±*	*9,2*	*25,4*	*±*	*6,0*	*22,2*	*±*	*9,2*	*15,4*	*±*	*7,7*	*3,3*	*±*	*2,8*
Saltpan- Avicenne and/or Rhizosphere	*21,7*	*±*	*7,4*	*4,3*	*±*	*3,2*	*19,1*	*±*	*9,7*	*19,7*	*±*	*6,9*	*16,1*	*±*	*8,7*	*12,1*	*±*	*7,3*	*7,1*	*±*	*4,9*

THE PRESENT RESEARCH WORK HAS BEEN (PREPARED TO BE) PUBLISHED IN:

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