THE COMMUNITY STRUCTURE OF BENTHIC MACROINVERTEBRATES IN THE INNER BRANCHES OF ESTERO SALADO ESTUARY AND GUAYAS RIVER IN ECUADOR

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To
Submitted for the degree of Doctor of Philosophy

HERIOT WATT UNIVERSITY
SCHOOL OF LIFE SCIENCES
SEPTEMBER, 2016.

ABSTRACT

Estuarine ecosystems and watersheds have suffered significant changes in its structure and functioning due to human activities as pollution generated by discharges of domestic and industrial wastewater. This study was conducted on water and sediments samples from the basin of Guayas River and the inner branches of Estero Salado (ES) in Guayaquil city, Ecuador during the period 2007 to 2012 in different phases. In this research were evaluated the relationship between several organic and inorganic pollutants as Total Petroleum Hydrocarbon (TPH), most toxic heavy metals (Pb, Hg. And Cd) and environmental parameters that may influence the invertebrate community structure in Protected and Urban Areas in superficial sediments of branches of Estero Salado. This survey contributes to knowledge of variation of polluted agents over space and time in estuarine sediments. To determine first the composition, abundance and diversity of macroinvertebrates in 43 water bodies in the Guayas province and evaluate the water quality by use the biotic index as (BMWP /Col).To determine changes in the macrobenthic community before and during the bioremediation process in sediments polluted by hydrocarbon in ES.To assess the feasibility of recolonization of benthic macroinvertebrates in artificial substrate on polluted areas in inner branches of ES. The main results showed that 72% of sites surveyed in the Guayas Basin are very polluted by organic matter, four different assemblages were detected in the subwatershed, 84 genera were identified, the most abundance specie was *Melanoides cf tuberculata*, this and others exotic species were registered such as *Pomacea canaliculata* and *Corbicula cf fluminea.* The highest diversity occurred in Bucay, El Triunfo, Simon Bolívar and Chongón counties, places being near of foothills of Coordillera Occidental de Los Andes and Chongón Colonche. The community structure of benthic macroinvertebrates in inner branches of Estero Salado is different along the time and space, the results of this research suggest a significant change in biotic ensemble in Urban Areas where decrease the diversity of native macroinvertebrates and proliferate opportunistic species have replaced characteristic species due to the physical and chemical alteration of the water column and sediment being some of the variables that significantly influence in this change were low salinities (0 psu), decrease of dissolved oxygen concentration (0 mg/L), high level of Total Petroleum Hydrocarbons (145872.7 mg/kg) and heavy metals especially lead (66.54 mg/Kg). This project aimed to develop restoration techniques as bioremediation of hydrocarbon sediment and determined that use of native bacterial consortia of ES did not affect macrobenthic communities and could be an efficient technique if there is removal of pressures and imposed on this habitat. Artificial substrate can increase the native bivalve populations in polluted area in ES.The results of this research have implications for management strategies, conservation and ecological restoration.Besides showed the role the Protected Area as Reserva de Producción Faunística Manglares El Salado as nucleus area in the conservation of few fragments of this emblematic estuarine ecosystem in Ecuador.

DEDICATION

All my effort to honor my God Jehovah and for my loved family Miguel, Arianna and Miguelito. To my parents Targelia and Humberto, my grandparents Targelia, Antonio, Margarita and Miguelito who thought that education and hard work allow achieving ours dreams and who started this route to have a better future. Today two generation happened to reach this degree sought and with God's blessing I am finalizing this stage of my life as a sign that My Lord keeps his promises because God always is faithful.

For my children with love as demonstrating that perseverance, dedication, clear goals in the life and correct decisions help us fulfill our dreams.

ACKNOWLEDGEMENTS

My PhD in UK was parcially funded by the Secretaría de Educación Superior, Ciencia, Tecnología e Innovación (www.senescyt.gob.ec) under the framework of Program Funds CEREPS (Cuenta de Reactivación productiva y Social, del Desarrollo Científico-Tecnológico y de la Estabilización Fiscal) of the Republic of Ecuador, during the presidential period of Dr. Alfredo Palacios in 2006 and sponsored by the Universidad de Guayaquil (UG) (www.ug.edu.ec). Financial support was also provided by a Heriot-Watt University Scholarship.This research work was supported mainly funded SENESCYT and the special upholder to specific topics by the Subsecretaria de Gestión Marina y Costera of Ministry of Environment (www.ambiente.gob.ec) and the Centro de Servicios Técnicos y Transferencia Tecnológica Ambiental de la Escuela Superior Politécnica del Chimborazo (CESTTA)(www.cestta.com.ec/) in relation chemical studies prior and during of bioremediation phase of contaminated sediments in the Estero Salado in the context of the consultancy code CDRE-SGMC-GE-13-2011 ***´´ Estudio a nivel piloto sobre tratamientos de bioremediación de lodos desarrollando y utilizando consorcios bacterianos nativos del Estero Salado´´*** developed during 2011-2012. The Gobierno Autónomo Descentralizado de la Provincia del Guayas (www.guayas.gob.ec) for the economical funds to development to the consultancy code CDC-GPG-DMA-001-12 called ***´´Sistema de Biomonitoreo Acuático de los ríos de la Provincia del Guayas´´*** and Research Center of the Universidad de Especialidades Espíritu Santo (UEES) (www.uees.edu.ec) for the funds for the studies of recolonization of macroinvertebrates using experimental artificial substrates in 2012.

Among the various people who helped in the collection of the specimens and surveys were Raul Zambrano, Julian Pérez, Gisella Andrade and Martha Maldonado. I especially thank my students of School Environmental Science of UEES in the period 2012 in my Ecology Restoration course who helped me with fieldwork.

For the laboratory analysis, there were several institutions that provided technical support (equipment, reagents and installations): ESPOCH-CESTTA assisted with the chemical and physical analysis. Bioelite S.A. (www.bioelite.org) and the Universidad de Especialidades Espíritu Santo provided support for equipment, laboratories and literature to identify macroinvertebrates. I would really like to thank Dr. Wills Flowers

(Prometeo-INIAP) for confirmations of species of freshwaters invertebrates, especially aquatic insects, Dr. Modesto Correoso (Universidad de las Fuerzas Armadas-ESPE) for confirmation of *Melanoides cf tuberculata,* Dra. Matilde Cornejo (UG) for the identification of isopods and amphipods and the Blga. Elba Mora and María Fernanda Arroyo (UG) for revision of some species of gastropods of the Estero Salado.

I also wish to give special thanks to Dr. Luis Troccoli (Prometeo del INP) for your advice, comments and support for statistic analysis. To Edlin Guerra (Universidad de Oriente) and Dr. Juan Cruz-Motta (Universidad de Puerto Rico) who taught me the use of Primer and multivariate techniques and Dr. Fernando Casanoves (CATIE) who attracted me to this beautiful world of biostatistics. To Dr. Carlos Rodríguez and Jahir Duque professors of the Universidad Nacional de Colombia and UEES for leader me in the use of ENDNOTE program and new techniques of research methodology.

I gratefully acknowledge the review of different versions of the manuscript to Dr. James M. Mair, Dr. Luis Troccoli, Dr. Gregorio Bigatti, Dr. Windsor Aguirre, Blga. Elba Mora, Virginia Salvador and the Blga. Milviana Maldonado.

To my friends and professors Blga. Elba Mora, Ing. Guillermo Baños, Dr. Segundo Coello and the Blgo. Xavier Romero because they provided moral sustain when I lost strenngh and pushed me forward to continue with words of encouragement.

I want to say many thanks especially to Dr. James M. Mair for giving me this great opportunity to work on this Ph.D. Thank you for your unconditional support throughout this process and for trusting me.

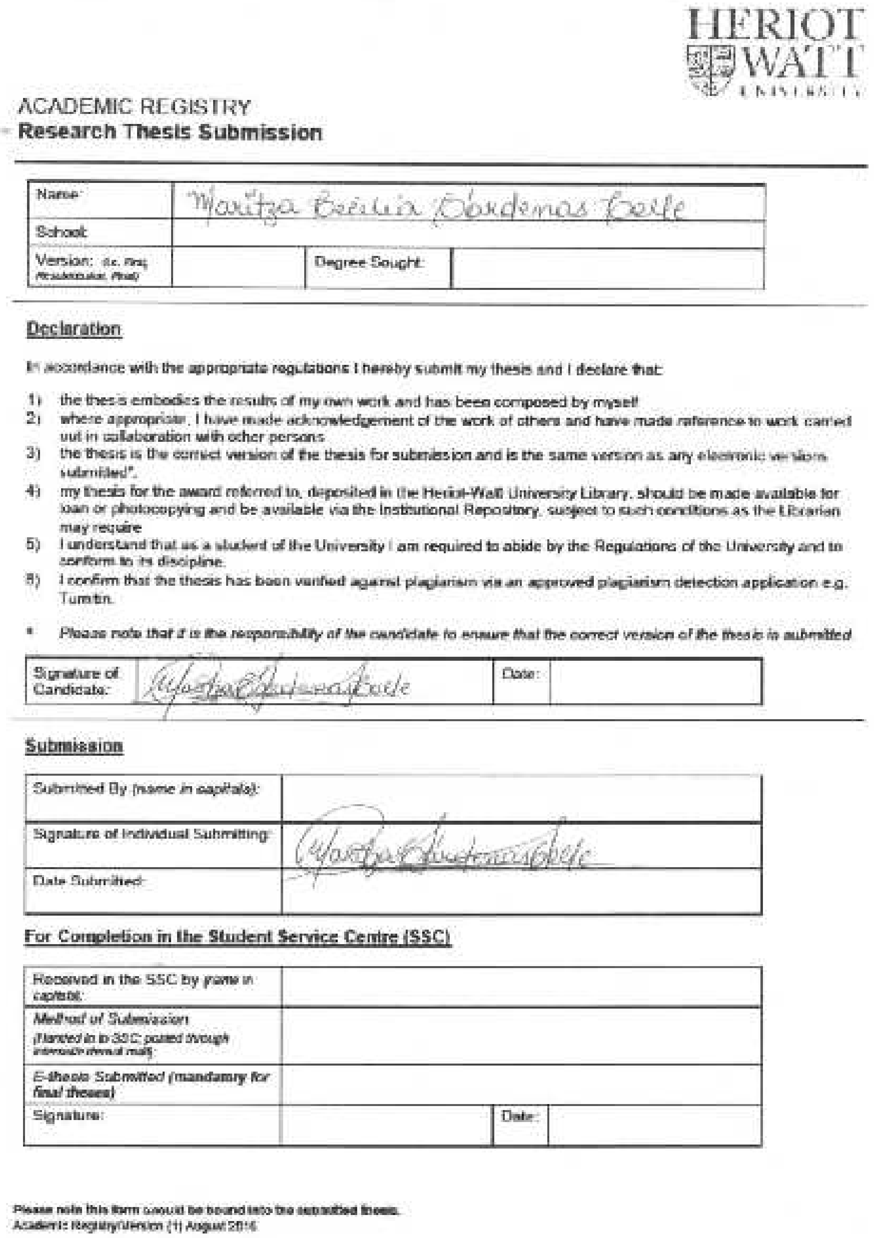
I must also include my sincere thanks to Ruth Duncan and Dra.Alba Lucía Mulford for the emotional hold during my last stay in Edinburgh. I also wish to thank my family in Christ: Sumi, Susan, Rubi, Julie, Emma, Edgard, Apolinario, Cecilia and Sam who gave me their warmth and shared their time with me in Scotland. To my family, my husband Miguel Triviño, my daughter Arianna Triviño and my son Miguelito Triviño that supported with love and patience. To my father Humberto Cárdenas, my dear uncle Flavio Calle and friends for their affection and prayers.

Finally, this work is the result of different financial support from state and private

institutions, physical, intellectual and emotional effort of many people. We worked as a team to get answers to environment problems that transcend beyond the biological knowledge as tangible realities such as the socio-economic area of the most vulnerable social strata, such as a artisanal fisherman and the farmer in Guayas province, who see natural resources losing their splendor, diversity, abundance because of the unsustainable use by those that have the political to make correct decisions and change that story to achieve the long-awaited status of good living (*Sumak Kawsay*).

This culminates only a part of learning, another begins and with humility. I am willing to undertake the new journey. Thank you very much every one.

DECLARATION STATEMENT



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- Cardenas-Calle, M. & Mair, J.M. 2014. Caracterización de macroinvertebrados bentónicos de dos ramales estuarinos afectados por la actividad industrial, Estero Salado-Ecuador. *Rev. Intrópica, Vol* (Acuerdo Ministerial No. 097A Publicado en el Registro Oficial edición especial No. 387), 118-128.

LIST OF PUBLICATIONS IN PREPARATION FOR JOURNAL SUBMISSION BY THE CANDIDATE

- Cárdenas-Calle, M.C., Zambrano, Carvajal, R. Flowers, W. & Mair J.M. 2017. Ensamblages of benthic macroinvertebrates and water quality of subwatershed of Guayas River-Ecuador during 2012-2013. (Chapter 3).Hydrobiology.
- Cárdenas-Calle, M.C., Erazo, R., Troccoli, L. & Mair J.M. 2017. Water and sediments quality and the relation with community structure of benthic macroinvertebrates in the inner branches of Estero Salado Estuary during 2007, 2009,2011 and 2012. (Chapter 4 and 5). Marine Pollution Bulletin.
- Cárdenas-Calle, M.C., Erazo, R., Bravo, V., Salazar, J. & Mair J.M. 2017. Bioremediation of hydrocarbon polluted sediments and the effects of benthic macroinvertebrates community in the inner branches of Estero Salado. (Chapter 5). Marine Pollution Bulletin.
- Cárdenas-Calle, M.C., Pérez, J., Bigatti, G., Troccoli, L. & Mair J.M. 2017. Recolonization of macroinvertebrates in the Estero Salado Estuary using experimental artificial substrates in polluted urban sites in Ecuador. (Chapter 6). Estuaries and Coasts.

Chapter 1. General introduction

The Gulf of Guayaquil is located in the south-west coast of the Republic of Ecuador in South America (Figure 1). This Gulf has an area of 13701km^2 that includes 1990 km^2 of islands and islets and 11711km^2 of water surface (according to studies by the Environmental Consultant Commission of the Presidency of the Republic (CAAM, 1996)). It is divided into two areas; the exterior and interior estuary. The outer estuary is between Boca de Capones and Punta Del Morro at latitude 03°23´33 96´´S and longitude 81°00´30´´W in the Pacific Ocean while the inner estuary is called "***Estero Salado***". The Gulf of Guayaquil is considered to be the most important estuary on the South American coast of the Eastern Pacific. This is due to the high primary productivity encountered there; with the values of annual average of chlorophyll a in the water column of 11.8mg/m^3 in the inner estuary and 54mg/m^3 in the outer estuary (Stevenson, 1981). The Gulf supports three important industrial fisheries; the Tuna fishery (*Thunnus albacores*), shrimp fishery (*Penaeus occidentalis, P. vannamei, P. stylirostris, P. californiensis, P. brevirostris, Trachypenaeus byrdi*) and the fishery of smaller pelagic fish (CAAM, 1996).

The Estero Salado extends from the Northwest of Puná Island in the Morro Chanel (South) near the open water of the Pacific and continues to the inner branches in Guayaquil city up at least 100 km in the continent, through the Babahoyo and Daule rivers (Montaño & Sanfeliú, 2008). It has a length of approximately 66 km (Osorio, 1984). It is influenced by the input of Guayas River (the confluence of Daule and Babahoyo rivers) 5 km at north of Guayaquil.

Figure 1. Ubication of Estero Salado estuary in the Ecuadorian coast.
Source: http://www.ecuaworld.com.ec/mapa_ecuador

The present work is focused on evaluating the composition, abundance, diversity and distribution of benthic macroinvertebrates in polluted zones of the inner branches of the Estero Salado (Guayaquil city), and the relation with physical and chemical parameters, to contribute to a better understanding of the structure of communities of macroinvertebrates in a tropical ecosystem heavily affected by anthropogenic activities with special attention in hydrocarbon petroleum and heavy metals were conducted from 2007 to 2012 in sediments. Besides this work, include research carried out to evaluate the water of quality of 43 rivers of Guayas province were surveyed and analyzed macroinvertebrates as bioindicators of health aquatic ecosystem.

Additionally, this study evaluated the potential effect of hydrocarbon bioremediation of native consortium bacterial on contaminated sediments and the macroinvertebrates communities in the most polluted area of the inner branches of Estero Salado in Guayaquil city, and analyzed the feasibility of recolonization of benthic macroinvertebrates in high anthropogenic disturbance zones using three types of artificial substrates on estuarine sediments in the inner branches of the Estero Salado (Figure 2).

This document is structured in three parts; the first part contains the general introduction, aims, specific objectives and the distribution of chapters (Chapter 1), and the review of state of the art regarding the physical, chemical and biological characteristics of Estero Salado estuary with emphasis on the community structure of macroinvertebrates and hydrocarbon pollution state. Also, it includes the literature review of physical, chemical and biological characteristics of estuaries; the studies which have been done in Estero Salado to the present day, and it explains the importance of the socioeconomic and environmental aspects of this work (Chapter 2).

The second parte includes the chapter 3 and 4. The Chapter 3 describe the results of the composition, abundance and distribution of macroinvertebrates in Guayas River Basin, and the evaluation the water of quality of 43 rivers of Guayas provinces using the BMWP/Col. biotic index modified to Neotropical Colombian. The Chapter 4 shows the pollutants agents' analysis (heavy metal and hydrocarbon), nutrients, benthic macroinvertebrates in sediments and parameters physical and chemical of water of the internal branches of the Estero Salado, to determine the gradient of hydrocarbon and heavy metals in the estuary between 2007 and 2012. In addition, there is an analysis of relationship of environmental variables with the biotic parameter.

The third part includes chapters 5 and 6 that evaluates the relation between community structure of macroinvertebrates and levels of hydrocarbon pollution, heavy metals, nutrients in the sediments in the inner branches in Estero Salado at 2009-2011-2012 and analyses the effects of bioremediation on hydrocarbon using a native bacterial consortium in polluted sediments and on benthic macroinvertebrate community structure in the 2012 (Chapter 5). Finally, there is an evaluation of the recolonization of aquatic macroinvertebrates feasibility in disturbance and bioremediation sites using artificial substrates in 2012 (Chapter 6).

In the final chapter (Acuerdo Ministerial No. 097A Publicado en el Registro Oficial edición especial No. 387) conclusions and recommendations are given for a sustainable management of aquatic resources in the inner branches of Estero Salado followed by references and appendices of whole work.

This research is a compilation of different research projects with the partial economic support of Fondos CEREPS grant (2006) of SENESCYT (Secretaría de Educación Superior, Ciencia, Tecnología e Innovación) and Heriot Watt University, which directly support of my PhD studies. As well as the Ministerio del Ambiente and the Universidad de Especialidades Espíritu Santo, for specific economic funds for the development of chapters 5 and 6 respectively.

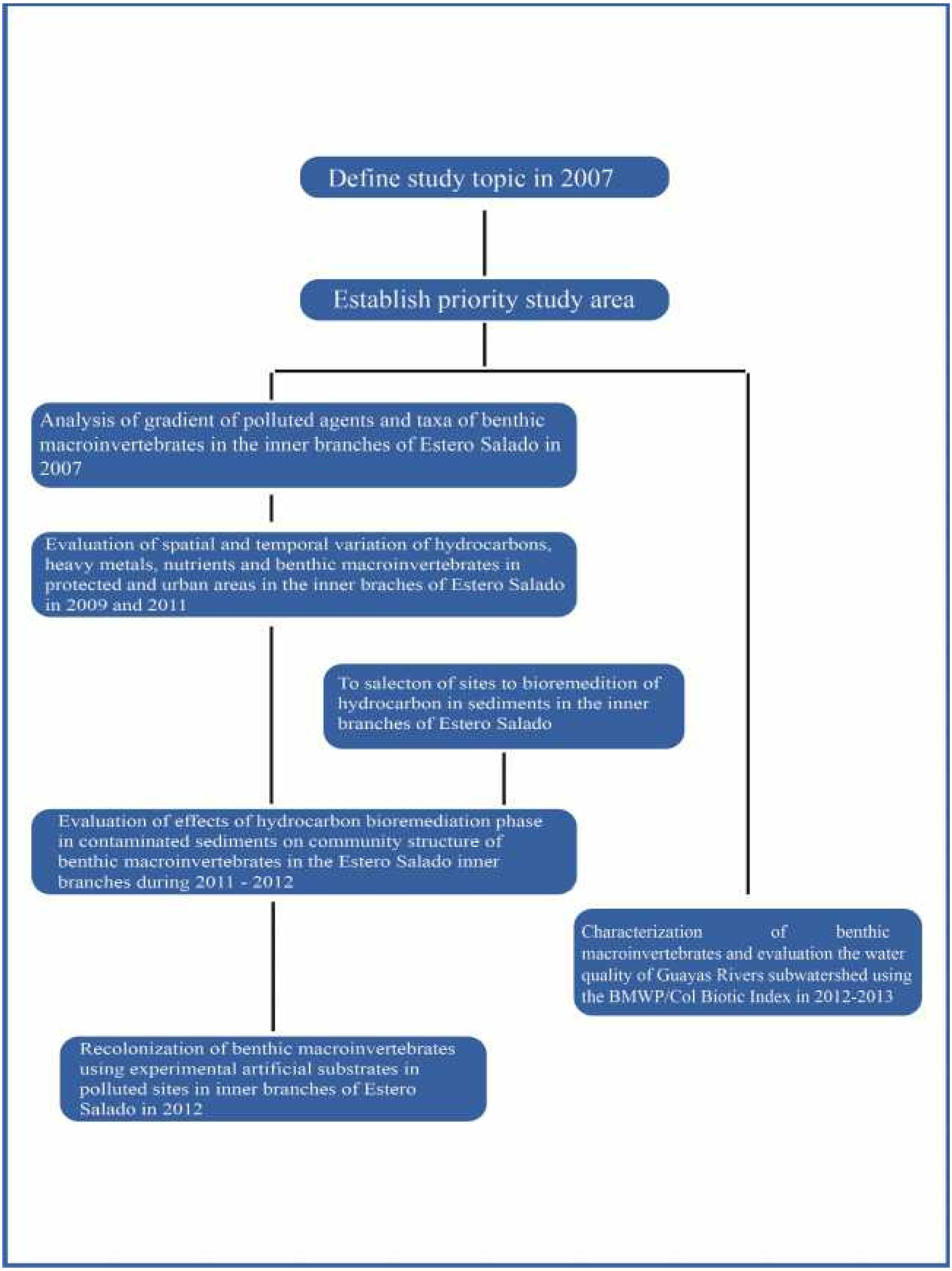


Figure 2. Overview of research chronologic sequence of development of this thesis.

Chapter 2. Overview of the general features of estuaries and main characteristics of Estero Salado Estuary

2.1. General features of estuaries

The definition of an estuary can change depending on the different aspects of physical, chemical and topographic features. Estuaries are complex systems and transition zones between land and sea; freshwater to salt water, and they are characterized by the gradient of salinity and density associated with mixture of rivers and seawater (Hansen & Rattray, 1966). Some of others factors than influence their characteristics are the strength of currents, tidal amplitude, wave strength, deposition of sediments, temperature, oxygen and nutrients (McLusky & Elliott, 2004). Therefore they all have variations of physical, chemical and biotic characteristic.

The most common estuary definition is the Pritchard "*An estuary is a partially enclosed coastal body of water which is either permanently or periodically open to the sea and within which there is a measurable variation of salinity due to the mixture of sea water with fresh water derived from land drainage"* (Pritchard, 1967). Estuaries form bodies of water along many coastlines, and are characterized by having mixtures of salt and freshwater masses, and generally dominated by fine sedimentary material carried into the estuary from rivers and sea (EPA, 2015; McLusky & Elliott, 2004). Certain plants and animals adapted to live in these highly changing conditions, however, the main features of estuaries are the tides that influence their water stages; thus, the limits of an estuary can be determined based on whether the water stage is tidal influence at the upstream river discharge (Chen, Kao, & Chiang, 2013).

Estuaries are usually divided into three sectors: (1) lower zone, free connection with the open sea; (2) middle zone, subject to strong salt and freshwater mixing and (Acuerdo Ministerial No. 097A Publicado en el Registro Oficial edición especial No. 387) upper zone, characterized by fresh water but subject to daily tidal action (Kaiser, Attrill, Jennings, Thomas, Barnes, Brieley, Hiddink, Kaartokallio, Polunin, & Raffaelli, 2011).

The variables that have strongly influence in the distributions of salinity and circulation in the estuary are the geomorphology, the freshwater flow and tides. The main biotic parameters that can affect the composition and distribution of macroinvertebrates species are salinity, texture of sediments, temperature, turbidity, and these can fluctuate drastically daily due to the rate of water flow and the size of the river-estuary interface (Bate, Whitfield, Adams, Huizinga, & Wooldridge, 2002). The gradient of salinity in an estuary influences both the pelagic and benthic invertebrate (and vertebrate) species assemblages.

Estuaries are considered one of the most important ecosystems creating more organic matter each year than comparably-sized areas of forest, grassland, or agricultural land. These ecosystems have different habitat types such as: Shallow waters, freshwater and salt marshes, swamps, sandy beaches, mud and sand flats, rocky shores, oyster reefs, mangrove forests, river deltas, tidal pools, and sea grasses (EPA, 2015). The mangrove forests are unique intertidal ecosystems that are fundamentally important in tropical and subtropical regions of the world (Field, 1999) such as: Asia, Africa, America and Oceania (Figure 3). The total mangrove area in the world is near of 18000000 ha (Spalding, Blasco, & Field, 1997). They have an important economic, scientific and cultural value for Latin America and the Caribbean (Yánez-Arancibia & Lara-Domínguez, 1999).

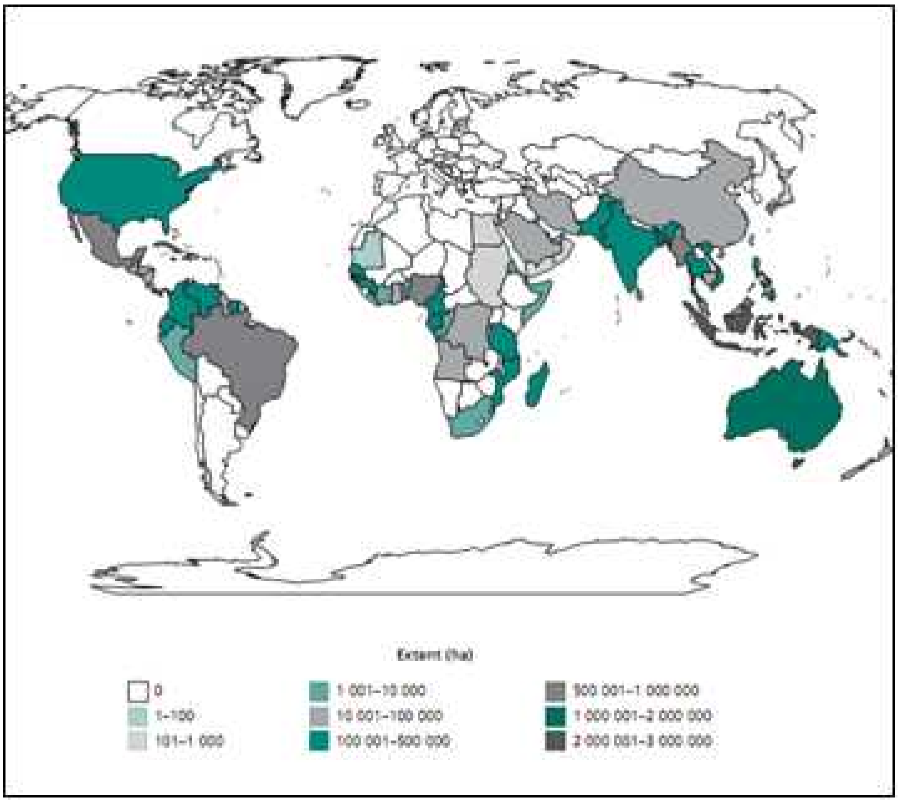


Figure 3. Distribution of mangrove forests in the world. Source: Food and Agriculture organization of the United Nations, 2007.

Mangroves are salt tolerant trees found in sheltered coastlines and in shallow water lagoons, rivers or deltas and estuaries between latitudes 25 ° N and 30° S. These plants grow in soft substrate, rocky shores and the mangroves may grow as tree or shrubs depending on salinity, tidal ranges, climate, soil composition, topography and edaphic features of zone in which they exist. Mangroves stabilize the soil and create a habitat which is exploited by a host of other organisms, their roots retain sediment and consolidate the soil thus facilitating accretion and retard coastal erosion (Hogarth, 2010). Only a few species of 'land plants' are able to adapt physiologically to the brackish water such as the families: Rhizophoraceae, Avicenniaceae, and Combretaceae. Nevertheless, there are around 50 to 70 species in the world and each mangrove ecosystem contains a range of biodiversity (FAO, 2007). There are 10 species of mangrove in South American countries which are: *Acrostichum aureum*, *Avicennia bicolor*, *A. germinans*, *A. schaueriana*, *Conocarpus erectus, Laguncularia racemosa, Pelliciera rhizophorae, Rhizophora harrisonii, R. mangle and R. racemosa* of which Colombia has nine species, and Ecuador, Brazil and Venezuela have seven species each (FAO, 2007). However other studies show that the total area of mangroves until the year 2000 was 137.760 km^2 distributed in 118 (Giri, Ochieng, Tieszen, Zhu, Singh, Loveland, Masek, & Duke, 2011). Approximately 75% of the world's mangroves are found in only 15 countries and only 6.9% is protected within the existing network of protected areas of IUCN and the highest percentage of mangroves is between latitudes 5 ° N and 5 ° S. The largest extent of mangroves is found in Asia (42%) followed by Africa (20%), North and Central America (15%), Oceania (12%) and South America (11%). While studies done between 1980 to 2005 by the Food and Agriculture Organization of the United Nations (FAO) showed there are mangroves in 124 countries (FAO, 2007).

There are mangrove forests in most of Latin America coastlines, except the three southernmost countries of the continent. More than 70% of the total area of mangroves is located in the Atlantic and the Caribbean coasts while a smaller percentage (~28%) is in the Pacific Coast. The total mangroves area in America is less that Asiatic Southwest but higher than Africa (Yánez-Arancibia & Lara-Domínguez, 1999) (Table 1).

In the Americas, specifically in the eastern Pacific coast, mangrove forests extend from the California Gulf in la Bahía de Los Angeles (28° 54´N, 113° 31´W) to the estuary of Tumbes river (3°30´S). The mangroves in Latin America are well developed along the

Ecuadorian coast due to the intense convective activity of the intertropical convergence zone characterized by high level of annual precipitation (>2000 mm).

Distribution and coverage of mangrove forests have been changed due the human pressure on coastal ecosystems with the consequent conversion of mangrove areas into urban, agricultural and aquaculture assets in the Africa, Australia and mainly in the Americas and Asia (Table 1). The destruction of mangrove forest is occurring globally due to land use changes and other human activities (Valiela, 2001).

Table 1. Mangroves area changed since 1921 until 1999 for Asia, Africa, Australia, Americas as well as total for the World.

Region	Original area (Km^2)	Present area until 1999 (Km^2)	Annual rate of loss ($Km^2\ y^{-1}$)^1
Asia	41208	26193	628
Africa	21847	14903	274
Australia	11617	10000	231
Americas	62242	38472	2251
Word Total	136914	89568	2834

Source: Valiela et al., 2001.

Original and area of mangroves for regions considered the data of regions with all theirs countries with available multiyear data.

In South America only five countries have large mangrove areas: Brazil with 50%, Colombia with 18%, Venezuela with 11%, Ecuador with 8% and Suriname with 6% (Figure 4).

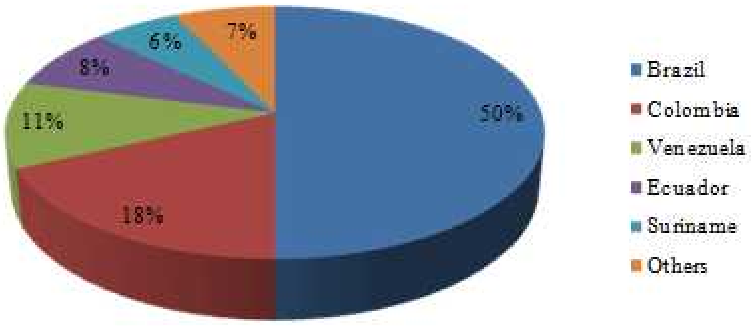


Figure 4. Five countries with the largest mangrove area in South America as of 2005. Source: FAO, 2007.

According to Cornejo (2014) the mangroves of Colombia, Ecuador and Peru are made up of 13 species corresponding to major mangroves 5, minor 2 and facultative 6. Between the major mangroves are *Rhizophora mangle*, *R. racemosa* and natural hybrid *R. x harrisonii, Avicennia germinans* and *Laguncularia racemosa*. Minor mangroves as

Pellicera rhizophorae and *Tabebuia palustris* and facultative mangroves such as: *Mora oleifera, Pterocarpus officinalis, Annona glabra, Conocarpus erectus, Talipariti tiliaceum var.pemambucense* and *Amphitecna latifolia.*

2.2. Types of estuaries

An estuary has characteristics of both river and sea, the riverine characteristic of an estuary are that it has banks, flowing water, sediment transport, occasional floods and, in the upper parts, has fresh water. Typical marine characteristics are the presence of tides and saline water (Table 2).

Table 2. Characteristics of an estuary.

Characteristics	**Estuary**
Shape	Funnel
Main hydraulic	Storage and transport
Flow direction	Dual direction
Bottom slope	No slope
Salinity	Brackish
Wave type	Mixed
Ecosystem	High biomass, productivity, high biodiversity

Source: Savenije, 2005.

The main factors affecting the estuaries are river discharge, mixing and currents tidal (Waterhouse, Valle-Levinson, & Winant, 2011). There are different types of classification of estuaries according different criteria such as (Table 3): Shape, tidal influence, river influence (Savenije, 2005), circulation, salinity distribution (Stommel & Farmer, 1952), topographic aspects, by convergence and friction; by morphology¸ structure of salinity (Pritchard, 1967). Geomorphology, freshwater, flow and tides are the dominant variables determining the distribution of salinity and circulation within the estuary (Hansen & Rattray, 1966).

Other classifications are based on river influence (riverine estuary and marine estuary), geology (Fixed estuary, Alluvial in a coastal plain), salinity (positive or normal estuaries and negative or hypersaline estuaries), stratification and circulation (salt wedge estuaries, estuaries well mixed, and partially mixed estuaries). The most general classification used is by Davies (1964) who distinguishes micro, meso, macro and hyper tidal estuaries on the basis of the tidal range. Other common classifications are based in topography (Hume & Herdendorf, 1988). This classification has ecological relevance because the association between topography and organisms (Kaiser et al., 2011). Tectonic activity and Tidal range (Hayes, 1975), summarized in four category: (1) microtidal (<2 m range), mesotidal (2-4 m), macrotidal (4-6 m) and hypertidal (>6 m). The tides have a greater importance in these cases by varying a range of physicochemical parameters that affected organism function, survival and distribution (Kaiser et al., 2011).

Estuaries traditionally have been classified according to their geomorphology and salinity stratification and tidal range (Cochran, 2014; Hansen & Rattray, 1966; Kaiser et al., 2011). Consistently the best criteria for defining the type of estuary are ones that allow a comparison with other estuarine systems. The table 3 shows the classification of the estuary according to the tidal wave type, river influence, geology and salinity.

Table 3. Estuary Classification in relation to the aspects: tide, river influence, geology and salinity.

Shape	Tidal wave type	River Influence	Geology	Salinity
Ria	Mixed wave	Small river discharge	Drowned drainage system	High salinity often hypersaline
Fjord	Mixed wave	Modest river discharge	Drowned	Partially mixed to stratified
Funnel	Mixed wave, large tidal range	Seasonal discharge	Alluvial in coastal plain	Well mixed
Delta	Mixed wave, small tidal range	Seasonal discharge	Alluvial in coastal plain	Partially mixed
Prismatic Channel	Progressive wave	Seasonal discharge	Man-made	Partially mixed to stratified

Source: Adapted from Savenije, 2005.

2.3. Division of estuaries into zones

There is a sub classification of estuaries in zones (Kaiser et al., 2011) based on environmental conditions by salinity, tidal reach and seasonal cycles variables. These zones are:

Zone 1 the estuary is dominated by river flow, salinity generally <5psu, strong river currents resulting in coarse sediments of stones and gravel.

Zone 2 (Upper reaches, main area of mixing of fresh and saline water, salinity highly variable (e.g. 5-18psu), currents resulting in fine and muddy sediments.

Zone 3 (Middle reaches), flow dominated more by tidal currents, salinity 18-25psu, extensive intertidal flats mainly muddy but with more sand present.

Zone 4 (Lower reaches) faster tidal currents, salinity 25-34psu. Sediments mainly are sand.

Zone 5 (Mouth), zone where estuary meets the sea, strong tidal currents and generally fully saline conditions with sediments mixed sand, biogenic deposit (shell fragments) and rock in some cases.

2.4. Fauna and macroinvertebrates diversity patterns in estuaries

The organisms that live in estuaries originate from two possible sources: species of freshwater that colonize the estuarine system or marine species colonizing estuaries after extinction of estuaries at higher latitudes during the last glaciations (Kaiser et al., 2011). There are some possible sources of recolonization of estuarine fauna derived in local refugia or remain in brackish water systems and lower latitudes and recolonized higher latitude systems. The high variability of environmental parameters produce high stress levels in biota that influence strong evolutionary selection forces and speciation in some estuarine habitats is very fast (e.g. amphipods, some of which have significant genetic differences between populations within the same estuary where they are found in different habitats (Stanhope, Hastwick, & Baillie, 1993).

The macroinvertebrates from estuarine and marine waters are composed of a high diversity of taxa including groups such as cnidarians, sponges, bryozoans, molluscs, crustaceans, echinoderms, annelids, insects and roundworms. By contrast, in freshwater, there tends to be a small diversity of invertebrates represented by, for example, flatworms, annelids, molluscs, crustaceans, and insects (APHA, 2005). These organisms play a significant role in estuarine ecosystems due to them being food sources for higher trophic consumers (Hutchings, 1998), whilst they also facilitate the decomposition of organic matter, recycling of nutrients and translocation of material through bioturbation and biodeposition (Mermillod-Blondin, 2011). However, the diversity of macroinvertebrates fauna changes along the estuaries, thereby change with depth, salinity and zone of estuary, for example, low diversity is common in the mid estuary zone and the number of species decrease in the head of an estuary and increase towards marine condition (Kaiser et al., 2011). The invertebrate community may use roots of vegetation as hard substrate for settlement, many organism depend of vegetative productivity directly or indirectly. The most representative phyla are: molluscs, arthropods, annelids, nematode, Nemertinen, platyhelminth and sipunculans. The most abundant are mollusks and crustaceans (Hogarth, 2010).

In tropical estuaries, mollusc´s inhabiting mangroves are mainly gastropods that include deposit feeders, herbivores which feed mainly on fallen leaves or algae. High numbers of snails live in mudflats and other few species live on trees, rocks or others hard surfaces. Common species in estuaries are *Thais* sp. and *Cerithidea* sp. *(*Hogarth, 2010).

The most common mangrove crustacea belong to Brachyura (true crabs) represented mainly for the families Grapsidae, Sesarmidae, Portunidae and Ocypodidae. The Ocypodidae include fiddler crabs represented by the species *Uca* spp., Sesarmidae by the genus *Sesarma*. In addition, hermit crabs, amphipods, isopods are common as are commercially important species of Penaeids shrimps.

Some Grapsid crabs are often general feeders, depending mainly on scavenging and opportunistic predation. However, Sesarmidae tends to herbivory as in the case of *Sesarma* that eats epiphytic algae from the surface of mangrove roots, leaves and reproductive products of mangrove trees. Some crab genera are locally important such as: *Cardisoma* in the Indian Ocean and *Ucides* in Central and South America: they grind food (leaves, debris) with arrays of ridges that are contained in the stomach, and secrete cellullase to break the material ingested to enable digestion and absorption. Some studies of stomach contents shows that 90% of the foregut consist of mangrove leaves (Ólafsson, Buchmayer, & Skov, 2002). However, other types of crabs are deposit feeders, such as *Uca* sp., that live at all levels of the shore and eat organic matter. They can be very abundant with densities of 70 crabs/m^2 (Macintosh, 1982).

Other types of arthropods live in terrestrial mangroves, these are insects, especially the Order Diptera such as *Aedes aegypti* (Venezuela, Colombia, Ecuador, Brazil), *A. amesii* (Australia) and these are vectors of diseases such as Dengue, Chicungunya, Zika. They live in shallow pools of water and mangroves provide a suitable habitat for egg laying and larval development. Nevertheless, different species of mosquito need different requirements to live. For that the macroinvertebrates have often been used in biological monitoring and environment surveillance in Europe throughout the last 100 years to detect alterations in aquatic ecosystems (Cairns & Dickson, 1973). Their assessment in all water body types: freshwater (Alba- Tercedor, Pardo, Prat, & Pujante, 2005; Alba-Tercedor & Sánchez-Ortega, 1988), estuarine (Rosenberg, Blomqvist, C Nilsson, Cederwall, & Dimming, 2004) and marine waters (Borja, 2004; Borja & Muxika, 2005; Borja, Muxika, & Franco, 2003; Reiss & Kröncke, 2005).

The main characteristics of these organisms (making them favourite indicators of water quality) are: easy to collect and identify, relatively good understanding of the biology of these animals, general sedentary lifestyle, and often being cosmopolitan species. They form part of the food chain of fishes and the resulting food for diets of humans. They can bioaccumulate contaminants, and sensitivity of some taxa to different contaminants can aid in the evaluation of environmental stress (Hawkes, 1979; Hellawell, 1978).

2.5. Main uses and services of mangrove

Mangrove forest ecosystems provide a wide range of goods and services to human society and coastal and marine systems (Giri et al., 2011). The mangrove help to stabilize shorelines, reduce the impact of natural disaster as tsunamis, hurricanes, cyclones, They provide breeding and nursery grounds for marine and pelagic species, food, medicine, fuel and building material for ocal communities (Giri et al., 2011; Twilley, Montaño, Valdivieso, & Bodero, 1999). They help provide productive activities as Aquaculture, agriculture, construction, fisheries, forest uses, tourism, recreation and others (Valiela, 2001). According to FAO (2007) the main uses of mangroves are divided by mangrove use for wood and non-wood forest products (Table 4). Mangroves provide important timber products and the ecological functions are very important as productivity and nutrient cycling, and they are considered an important link to the support of economically important fisheries (Twilley & Day, 1999).

Estuaries and mangrove forests are an extremely valuable natural resource due to their high productivity and essential role in the maintenance of the biological diversity of coastal and marine environment (Barbier, Hacker, Kennedy, Koch, Stier, & Silliman, 2011). They hold a great diversity of aquatic species, perform sequestration of carbon (at rates up to 50 times higher than tropical rainforests and provide forest products energy sources, food, medicine, fishing, construction, textiles, papers and a wide range of ecosystem services to humanity (Bouillon, 2011).

The sustainable management and conservation of estuaries and mangroves habitats are fundamentally important to ecological, functionality and equilibrium of coastal zones to the support the natural resources and ecological services (Saenger, Hegerl, & Davie, 1983). In Ecuador, they sustain socio-economic activities, such as artisanal and commercial fisheries, aquaculture, agriculture, tourism and others subsistence activities (Cruz, Gaibor, Mora, Jiménez, & Mair, 2003).

Mangrove forests in South America have traditionally been used for a great variety of uses such as: timber and fuel wood in Brazil, Colombia, Ecuador, Guyana, Perú and Venezuela. There is production of charcoal in Guyana, Colombia, Ecuador and Perú. There is extraction of tannin from bark, especially of the mangrove species Rhizophora mangle. Besides this, are developed commercial and subsistence fishing and aquaculture (FAO, 2007) (Table 4).

Table 4. Main Uses of Mangrove forest in South America

Uses	Guyana	Venezuela	Colombia	Ecuador	Perú	Brazil
Source of food	x	x	x	x	x	x
Fishing activities	x	x	x	x	x	x
Aquaculture		x	x	x		x
Timber	x	x	x	x	x	x
Fuelwood	x	x	x	x	x	x
Production of Charcoal	x		x	x	x	
Extraction of tannin	x		x	x		x

Source: FAO, 2007.

2.6. Estuaries in Ecuador

The most important estuaries in terms of the surface area in the Ecuador are: Cayapas-Mataje, Estuary of River Esmeralda, Estuary of River Atacames, Estuary of River Muisne, Estuary of River Cojimíes, Estuary of River Chone, Estuary of River Guayas and Archipelago of Jambelí including Jubones-River Zarumilla (Table 5; Figure 5) being the Gulf of Guayaquil estuary the biggest. While the estuaries more important by their primary productivity are: Gulf of Guayaquil, Muisne, Cojimíes, Chone and Jubones (Arriaga, 2000).

In the estuaries of Ecuador there are one hundred and seventy nine species of plants are associated in the mangroves areas (Cornejo, 2014), that includes thirteen species of mangroves of wich five are major mangrove type such as: the red mangrove *Rhizophora mangle, R. racemosa, R. harrisonii*; the black mangrove *Avicennia germinans;* the *mangle jelí Conocarpus erectus* and the white mangrove *Laguncularia racemosa* (Cornejo, 2014; Valverde & Pérez, 2012) and others trees species as *Pelliciera rhizophorae,* (Bodero & Robadue, 1995), *Tabeuia palustris*, *Pterocarpus officinalis* and *Mora oleifera* are distributed along the Ecuadorian coast in the intertidal zone with salt flats 15 estuaries (Bravo, 2010) mainly restricted at the northwestern of Ecuador (Cornejo, 2014). All these located in the Esmeraldas, Manabí, Guayas, Santa Elena and El Oro provinces. Physiographically there are three types of mangroves and vegetation have been occurred in Ecuador (Border, river and basin mangrove swamps), the largest extensions of border and river mangrove swamps there are in the Guayas Province, and are mostly located along Guayas River and Gulf of Guayaquil (Cornejo, 2014).

Table 5. Surface area covered by mangroves in the main estuaries of Ecuador during the period between1969 to 2006) expressed in hectares.

Estuaries	**1969**	**1984**	**1987**	**1991**	**1995**	**1999**	**2006**
Cayapas Mataje	23677	23650	23507.7	22863.3	21947.9	22057.9	21400.8
Muisne	3282	2702	2445.9	1340.3	830.8	1187.8	1557.5
Cojimíes	13123.8	9917	8466.9	6028.8	3651.5	1921.4	2742.3
Chone	3973	1673.5	1040	784.9	391.6	705	932.5
Gulf of Guayaquil	159032.8	143870	139355	130701	122438	104715.5	10513.4
Archipelago of Jambelí	34712.5	24592.3	23570.3	21092.7	17697.8	19111.4	15207.6

Source:(Arriaga, 2000; CLIRSEN-PMRC, 2007).

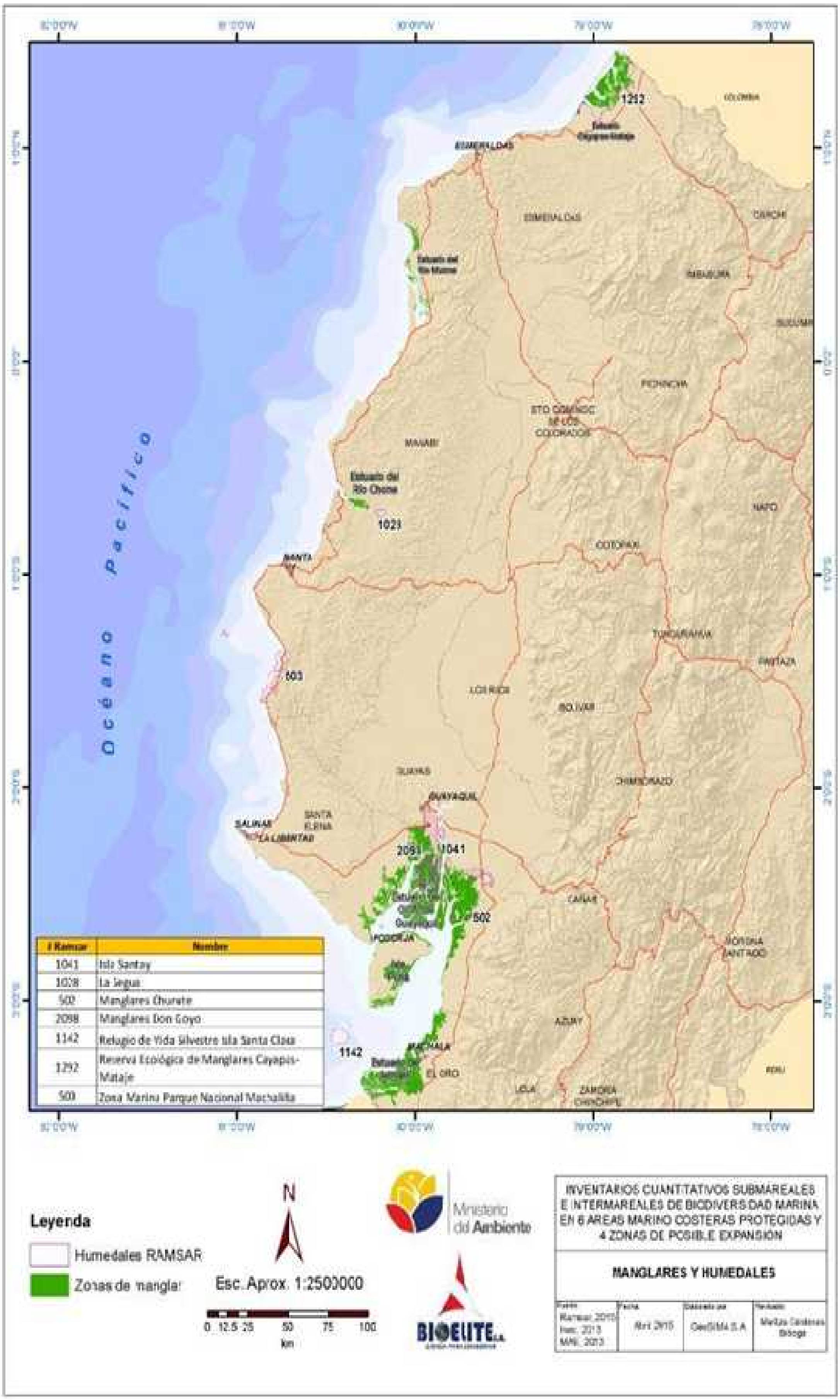


Figure 5. Estuarine Ecosystems of the Ecuadorian coast. Source: Ministerio del Ambiente, 2015.

2.6.1. *Description of Gulf of Guayaquil and the Estero Salado Estuary*

The Gulf of Guayaquil is the largest estuary along the South American coast on the eastern Pacific. It is located at the Republic of Ecuador in the Guayas and El Oro provinces (Figure 6). The Gulf extends landward approximately 120 km. The Gulf of Guayaquil is naturally divided inner and outer estuary, which originates on the western side of Puná Island (80º 15´W) and ends along 81^{o} W longitude (Stevenson, 1981) and the inner estuary, which extends northeastward from the western end of Puná Island for another 74 km before going into the channel of the Guayas River, this Gulf including the Cascajal Channel, Jambelí Channel, the Estero Salado and Guayas River systems. The outer estuary only is formed by the area next to Playas (Pesantes, 1998).

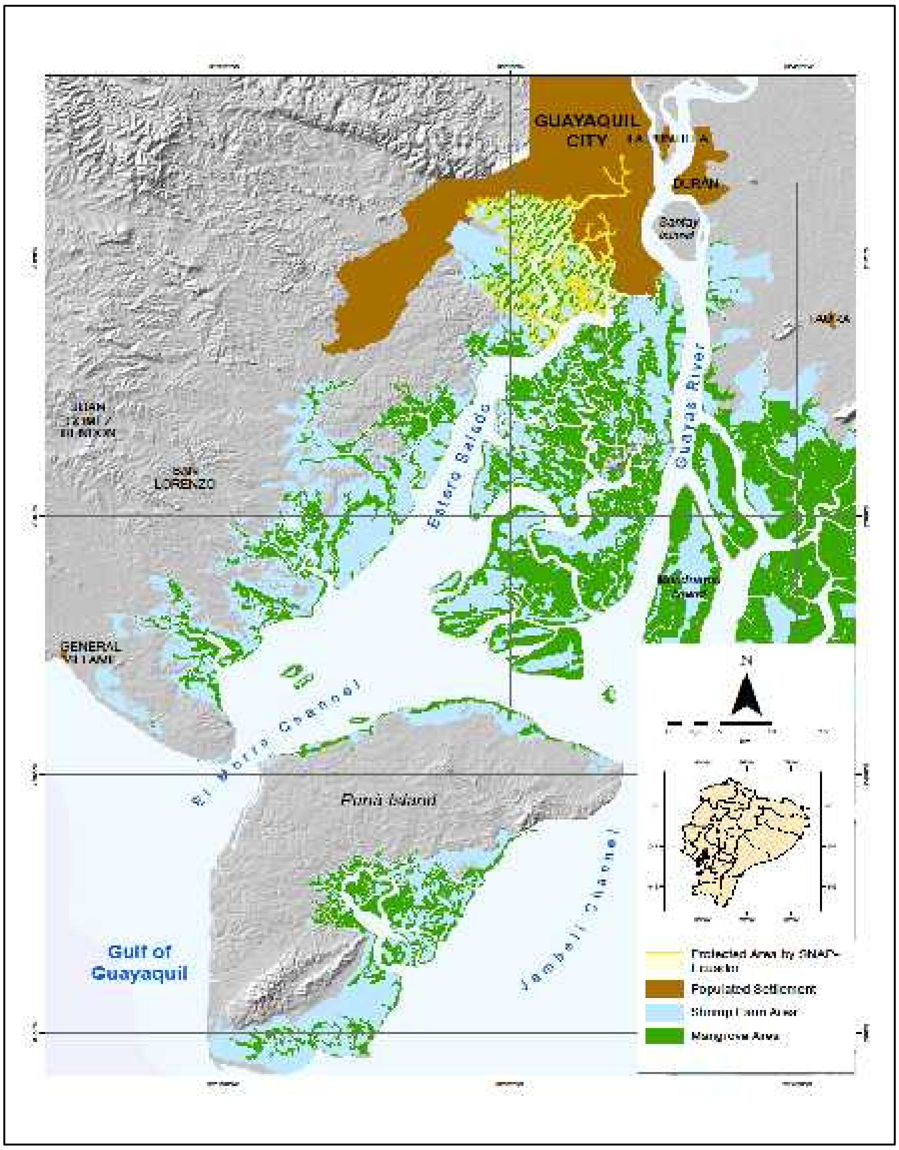


Figure 6. Geographic location of Gulf of Guayaquil in the Ecuadorian coast.

The Gulf of Guayaquil has a extension of 13701 km^2 including 11711km^2 of water surface and 1900 km^2 of islands and small barren islands (CAAM, 1996). It has a high degree of biological productivity: its status as a habitat for a diverse and rich biota, which supports the country's most important fisheries, the presence of mangroves on all the edges of the estuaries, the large amounts of organic material deposited in it by inflowing rivers, the influence of various water masses, the predominant estuarine conditions combining marine and fluvial characteristics, the large area and shallowness of the inner platform. Thus it is considered as a special conservation zone by the Ecuadorian Environmental Ministry and '*Ecologically or Biologically Significant Marine Areas*' according to the eighteenth report of the subsidiary body on scientific, technical and technological advice to Convention on Biological Diversity (UNEP-CBD-COP, 2014).

The Gulf has an oceanic influence due the convergence of warm tropical of low salinity water from the north and subtropical cold water and salt from the south. Besides the contribution of organic material transported by rivers, the action of ocean currents, the convergence of different types of water and the shallowness of the inner shelf makes it a very complex environment (CAAM, 1996). The winds in this area variable and influenced by the Inter Tropical Convergence Zone (ITCZ), the dominant winds are from the south (1.5 m s^{-1}) (Twilley, Cárdenas, Rivera- Monroy, Espinoza, Suescum, Armijos, & Solorzano, 2001); in the wet season northeast winds dominate (1.8 m s^{-1}) compared to southwest winds during the dry season (3.5 m s^{-1}). Depending on the location of dominant oceanography current off the coast (Twilley et al., 2001).

This Gulf receives runoff from 23 watersheds of the 79 that Ecuador has with an area of about 51,230 Km^2, these rivers are Salado, La Seca, Zapotal, Daular, Chongón, Guayas, Taura, Churute, Cañar, Naranjal, San Pablo, Jagua, Balao, Gala, Tenguel, Siete, Pagua, Jubones, Santa Rosa, Arenillas y Zarumillas and the estuaries Estero del Morro and the Estero Motuche. The biological productivity of this ecosystem is due to the nutrients which are transported through the rivers, wedge seawater enriched water generated by various productive activities taking place in the Gulf and environmental factors that influence the wealth and characteristics of the water such as rainfall, temperature, salinity, pH, organic matter, among other nutrients that are transported through the estuary due to tidal flow in a net movement toward the open sea. The net productivity calculated for twelve hours days differed more from the inner estuary to outer estuary than from dry season to wet season. The production in the outer estuary was 200 mg/C m^3 /days or less compared to inner estuary values of up to 600-800 mgC/$m^{2/}$day in the dry season and to 800-1000 mg/C/m^2/day in the wet season (Stevenson, 1981).

2.6.2. Estero Salado Estuary

The Estero Salado Estuary forms part of inner estuary of Gulf of Guayaquil, It extends east of the line between Boca de Capones and Punta del Morro (CAAM, 1996) across the island Puná to internal branches that enter the city of Guayaquil (EMAG, 1978), and is nearly 75km in length (Cruz, 1992).The inner branches of this estuary start in Guayaquil city, in the Guayas provinces (central zone of the Ecuadorian coast), which has a population of 2350915 according to the latest census made in 2010 by the National Institute of Statistics and Census of Ecuador (INEC). This estuary is in Guayaquil and it is considered one the most populated cities in the Ecuador (INEC, 2010a). It´s classified geologically as an estuary of tectonic type and according to the pattern of circulation and stratification in a partially mixed estuary due that it has a characteristics intermediate between salt wedge estuaries (Stevenson, 1981) and of type 2B (Murray, Conlon, Seripong, & Santoro, 1975) where *´´the average of caudal, the net flow reverses and depth, the transfer flow upstream salt is by advection plus diffusion´´*(Hansen & Rattray, 1966).

2.6.3. Physical and chemical characteristics of Estero Salado Estuary

a) Geology

Geologically the Estero Salado consists of silty clay sediments (INOCAR, 2012; Rada, 1986; Stevenson, 1981). The inner branches have sediments formed by silt and silt-clay mixtures or clay-loam come from Guayas River, this sediment suspended is transported across the Cascajal Channel to outer estuary of Gulf of Guayaquil (INOCAR, 2012).

b) Bathymetry and seabed features

The water depth throughout the Estero Salado has less than 18 m except in Jambelí and Morro Channel (22 m) in the most external branches, to the north and south of Puná Island, respectively (Stevenson, 1981). However, it has a depth average of 10 m between Canal de Cascajal and Guayaquil city (Cruz, 1986), the depth decreases in internal branches near of Guayaquil city until less 1 m (INOCAR, 2012).

c) Water Circulation

Water circulation during the dry season the surface water enters the outer Gulf from the northwest and flows southward in the Gulf before veering to the southwest and leaving the Gulf. While in the wet season the circulation is complex, where the surface water enters the outer Gulf across the northern half of the entrance. The circulation is more rapid than in the dry season due river discharge (Stevenson, 1981).The circulation of water masses in this area, are influenced by the contribution of river discharge, tidal currents (CAAM, 1996) and by the action of the winds there a slow stream bottom, up and saline water displacing water from rivers.

d) Climate

In this area the climate according the Koppen Classification is *´´Tropical Sabana´´* in Guayaquil. The average annual rainfall recorded between 1961 and 2005 was 1109.2 mm, with maximum values of precipitation 4230.76 mm (Pires, 2000) although in period of the El Niño Southern Oscillation (ENSO) phenomenon it has reached 2947 and 3603 mm in 1997 (INAMHI, 2001) and 1998 (INAMHI, 2000) respectly. The Guayaquil climate is characterized by a marked seasonality, it has two seasons: a dry season (June to December) and the rainy season (January to May).

In accordance with the meteorological information from The National Institute of Meteorology and Hydrology from Ecuador (INAHMI) during the period 2007 and 2012, the rainfall in Guayaquil was between 840 and 1650 mm with average annual of 1172 mm. The multiannual average values of evaporation was 1460 mm, the heleofania was 1206 hours, the cloudiness was 7/8 and the winds velocity was 12Km/h with predominance southwest winds for this period (INAMHI, 2010a, 2010b, 2012a, 2012b, 2014, 2015).

e) Currents

The Ecuadorian coast is influenced by the Humboldt Current from southern South America and the Panama Current (Figure 7). These two bodies of water have a transition zone called Equatorial Front with intermediate characteristics; and the existence of an undercurrent known as Cromwell (Cucalón, 1987).

The Panama Current is characterized by its waters being warm and low salinity flowing along the Ecuadorian coast from the Bay of Panama. It is characterized by temperatures between 25 °C and 27 °C, salinities under 34 psu and low nutrient concentration. This trend is influenced annually in the varying intensity of rains in the coastal region between the months of January to April with varying intensity. While Humboldt Current is a body of surface water and cold saline subtropical, which flows north from the Peruvian coast, characterized by temperatures between 19 and 20 °C, salinity around 35 psu, high concentration of nutrients and influence dry season from July to October (Ministerio del Ambiente, EcoCiencia, & Unión Mundial para la Naturaleza, 2001).

The ´Equatorial Front´ is the transition zone between water masses transported by El Nino and the Humboldt Current is characterized by an intense thermohaline gradient, normally it is located between 0^{o} to 3^{o}S which has its greatest development during the dry season. The position of the Equatorial Front is a determinant in the rainy season as its geographical position determines the rainy areas in the coastal region (Jiménez, 2008), can be strong in the east with temperatures very cooled lower of the South Equatorial Current (SEC) by influx of Peru Current water and salinity,nutrients,carbon dioxide, chlorophyll values higher than in the North Equatorial Countercurrent (NECC) (Pennington, Mahoney, Kuwahara, Kolber, Calienes, & Chavez, 2006)

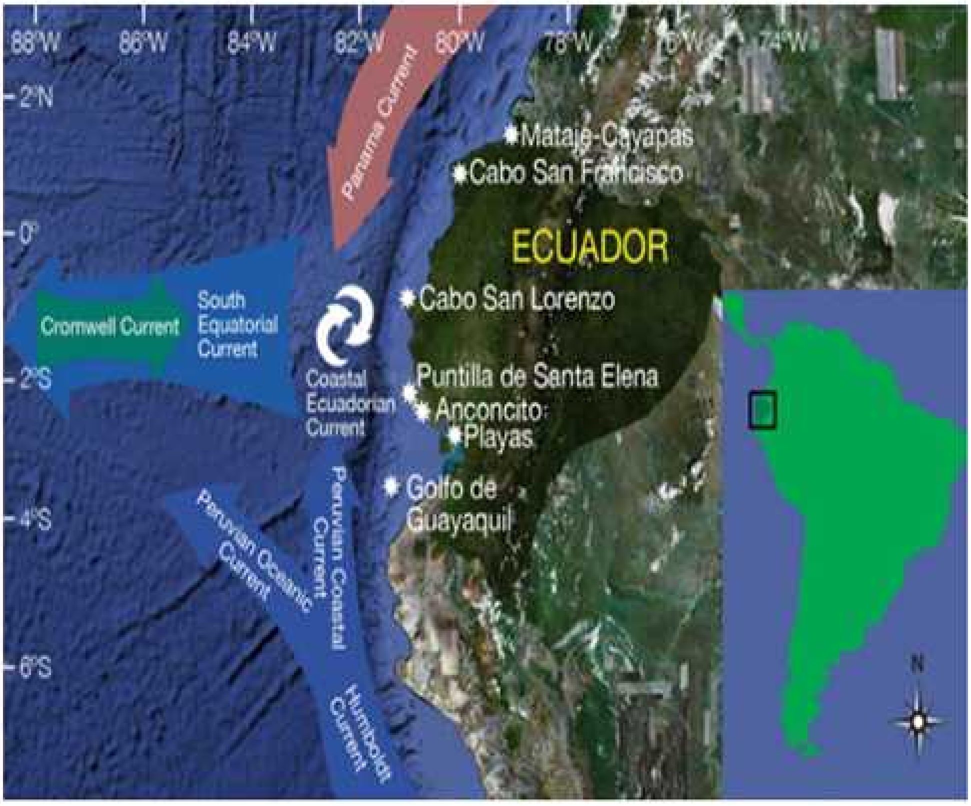


Figure 7. Main currents in the Ecuadorian Coast. Source: (Alvarado & Solís-Marín, 2013).

f) Temperature

In the city of Guayaquil the mean multiannual value is 26^{o} C considering the period between 2007 and 2012, with minimum temperatures registered 26.1 ^{o}C and maximum of 26.5 in 2007 and 2012 respectly (Annex 1). In the lowland region there are no drastic temperature fluctuations.

g) Humidity

The annual average of humidity in the lower part of Guayas basin is 81%. The monthly averages oscillate between 65 and 89% according data from the National Institute of meteorology and Hydrology (INAHMI).

h) Salinity

The inner branches of Estero Salado have reported salinities of between 22 and 24 psu in the rain season and 30 to 31psu in the dry season (Pesantes, 1975). This estuary is of high salinity with few changes in the lower branches of the estuary in relation with upper branches, but with significance changes between years depending on the intensity of rainfall (Cruz, González, Gualancañay, & Villamar, 1980). However, there is a seasonal salinity pattern in the Guayas (0.08-33psu, mean 18 psu), Estero Salado (19-36, mean 28) and sub-estuaries reflecting fresh water discharge (Cárdenas, 1995). The averages the pH in the sub-estuaries has a range from 7.5 to 7.7 at high and low river flow but pH increases significantly between the upper. In the dry season, the pH values tend to increase from inner to the outer Gulf and changes (of up to 0.5) during the wet season and lower (8.01±0.2) Guayas river estuary (Cárdenas, 1995).

i) Tide

The tide is semi-diurnal (M2= 12.42h) with currents in excess of 3.5 m s^{-1} in the river and channels of the estuary. Tidal differences are 1.8 m near the upper boundary of the Gulf of Guayaquil and increase to 3-5 m in the Guayas River estuary near of Guayaquil city (Twilley et al., 2001). However, the amplitude of tidal commonly oscillate from 2.5 to 3 m, reaching up to 5 m in the Gulf (Cornejo, 2014).

The diurnal tides changes to 3 m near Posorja, 3.5 m in the south of Guayaquil (Puerto Nuevo) and 4 m in Guayaquil city. The tide current is very strong in the Guayas river to above 100 cm $s-^{1}$ (Murray et al., 1975). Rainfall influences the behavior of tides, being refluxe and flow tides.

2.6.4. Biological Characteristic: Benthic Macroinvertebrates of Estero Salado

The main estuarine habitats and communities of Estero Salado are formed by mangroves, saltworks zones and shrimp ponds. The dominant mangrove in the Gulf of Guayaquil is *Rhizophora harrisonii* followed by *Rhizophora mangle, Avicennia germinans, Laguncularia racemosa* and *Conocarpus erectus.* Therefore, all species of major mangroves are in Guayas (Molina-Moreira, Lavayen-Tamayo, & Fabara-Suárez, 2015), other types of vegetation occur in salt marshes such as: *Salicornia fruticosa*, *Maytenus octogona*, *Acrostichum aerum*, *Cryptocarpus pyriformis* and *Batis maritima* (Pérez, 2012; Twilley et al., 2001; Valverde & Pérez, 2012).

In relation to the macro benthonic fauna a total of 161 species have been identified in the inner branches of Estero Salado based in the analysis of 22 benthic samples, these samples were collected in two cruises in October of 1978 (Cruz et al., 1980). However, there are few and isolate information sources about some studies done with different objectives, time, seasonal stations and spatial distribution inside the Estero Salado.

Regarding studies on macroinvertebrates in the Estero Salado, the first contributions were from foreign expeditions that contributed to the knowledge of certain biological groups in the Pacific, including mollusks, that were studied and described by Cuming (1836); Hinds (1841); Agassiz (1904); D'Orbigny (1926), according to historical documents reviews by Olsson and Keen (Keen, 1971; Olsson, 1961). In the decade of 60´s and 70´s, new researches were made by Ecuadorian scientist to asses the status of aquatic resources and ecosystems along the Ecuadorian coast. Through the creation of two important research institutes such as: National Institute of Fishing (INP) in 1960 and the Oceanographic Institute of the Navy (INOCAR) in 1972.

Some of these studies included work of the National Institute of Fishing (INP for its acronym in spanish) relate to know the status of fishery resources of important species of macroinvertebrates in the estuarine zone in Ecuador, as shell (*Anadara tuberculosa, A. similis*), crabs such as: red crab (*Ucides occidentalis*), blue crab *(Cardisoma crassum*) and experimental test developed for crop development of larval and juvenile shrimp *Litopenaeus vannamei*. While the Oceanographic Institute of Naivy (INOCAR for its acronym in Spanish) is focused on the hydro-oceanographic research in aquatic and maritime spaces in the Ecuador.

The most of the biological information about diversity of macroinvertebrates in the Gulf of Guayaquil and external branches Estero Salado has been produced by INOCAR. However, there are few studies in the inner branches of Estero Salado, some are limited to the important descriptions of lists of benthonic sublittoral species (Cruz et al., 1980), and evaluation of the composition of taxa of specific groups such as: Polychaetes (Villamar, 1986, 1989); mollusks (Cruz, 1983, 1986, 1992, 2003; Mora & Reinoso, 1981) realized on the interrelationship between physical and chemical parameters with the macroinvertebrates such as studies of mercury in bivalves (Ayarza, Coello, Chalén De Padilla, Garcés, García, García, Ormaza, Pérez, Pesantes, & Solórzano, 1993; Chalén, 1989)

In the 1990s, others institutions as universities began to study specific topics about of heavy metals, pesticides in separate sites, years and programs such as: The Universidad de Guayaquil (UG) and the Escuela Superior Politécnica del Litoral (ESPOL). Between the years 2000-2010 further investigations were developed by undergraduate and graduate students from national and international universities; and private consulting studies about water quality. With the economic support of public institutions as

Municipalidad de Guayaquil who evaluated the feasibility of a integrated plan for the recovery of Estero Salado (Lahmeyer-Cimentaciones, 2000) and Ministerio del Ambiente (MAE), has been conducted a specific project known as´´ Guayaquil Ecológico´´ during the period (2010-2017) with the aim of making social and ecological restoration in sensitive habitats, including El Estero Salado in Guayaquil city.

The most relevant studies in the Estero Salado are detailed below:
Cruz (1980) analyzed 22 sediment samples of Estero Salado lower branches without actually entering the city of Guayaquil during the dry season (October) in 1978 and identified three epibenthic communities of Sabellarids formed by reefs, gravel bottoms formed by shells and *Mytella* reefs and listed a total of 161 species distributed in 63 species of bivalves, 36 species of gastropods, 36 arthropod species, 13 species of polychaetes, 5 species of cnidarians, 3 species of bryozoans, 3 species of echinoderms, 1 species of turbelaria, 1 species of tunicate (Cruz et al., 1980).

The benthonic sublitoral fauna is rich and diverse in the outer estuary of Estero Salado (Cruz *et al*., 1980), and composed of mollusks such as: *Anadara tuberculosa*, *A. grandis, A. similis; Mytella guyanensis, M. strigata, Corbula amenthisima and others;* crustaceans such as: *Penaeus occidentalis, P. vannamei, P. californiensis, P. brevirostris, P. stylirostris; Trachipenaeus riveti; Callinectes toxotes; Ucides occidentalis*. In the inner estuary the diversity of biota decreases and the presence of *Crucibulum spinosum*, *Corbula* sp, *Callinectes arcuatus*, *Penaeus vannamei* were registered (Estrella, 2000); *Ostrea columbiensis*, *Mytella strigata*, *Mytilopsis trawtuineana*, *Protothaca asperrima*, *Macoma silique, Tellina* sp., *Polymesoda* sp., *Theodoxus luteofasciatus, Cerithidea mazatlanica, Littorina varia* and *Tralia panamensis* were present in the intertidal zone (Cruz, 2003).

Other studies were focused on the knowledge of the state of populations of invertebrates of commercial interest such as oysters (*Ostrea columbiensis*) in the inner estuary of the Gulf of Guayaquil including the southwest part of the city of Guayaquil during the period between August and October 1978, the highest average density was 95.89 Ind / m^2 and recorded in zone 1 corresponding to the branches entering the city of Guayaquil on June 5 bridge to the south of the island of Santa Ana.

Cruz (1983) conducted a study on bivalves of the Gulf of Guayaquil. Later Cruz in 1986 contributed to the knowledge of live bivalves in the El Salado and Cascajal Gulf of Guayaquil Interior based on the analysis of three cruises in 1978, 1983 and 1985 by INOCAR (Cruz, 1986), and identified 19 species, the most frequent and abundant being *Mytella strigata* and determined that one of the most representative families was Corbiculidae, one of the species more common was *Corbula amethystina,* and also made the first report of the presence of *Cumingia adamsi* for the study area.

Cruz in 1992 presented the results of six cruises in the subtidal zone of the Gulf of Guayaquil and published on the status of malacological resource (bivalves and gastropods) from the collection of 144 samples of sediment carried in Estero Salado, Channel gravel pit, Jambelí, the Morro and the island Puná, identifying 52 species: 31 bivalve molluscs and 21 gastropods, being those with high potential economic and food; *Mytella strigata*, *Mytella guyanensis, Crassostrea columbiensis* and *Anadara tuberculosa*, of which *M. strigata* was the most abundant and characteristic of estuarine environment (Cruz, 1992). The same author made a study of the existing malacofauna in estuarine benthic branches around the city of Guayaquil including Estero Salado and the Rio Guayas in 11 sites and recorded 11 species of molluscs; corresponding to 7 species of bivalve families Ostreidae, Mytilidae, Dressenidae, Veneridae, Tellinidae and Corbiculidae; and 4 species of gastropods as Neritidae, Potamididae, Littorinidae and Melampidae, registering the most common families Ostreidae, Dressenidae, Potamididae and the area with the greatest diversity around the bridge Portete and the least diversity was observed in the Guayas River.

One of these studies was the evaluation of water quality in the Puerto Hondo estuarine branches (Estrella, 2000), where the following species of mollusks were recorded: *Littorina varia*, *Cerithidea mazatlanica*, *Cerithidea valida, Plekobeilus sp. Sphenia fragilis Leptopecten velero*, *Corbula amethystina Anadara grandis, Anadara tuberculosa;* and Crustaceans: *Litopenaeus occidentalis, L. californiensis, L. brevirostris, L. stylirostris, Trachipenaeus riveti, Callinectes toxotes, Ucides occidentalis, Uca* sp. In addition other taxa were registered in the inner branches of Estero Salado as echinoderms, anemones, nematodes, polychaetes and other taxa (Lahmeyer-Cimentaciones, 2000).

Studies conducted in 2003 in different sections of Estero Salado, determined the presence of the intertidal benthic fauna, mainly consisting of polychaetes (43%), gastropods (34%), bivalves (22%), insects (1.21%) and less abundance was recorded of arachnids, crustaceans and nematodes. Also, it was determined that in the section B (Barbier et al.) the presence of five species *Cerithidea* sp., *Psychoda*, *Polymesoda inflata*, and *Capitella* sp., *Hydrophorus* sp., this last species was the dominant species (Hidroestudios, 2003).

Eighteen species were recorded in the preliminary inventory of mollusks from the Wildlife Reserve Mangroves among which are found: *Ostrea columbiensis, Mytella guyanensis, Mytella strigata, Prothothaca asperrina, Prothothaca ecuatoriana, Littorina fascista, Marinula* sp, *Mellampus* sp. (Fundacion Natura, 2006).

Some the macrobenthic species of commercial importance are: the oyster *Crassostrea columbiensis*, *C. iridescens*, *Mytella guyanensis*, *M. strigata, Alabina* sp*.,* the mangrove crab *Ucides occidentalis*, which lives in intertidal mangrove habitats in the Guayas River estuary (Twilley et al., 2001). There are five species of Penaeid shrimp: *Penaeus occidentalis, P. stylirostris, P. brevirostris, P. vannamei* and *P. californiensis* the last two being the most abundant in the Gulf (García-Saénz & Peláez, 1998).

The main research related to macroinvertebrates in the Estero Salado and Gulf of Guayaquil is showed in the Table 6. Which includes a project for Ministry of Environment of Ecuador (MAE) ***´´The Pilot study level on treatment sludge bioremediation developing and using native bacterial consortia of Estero Salado´´***, some datum of this project are part of this Ph.D. thesis.

Table 6. Chronology the main studies relate with of macroinvertebrates biodiversity in the Estero Salado and Gulf of Guayaquil.

Year	Autor	Institution	Research
1826	D´Orbigny	Museum National d´Histoire Naturelle de Paris	Expedition to South America Brazil, Argentina, Bolivia, Chile y Norte del Perú.
1831	Hugh Cuming	British Museum Natural History	Exploration the coast of Perú, Ecuador, Galápagos, Panamá, Costa Rica, México. In Ecuador collected shells at Isla del Muerto, Puná Island in the **Gulf of Guayaquil**, Santa Elena, Puerto cayo, Salango, Isla de la Plata , Bahía de Caráquez and Atacames. Cuming collected marine mollusks
1841	Richard B. Hinds	British Institution	Survey of coast from Gulf of Guayaquil to Panamá to collect shells. Hinds found 50 species of mollusks in Bay of Guayaquil
1887	A. Cousin F. Jousseaume	Societe Zoologique de France	Mollusques nouveaux de la Republique de I´Equateur.
1904	Alexander Agassiz	Museum of Comparative Zoology at Cambridge	Extensive dredging operations along the coasts of Perú, Ecuador, Galápagos, Panamá and Mexico (First report about deep-sea mollusks of The Eastern Pacific)
1961	Axel A. Olson		Collected mollusks since Panamá to Perú
1967	D. Bonilla	Universidad de Guayaquil- Facultad de CCNN	Study the family Mytilidae in ecuadorian coast
1971	Myra Keen		Shells of Tropical West America
1974	Manuel Cruz	Instituto Oceanográfico de la Armada	Registred the presence of shells *Crassostrea corteziensis* at Estero Salado
1978	Guillermo Castillo	Empresa Municipal de Alcantarillado de Guayaquil	Recuperación del Estero Salado. Plan de Trabajo
1980	Manuel Cruz, Matilde González, Elena Gualancañay & Francisco Villamar	Instituto Oceanográfico de la Armada	Fauna sublitoral bentónica del Estero Salado Interior incluyendo moluscos, anélidos, crustáceos y foraminíferos
1981	Elba Mora & Blanca Reinoso	Instituto Nacional de Pesca	Estado actual de las poblaciones de ostiones (*Ostrea columbiensis*) en el estuario interior correspondiente al sector suroeste de la ciudad de Guayaquil

1986	Manuel Cruz	Instituto Oceanográfico de la Armada	Contribución al conocimiento de los bivalvos vivos en los esteros de El Salado y Cascajal del Golfo de Guayaquil Interior
1986	Francisco Villamar	Instituto Oceanográfico de la Armada	Distribución de poliquetos bentónicos en el Golfo de Guayaquil
1988	Norma Chalén	Instituto Nacional de Pesca	Mercurio en sedimentos y organismos marinos
1989	Norma Chalén	Instituto Nacional de Pesca	Concentración de mercurio en organismos bivalvos
1992	Manuel Cruz	Instituto Oceanográfico de la Armada	Estado actual del recurso malacológico (bivalvos y gasterópodos) de la zona infralitoral del Golfo de Guayaquil
2000	Asociación Lahmeyer Cimentaciones	M.I. Municipalidad de Guayaquil	Estudios de Prefactibilidad, factibilidad y selección de la mejor alternativa del Plan Integral de la Recuperación del Estero Salado. Informe Final parte II
2001	Petroecuador	Esingeco	Diagnóstico y Plan de manejo Ambiental del terminal Fuel Oil de Petrocomercial en la ciudad de Guayaquil
2003	Hidroestudios Cía. Ltda.	M.I. Municipalidad de Guayaquil	Plan Integral de recuperación para el Estero Salado-Fase I Guayaquil (PIRES). EIA de los desvíos temporales de los tramos Ay B del Estero Salado
2004	W. Lobato Paraense	Pan American Health Organization	Planorbidae, Lymnaeidae and Physidae of Ecuador (Mollusca: Basommatophora
2005	Hidroestudios Cía. Ltda.	M.I. Municipalidad de Guayaquil	Plan Integral para la recuperación del Estero Salado-Fase I Guayaquil (PIRES)- Estación experimental de aireación
2006		Fundación Natura. Capítulo Guayaquil	Zonification of the Faunistic Production Reserve Manglares El Salado.
2007	Nelson Zambrano	M.I. Municipalidad de Guayaquil	Estudio de Impacto Ambiental del Parque Ecoturístico Estero Salado Norte.
2010	Rosa Sigüenza	Universidad de Guayaquil- Facultad de CCNN	Niveles de Coliformes totales y *Escherichia coli* en Bivalvos de interés comercial *Ostrea columbiensis* y *Mytella guyanensis* (Mollusca: Bivalvia) como indicador de contaminación microbiológica en el Estero Puerto Hondo. Provincia del Guayas-Ecuador.
2011-2012	Roberto Erazo, Maritza	CESTTA-ESPOCH-Ministerio del Ambiente	Estudio a nivel Piloto sobre tratamientos de

	Cárdenas, Verónica Bravo y John Salazar		biorremediación de lodos desarrollando y utilizando consorcios bacterianos nativos del Estero Salado.
2012	INOCAR	Instituto Oceanográfico de la Armada	Estudio y Levantamiento de la batimetría y caracterización del sedimento en el Estero Salado
2013	David Jiménez	Universidad de Guayaquil- Facultad de CCNN	Cuantificación de metales pesados (Cadmio, cromo, niquel y plomo) en agua superficial, sedimentos y organismos (*Crassostrea columbiensis*) Ostión de mangle en el Puente Portete del Estero Salado (Universidad de Guayaquil).
2013	Alfonso Kuffó	Universidad de Guayaquil- Facultad de CCNN	Niveles de cadmio, cromo y plomo y su bioacumulación por *Mytella strigata* delimitando la zona urbano-marginal en el Estero Salado de Guayaquil

2.7. Uses, importance of the Estero Salado and justification of the work

In Ecuadorian coasts the main goods and main uses are: aquaculture, fishing, tourism and recreation, human settlements, industrial building, agriculture, livestock farming, scientific research, conservation of protected areas, navigation, commercial activities, mining, wood exploitation, salt production and dumping of wastes (Arriaga, 2000). However, other different activities (Figure 8) developed without sustainable management of the natural resources in the estuaries has had negative effects since some of these activities are causes of estuarine pollution (EPA, 2006).

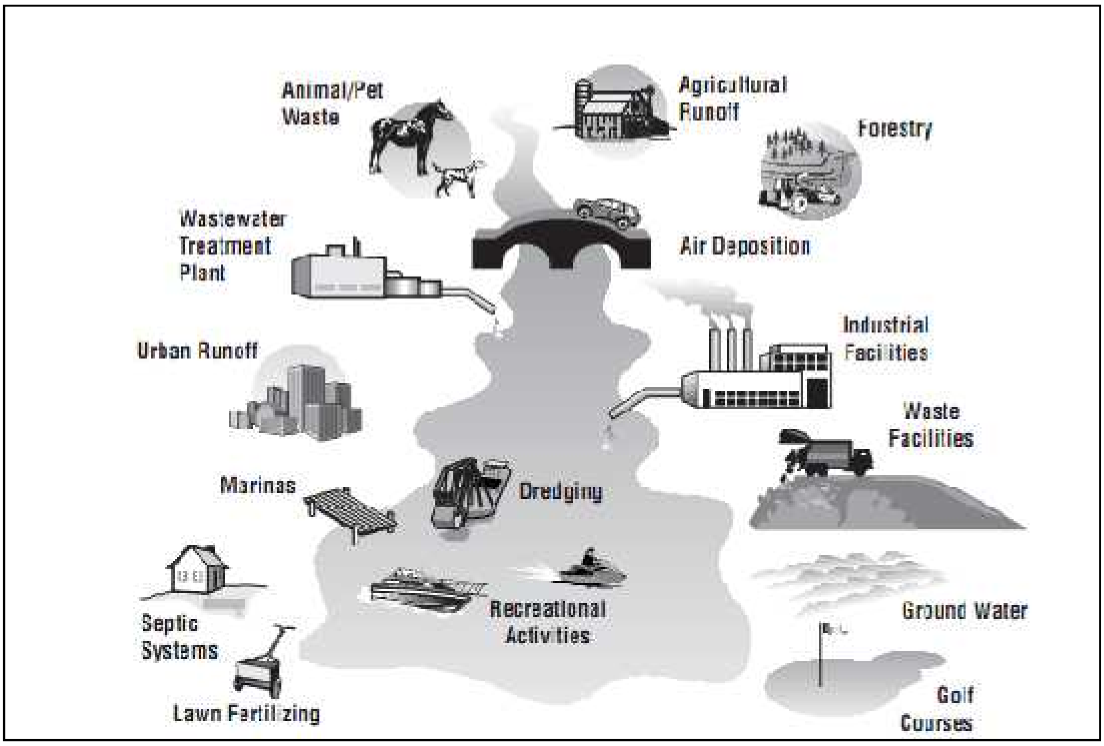


Figure 8. Source of estuarine pollution. Source: EPA, 2006.

The main productive activities that have developed in the Estero Salado influence area are: agriculture, livestock farming, aquaculture, fishing, commercial activities, manufacturing industries, food and beverages, tourism, shipping, wood exploitation, mining, scientific research, transfer of liquefied petroleum gas, electricity generation, thermoelectric plants (Fundacion Natura, 2006). The traditional fisheries have developed in the outer and inner estuarine branches, in the inner branches the fisheries sites are mainly Puerto Hondo, Plano Seco, Mongón and Southwest of Guayaquil city, where there are at least 102 fishermen, according to studies in 2006, of molluscs, crustaceans and fish through the use of different fishing gears. The main species of fishes in the inner branches present in Guayaquil city, specifically in the estuaries of Plano Seco, Mongón, Puerto Hondo are *Diapterus peruvianus*, *Mugil cephalus*, *Centropomus viridis*, *Micropogonias* sp, *Eleoctris picta*, *Bagre panamensi*, *Poecilia* spp, *Pomadasys panamensis, Oligoplites altus*, *Bairdiella ensifera*, and others species (Fundacion Natura, 2006).

There is unsustainable management of natural resources of Estero Salado estuary due to the pollutant contribution which come from industries and residual waters mainly of organic discharge, solids in suspensions, fat and oils, foods, tobacco, textiles, paper and paperboard (Zambrano, 2007), and also domestics waters, sedimentation produce by deforestation of mangrove forest, unplanned human settlement, over-fishing of commercial species, inadequate management of solid waste mainly of plastic, change of natural forms of estuarine inner branches, affecting the water quality from the early 1900s with the channeling and sanitation of Guayaquil, alteration of habitat, alteration of physical, chemical and microbiological of water, where the dissolved oxygen have been depleted over time reaching concentrations between 4.5 to 0 mg/L in sites close to Guayaquil city (CAAM, 1996). Hence, there is negative impact on the native biota of the inner branches of Estero Salado that enter to Guayaquil city. Therefore, is well understood that there is an increasing deterioration of Estero Salado due to different human activities which are intensive and increasing over time (EMAG, 1978; Hidroestudios, 2003; Holden, 1978; Lahmeyer-Cimentaciones, 2000).

There is few information about the concentration of persistent organic pollutants (Total Petroleum Hydrocarbon) and trends in metals (Fernández-Cadena J.C., Andrade, Silva-Coello, & De la Iglesia, 2014; INOCAR, 2012) , most toxic heavy metals such as: Pb, Cd, and Hg in sediments and their relationship on the community structure of macroinvertebrates. These are considered very important in the functionality of this estuarine ecosystem considering that they are the main source of food and work to local fisherman who live around of this estuary.

This thesis includes an analysis of the interrelation between contaminants with biological parameters and organism such as macroinvertebrates to evaluate the status of community structure as a way to measure the health of aquatic systems of Estero Salado inner branches. This research will add knowledge to the assessment of if there is an increasing loading of pollutant agents in time and space between 2007 and 2012 and to measure if there is a decrease of diversity of benthic macroinvertebrates in the intertidal zone of mangrove. The thesis will also provide technical information that supports changes in the management and environmental public policy of this emblematic and very important estuary of Ecuador.

2.8. Area of study

This study was conducted in the internal branches of the Estero Salado in Guayas province, it included protected areas by The National System of Protected Areas (SNAP) in the part of Subsystem Heritage State Natural Areas (PANE) know as Reserva de Producción de Fauna Manglares El Salado with a surface of 15520.95 ha located Southwest of Guayaquil according the official register of Ministry of Environment 072 (28 June of 2016) and part the Private Protected Areas Subsystem (APPRI) called Bosque Protector Estero Salado Norte with 30.10 ha (Zambrano, 2007). As well as the branches A and B section of zone I located in the Northeast of Guayaquil city (Figure 9).

The inner branches of Estero Salado are divided in three aquatic zones in Guayaquil city, these zones are: I, II, III, (Table 7; Figure 9) according to the classification made by the Municipal Company of Drainage Systems called Empresa Municipal de Alcantarillado de Guayaquil (EMAG, 1978) and accepted in 2000 by the Municipality of Guayaquil and the Environment Ministry of Ecuador in 2012. The zoning of areas consider the geographic situation, the physical configuration, hydraulic regime, degree of pollution and land use. Each zone includes interior sections of Estero Salado (Lahmeyer-Cimentaciones, 2000).

Zone I corresponds to urban areas with services of drainage and drinking water systems,, these are residential and industrial zones discharging their domestic waters and serve to channel rainwater and the estuary. This zone extends from Urdesa, Kennedy until the bridge 5 de junio and the 17th street.

Zone II is formed by recent urban areas, due to unplanned urban settlements, which were invaded by people of low economic resources that filled up the estuarine branches to build their homes; most homes have drainage systems and drinking water. In this zone is situated since 17th. Street and the Portete bridge until the south of Guayaquil city.

Zone III includes the estuarine branches called Estero Plano Seco, Estero Mongón, Madre de Costal and the Salado located in Southwest of Santa Ana Island and the Northwest the Esperanza Island. In this zone there is the lowest anthropogenic activity of Guayaquil city, and it is situated in a protected natural area by the Ecuadorian State called Reserva de Producción de Fauna Manglares El Salado (RPFMS) since 2003 (Fundacion Natura, 2006).

Table 7. Description of Zones and Section the inner branches of Estero Salado.

Zone	Section	Description
Zone I North of Guayaquil city	Section A	Interior branches in the north area of Guayaquil city between urban areas Urdesa and Kennedy.
	Section B	Interior branches in the northeast of Guayaquil between sports park Miraflores and the bridge located between Kennedy and Urdesa.
	Section C	Section the confluence between section A and section B.
	Section D	Section between the bridge 5 de junio and the seventeen street.
Zone II Southeast of Guayaquil city	Section E	Section between seventeen street and the bridge Portete.
	Section F	Section between the Portete bridge and the Third bridge of the Perimetral
	Section G	Section between Estero Santa Ana and south of Trinitaria Island.
	Section H	From Puerto Lisa to Cuatro Bocas.
	Section I	Section between Cuatro Bocas and Estero Cobina (It is located between Guayas river and Trinitaria Island) in the south of Guayaquil city.
Zone III Southwest of Guayaquil city	Section Puerto Hondo	Inner branch of Puerto Hondo to Estero Plano Seco, Estero Mongón and Estero Madre de Costal at northwest of Guayaquil city.
	Several Branches	Inner branches situated in west and south of Santa Ana Island and west of Esperanza Island.

Source: Ministerio del Ambiente, 2012.

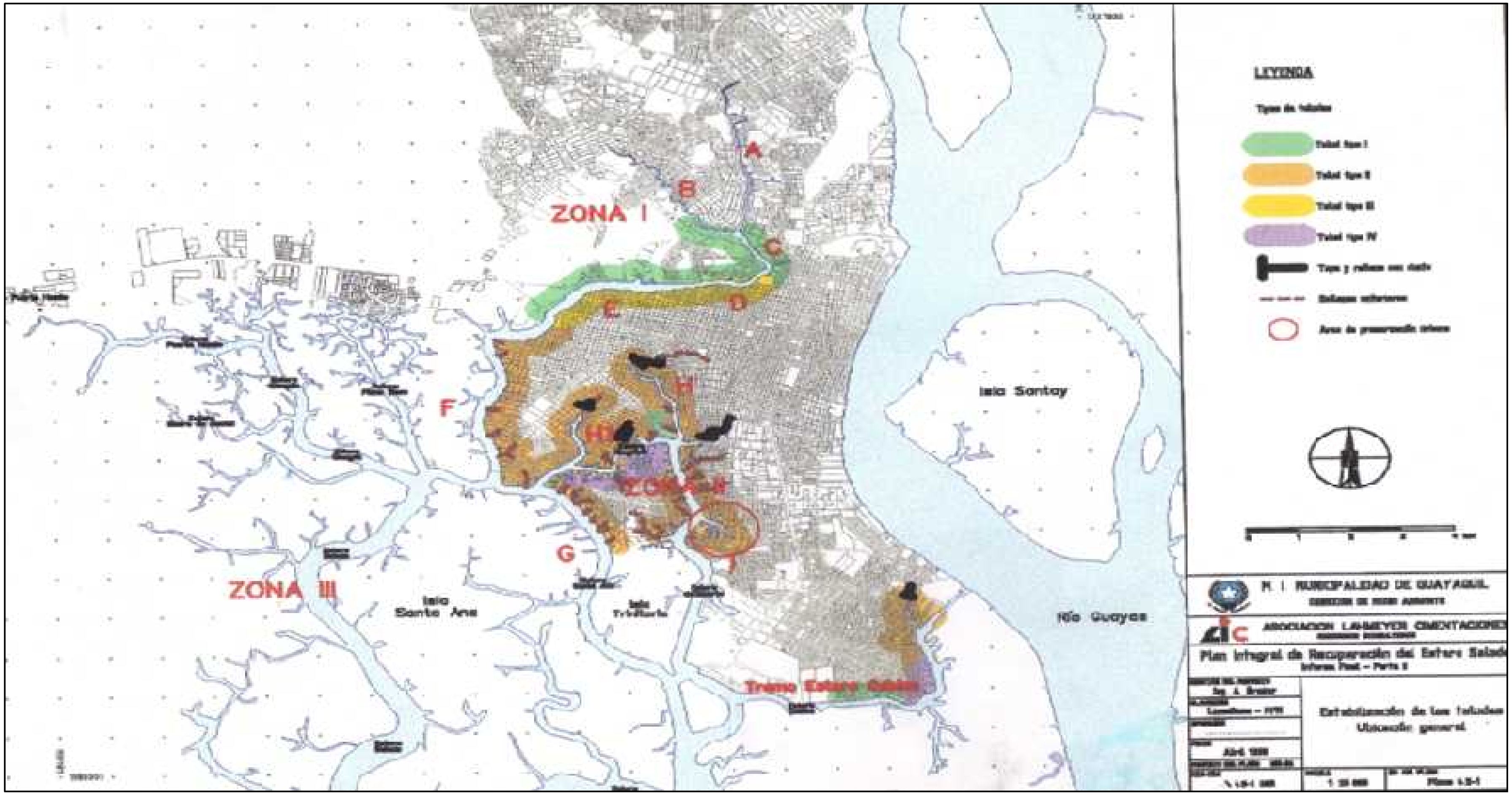


Figure 9. Map of ubication of zones and sections of Estero Salado Inner branches in Guayaquil City. Source:(Lahmeyer-Cimentaciones, 2000).

Chapter 3. Composition, abundance, diversity and distribution of Macroinvertebrates in the Guayas River at 2012-2013

3.1 Introduction

The Guayas River watershed is situated center-west of Ecuador in the Guayas province. It has the highest population of Ecuador (3573003 inhabitants (INEC, 2010b)). The Guayas River is one of the most important rivers of the Pacific Coast of South America for its high productivity and hydrological characteristics (CAAM, 1996). Adjoining it are the Daule and Babahoyo rivers. It drains a basin area of about 32116.84 km^2 (64% of total drainage basin) and is the major source of freshwater (20 km^3 year^{-1})(Stevenson, 1981). The watershed of Guayas River width ranges from 1.5 km and 3 Km except in front of the city of Guayaquil in the sheltered area called Area Nacional de Recreación Isla Santay that reaches 5 km in width (CAAM, 1996). The Guayas River watershed has an extension of 36000 km^2 (Gobierno Provincial del Guayas, 2012) and it is composed of seven sub-basins from the rivers Daule, Babahoyo, Yaguachi, Vinces, Macul, Jujan according to studies by the National Institute of Meteorology and Hydrology for its acronym in Spanish (INAMHI).

This River is considered part of **Ecosystem Guayas** together with the Gulf of Guayaquil and the estuary of Guayas River (Montaño & Sanfeliú, 2008). The Guayas River and Estero Salado are interconnected by channels between mangrove islands north of Puná Island, (Ayón, 1987) in´´ Gobierno Provincial del Guayas´´, 2012. It has the largest economic development of the country due to its agricultural potential generated by the Guayas River Basin with subsistence crops such as cassava, vegetables and fruit trees and export crops such as bananas, rice, coffee, and cocoa, over 300000 hectares of easily irrigable extent, with cattle raising and shrimp farming (Gobierno Provincial del Guayas, 2012; Montaño, 2010; Rossel, Cadier, & Gómez, 1996). However, since the decade of the 1980s, there is evidence that domestic and industrial activities have caused contamination in the Guayas River basin, due to nutrient enrichment, traces of pesticides, heavy metals concentrations such as iron, cooper, cadmium, mercury in water and sediments (Pin, García, & Castello, 1998; Solórzano, 1989; Suéscum, Maridueña, Castro, Moncayo, Morán, Estrella, Guale, Sonnenholzner, Freire, & Massuh, 1998); organic load, toxic substances, hydrocarbons and pathogenic microorganisms (Gobierno Provincial del Guayas, 2012).

High concentrations of fecal coliforms (organic pollution) have been registered in some tributaries of the Guayas River watershed, e.g. Balzar (El Empalme County and downstream of the population centre of Daule (Daule county) with mean values in the Daule River of 93NMP /100 ml. As well as pesticides such as DDT, dieldrin, lindane, heptachlor, benomyl among others organochlorine and organophosphate pesticides, biocides derived from agriculture activities in the rivers Daule, Chimbo, Chanchán, Barranco Alto, Bulubulu and Culebras river in the years 1997 ,1998 and 2009 (Gobierno Provincial del Guayas, 2012; Universidad Agraria del Ecuador, 2009, 2010).

Water pollution is one the most important anthropogenic causes of global change in freshwater ecosystems. In the case of Daule and Babahoyo River, research in April 2012 during the rainy season, showed that certain human activities are unfortunately leading to increases in aluminium, ammonia, fecal coliforms, oils and fats levels and decrease oxygen dissolved (<5mgL) in the surface waters. These parameters are in non-compliance of Ecuadorian environmental regulation of waters. ¨*Criteria of water admissible to preservation of flora and fauna in cold and warm waters and marine and estuarine waters of TULSMA¨* (Ministerio del Ambiente, 2003b) . All sites surveyed had water with regular category according to application of the ICA index (50-70) and produced a decrease in the diversity of aquatic organisms and generate algal growth demonstrating the lack of urban wastewater treatment of waters that are discharged into rivers (CESTTA, 2012). Therefore, unsustainable management of natural resources in intensive agriculture, industrialisation and urbanization in this watershed produce high stress levels on this ecosystem.

Given that anthropogenic stressors usually alter the biotic and abiotic components of freshwater ecosystem, the use of biological methods to assess their impacts on these ecological systems has been promoted over the last few decades as a useful and complementary technique to water physicochemical analyzes (Gonzalo & Camargo, 2013; Hellawell, 1986). Macroinvertebrate communities have been extensively used for biomonitoring of freshwater systems in the world (Alba- Tercedor, 1996; De Pauw & Hawkes, 1993; Domínguez & Fernández, 2009; Hellawell, 1986; Rosenberg & Resh, 1996; Rosenberg & Vincent, 1993). These organisms have been used as a biological group for the assessment of the quality of inland waters (Hellawell, 1986). Therefore they are one of the widest bio-indicators which have been used in Europe and the USA in the monitoring and the assessment of the water quality of rivers since 1920.

Macroinvertebrates have been used as excellent bio-indicators of contamination of water ecosystems because they are of wide geographical distribution, they are visible to the naked eye (>1mm), their taxonomy is well known, high species richness, sedentary habits which allows the evaluation the effect of pollution at a site, long lifecycles, easy to sample, and some taxa are sensitive to different pollution types (Domínguez & Fernández, 2009). The use of aquatic macroinvertebrates as indicators of water quality is becoming more widespread at the global level and techniques are still widely used for assessing the environmental impacts on inland water ecosystems. These organisms have been adopted in the monitoring and evaluation system for the quality in 27 nations of the European Union (Twelve, 2000) and other countries of the American continent as United States, México, Costa Rica, Colombia, Bolivia, Argentina and others sites (Domínguez & Fernández, 2009; López-Hernandez, G., & Hernández-García, 2005; Moya, Domínguez, Goitia, & Oberdorff, 2011; Roldán, 1988; Springer, Ramírez, & Hanson, 2010).

In the Ecuadorian coast rivers, studies of quality of water using macroinvertebrates have been done in the Baba River conducted by the National Institute of Fishing during the period 2009 and 2012 (Instituto Nacional de Pesca, 2009). Another study reported the registration of a new species of Ephemeroptera to Ecuador (*Traulodes quevedoensis)*, it was found in a moderately polluted area by urban and agricultural activities in the city of Quevedo (Flowers, 2009). Studies of community structure have been done in the rivers of Chone and Portoviejo during the rainy season in 2009, where low diversity and abundances of species was registered as well as the presence of the exotic species of gastropods *Melanoides cf tuberculata (*Cárdenas-Calle & Coello, 2009).

In The Guayas River, the communities of macroinvertebrates have been relatively little studied as indicated by Daule 2009 (Universidad Agraria del Ecuador, 2010) and the few sources information about it in the most of cases were not widely published but form part of environmental impact assessment studies (Long, Tull, Jeppe, De Souza, Dayalan, Pettigrove, McConville, & Hoffmann, 2015) did carried out by environmental consulting companies. Most of these studies have been related with others biological groups as phytoplankton and zooplankton (Cajas de, Coello, & Domínguez, 1998; Cajas de, Coello, & Moya, 1998) and there are some studies about chemical, physical and biological parameters in tributaries of Guayas Rivers as Daule and Babahoyo (Suéscum et al., 1998; Universidad Agraria del Ecuador, 2010). The most recent publication about the water quality in Guayas river basin using BMWP/Col and Neotropical Low-Land Stream multimetric index (NLSMI) and physical-chemical parameters showed that in the dry season (October to November) from 2013 bad water quality was found in residential areas and good quality in upstream forested locations. The main environmental variables that determined the ecological water of quality were flow velocity, chlorophyll concentration, conductivity, land use, sludge layer, and sediments type (Damanik-Ambarita, Lock, Boets, Everaert, Nguyen, Forio, Musonge, Suhareva, Bennetsen, Landuyt, Dominguez-Granda, & Goethals, 2016).

This study establishes a baseline of the composition, abundance and diversity of macroinvertebrates along 43 contiguous water bodies of the fluvial section of Guayas River watershed contained in sub-basins such as: Daule (1); Yaguachi (Acuerdo Ministerial No. 097A Publicado en el Registro Oficial edición especial No. 387); Macul (5); Macul (6); Drenajes menores (Acuerdo Ministerial No. 097A Publicado en el Registro Oficial edición especial No. 387) and an area out watershed (Acuerdo Ministerial No. 097A Publicado en el Registro Oficial edición especial No. 387), where is examined the use the macroinvertebrates as bioindicators of the water quality through the diversity and biotic index BMWP/Col (Biological Monitoring Working Party) modified to the Colombian neotropical zone (Zúñiga De Cardoso, 1997).

3.2 Aim

• To determine the composition, abundance and diversity of the communities of macro invertebrates of 43 water bodies in the Guayas province to evaluate the quality of their waters.

3.2.1 Specific Objectives

• To determine the composition and assemblages of benthic macroinvertebrates

• To establish the abundances, diversity and distribution of the aquatic macroinvertebrates.

• To assess the quality of waters from the subwatershed of Guayas river using the BMWP/Col biotic index.

3.3 Study area

The study area was located in the province of Guayas and covered approximately a 205 km long section of the fluvial section of Guayas River watershed from El Empalme (Site N_o 1) in the north to the south in the site Balao-Tengel (Site N_o 43) (Table 8). Sampling stations were selected in the 25 counties of Guayas province considered subwatersheds of Guayas River, the altitude, fast or slow water, type of sediments, and anthropogenic activities such as dredging, organic and inorganic contamination, and industrial effluents (Figure 10). A total of 43 stations were visited, 34 stations were surveyed during the rainy season 2012 and 2013 and 9 stations were survey during the last part the dry station (November of 2012), and these were the first nine sites mentioned in Table 8. The stations were divided in four areas (North, South, West and East). In the North from El Empalme to the South in Tengel and the West in Playas county to Bucay in the Eas. This last area is part of transition zone between the highlands and lowlands in the western mountains of Ecuador called Cordillera Occidental de los Andes (Gobierno Provincial del Guayas, 2012).

The sites were selected considering three scales: I) Regional scale based in altitudinal levels and vegetation type presented in the Guayas province due to their closed with to temperature and precipitation and that they play a important role in the establishing of aquatic plant communities as macrophytes that represented a greater surface area of settlement of macroinvertebrates (Hellawell, 1986). II) Local scale considering subwatershed (SENPLADES, CLIRSEN, MAGAP, & SINAGRO, 2009) and land use in Guayas provinces for the connection with pollutant agents produced by the discharges of domestic water, industrial or agriculture effluents and proximity of local populations (Gobierno Provincial del Guayas, 2012) and III) Punctual scale that includes different aquatic ecosystem and micro-habitat as different type of river, type of substrate and marginal vegetation (Domínguez & Fernández, 2009) (Figure 10-11-12).

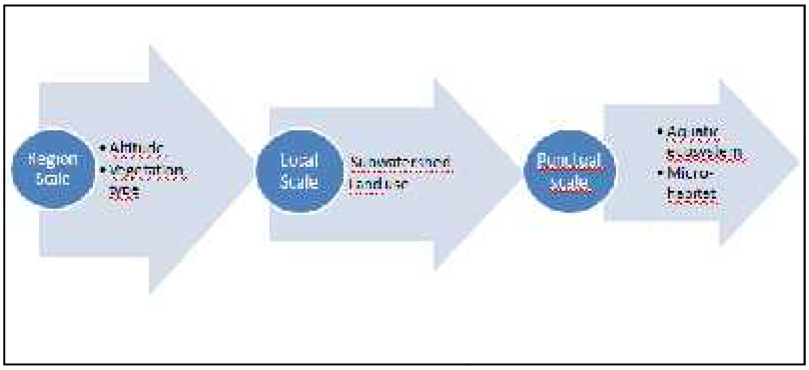
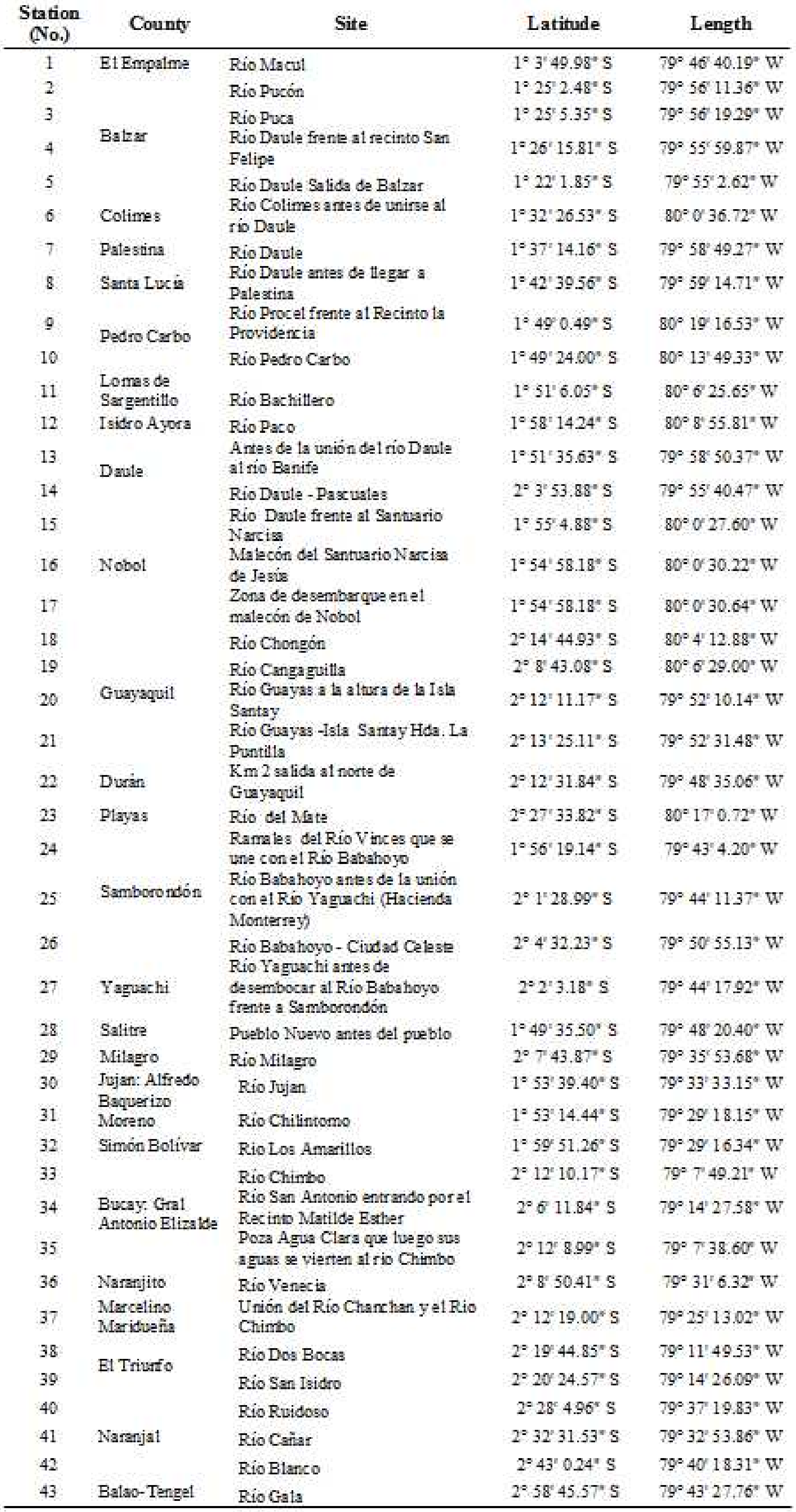


Figure 10. Outline the criteria considered to determine the sampling sites.

Table 8. Geographical position of the study sites in the province of Guayas.



Station (No.)	County	Site	Latitude	Length
1	El Empalme	Río Macul	1° 3' 49.98" S	79° 46' 40.19" W
2	Balzar	Río Pucón	1° 25' 2.48" S	79° 56' 11.36" W
3		Río Puca	1° 25' 5.35" S	79° 56' 19.29" W
4		Río Daule frente al recinto San Felipe	1° 26' 15.81" S	79° 55' 59.87" W
5		Río Daule Salida de Balzar	1° 22' 1.85" S	79° 55' 2.62" W
6	Colimes	Río Colimes antes de unirse al río Daule	1° 32' 26.53" S	80° 0' 36.72" W
7	Palestina	Río Daule	1° 37' 14.16" S	79° 58' 49.27" W
8	Santa Lucía	Río Daule antes de llegar a Palestina	1° 42' 39.56" S	79° 59' 14.71" W
9	Pedro Carbo	Río Procel frente al Recinto la Providencia	1° 49' 0.49" S	80° 19' 16.53" W
10		Río Pedro Carbo	1° 49' 24.00" S	80° 13' 49.33" W
11	Lomas de Sargentillo	Río Bachillero	1° 51' 6.05" S	80° 6' 25.65" W
12	Isidro Ayora	Río Paco	1° 58' 14.24" S	80° 8' 55.81" W
13	Daule	Antes de la unión del río Daule al río Banife	1° 51' 35.63" S	79° 58' 50.37" W
14		Río Daule - Pascuales	2° 3' 53.88" S	79° 55' 40.47" W
15	Nobol	Río Daule frente al Santuario Narcisa	1° 55' 4.88" S	80° 0' 27.60" W
16		Malecón del Santuario Narcisa de Jesús	1° 54' 58.18" S	80° 0' 30.22" W
17		Zona de desembarque en el malecón de Nobol	1° 54' 58.18" S	80° 0' 30.64" W
18	Guayaquil	Río Chongón	2° 14' 44.93" S	80° 4' 12.88" W
19		Río Cangaguilla	2° 8' 43.08" S	80° 6' 29.00" W
20		Río Guayas a la altura de la Isla Santay	2° 12' 11.17" S	79° 52' 10.14" W
21		Río Guayas -Isla Santay Hda. La Puntilla	2° 13' 25.11" S	79° 52' 31.48" W
22	Durán	Km 2 salida al norte de Guayaquil	2° 12' 31.84" S	79° 48' 35.06" W
23	Playas	Río del Mate	2° 27' 33.82" S	80° 17' 0.72" W
24	Samborondón	Ramales del Río Vinces que se une con el Río Babahoyo	1° 56' 19.14" S	79° 43' 4.20" W
25		Río Babahoyo antes de la unión con el Río Yaguachi (Hacienda Monterrey)	2° 1' 28.99" S	79° 44' 11.37" W
26		Río Babahoyo - Ciudad Celeste	2° 4' 32.23" S	79° 50' 55.13" W
27	Yaguachi	Río Yaguachi antes de desembocar al Río Babahoyo frente a Samborondón	2° 2' 3.18" S	79° 44' 17.92" W
28	Salitre	Pueblo Nuevo antes del pueblo	1° 49' 35.50" S	79° 48' 20.40" W
29	Milagro	Río Milagro	2° 7' 43.87" S	79° 35' 53.68" W
30	Jujan: Alfredo Baquerizo Moreno	Río Jujan	1° 53' 39.40" S	79° 33' 33.15" W
31		Río Chilintomo	1° 53' 14.44" S	79° 29' 18.15" W
32	Simón Bolívar	Río Los Amarillos	1° 59' 51.26" S	79° 29' 16.34" W
33	Bucay: Gral Antonio Elizalde	Río Chimbo	2° 12' 10.17" S	79° 7' 49.21" W
34		Río San Antonio entrando por el Recinto Matilde Esther	2° 6' 11.84" S	79° 14' 27.58" W
35		Poza Agua Clara que luego sus aguas se vierten al río Chimbo	2° 12' 8.99" S	79° 7' 38.60" W
36	Naranjito	Río Venecia	2° 8' 50.41" S	79° 31' 6.32" W
37	Marcelino Maridueña	Unión del Río Chanchan y el Río Chimbo	2° 12' 19.00" S	79° 25' 13.02" W
38	El Triunfo	Río Dos Bocas	2° 19' 44.85" S	79° 11' 49.53" W
39		Río San Isidro	2° 20' 24.57" S	79° 14' 26.09" W
40	Naranjal	Río Ruidoso	2° 28' 4.96" S	79° 37' 19.83" W
41		Río Cañar	2° 32' 31.53" S	79° 32' 53.86" W
42		Río Blanco	2° 43' 0.24" S	79° 40' 18.31" W
43	Balao-Tengel	Río Gala	2° 58' 45.57" S	79° 43' 27.76" W

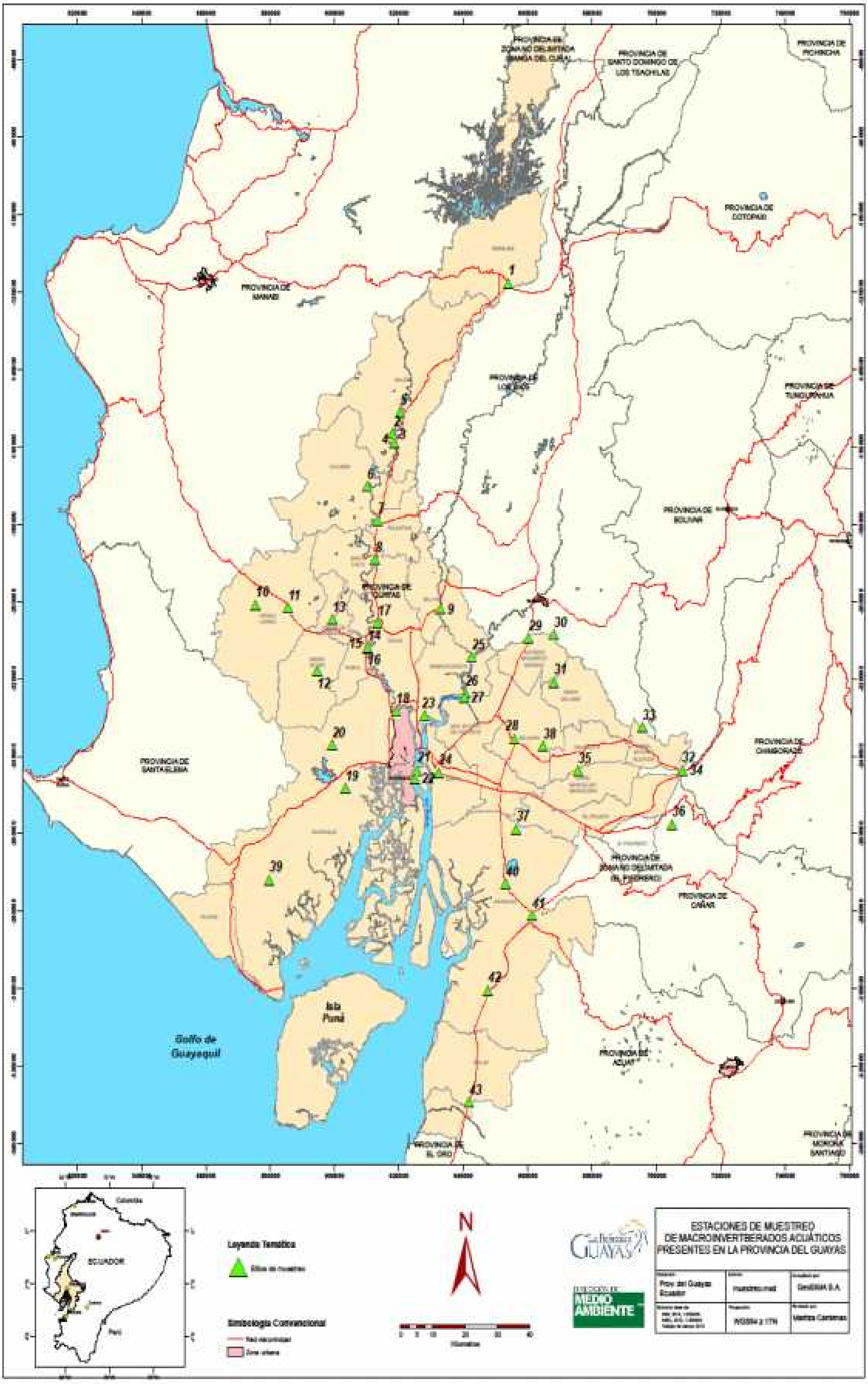


Figure 11. Geographical locations of the sampling stations of macroinvertebrates present in Guayas River watershed during November 2012 and March 2013. Source: (Gobierno Autónomo Descentralizado Provincial del Guayas, 2013). Source: (M. Cárdenas, Flowers, Zambrano, & Maldonado, 2013).

Figure 12. Descriptive pictures showed the characteristic of study sites in Guayas Provinces: a) High watershed; b) Low watershed; c) Polluted sites by anthropogenic activities in the low watershed of Guayas province during 2012-2013.

3.4 Methodology

3.4.1 Field work

Three samples of macroinvertebrates were randomly collected from shallow sediments, at each station using a semi-quantitative and multihabitat sampling method using a D net (500 µm mesh) on all sampling occasions (Springer et al., 2010) for fifteen minutes (five minutes by each replicates). Macroinvertebrates were collected from different habitats (e.g. gravel, silt, weed beds) at representative sites of river stretches. Organisms were removed from 0.1 m^2 area in the sampler down to a depth of ca. 10 cm by: 1) picking up the rocks and scrubbing off the attached animals with a bristled brush; 2) washing the bottom and walls of the net; 3) checking that the area had been sampled efficiently by examining the sediments for remaining animals. All material collected with the D net was deposited in plastic boxes to separate the invertebrates from the rocks and sediments and prevent the destruction of their body structures for ca. 5 minutes in each station. Macroinvertebrates were quickly extracted with forceps and placed in vials of 25 ml and other part of the samples was preserved in plastic bags, twices samples preserved with 80% ethyl alcohol.

3.4.2 Laboratory analysis

In the laboratory the samples were washed with potable water, the organisms were separated from sediment, roots, stems, leaves and stones and they were separated at the level of orders and placed in plastic vials of 25 ml of capacity. The taxonomic identification until most low level was done using different freshwater macroinvertebrates keys for the region (Domínguez & Fernández, 2009; Pennak, 1989; Roldán, 1988; Springer et al., 2010; Thorp & Covich, 2001). For trichoptera larvae (Springer, 2006), Palaemonidae (Holthuis, 1952); bivalves and gastropods (Ituarte, Cuezzo, & Ramírez, 2008; Ituarte, 1995) and leeches (Klemm, 1995).

3.4.3 Statistical analyses

A variety of univariate and multivariate analyses were employed. The data exploration and descriptive statistic were done using Statgraphics plus software version 4.1. To assess whether those data satisfied assumptions of normality and constant variance, it was used the Kolgomorov-Smirnov and Bartett´s test respectly (Legendre, 1998). In order to describe the spatial patterns of composition of macroinvertebrate assemblages of subwatershed was performed a non-metric multidimensional scaling (MDS). Biological variables were standardized with square-root. The Bray-Curtis similarity coefficient was employed to construct a similarity matrix, and a dummy variable was added. This matrix was then subjected to (MDS), followed by cluster analysis to identify the similarity between stations using abundance and presence-absence data at genus levels using Primer 6 package version 6.1.14 (Clarke & Gorley, 2006).

The Bray Curtis similarity matrix was subjected to a two-way crossed PERMANOVA designed to determinate whether there was a significant difference in composition and abundance of invertebrates considering subwatershed, seasonal stations and main human activity. Subsequently this test examined the null hypothesis that *a priori* there were no significant differences and it was rejected if the significance level (P) was >1% (Clark & Warwick, 2001). The Global and pairwise R statistic values were employed to evaluate these differences. The R statistic has a range between 1,when the composition of the samples within each group are more similar to each other than to that of any of the samples from other groups, down to about 0,when within-group and between group similarities do not differ (Clarke & Gorley, 2006).

3.4.4 Biological metrics and indices

Furthermore, combinations of statistics were used for macroinvertebrate analysis diversity as: species richness (S), total number of individuals (N), Shannon-Wiener index (H´), Simpson index (1-lamda´) and Pielou´s evenness index (J´) were calculated due to their widespread use in the characterization of benthic communities and their inclusion in the multimetric indices applied. The diversity indices were calculated using the DIVERSE routine with Primer 6 soft-ware (Clarke & Gorley, 2006). To evaluate the water quality was use the biotic index Biological Monitoring Working Party (BMWP) (Armitage, Moss, & Wright, 1983) modified for neotropic Colombian (BMWP/Col) (Zúñiga De Cardoso, 1997).

In addition, the following macroinvertebrate metrics were calculated; abundance and percentage of families of macroinvertebrates. Significant changes in the value of these metrics can indicate environmental disturbances, including freshwater pollution (Hellawell, 1986). Besides the Shannon-Wiener was performed because is a good measure to know the environment stress level (Masson, 2002; Puente & Diaz, 2008; Roldán, 1992), where low diversity score (H´) were considered indicative of higher stress and poorer environmental conditions, a value less than 1 was interpreted to signify substantial pollution (Chapman, 1996). The BMWP/Col index which allowed the valuation of habitat condition and freshwater quality, particularly organic polluted, through of sensitivity of certain families of invertebrates to assess the impact of pollution in rivers and streams. The families are assigned a score between 1-10 (Table 9) for each study site by adding up the score of all families present at a site.

These indices were used for effect of comparison with other studies in Central, Southamerica (Domínguez & Fernández, 1998; Junqueira & Campos, 1998; Rocabado & Goitia, 2011; Zúñiga De Cardoso, 1997; Zúñiga, Rojas, & Mosquera, 1993) and Europe (Alba- Tercedor et al., 2005; Hellawell, 1986; Wenn, 2008) and the combined approaches use of indices is necessary for the assessment of naturally stressed communities (Puente & Diaz, 2008) and the intensity of the environmental stress (Hellawell, 1986).

Table 9. Biological Monitoring Working Party (BMWP/COL) modified to Neotropical Colombian-Score.

Families	**Score**
PLECOPTERA: Perlidae EPHEMEROPTER: Oligoneuriidae TRICHOPTERA: Calamoceratidae COLEOPTERA: Psephenidae DIPTERA: Blepharoceridae ODONATA: Polythoridae	10
EPHEMEROPTERA: Euthyplociidae TRICHOPTERA: Helicopsychidae .Odontoceridae.Philopotamidae-Anomalopsychidae COLEOPTERA:Ptilodactylidae MEGALOPTERA: Corydalidae	9
EPHEMEROPTERA: Leptophlebiidae-Polynitarcyidae-Caenidae TRICHOPTERA: Leptoceriidae-Hidrobiosidae- Xiphocentronidae-Hydroptilidae ODONATA:Gomphidae	8
EPHEMEROPTERA: Leptohyphidae TRICHOPTERA: Glossosomatidae-Polycentropodidae COLEOPTERA:Elmidae ODONATA:Aeshnidae-Calopterygidae	7
COLEOPTERA: Scyrtidae ODONATA: Coenagrionidae DIPTERA: Simuliidae HEMIPTERA:Corixidae-Gerridae-Vellidae GASTROPODA: Ancylidae	6
EPHEMEROPTERA: Baetidae TRICHOPTERA: Hydropsychidae COLEOPTERA: Staphylinidae ODONATA: Libellulidae	5
COLEOPTERA: Curculionidae-Crysomelidae-Hydrophilidae-Gyrinidae DIPTERA: Tabanidae-Ceratopogonidae-Psychodidae- Dixidae-Empididae HEMIPTERA:Pyrolidae TRICLADIDA: Planariidae GASTROPODA: Planorbidae-Lymneidae-Thiaridae	4

COLEOPTERA: Dytiscidae HEMIPTERA:Hydrometridae GASTROPODA: Physidae BIVALVIA: Sphaeriidae HIRUDINEA: Glossiphonidae	3
DIPTERA: Chironomidae -Culicidae-Syrphidae	2
OLIGOCHAETA: Tuficidae	1

The organisms were identified to genus level; however, the family level was allocated a score between one to ten. The most sensitive such as the family Perlidae (Plecoptera); Oligoneuriidae (Ephemeroptera) and other families scored ten, Baetidae (Ephemeroptera) score five, molluscs score four and the least sensitive worms (Oligochaeta) score one. The BMWP index modified for Colombia (Zúñiga De Cardoso, 1997) is calculated by adding the scores for each of the families represented in the sample. The number of taxa gives an indicator of the diversity of the community. For each station sampled the sum of scores based on the presence of the families of invertebrates recorded at each station was obtained and scores of the BMWP index modified for Colombia were interpreted according to the assessment of the quality of the water (Table 10).

Table 10. Interpretation of the quality of the water through the application of the index BMWP modified to the Neotropical Colombian.

Class	Quality	Value	Meaning	Color
I	Good	>101	Water very clean	Blue
II	Acceptable	61-100	Obvious effect of pollution	Green
III	Doubt	36-60	Contaminated Water	Yellow
IV	Critical	16-35	Very Polluted Water	Orange
V	Very Critical	>15	Strongly Polluted Water	Red

3.5 Results

Among the major human abnormalities observed of the study sites were changes in the riverbed and the rivers flow, loss of the marginal aquatic vegetation, deforestation of the nearby slopes to the rivers, removal of sediment of rivers, human settlements and factories built on the edges of rivers, domestic effluents that directly are discharged in the river, we observed solid waste at the surface of water as plastic dishes and bottles, dumping of garbage on the banks of rivers and cow manure (Figure 13).

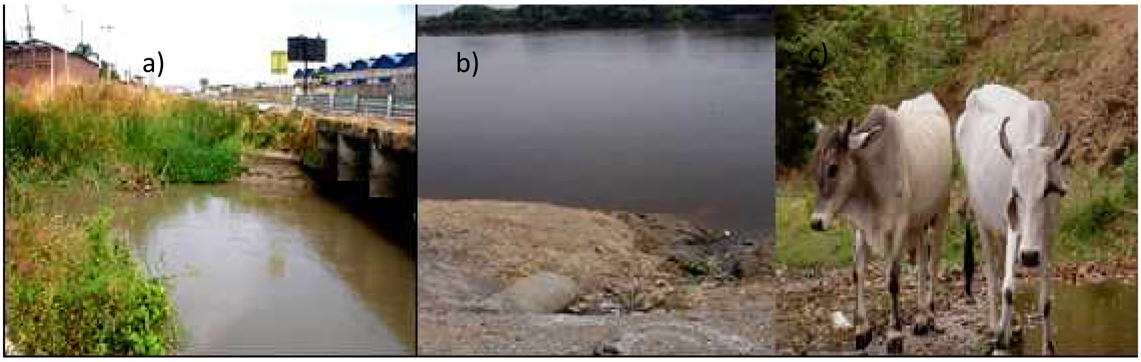


Figure 13. Some sources of pollution that change the water quality in Guayas River watershed are: a) industrial zona in Durán (station 22); b) domestic effluents; c) organic waste derived of livestock activity (cow manure) deposited in water bodies.

3.5.1 Composition and abundance of benthic macroinvertebrates fauna

A total of 6831 organisms were collected belonging to three phyla, i.e., Mollusca , Arthopoda and Annelida, 58 families and 84 genera were identified (Annex 2). The most abundant taxa were Gastropoda, Ephemeroptera and Hemiptera. Others taxa abundant were Diptera, Bivalvia, Ostracods, Collembola, Trichoptera and Coleoptera. Low abundance (<100 individuals) was found in: Decapoda, Odonata, Amphipoda, Hirudinea, Oligochaeta, Lepidoptera, Megaloptera, Homoptera and Isopoda (Figure 14). One specie belonged to Gastropoda, which was the numerically dominant taxa was *Melanoides cf tuberculata*.

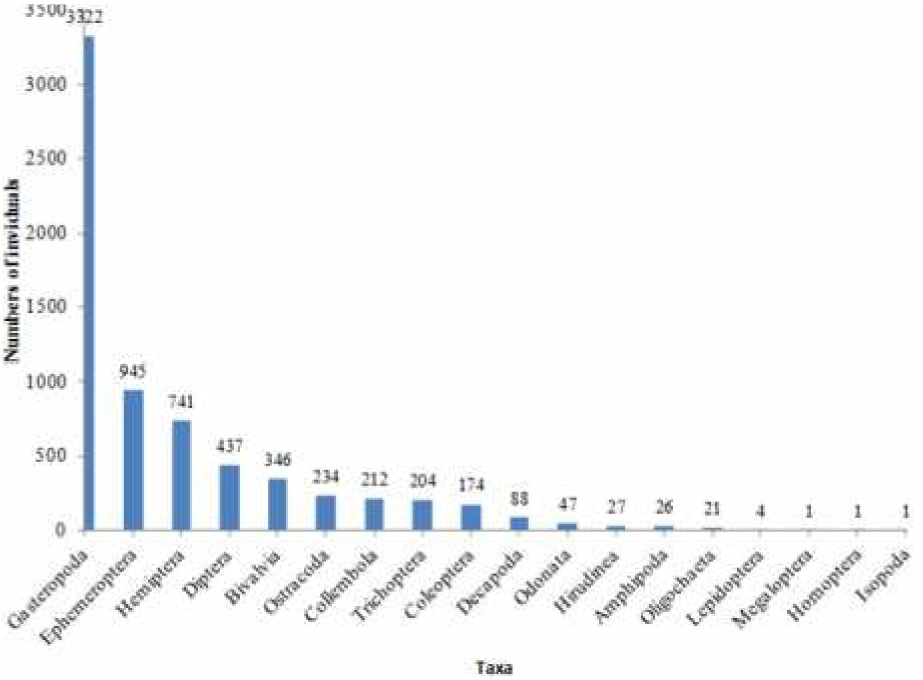


Figure 14. Abundance the main macroinvertebrates taxa registered in the Guayas Watershed River in Guayas provinces during November of 2012 to March of 2013.

The mollusks were represented by snails of the family Thiaridae with 76%, with the presence of *Melanoides cf. tuberculata*, followed by the family Hydrobiidae with 12% represented mainly by *Heleobia* sp. and the family Sphaeriidae (Bivalve) with the 9% (Figure 15a). Others families less abundance were Ancylidae, Physidae, Planorbidae and Corbiculidae (Annex 2)

The most abundant insects' family pertained to Ephemeroptera order with the Leptohyphidae Baetidae and Leptophlebiidae families. The family with the lowest abundance was Polymitarcyidae with less than 1% (Figure 15b). The Leptohyphidae family was mainly represented by the genus *Tricorythodes* and *Leptohyphes*, only a few individuals were registered of the genus *Traverhyphes*, *Leptohyphodes*, *Allenhyphes* and *Yaurina*. The Polymitarcyidae family was represented only by *Campsurus*.

The Baetidae family was mainly represented by the genus *Callibaetis*, *Baetodes* and *Camelobaetidius* and with fewer individuals *Americabaetis*. The Leptophlebiidae family was represented by the genus *Thraulodes* and *Traverella.*

Among the leading families of insects of the Order Hemiptera were: Mesoveliidae, Veliidae, Corixidae, Naucoridae, Notonectidae, Ochteridae, Belostomatidae, Hidrometridae, Hebridae and Gerridae. The most abundance were Corixidae and Mesoveliidae with few abundance were registred Mesoveliidae, Naucoridae, Gerridae, Ochteridae, Notonectidae, Hebridae, Belostomatidae e Hidrometridae (Figure 15c). The Corixidae family was represented only by the genus *Tenagobia*, Veliidae was mainly represented by the genus *Rhagovelia* and M*icrovelia*. The family Naucoridae was represented by the genus *Limnocoris* and *Cryphocricos*.

The Diptera were represented mainly by the Chironomidae, Ceratopogonidae and the Culicidae families (Figure 15d), with few abundance were registred the families Stratiomyidae, Psychodidae, Simulidae and Tipulidae. The family Chironomidae was represented mainly by genus *Chironomus*, *Tanytarsus*, and *Polypedilum*, while less abundant genera were *Paratanytarsus*, *Dicrotendipes* and *Pseudochironomus*. The family Ceratopogonidae was represented by the genera *Stilobezzia* and *Alluaudomyia*. The family Culicidae presented two genera *Anopheles* and *Culex*. While the family Tipulidae only was represented by *Tipula*. Others Diptera were identified only at the family level, and these were: Psychodidae, Simuliidae and Stratiomyidae due to incomplete structures preventing further identification.

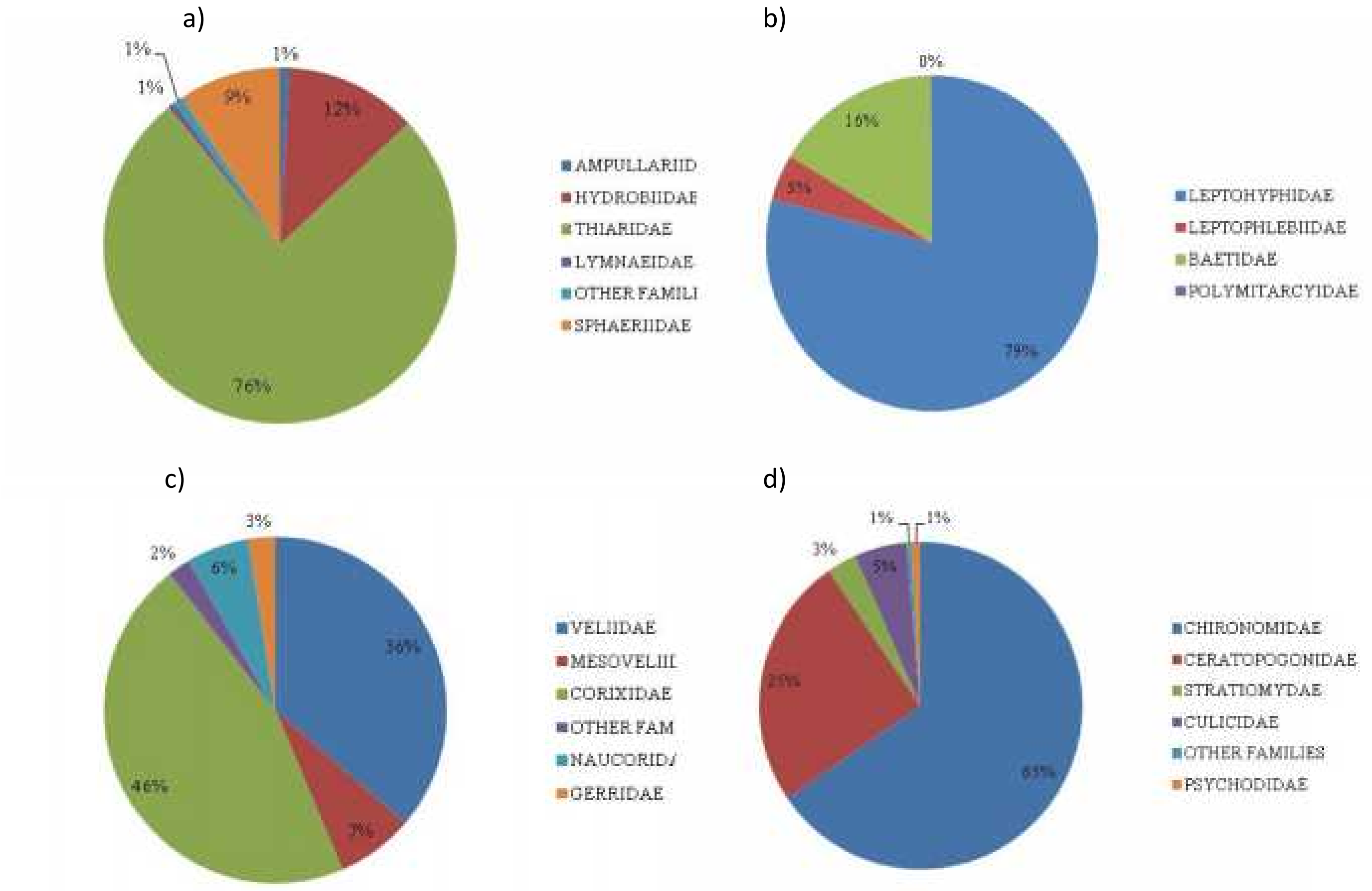


Figure 15. Relative abundance of most representative families of: a) Molluscs; b) Ephemeroptera; c) Hemiptera and d) Diptera recorded the Guayas Watershed River in the Guayas province during November of 2012 to March 2013.

The Odonata (dragonflies) were represented by the families Calopterygidae, Gomphidae, Libellulidae and Megapodagrionidae. Less abundant families were: Coenagrionidae and Protoneuridae (Figure 16a).

The Libellulidae family was represented by the genus *Elasmothemis*, *Brechmorhoga* and *Orthemis*. The Family Gomphidae recorded two genera *Progomphus* and *Phyllocycla*. Family Calopterygidae presented only the genus *Hetaerina*. Protoneuridae with *Neoneura* and the Coenagrionidae family recorded the presence of *Leptobasis* and *Ischnura*.

The Coleoptera was represented by ten families; the most abundant was Elmidae and the less abundant were Curculionidae, Psephenidae, Dytiscidae, Hydrophilidae, Staphylinidae, Noteridae, Chrysomelidae, Leptoceridae and Buprestidae (16b).

The Trichoptera registered four families; the most abundant were Hydropsychidae and Hydrophilidae. The less abundant were Hydroptilidae and Leptoceridae (Figure 16c).

Other important biolgical group were the crustaceans represented by the family Palaemonidae (river shrimp) with 72%, followed by the Amphipods of the family

Hyalellidae and the family Haustoriidae, with less abundant we registered the crabs family Ocypodidae and the Isopod family Bopyridae (Figure 16d). The Decapoda were represented by mainly by the family Palaemonidae with the species *Macrobrachium gallus* and *Macrobrachium carcinus*. Less abundance registered the specie *Macrobrachium panamense* and *Uca panamensis*. The amphipods registered two species being the most abundant the specie *Hyalella* sp. and the isopods were represented by only by the specie *Probopyrus* sp.

Other insect Order registered were Homoptera, Megaloptera, Collembola and Lepidoptera represented by the families Cicadellidae, Corydalidae, Isotomidae and Pyralidae respectively. The most abundant was the family Isotomidae and the lowest abundant were Corydalidae and Cicadellidae. Besides the presence of Ostracods were registered (Annex 2).

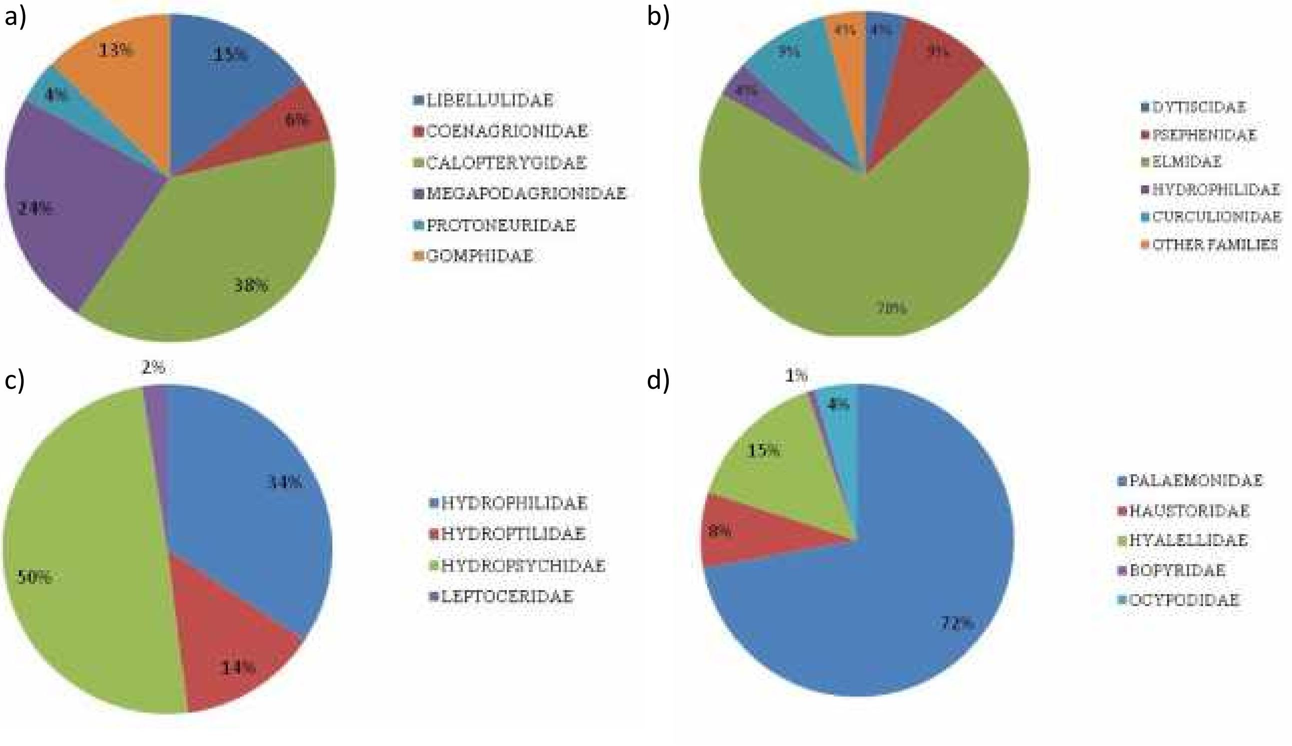


Figure 16. Relative abundance of less representative families: a) Odonata; b) Coleoptera; c) Trichoptera and d) Crustaceans recorded the Guayas River Watershed in the Guayas province during November of 2012 to March 2013.

The annelids were represented mainly by the Hirudinea (leeches) with 56% and oligochaeta with 44%. Leeches belonged to families Glossiphonidae and Piscicolidae.

3.5.2 Composition of benthic macroinvertebrates assemblages

The Non-metric multidimensional scaling ordination of taxa and abundance of macroinvertebrates shown two zones First zone conformed only for one station (E22) and other zone where the rest of stations are grouped. Nonetheless, it seems that there is a spatial pattern between the abundances of invertebrates in relation with the subwatersheds of Guayas River specialty in Daule and Drenajes menores. The assemblages of almost stations located out the watershed, Yaguachi and Jujan were more related between them (Figure 17).

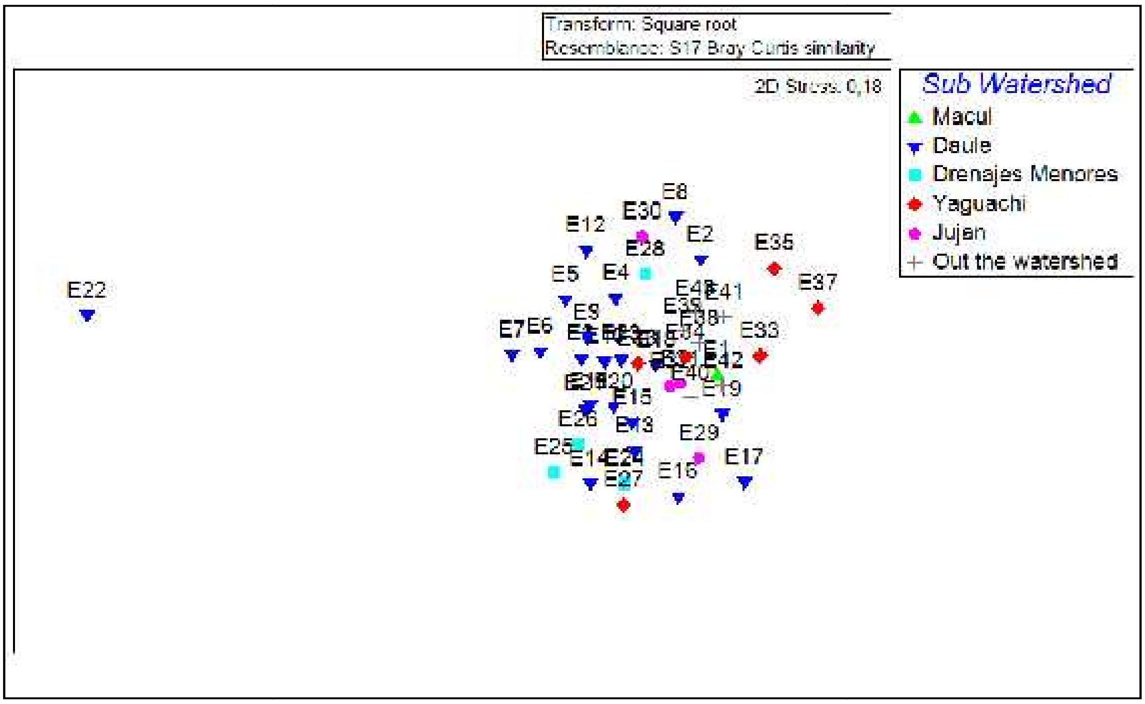


Figure 17. Non- metric multidimensional scaling ordination, derived from the Bray Curtis similarity matrix constructed using fourth root transformed the abundances of genus of benthic macroinvertebrates at the 43 sampling sites considering sub watershed between November of 2012 and March of 2013.

The figure 18 displays the results of a cluster analysis on the Guayas River watershed. The first group comprised in only the station E22 (San Camilo) located in Daule subwatershed in the downstream and industrial zone closest of Guayaquil city. The Second group comprised the rest of stations, Daule and other subwatershed. This group contained in two subgroups: 1) formed by sites located in upstream of Guayas province as Bucay and Marcelino Maridueña (E35 and E37) both stations belonging the Yaguachi subwatershed 2) comprised Daule River (E8 and E12) (Daule River before to arrive to Palestina town) and E12 (Paco River) pertain similar subwatershed Daule and in this area develops agricultural activities. Third group comprised the remainder of stations. In fact, at a similarity of around 45% it was observed only the stations E35, E37 (Poza de Agua Clara and Unión del Río Chanchán y el Río Chimbo); E30, E4 (Río Jujan and Río Daule frente al Recinto San Felipe) and E31 and E32 (Río Chilintomo and Río Los Amarillos).The dendrogram show that almost all stations there aren not similar between them and the only the station E24 and E27 (Vinces and Yaguachi Rivers) are significantly similar in 50%.

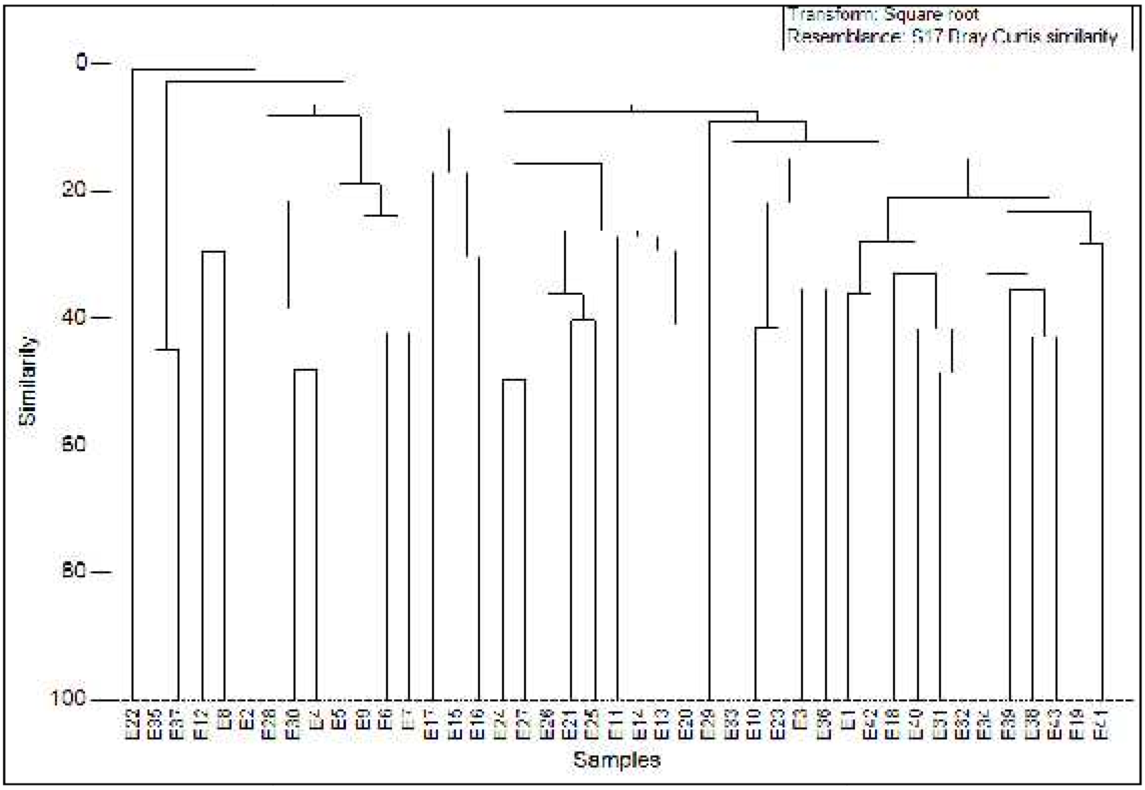


Figure 18. Dendrogram for hierarchical clustering from each of study sites, based on the Bray-Curtis similarity matrix constructed using square root transformed total abundances of the species of benthic macroinvertebrates in each site in Guayas River watershed between November of 2012 and March of 2013.

The non-metric multidimensional scaling ordination shows that there are no patterns of abundance of taxa found considering the weather season. Nonetheless, it seems that there is a spatial pattern between the abundances of invertebrates in relation with main human activities (Figure 19). The sites with greater similarities were the stations influenced by human activities such as settlement, urban zone, fishing and tourism, while the opposite were the stations influenced by industrial activity.

PERMANOVA demonstrated that there was significance in the abundance of macroinvertebrates in subwatershed. A one – way ANOSIM showed that composition and abundance was influenced for subwatershed (P = 0.1%) only. The global R statistic was low, reflecting the large variability in fauna composition across the scores. The comparison between the composition in each pair of watershed vs. main human activity were significantly different (P = 0.1%) and the significance level between groups was shown by Daule and the area out the watershed, the R statistic for all significant pairwise comparison ranging from 0.1 to 0.54.

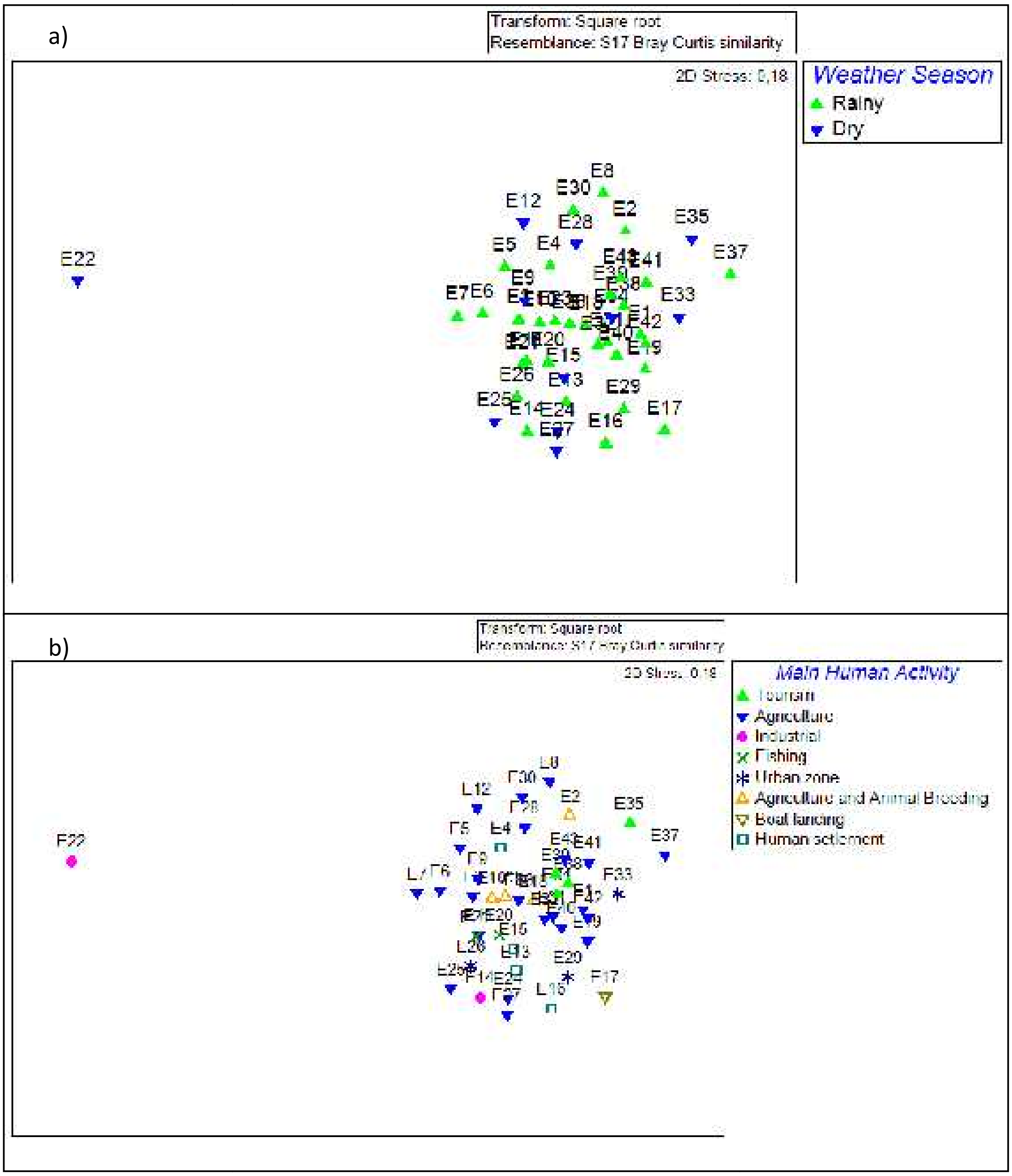


Figure 19. Non-metric multidimensional scaling ordination, derived from Bray-Curtis similarity matrix constructed using fourth root transformed total abundances of the genus of benthic macroinvertebrates at 43 sampling sites considering: a) weather season: dry and rainy season and b) Main Human activity between November of 2012 and March of 2013.

3.5.3 Number of species and diversity of benthic macroinvertebrates

The total number of individual (S) registered a range from 1-32 taxa. The median of species richness was 11 taxa (Annex 3). The subwatershed station with highest median of species richness value were Macul (30), the sites were out the watershed (16) and Jujan (15).

The highest species richness were seen in the stations E1 (Macul River) and E34 (San Antonio River) with the 30 and 32 taxa respectively. Most stations registered between 20 and 5 taxa. The lowest species richness values (≤ 5taxa) were seen in the stations E2, E6, E7, E8, E12, E13, E14,E17, E25, E28, E30, E35 and E37 and the station E22 registered only one taxa (Annex 4).

Community diversity, measured Shannon Wiener´s index (H´), ranged from 0 to 2.73 for the period 2012- 2013. The median value was 0.67 and the station with the highest index was E18 (Chongón River), others sites with values above of 2 were E3 (Puca River), E11 (Bachillero River), E15 (Daule River), E21 (Guayas River), E23 (Mate River), E32 (Los Amarillos River), E34 (San Antonio River), E36 (Venecia River), E38 (Dos Bocas River) and E39 (San Isidro River). The sites with values of diversity less than 1 were E2 (Pucón River), E5 (Daule River), E17 (Boat landing), E29 (Milagro River) and E30 (Jujan River). The Estero San Camilo (E22) located at Durán did not register value of diversity (Annex 4). A similar trend to that of the Shannon-Wiener Index was observed with the Simpson Index.

The equitability was high in twenty stations, these were E3, E6, E7, E8, E11, E12, E13, E14, E15, E18, E19, E21, E24, E25, E26, E35, E36, E37 and E39. Inversely were observed in the stations E2 (Pucón River) where there were dominances of *Tenagobia* sp. (Hemiptera), *Chironomus* sp. (Diptera), in the station E5 (Daule River), *Melanoides cf tuberculata* in the E22 (Estero San Camilo) and the specie *Callibaetis* sp., in the station E30 (Jujan River). No significant differences were obtained with richness of species, Shannon-Wiener index and Evenness between subwatersheds (Figure 20).

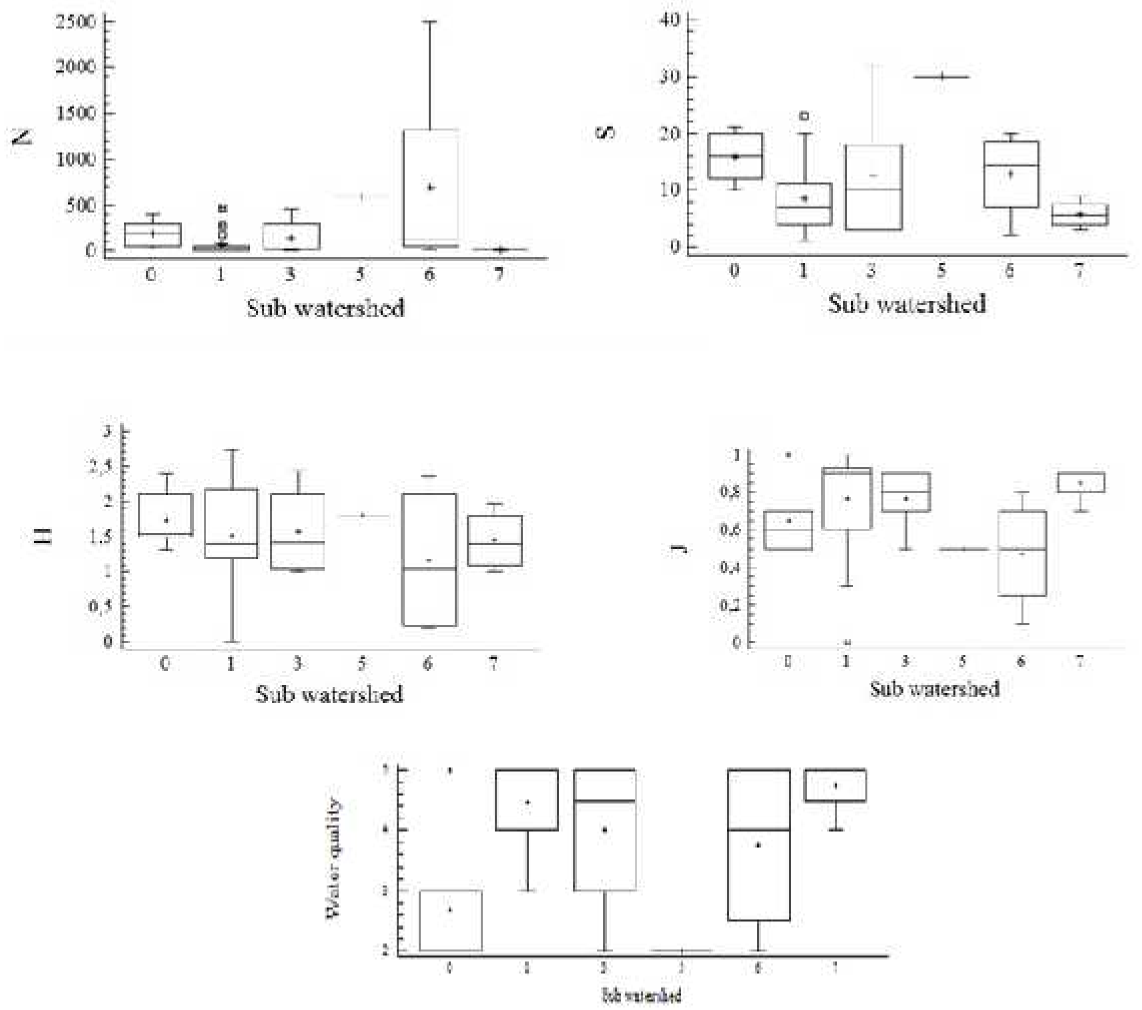


Figure 20. Box and Whiskers for number of macroinvertebrate taxa (S), total number of individuals (N), diversity index (H´), Eveness (J´) and water quality using BMWP/COL index among the Subwatershed. Each box encloses 50% of the data with the median value of t he variable displayed as a line. Top (upper quartile) and bottom quartile) of the box mark the limits of ±25% of the variable population. Lines extending from the top and bottom of each box mark the minimum and maximum values that fall within acceptable range. Outliers are displayed as individual point in the area o (out watershed) and sub-watersheds 1(Daule); 3 (Yaguachi); 5 (Macul); 6 (Jujan); 7 (Drenajes menores).

The dominant taxon was the snail of the family Thiaridae: *Melanoides cf tuberculata* which presented a very highest number of individuals with the 41% of the total individuals (Annex 2) and the most abundance was registered in Milagros River (E29). Other sites where it was registered with less abundance were Blanco River (E42), Daule River (E16), Cangaguilla River (E19), Los Amarillos River (E32) and The Ruidoso River (E 40) (Figure 21).

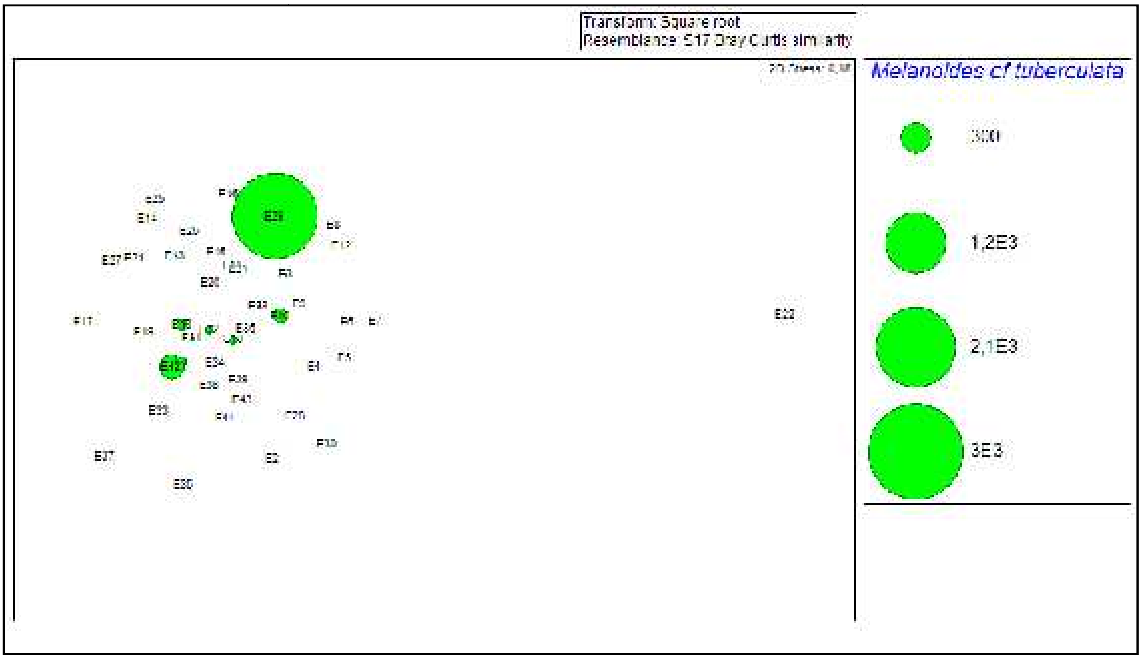


Figure 21. 2D bubble MDS configuration, derived from Bray-Curtis similarity matrix constructed using square root transformed of total abundances the exotic specie *Melanoides cf tuberculata* in each site surveyed in Guayas province between November of 2012 and March of 2013.

Another dominant taxon was the snail from family Ampullariidae, *Pomacea canaliculata*, in El Mate River (E23), in Playas County. However, it was present at the stations Pedro Carbo River (E10), Venecia River (E36), Babahoyo River near to Ciudad Celeste urbanization (E 26) and in the Yaguachi River (E27) (Figure 22).

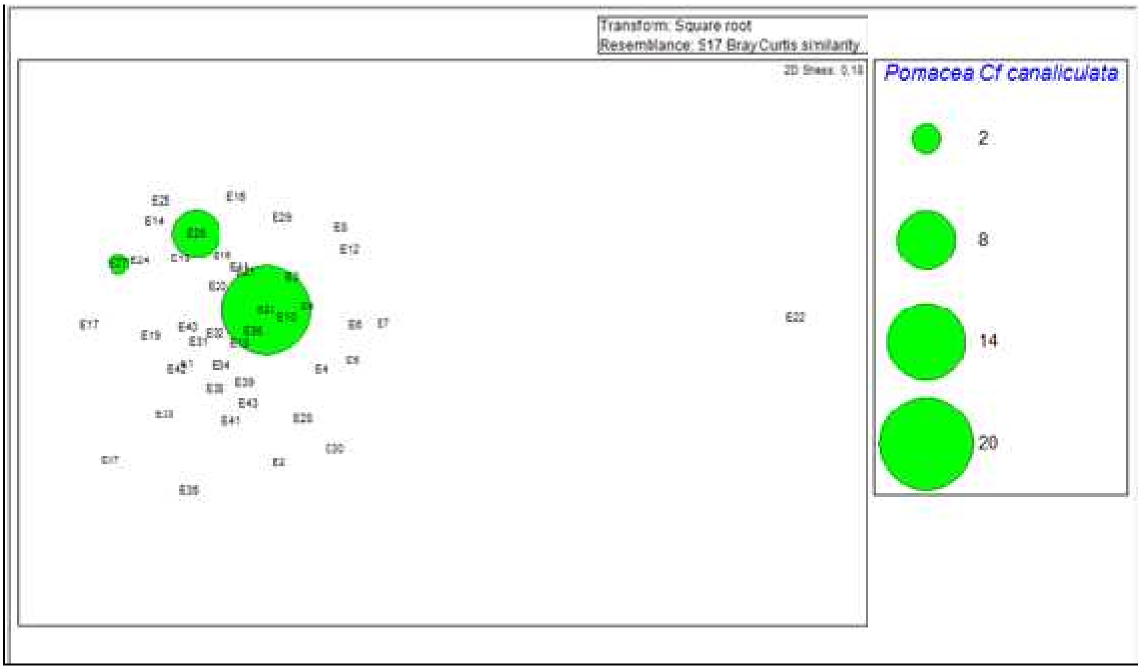


Figure 22. 2D bubble MDS configuration, derived from Bray-Curtis similarity matrix constructed using square root transformed of total abundances the exotic specie *Pomacea canaliculata* in each site surveyed in Guayas province between November of 2012 and March of 2013.

The one-way SIMPER showed that, abundance among the subwatersheds there were high percentage of dissimilarity between 78.92% to 97. 63%. The watersheds with most dissimilarity were Macul – Drenajes menores with the 97.63%, Drenajes menores - Out watershed and Daule - Yaguachi menores with 96% (Table 11). Among the taxa that distinguished from each watershed were the gasteropod *Amnicola* in the Macul and Drenajes Menores subwatersheds, the insect *Tricorythodes* sp., in the area out the watershed and the Hemiptera *Trepobates* was characteristic in the Yaguachi watershed.

Table 11. Summary of taxa detected by SIMPER as distinguishing between the benthic macroinvertebrate assemblages in the Guayas River watershed in 2012/2013. The subwatershed in which the abundances were greatest percentage of dissimilarity, average abundance, their contribution (%) to the within-group similarity, and cumulative total (%) of contribution (90% cut-off).

Subwatersheds	Distinguishing Species	Average abundance		Contribution%	Cum. %
		Macul	Drenajes menores		
Macul & Drenajes Menores (Average dissimilarity 97.63%	*Amnicola* sp.	17.80	0.25	18.05	18.05
	Yaurina sp.	9.22	0.00	9.48	27.53
	Melanoides cf tuberculata	5.29	0.00	5.44	32.97
		Drenajes menores	Out the watershed		
Drenajes Menores & Out the watershed (Average dissimilarity 96.22%)	*Tricorythodes* sp.	0.00	3.19	7.97	7.97
	Tenagobia sp.	0.00	4.17	7.45	15.42
	Melanoides cf tuberculata	0.00	3.37	6.75	22.16
		Daule	Yaguachi		
Daule & Yaguachi (Average dissimilarity 93.75%)	*Trepobates* sp.	0.09	0.79	5.22	5.22
	Chironomidae	1.00	1.74	4.76	9.98
	Leptohyphes sp.	0.00	3.40	4.74	14.72

3.5.4 *Water quality: application of BMWP/Col. biotic index*

The BMWP/Col index showed that the 49% of sites surveyed had very polluted water, 23% of sites had a strongly polluted water, 16% of sites had evident effects of pollution and a 12% of sites registered acceptable quality water. Significant differences were obtained on water quality between subwatersheds (P<0.001) see Figure 23.

Patterns of change in assemblages were observed between stations considering different water quality generated by BMWP/Col. The stations with acceptable water quality (blue color) and doubtful quality were located relatively closely together (Figure 23) being located all sites along the Coordillera Occidental de los Andes in the North-East in Macul River, East in San Antonio River up to South-East Gala River the Guayas province. These sites were characterized by being in the transition zone between lowland and highland, had on altitude over a 300m, and flowing (lotic) waters. These stations were: E1 (Macul River), E32 (Los Amarillos River), E34 (San Antonio River). E38 (Dos Bocas River) and E 43 (Gala River). Althout the station with critical and very critical water quality showed some differences in your emssamblage spetially the station E22 (San Camilo) located in the lowland, lower to 50 m of altitude and lentic water.

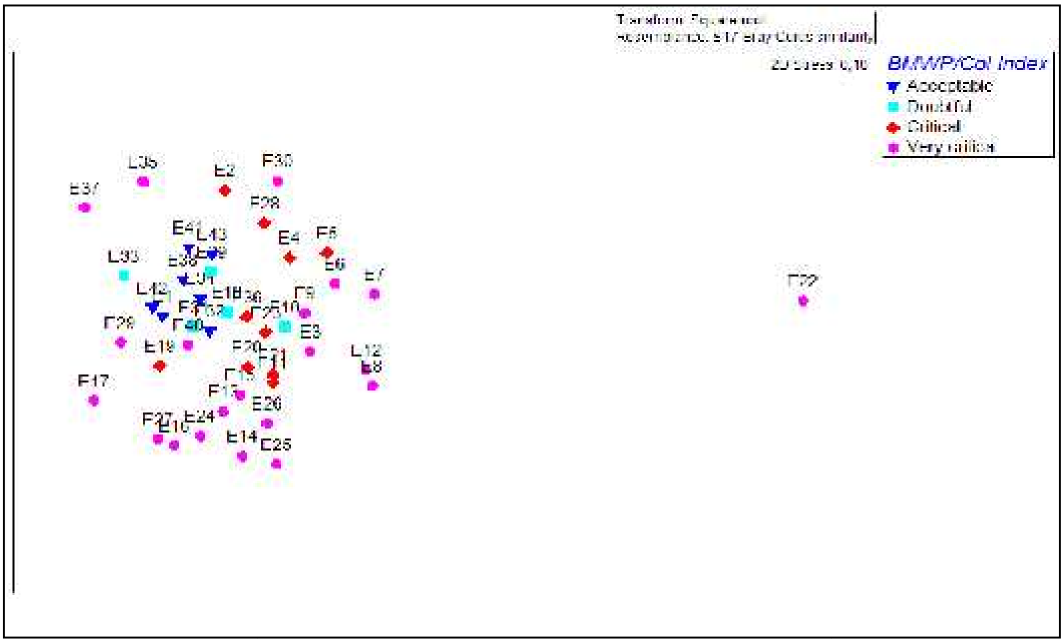


Figure 23. Non-metric multidimensional scaling ordination derived from Bray-Curtis similarity matrix constructed using square root transformed of total abundances of the families of benthic macroinvertebrates in each sample from each site considering the water quality criteria based in BMWP/Col biotic index between November of 2012 and March of 2013.

The very critical quality corresponded to watershed Daule, Jujan, Yaguachi and Drenajes Menores and is related to the human activities as: Urban and industrial area, human settlement, boat loading, and agriculture (Figure 24).

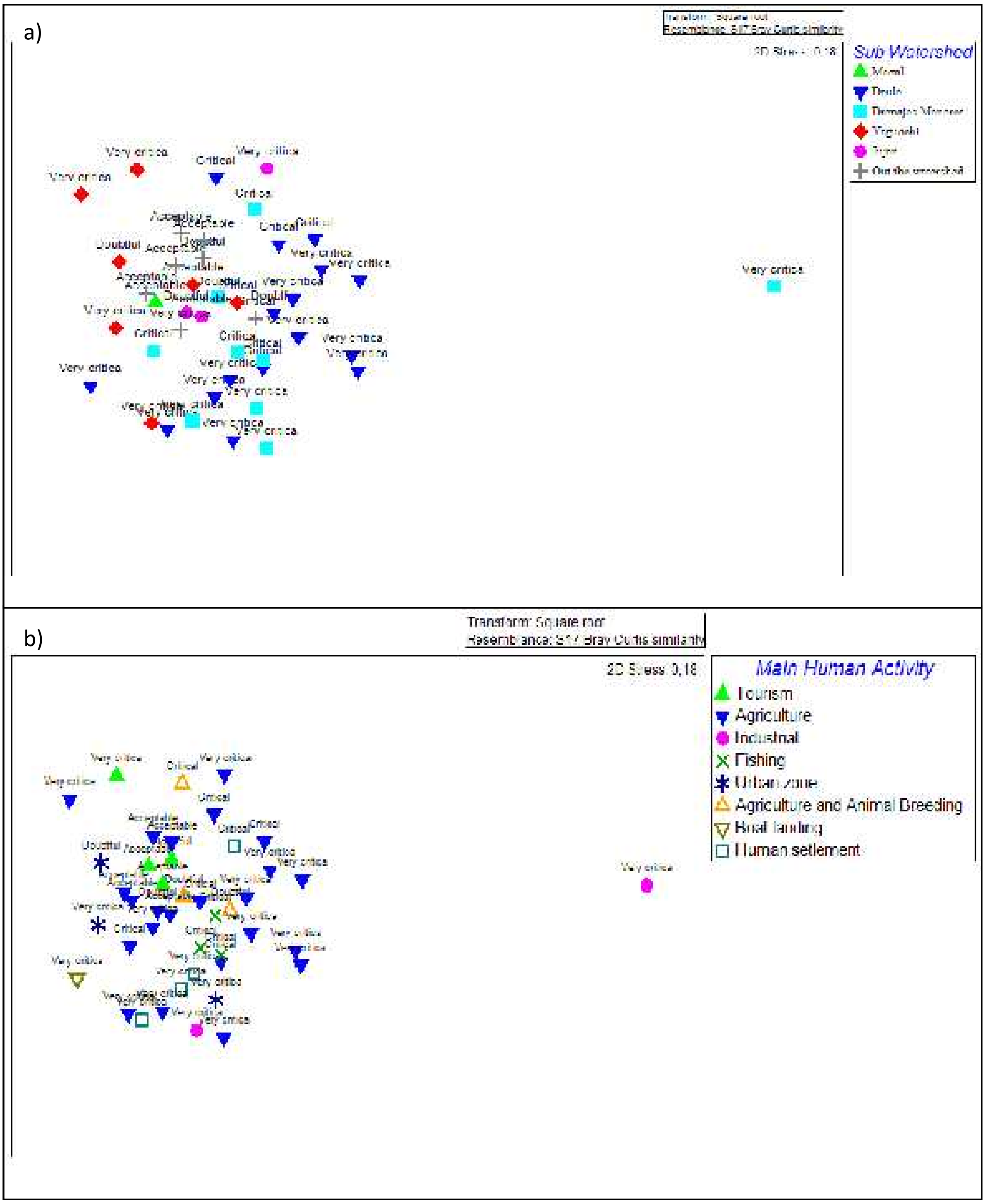


Figure 24. Non-metric multidimensional scaling ordination, derived from Bray-Curtis similarity matrix constructed using square root transformed of total abundances of the families of benthic macroinvertebrates in each sample from each site considering the water quality criteria based in BMWP/COL biotic index in relation to a) Subwatershed and b) Main human activity in Guayas Watershed River.

Based on water quality class discriminated by the BMWP the following groups were obtained see Figure 25 and Annex 5:

- *Class I. Good quality water*

No one station was registered with this water quality.

- *Class II. Acceptable quality water*

River: Macul, Los Amarillos, San Antonio, Dos Bocas, Cañar, Blanco and Gala.

- *Class III. Doubtful quality water*

Rivers: Pedro Carbo, Chongón, Chilintomo, Chimbo and San Isidro.

- *Class IV. Critical quality water*

Rivers: Pucón, Daule, Bachillero, Cangaguilla, Guayas, Mate, Salitre and Venecia.

- *Class V. Very Critical water quality*

Rivers: Puca, Colimes, Daule (To level of Palestina, Nobol, Pascuales and St. Lucia) Procel, Paco, Procel, Estero San Camilo, Babahoyo (To level the junction with the Yaguachi river Milagro, Jujan, Ruidoso and the Poza de Agua Clara.

The BMWP index showed the quality of the waters were that 49% very critical (water very polluted), 23% critical (heavily polluted water), 16% acceptable water (with evident effects of pollution) and the 12% Doubtful water (Acceptable water)

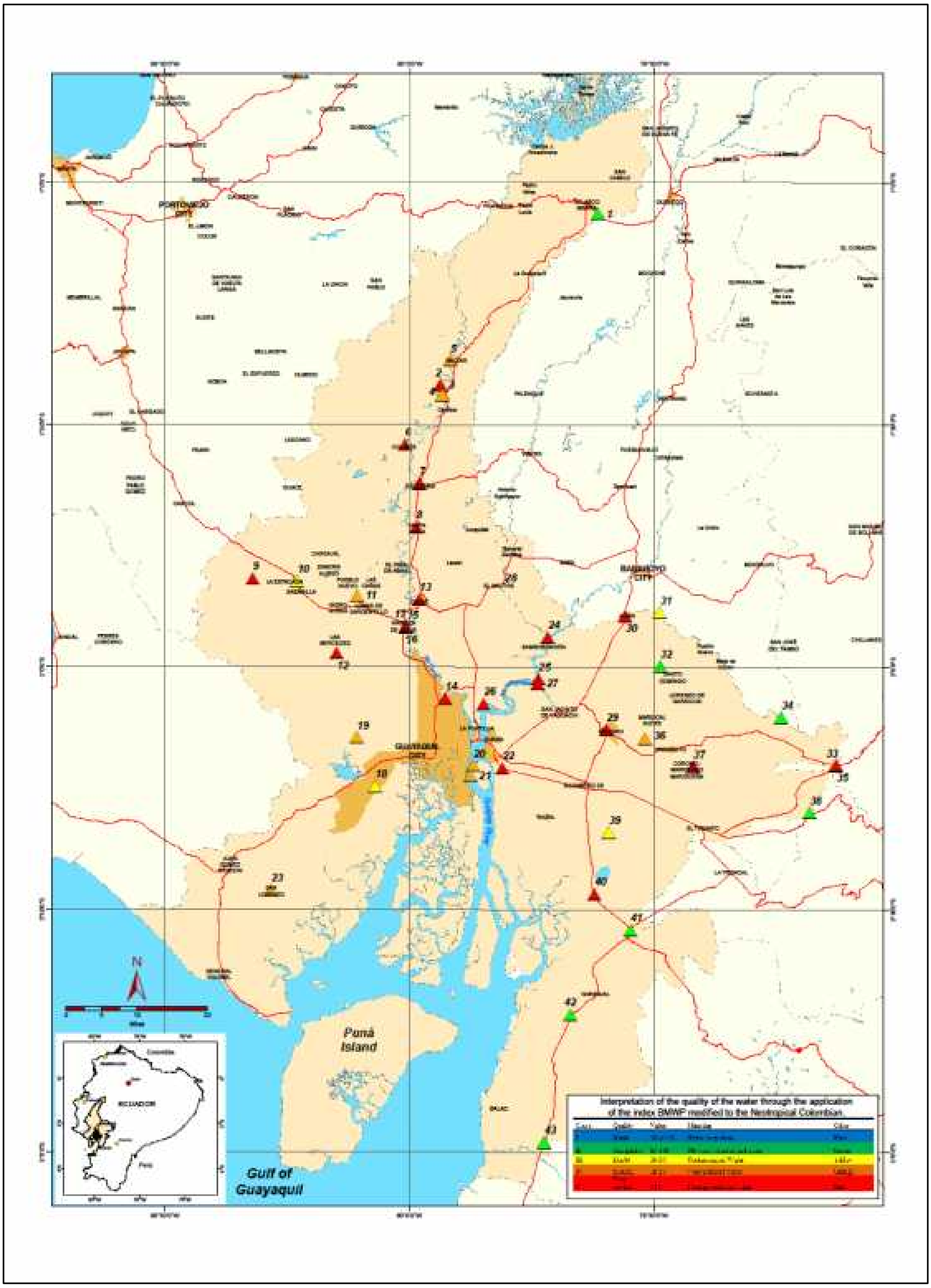


Figure 25. Geographical locations of the each station surveyed in Guayas province between November 2012 and March 2013 in each site is shown the different water quality using the BMWP/Col Index. Source: (M. Cárdenas et al., 2013),

3.6 Discussion and conclusions

This study showed that two different zones, of which one zone grouped the most sampled sites showing different assemblages of macroinvertebrates in the fluvial section of the Guayas river watershed. While that another zone only was represented by the station Estero San Camilo (E22), located in Durán in the industrial zone closest to Guayaquil city, where registered the family Ceratopogonidae with the specie *Stilobezzia* sp.The stations most similar by the Poza de Agua Clara (E35) and the join between Chimbo and Chanchan Rivers (E37), located in Bucay (small waterfall) and Marcelino Maridueña counties, where were registered three families of Hemiptera Corixidae, Naucoridae and Gerridae with the species *Tenagobia* sp., *Limnocoris* sp., and *Trepobates* sp. The other stations were similar in 45% were formed by Daule (E8) and Paco Rivers (E12) located in Santa Lucía and Isidro Ayora counties respectively. In the Daule River were found the diptera *Polypedilum* sp. and *Chironomus* sp. the Coleoptera *Corydalus* sp. In Paco River registered Ostracods, Baetidae and the family Belostomatidae with the specie *Belostoma* sp.

The Macul watershed were characterized by Hidrobiidae (*Amnicola* sp.) that inhabit variety of water bodies including lentic waters and strong currents (Pyron & Brown, 2015) and the Leptophlebiidae (*Tricorythodes* sp., and *Yaurina* sp.) are abundant in tropics and warm temperate regions (Sartori & Brittain, 2015).This last specie characteristic in the mountain forests from Ecuador (Domínguez & Fernández, 2009). While in Daule watershed were characterized by Diptera (Chironomidae and *Chironomus* sp.) cosmopolitan insects (Petsch, Pinha, Dias, & Takeda, 2015) recognized to be representative of oligotrophic sities (Pinilla, 2000) and the gastropod *Melanoides cf tuberculata.*

The aquatic insect taxa were more representative of Guayas river watershed, the Ephemeropthera, Hemiptera and Diptera were orders the most abundant; the families most representative were Leptohyphidae, Corixidae, Vellidae, Chironomidae, Ceratopogonidae, Calopterygidae, Megapodagrionidae, Elmidae and Hydropsychidae.

The Ephemeroptera were registered mainly in river located with most altitude and associated to western mountains (upstream) especially in the Empalme, Bucay, Triunfo and Balao counties with the families Leptophypidae, Leptophlebiidae, Baetidae and

Polymitarcyidae. While the Hemiptera (*Trepobates* sp; *Tenagobia* sp; *Rhagovelia* sp.); Diptera (Chironomidae; Ceratopogonidae, and *Chironomus* sp.); the gastropod (*Melanoides cf tuberculata*) were more frequently and abundances in downstream.

The taxon with the highest abundance was gastropoda with the family Thiaridae with the specie *Melanoides cf tuberculata* contributed significantly to this abundance specifically in Milagro River. However, was registered in 10 of 25 counties from Guayas province such as: El Empalme, Balzar, Pedro Carbo, Daule, Guayaquil, Playas, Milagro, Jujan, Simón Bolívar and Naranjito. It is a invasive specie, native from Asia and Africa introduced in streams of Northern California, Washington and Florida by the commercialization of aquariums (Pennak, 1989). Now present in Neotropical region spreading since southern of U.S.A. and Argentina (Domínguez & Fernández, 2009; Peso, Vogler, & Pividori, 2010) and some coastal watershed from Ecuador as Chone River and Portoviejo River (Cárdenas-Calle & Coello, 2009) and Baba River watershed (Cárdenas, 2012).

Other exotic specie was found *Pomacea canaliculata* (Ampullariidae) commonly known as apple snail, registered only in three counties Yaguachi, Samborondón and Playas, this specie has caused severe damage in the low basin in the Ecuadorian coast. In 2011the plague apple nail reduced the rice production in 40% from 398152 ha to 200000 ha affecting mainly Guayas and Los Ríos provinces (Mena, 2012).

Three commercial species of river shrimp were found and these were *Macrobrachium panamense*. *M. gallus* and *M. carcinus,* in addition to the clam *Corbicula cf fluminea* which are consumed by local fishermen.

The sites with as highest macroinvertebrates were Milagro, Macul and Pedro Carbo with 2500, 595 and 470 individuals each.

The analysis of similarity of sampled stations based on composition and abundance of species showed that 95% (41 sites) of stations were dissimilar and only 4.65% of the stations (Vinces and Yaguachi Rivers) were similar at the level of 50%.

The highest diversity occurred generally in the transition zone between highlands and lowlands in Guayas province; San Antonio River (E34) in Bucay, San Isidro River

(E39) in El Triunfo, Los Amarillos River (E32) in Simon Bolívar and Chongón River (E18) zone near of foothills of western mountain and Coordillera Chongón Colonche. This diversity could be related with the altitude and temperature, of these sites. The presence of running water is normally associated with high oxygen dissolved concentration, which provides ideal habitat conditions for colonization and establishment of a wide variety of species. The presence of *Psephenus* and the a variety of Ephemeroptera (*Leptohyphes* sp., *Tricorythodes* sp., *Traverhyphes* sp.,*Yaurina* sp.) sensibles taxa to pollution and eutrofization (Yee & Kehl, 2015) showed still there are good water quality in these rivers.

The sites with most diversity were in decreasing order Chongón (Universidad de Guayaquil), San Antonio (Bucay) and Macul (El Empalme) associate to Chongón Colonche and Cordillera Occidental.

In relation to water quality the Guayas River watersheds based in BMWP/Col showed that the most of sites surveyed are very polluted by organic matter (72%) and only 16% of sites were acceptable but with obvious effects of contamination. This suggests that macroinvertebrates determined the state of environmental quality of rivers studied and some many natural (natural environmental stress) or anthropogenic factors (imposed environmental stress) are affecting the biodiversity of macrofauna.

Significant differences were found in the macroinvertebrates abundances and water quality in subwatersheds and productive activities. Most stations with very critical quality were located in the lower coastal areas of the basin of Guayas in Daule, Yaguachi, Jujan, Drenajes menores had less numbers of macroinvertebrates. This may be related with the low level of oxygen dissolved registered in Daule River (less 5mg/L) and measured concentrations less 3 mg/L in sites as Balzar, Santa Lucia, and Nobol.
This is caused by organic wastes that have oxygen used by bacteria in the decomposition process and there is a dramatic decrease and change of species in waters deoxygenated by heavy sewage pollution (Rand, 1995).

In addition, the presence of fecal coliforms (100 – 40000 MPN/100ml) , aluminium (2.22 - 30.32 mg/L), ammonium (0.44-2.43 mg/L) tended to be higher in Daule and Babahoyo Rivers in April 2012 (CESTTA, 2012). Other harmful substance as Total petroleum hydrocarbon (<0.3 mg/L); lead (<0.3 mg/L), cadmium (<0.4 mg/L) and 38 different type of pesticides with organochlorine and phosporus were found in Daule and Babahoyo River, which could be negatively influencing the aquatic biota. So these might be some of the factors causing a decline of abundance and diversity and further decrease.

Some pesticides are prohibited because they produce chronic toxicity and acute effects on aquatic and human health. e.g., pesticides organophosphorus (etil parathion; dimethyl (E)-1 methyl-2 (methylcarbamoy) vinyl phosphate （monocrotophos); pesticides organochlorine (Lindane, ppDDT) and carbamates (aldicarb, carbaril, carbofuran, oxamyl). The use of pesticides in permanent agriculture culture such as banana, African palm, cocoa and cugar cane show percentages ranging between 20.7% and 25.9% of toxic plaguicides and between 9.8% and 14.6% of very toxic plaguicides according to descriptive analysis of plaguicides use in agriculture done in 2013. This is in spite of some pesticides being prohibited from use in Ecuador (INEC, 2013; MAGAP, s/f) because they have carcinogenic, mutagenic. reproductive and endocrine disruptor effects (UTZ, 2015).

The stations with acceptable quality were found in the highlands of the northeast and southeast of Guayas provinces in Macul. Jujan and Yaguachi sub-basins located within the limits of the foothills of the cordillera of the Andes. These were adjacent in the north of Guayas provinces with the provinces of Pichincha and the centre and south in the El Triunfo, Simon Bolivar, Bucay, Cañar and Azuay provinces. These results suggested that composition and diversity of macroinvertebrates species were influenced by environmental factors such as altitude, temperatures, sediments, caudal and velocity water, depth of river. Therefore, these stations showed different ecological characteristics from the other stations downstream mainly since these sites were characterized by flowing (lotic) water noticeably influencing the residence time of any pollutants with significant implications for the release of the same in the effluent downstairs because the main factor is the rate of exchange of water through the system would allows the possibilities of their loss (Hellawell, 1986).

The biotic index applied in this study relates very well with the levels of organic pollution given by the presence of fecal coliforms especially in the basin of the river Daule recorded in the rainy season of 2012 and waters of regular quality obtained through of physical and chemical parameters of ICA index (CESTTA, 2012). However, BMWP / Col indicated that water quality is not only fair but the quality of the water in the basin of Daule varies at several localions in critical and very critical water quality suggesting that these are highly polluted or heavily polluted waters. An important fact is that sensitive macroinvertebrates and high scores sensitivity list BMWP / Col. were found in areas where there was low abundance and diversity of organisms such as the families Elmidae, Psephenidae and Corydallidae.

The richness, Shannon diversity and BMWP/Col indices were able to identify the most polluted sites as the Estero San Camilo (E22) located in Durán County. Milagro River (E29). Jujan River (E30). Pucón River (E2). Daule River (E5; E17) and these sites were located in downstream of Guayas River watershed. The most sensitive invertebrates with the highest scores in BMWP/Col were Psephenidae (Coleoptera). Corydalidae (Megaloptera). Leptophlebiidae (Ephemeroptera). Leptoceridae and Hydroptilidae (Trichoptera) that disappear immediately when there is an alteration a (Domínguez & Fernández, 1998).

In conclusion, the results indicate that macroinvertebrates communities in Guayas River Watershed differed along the fluvial sections during November 2012 and March of 2013 in certain ways that would be expected in a system with different environmental characteristic and human activities. These included a pronounced change in species composition, abundance and diversity of macroinvertebrates, especially with a marked decrease in the number of organism and water quality at the level of sub-basins and downstream of the basin. The Daule and Drenajes menores were characterized by similarities in critical and very critical water quality with bad and poor benthic macroinvertebrates diversity. While that most stations located in upstream in the out the watershed, Macul and some stations from Yaguachi and Jujan were characterized by acceptable waters and moderate diversity of macroinvertebrates. Similar results were found in where highest richness was observed in locations situated in mountains areas between in the months October and November in 2013 (Damanik-Ambarita et al., 2016).

The presence of families sensitive to pollution area (score10-8) as Psephenidae Corydalidae, Leptophlebiidae, Leptoceridae and Hydroptilidae prefer cold water with high oxygen concentration and running water seems to be one of the principal factors control, the distribution of organisms (Damanik-Ambarita et al., 2016; Hellawell, 1986), The sites with greater similarities were the stations influenced by human activities mainly with settlement, urban zone, fishing and tourism. The stations with industrial activity showed differences between them probably generated for the spatial localization in Daule and Durán counties but both with very critical water quality.

The Odonata, Megaloptera, Lepidoptera, Crustaceans, Amphipods, Isopods, Hirudinea. and oligochaetes were in low abundances, from them crustaceans and amphipods are susceptible to the effects of environmental perturbation (Wildsmith, Rose, Potter, Warwick, & Clarke, 2011) and an increase in the abundance of species more tolerant of such perturbation such as *Melanoides cf tuberculata* they become dominant and which represented c.a. of 50% of the total abundance of organisms in this study. This specie is the first register for Guayas River Watershed, dominant in Milagro River. It is a competitor of native mollusks and inhabiting different substrata and environments (organic matter, sand, stone, marginal vegetation) and it has been found in Argentina. Paraguay and Brazil where native population of *Aylacostoma tenuilabris* in the Tocantins River were replaced by population of *M. tuberculata* (Fernadez, Thiendo, & Simone, 2003) affecting the biodiversity in freshwater system in South America.

It is recommended that integrated biomonitoring which includes physical (altitude. temperature. sediments. velocity of river. turbidity) chemical (oxygen, pH, salinity, nitrate, nitrite, phosphate, ammonium, conductivity total hardness, most toxic pesticides, heavy metal, total petroleum hydrocarbon, oils and fats) and biotic parameters (fecales coliformes, phytoplankton, zooplankton, macroinvertebrates and fishes communities) is done at least twice a year, preferably in the months more representative of each season stations (February and July) in the sub-basins, different productive activities, land-use especially in agriculture area where the use toxic pesticides is common in permanent crops such as sugar cane, African palm, banana and cocoa and temporary crops as rice; corn and other products in the Ecuador (INEC, 2013) and industrial areas. Further studies on the populations of alien species such as *Melanoides cf tuberculata* and *Pomacea canaliculata* are needed to avoid loss of macrobenthic diversity and economic losses in the agricultural sector linked to rice production.

It is necessary the evaluation of environmental parameters in polluted area mainly in the lowlands basin in Daule and Babahoyo rivers watershed the Estero San Camilo station located on the route Durán Tambo E22 station (that was presented was completely alienated from the other stations)t with very critical quality, it is important to monitor the quality of the waters in that area for being in a place with direct influence of industrial activities as a waste receiving area.

New questions are originated from this study as to which human activities have highest negative impact on the community structure of macroinvertebrates, what contaminants and concentrations are affecting aquatic species (sub-lethal effects. reproductive and growth. effects on prey-predator). What species are being displaced by alien species, which environmental factors impact on the reproductive development of the same, which species are bioaccumulating harmful substances such as pesticides, which are generating sources of diffuse pollution and why Guayas province (despite being of great socioeconomic importance) does not have an environmental monitoring program that integrates physical, chemical and biological parameters that would enable sustainable use of bio-aquatic resources in the province of Guayas.

Chapter 4. Analysis of water and sediment quality in inner branches of the Estero Salado Estuary during the dry season of 2007 with emphasis in distribution of Total Petroleum Hydrocarbon and Heavy Metals.

4.1 Introduction

Estuaries and mangrove forests are essential natural resources due to their high productivity and major role in the maintenance of the biological diversity of coastal and marine environments (Barbier et al., 2011). They hold a great diversity of aquatic species, perform sequestration of carbon (at rates up to 50 times higher than tropical rainforests and provide forest products energy sources, food, medicine, fishing, construction, textiles, papers and a widespread range of ecosystem services to humanity (Bouillon, 2011). However the estuaries endure high levels of stress produced by human activities such as: shoreline modification (Bulleri & Chapman, 2010), habitat fragmentation, removal of original to riparian vegetation (Peterson & Lowe, 2009) poor urban development planning, industrial growth, agriculture activities, all of which change the natural balance of physical and chemical features of water and sediments of estuaries.

Oil pollution inputs in estuaries are from many sources including: tankers accidents, wild wells (Menzel, 1979), oil spills (diesel, fuel oil, Polycyclic Aromatic Hydrocarbons (PAHs), marine operations, run-offs by refineries and industry (Zrafi-Nouira, khedir-Ghenim, Zrafi, & Bahri, 2008). The sediments are an excellent indicator of the magnitude of environmental pollution resulting from these events (Kingston, Runciman, & McDougall, 2003). Other main stressor sources are the heavy metals and Persistent Organic Pollutants (POPs) which accumulate in the surfaces of sediments of estuarine ecosystems (Bayen, 2012) and all the associated aquatic invertebrates which take up and accumulate trace metal (Rainbow, 2002).

The Estero Salado (ES) Estuary is located in the southeast coast of the Ecuadorian Republic and it is one of the biggest estuaries in the Pacific Coast of South America. The Estero Salado is part of an inner estuary of the Gulf of Guayaquil known for its highest biological productivity zone of around 20.000Kcal/m^2/year (Montaño & Sanfeliú, 2008) and it is considered as a special zone of conservation by the Ecuadorian Environment Ministry and Ecologically or Biologically Significant Marine Areas in the

18th report of the subsidiary body on scientific, technical and technological advice to Convention on Biological Diversity (UNEP-CBD-COP, 2014).

However, hydrocarbon substances are present in the water and sediments of Estero Salado, and studies done in the area surrounding the docks of the Guayaquil Port Authority (APG) during the period 1984-2004 by the Instituto Ocenaográfico de la Armada (INOCAR), showed the presence of Total Petroleum Hydrocarbon (TPH), produced mainly by oil spills, fuel oil leakages and from bilge water of merchant ships, fuel transport, navigation activities (Rodríguez, 2005). High concentrations of aliphatic hydrocarbon were found in the sediments of the Puerto Marítimo (South of Guayaquil) and The Terminal Tres Bocas (oil, diesel, jet fuel and gas terminal) in 2000, with concentrations of 762.85 and 22.566 mg/kg dry weight respectively (Thiakos, 2000), and Heavy metals considered highly toxic were found in the Estero Salado sediments, such as Ba, Cd, Cu, Se, V, Pb and Zn levels exceeded the levels of international environmental quality standards (Fernandez-Cadena, Andrade, Silva-Coello, & De la Iglesia, 2014).

The Estero Salado is inhabited by phytoplanktonic organisms (diatoms, cyanobacteria and dinoflagellates), zooplankton organisms (copepods, cladoceran, crustaceans, early stages of invertebrates such as eggs, nauplio, larves) , aquatic fungi like *Phycomycetes* and pathogenic bacteria of marine fauna such as *Enterobacter cloacae*, *Interobacter agglomerans*, *Citrobacter freundu*, *Escheria spp.*, *Salmonella sp.*, *Proteus sp.*, *Vibrio cholerae*, *V. alginolyticus*, *V. vulnificus* and macroinvertebrates (Hidroestudios, 2003). The benthonic sublittoral fauna is rich and diverse in the outer estuary of Estero Salado (Cruz et al., 1980) and composed of mollusks as such *Anadara tuberculosa*, *A. grandis, A. similis; Mytella guyanensis, M. strigata, Corbula amenthisima* and others*;* crustaceans such as: *Penaeus occidentalis, P. vannamei, P. californiensis, P. brevirostris, P. stylirostris; Trachipeneus riveti; Callinectes toxotes; Ucides occidentalis*. In the inner estuary the diversity of biota decreases (Ayarza, Coello, Chalen, Garces, García, Ormaza, Pérez, Pesantes, & Solorzano, 1993) and there is the presence of *Crucibulum spinosum*, *Corbula* sp., *Callinectes arcuatus*, *Penaeus vannamei* (Estrella, 2000); *Ostrea columbiensis*, *Mytella strigata*, *Mytilopsis trawtuineana*, *Protothaca asperrima*, *Macoma silique, Tellina* sp., *Polymesoda* sp.,*Theodoxus luteofasciatus, Cerithidea mazatlanica, Littorina varia y Tralia panamensis* present in the intertidal zone (Cruz, 2003).

This study examined the state of pollution in the sediments considering Total Petroleum Hydrocarbon (TPH) and the most toxic heavy metals such mercury, cadmium and lead (Hg, Cd and Pb) in sediments from ES, to characterize the spatial distribution and to determinate the pollution gradient of hydrocarbons in the inner branches of ES that get in Guayaquil city thus, this study provide technical criteria for the implementation of environmental and biomonitoring design performed later between 2009-2012 in the protected area Reserva de Producción Faunística Manglares El Salado (RPFM) and urban estuarine zone in Guayaquil, Ecuador.

4.2 Aim

- Determine the pollution state of the water and sediment in inner branches of Estero Salado estuary through of physical, chemical aspects of estuarine water and the presence of persistent substances as the Total Petroleum Hydrocarbon and the most toxic heavy metals (Pb, Cd. Hg) in the sediments during June to July 2007.

4.2.1 Specific objectives

- To characterize physical and chemically the water in the inner branches of Estero Salado Estuary. To evaluate the concentration and distribution of most toxic heavy metals (Lead, Mercury and Cadmium), Total Petroleum hydrocarbon, fats and oils in the inner branches of Estero Salado Estuary.
- To assess the gradient and the spatial distribution of Total Petroleum Hydrocarbons in the inner branches of Estero Salado.
- To identify the composition and abundance of taxa in the inner branches of Estero Salado

4.3 Study area

The sampling sites were located in the inner branches of Estero Salado. The areas under study were Reserva de Producción Faunística Manglares El Salado (RPFMS) (protected area: Area I) and inner branches located in the vicinity of Guayaquil city (urban area: Area II).

Nine stations were studied which had been chosen to represent different human productivity activities (aquaculture, agriculture, landing dock of company producing cement, port of boats, tourism, and main oil, diesel, jet fuel and gas terminal) along the Estero Salado Estuary basin. These sites were: Puerto Hondo Camaronera (PHC), Muelle de Puerto Hondo (MPH), Puerto Azul (PA),Terminal Portuario Internacional (TPI), Estación de Transferencia Tres Bocas (ETB), Estero del Muerto (Spalding, Fox, Allen, Davidson, Ferdaña, Finlayson, Halpern, Jorge, Lombana, Lourie, Martin, McManus, Molnar, Recchia, & Robertson) located inside protected area and the stations Miraflores (MIR) and Albán Borja (ALB) located at the urban area in north of Guayaquil city corresponding to residences and industrial zones which discharge domestic waters, industrial effluents and rainwater to the estuary (Table 12, Figure 26-27). Sample collection occurred in later June and early July 2007 (dry season) in the lower intertidal and high tide.

Table 12. Geographic location of the Estero Salado sampled stations.

Station	**Area**	**Site**	**Abbrev.**	**Type of Analysis**	**Latitude**	**Length**
1	I	Puerto Hondo Camaronera	PHC	Water	2 ^{o} 11’968”S	80^{o}00´733´´W
2		Puerto Hondo	MPH	Water - Benthos	2 ^{o} 11´556´´S	80^{o} 00´143W
3		Internacional	TPI	Water - Sediment - Benthos	2^{o}11’701”S	80^{o} 00’970”W
4		Muelle de Puerto Azul	MPA	Water - Sediment	2^{o}11”899”S	79^{o}58’062”W
5		Puerto Azul	PA	Water - Sediment - Benthos	2º11’781”S	79^{o}57’857”W
6		Estación Tres Bocas	ETB	Water - Sediment - Benthos	2º 13’655”S	79^{o} 57’604”W
7		Estero del Muerto	EDM	Water - Benthos	2º 16’695´´S	79^{o} 54’753”W
8	II	Miraflores	MIR	Water - Sediment - Benthos	2º 09´751´´S	79^{o}55´145´´W
9		Albán Borja	ALB	Water	2^{o}10´026´´S	79^{o}55´003´´ W

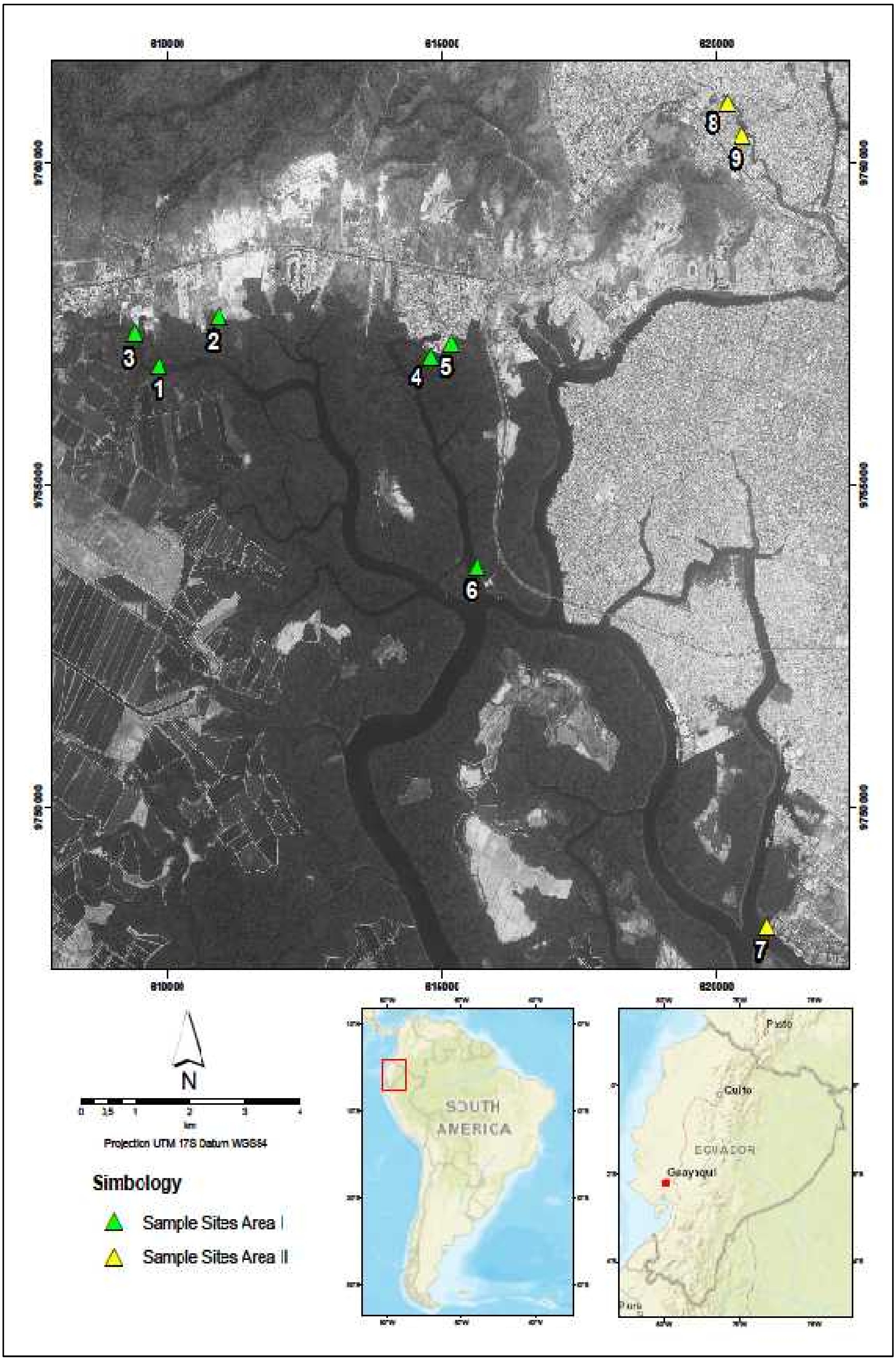


Figure 26. Localization of sampling sites analyzed in this study.

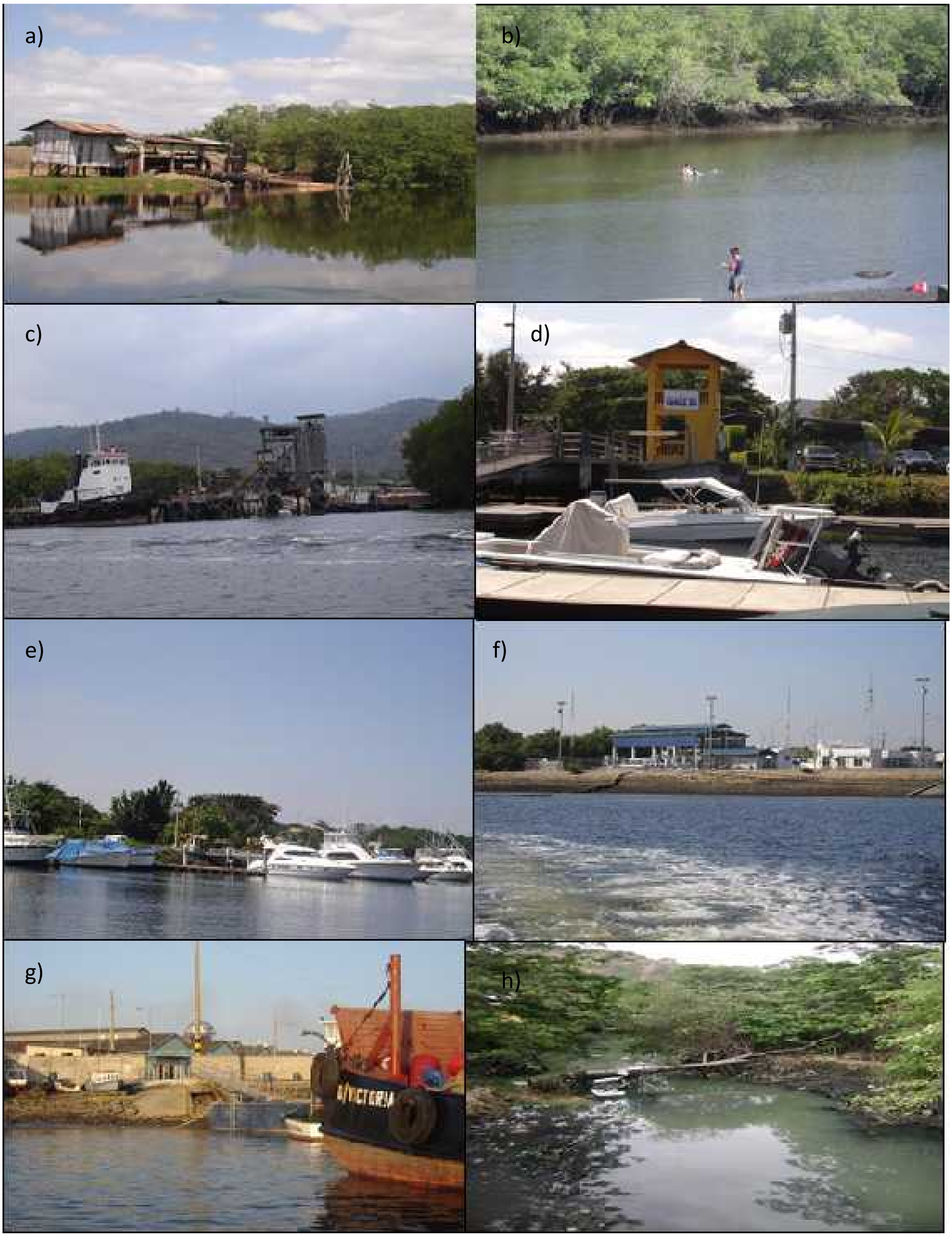


Figure 27. Descriptions pictures showed the study sites except Alban Borja a) Pto. Hondo Camaronera: view shrimp pond water intake;b) Muelle de Puerto Hondo: watering and recreational place; c) Terminal Portuario Internacional; navegation and landing area of raw materials for cement company d) Muelle de Puerto Azul:navegation and boat dock; e) Puerto Azul: urbanized area; f) Estación Tres Bocas: station of reception and pumping of the fuel oil that comes from Esmeraldas and La libertad through ships; g) Estero del Muerto: estuarine branch located south of Guayaquil affected by pollution; h) Miraflores: estuarine branch affected by industrial zone and urban settlement in the northwest of Guayaquil city.

4.4 Methodology

4.4.1 Field work

a) Water

Some common water quality were measured on the water surface of estuary, such as: water temperature, pH, dissolved oxygen (DO), biochemical oxygen demand (BOD_5) and total suspended solids (TSS) parameters (EPA, 2006). The water temperature, pH and dissolved oxygen concentration were recorded at nine locations at each site on each sampling occasion using Multiparameter Hand Held Meter YSI model 556 MPS.
Other parameters (BOD_5, TSS) were determinate in the certificate chemical laboratory called Laboratorio Marcos, under accreditation Agency Ecuador (OAE) of ISO 17025 by the O.A.E. No. OAE LE 2C 05-001 using methods based on standard methods (American Public Health Association, American Water Works Association, & Federation, 1999). Three randomly-replicates of water were survey in each one of nine stations (Table 12), by obtained 18 water samples were stored in plastic bottles and were kept at 4^{o}C until its later analysis in the laboratory.

b) Sediments

For each sampling site environmental (total petroleum hydrocarbon, heavy metals and nutrients) and biological (benthic macroinvertebrates) variables were measured (FAO, 1981). Three replicates of sediment for chemical were surveyed in each one of five stations and six stations were survey to biological samples were took (Table 12) with three replicates each one. The sediments samples were sampled by using a stainless steel Van Veen grab (0.1 m^2) in sludge layer with maximum depth of 10 cm (Eleftheriou & McIntyre, 2005). In the case of chemical samples were kept at 4^{o}C until arrival at the laboratory.

The biological samples were fixed in 10% formalin buffered in estuarine water and subsequently wet-sieved through a 1mm mesh.

4.4.2 Laboratory analysis

Using a dissecting microscope, the benthic macroinvertebrates were removed from any sediment that was retained on the mesh, identified to the lowest possible taxon and stored in 70% ethanol (Mair, Mora , Cruz, Calles, Arroyo, & Merino, 2000). To identify the species of benthic macroinvertebrates the following taxonomic keys were used: for mollusks (Cruz, 2004; Keen, 1971), polychaetes (Hartman, 1968, 1969; Villamar, 1983; Villamar & Cruz, 2007), crustaceans (Barnard & Karaman, 1991; Gosner, 1971), aquatic insects (Domínguez & Fernández, 2009). The biological samples were analyzed in a laboratory by a stereo-microscopic (Globe brand) and compound microscope (BOECO brand). Composition abundance (number of individuals) and diversity (species richness) were determined for each replicate.

4.4.3 Statistical analyses

The means, standard deviations and distributions fitting data were calculated using Statgraphics plus version 4.1, to assess whether those data satisfy assumptions of normality and constant variance used Kolgomorov-Smirnov and Bartett´s test (Legendre, 1998). Analysis of variance (ANOVA) test were used to determine whether the environmental variables (Water temperature (Temp), Dissolved oxygen concentration (O_2), Biochemical Oxygen Demand (BOD), Total Suspended Solids (TSS), Total Petroleum Hydrocarbon (TPH), lead (Campbell & Hewitt) and total mercury (Hg)) and biotic variables (number of species, abundance and diversity) significantly differed among areas (protected area: I and urban area: II). Kruskal – Wallis test were used which compares medians due the data which did not satisfy assumptions of normality and constant variance.

For that, multivariate analyses were carried out using the PRIMER V6 statistical package (Clarke & Gorley, 2006) to explore whether the composition and abundance of benthic macroinvertebrates differed significantly among sites. The Bray-Curtis similarity coefficient was employed to construct a similarity matrix from the log (n+ of the various macroinvertebrates species recorded for each replicate sample at each station. This matrix was then subjected to non-metric multidimensional scaling (MDS) ordination. In addition Cluster analysis was done between environmental variables using Euclidean distance to assess the similarity between the sites of study.

Other statistical analysis were done using a routine of multivariate analysis: the ordination of samples by Principal Components Analysis (PCA) analyzing eleven variables (temperature, pH, dissolved oxygen, biochemical demand of oxygen, total suspended solids, total petroleum hydrocarbon, mercury, lead, cadmium, total abundance of macroinvertebrates and richness of species) to determinate the variables which had more influence in the structure community of macroinvertebrates.

4.5 Results

4.5.1 Environmental measurements

a) Water

Data concerning the measured physicochemical factors at the two zones I and II in the Estero Salado inner branches are shown in Table 13 and Figure 28. There were fluctuation of temperatures due difference in the time collecting (midday or late afternoon) (Annex 6).

The pH was similar between the stations (Figure 28), the alkalinity sediments were observed at Puerto Azul (8.0) and acid sediments in Terminal Portuario Internacional (TPI) station (6.2). This value was lower than the permissible limits of estuarine and marine water for preserving the fauna and flora (6.5 to 9.5 according the environmental law in Ecuador (Ministerio del Ambiente, 2003a).

The biochemical oxygen demand had an average value of 9.56 mg/L. The highest values were in zone II at Miraflores and AlbanBorja with 22 and 64 mg/L and the lowest values were in zone I with values between 0.17 to 0.19 mg/L. Suspended solids had an average of 157 mg/L. The maximum value was 293 mg/L at the Tres Bocas station and the minimum value was 22 mg/L (Table 13) at Miraflores Station.

Table 13. Statistical parameter (mean, 95% confidence level for the mean, minimum and maximum value) of temperature, pH, oxygen, Biochemical Oxygen Demand and Totals Suspended Solids of the all water stations survey.

Physicochemical Parameters	Mean	Minimum - Maximum		95% Confidence Interval for Mean
Temperature (°C)	26.45	25.1	29.1	18.788- 34.123
pH	7.51	6.23	8	4.462- 10.558
Oxygen (mg/L)	2.56	0	5	-0.198- 0.198
BOD_5 (mg/L)	9.56	0	64	-6.913- 26.301
TSS (mg/L)	157	22	293	-222.681- 536.681

The average dissolved oxygen was 2.60 mg/L, the station located in the Estero del Muerto (Area I) showed high oxygen values (4.97 mg/L), while the low values occurred in the area corresponding to Miraflores and Albán Borja Sites (Area II) with values of 0.06 and 0.05 mg/L respectively (Annex 6). There were significant spatial differences ($p<0.05$) between oxygen concentration between two zones (Figure 29).

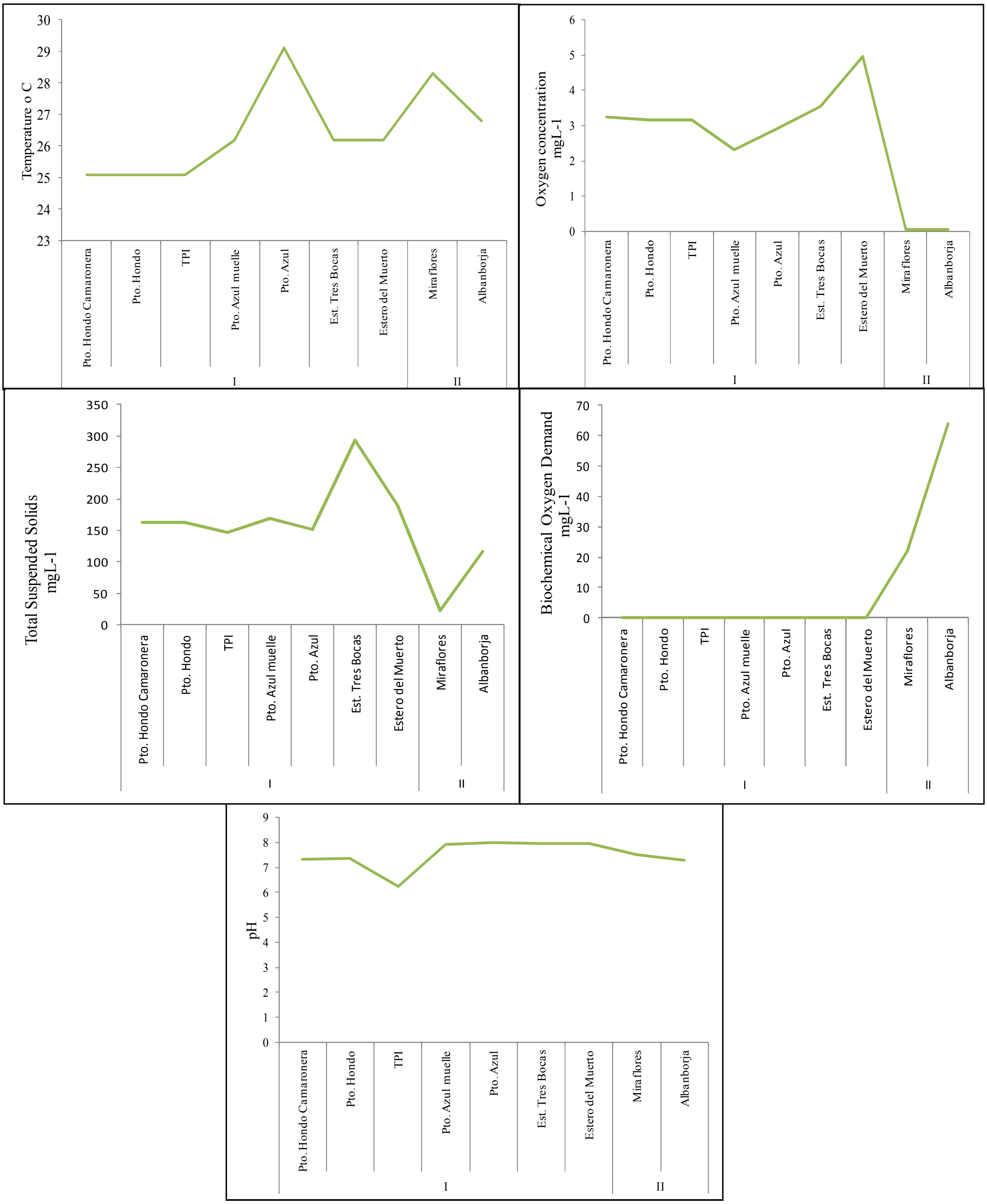


Figure 28. Spatial variation of main variables (Temperature, oxygen concentration, total suspended solids, biochemical oxygen demand and pH) in water surface in protected area (I) and urban area (II) during June and July of 2007 at inner branches of Estero Salado-Guayaquil.

Variance analysis showed that only environmental variables significant differences ($p<0.05$) were oxygen concentration, biochemical oxygen demand, temperature, pH and total suspended solids there between two protected and urban (Figure 29).

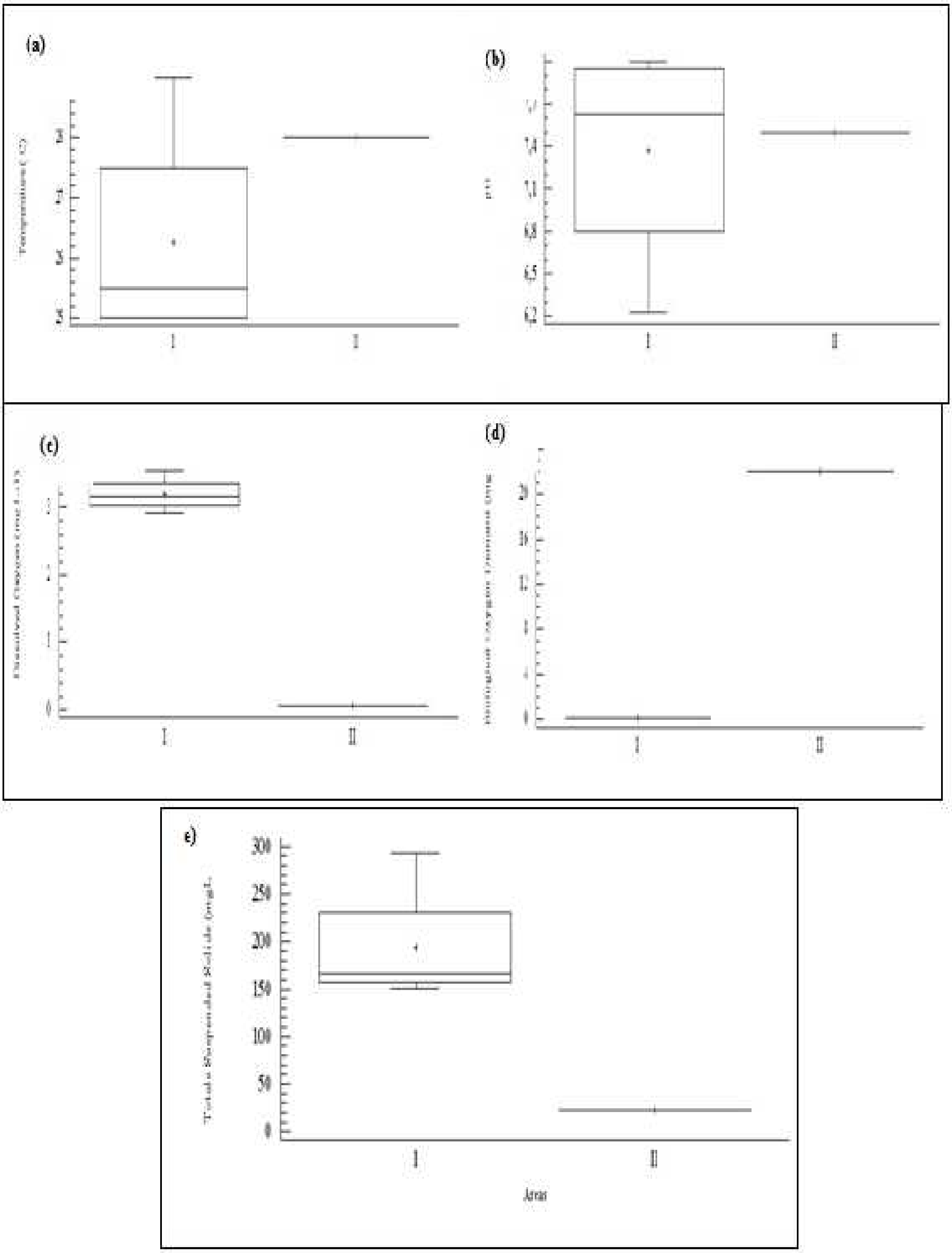


Figure 29. Medians ± 95 % confidence limits for (a) temperature, (b) pH, (c) Dissolved Oxygen, (d) Biochemical Oxygen Demand and (e) Total Suspended Solids at the bottom of the water column at the two sampling areas at July of 2007.

b) Sediments

The distribution of mercury (Hg) in sediment ranked between 0.3166 to 0.5365 mg/kg dry weight, there was an increase from the farthest stations from Puerto Hondo until the inner branches of estuary at Miraflores (Table 14).

Cadmium showed a median of 0.836 mg/Kg. A consistent trend was observed from Puerto Hondo to the Terminal de Transferencia Tres Bocas and an increase in the internal branch that enters to Miraflores area with a concentration of 0.98 mg /kg. Lead values showed the same trend as Cadmium with a concentration of 20mg /Kg while at Miraflores station, the lead concentration was 66.54 mg /Kg (Table 14; Figure 30).

Total concentrations of hydrocarbons showed a spatial variability increasing the values from 70 mg/kg especially in the northwest concentration zones were up to 145.872 mg/ kg in the domestic branch that enters to Guayaquil city up to Miraflores (Table 14). The total petroleum hydrocarbon concentrations in the Estación Tres Bocas and Miraflores stations exceeded the maximum permissible levels of environmental quality of soils for the industrial and residential area according the Ecuadorian law (Ministerio del Ambiente, 2015c).Whilst the sediments lead level were elevated above the upper ecotoxicological assessment criteria (EACs) to marine sediments according OSPAR (OSPAR Commission, 2000).

Table 14. Total petroleum hydrocarbon and heavy metal concentrations (mg/Kg^{-1} dry weight) in superficial mangrove sediments in the Estero Salado in Guayaquil.

Chemical Parameters (mg/Kg-1)	Sites					*Environmental Quality Standard - Ecuador to remediation of soil polluted according soil uses		** OSPAR EACs for metals
	Muelle de Puerto Hondo	Terminal Portuario Internacional	Puerto Azul	Estación Tres Bocas	Miraflores	Maximun Chemical Exceedance Level to Residence Zones	Maximun Chemical Exceedance Level to Industrial Zones	Marine sediments (mg/Kg-1 dry weight)
TPH	70	157.07	70	1545.42	145872.77	230	620	
Hg	0.3166	0.3794	0.4584	0.4603	0.5365	1	10	0.05-0.5
Cd	0.8	0.8	0.8	0.8	0.98	4	10	0.1-1.0
Pb	20	20	20	20	66.54	140	150	5-50

* **EQS-ECU:** Environmental quality standard – Ecuador. Anexo 2 Del Libro VI del Texto Unificado de Legislación Secundaria del Ministerio del Ambiente: Norma de Calidad Ambiental del Recurso Suelo y Criterios de Remediación para Suelos Contaminados Tabla 2 Criterios de Remediación (Valores máximos permisibles).

** EACs (Ecotoxicological Assessment Criteria) in marine sediments according OSPAR (OSPAR Commission, 2000).

The concentration of the TPH were lower in protected zone (Area I) with concentrations between 70 to 1545 mg/Kg while there was an increase in urban zone (Area II) in Miraflores that showed a concentration of 145872 mg/Kg (Table14).The concentration of mercury and lead were similar trends that TPH (Figure 30a-b.c) with highest concentrations in the urban zone and lowest concentrations in protected zone. The variables measured in sediments showed significant difference between areas ($p< 0.05$) (Figure 30a-b).

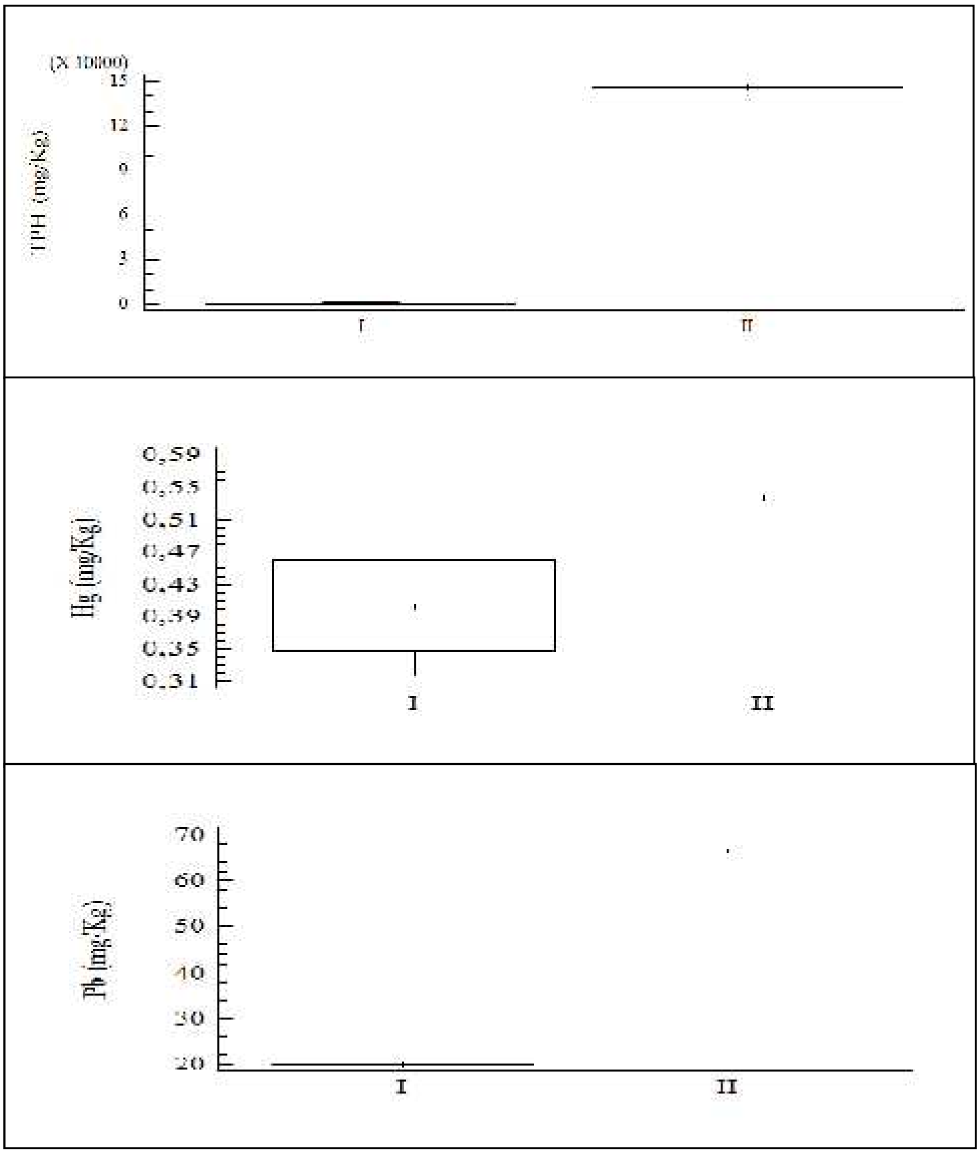


Figure 30. Medians for (a) total petroleum hydrocarbon, (b) mercury and (c) lead at the sediments at the two sampling areas in Estero Salado Estuary Protected area (zone I) and urban area (zone II) at June to July of 2007.

The non-metric multidimensional scaling ordination of environmental variables showed three zones: First zone conformed by Muelle de Puerto Hondo (MPH) and Terminal Portuario Internacional (TPI), the second zone conformed by Estación Tre Bocas and Puerto Azul (ETB and PA) and one third zone conformed only by Miraflores (MIR) (Figure 31).

The station Miraflores (MIR) located in the urban area is totally different than the others stations of Puerto Azul (PA), Muelle Puerto Hondo (MPH), Terminal Portuario Internacional (TPI), Estación Tres Bocas (ETB) located in protected area. However, MPH and TPI stations are more similar between each one due to the two estuary branches being connected, and with similar human activities development in this area such as: Fishing, ecotourism, aquatic sport as kayaks and these sites are landing area. The stations ETB and PA were similar, the ETB is a transference station of petroleum where oil, diesel, jet fuel and gas terminal and all products from the La Libertad (Santa Elena province) and Quito refineries arrive to this terminal through a system of pipelines (Thiakos, 2000).

The site Miraflores was different from the others because it is an inner branch that enters the city of Guayaquil and where there is high influence of industrial and domestic effluents and human settlements.

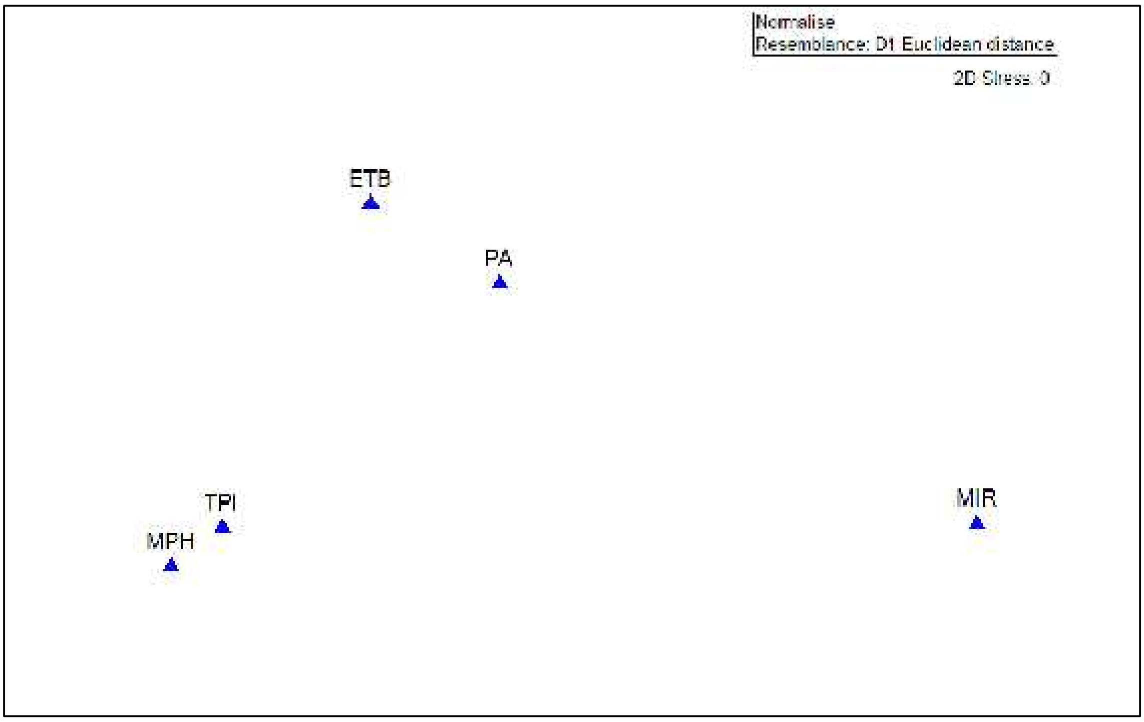


Figure 31. Non- metric multidimensional scaling ordinations, derived from the Euclidean distance matrix constructed using normalize variables at each sampling site in July of 2007.

The trends were more evident with the dendrogram where the sites were more similar in a 50% (Figure 32), the Muelle de Puerto Hondo (MPH), Terminal Portuario Internacional (TPI), Puerto Azul (PA) and The Estación Tres Bocas (ETB). But Miraflores (MIR) was the site totally different in relation with others sites.

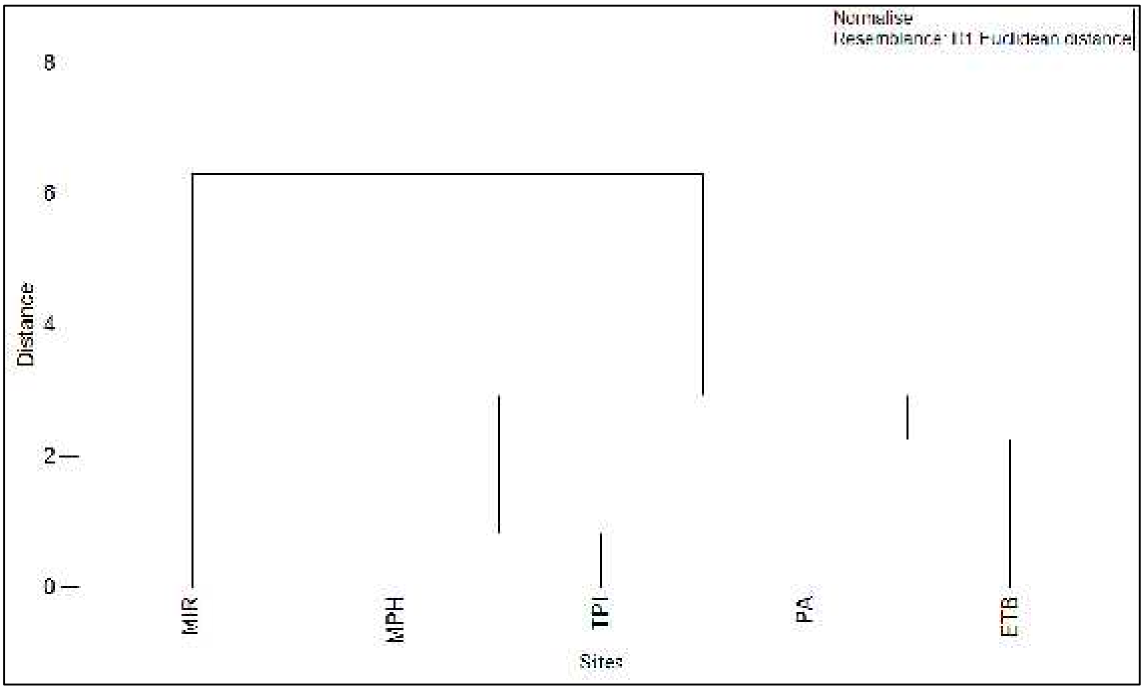


Figure 32. Similarity between stations according to the environmental variables.

4.5.2 Composition and abundance of benthic macroinvertebrates

A total of 1181 organisms were obtained containing seven phyla, i.e. Porifera, Bryozoa, Nematode, Annelida, Molluska, Arthopoda and Chordata (Urochordata). Annelida (77%) and Arthropoda (15%), which were the richest phyla during the survey in dry season, were represented by Polychaeta and aquatic insects. The most abundant species were *Capitella cf capitata* (53.9%) and *Stilobezzia* sp. that contributed 8.5% to the total number of individuals (Tables 15-16).

The polychaeta was the taxa present in all study sites, followed by Mollusca represented especially by gastropods, although this was not registered in Miraflores (industrial and urban zone).The Porifera was only present in the more estuarine branches in Puerto Azul, Estación Tres Bocas and Estero del Muerto (protected natural area called Reserva de Producción Faunística Manglares El Salado). The colonial Bryozoa and colonial tunicates were found only in the Estero Del Muerto (zone with more marine influence).

The annelids were represented by the family Phyllodocydae (Station Tres Bocas) and the species *Lumbrinereis* sp. (Miraflores) and *Micronereis* sp. (Estero Del Muerto). Nematodes and other worm were not identified further and were present in Terminal Portuario Internacional, Estación Tres Bocas y en el Estero Del Muerto.

The molluscs were represented by two classes Bivalvia and Gastropods, this last one was the most representative mainly in Puerto Hondo, Terminal Portuario Internacional, Puerto Azul and Estero Del Muerto.

The Arthropods were represented mainly by the Class Insect and to a lesser extent by barnacles. For insects the Order Diptera dominated in the estuarine areas more polluted by industrial and domestic sources, with the families Dolichopodidae and Ceratopogonidae, the species *Psychoda* sp., *Alluaudomyia* sp., *Stilobezzia* sp.

The predominance of annelids such as Capitella *cf* capitata and the presence of insect larvae of the families Ceratopogonidae, Dolichopodidae, Psychodidae indicate the presence of a deteriorated environment where there are no species species of a healthy estuarine environment (Table 15-16).

Table 15. Mean individual, standard deviations, confidence limits and coefficients of variation of the benthic macroinvertebrate taxa in samples collected at six sites in the basin of the Estero Salado during 2007.

TAXA	PH	TPI	PA	ETB	EDM	MIR
Polychaeta	19	14	2	64	160	4
Phyllodocydae	0	0	0	1	0	0
Capitella cf *capitata*	0	0	0	0	0	637
Gastropods	3	2	3	0	1	0
Bivalve	0	0	0	1	0	0
Tunicate	1	0	0	0	12	0
Insecta	0	0	0	0	45	15
Stilobezzia sp.	0	0	0	0	100	0
Nematoda	0	2	0	13	32	0
Balanus sp.	0	0	19	0	0	0
Porifera	0	0	1	2	4	0
Bryozoa	0	0	0	0	6	0
ND	3	3	0	0	12	0
Mean	1.62	1.31	1.56	5.06	23.25	41
Desv.Standard	4.48	3.03	4.73	11.47	43.13	158.9
Upper 95% Confidence limit	16.99	12.14	17.91	39.27	141.63	470.11
Lower 95% Confidence limit	-13.75	-9.52	-14.79	-29.15	-95.13	-388.1
Coeficient of variation %	2.77	2.31	3.03	2.27	1.86	3.88

The most abundant of macroinvertebrates was in Miraflores and the Estero Del Muerto with 56% and 31% respectively. The lowest numbers of invertebrates were found in west branches Puerto Hondo, Terminal Portuario Internacional and Puerto Azul (Figure 33).

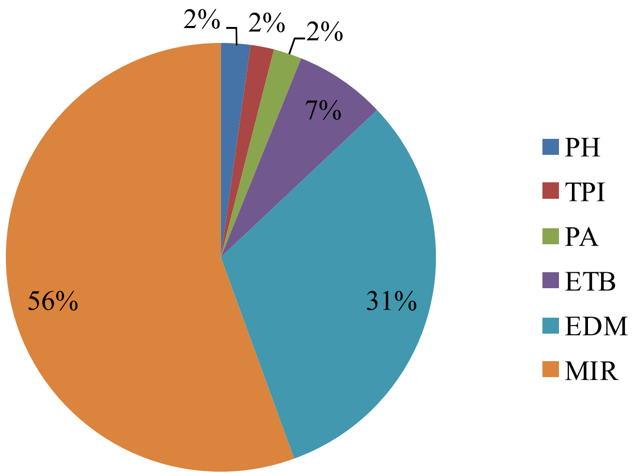


Figure 33. Relative Abundance of macroinvertebrates presents in study sites PH: Puerto Hondo; TPI: terminal Portuario Internacional; PA: Puerto Azul; ETB: Estación Tres bocas; EDM: Estero Del Muerto; MIR: Miraflores during 2007.

Table 16. List of all taxa of macroinvertebrates encountered in the Estero Salado branches in June 2007.

Taxa	Puerto Hondo	Terminal Portuario Internacional	Puerto Azul	Estación Tres Bocas	Estero Del Muerto	Miraflores
PORIFERA			x	x	x	
Sponge ND						
BRYOZOA					x	
Colonial Bryozoa						
ANNELIDA						
Polychaeta ND	x	x	x	x	x	
Phyllodocidae				x		
Capitella cf capitata						x
Micronereis sp.					x	
MEMATODA		x		x	x	
MOLLUSKA						
Vitrinellidae	x					
Bivalve				x		
Gastropod	x	x	x		x	
ARTHROPODA						
Crustacea						
Balanus sp.			x			
Insecta						
Dolichopodidae						x
Ceratopogonidae						x
Psychoda sp.						x
Alluaudomyia sp.						x
Stilobezzia sp						x
UROCHORDATA						
Colonial tunicate	x				x	

The zone with most abundance of invertebrates was urban area (Area II) in the Miraflores site (MIR) represented mainly by the polychaeta (*Capitella cf capitata*) and larvae of insects such as: the families of Dolichopodidae and Ceratopogonidae, the species *Psychoda* sp*., Alluaudomyia* sp., and *Stilobezzia* sp. While that in the protected area had the lowest abundance of macroinvertebrates but with the composition of species more characteristic of estuarine zones such as: sponges, bryozoa, annelida, mollusk, tunicates, nematodes and crustaceans.

Another site with important numbers of individual was the Estero Del Muerto located in the limit in south of the natural reserve (RPFMS) where the insect larvae *Stilobezzia* sp., and polychaetes (*Capitella cf capitata*) were the most dominant taxa (Figure 34).

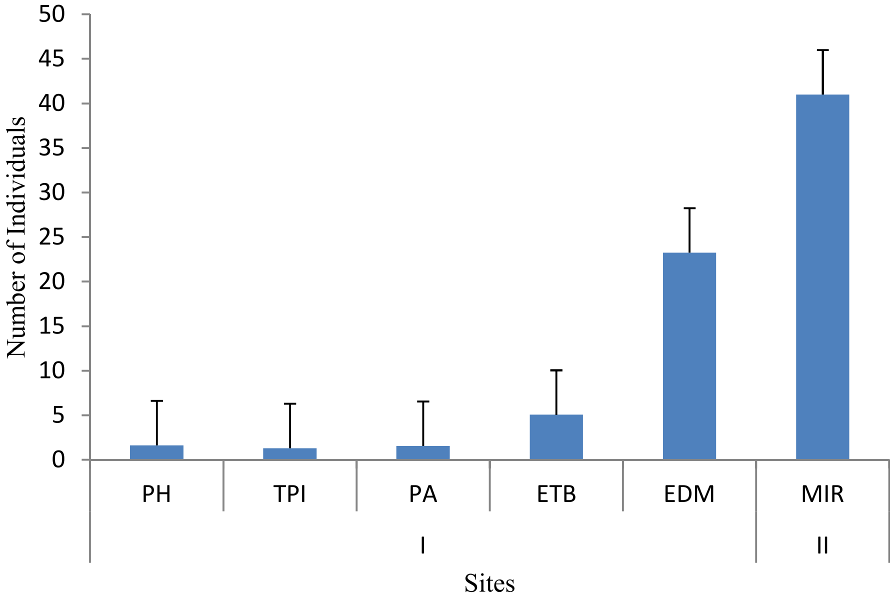


Figure 34. Mean (+ SE) abundance of benthic macroinvertebrates in protected area (I) and Urban area (II).

The polychaetes and diptera larvae were the group that were present both in the urban area and the protected area, the insects were present in sites with influence of anthropogenic activities specially influenced with domestic and industrial effluents. While the gateropods, bryozoa bivalves, tunicates and crustaceans were present only in the protected area with more tidal influence (Figure 35).

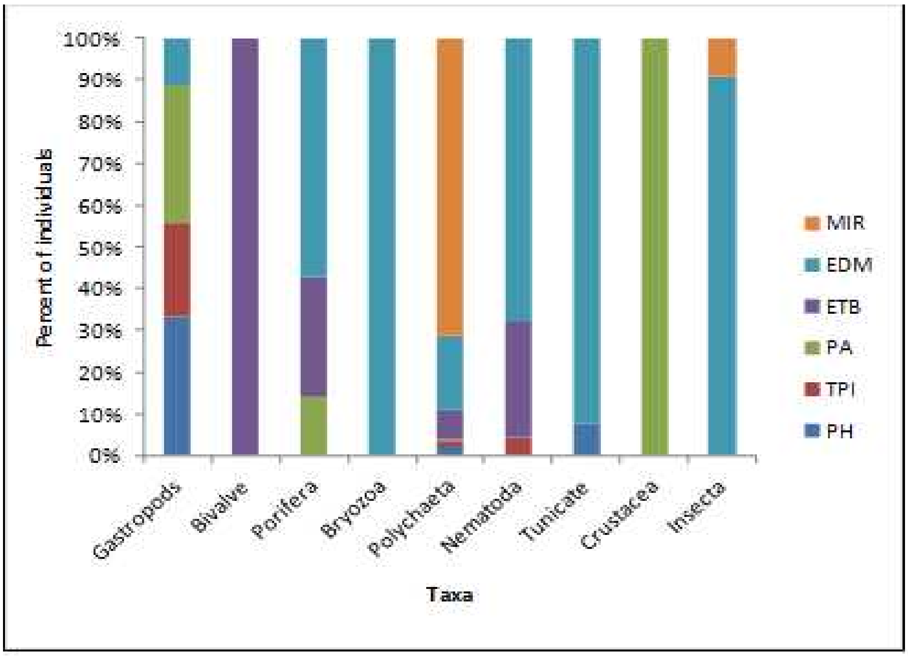


Figure 35. Distribution of Percentage of taxa registered in six sites of Estero Salado during July of 2007.

4.5.3 Interrelation between environmental and biological variables

The analysis of the main components (PCA) and results of eleven variables showed there are two areas well defined in the Estero Salado, one is formed by the Station Miraflores (Area I: North of Guayaquil city) and the other is formed by Puerto Hondo (2), Terminal Portuario Internacional (Acuerdo Ministerial No. 097A Publicado en el Registro Oficial edición especial No. 387), Puerto Azul (5), and Tres Bocas Station (Acuerdo Ministerial No. 097A Publicado en el Registro Oficial edición especial No. 387), all situated in Area II: Southeast of Guayaquil city (Figure 36).

The two first main components (CP_1 and CP_2) that explain 91% of the total variability of the environmental observation in the stations observed in the Estero Salado branches (Table 17: Figure 36). The first Principal Component (CP_1) has a variance (Eigen value) of 8.12 and accounts for 74% of the others variables (cumulative value= 74%) see Table 17. The second component (CP_2) has a variance of 1.83 and contains 17% of the other variables (cumulative value= 91%). The results of table 17 show that the variables have more influence in the spatial distribution of sites through TPH, O_2 BOD_5, Hg, Cd and Pb. In Component 2 (CP_2) the oxygen levels explain the variability and there is a positive association with the taxa represented mainly by polychaetes and Total Suspended Solids (TSS) in the Estación Tres Bocas (ETB) because they are more tolerant of environmental perturbations (Wildsmith et al., 2011)

There is a distinct separation between Area I= (stations 2, 3, 5 and 7) and the Area II (station 8). The Miraflores station located in zone II is influenced by Hg, Pb and temperature. There is a positive association between lead and the numbers of individuals of macroinvertebrates (abundance). However this abundance is caused by the presence of *Capitella cf capitata* (Zabbey & Uyi, 2014) and larva of Diptera species typical of organic pollution (Pinilla, 2000).

Area I is represented by four stations Puerto Azul (Centro Ecuatoriano para la Defensa de la Naturaleza y el Medio Ambiente), Estación Tres Bocas (ETB), Muelle de Puerto Hondo (MPH) and Terminal Portuario Internacional (TPI). Nevertheless, the MPH and TPI stations are similar to each other because have the same physical, chemical and biological characteristics and similar to Puerto Azul, a big impact between them are the temperature and the depth, while the Tres Bocas Station shows an association with the total hydrocarbons, suspended solids and abundance total of macroinvertebrates, pH, mercury and dissolved oxygen (Figure 36).

Table 17. Eigen value and autovectors of PCA for the main factors that explain the spatial distribution of stations study in the Estero Salado branches.

Lambda	**Eigenvalue**	**Proportion**	**Acumulate Proportion**
1	8.12	0.74	0.74
2	1.83	0.17	0.91
3	0.72	0.07	0.97
4	0.33	0.03	1.00

Autovectors

Variables	**e1**	**e2**
Temperature	-0.19	-0.50
TPH	-0.35	-0.04
pH	-0.04	0.67
O_2	0.35	0.05
BOD_5	-0.35	-0.04
TSS	0.30	0.23
Hg	-0.26	0.41
Cd	-0.35	-0.04
Pb	-0.35	-0.04
Abund.Total	-0.29	0.28
S	-034	-0.01

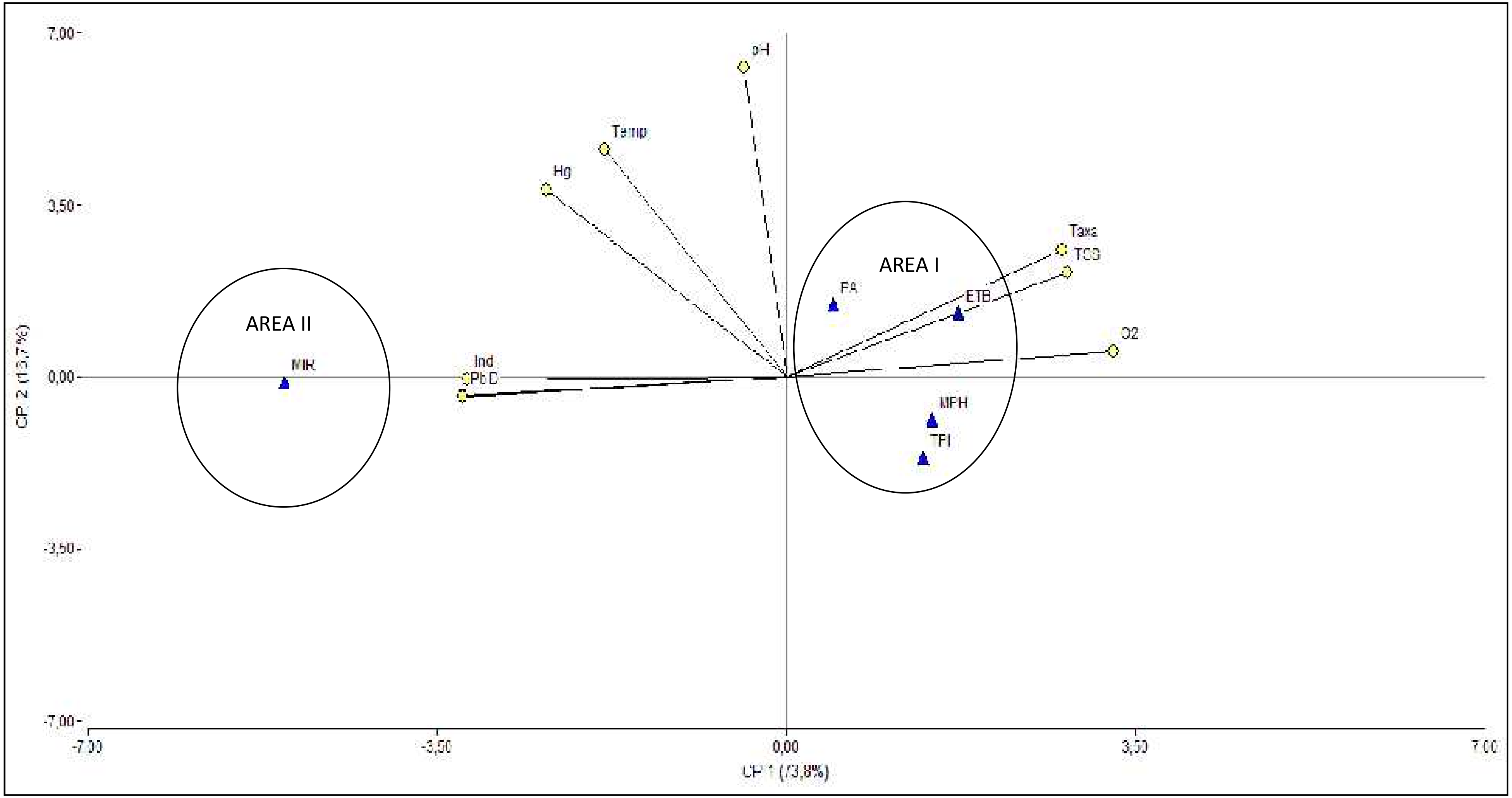


Figure 36. PCA for the study stations in the Estero Salado inner branches during dry season in 2007.

4.6 Discussion and conclusions

The water and sediments of Estero Salado inner branches entering the city of Guayaquil showed deterioration of quality of its environment.

The level of oxygen dissolved concentration decreased considerably in the north of city to anoxic levels in the urban area (Miraflores and Alban Borja). This confirms the continued deterioration of the quality of the adjacent waters in section B of Estero Salado. Studies did between 1999-2001 in this section which included urbanizations as Bellavista, Los Ceibos, Miraflores, Mapasingue Este and Ciudadela Guayaquil in Mapasingue Oeste showed a range of oxygen between 0-1 mg/L (Hidroestudios, 2003). While, the high level of oxygen were found in the Reserva de Producción de Fauna Manglares El Salado (sheltered area), where the lowest oxygen concentration were in landing area (Muelle Puerto Azul) and human settlements (Puerto Azul) and it was increasing to te south of the city in the Estacion Tres Bocas and Estero Del Muerto.

The low levels of dissolved oxygen support the hypothesis that there are high environment deterioration in seccion B of Estero Salado producing a considerable decrease diversity of benthic organisms and presumably affects the growth, metabolism, behavior of invertebrates and specially fishes with concentrations between 4-2 mgL and producing mortality with concentrations engaging 2-0.5 mgL(Gray, Wu, & Or, 2002).

All concentrations of dissolved oxygen were outside permissible limits of Ecuadorian environmental law for estuarine and marine water and for preserving the fauna and flora (NO < 5 mg/L according the TULSMA (Ministerio del Ambiente, 2003a). The Figure 28 shows the results for each study site.

The concentrations of Biochemical Oxygen Demand were higest in sites where oxygen concentrations were low in the north of Guayaquil mainly in Alban Borja and Miraflores, similar values were recorded in seccion B during April 1998 (Hidroestudios, 2003). While, en the sheltered area the biochemical oxygen demand were low. The total suspended solids concentration showed the higher salinity occurs in the south of the protected area in the Estación Tres Bocas and the lower salinity was registered in Miraflores due probably to the water supply domestic and industrial effluents.This concentrations were lower than those recorded between 1999 and 2000 (417 mg/L) in the section B (Hidroestudios, 2003).

The pH of water registered normal values for this estuary but showed more acid values in the Terminal Portuario Internacional. The temperature was warm with the maximu values in Puerto Azul possibly influenced by sampling time (afternnon). However, the section B in Miraflores registered high value influenced by time survey and few depth of bottom soil (0.5 m). The variables oxygen, biochemical oxygen demand, temperature, pH and total suspended solid showed significant differences between the study zones.

This study has demonstrated that the surface sediments of the Estero Salado in the inner branches specifically in urbanized area (Miraflores station: MIR) showed that there are high concentrations of total petroleum hydrocarbon, mercury, lead and cadmium in comparison with the sheltered area called Reserva de Producción Faunística Manglares El Salado. However, in this last one area the sites with highest concentrations of total petroleum hydrocarbon were the Estación Tres Bocas and the Terminal Portuario Internacional and in relation to heavy metal the highest concentrations were registered in Estación Tres Bocas and Puerto Azul.

The heavy metal with highest concentrations was lead and with lower concentration were mercury and cadmium. All heavy metal surveyed were highest in urbanized zone (Miraflores station). The cadmium and lead presented the same concentration in sheltered zone (RPFMS) but mercury presented a gradient of low to high concentrations since RPFMS to the urbanized zone. All concentrations were inside the Ecuadorian environmental law (Ministerio del Ambiente, 2015c). However, these concentrations of lead were elevated above the upper ecotoxicological assessment criteria according the OSPAR in marine sediments (OSPAR Commission, 2000).

Additionally, a gradient in the spatial distribution was observed in hydrocarbon with an increased concentration toward the urbanized area influenced by industrial effluents which could have adverse effects on typical structure of macroinvertebrates community, as by reduction of richness of taxa and an increment of the abundance of species characteristic of polluted water. This seems to be influenced by low water renewal derived of tidal, the contribution of effluents with total petroleum hydrocarbon, lead and mercury probably from industrial and commercial sectors. Only lead showed significant differences between the protected and urban zones.

The results of PCA showed two well differentiated areas, the area I (Puerto Azul, Estación tres Bocas, Muelle de Puerto Hondo and Terminal Portuario Internacional) characterized by the high oxygen dissolved concentration and total suspended solids with higher concentrations of salts, essential factors that affect the variety of taxa in the Reserva de Producción Faunística Manglares El Salado. The area II (Miraflores) was influenced by variables pH, temperature and the highest concentrations of pollutants with poor diversity of taxa, with dominant of *Capitella cf capitata* and larvae of insecta characteristics of sites with organic contamination.

In all of the sampled stations taken from the Estero Salado, from the northwest sector up to the south of Guayaquil city and the downtown, the oxygen concentrations were below of permissible level to Ecuadorian environmental law for the protection of flora and fauna of freshwater, warm waters, marine and estuarine waters. The oxygen concentrations showed a gradient with a decrease in oxygen levels from the internal branches of the nature reserve (RPFMS) at the Muelle de Puerto Azul level increasing towards the south of the city of Guayaquil especially in Estero del Muerto, and decreasing the oxygen concentration in Miraflores Station. The water oxygen concentrations measured showed the (MIR) is polluted and it is considering anoxic and the other sites of protected area were hypoxic although there was still development of aquatic life.

The presence of pollutanting agents increased the environmental stress that affected the common composition, abundance and richness of the inner branches of Estero Salado, reducing the species and densities of crustaceans which are susceptible to environmental stress and allowing the dominance of species tolerant of such perturbation as polychaetes and Diptera larva.

The sheltered protected RPFMS had better environmental quality conditions in comparison with urban zone there was observed elevation of the organic and inorganic substances. There is a need to implement a permanent biomonitoring program with Ministry of Environment (MAE) stakeholder and universities to have a major control of effluents and prevent environmental degradation of this important estuary and to have best management procedures in placof natural resources, mainly for the maintenance of local fisheries, and recreation activities.

The Environmental Ministry should generate studies that promote a new law referenced to sediment quality criteria for the preservation of flora and fauna associated with bottom of marine and estuarine waters, where normally in this last one ecosystem there are bioaccumulation and biomagnifications of persist contaminant substances such as hydrocarbon and heavy metals derived of anthropogenic activities.

Chapter 5. Relation between community structure and levels of hydrocarbon pollution in the Estero Salado Estuary at 2009-2011-2012 and the evaluation of the effects of bioremediation on hydrocarbon polluted sediments and on benthic macroinvertebrate community structure in the inner branches in Estero Salado at 2012

5.1 Introduction

The Hydrocarbons are common anthropogenic pollutants in the water and sediments of urban waters that are mainly derived from crude oil such as gasoline, kerosene, fuel oil, mineral oils, naphtha from mainly by municipal and industrial waste, urban run-off (Pon & Albaigés, 2012; Yang, Wang, Ma, Yu, & Martín, 2016). It is estimated that 1.7 to 8.8 million metric tons of petroleum oils are released into the environment annually (Clark, 1997). Main contributory sources of petroleum hydrocarbon pollution include atmospheric fall-out (Clark, 1997) and deposition from vehicles, industrial emissions, asphat degradation. Crude oil is a combination of many and different chains of hydrocarbon mainly of satured straight-chain alkanes and little amounts of alkanes, cycloalkanes and aromatics (Pettigrove & Hoffmann, 2005).

Polycyclic aromatic hydrocarbons (PAHs), are most toxic component of petroleum hydrocarbon and essential parameter in assessing the effect of industry in waters due the negative effect on aquatic organism are exposed to this chemical substances and other as heavy metals present in water and sediments. Many of these components are hydrophilic (water soluble) so easily available to organism than hydrophobic (water insoluble) that are narrowly absorbed to joined to organic matter or biological system (Rand, 1995). There are a wide variety of criteria to assess the effect of oil pollution in a aquatic system, such as; aquatic ecology, behavior, physiology, histology, biochemistry and genetic aspects. One of the most used in SouthAmerica is ecology of benthic macroinvertebrates as be good bioindicators of enviromental health (Domínguez & Fernández, 2009) based in the community structure where usually the species composition, species richness, abundance and diversity are good measures to estimate any alteration to aquatic biota due to the lack of economical funds to deeper level ecotoxicological studies and long time research.

The purpose of this research was to determinate the spatial and temporal variation contaminants, nutrients and benthic macroinvertebrates characteristics prior and after of hydrocarbon polluted sediments bioremediation in inner branches of Estero Salado during 2009-2012.

5.2 Aim

- To determine the spatial and temporal variation of nutrients, contaminants and biological characteristics in inner branches of Estero Salado sediments as part of the study on bioremediation of hydrocarbon contamination: 2009-2012.

5.2.1 Specific objectives

- To evaluate the concentration of nutrients and contaminants in sediments during 2009-2011
- To determine the composition of the community of aquatic macroinvertebrates during 2009-2011
- To establish the area designated for bioremediation of oil polluted sediments.
- To establish the reduction of contaminants after the bioremediation and possible changes on benthic macroinvertebrates community during 2012.

5.3 Study area

The sites studied were located in the inner branches of Estero Salado including protected (Reserva de Producción Faunística Manglares El Salado), and Urban area (Residence and industrial zones) in the area I and II (Table 18; Figure 37).

In total 25 sites were sampled (Table 18), of which 22 sampling sites were located at prior bioremediation, phase (PBP). The three remaining sites were surveyed at the bioremediation phase (BP). The PBP sites were selected according the gradient of total petroleum hydrocarbon (TPH) pollution along the total petroleum hydrocarbon gradient in the sediments of Estero Salado Estuary basin in 2007 (see chapter 4 study sites) and the expected disturbance gradient in residence and industrial zones. Although the BP sites were selected according level of hydrocarbon concentration obtained in previously phase of bioremediation during 2009 and 2011 and area proposed by the technical staff Ministry of Environment of Ecuador.

All sites in 2009 were surveyed in November (dry season) were focused to confirm the gradient of disturbance between protected and urban area, while the sites surveyed in 2011 in January (rainy season) were guided to assess the differences between residential and industrial zone only in urban area (Figure 37). The sites studied during the bioremediation period were done between Decembers to March of 2012 distributed in three stations in five time period. Time 0: December 2011, characterize sites, Time 1: February 2012, first bioremediation period was done in 9 - 10 of February, Time 2: March 2012, the second bioremediation period was done in 5 - 6 of March, Time 3: March 2012, third bioremediation period was done in 19 - 20 of March and the Time 4: March 2012, last period was done 30 of March in the intertidal zone and low tide in Aventura Plaza (Figure 38).

Table 18. Geographic locations of the Estero Salado sampled stations.

Phase of survey	Area	Station (No)	Site	Abrev.	Latitude	Length
Prior Bioremediation (2009)	Protected Area	1	TPI antes muelle	TPIm	80° 0' 57.66" W	2° 11' 51.55" S
		2	TPI muelle centro	TPIc	80° 1' 3.43" W	2° 11' 37.81" S
		3	TPI Puerto Hondo	TPIh	80° 1' 8.84" W	2° 11' 36.58" S
		4	Estación Tres Bocas Boya 19	ETBb19	79° 57' 44.05" W	2° 14' 9.56" S
		5	Estación Tres Bocas Puerto Azul	ETBpa	79° 57' 36.02" W	2° 13' 38.16" S
		6	Estación Tres Bocas Muelle Suinsa	ETBs	79° 57' 30.05" W	2° 13' 52.36" S
		7	Cuarentena centro	CUAc	79° 57' 7.91" W	2° 19' 45.79" S
		8	Cuarentena este	CUAe	79° 57' 5.15" W	2° 19' 56.34" S
		9	Cuarentena oeste	CUAo	79° 57' 34.02" W	2° 19' 20.15" S
	Urban Area	10	Miraflores	MIR	79° 55' 19.32" W	2° 9' 36.83" S
		11	Kennedy-Universidad de Guayaquil	KUG	79° 54' 3.78" W	2° 10' 52.26" S
		12	Urdesa	Urd	79° 55' 9.61" W	2° 9' 45.29" S
Prior Bioremediation (2011)	Urban Area: Residence zone	13	Parque Ecológico Urdesa	PEU1	79° 54' 58.50" W	2° 9' 55.57" S
		14	Parque Ecológico Urdesa	PEU2		
		15	Bosque Salado Norte	BPSN1	79° 54' 8.24" W	2° 8' 57.31" S
		16	Bosque Salado Norte	BPSN2		
		17	Bosques El Salado	BES1	79° 54' 13.11" W	2° 9' 17.18" S
		18	Bosques El Salado	BES2		
	Urban Area: Industrial zone	19	Puente Miraflores-Urdesa	PMir1	79° 55' 6.08" W	2° 9' 44.57" S
		20	Puente Miraflores-Urdesa	PMir2		
		21	kennedy -Universidad de Guayaquil	KUG1	79° 54' 3.56" W	2° 10' 52.19" S
		22	kennedy -Universidad de Guayaquil	KUG2		
Bioremediation (2011-2012)	Urban Area: Residence and Commercial zone	23	Aventura Plaza 1	AP1	79° 54' 47.02" W	2° 10' 19.07" S
		24	Aventura Plaza 2	AP2	79° 54' 47.09" W	2° 10' 18.88" S
		25	Aventura Plaza 3	AP3	79° 54' 47.54" W	2° 10' 17.22" S

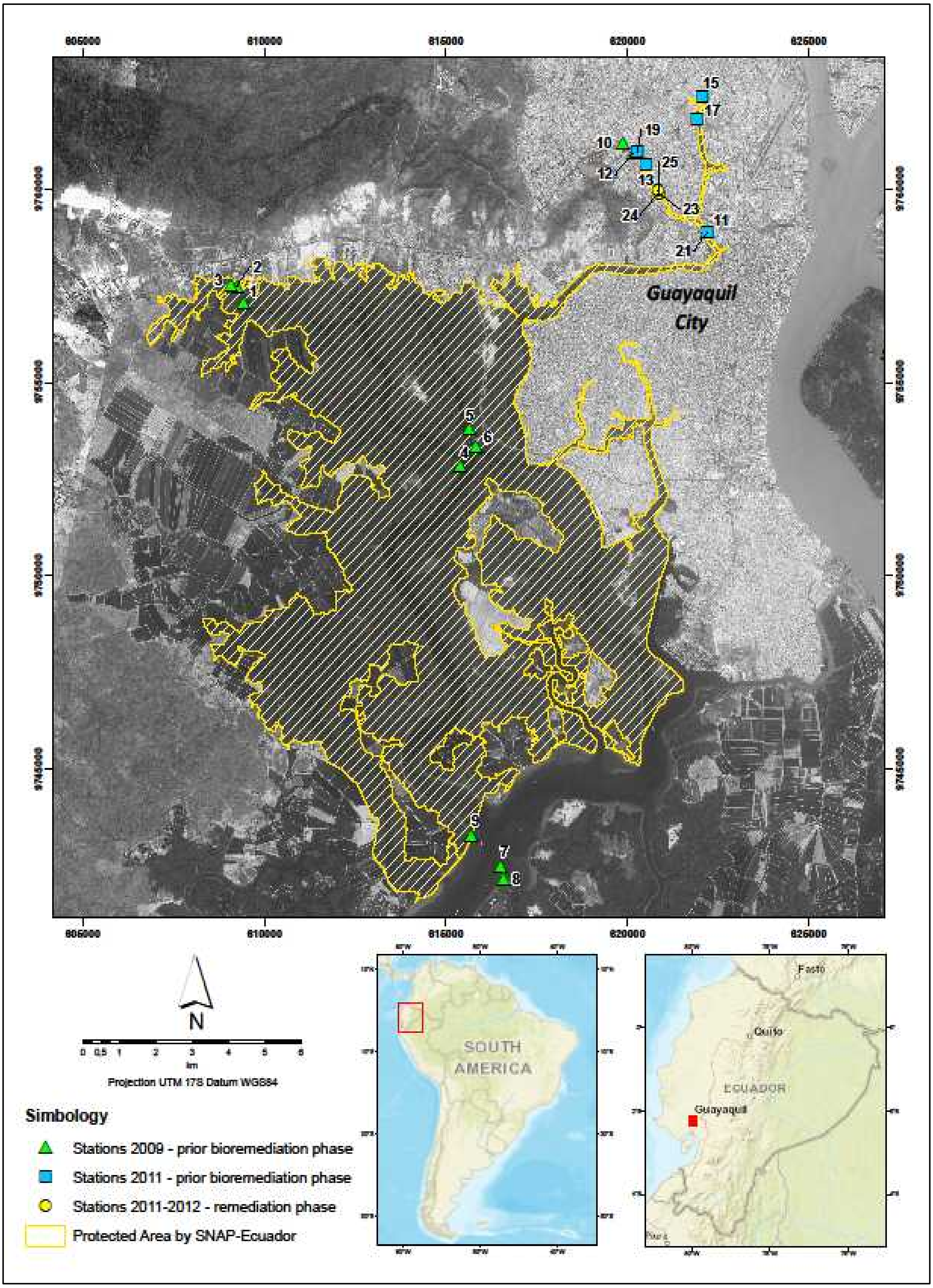


Figure 37. Localization of sampling sites surveyed in 2009 and 2011 in inner branches of Estero Salado

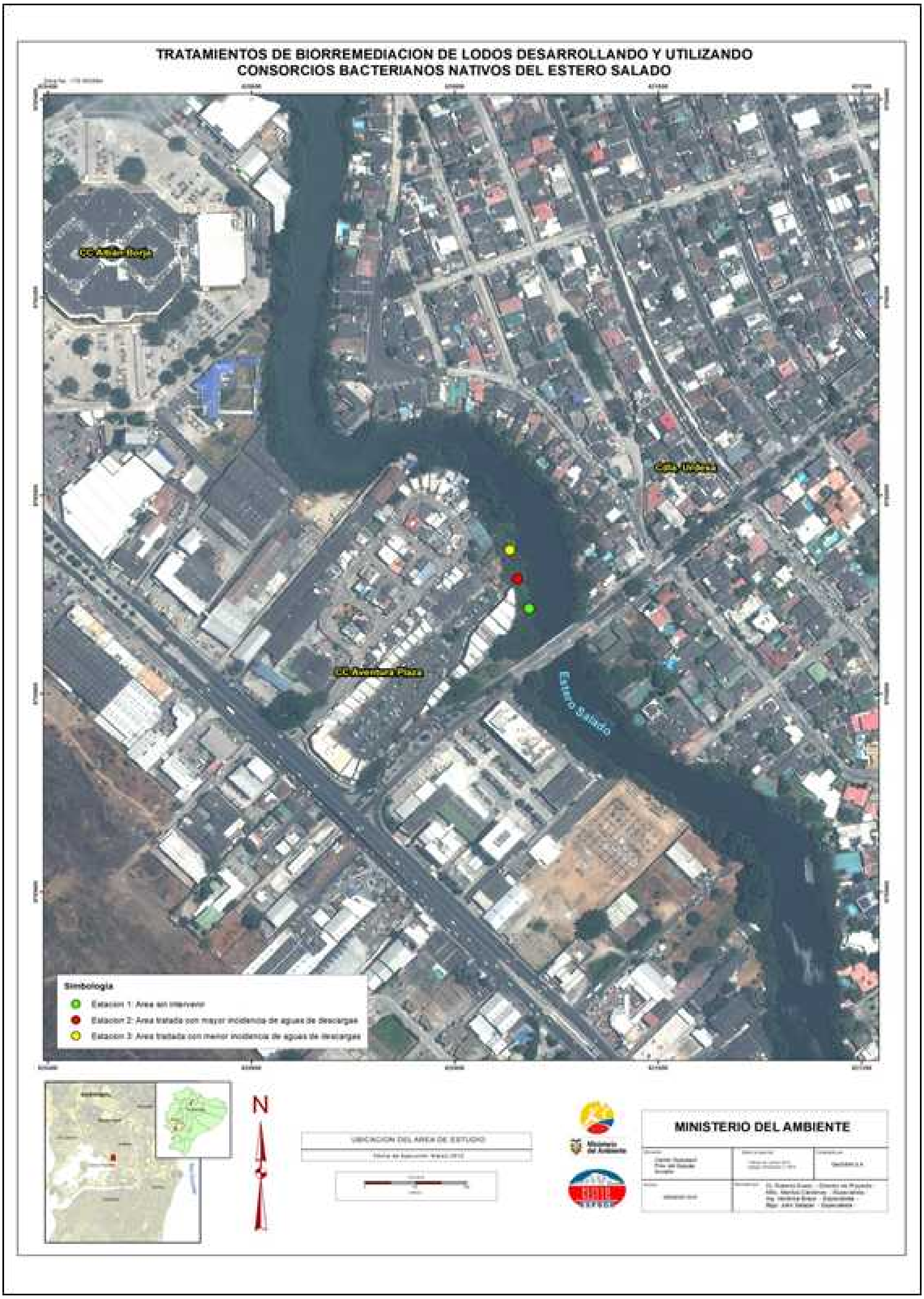


Figure 38. Localization of sampling sites surveyed in Aventura Plaza at 2011 and 2012 in inner branches of Estero Salado.

5.4 Methodology

5.4.1 Field work

The biological and environmental variables were measured during two phases one prior to the bioremediation of hydrocarbons present in the estuarine sediments during 2009 and 2011 and during the implementation of the bioremediation phase that began at the end of 2011 and ended in March 2012.

The integrated sediments sampling (chemical and biological parameters) took place since 2009 to 2012. The water analyzes were taken in function to the information needs and study objective for each year during 2009, 2011 and 2012. The water parameters such as: dissolved oxygen (DO) concentration, water temperature, pH and salinity were recorder in 2009; salinity in 2011 and biochemical oxygen demand (BOD_5), temperature and pH were registered in 2012.

In the 2009 the samples were analyzed by the certificate chemical laboratory called Laboratorio Marcos, under accreditation Agency Ecuador (OAE) of ISO 17025 by the O.A.E. No. OAE LE 2C 05-001. The samples of 2011 and 2012 for water and sediments were analyzed in their entirety in Lab-Cestta Laboratory, which is accredited by the Ecuadorian Accreditation Agency (OAE) with accreditation N ° OAE LE 2C 06-008 under the ISO 17025 standard that guarantees the technical competence of the laboratories using methods based on standard methods (American Public Health Association et al., 1999).

a) Water

Dissolved oxygen (DO) concentration, water temperature and salinity were recorder at surface of the water column were recorded *in situ* at three times at each site using a Multiparameter Hand Held Meter YSI model 556 MPS in 2009. The pH and DBO_5 were measured later in the laboratory by Lab-cestta during 2011 and 2012.

b) Sediments

The sediments samples were sampled at using a stainless steel Van Veen grab (0.1 m^2) in sludge layer with maximum depth of 10 cm (Eleftheriou & McIntyre, 2005). In each station, three replicates of the sediment were randomly collected in the sampling zone and placed in polyethylene bags. All the bags were placed on ice in cooler box for 24 h until their arrival at the laboratory where they were stored at 4°C in the case of chemical analysis.In each sediment sample, we measured the concentration of total petroleum hydrocarbon (TPH), metals (Hg, Pb and Cd), nitrate, nitrite, phosphate, hydrogen sulphide, organic matter and pH. All the chemical methods were based on quality assurance by use of protocols developed and validated at the Lab Cestta.

The macroinvertebrates were randomly collected at each station using stainless steel Van Veen grab (0.1 m^2) (FAO, 1981) and placed into wide-mouth plastic containers. Samples were preserved on site in 10% formaldehyde solution and later followed up with the same routine to preservation and taxonomic identification of organism in the laboratory was done, following the methodology described in the chapter 4 (see methodology). Three replicates of sediment were survey in each site. 91 chemical and 96 biological samples were took, distributed in 72 samples (36 biological and 36 chemical) in 2009, 25 samples (15 biological and 10 chemical) in 2011 and 90 samples (45 biological and 45 chemical) during period of bioremediation phase 2011-2012.

During the bioremediation phase sampled three stations were selected in Aventura Plaza with extension of 100 m of long parallel to the border of estuary ion the intertidal zone. This area was divided in three stations, each one with an extension of 33m. The station 1 (AP1), the station 2 (AP2) and the station 3 (AP3), the sations were called according to bioremediation time in APE11, APE21, APE31 for the first period of bioremediation in February of 2011, APE12, APE22, APE32 and so on until time 4 of bioremediation in March 30 of 2012.

Each of the stations despite found by (25 m) had different characteristics. All were influenced by combination stressor factors such as industrial and domestic effluent discharges, bridge construction activities, disposal of solid waste. At Station 1 there was a discharge zone it was also near the construction area of Urdesa Bridge (Figure 39), in this station did not applied biostimulation, bioaugmentation neither nutrients. It station was considered as reference site.

Figure 39. Descriptions pictures showed the station 1 situated near of bridge Urdesa during 2011 and 2012.

The station 2 had a higher incidence of waste waters where rainwater,domestic wastes are discharged and a lot of chlorophytes, insects and algae were observed on the substrate. In this station applied biostimulation (addition of nutrients) and Bioaugmentation (addition of degraders) was applied using the native bacterial consortium A. Finally the station 3 had a low incidence of water discharge and had the same bioremediation techniques that station 2 only was different that used the bacterial consortium B (Figure 40).

Figure 40. Descriptions pictures showed the station 2 and station 3 (right) in Aventura Plaza in Urdesa – Section B of Estero Salado during 2011 and 2012.

5.4.2 Laboratory analysis

All bacterium consortiums were sludge extracted from the sediments of Estero Salado and native strains was purified in two specialized laboratories. After the obtaining consortia bacteria through different phases in the laboratory (ESPOCH, 2012) (Figure

41). The bacterium consortiums were attached to an inert substrate that could not be dragged by the tide and have more exposition time and more hydrocarbon degradation time. The biostimulation of sediments were done with the addition of ammonium nitrate as a fertilizer. This was applied prior to application of the substrate on the sediment about two days earlier. The substrates with bacterial consortium were buried in the sediment at a depth of 20 cm to 30 cm in the station 2 and 3. To evaluate the effectiveness of bioremediation techniques monitoring of physical, chemical and biological parameters in water and sediment monitoring was performed.

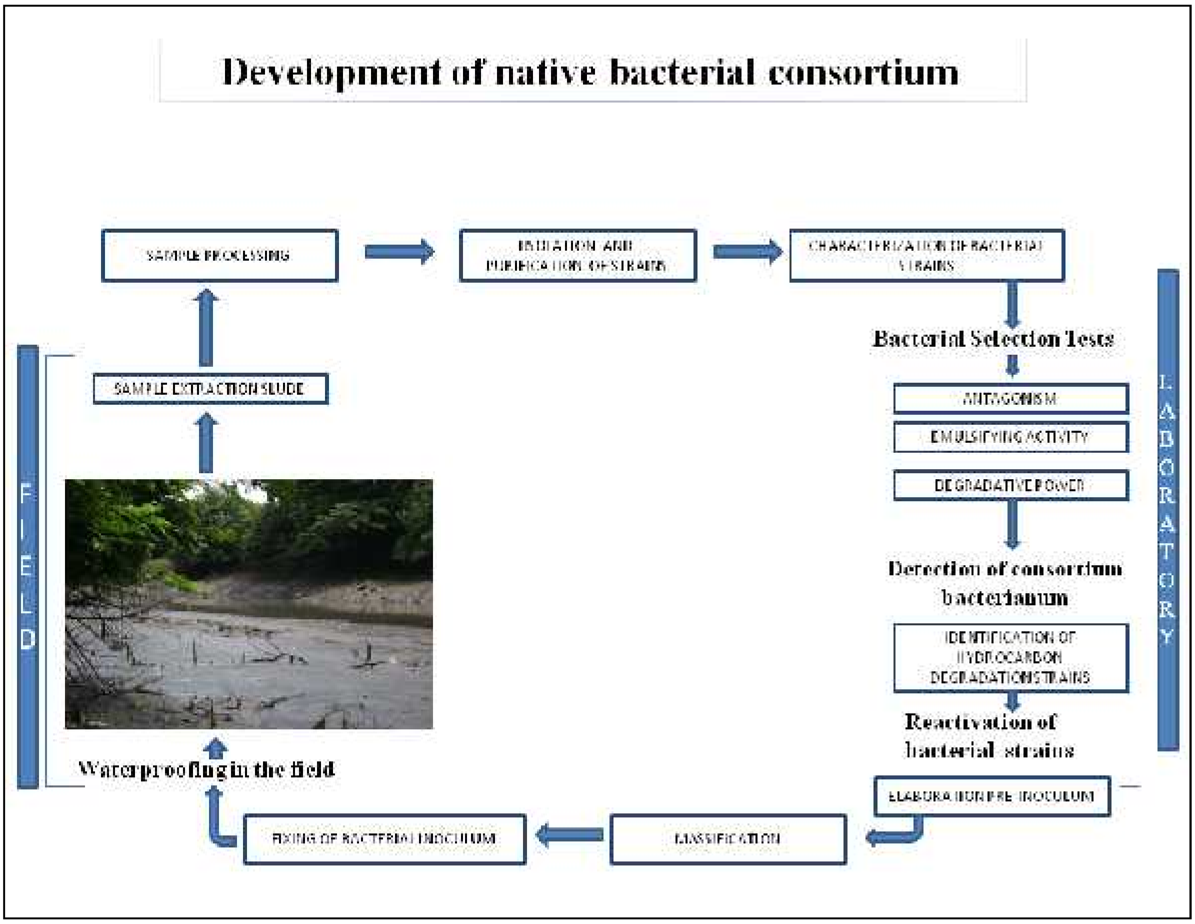


Figure 41. Laboratory phases of development of native bacterial consortium of inner branches from Estero Salado Estuary. Source: Diagrame elaborated according report generated by Centro de Servicios Técnicos y Transferencia y Tecnológica Ambiental (CESTTA) to Ministry of Environment.

5.4.3 Statistical analyses

The data exploration and descriptive statistic were done as discussed in chapter 3 (see. Statistical analyses) using Statgraphics plus version 4.1. To assess whether those data satisfy assumptions of normality and constant variance used Kolgomorov-Smirnov and Bartett´s test respectly (Legendre, 1998).

Some multivariate analysis were done for environmental variables (dissolved oxygen, total petroleum hydrocarbon, lead, cadmium and mercury), nutrients, other environmental variables and biotic variables (number of species, abundance and diversity) with PRIMER V6 statistical package. In order to describe the spatial patterns of composition of macroinvertebrate assemblages of subwatershed was performed a non-metric multidimensional scaling (MDS). Biological variables were standardized with square-root. The Bray-Curtis similarity coefficient was employed to construct a similarity matrix, dummy variable was added. This matrix was then subjected to (MDS). Followed by cluster analysis to identify the similarity between stations using abundance and presence-absence data at genus y/o species levels (Clarke & Gorley, 2006).

The biological indices (Abuntot), species richness (S) and diversity indices (Shannon-Wienner and Simpson Index) were calculated using the DIVERSE routine. The Principal components analysis (PCA) was used to ordinate 10 sediments and water variables: pH, total petroleum hydrocarbon (TPH), mercury (Hg), lead (Campbell & Hewitt), cadmium (Cd), nitrate ($NO3^-$), nitrite ($NO2^-$), phosphate ($PO4^-$), hydrogen sulphide (HS), organic matter (OM) and oxygen dissolved that were indicative of organic pollution (organic matter, hydrocarbons and nitrogenous substances and heavy metal) were used to represent contamination from industrial sources and to determinate the variables have more influence in the structure community of macroinvertebrates between years and areas. Besides, with quantitative matrices of the community descriptors (Shanon Wienner Diversity, Index of Dominance, species richness, number of individuals and equitability), nutrients and pollutants present in the sediment were performed classification analyzes to establish the relationship between the variables through of a Canonical Correspondence Analysis (Legendre, 1998).

Analysis of variance (ANOVA) test were used to determine significantly differed among areas (protected and urban area); among zones (residential and industrial zones) and between years (2009-2011 and 2012) and period of bioremediation. Kruskal-Wallis test were used which compares medians.

The SIMPER analysis was used to identify wich species made the greatest contributions among the period of study (Characterization and Bioremediation phases).

The criteria for determining the status of sediment quality and the maximum permissible levels of total hydrocarbon, mercury, cadmium and lead were established according to the Table 3 from TULSMA. Criteria remediation or restoration of soils, because

Ecuador does not have any regulations that control (TPH, Hg, Cd, Pb) or other pollutans agents in sediment of estuaries. Also use the ecotoxicological assessment criteria (EACs) to marine sediments according OSPAR (OSPAR Commission, 2000) for heavy metals. For the water was taken as reference Table 3 TULAS. Criteria eligible for the preservation of the flora and fauna in fresh, cold or warm water quality and marine waters and estuary

5.5 Results

5.5.1 Phase of survey prior to bioremediation of hydrocarbon contaminated sediment 2009-2011

a) Contaminants

Total Petroleum Hydrocarbon

The Total Petroleum Hydrocarbon (TPH) in sediments collected in 2009 and 2011 were ranged from 69 to 2825.20 mg/kg (Table 19). The highest TPH concentrations were found in Miraflores (station 10) in the 2009 and the Parque Ecológico de Urdesa (station 14) in 2011. Mean TPH concentration in 2009 was 315.96 mg/kg compared to 1249.45 mg/kg in 2011 (Table 19-Annex 7) .

The spatial distribution of TPH concentration in 2009 in the protected area showed high values in the Terminal Portuario Internacional station (station 3) and Tres Bocas Stations (stations 4 and 5), whilst decreasing toward Cuarentena (stations 7, 8 and 9). In 2011 highest concentrations of TPH were registered in residential zone in the stations Parque Ecológico de Urdesa (stations 13 and 14) and Bosque Protector Salado Norte (stations 15 and 16). The 64% (14) of stations exceeded the maximum allowable TPH concentrations level in sediments to Residence and Industrial zone according Ecuadorian environment law (EQS-Ecuador) mainly in 2011.

In general the urban area registered the higher concentration in relation with protected area, being the residence area, which had the higher concentrations. There was an increase in the concentrations of TPH in 2011 compared to 2009.

Heavy metals

The mercury (Hg) concentrations were ranged from 0.10 to 0.89 mg/kg. The highest concentration was in Cuarentena (station 7) in 2009 (Table 19). The mean concentration was 0.20 mg/kg in 2009 and 0.30 mg/kg in 2011 (Annex 7).

The Hg concentration in both years were almost constant with increased concentrations in Cuarentena stations (stations 7 and 8) in 2009. However, there was an increase in the concentrations in 2011 compared to 2009.

Cadmium (Cd) registered a range from 0.70 to 4.88 mg/kg. The highest concentration was in the Estación Tres Bocas (station 4) in 2009 and Bosques El Salado (stations 17 and 18) in 2011. The mean concentration was 0.78 mg/kg in the 2009 and 3.78 mg/kg in the 2011. Higher concentrations of Cd were registered in 2011 comparate with 2009, especially in residence zone. Which one Bosque El Salado (stations 17 and 18) exceeded the maximum allowable Cd concentrations level in sediments (4 mg/kg) to Residence zone according Ecuadorian environment law (EQS-Ecuador).

Lead (Campbell & Hewitt) registered concentrations between 19.00 and 59.33 mg/Kg (Table 19). The highest concentrations were in Estación Tres Bocas (station 4) in 2009 and Bosques El Salado (station 18) in 2011. The mean concentration was 19.00 mg/Kg in 2009 and 39.58 mg/kg in 2011.

Nutrients

The predominant nitrogen form was nitrate (NO3). The range fluctuated between 0.10 to 6.32 mg/kg. The highest concentration was registered in Parque Ecológico de Urdesa (stations 13 and 14) in 2011. The mean concentration was 0.1mg/kg in 2009 and 0.89 mg/kg in 2011 (Annex 8).The urban area registered the higher concentration in relation with protected area, especially in the residence zone, similar trend was the nitrite.

The phosphate concentrations were ranged from 0.06 to 15.90 mg/kg. The highest concentration was registered in Kennedy-Universidad de Guayaquil (stations 21 and 22) in 2011. The mean concentration was 0.60mg/kg in 2009 and 5.75 mg/kg in 2011.

Other chemical parameters of sediments and water

The Table 19 shows complete details of all environmental variables and the upper chemical exceedance level to sediments and water proposed by the Environmental Quality Standard for contaminated soils of Ecuador (EQS-EC: a) and preservation of aquatic life and wildlife in freshwater, marine and estuarine of Ecuador (EQS-EC: b). Principal component axis 1 (PC1) accounted for 82% of the variance and appeared to represent the influence of mercury (Hg) and pH (Figure 42). Along the PC2 (1.18% of the variance) locations spread according to the concentration of phosphate (PO4) and total petroleum hydrocarbon (TPH). Based on this analysis, we established two groups of area according to their potential degree of disturbance: (I) protected and urba area (residential zone) sites affected with organic pollution (OM) and (II) urban area (industrial zone) sites affected from industrial sources (heavy metals) and pH mainly. In the Annex 9 shows the results of eigenvalues and autovectors.

The concentrations of hydrogen sulphide were less than 1 mg/Kg. The pH of sediments was ranged from 6.20 to 8.35 and the levels of oxygen dissolved only were registered only in 2009 and registered values from 0.19 to 4.22 mg/L (Table 20).

The analysis of canonical correspondence between the ecological indexes and contaminants in the sediments of protected area showed a lowest percentage of explained variance with only a cumulative variance in the first two axes only of 37%. The highest percentages of explained variance in macroinvertebrates community structure were obtained when the relation between ecological indeces and nutrients with a cumulative variance of 70.68% with the two first components (Figure 42b). This result shows the inverse relationship between sulfur and contaminants. It is probably due to anoxia in the sediments.

The cumulative variance in the first two axes only of 37%, of which 0.73 is explained by the component 1 and 0.27 in component 2 (Figure 42a). The relation between ecological indeces and nutrients with a cumulative variance of 70.68% with the two first components, explained by 0.81 in axi 1 and 0.95 in the axi 2 (Figure 42b). Which would indicate that other environmental variables and pollutants influence much more than the pollutants sampled? The variables expressed as vectors indicate the relation of the ecological indexes is associated to the mercury what suggested that species are adapted to the level of contamination of this heavy metal. The canonical correspondence analysis for urban area only showed the relation pollutans and nutrients where the cumulative variance was the 99% explained by the first axi 1 (Figure 42 c). Finally the relation between ecological indexes and nutrients were explained by the 99.9% explained by the first axi with 97% and axi 2 with 2.79% (Figure 42d)

In both zones (protected and urban) the presence of sulfhydric is inversely related to the ecological indexes and is therefore exclusive to diversity.

Table 19. Contaminants concentration (mg/Kg/dry weight) for sediments samples collected in the inner branches of Estero Salado during 2009 and 2011.

		Stations	**Hydrocarbon**	**Mercury**	**Cadmium**	**Lead**
Years	**2009**	1	123.21	0.10	0.70	19.00
		2	605	0.10	0.70	19.00
		3	*627.12	0.10	0.70	19.00
		4	*233.41	0.10	1.84	19.00
		5	*369.56	0.10	1.50	19.00
		6	213.64	0.10	1.66	19.00
		7	74.74	0.89	0.85	19.00
		8	69	0.31	1.07	19.00
		9	72.15	0.10	1.32	19.00
		10	*1124.30	0.10	0.70	37.89
		11	87.5	0.10	0.70	19.00
		12	191.87	0.10	0.70	19.00
	2011	13	*2748.89	0.30	2.82	40.01
		14	*2825.20	0.30	2.57	39.15
		15	*2779.85	0.30	3.72	29.9
		16	*2772.06	0.30	3.61	30.20
		17	*822.77	0.30	*4.36	56.2
		18	*677.14	0.30	*4.88	59.33
		19	*236.93	0.30	3.93	41.63
		20	*245.66	0.30	4.01	40.17
		21	*860.39	0.30	3.85	33.14
		22	*872.71	0.30	3.44	32.11
Upper Chemical Exceedance Level	**EQS-EC (mgKg-1)**	**Residence zones**	230	1.00	4	140
		Industrial zones	620	10	10	150
	<ERL-EPA (ug/Kg)		ns	150.00	1200	47000
	EAC s (mg/Kg-1)			0.05-0.5	0.1-1.0	5.0-50

EQS-ECU: Environmental quality standard – Ecuador. Norma de Calidad Ambiental del Recurso Suelo y Criterios de Remediación para suelos contaminados. Anexo 2 Del Libro VI del Texto Unificado de Legislación Secundaria del Ministerio del Ambiente. Tabla 2 Criterios de Bioremediación (Valores máximos permisibles)
(Ministerio del Ambiente, 2015c).

<ERL: Effect Range Low Concentration below of the contaminants in sediments (e.g. PAHs and metals) that rarely cause adverse effects in marine organism. ERL were development by the Unites States Environmental Protecction Agency.https://www3.epa.gov/

EACs (Ecotoxicological Assessment Criteria) in marine sediments according OSPAR (OSPAR Commission, 2000).

*Values exceeding the Ecuadorian environment law **(EQS-ECU)**

Table 20. Nutrients concentration (mg/Kg dry weight), Hydrogen sulphide (mg/Kg dry weight), Organic matter (%), pH for sediments and Oxygen dissolved (mg/L), pH and Salinity (ups) samples collected in the inner branches of Estero Salado during 2009 and 2011.

	Sediments							Water		
Year	**Stations**	**Nitrate**	**Nitrite**	**Phosphate**	**Hydrogen Sulphide**	**Organic Matter**	**pH**	**Oxygen dissolved**	**pH**	**Salinity**
2009	1	0.10	0.028	0.56	0.06	10.55	7.00	1.98	7.4	36
	2	0.10	0.033	0.55	0.06	8.55	6.90	1.86	7.4	35
	3	0.30	0.012	1.16	0.07	16.00	6.40	1.84	7.4	36
	4	0.10	0.010	0.63	0.04	10.91	7.00	2.73	7.5	35
	5	0.10	0.010	0.70	0.10	19.02	7.20	2.64	7.5	34
	6	0.10	0.032	1.18	0.07	10.01	6.20	2.64	7.5	32
	7	0.80	0.488	0.06	0.07	7.24	7.00	3.00	7.5	32
	8	0.50	0.010	0.37	0.02	13.41	6.80	4.22	7.6	34
	9	0.10	0.027	0.67	0.43	7.23	6.20	3.71	7.6	34
	10	0.20	0.007	0.12	0.03	10.53	7.00	0.19	7	10
	11	0.10	0.323	1.00	0.14	5.94	6.30	1.46	7	25
	12	0.20	0.013	0.43	0.04	5.94	7.00	0.35	7	0
2011	13	6.32	2.22	5.70	0.04	5.75	8.35			
	14	5.29	2.22	6.60	0.04	5.91	8.15			
	15	0.50	0.099	9.00	0.04	23.48	7.75			
	16	0.50	0.098	8.90	0.04	22.91	7.82			
	17	0.50	0.033	5.70	0.10	9.71	7.76			
	18	0.50	0.029	5.80	0.10	8.99	7.79			
	19	0.98	0.046	2.10	0.06	3.95	7.53			
	20	0.79	0.049	2.00	0.06	3.97	7.39			
	21	1.16	0.490	15.90	0.14	10.06	7.79			
	22	1.28	0.510	15.80	0.14	9.15	7.9			
Upper Chemical Exceedance Level	EQS-EC : a) Sediments (mgKg-1) b) Estuarine water (mgL)						a) 6 to 8	b)>60 % de saturación	b) 6.5-9.5	ND

EQS-ECU: Environmental quality standard – Ecuador.

a) Norma de Calidad Ambiental del Recurso Suelo y Criterios de Remediación para suelos contaminados. Anexo 2 Del Libro VI del Texto Unificado de Legislación Secundaria del Ministerio del Ambiente. Tabla 2 Criterios de Bioremediación. (Ministerio del Ambiente, 2015c).

b) Norma de Calidad Ambiental y de Descargas de Efluentes: Recurso Agua. Anexo 1 Del Libro VI del Texto Unificado de Legislación Secundaria del Ministerio del Ambiente. Tabla 2 Criterios de Calidad Admisibles para la Preservación de la Vida Acuática y Silvestre en Aguas Dulces, Marinas y de Estuarios. (Ministerio del Ambiente, 2015b).

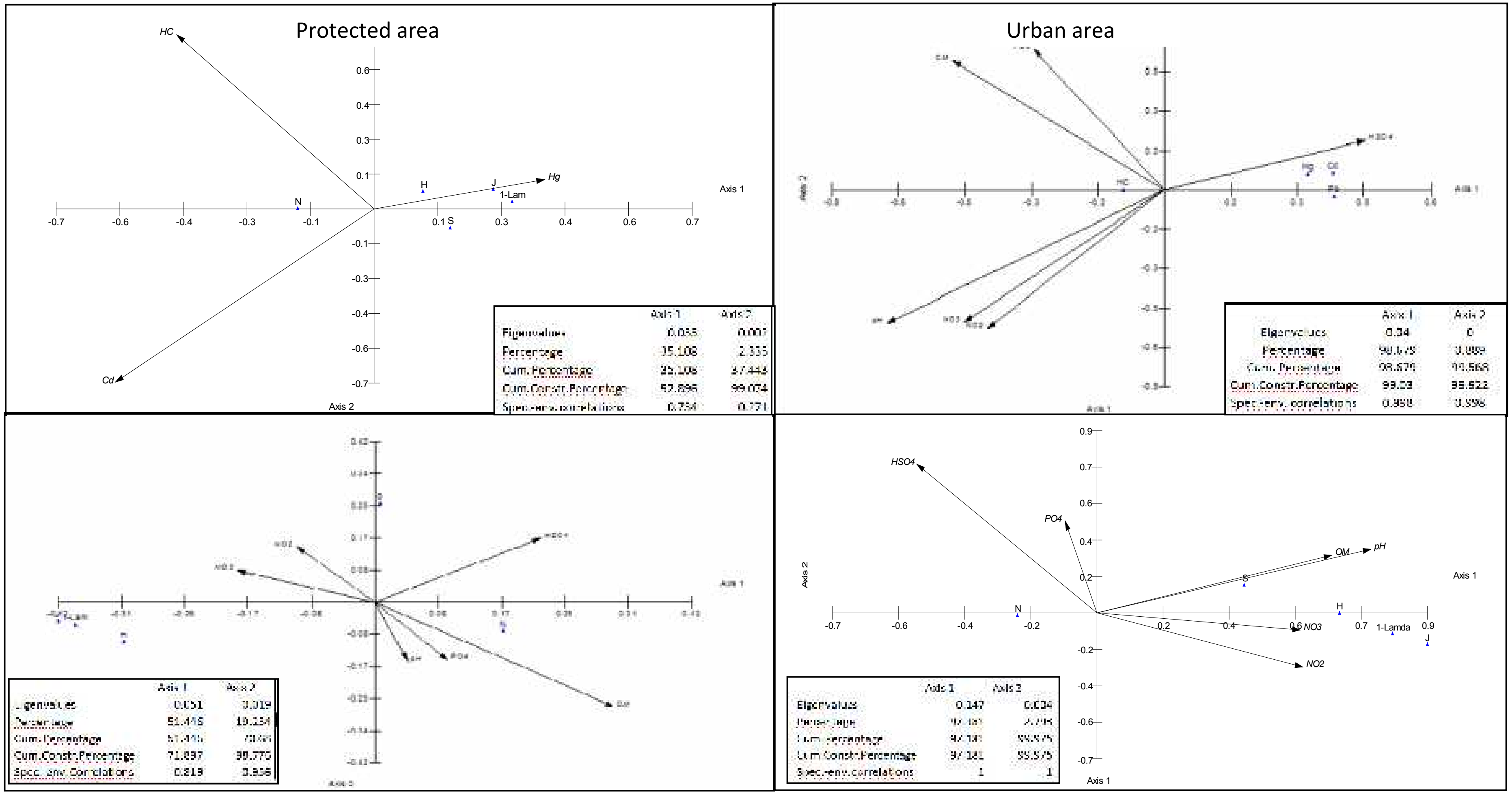


Figure 42. Biplot of the first two axes of the canonical correspondence analysis of the pollutanst, environmental variables and ecological indeces in the inner branches of Estero Salado during 2009 and 2011. Results for the first two components a) that explain 37% of the variability explained between pollutans and ecological indeces in protected area.The eigen value for axis 1 contains 35% and the axis 2 the 2.34%. b) The eigen value for PC2 (1.80) contains 1.18% of the other variables (cumulative value = 1).

b) **Biological variables**

Overall benthic macroinvertebrates fauna in 2009 and 2011.

The samples obtained during 2009 contained 1522 individuals contained in 28 species representing five phyla. i.e. Annelida (45%) Arthopoda (33%), Mollusca (20%), Echinodermata and Nematode (1%) each one (Annex 10). While whereas those collected during 2011 yield 2096 individuals in 16 species were survey contained five phyla.i.e.. Arthopoda (42%), Annelida (33%), Mollusca (24%) and the 1% represented by Nemertine and Nematode.

The most abundant taxa in 2009 were Polychaeta, Insecta, Gastropoda and Entognatha. Low abundance was registered by Bivalvia. Ophiuroidea, Nematode. Malacostraca and Arachnida (Figure 43a). While in 2011 the taxa most abundant were similar to registered in 2009 (Figure 43b; Annex 10).

In the 2009 the Annelida were represented by polychaetes of the family Capitellidae with 97%, followed by the family Nephtyidae with 2% represented by *Nepthys squamosa* and the family Eunicidae with the 1% represented by *Eunice* sp.

The Arthropoda were represented mainly by insects of Orden Diptera (66%) with the families Psychodidae with the species *Pericoma* sp., Ceratopogonidae represented by *Probezzia* sp. and Tipulidae. Other abundant family was Poduridae represented by *Podura aquatica* (33%). Others insects were few representatives such as Dolichopodidae, Phoridae and Ephydridae. The decapods were scarce and were represented by the family Ocypodidae (1%) with the specie *Uca* sp.

The mollusk were represented mainly by gastropods with the family Collumbellidae (49%) represented by *Cosmioconcha* sp., *Costoanachis nigricans* and *Anachis* sp., Littorinidae (15%) with the specie *Littoraria aberrans*, Cylichnidae (13%) with the specie *Cylichna* sp., Potamididae (11%) with species *Cerithidea mazatlanica* and *Cerithidea valida.* Low abundances were the families Thiaridae, Tornidae, Epitoniidae, Naticidae, Planorbidae and Calyptraeidae. The Bivalve was lower abundance represented by two families Tellenidae and Veneridae with 54% and 46% respectively. Finally Echinodermata (Ophiuroidea) and Nematode sp1 were only identify to orden level were lower abundant Annex 11.

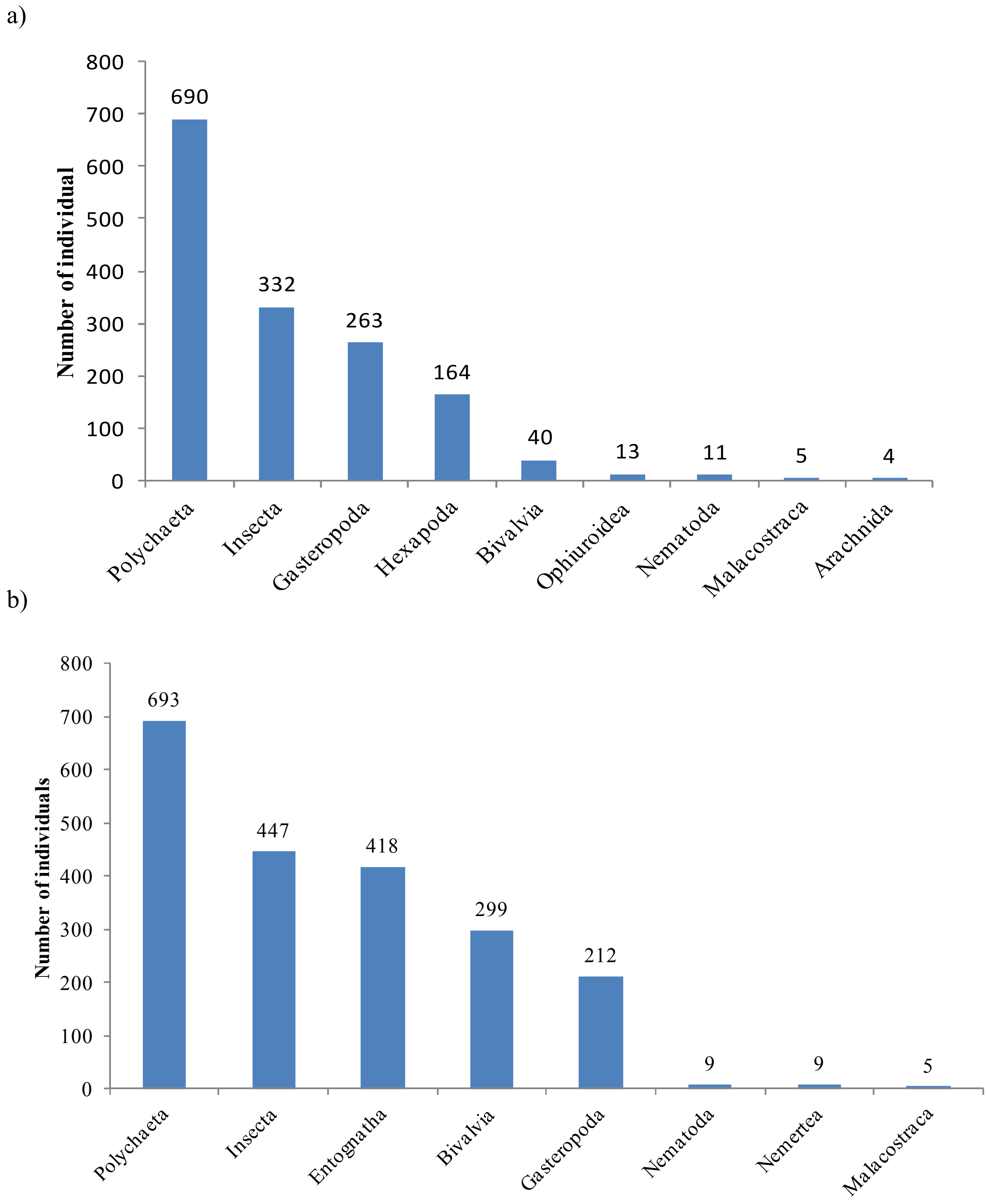


Figure 43. (a) Abundance the benthic macroinvertebrates taxa registred in Protected area (I) and urban area (II) at November 2009 and (b) Urban area in Estero Salado Estuary: Residence and Industrial zone in sediments of Estero Salado at January of 2011.

The Figure 44 shows the most abundance species and families registered in protected and urban area, Capitellidae (*Capitella cf capitata*), Psychodidae (*Pericoma* sp.) and Poduridae (*Podura aquatica*) were registered only in urban area. While the family Columbellidae with the species *Costoanachis nigricans* and *Cosmioconcha* sp., and the family Cylichnidae (*Cylichna* sp.) registered in the protected area.

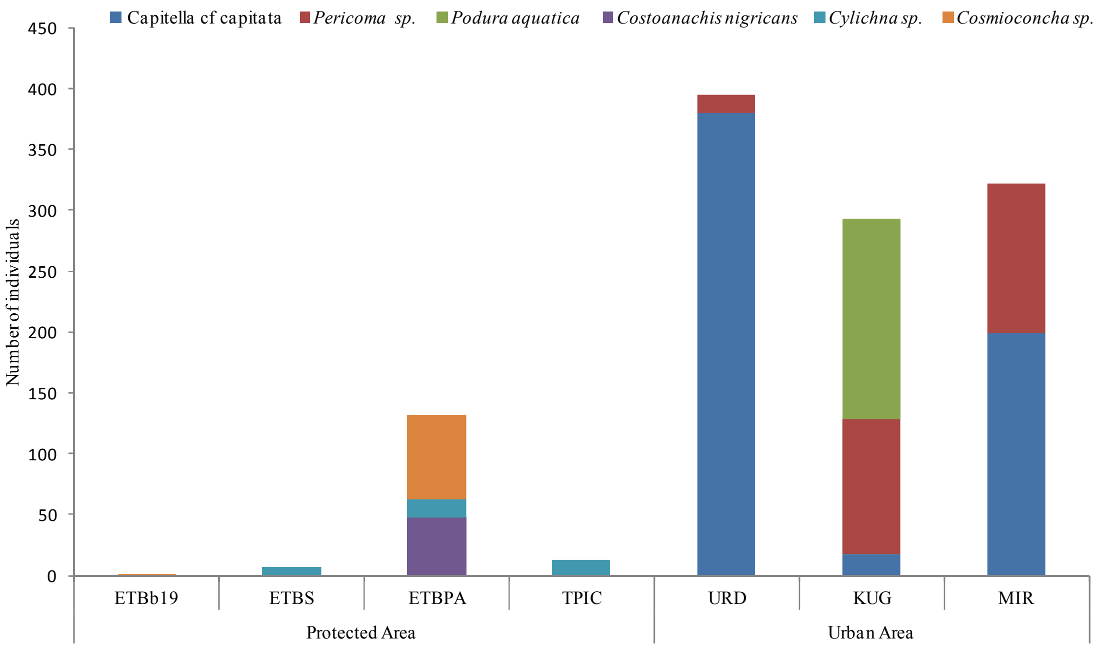


Figure 44. Number of individuals of the most abundance species registered in sediments of Protected Area (I) and Urban area (II) from inner branches of Estero Salado in November 2009.

In 2011 the Annelida were represented only for two families Capitellidae with 378 individuals represented by *Capitella cf capitata* and the family Nereidae with the specie *Micronereis* sp. with the 315 individuals (Figure 45).

The Arthropoda were represented by Collembola with the family Poduridae with (418 individuals) with the specie *Podura aquatica,* the insects represented by the families Ceratopogonidae represented by *Probezzia* sp. and *Culicoides* sp., Dolichopodidae (*Hydrophorus* sp.), and Psychodidae (*Pericoma* sp.) with 79 and respectively. The amphipds were represented by the family Talitridae (1%).

The mollusk were represented mainly by gastropods with the family Thiaridae (110 individuals represented by *Melanoides cf tuberculata*. The family Hidrobiidae (42), Ellobiidae (35) with the specie *Melampus carolianus*, *Marinula concinna* and *Detracia graminea.* The family lower abundant was Planorbidae with 13 individuals. The Bivalve were lower abundance represented by two families and Cyrenoididae with the specie *Cyrenoida inflata* (207) and the family Mytilidae with the specie *Mitylopsis* sp., with 92 individuals. The Entognatha was represented by the family Poduridae with the specie *Podura aquatica.* Other taxa lower abundant were Nemertine and Nematode (Annex 11).

The Figure 45 show the most abundance species registered in residence and industrial zones, all species were abundant in industrial zone in comparison with residential zone where *Capitella cf capitata* wasn't register.

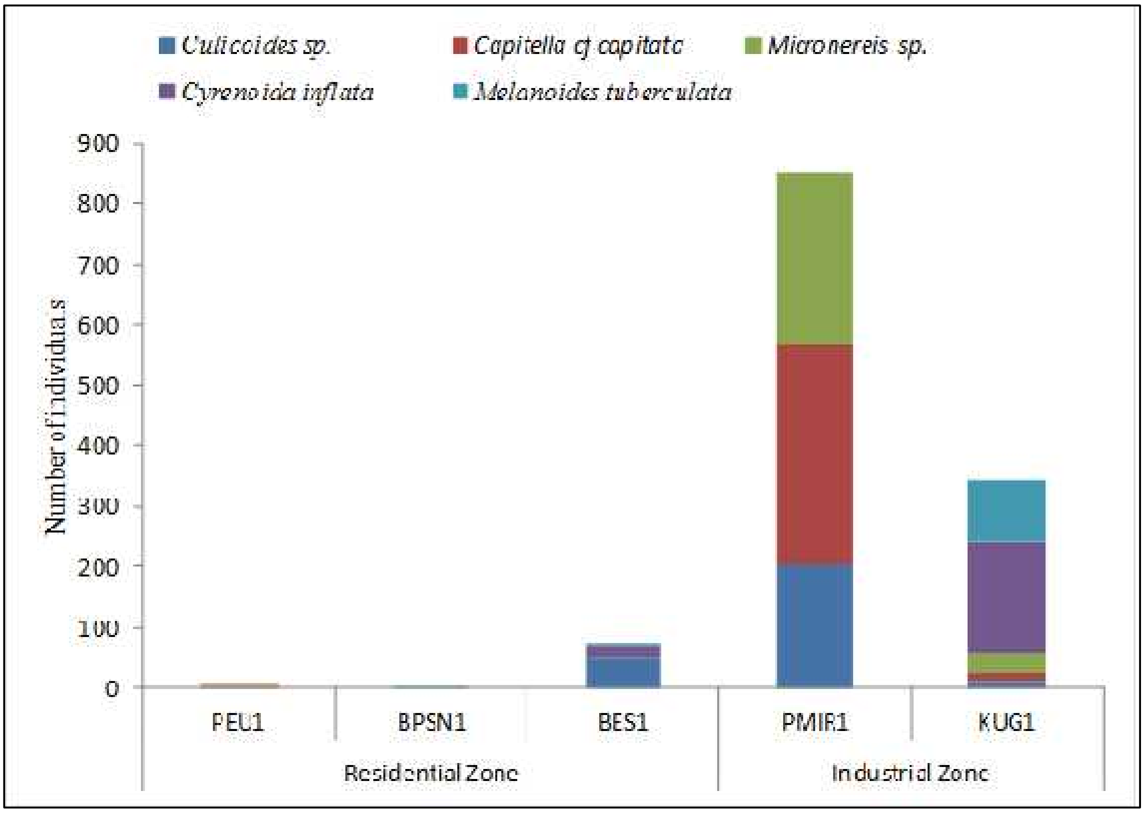


Figure 45. Number of individuals of the most abundance species in sediments at the urban area in Estero Salado Estuary: Residence and Industrial zone at January of 2011.

Composition of benthic macroinvertebrates assemblages

The non-metric multidimensional scaling ordination of taxa and abundance of macroinvertebrates from 2009 showed two areas (Figure 46); one represented principally by urban area. Conformed by the station Urdesa (Colcha & López), Miraflores (MIR) and Kennedy-Universidad de Guayaquil (KUG), where there were dominant of capitelids and insects of Orden Diptera represented mainly by the families Psychodidae and Tipulidae. The others groups of stations were in protected area (Figure 46) there were more abundance of mollusks owner of mangrove as *Cerithidea mazatlanica*, *Cerithidea valida*, *Anachis* sp., *Cylichna* sp., *Tellina* sp., Polychaetes *Nepthys squamosa*, *Eunice* sp., decapods as *Uca* sp. and ophiuroideos. However, this area showed three subgroups of stations with clear differentiation between them, one sub-group formed by Estación Tres Boca Muelle (ETBm) and boya 19 (ETBb19) and

Cuarentena centro (CUAc), second sub-group formed by Cuarentena oeste (CUAo) and Cuarentena este (CUAe), the third sub-group formed by all stations of Terminal Portuario Internacional (TPI) but and the last station Estación Tres Bocas Puerto Azul (ETBPA) far of the others station of this area.

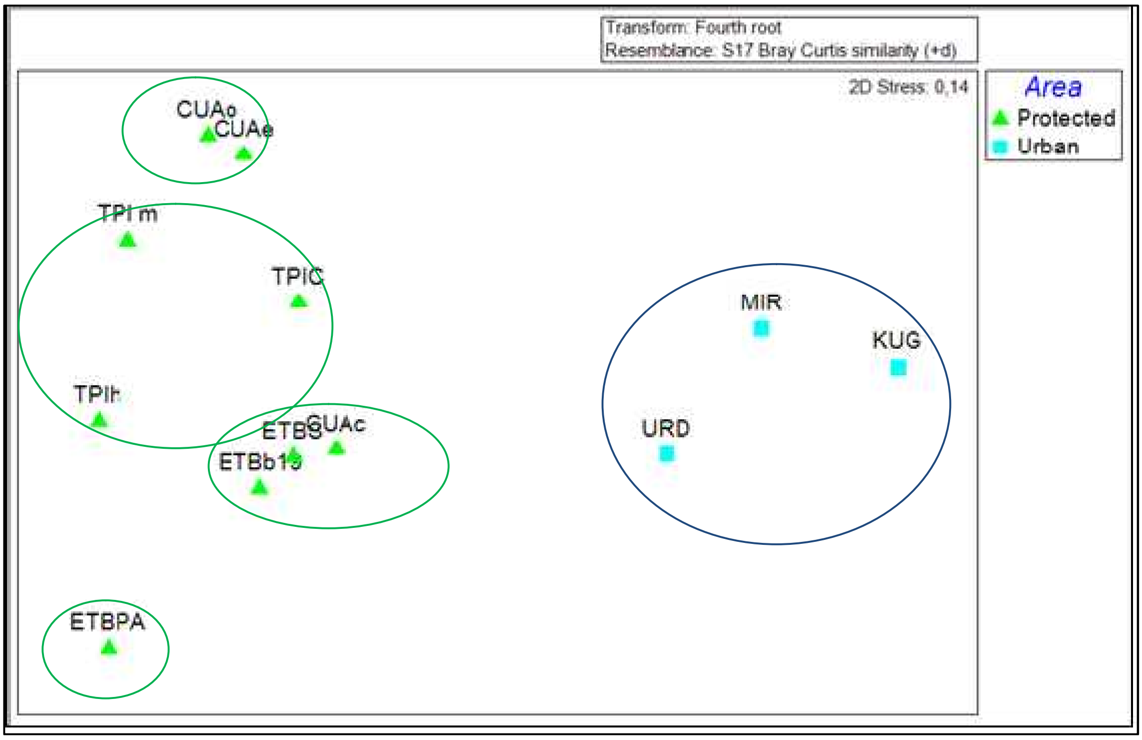


Figure 46. Non-metric multidimensional scaling ordination, derived from the Bray Curtis similarity matrix constructed using fourth root transformed the abundances of genus of benthic macroinvertebrates in sediments at the two areas in Estero Salado Estuary: Protected area and urban area at November 2009.

The analysis of similarity of stations (Figure 47) based on taxa and abundance of invertebrates of 2009 determined clearly the separation of urban and protected area, the stations more similar in 60% were Urdesa (UG) and Miraflores (MIR). These stations were characterized by domestic and industrial effluents and the presence of Psychodidae larvae and dominance of *Capitella cf capitata*.

In the protected area was observed three subgroups of which the stations more similar to each other in 50% were Estación Tres Bocas b19 (ETBb19) and Cuarentena c (CUAc), these stations were characterized for the influenced of seawater and the presence of Polychaeta sp 1 (Figure 47).

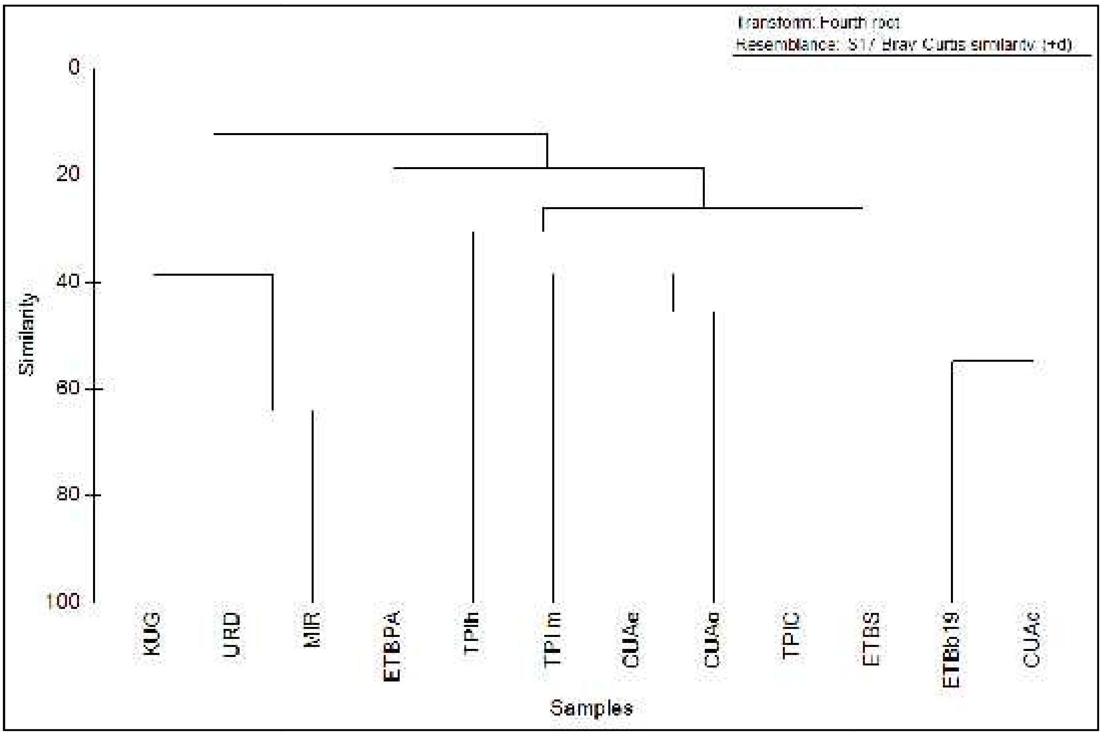


Figure 47. Dendrogram for hierarchical clustering from each of study sites, based on the Bray- Curtis similarity matrix constructed using fourth root transformed total abundances of the species of benthic macroinvertebrates in sediments at the two areas in Estero Salado Estuary: Protected area (I) and urban area (II) at November 2009.

In 2011 the non-metric multidimensional scaling ordination of taxa and abundance of macroinvertebrates showed three groups (Figure 48); One represented by the stations Bosque Protector Salado Norte (BPSN) and Parque Ecológico Urdesa (PEU) twice located in residence zone where the common fauna was conformed by gastropods *Melanoides cf tuberculata* and hidrobids. Other group formed by the stations Kennedy Universidad de Guayaquil (KUG) located in Industrial zone and Bosque El Salado (BES) located in residence zone, both sharing a fauna of aquatic insects as Diptera (*Pericoma* sp., *Culicoides* sp., *Hydroporus* sp.), Nematode, Annelida (*Capitella cf capitata*) and Mollusks as *Cyrenoida inflata*, *Marinula concinna*, *Melanoides cf tuberculata* and hidrobids. Other station totally different was Puente Miraflores (PMIR) characterized by the presence of polychaetes (*Capitella cf capitata* and *Micronereis* sp.) and by the insect *Culicoides* sp., so too as larve and pupa of diptera and high concentrations of total petroleum hydrocarbon and cadmium.

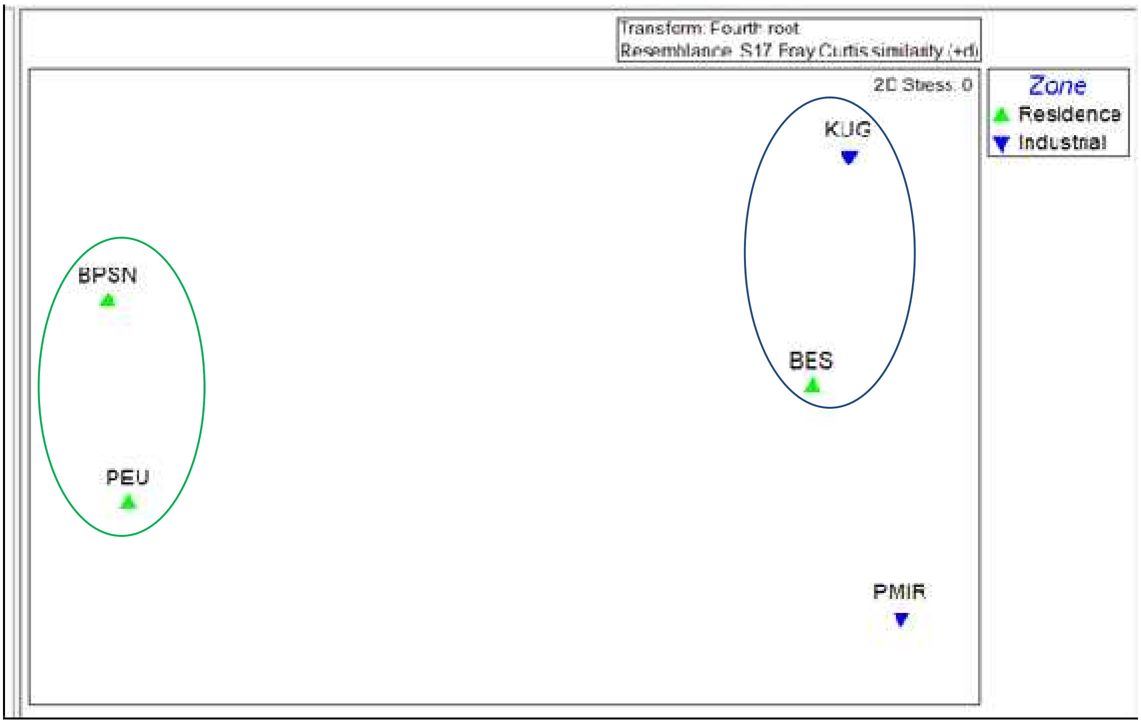


Figure 48. Non-metric multidimensional scaling ordination, derived from the Bray Curtis similarity matrix constructed using fourth root transformed the abundances of genus of benthic macroinvertebrates in sediments at the urban area in Estero Salado Estuary: Residence and Industrial area at January of 2011.

In 2011 also the majority of stations were similar in a 50% except Puente Miraflores (PMIR) (Figure 49), the stations more similar were Parque Ecológico Urdesa (PEU) and Bosque Protector Salado Norte (BPSN) are affected by urban growth. The Kennedy Universidad de Guayaquil (KUG) and Bosque El Salado (BES) have a major influence of tidal however represented two different section of inner branches of Estero Salado such as Section C and B forming part of residence and industrial zone.

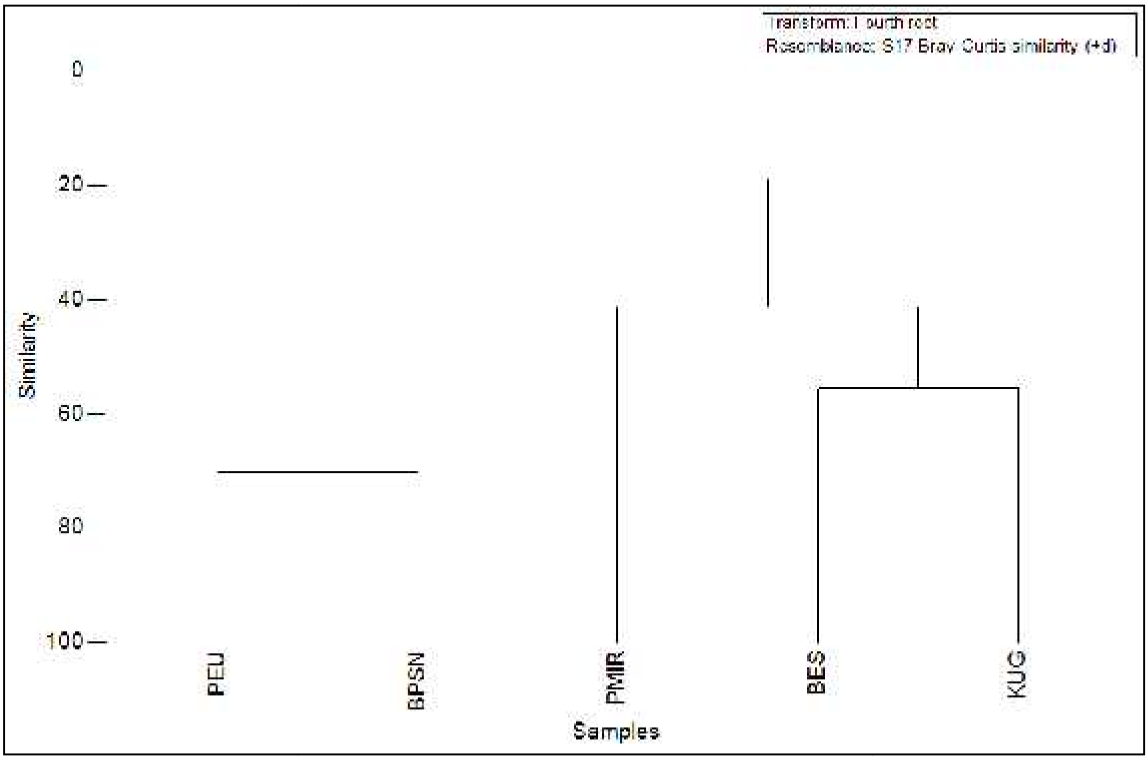


Figure 49. Dendrogram for hierarchical clustering from each of study sites, based on the Bray- Curtis similarity matrix constructed using fourth root transformed total abundances of the species of benthic macroinvertebrates in sediments at the urban area in Estero Salado Estuary: Residence and Industrial zone at January of 2011.

Species richness and diversity

The Shannon´s index (H´) between sites in 2009 show a ranged from 0 to 1.9. The median value was 0.72 and the station with the highest index was Estación Tres Bocas Puerto Azul. Simpson index was the same trend of Shannon-Wiener Index (Table 21). The higher diversity was registered in urban area (II) generated mainly by insects' larves (Annex 10). The eveness mean was 0.47 with dominance of species of *Pericoma sp.,* and pupas of Psychodidae (Diptera), Capitellidae (Polychaeta) and Pupas of in Urdesa and Miraflores. One - way ANOVA showed that the number of species (S) and total number of individuals (N) each differed significantly among areas in the 2009 (Annex 12). The median area number of benthic macroinvertebrates species was greater in urban area, declining markedly in protected area (Figure 50). The median of number of individuals (abundance) followed similar trends index of diversity showed macroinvertebrates community (Annex 13). No significant differences were found with Eveness, Shannon-Wiener and Simpson index between areas in 2009 (Figure 50).

The Shannon-Wiener Index registered between sites in 2011 a ranged from 1.01 to 2.00. The median value was 1.39 (Annex 14) and the station with the highest index was Bosques El Salado, Simpson index was the same trend (Table 21). The higher diversity was registered in residential zone (I) generated by insects' larves, gastropods, bivalve, nematodes and Nemertine. The eveness mean was 0.81 with dominance of species of *Podura aquatica*, *Cyrenoida inflata*, *Mytilposis* sp., and *Melanoides cf tuberculata* in kenendy-Universidad de Guayaquil.

Significant differences were detected in 2011 between the total number of individuals (N) (H=7.11; P-Value 0.008) and Eveness (H=6.75; P-Value 0.009) among zones (Annex 15). The median area number of individuals of macroinvertebrates was greater in industrial zone and lower in residential zone. The median of eveness was different trends, with a median greater in residence zone and lower in industrial zone where there are dominance of Diptera and Polychaetes. No significant differences were found with Richness of species; Shannon-Wiener and Simpson index between zones twices areas were poor in diversity of invertebrates (Figure 50-51-52).

Table 21. Diversity Indices of benthic macroinvertebrates: Species Richness (S); Total number of individuals (N); Shannon Wiener (H´); Simpson (1-Lamda´) and Equitability (J´) in sediments at Protected area (I) and Urban area (II) at November 2009 and Residence and Industrial area at January of 2011 in inner branches of Estero Salado Estuary.

Year	Area	Abrev.	Station No.	S	N	H´ (log e)	1-Lamda´	J´
2009	Protected Area	TPIm	1	2	2	0.69	1	1
		TPIc	2	5	27	1	0.68	0.77
		TPIn	3	2	16	0.38	0.23	0.54
		ETBb19	4	3	12	0.57	0.32	0.52
		ETBPA	5	12	198	1.90	0.8	0.77
		ETBS	6	4	23	0.94	0.56	0.68
		CUAc	7	3	8	0.74	0.46	0.67
		CUAe	8	1	2	0	0	0
		CUAo	9	1	2	0	0	0
	Urban Area	LFD	10	6	444	0.55	0.26	0.3
		KUG	11	15	150	1.88	0.78	0.7
		MIR	12	5	330	0.78	0.5	0.49
2011	Urban Area: Residendial Zone	PEU1	13	3	6	1.01	0.73	0.92
		BPSN1	15	3	6	1.09	0.8	1
		BES1	17	13	165	2.00	0.83	0.78
	Urban Area: Industrial Zone	MIR1	19	8	945	1.39	0.71	0.71
		KUG1	21	19	970	1.89	0.76	0.64

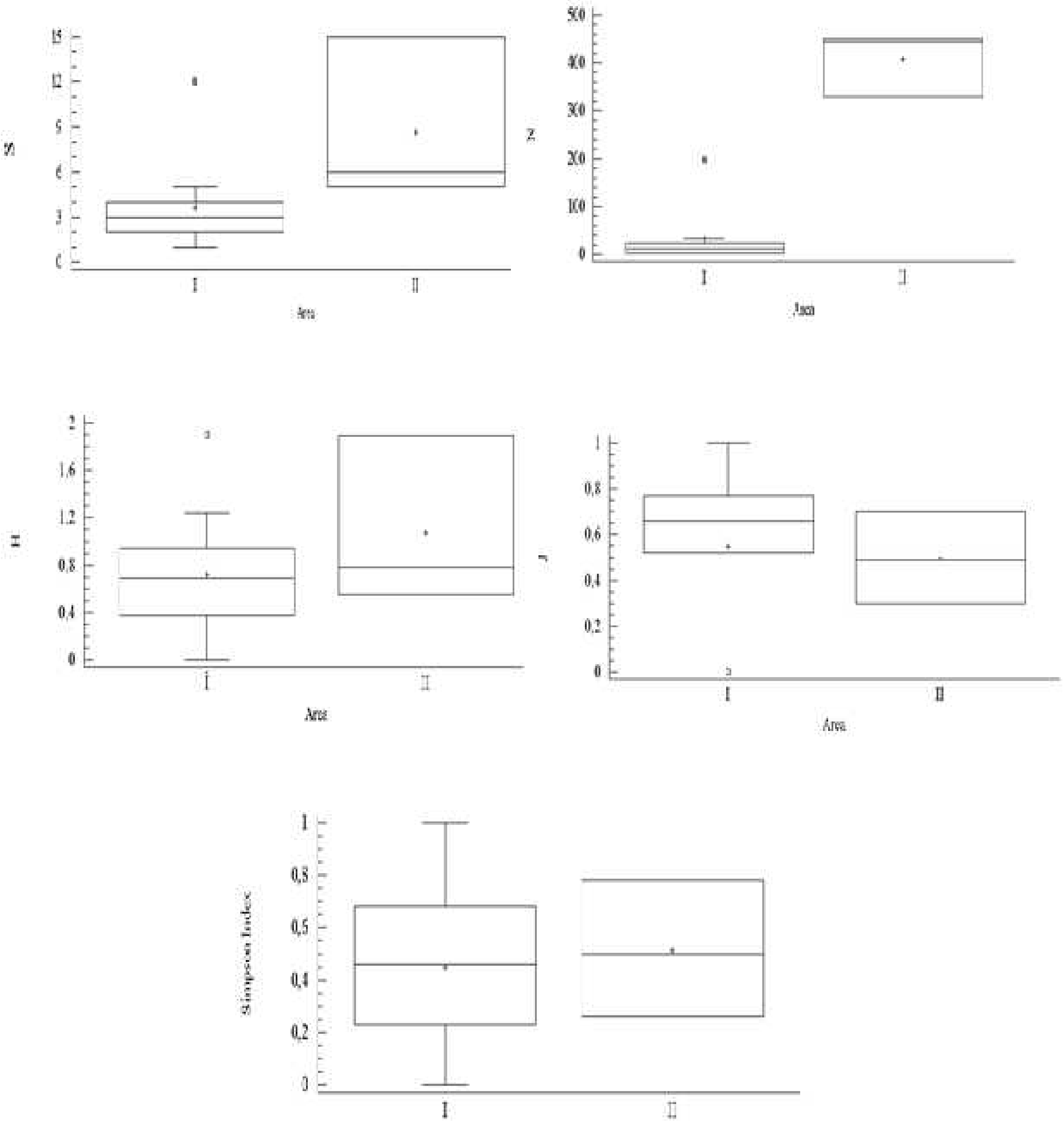


Figure 51. Box and Whiskers for number of macroinvertebrate taxa (S), total number of individuals (N), diversity index (H´), Eveness (J´) and Simpson Index among the areas Protected area (I) and urban area (II) in Estero Salado Estuary at November 2009. Each box encloses 50% of the data with the median value of the variable displayed as a line. Top (upper quartile) and bottom quartile) of the box mark the limits of ±25% of the variable population. Lines extending from the top and bottom of each box mark the minimum and maximum values that fall within acceptable range. Outliers are displayed as individual point.

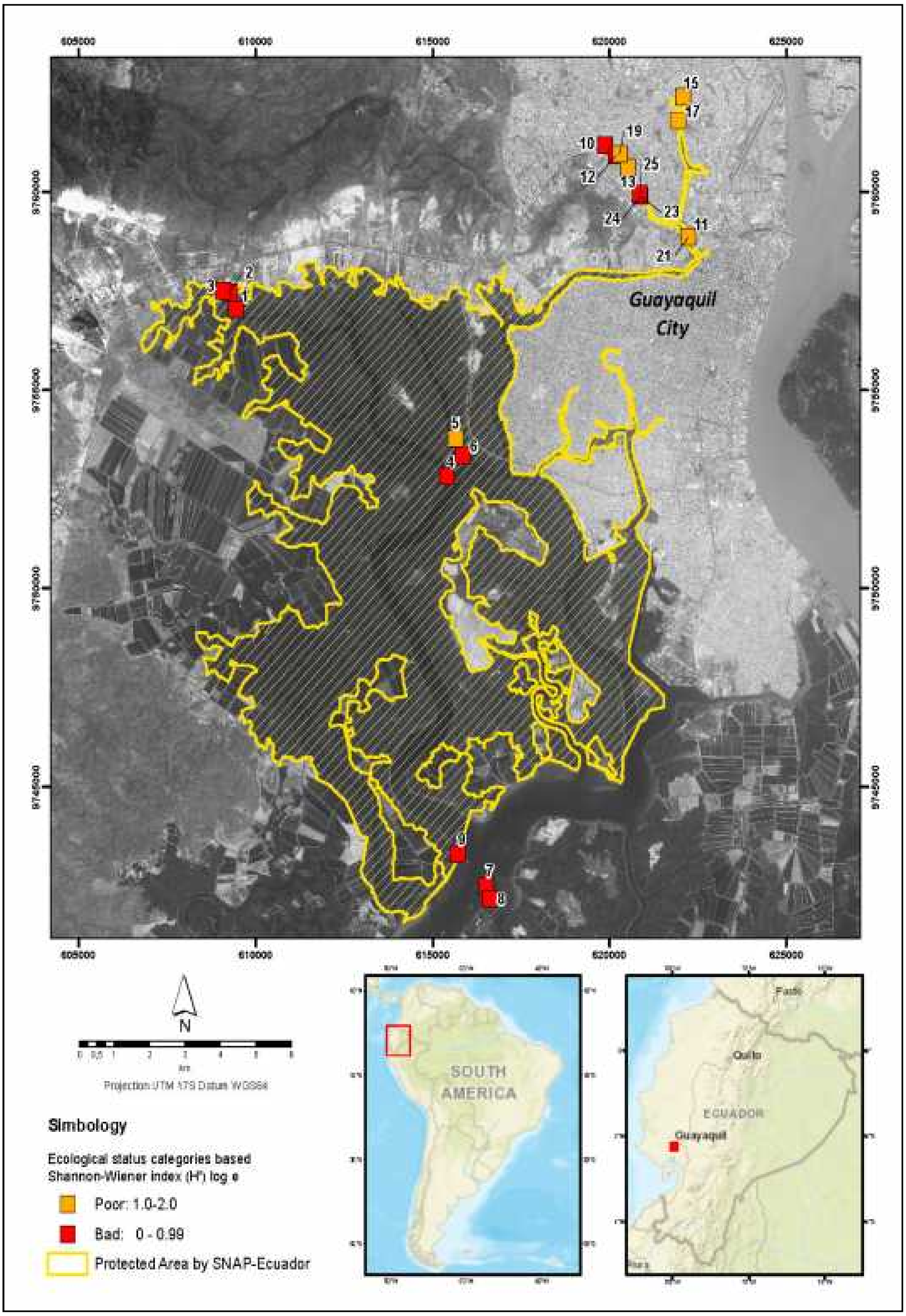


Figure 51. Map of diversity of benthic macroinvertebrates registered in 2009 and 2011 in the inner branches in the Estero Salado.

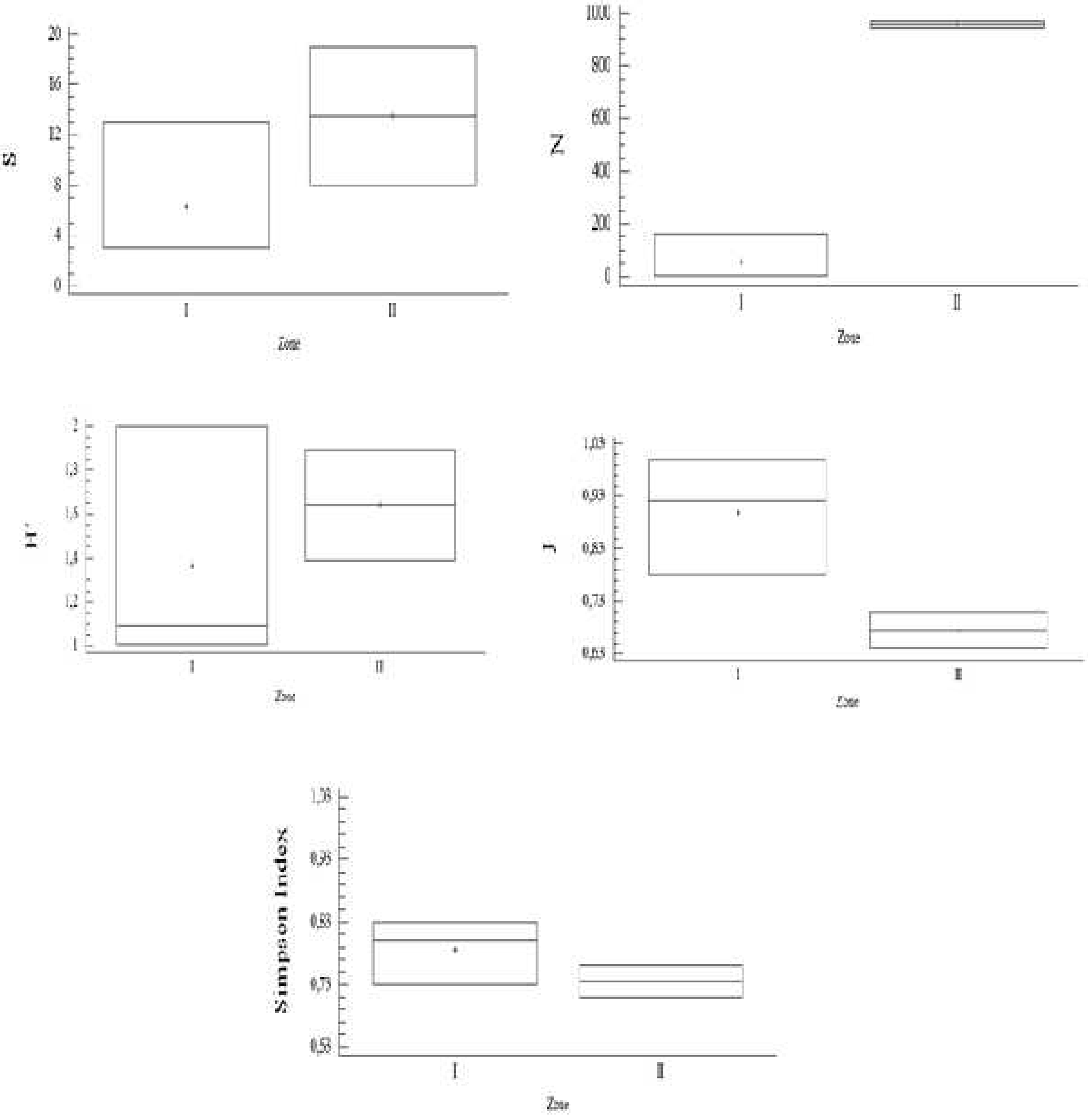


Figure 52. Box and Whiskers for number of macroinvertebrate taxa (S), total number of individuals (N), diversity index (H´), Eveness (J´) and Simpson Index among the zones Residence (I) and Industrial (II) at the urban area in Estero Salado Estuary at January of 2011. Each box encloses 50% of the data with the median value of the variable displayed as a line. Top (upper quartile) and bottom quartile) of the box mark the limits of ±25% of the variable population. Lines extending from the top and bottom of each box mark the minimum and maximum values that fall within acceptable range. Outliers are displayed as individual point.

5.5.2 Phase of bioremediation of hydrocarbon contaminated sediment 2011-2012

a) Environmental variables

Contaminants

Total Petroleum Hydrocarbon

The Total Petroleum hydrocarbon concentrations in the sediments showed a range between 227 to 994 mg/kg (Annex 16).The highest values were registered in station 1 in the fourth period of bioremediation (Time 4) with 994 mg/kg of TPH and the station 2 in the second period of bioremediation (Time 2) with 979 mg/kg. The lowest value was registered in the station 3 in the last phase of bioremediation (Time 4) with 227 mg/kg. The station 1 registered a TPH increase during the three time period of study, reaching the maximum value during Time 4 (Figure 53).

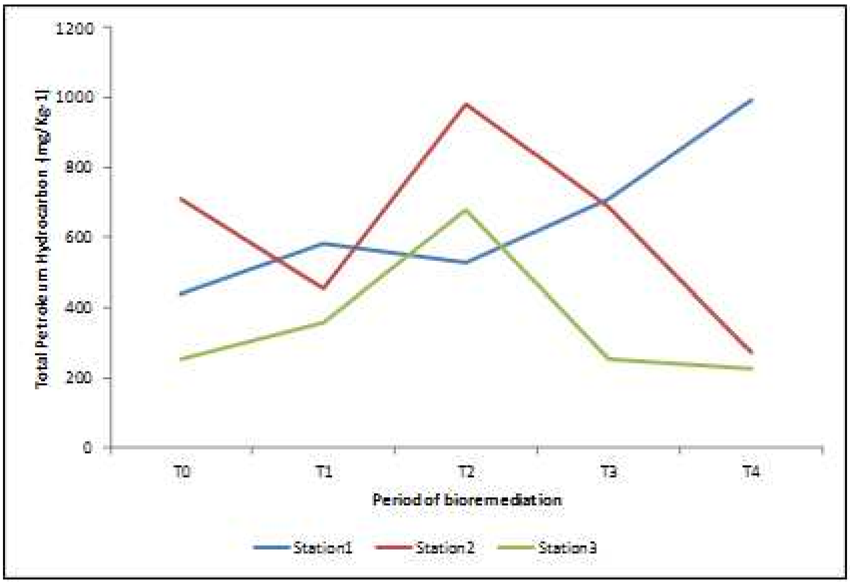


Figure 53. Temporal variation of the sediment TPH concentrations at characterization time (To) and bioremediation time (T1-T4), from December 2011 to March 2012, at Aventura Plaza – Estero Salado inner estuary.

Heavy metals

The concentration of total mercury fluctuated between 0.1 and 0.34 mg/Kg. Stations 2 and 3 were registered the highest concentrations during the period two and three of bioremediation. Station three registered the highest value during the time 2 during the bioremediation period (Figure 54; Annex 16). The mercury value declined in all stations during the last time period (Time 4), especially in the station 1 where no bioremediation techniques were applied.

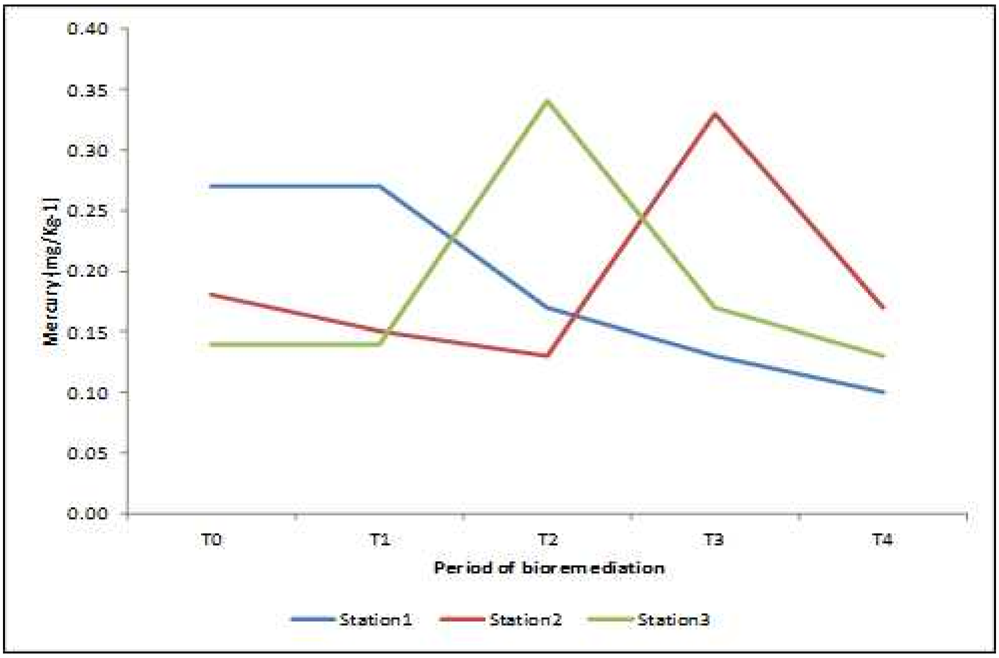


Figure 54. Temporal variation of the sediment total mercury concentrations at characterization time (To) and bioremediation time (T1-T4), from December 2011 to March 2012, at Aventura Plaza – Estero Salado inner estuary

Cadmium concentrations were between 0.10 to 0.72 mg/Kg and lead concentrations were between 12.02 to 53.9mg/Kg (Figure 55). Cadmium reached the highest concentration in the station 2 during the time 3 of bioremediation period with 0.72mg/kg. While the lowest concentration was recorded in the station 3 during the time 3 and 4 of the bioremediation. The highest concentration of lead was 53.9 mg/kg in the station 2 during the Time 1 and the lowest concentration was recorded in station 3 in last period of bioremediation (Time 4).

All heavy metals were inside of maximum level permitted by Ecuadorian environmental legislation for sediments after of bioremediation process more details are showing in the Annex 16. Except hydrocarbons where most of the measurements exceeded the maximum limits allowed by the environmental quality standard of the soil resource and remediation criteria for contaminated from the Annex 2 of Book VI of TULSMA.

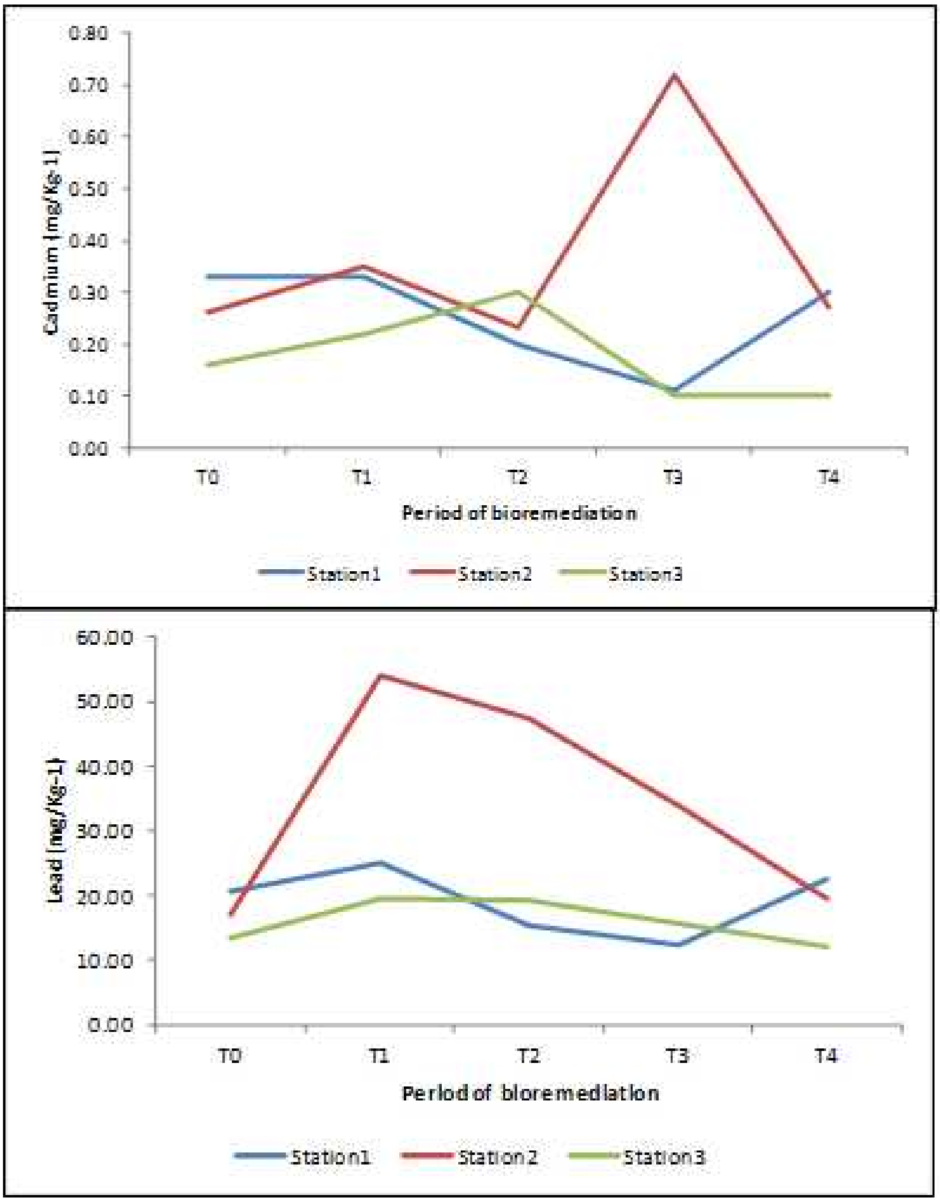


Figure 55. Temporal variation of the sediment cadmium and lead concentrations at characterization time (To) and bioremediation time (T1-T4), from December 2011 to March 2012, at Aventura Plaza – Estero Salado inner estuary.

The differences between TPH, Hg, Cd and Pb concentration during different time of bioremedition are showed in Figure 56. There weren´t found significant differences with any of these variables.

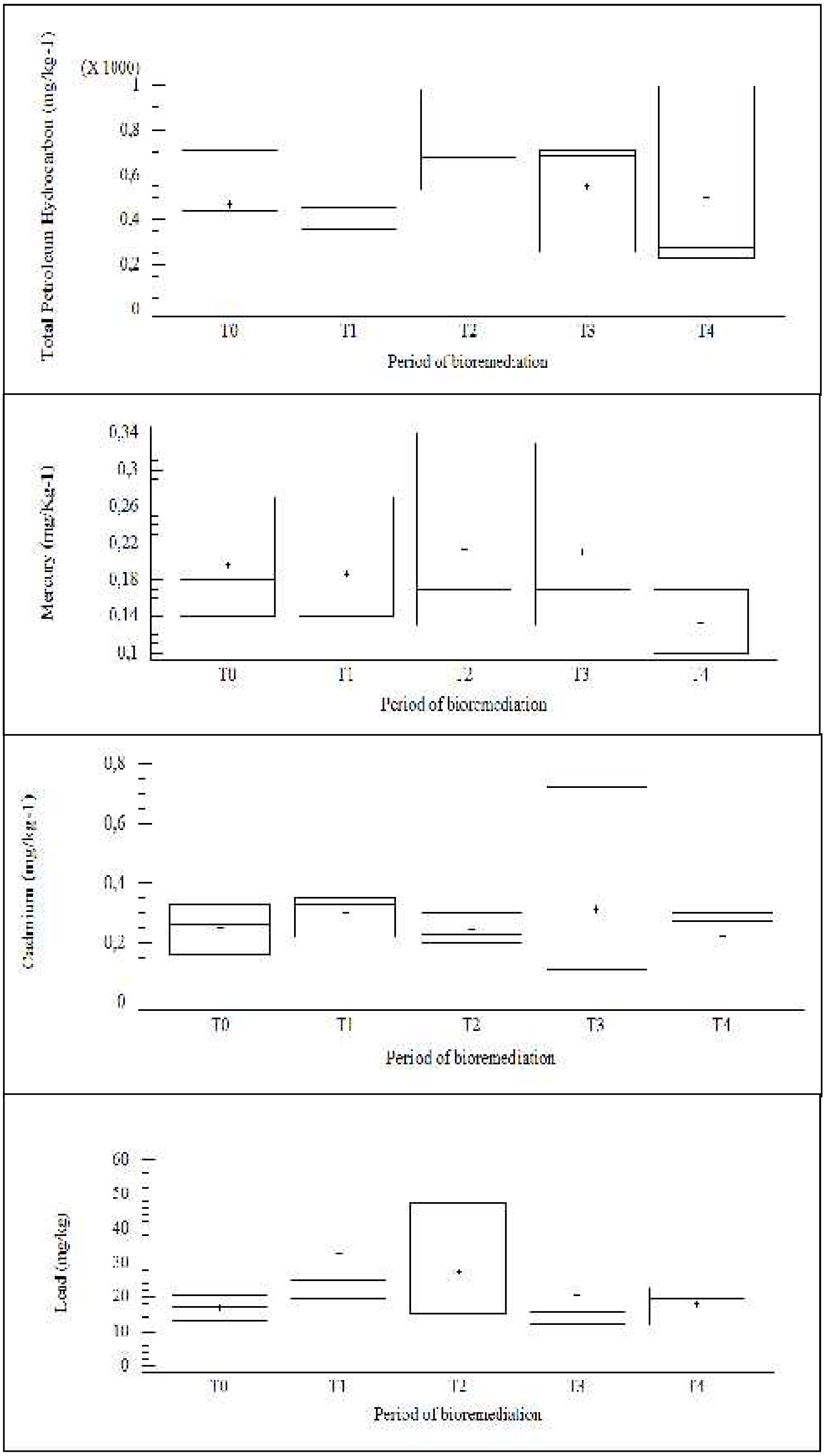


Figure 56. Medians for Total Petroleum Hydrocarbon; Mercury; Cadmium; and Lead in sediments at characterization time (To) and bioremediation time (T1-T4), from December 2011 to March 2012, at Aventura Plaza – Estero Salado inner estuary.

Nutrients

The Nitrate concentrations fluctuated between 0.5 to 30 mg/Kg. The highest concentrations were registered in the station 3 during the second months of bioremediation. However, this trend was same in this station in the third month but decreased in the last month. The lower value (<1mg/kg) were registered during the characterization and the first month of bioremediation. The Nitrite concentrations registered low values). The highest concentration was registered in the station 1 during the second months of bioremediation. The lowest value were observed before and the first month of bioremediation (Annex 17).

The phosphate concentration obtained presented high values that ranging from 8.3 to 151.23 mg/Kg. The highest concentration were registered in station three during the characterization phase the same trend of high values were observed in the others stations in this phase. Lowest values were registered in the first period of bioremediation in stations 2 and 3.

Other chemical parameters of sediments and water

The concentrations of H_2S, pH, organic matter and water are shows in the Annex 17. In generall the hydrogen sulfide concentration in sediments were cero, the pH were high with clear trend to the alkalinity along during all phases of study, with a mean 8.54. The highest value (9.44-9.29) was registered in the station 2 and 3 during the characterization phase. All stations during bioremediation phase the sediments registered pH exceeding the maximum values according to Ecuadorian environmental standard (Annex 17). The organic matter percent was alterative between stations with a mean of 6.66. The high percentage was registered in the station 2 during the first month of bioremediation period the same trend were observed in the others stations. The lowest value was registered in the station 3 during the characterization period.

The level of dissolved oxygen in general were lower with mean of 1.91 mg/L, the highest concentrations were registered in the stations 2 and 3 during the second period of bioremediation, while the lowest concentrations were found in stations all stations during the second and four period of bioremediation (Figure 57).

At characterization (To) and the first time of bioremediation period (T1) 5, significant differences between period of study were detected :Nitrate (H=12.1216; P-Value: 0.016); Nitrite (H=10.6716; P-Value: 0.030) and Phosphate (H=12.4; P-Value: 0.01) (Annex 17). No significant differences was detected in hydrogen sulphide (H=4.0; P-Value: 0.5) (Annex 18).

At the first time of bioremediation period (T1) there was a significant difference between the period of study were detected: pH (H=11.6; P-Value: 0.02) but no significant differences was detected in organic matter (H=6.9; P-Value: 0.14) (Annex 19).

There were significant differences between the period of bioremediation (H=13; P-Value: 0.01), the concentrations of oxygen dissolved were low during almost all time of study, showed hypoxia condition , only in the time T1 the level of oxygen increased to 4 mg/L due the change the tidal to pleamar and other periods were registered during low tide (Figure 57).

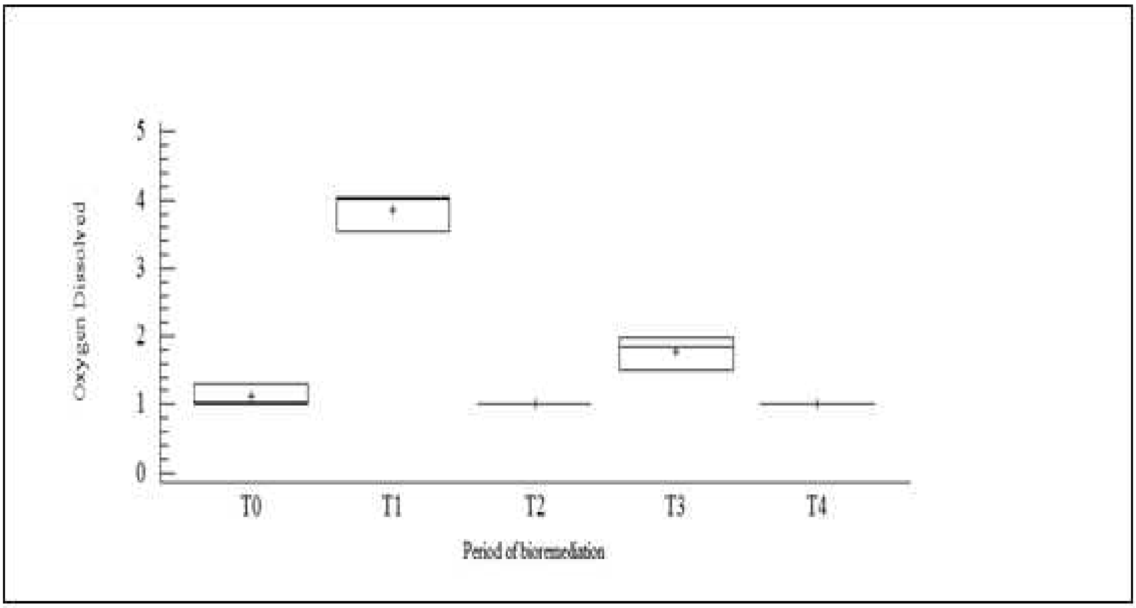


Figure 57. Medians for Oxygen dissolved in superficial water at characterization time (To) a bioremediation time (T1-T4), from December 2011 to March 2012, at Aventura Plaza – Estero Salado inner estuary.

b) Biological variables

Overall benthic macroinvertebrates fauna in 2009 and 2011

The most abundant taxa were Oligochaeta with 5386 individuals, Gastropods (1154), Insects (206) and the less abundant (less 25 individuals) were Bivalvia, Polychaeta, Amphipoda, Isopoda, Coleoptera, Nematode, Nemertina and Arachnida (Figure 58. Annex 20).

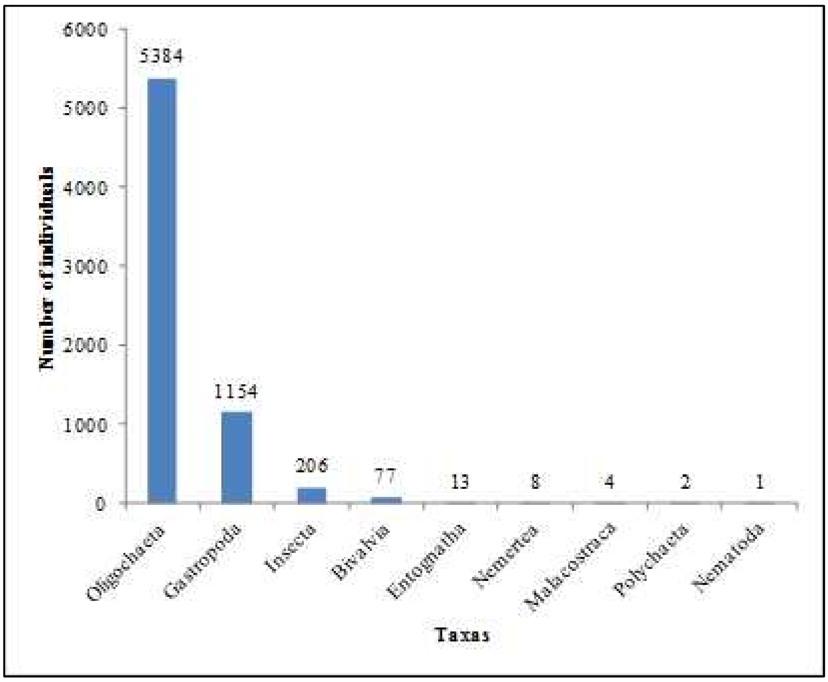


Figure 58. Abundance the main macroinvertebrates taxa registered in superficial mangrove sediments in Aventura Plaza in the inner branches of Estero Salado from December 2011 to March of 2012.

The Annelida were represented almost interily by oligochaeta (99.9%) and by the family Nereidae (0.1 %) represented by *Nereis* sp., (Annex 20).

The mollusk were represented mainly by gastropods with the family Thiaridae (89%) represented by *Melanoides cf tuberculata*. The family Hidrobiidae (9%), Ellobiidae (2%) with the specie *Marinula acuta, Marinula* sp. *Heleobia* sp. (Annex 20). The Bivalve were poorly represented for the families Cyrenoididae with the specie *Cyrenoida inflata* (92%) and the family Mytilidae (8%) with the specie *Mytella guyanensis*.

The Arthropoda were represented mainly by insects such as Psychodidae (72%) represented by *Pericoma* sp. and Dolichopodidae (14%) and the Etnognatha represented by the family Poduridae (7%). Others families of Diptera were found as Chironomidae represented by species *Ablabesmyia* sp., *Chironomus* sp., the family Ceratopogonidae with the specie *Probezzia* sp., and the Coleoptera with the family Staphylinidae. Other arthropods as isopods with the family Tylidae (2%), Palitridae, Staphylinidae with the 1% (Annex 20).

The Entognatha was represented by the family Poduridae with the specie *Podura aquatica* and other taxa lower abundant as Nemertine and Nematode (Annex 20). Most abundant taxa in characterization and bioremediation phases show in Figure 59.

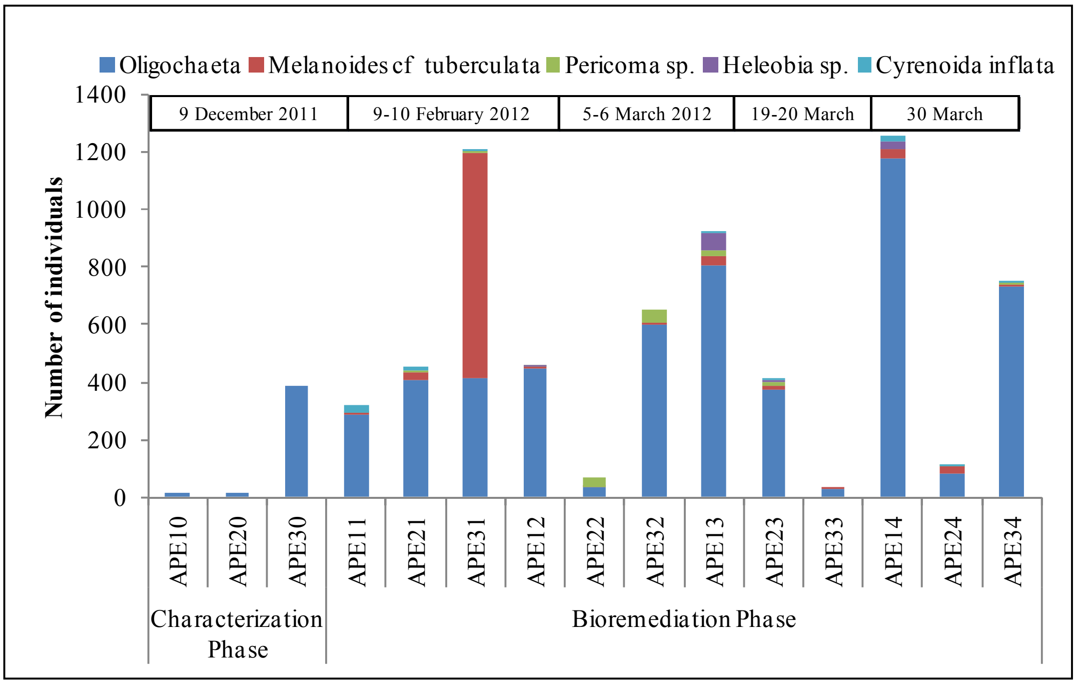


Figure 59. Number of individuals of the most abundance species in sediments water during Characterization and Bioremediation phase in Aventura Plaza in the inner branches of Estero Salado from December 2011 to March of 2012.

Composition of benthic macroinvertebrates assemblages

The MDS ordinations, the samples for each station during characterization and different period of bioremediation formed distinct groups, regardless of belonging to a station and only two tight group were plotted, the station 1 and 2 during the characterization time (APE1- APE2) and the station 1 during the bioremediation time 3 and 4 (APE13-APE14) all them were sites where didn´t use bioremediation consortium. However the groups were more defined in the different time period of bioremediation, formed distinct groups in the plot (Figure 60).

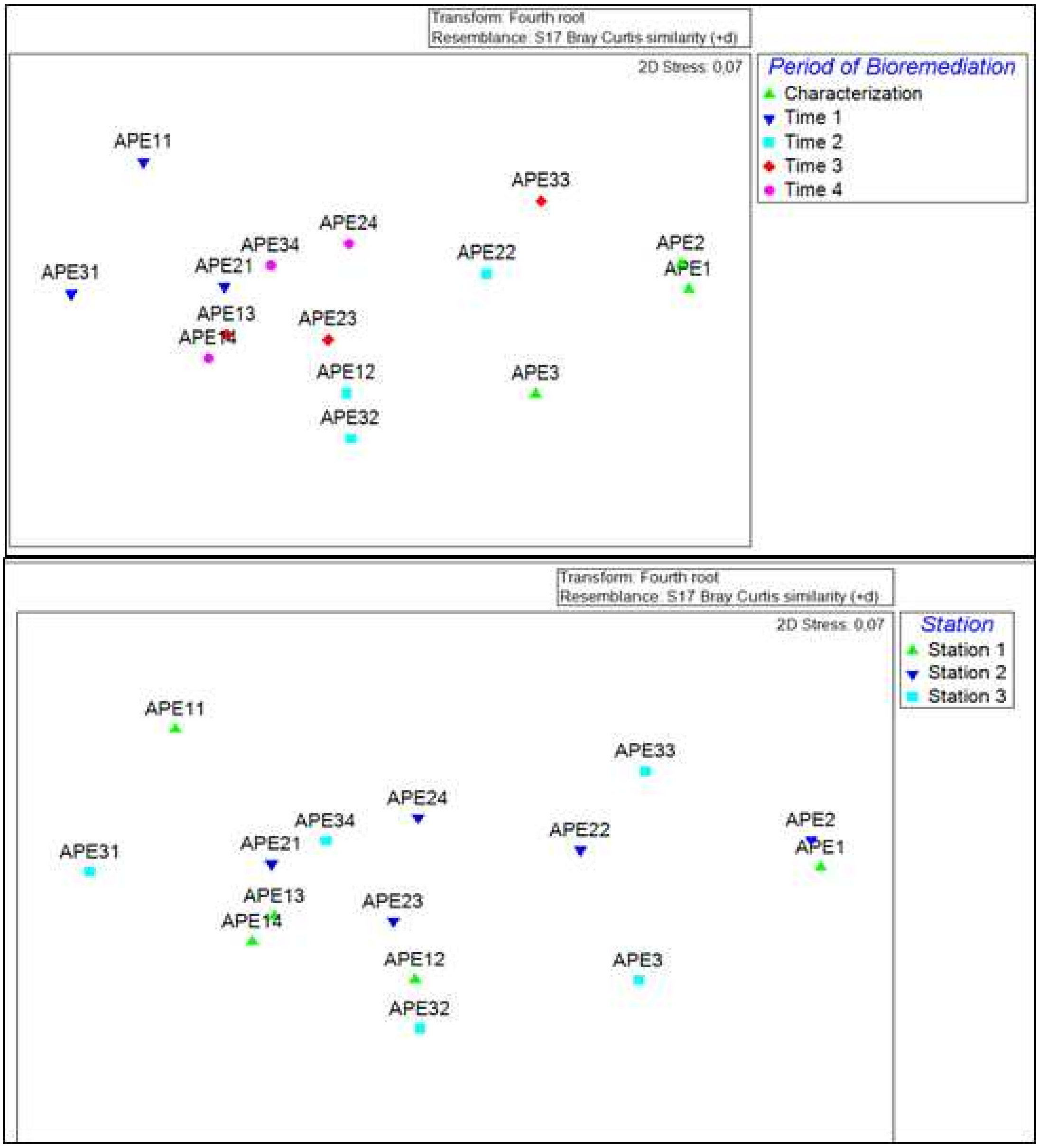


Figure 60. Non-metric multidimensional scaling ordination, derived from the Bray Curtis similarity matrix constructed using fourth root transformed the abundances of genus of benthic macroinvertebrates in superficial mangrove sediments in Aventura Plaza in the inner branches of Estero Salado from December 2011 to March of 2012.

The Figure 61 shows that all stations were similar in a 50%, two big groups were formed. One group formed mainly for the three stations (AP1-AP2-AP3) that showed similar composition of macrofauna give by oligochaeta but the with the largest increase in abundance in Station 3 (AP3), all them evaluated previous to bioremediation time. In this group too was formed by the station 2 and 3 during the time 2 and 3 of bioremediation and that were in common species as Oligochaeta, *Melanoides cf tuberculata, Pericoma* sp., and pupas of insects. The second group was formed by three subgroups where the station more similar were along the time of bioremediation was station 1 and the stations more different were the station 3 and station 1 during the first bioremediation phase (Time 1).

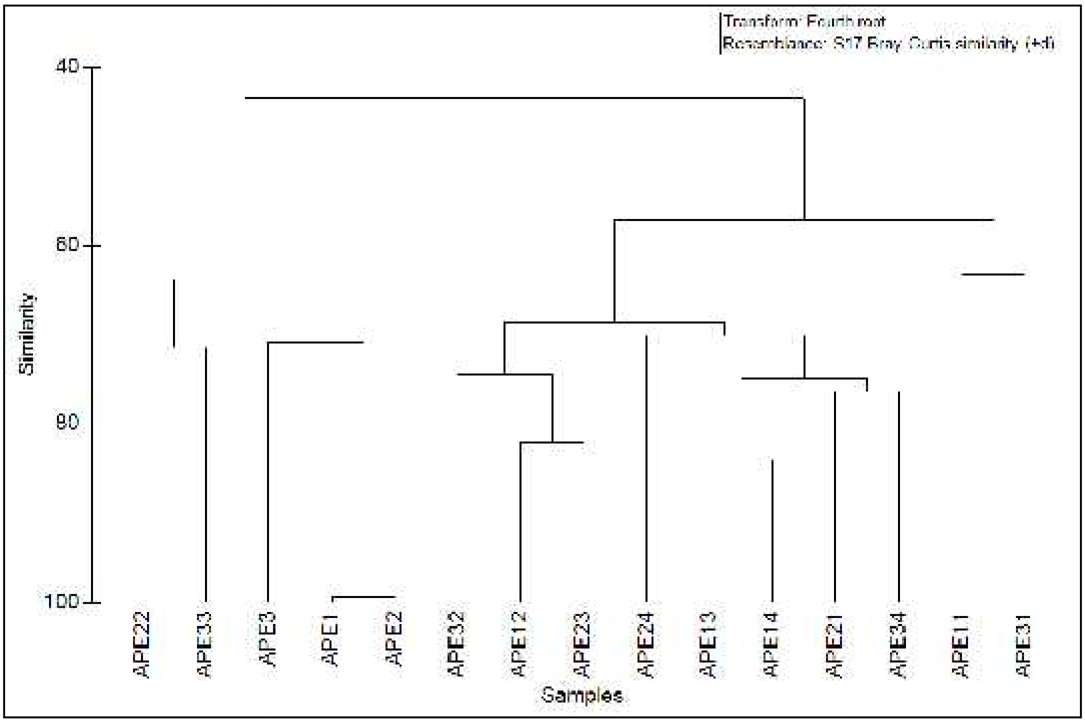


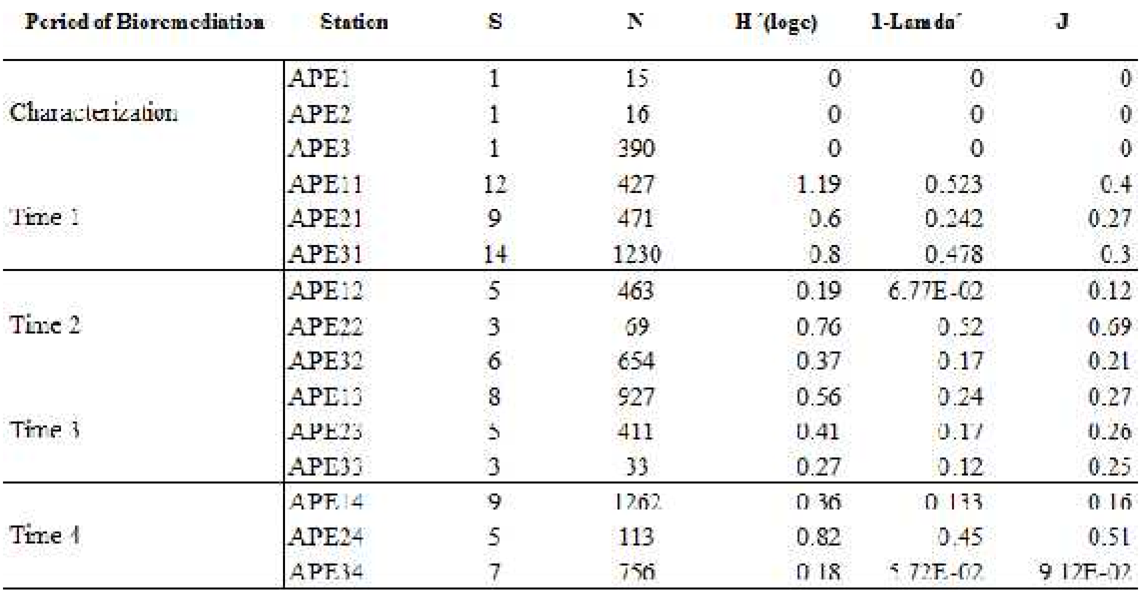
Figure 61. Dendrogram for hierarchical clustering from each of study sites, based on the Bray- Curtis similarity matrix constructed using fourth root transformed total abundances of the species of benthic macroinvertebrates in superficial mangrove sediments in Aventura Plaza in the inner branches of Estero Salado from December 2011 to March of 2012.

Species richness and diversity

The median of diversity according to Shannon-Wiener Index varied between 0 to 1.19 with a median of 0.37, the high diversity was registered in station 1 during the first time of bioremediation, Simpson index was the same trend (Table 22). This diversity was generated mainly by insects and gastropods (Annex 20). The eveness mean was 0.25 with dominance of species of Oligochaeta, *Melanoides cf tuberculata* and *Pericoma* sp. The abundance was greater in Time 4 in comparison with other period of time (Figure 62).

Significant differences were detected between the richness of specie (S) and Shannon-Wiener Index $p<0.05$ among times of bioremediation of hydrocarbon (Annex 21). On the contrary, no significant differences were found for abundance, eveness and Simpson index. The median of richness was greater in Time 2 of bioremediation phase. The median diminish in the other time of bioremediation. However the Richness was higher in all phases of bioremediation than characterization time (Figure 62).

Table 22. Diversity Indexes of benthic macroinvertebrates: Species Richness (S); Total number of individuals (N); Shannon Wiener (H´); Simpson (1-Lamda´) and Equitability (J´) in superficial mangrove sediments in Aventura Plaza in the inner branches of Estero Salado from December 2011 to March of 2012.



Period of Bioremediation	Station	S	N	H´(loge)	1-Lamda´	J
Characterization	APE1	1	15	0	0	0
	APE2	1	16	0	0	0
	APE3	1	390	0	0	0
Time 1	APE11	12	427	1.19	0.523	0.4
	APE21	9	471	0.6	0.242	0.27
	APE31	14	1230	0.8	0.478	0.3
Time 2	APE12	5	463	0.19	6.77E-02	0.12
	APE22	3	69	0.76	0.52	0.69
	APE32	6	654	0.37	0.17	0.21
Time 3	APE13	8	927	0.56	0.24	0.27
	APE23	5	411	0.41	0.17	0.26
	APE33	3	33	0.27	0.12	0.25
Time 4	APE14	9	1262	0.36	0.133	0.16
	APE24	5	113	0.82	0.45	0.51
	APE34	7	756	0.18	5.72E-02	9.12E-02

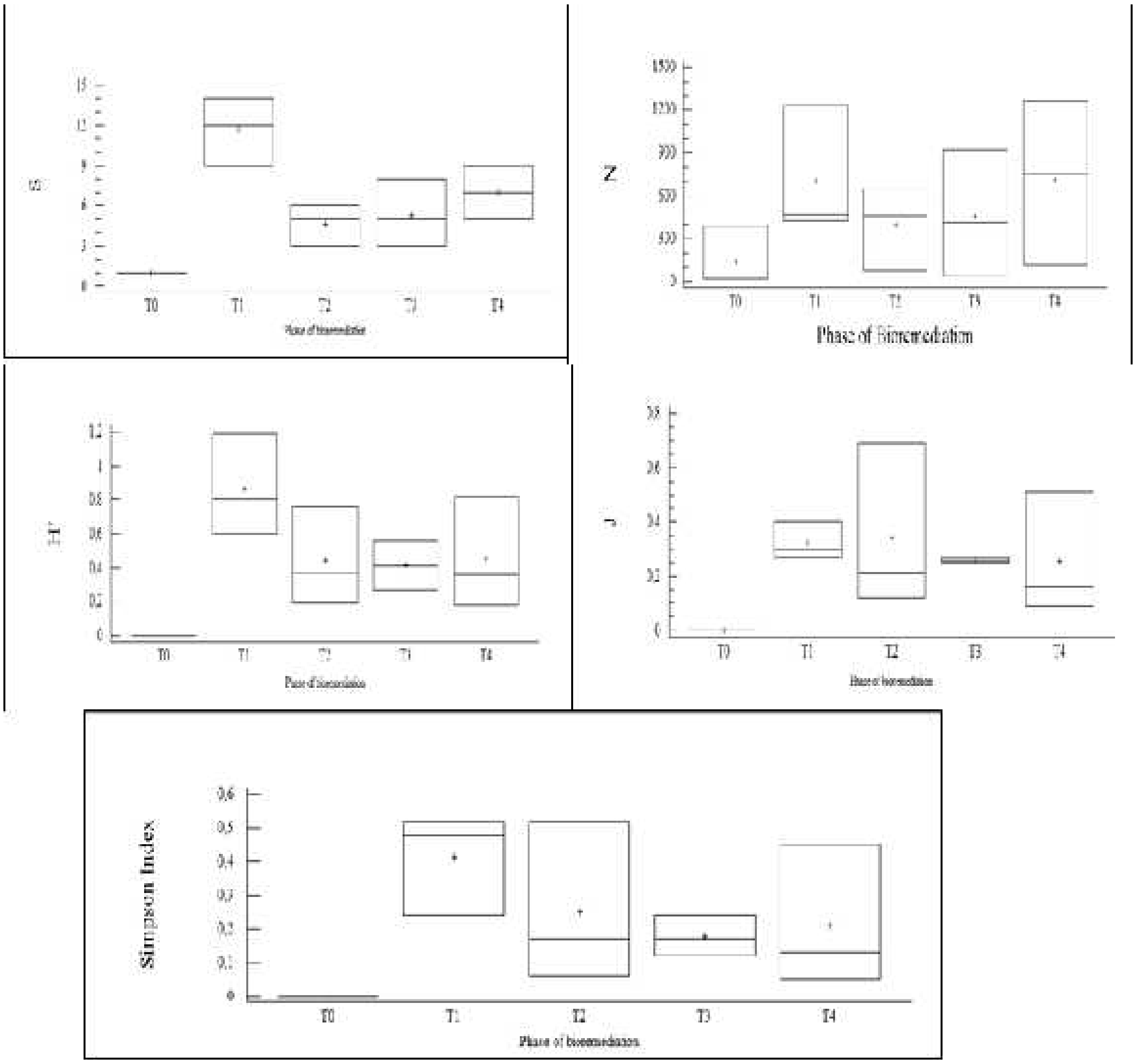


Figure 62. Box and Whiskers for number of macroinvertebrate taxa (S), total number of individuals (N), diversity index (H´), Eveness (J´) and Simpson Index among in superficial mangrove sediments in Aventura Plaza in the inner branches of Estero Salado from December 2011 to March of 2012. Each box encloses 50% of the data with the median value of the variable displayed as a line. Top (upper quartile) and bottom quartile) of the box mark the limits of ±25% of the variable population. Lines extending from the top and bottom of each box mark the minimum and maximum values that fall within acceptable range. Outliers are displayed as individual point.

The SIMPER showed that composition of the benthic macroinvertebrate was similar between different time of bioremediation of hydrocarbon in sediments mainly for the presence of Oligochaeta, *Melanoides cf tuberculata*, *Pericoma* sp. and others larva of aquatic insects (Annex 18). However, the average of dissimilarity of abundance of macroinvertebrates changed between period of bioremediation. Thereby, the average of dissimilarity of abundance during the Characterization time (Time 0) and the Time 4 of bioremediation phase was 66.56%. In the Time 0 was lower abundant and diverse in relation to the others period of bioremediation, due the presence of oligochaeta (Figure 63: Annex 22).

The overall composition of the benthic macroinvertebrate fauna during the Time 1 was distinguished from that Time 2 by greater abundance of oligochaeta, the gastropod *Melanoide tuberculata* and the diptera *Pericoma* sp., *Probezzia* sp., Larvae of Dolichopodidae, *Podura aquatica, Cyrenoida inflata, Marinula acuta, Mytella guyanensis* and Nemertine. In contrast, *Pericoma* sp. and *Heleobia* sp., were more abundant in the Time 2. The Time 1 was distinguished from Time 3 for the greater abundance of particularly Oligochaeta (Figure 63), *Melanoides cf tuberculata* and gastropods sp.1, while the Time 3 the species more abundant were Oligochaeta, *Heleobia* sp and *Pericoma* sp. The Time 1 was distinguished from Time 4 for the greater abundance mainly of *Melanoides cf tuberculata* (Figure 64) and gastropods sp 1.While, in the time 4 Oligochaeta and *Heleobia* sp. were more abundant than Time 1 (Annex 20). The Time 2 was more abundant that Time 3 and Time 4 only in abundant of *Pericoma* sp. In contrast Time 3 and 4 the greatest abundance was generated by Oligochaetas. Finally the Time 3 in comparison with Time 4 was distinguished for greater abundance of *Heleobia* sp. and *Pericoma* sp. In contrast, the Time 4 the greater abundance was given by Oligochaeta, *Melanoides cf tuberculata* and gastropods sp.1 (Annex 20).

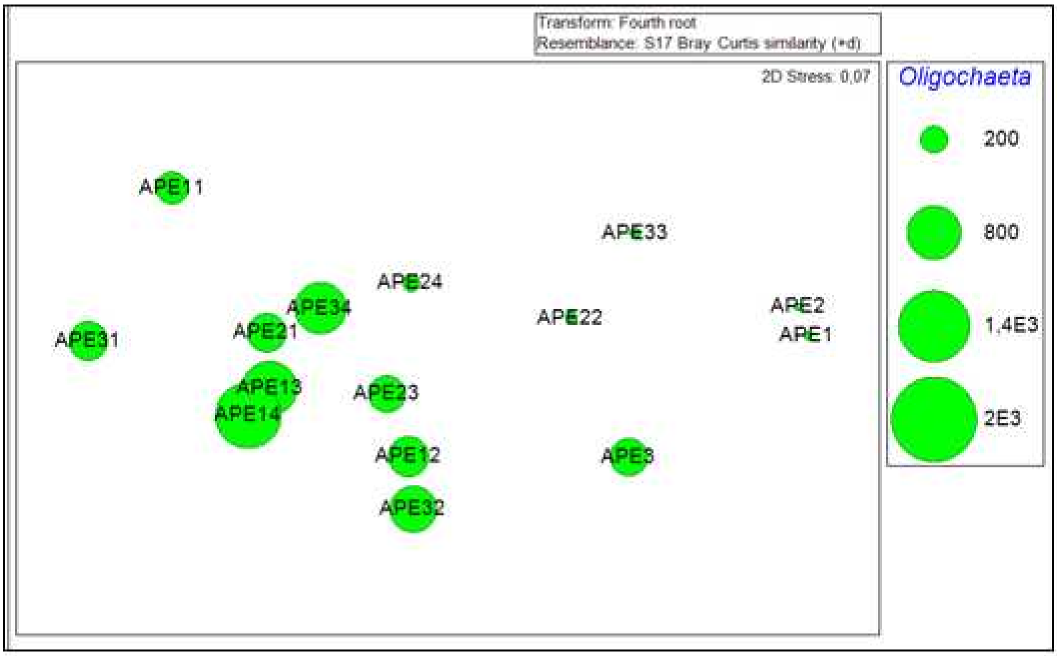


Figure 63. 2D bubble MDS configuration, derived from Bray-Curtis similarity matrix constructed using fourth root transformed of total abundances the oligochaeta in Aventura Plaza sediments in the inner branches of Estero Salado from December 2011 to March of 2012.

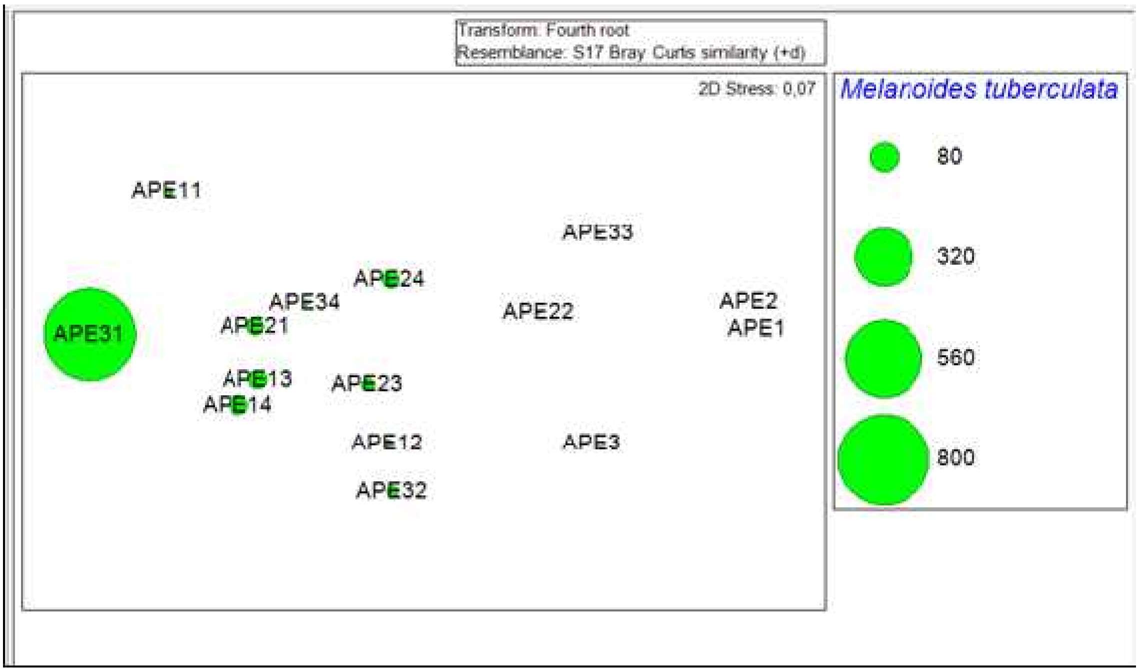


Figure 64. 2D bubble MDS configuration, derived from Bray-Curtis similarity matrix constructed using fourth root transformed of total abundances the Melanoides cf tuberculata in Aventura Plaza sediments in the inner branches of Estero Salado from December 2011 to March of 2012.

The branch of the Estero Salado adjacent to Aventura Plaza shopping mall, has an adjoining the estuary characterized by the presence of mangroves, legumes (Caesalpinacea), palms, followed by a submerged area where the substrate is a mixture of mud and rocks mainly (subzone margin a), as is advanced into the estuary a mixed mud, rocks and logs smaller (sub B) and in the middle of the estuary a predominantly muddy substrate (sub C) was found area was observed (Figure 65).

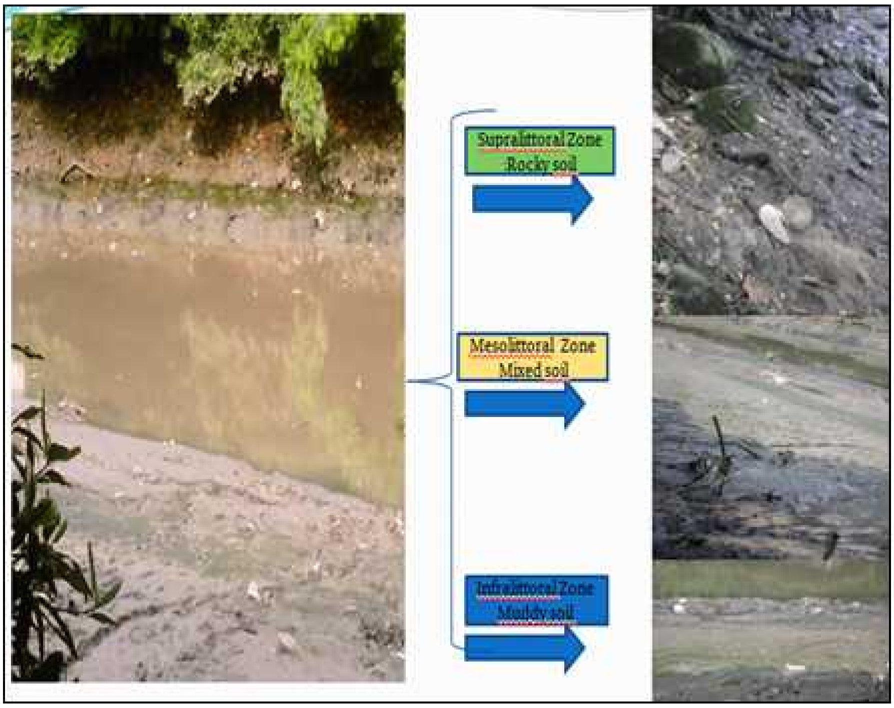


Figure 65. Biotic zonation of intertidal estuarine sediments in the Aventura Plaza –Estero Salado in 2012.

c) Influence of sediments quality and environment on macroinvertebrates communities during bioremediation phase

PCA results with the sixteen variables measured for sediments showed that there are three main components that explain 66% of the distribution of stations, environmental and ecological variables in different period of bioremediation (Figure 66). The first Principal Component (PC1) has a variance (eigen value) of 5.83 and accounts for 36% of the total variance (contains 36% of the components necessary for the distribution de stations. The second component (PC2) has a variance of 2.54 and contains 16% of the other variables (cumulative value =0.52).The third component (PC3) has a variance of 2.25 and contains 14% of the other variables (cumulative value =0.66) (Annex 24).

Principal component axis 1 (PC1) appeared to represent the influence of pH (pHs), phosphate (PO4) with negative loadings related to richness of specie. Along the PC2 the stations spread according to the nitrate (NO_3), nitrite (NO_2) (positive loadings), dissolved oxygen (O_2) and abundance of invertebrates (N) (negative loadings).The third component (PC3) are influenced by cadmium (Cd), Lead (Campbell & Hewitt) (positive loadings) and (NO_2) (negative loadings). Based on this analysis, we established that variables more influence have in the community structure are Cd, NO_2, NO_3, Pb and O_2 (Annex 24).

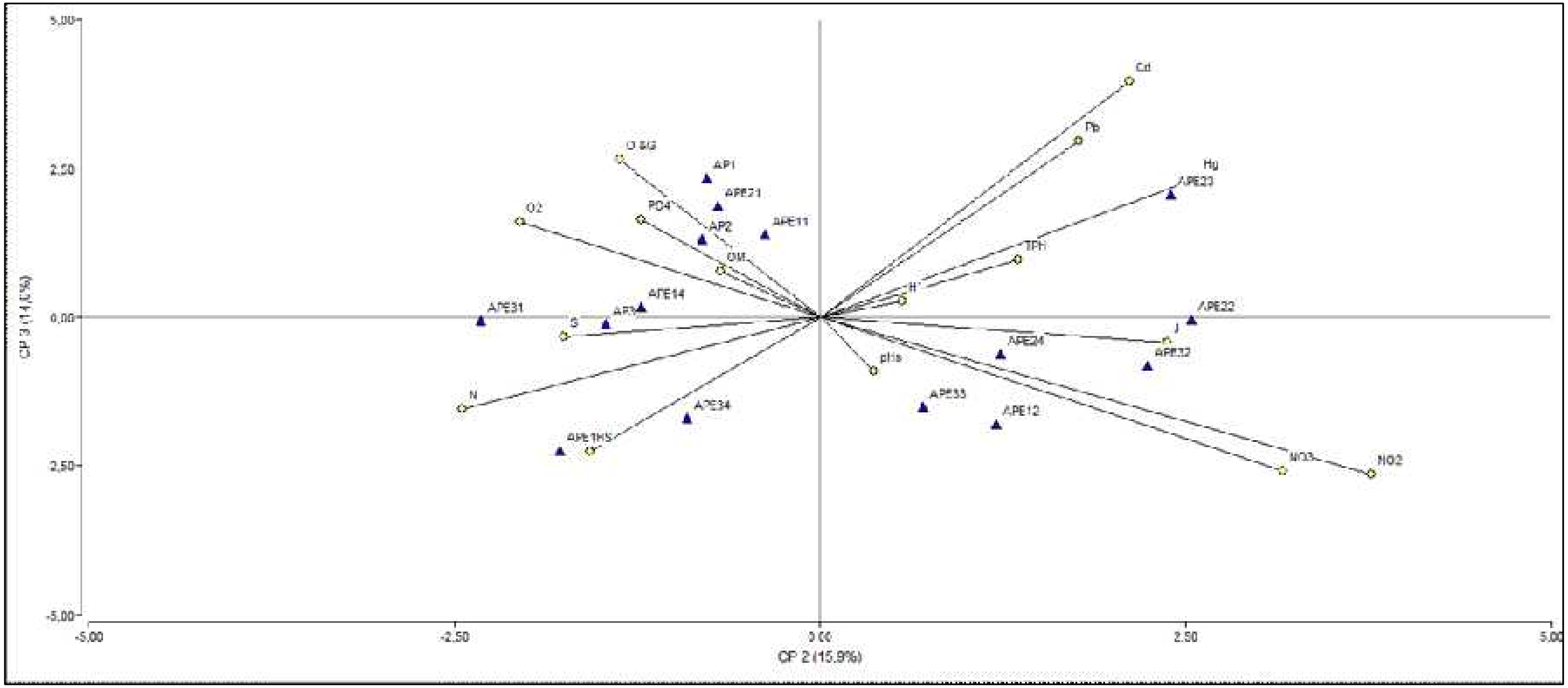


Figure 66. PCA for the environmental and ecological variables. Results for the first two components that explain 52% of the variability for the distribution of stations. The eigen value for PC1 (5.83) contains 36% of the components necessary for the separation of sites surveyed. The eigen value for PC2 (2.54) contains 16% of the other variables (cumulative value = 0.52). See Annex x for complete results of PCA for the community structure and environmental variables - observed in sediments in Aventura Plaza in the inner branches of Estero Salado from December 2011 to March of 2012.

5.6 Discussion and Conclusions

A spatial gradient was observed in concentrations of total petroleum hydrocarbons (TPH) in sediments of the inner branches of Estero Salado, both the RPFMS (protected area) and urban areas. The highest concentrations of TPH were in the urban area in residence zone belonging to section A (Parque Ecológico de Urdesa, Bosque Protector Salado Norte) and industrial area in section B (Miraflores and Aventura Plaza).

The high concentrations of TPH show the influence of industrial wastewater in these sections A and B of Estero Salado which are not only receiving bodies of domestic wastewater from the Juan Tan Camarengo and Mapasingue-Prosperina areas but also of the industrial zone, confirming that these areas are the sources of industrial pollutants as detected before in 2000 (Lahmeyer-Cimentaciones, 2000). The main industrial activities in these areas produce food, beverages, tobacco, textiles, paper, cardboard and generate significant organic load, suspended solids, oil and grease to the receiving waterbody (Lahmeyer-Cimentaciones, 2000). Significantly lower concentration of TPH were registered in these sections A, B and C in June of 2012 with values <0.04mg/Kg, below minimum detectable, however phenanthrene was detected with a concentration of 2.76 mg / kg, in the section B (INOCAR, 2012).

In the protected area the concentration of TPH were lower than urban areas, the highest concentrations were recorded in the Terminal Portuario Internacional and Terminal de Transferencia Tres Bocas; both sites characterized by active route of navegation where nearby areas (Autoridad Portuaria) have already had oil spills from bilge water, fuel oil, diesel, bunker, sludge, especially in the years 1999, 2000 and 2004. These are generated by berthing of vessels, bilge, loading and unloading fuel, from which the two main sources of pollution to the estuarine water were bilge water and fuel oil produced by maneuvering load and transfer of this fuel (Rodríguez, 2005). So too the data observed in the Tres Bocas Station in June 2007 showed a concentration of 1545mg / kg of TPH and similar values were recorded in December the same year with a concentration of 1094 mg / kg (Universidad de Guayaquil, 2008).

The results of concentrations of Total Petroleum Hydrocarbon indicated that there was spatial variability at the level of area and sections of inner branches of ES with temporary changes in the concentrations with occasional severe shocks to the water body as recorded in Miraflores in 2007 and other important concentrations exceeding the maximum levels permitted by Ecuadorian environmental standard laws, as noted in field disturbance observed in the intense black color of bodywater, the viscocity,

density and physical change of estuarine waters mainly in Miraflores. These may be suspected as clandestine discharges of fuel and burned oils that are deposited directly in this area. However, there are high TPH concentrations (above environmental law limits) in the section A so it is clear that the source is of industrial origin. It would seem that it is most logical to monitor effluents in areas where there are repair shops of machinery such as: cars, trucks, electric generators, tractors and road equipment, agricultural vehicles, power stations, lubricants and other companies using processes involving hydrocarbons.
The highest concentrations of mercury and lead were registered in urban areas as opposed to protected areas. Each branch was affected with one or other heavy metals in particular, thereby high mercury concentrations are associated with section B in Aventura Plaza station, while in protected area the highest concentration was registered in south of RPFMS at the Cuarentena zone. In general the values were less than 0.9 mg/Kg, the concentration recorded in the central and deepest part of the navigation channel and values from 0.3 to 0.1 mg/Kg on the flanks of the zone. The low concentrations could were related with areas of dredging carried out in this area by the Autoridad Portuaria in previous years.

Cadmium and lead registered high concentration in 2011 in the section A of inner branches of Estero Salado (Bosque El Salado) receives wastewater from the industrial zone Mapasingue-Prosperina (Lahmeyer-Cimentaciones, 2000). In this zone the Ministry of Environment identified 190 companies that violate environmental management processes and that discharge wastewater into the Estero Salado and pollute it. Most of them are engaged in the production of plastic, paper, beverages, storage of agricultural produce, preparation of food products, pesticides, casting, car washing, car mechanic shops, manufacture of electrical conductors, printing, dyeing fabrics, associated electricals, storage of food, smelting, fertilizer, manufactured of plastics and textiles and industrialization of glass and other industries (MAE). The last eight of them related to the use of Cd and Pb at some stage of their processes (Shi, Lv, Cai, Liu, Wang, Feng, & Zhao, 2010).

There were significant differences between concentrations of total petroleum hydrocarbon, mercury, lead, among protected area (I) and urban area (II). The high values were found in urban area.

The nitrate is the main form of nitrogen in the inner branches of Estero Salado presented in generally low concentrations, the high concentration prior to the bioremediation were located in section A, decreased in section C, until almost nonexistent levels in the section B. For that reason during the bioremediation phase, nitrogen compounds were added to stimulate bacterial activity in sediment; hence the maximum values of nitrate were recorded in Aventura Plaza during the third and fourth months of experimentation. The nitrate concentrations in urban area were higher than protected area probably because of the major influence of discharge of domestic effluents as registered in residential zone Parque Ecológico de Urdesa but, despite this contribution, there does not seem to be evidence of eutrophication.

The Phosphate concentration was very low in the protected area and high in urban area in the section B and C (Aventura Plaza and University of Guayaquil respectively). The most sites registered major proportion the phosphorus that nitrogen. Oppositive relation in the proportion between N:P that observed in Bosque El Salado and University of Guayaquil one year before to our sampling in 2011 (INOCAR, 2012). So it is important to avoid the increase because their emissions must be controlled to avoid eutrophication.

The pH of sediments oscillated from 6.2 to 9.44. The pH was more acid in protected area where there area influence of the descomposition of organic matter typical of mangrove forest. While in urban area the sediments were more alkaline in special section A and B Aventura Plaza exceeding the maximum value to residence and industrial zone according the Ecuadorian environment law to soils remediated in the Parque Ecológico de Urdesa and Aventura Plaza. This data confirm the presence of industrial effluents in these area and changes in pH sediments registered before in 2002 by Lahmeyer-Cimentaciones that registered pH between 5.5 to 6.1. An increase in pH of neutral to alkaline can promote the growth of pathogenic strains.

The hydrogen sulphide concentrations were lower in general. Nevertheless, high concentrations were registered in Universidad de Guayaquil in 2011 and similar results were observed in 2012 near of this station by INOCAR and are relationeds with degradation of organic matter that is produced in this site. High concentrations of sulphur are normally associated with low levels of oxygen and with species more tolerant to hypoxia that can live in high concentrations of Sulphur, such as Diptera and gastropods.

This macroinvertebrate community structure confirms the alteration of Estero Salado estuary due the low diversity and change in the composition of biota where the dominant taxa are the oligotrophic environments in the urban area. While that in the protected area the composition is more of estuarine zones, more diverse in taxa due the influence of environmental variables as salinity, currents, tidal, sediments, more oxygenated waters, less presence of pollutings agents and the wide limits of tolerance of aquatic organism in special benthic macroinvertebrates. In the Reserva de Producción Faunística Manglares El Salado there are a taxacenosis characteristic of the estuarine area where the conditions and diversity of taxa allowed the presence of bryozoans, tunicates, sponges, bivalves, gastropods, crustaceans, polychaetes, nematodes.

In the RPMS, common species were *Cosmiconcha* sp., *Costoanachis nigricans*, *Cylinchna* sp., *Balanus* sp., *Micronereis sp.* While in the urban area were: larvae of Dolichopodidae and Ceratopogonidae, the species *Psychoda sp*., *Pericoma* sp., *Culicoides* sp., *Alluaudomyia sp*., *Stilobezzia sp., Melanoides cf tuberculata, Heleobia* sp*.,* and *Capitella cf capitata.* These are confirmed as bioindicators of aquatic polluted environments in tropical zones and can live there due physiological adaptations to anoxia such as *Chironomus* producing haemoglobin or changing movements (Stief, Nazarova, & Beer, 2005).

The Protected Area 'Reserva de Producción Faunística Manglares El Salado is an important habitat that allows the shelter, growth, reproduction and flow of larvae for dispersal to more contaminated areas, hence the importance of monitoring the status of their diversity and potential contaminants that may be affecting that area, so this site is a priority for the conservation of aquatic biodiversity of salt marsh and is a reference area for future environmental restoration projects at the level of species, communities, populations and the closest to the pristine system that had urban areas in the 1940s. It is suggested that it would be helpful to target the control effort at stations: Estación Tres Bocas, Terminal Portuario Internacional and the southern zone (Cuarentena and Autoridad Portuaria) for its environmental protection.

In relation to the techniques of bioaugmentation and biostimulation there was no significant difference in the degradation of hydrocarbons considering that the main factor that led to this was the lack of control variables in the natural medium as a continuous steady input of hydrocarbons and heavy metals was happening as evidenced in Aventura Plaza´s sediments. This and any other environmental restoration applied to contaminated sludge, will not be effective if stressful pressure do not stop first. Hence, it is recommended to try the Estero Salado with the bioremediation because it is a useful tool in the detoxification of soils contaminated with petroleum. However, is important consider no only microbial properties but also environmental factors such as: temperature, pH, salinity,nutrient and water availability, presence of terminal electron acceptors and osmotic stress and properties of the hydrocarbon such as: concentration, solubility, hydrophobicity,volatility and molecular mass (Gkorezis, Daghio, Franzetti, Van Hamme, Sillen, & Vangronsveld, 2016).

Sediments from Aventura Plaza showed very poor levels of macrobenthic diversity, the composition was given mainly by the presence of species of Diptera and other taxa characteristic of organic pollution and oligotrophic environments such as: Ceratopogonidae, Dolichopodidae*,* *Pericoma* sp, *Probezzia* sp., *Ablabesmyia* sp., *Chironomus* sp., oligochaeta and *Melanoides cf tuberculata* (exotic specie). There was an increase in the diversity of invertebrates throughout the period of bioremediation especially in station 3 but this diversity was produced by the presence of insects generated by contribution of domestic waste waters in the study area. Similar composition and abundance of organisms were found along the bioremediation time. So the contribution of effluents from domestic and industrial wastes contribute in an important way to the community structure of macroinvertebrates, probably much more at low tide where there is less incidence of dilution in estuarine waters, so this would be a factor on the macrobenthic community more than the technique of bioremediation.

This study confirms that the section A and B are continuing to be affected by several sources of domestic sewage inputs, urban activities, discharges of industrial activities as happen in others estuaries in China (X. Yang et al., 2015)¸Malaysian (Masood, Zakaria, Halimoon, Aris, Magam, Kannan, Mustafa, Ali, Keshavarzifard, Vaezzadeh, Alkhadher, & Al-Odaini, 2016), The greater magnitude of TPH and heavy metals and the spatial variability indicated that the contents of Cd, Pb and total petroleum hydrocarbons were primarily contributions of anthropogenic sources and are affecting severely the composition, abundance and diversity of benthic macroinvertebrates mainly in Bosque El Salado, Bosque Protector Salado Norte, Parque Ecológico de Urdesa, Miraflores y Aventura Plaza. The continuing and intense industrialization and lack of control of emissions of industrial effluents (unless controlled) will continue to cause loss of biodiversity in the inner branches of section A and B of Estero Salado.

In addition, the presence of hydrocarbons such as PAHs and heavy metals such as lead tend to be associated with the particulate matter and because of their hydrophobic nature they are deposited in the sediment (Zhang, Zhang, Guan, Shu, Shen, Chen, & Li, 2016), their enter the food chain and are absorbed by the invertebrates (Masood et al., 2016) producing carcinogenic and endocrine disruption in the fauna that lives in aquatic systems (Singare, 2016). Hence, the potential ecological risk for invertebrates and fish as well as for public health due to the bioaccumulation and biomagnification capacity of contaminants in species of consumption by local communities that still live from subsistence fishing mainly.

Chapter 6. Recolonization of macroinvertebrates in Estero Salado Estuary using experimental artificial substrates in polluted sites during 2012

6.1 Introduction

Mangrove forests are very important ecosystems due to their high productivity (10 to 20g/m^2/day) and their ecological role in the following: flood control, shoreline stabilization, erosion control, sediment and toxic substances retention, storm protection and desalination of water entering the mainland (Twilley & Day, 1999) They hold a great diversity of aquatic species, perform sequestration of carbon (at rates up to 50 times higher than tropical rainforests) and provide energy sources, food, medicine, fishing, construction, textiles, papers and a wide range of ecosystem services to humanity (Bouillon, 2011).

Despite its considerable ecological economic and social importance this ecosystem is being threatened by human activities, which have reduced mangrove forest cover by more than 35% on a worldwide scale in the last few decades. Such human activities include: conversion of mangroves to mariculture; agriculture; forestry; urbanization; (Valiela, 2001); watershed deforestation and other down-estuary transformations (Valiela et al.. 2013). In addition the health and functioning of mangrove ecosystems are impacted by the accumulation of a wide array of toxic substances such as heavy metals, hydrocarbons, pesticides and other pollutants from industrial and municipal wastewaters, urban runoff, dredging activities, sewage sludge, ground water seepage and atmospheric deposition (Kennish. 1996).

The Estero Salado estuary (Figure 1) within the Gulf of Guayaquil.-Ecuador forms part of the most important estuarine systems of the west coast of South America (CAAM, 1996). The inner estuarine channels enter into the city of Guayaquil which is the main port and most populated city of Ecuador with more than 2.35 million inhabitants according to the 2010 census of INEC (National Institute of Statistics and Census). The main productive activities of the Guayaquil region are agriculture, aquaculture, fishing, commercial activities, manufacturing industries (food and beverages), tourism and maritime transport.

The surrounding coastal ecosystems are affected by contaminants in wastewaters from industries: mainly organic discharges, solids in suspension, fats and oils and by products from food, tobacco, textile, paper and paperboard industries (Zambrano, 2007). Other impacts are derived from unplanned human settlement resulting in untreated domestic waste waters discharges, sedimentation produced by mangrove deforestation, inadequate management of plastic solid waste; and alteration of natural forms of estuarine inner branches, affecting the biota composition water and sediments quality. The few studies about benthonic macroinvertebrates diversity existing are in the inner branches of Estero Salado affected by industrial pollution in Guayaquil city (Cárdenas-Calle, 2010; Cárdenas-Calle & Mair, 2014; Ministerio del Ambiente, 2012). Most studies have been in the external branches of this estuary (Cruz, 1983, 1986, 1992, 2003; Cruz et al., 1980; Mora & Reinoso, 1981; Thiakos, 2000).

Highly toxic heavy metals such as Lead (Campbell & Hewitt), cadmium (Cd) and mercury (Hg) were found during the 2009 dry season in the Estero Salado sediments with concentrations of Pb up to 37.82 mg/kg Cd up to 1.84 mg/kg dw., and Hg up to 0.89 mg/kg dw (Chapter 5). Other studies confirm the presence of these and other heavy metals such as: B, Cd, Cu, Se, V, Pb and Zn in the Estero Salado sediments with levels exceeding the international environmental quality standards (Fernández-Cadena J.C. et al., 2014). Total hydrocarbon were found in both inner and outer branches of Estero Salado (Miraflores. Terminal Portuario Internacional. Tres Bocas and Cuarentena Stations) and the diversity of benthic macroinvertebrates was found to be lower in the areas with higher concentrations of hydrocarbons. These previous studies indicate the negative influence of industrial wastes on sediments and aquatic biotic in this estuary (Cárdenas, 2010).

Currently the Ministry of Environment of Ecuador is trying, through different means, to recover ecosystem functionality of the emblematic Estero Salado through the 'Guayaquil Ecological Program' through which studies have been funded aimed at ecological restoration of this estuary. Some of the projects completed include: superoxigenation, sediment bioremediation, mangrove reforestation, domestic and industrial waste discharge control, chemical characterizations of sediments. and environment education (Ministerio del Ambiente, 2015a). Net productivity in surface waters of the inner estuary was measured during 1962-1964 and the estimates of surface net carbon for 12 hour days show that the average annual inner estuary productivity was 401mg/C/m^3/day [12] . it was four time greater than for the outer part of the Guayaquil Gulf (98 mg /C/m^3/day [12]) and surface measurements of chlorophyll-a were made at 46 stations showed that the average concentration of chlorophyll-a was greater in the inner estuary (near 4 mg/m^3) than outer estuary (near 2 mg/m^3) (Stevenson, 1981).

6.2 Aim

To assess the feasibility of recolonization of benthic macroinvertebrate in different types of artificial substrates on polluted areas in inner branches of Estero Salado to design future strategies of ecological restoration to recover native macroinvertebrates species.

6.2.1 Specific objectives

- To describe the benthic macroinvertebrate community structure in two polluted estuarine sites with different pollution souces and tidal influence.
- To determine composition, abundance and diversity of macroinvertebrate in the different types of artificial substrates.
- To evaluate and compare the speed and efficiency of settlement of macroinvertebrates useful to recovery of native species.

6.3 Study area

The study was performed at the inner branches of Estero Salado Estuary around of Aventura Plaza Commercial Center (2°10´16.37´´S -79° 54´47.57´´W) and Guayaquil University (2°10´54.15´´S-79°54´2.75´´W) (Figure 67). The Aventura Plaza station corresponds to a site which did hydrocarbon bioremediation during January of 2011 to April. 2012 previously (see Chapter 5). While the Guayaquil University station was not bioremediated area. Both sites were chosen because their superficial sediments are strongly affected by heavy metal and hydrocarbon contamination. At the University of Guayaquil sites the following compounds were previously detected: Lead with concentration of 33.14 (mg/kg dw), Cadmium (3.80 mg/kg dw). Zinc (149.14 mg/kg) and others metals as Hg. Ni. Cr. Al and As in January 2011 (Cárdenas & Erazo. 2011) with some of them repeated again in 2014 in the Estero Salado mangrove (station 8) near Guayaquil University, with higher concentrations of Al. As. Cr. Ni Zn and Pb (Fernández-Cadena et al., 2014). There is evidence of presence of total petroleum hydrocarbons (TPH) with concentrations 872.71 mg/kg. polycyclic aromatic hydrocarbons (PAHs) with concentrations less than 0.3 mg/kg oils and lipids 0.53 mg/kg at the Universidad de Guayaquil station (Cárdenas & Erazo. 2011).

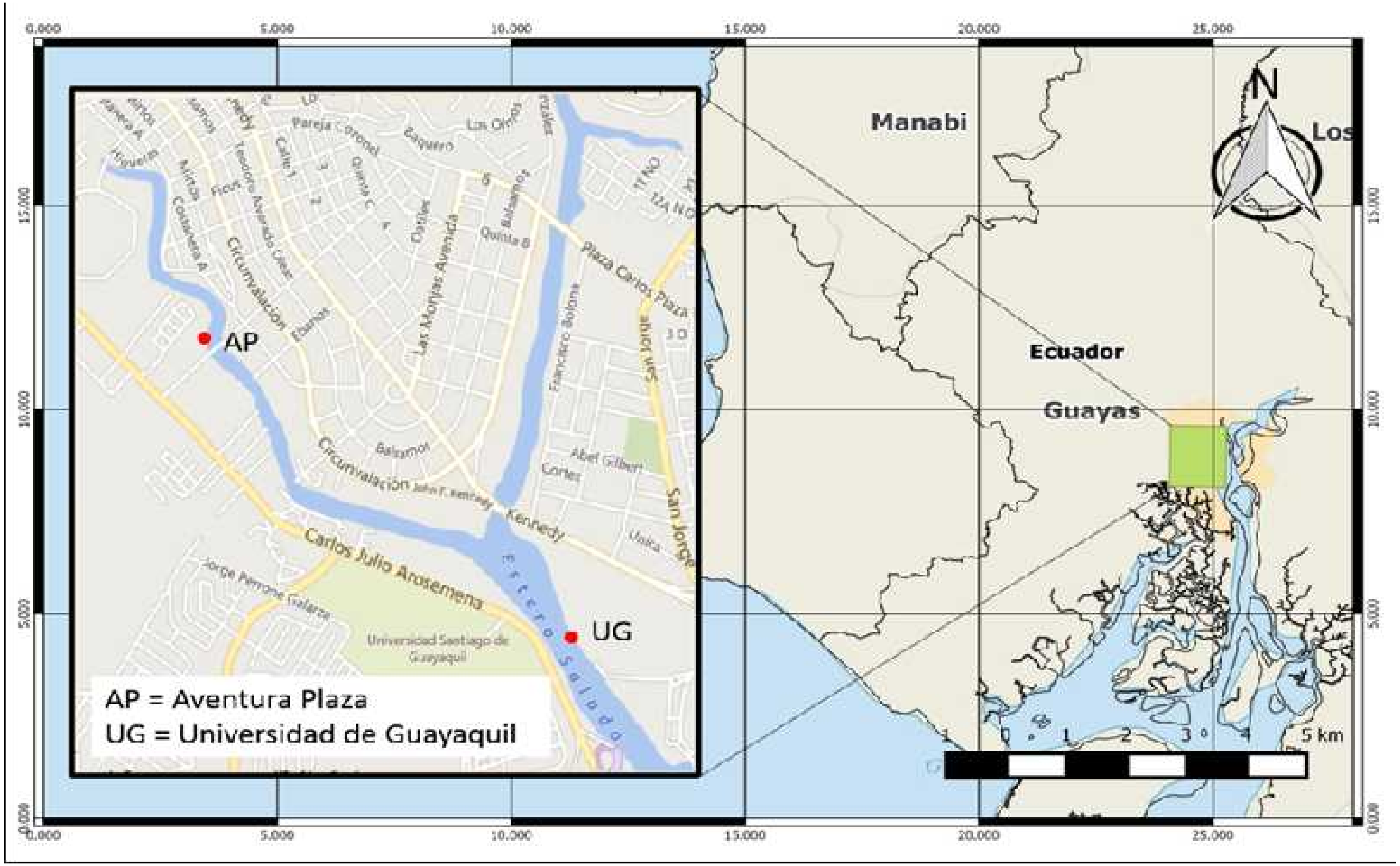


Figure 67. Geographic localization of the two sites the study

6.4 Methodology

6.4.1 Field work

Three samples of macroinvertebrates were randomly sampled at two sites (Aventura Plaza and Guayaquil University) in the Estero Salado during the middle of each month of four surveys between September to December of 2012. These were the same sites as those sampled for hydrocarbon bioremediation early in first months of 2012 in Aventura Plaza and January of 2011 in Guayaquil University and which had been chosen to represent different habitats, diverse source of pollution as industrial zone in Guayaquil University and residence & commercial zone in Aventura Plaza and distinct wave influences in the basin of the estuary.

Three randomly-located sediment samples were collected from each site during the day in each month. Each site comprised the intertidal zone 100 along and parallel the shoreline in low tidal.The sediments were taken using a Van Veen grab (0.10 m-^{2}) (Eleftheriou & McIntyre, 2005) and placed into a plastic wash tank with water from the estuary.The samples were rinsed with estuarine water on site inside of a sieve of 500 µm and afterwards the samples were placed into a clear food grade polyethylene bags. The samples were preserved in 10% formaldehy (Mair, Mora, Cruz, Calles, Arroyo, & Merino, 2000) with a solution buffered with sodium tetraborated and subsequently analyzed in the biology laboratory of Environment Sciences School of UEES. The taxonomic analysis of macroinvertebrates was carried out using a stereomicroscope, the macroinvertebrates were removed from any sediment that was retained on the mesh, identified to the lowest possible taxon and stored in 70% ethanol. For taxonomic identifications used relevant identification keys (Keen. 1971; Pennak. 1989; Cruz & Jimenez. 1994; Thorp & Covich. 1997; Rouse & Pleijel. 2001; Correoso. 2008; Dominguez & Fernández. 2009).

Some variables were measured *in situ* as temperature sediments, salinity, pH of water and soil pH. The temperature and pH in sediments were measured in the first 5 cm of sediment using thermometer and Kelway soil pHmeter.

The Salinity and water pH at the surface of water column were recorded at two locations at each site on each sampling occasion using a salinity refractometer Oakton model STX-3 and a pHmeter model ecotestr pH2.

Settlement experiments

Three types of substrates were used in settlement experiments: bricks made of clay (L); artificial sponge (E); and organic substrate (O) made of a nylon mesh bag containing fresh native plant leaves. These substrata were chosen because they are cheap, easy to obtain and residual material commonly meet in development and setlement urbanistic zones in the riverine areas along the inner branches of Estero Salado. All of the 3 substrates had the form of blocks of 25 cm length x 25 cm wide x 7 cm of high. The surface of the brick and sponge substrates were abraded with holes to facilitate larval settlement and each one was put inside of a nylon bag (35 x 20 cm) with 1 cm mesh. Three replicates of each type of substrate (biological aggregater) were attached in random order 150 cm-long to a stainless steel stick using nylon filament of 0.5 inches of diameter, the in the sediments during low tide.The distance between biological aggregate on a single stick was 300 mm and the distance between different stick was 25 cm. This design allowed that substrate was maintainted 25 cm water above the sediments.

One sticks contained a nine replicates with 3 substrata of each type. Three sticks (27 replicates or blocks) were ubicated between roots of mangroves to avoid their lost with the change of tidal for each site.

Sampling was designed to evaluate the speed and efficacy of benthic macroinvertebrate recruitment in the three types of substrate. In total a set of 54 blocks (27 blocks at each station were placed in the water column during September 2012 with three blocks of each of the three substrate types (three replicates for each substrate). Blocks were removed in October 2012 (after one month). November (after two months) and December (after three months). Prior to placement of the biological aggregators, characterization of invertebrates (Time 0) present in the sediments was done at both research sites as reference previously to implementation of biological aggregaters. Three replicates were sampled at each station with the Van Veen grab (Eleftheriou & McIntyre, 2005). These samples were used to give an indication of the faunal composition prior to the experiment.

All samples were collected during low tide on 15th day of the each month. The blocks were carefully removed from the sticks at each site and placed into plastic bags with 10% formaldehyde in seawater.

6.4.2 Laboratory analysis

Each substrate with macroinvertebrates was washed carefully into a plastic wash tank with potable water to dislodge the organisms. Water and organisms were filtered in sieves of 1 mm mesh; afterwards the samples were transferred into plastic vials and preserved with 10% buffered formaldehyde with sodium tetraborate. Only in the case of sponge substrate only a fraction was considered 10 x 10 cm due to the excessive number of individuals and the degree of difficulty to extract them. The samples were sorted under a dissecting microscope and stereomicroscope, identified to the lowest possible taxonomic resolution and counted. For the identification of benthic macroinvertebrate we used the specific taxonomy keys for aquatic insects and estuarine biota as described in the chapter 5.

6.4.3 Statistical analyses

The abundance data were transformed to the 4th root (down-weighting the importance of the abundant species) allowing the mid-range and rarer species to exert some influence on the calculation of sample similarity (Clarke & Warkick.2001). A similarity matrix between samples was constructed using the Bray- Curtis similarity coefficient (Bray &Curtis.1957). Analysis of similarity was tested with ANOSIM. Two-way crossed design was used to test null hypotheses of no differences between assemblages on different substrata and at each site (Clarke. 1993; Clarke & Warwick. 2001). Cluster analysis was used to determinate the similarity between samples. In addition, Non-metric Multidimensional Scaling (MDS) was utilised to assist in the visual representation of composition and abundance of species (Clarke. 1993). Shannon-Wiener index, richness species and evenness were calculated using the DIVERSE routine to evaluate the community structure of macroinvertebrates.

The SIMPER analysis was used to identify wich species made the greatest contributions among natural conditions during the characterization time and the different type of substratum used as biological aggregator. All multivariate techniques were undertaken using the PRIMER V6 computer program.

Each sampling unit was coded to facilitate the entry of the data in the statistical packages, the first letter being the initial one of the sampled month being September (S), October (O), November (N) and December (D), second and third letters represent the initials of the Aventura Plaza (AP) and Universidad de Guayaquil (UG) sampling station, the fourth letter represents the sample replica (A), (B) or (C) and the fifth letter represents the characterization phase (C) ot type of substratum: sponge (E), brick (L) and biological (O).

6.5 Results

6.5.1 Environmental variables

In the Annex 25 showed the temperature, salinity of Aventura Plaza and Univerisdad de Guayaquil.

6.5.2 Benthic macroinvertebrate characterization prior to experiment implementation phase

Prior to the installation of artificial substrates (month 0) a total of 461 individuals (the most common being *Pericoma*, *Podura aquatica*, *Heleobia*, *Polymesoda, Melampus, Capitella* and Oligochaeta) were recorded during the characterization of benthic macroinvertebrate (Annex 26). Annelida constituted 76%, Arthropoda 16% and Mollusca with 8%. The University of Guayaquil station had the highest richness with 379 individuals while 82 individuals were registered at the Aventura Plaza station (Table 23).

The annelids were represented mainly by Oligochaeta (family undetermined) with 348 individuals and Polychaeta of the family Capitellidae (4 individuals), the arthropods were represented by the insects with 72 individuals mainly Diptera of family Psychodidae (*Pericoma*) with 62 individuals, Dolichopodidae with 7 individuals and Poduridae (*Podura*) with 2 individuals.

The Molluscs were represented mainly by gastropods of the families Ellobiidae (*Mellampus*), Thiaridae (*Melanoides*) each one with 2 individuals and Hydrobiidae

(*Heleobia*) with 31 individuals and the bivalve family Corbiculidae (*Polymesoda*) and one unidentified bivalve.

Analyses of diversity (Table 23) demonstrated that Aventura Plaza and Universidad de Guayaquil stations had low Shannon-Wiener indices (0.62 to 0.13) with low species richness (*d)* and number of species (*S*). The Aventura Plaza Station had the highest diversity and evenness with a mean evenness of 0.528 where the dominant invertebrate was the Diptera *Pericoma*. Whilst at the Universidad de Guayaquil station mean evenness was 0.332 with the Oligochaeta group being the dominant invertebrate.

Table 23. Diversity indices for Aventura Plaza and University of Guayaquil during characterization of benthic macroinvertebrates.

Stations	Replicates	No of species (*S*)	Species Richness (*d*)	Shannon – Wiener Diversity index (H´)	Species Evenness(*J´*)
Aventura Plaza	SAPAC	3	0.56	0.1507	0.3159
	SAPBC	5	1.34	0.6213	0.8889
	SAPCC	3	0.6068	0.1822	0.3819
Universidad de Guayaquil	SUGAC	4	0.5933	0.1290	0.2143
	SUGBC	4	0.7015	0.1413	0.2348
	SUGCC	2	0.1996	0.1650	0.5482

Meaning of the replication coding: First letter=month: September; second and third letter = site where Aventura Plaza is AP and Universidad de Guayaquil UG; fourth letter is a sample replicate A-B-C and fifth letter represents the characterization phase (C) of type of substratum: sponge (E), brick (L) and biological (O).

The pairwise comparison in the ANOSIM test for the diversity indices of two stations indicate that species diversity was not significantly different (R= 0.04; p>0.182).

6.5.3 Benthic macroinvertebrate community structure during the experimental phase

Overall benthic macroinvertebrate fauna

A total of 36676 individuals representing 13 genera, 18 families, 8 classes and 4 phyla were recorded within the 54 blocks analysed (Annex 26). The most abundant phylum was Arthropoda with 41% followed by Annelida with 38%, Mollusca with 17% and Nematode with 4%. An increase of macroinvertebrates individuals was evident during the period of submersion of artificial substrata in the interface water and sediments in both stations and also compared to the original in-sediment numbers measured in Month 0 (Figure 68).

The pairwise comparison in the ANOSIM tests showed that macroinvertebrates assemblages at the two sites during characterization (Time 0) there were not a statistically significant differences (R= 0.17, p>0.001). However, the high abundance was registered at Aventura Plaza.

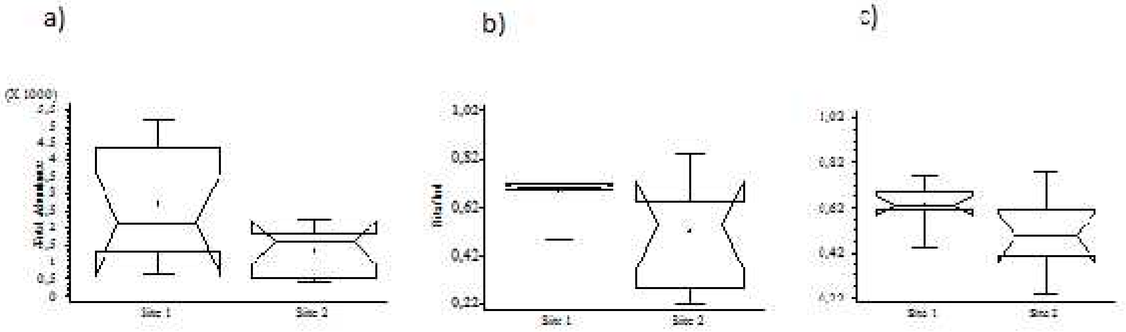


Figure 68. Spatial distribution of abundances (a) Shannon Diversity Index (b) and evenness (c) in the Estero Salado. Site 1: Aventura Plaza; Site 2: Universidad de Guayaquil during characterization phase in September of 2012.

The highest macroinvertebrate abundance was found in the replicate corresponding to sponge substrate at Aventura Plaza station in December (DAPCE) with a maximum number of 5174 individuals (months 3) composed mainly by Oligochaeta (1946 individuals), *Podura* (1130 individuals) and Hydrachnidae (1091 individuals). However the second replicate with high number of macroinvertebrates was the replicate corresponding to brick substrate at Aventura Plaza in November (NAPCE) with the dominance of Diptera *Pericoma* (1553 individuals), Hydrachnidae (1250 individuals) and Oligochaeta (541 individuals).

The pairwise comparison in the ANOSIM tests for all substrata indicated that assemblages and abundance of macroinvertebrates between sponge and organic substrate were significantly different (p<0.001) and among the abundance and composition of species during the characterization and each type of substrate (Table 24).

Table 24. Summary of pairwise percent significance levels among three substrata submersed between October to December (1 to 3 months) and benthic macroinvertebrates characterization (0 month).

	Characterization	Clay Brick	Sponge Artificial	Biological Substrate
Characterization		0.01	0.01	0.01
Clay Brick			0.02	0.042NS
Sponge Artificial				0.01
Biological substrate				

Total number of permutations 999. Test were significant except where indicated by NS .p 0.05

There were significant differences (p<0.01) among characterization period (Time 0) and periods of submersion: 0-30 days (1 month); 0-60 days (2 months) and 0-90 days (3 months). However, there were no significant differences (p>0.01) between 30 to 60 days, 30 to 90 days and 60 to 90 days. This trend was also evident in the hierarchical cluster analyses (Figure 69) where the abundance and diversity of macroinvertebrates were different. Showing a clearer pattern after the first month (biotic characterization).

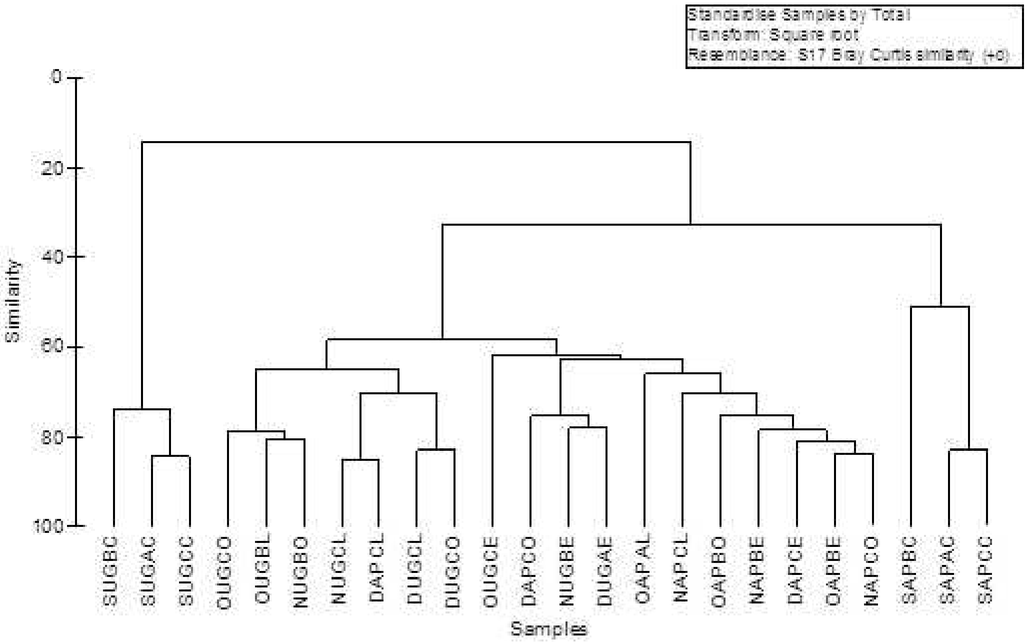


Figure 69. Cluster of benthic macroinvertebrates during all the period of study in 2011 and 2012.

Composition of benthic macroinvertebrates assemblages

The MDS of both stations and four periods of study demonstrated that abundances of invertebrates were different in the two stations surveyed during the benthic macroinvertebrates characterization (month 0). The MDS showed that assemblages of different substrata and submersion period remained similar (30, 60 and 90 days) in twice stations were similar in the 60 and 90 days (Figure 70).

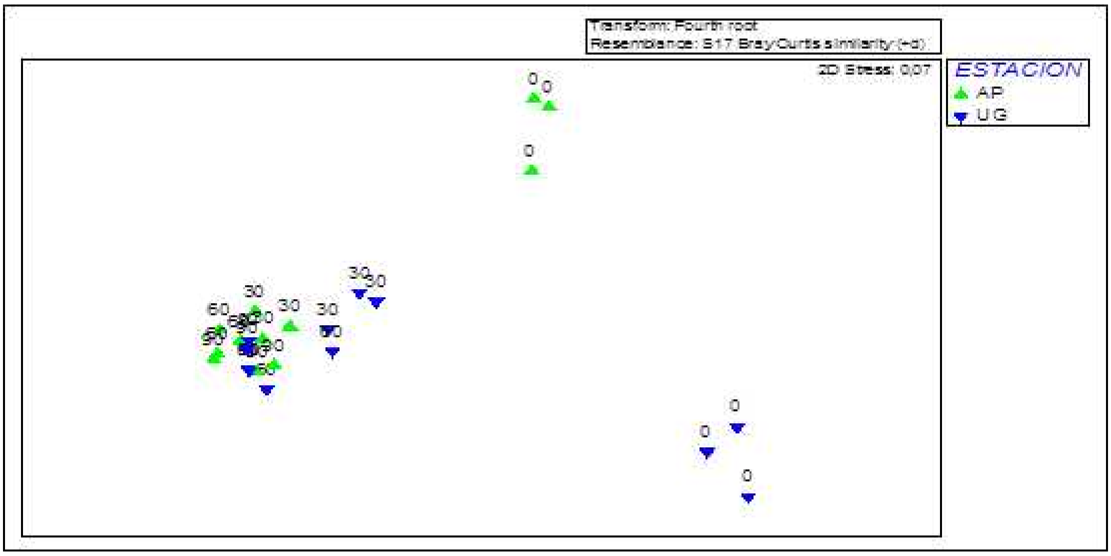


Figure 70. Two dimensional two- factor MDS ordinations of assemblages on stations and study period.

The abundance of macroinvertebrates on artificial substrata increased with increasing period of submersion up to 2 months (Annex 26). There were significant differences between the abundance in the sponge and organic substrata (ANOSIM. Test for differences between substrata: $p<0.001$).

The most common macroinvertebrates in the three types of substrata were insects and oligochaeta corresponding to 76.5% of all macroinvertebrates. Smaller percentages were found in molluscs and nematodes (23.5%).

The most common genera were *Pericoma*, *Podura*, *Stilobezzia*, *Capitella*, *Heleobia*, Hydrachnidae, Dermaptera and Oligochaeta present in all types of substrata. The most abundant species were Diptera *Psychoda* and Dolichopodidae present in the three substrata. The gasteropod *Melanoides* was present in the three substrates but specially in brick substrate. The bivalve *Mytilposis* and nematodes were registered only in sponge and organic substrata.

The uncommon species were *Penaeus, Balanus, Nereis, Melampus, Detracia, Polymesoda* and amphipods. Zoea of Brachyura indeterminate and ostracods were also present. All these were observed mainly in sponge and brick substrata. The shrimp *Penaeus* sp., *Balanus* sp., and *Nereis* sp., were registered only in brick substrata (Table 25).

The gastropods *Melampus* were present only in brick substrata and *Detracia* was present in brick and sponge substrata with higher frequency in sponge substrata. The bivalves *Mytilposis* and *Cyrenoida* were registered in the three kind of substrata especially in sponge substrata (Table 25).

The SIMPER analysis (Table 26) demonstrated that average similarity between the species found in the sponge substrata was 69.39% and the species that contributed in the similarity between the replicates were Oligochaeta (22.20%) and the insect *Podura* (Collembola) (18.64%). While the species that contributed with less similarity were polychaetes (5.55%) and *Pericoma* with (8.14%).

The average similarity between the species found in the organic substrata was 64.51% and the species that contributed in the similarity between the replicates were Oligochaeta (45.36%) and the gastropods *Heleobia* with 15.92%. The species that contributed with less similarity were Hydrachnidae (4.96%) and Nematode with 5.43%. The brick substrata replicates had an average similarity of 60.45% the species that contributed in the similarity were Oligochaeta (33.06%) and *Heleobia* with 22.61%. While the species that contributed with less similarity were the Diptera *Stilobezzia* (2.92%) and Dermaptera with 2.75% (Table 26).

Table 25. Summary of species composition by substrata as presence and occurrences of taxa in each substratum submersed in September 2012 (n= 18).

Occurrences		Sponge Substrate						Brick Substrate						Organic Substrate					
	Station	Aventura Plaza			Guayaquil University			Aventura Plaza			Guayaquil University			Aventura Plaza			Guayaquil University		
	Period of submerged (days)	30	60	90	30	60	90	30	60	90	30	60	90	30	60	90	30	60	90
	Taxa	OAPBE	NAPBE	DAPCE	OUGCE	NUGBE	DUGAE	OAPAL	NAPCL	DAPCL	OUGBL	NUGCL	DUGCL	OAPBO	NAPCO	DAPCO	OUGCO	NUGBO	DUGCO
Uncommom	Amphipoda	0	1	0	0	1	0	0	0	1	0	1	0	0	0	1	0	0	0
	Penaeus	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	Zoea de Brachyura	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0
	Ostracoda	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
	Balanus	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Nereis	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	Melampus	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
	Detracia	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	Cyrenoida	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1
Common	*Psychoda*	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1	0	0	1
	Dolichopodidae	1	1	1	0	0	0	1	1	0	1	0	1	1	1	0	0	0	1
	Melanoides *	1	0	0	0	0	0	1	1	1	0	1	1	0	1	0	0	0	0
	Mytilopsis	1	1	1	1	1	1	0	0	1	1	1	0	0	0	1	0	1	0
	Nematodo	0	1	1	0	1	1	0	0	0	0	0	1	1	1	1	0	1	1
Very commom	*Pericoma*	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Podura	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Hydrachnidae	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1
	Dermaptera	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	0	1
	Stilobezzia	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	0	1
	Oligochaeta	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Capitella	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
	*Heleobia**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

The highlighted number corresponded to taxa characteristic of the estuarine ecosystem.
*** Species reported in freshwater.**

Table 26. Summary of Simper results for natural condition of sediments and artificial substrate for Estero Salado estuary: average abundance of typifying taxa in each substrate, their contribution (%) to the withing-group similarity, and cumulative total (%) of contributions (90% cut-off).

Characterization	**Average similarity: 29.09**			
Species	**Av.Abund**	**Av.Sim**	**Contrib%**	**Cum.%**
Enchitraeidae	4.78	13.85	47.61	47.61
Pericoma	4.08	8.69	29.88	77.5
Heleobia	1.88	2.96	10.16	87.66
Dolichopodidae	1.46	2.57	8.82	96.47
Brick	**Average similarity: 60.45**			
Species	**Av.Abund**	**Av.Sim**	**Contrib%**	**Cum.%**
Oligochaeta	6.26	19.99	33.06	33.06
Heleobia	4.58	13.67	22.61	55.67
Pericoma	2.8	7.38	12.21	67.89
Podura aquatica	2.13	6.27	10.37	78.26
Hydrachnidae	2.27	5.13	8.48	86.74
Stilobezzia	0.72	1.77	2.92	89.66
Dermaptera	0.65	1.66	2.75	92.42
Sponge	**Average similarity: 69.39**			
Species	**Av.Abund**	**Av.Sim**	**Contrib%**	**Cum.%**
Oligochaeta	4.85	15.4	22.2	22.2
Podura aquatica	4.38	12.94	18.64	40.84
Heleobia	3.08	9.22	13.29	54.13
Mytilopsis	3.42	8.1	11.67	65.8
Hydrachnidae	3.02	7.6	10.95	76.75
Pericoma	2.23	5.65	8.14	84.89
Polychaeta	1.42	3.85	5.55	90.44
Biological substrate	**Average similarity: 64.51**			
Species	**Av.Abund**	**Av.Sim**	**Contrib%**	**Cum.%**
Oligochaeta	7.39	29.26	45.36	45.36
Heleobia	3.01	10.27	15.92	61.27
Pericoma	1.81	5.61	8.7	69.97
Podura aquatica	2.36	4.6	7.13	77.1
Polychaeta	1.26	4.3	6.66	83.76
Nematodo	2.02	3.5	5.43	89.19
Hydrachnidae	1.49	3.2	4.96	94.15

Species richness and diversity

The diversity analysis during the period of submersion of artificial substrata in the interface water and sediments in the two stations demonstrated there was a low Shannon - Wiener index in general (Table 27), with values between (0.2210 to 0.8728). The highest Shannon- Wiener index corresponded to the Sponge substrate at Aventura Plaza Station during November (NAPBE) and the lowest Shannon - Wiener index corresponded to the Brick substrate at Universidad de Guayaquil during October (NUGBO).

The highest diversity of macroinvertebrates were found in the sponge substrate in the both stations during November and December (60 and 90 days of submersion of artificial substrata in the field) corresponding to replicates NAPBE, NUGBE and DUGAE. While lowest diversity of macroinvertebrates was found in the organic and brick substrate during October and November but specially in October (30 days of submersion of artificial substrate) corresponding to replicates NUGBO, OUGBL and OUGCO all these met at Universidad of Guayaquil station.

The most species richness (*d) and* number of species (*S*) were found in the replicates NUGCL. DAPCL and DAPCE corresponding to the brick and sponge substrates (Annex 25). Universidad de Guayaquil and Aventura Plaza stations during the months of November and December. The brick substrate had a richness of 2.3060 and a species number 18 and the sponge substrate was a richness of 1.7545 and species number 16; the station with most species richness was Guayaquil University during November and December.

The highest evenness was found in the sponge substrate in the Universidad de Guayaquil Station during November (months 2) corresponding to replicate NUGBE with a value of 0.7811. The replicates with a trend to homogeneous distribution were mainly the sponge substrates at both stations during November (months 2) corresponding to NUGBE and NAPBE replicates. So too the organic substrate showed similar trends during October at Aventura Plaza (replicate OAPBO). The replicates where there was dominance of species were the replicates NUGBO and OUGBL corresponding to organic and brick substrate at Universidad de Guayaquil during October and November the dominant invertebrate was Oligochaeta with 1161 individuals at the NUGBO replicate and 446 individuals at OUGBL (Table 27- Annex 25).

Table 27. Diversity indices of benthic macroinvertebrates presented on three substrata submersed in October for 1 month. November for 2 months and December for 3 months in the Aventura Plaza and Universidad de Guayaquil Stations.

Station	Replicates	No of species (*S*)	Species Richness (*d*)	Shannon diversity (H´)	Species Evenness (*J´*)
Aventura Plaza (October)	OAPAL	9	1.1211	0.4858	0.5091
	OAPBE	11	1.3221	0.7161	0.6877
	OAPBO	9	1.2334	0.6950	0.7284
Universidad de Guayaquil (October)	OUGBL	9	1.2869	0.2280	**0.2389**
	OUGCE	11	1.6663	0.6421	0.6166
	OUGCO	5	**0.6361**	0.2848	0.4075
Aventura Plaza (November)	NAPCL	13	1.4298	0.7009	0.6292
	NAPBE	14	1.5523	**0.8728**	0.7615
	NAPCO	12	1.3616	0.7201	0.6673
Universidad de Guayaquil (November)	NUGCL	18	**2.3060**	0.5309	0.4230
	NUGBE	12	1.4457	0.8429	**0.7811**
	NUGBO	8	0.9749	**0.2210**	0.2447
Aventura Plaza (December)	DAPCL	16	2.0808	0.5339	0.4434
	DAPCE	16	1.7541	0.7340	0.6096
	DAPCO	13	1.5627	0.6944	0.6234
Universidad de Guayaquil (December)	DUGCL	13	1.6043	0.6042	0.5424
	DUGAE	14	1.7357	0.7649	0.6673
	DUGCO	13	1.5549	0.5461	0.4902

Meaning of the replication coding: First letter is the period of submersion of artificial substrata in the interface water and sediments expressed in months O= October. N= November. D= December; Second and third letter represent the initials of station´s name AP= Aventura Plaza. UG= Universidad de Guayaquil; Fourth letter represent the sample replicates A-B-C and the fifth letter represent the type of substrata: L= Brick. E= Sponge and O= Organic.

6.6 Discussion and conclusion

The composition of benthic macroinvertebrates was similar in the two study sites where the following were recorded: phyla Arthropoda, Annelida, Mollusca and Nematode (Nematode were additionally registered at the University of Guayaquil).

The station with the highest abundance of macroinvertebrates during the characterization was Universidad de Guayaquil, influenced mainly by the presence of oligochaeta registered too in July of 2012 in the Zig Zag Bridge area relatively near to this station but in low amounts during dry season (INOCAR, 2012). Also others taxa were registered: *Capitella, Melampus, Polymesoda*, this last species recorded in previous studies in 2003 (Cruz, 2003) and 2009 (Cárdenas-Calle, 2010) in this station and they appear adapted to live in low salinity environment (Cruz, 2003). In the Aventura Plaza station the dominant taxa were Diptera *Pericoma and* other insects' larvae from family Dolichopodidae and Poduridae characteristic of water with organic pollution.

There was a rapid recolonization of the fauna in the substrate mainly in the Aventura Plaza during all the survey period. However, these macroinvertebrate communities were formed by aquatic insects and oligochaetas. The Shannon-Wiener index demonstrated both stations had a poor diversity of benthic macroinvertebrates in the characterization and recolonization phase. Nevertheless, the greatest variety of taxa was found in Aventura Plaza due to the presence of wide type of insect larvae. This indicated that native macroinvertebrate composition and diversity have changed by opportunistic species that are pollution tolerant such as oligochaeta and insects (Hellawell. 1941) due to the physical and chemical alterations of sediments of Estero Salado estuary, as the change of aerobic environments to anaerobic generate significant changes in the biogeochemistry of the ecosystem. Sediments vary from oxidized to reduced with negative effect in assemblage and community structure causing the loss of aquatic wildlife (Valiela, 2015). Conditions of hypoxia (1.46 mg/L) were recorder in Universidad de Guayaquil in 2009 and concentrations between 1 to 1.99 mg/L were registered in Aventura Plaza in 2012 (Chapter 5). While lower values were recorded in this same section B of ES in Miraflores and AlbanBorja with values <0.1mg/L in 2007 (Chapter 4) reflecting states of anoxia.

No significant differences in abundance, diversity and evenness were observed among sites. The assemblages of macroinvertebrate, and dominance of Pericoma and oligochaete, are recognized bio-indicators representative of oligotrophic ecosystem (EFFICACITAS; Pinilla, 2000). These biological effects can be related with the low salinity (14 UPS) specially in Aventura Plaza (Cárdenas-Calle & Mair, 2014) and the presence of contaminants from anthropogenic sources such as hydrocarbon registered in 2009 and 2011 in sediments of Universidad de Guayaquil (Chapter 5) and heavy metals Ag, Cd, Cu, Se and Pb associated with industrial and wastewater discharges in the section A and B of inner branches of Estero Salado (Fernández-Cadena J.C. et al., 2014). These variables could be generating the low diversity of benthic macroinvertebrates but mainly also salinity, considering the species are mainly from freshwater.

The assembly of various macroinvertebrates in sediments of the sites prior to placing artificial substrates and registered taxa such substrates characterized. This variation was given by an increase of 5 taxa (characterization phase) to 8 taxa (experimental phase).The taxa were recorded as Oligochaeta, Polychaeta, Gastropoda, Bivalvia, Malacostraca, Maxillopoda, Ostracods (these last three additionally registered in surface substrates).

An increase of numbers, abundance and diversity of macroinvertebrates was evident during the period of submersion of artificial substrata in both stations in comparison with the benthic macroinvertebrate characterization registered. The abundance increases were generated by insect´s larvae and oligochaetes which increased significantly in the second month especially in Aventura Plaza. These taxa are common in rivers and some species are often associated with degraded conditions (Fründ, Graefe, & Tischer, 2011) where the oxygen is reduced (Hellawell, 1986) and where organic enrichment is important (Burt, McKee, Hart, & Kauss, 1991).

The insect larvae, polychaetes, gastropods and oligochaetes were present in all types of substrata. The bivalve *Mytilposis* and nematodes were registered mainly in sponge and organic substrata. *Melanoiodes cf tuberculata* was very abundant in the brick substrate.

The species typical of estuarine environments such as: *Penaeus* sp.*, Balanus* sp.*, Nereis* sp.*, Melampus* sp.*, Detracia* sp.*, Polymesoda* sp.*,* and amphipods*,* zoea of Brachyura and Ostracods were uncommon species and were registered in the artificial substrata especially in sponge and brick substrata. This increase is due to the porosity of the substrates from sponges and brick, that larvae generally prefer this porous surface (Anderson & Underwood, 1994). Besides the accumulation of phytoplankton, this porous material facilitates the availability of food for the larvae of invertebrates.

The highest taxa number was registered only in brick substrata and *Penaeus* sp., was registered only in this substrate. Therefore, this material could be useful in the future to ecological restoration of macrobenthic fauna of Estero Salado. The high diversity was registered in sponge substrate mainly in Aventura Plaza where there was low salinity. It allows fixing of a variety of insect larvae, and it seems that there is a selection of some species by the type of substrate and *Capitella* prefer muddy sediments more than artificial substrates susch as glass beads, others depend on the renewal of the water by tidal action which extends the conditions for survival and for movement as many larvae are usually small and have a slow shift (Valiela, 2015).

Significant differences in abundance, diversity was registered between characterization phase and experimentation with artificial substrate also between organic and sponge substrate and between periods of submersion (months 1, 2 and 3).

In this study the increased abundance of macroinvertebrates was highly significant in the 60 days of time of submersion of substratum but decreased after 90 days; similar results were found by Anderson & Underwood (1994) where the assemblages on different substrata were very different after 1 or 2 months and were not significantly different after longer periods.

In conclusion, the results indicate that use of sponge and brick are materials that help the fast colonisation of native macroinvertebrates of Estero Salado. Bbest results were in areas with most influence of tidal elevations (Bolam & Whomersley, 2003) for settlement of common species from the estuary. However, it is essential to consider variables such as the hydrodynamics in new studies, the incidence of wave and velocity current (De Montaudouin, Bachelet, & Sauriau, 2003) are factors that affect the composition of benthic macrofauna, high speeds current produced erosion to the larvae while currents of low intensity are avoided by adult organisms, generating a selective behaviour of taxa towards the substrate (Valiela, 2015). Other factors important in the recolonization in substrates are related to predator activity, availablity of food, prior bacterial colonization substrate (biofilm) providing nutrients such as polysaccharides and proteins, early succession of other organisms that facilitate the settlement of later species. In total there has to be acomplex set of factors to consider in assessing effectiveness of the rate of recovery of invertebrates.

As in all ecological restoration projects the phase of identification of causes and processes responsible for ecosystem degradation is very important for the success of any project. Hence the hydrographic factor is very important in the Aventura Plaza, influenced by anoxic, hypoxic and eutrophication environment and which affect community structure, distribution, growth, reproduction, physiology, mortality of macroinvertebrates. For example, factors such as: concentrations of O_2 between 1-1.6 mg/L produce mortality in isopods associated of bottom; 1-1.5 mg/L produce changes in the growth in bivalves and values between 1-1.2 have the same effect in annelids (Gray et al., 2002).

For this reason, alongside ecological restoration projects there must be a major control and reduction of industrial waste and domestic water in the estuary which could influence significantly in the reduction de opportunist fauna and could increase the potential of rehabilitation of Estero Salado with a realistic and cost-effective approach.

If emissions of industrial wastewater and domestic are not controlled, and if the amounts were to exceed capacity of estuarine water to the branches of the section A and B, then this could severely impact the composition, diversity (not only of macroinvertebrates but also of other organisms of trophic links) and alter the ecosystem functionality of these areas. There would therefore not be any improvement in the environmental management of these branches and, in the short time, the rate of natural purification would be overtaken by the rate of emissions of industrial water and tidal action will have a minimal effect on water renewal which will decrease the tolerance limits of species of fish, crustaceans, molluscs whose niches will be replaced by species such as opportunists that have the ability to adapt to these media.

Chapter 7. General conclusions and recommendations

Guayas River watersheds

In conclusion, the results reported in this thesis indicate that benthic macroinvertebrates provide useful information about the health status of Guayas River Basin and Estero Salado Estuary and, therefore, can become a key tool in monitoring water quality. Hence these organisms should be included in the parameters list of EQS and effluent discharge: Water Resource in TULSMA. The estimation of abundance, diversity and biotic indices and evenness show close relationships to sediment quality in the freshwaters and estuarine waters.

The results of composition, diversity and the interpretation of biotic index BMWP / Col provide evidence of deterioration in water quality especially in the lower basin of the Guayas River and Daule sub-watershed. There are significant differences in abundance and assemblages of benthic macroinvertebrates and these are associated with subwatersheds and the main productive activities in the sampling sites.

The composition of macroinvertebrates throughout the Guayas basin is represented fundamentally by aquatic insects especially by Ephemeroptera, Hemiptera and Diptera. This composition changes spatially along the altitudinal gradient and the results seems to be influenced by temperature; oxygen dissolved levels, type of current and substrate since this study showed the presence of species tolerant to organic pollution are mainly found downstream such as oligochaetes, Chironomidae, *Chironomus*, *Melanoides cf tuberculata* and *Trepobates* sp.

The diversity of macroinvertebrates in the Guayas river watershed showed variations from bad to moderate. It was high in sites closely to mountains (Cordillera Occidental de Los Andes and Chongón Colonche) and upstream. While the diversity decreases in the lower basin.

The BMWP/Col and diversity indices were shown to be sensitive to types of human activity and they provided information about the quality of water. Of the survey sites, 72% were shown to have critical and very critical waters (very and heavily polluted), these sites were located in the lower basin. While 29% of sites were doubtful and acceptable waters (with evident effects of pollution and acceptable waters) and were located in the higher basin. Hence, the implementation of a long term biomonitoring programme is recommended that includes not only benthic macroinvertebrates, but also: phytoplanckton, zooplankton, ictioplanckton and fishes for their importance in the food chain; status of populations of commercial and local consumption species in the case of clams, river shrimps and fishes of freshwater important in the aquatic resources conservation of Guayas River Basin.

It is recommended that integrated biomonitoring of other environmental variables is done, such as: Physical (altitude. temperature, particle size, velocity of river, turbidity); chemical (oxygen, pH, salinity, nitrate, nitrite, phosphate, ammonium, conductivity, total hardness), contaminants such as pesticides, heavy metal, total petroleum hydrocarbon, oils and fats and fecal coliforms considering altitude, sub-basins, productive activities (mining, agriculture, breeding, fishing, tourism), land-use, as an information base to choose the permanent sites of monitoring.

Results suggested the importance of conservation of the aquatic ecosystem in the rivers: Macul, Los Amarillos, Chongón, Dos Bocas, San Isidro and San Antronio as special areas where there should be special sustainable management because these sites constitute good reference sites for future studies to control the health if the aquatic environment and its diversity and can provide information as base line for ecological restoration projects in the future. Besides the three last sites are used for developing tourist activities associated directly with these rivers and generate economic sustainability for local families.

There should be development a special programme of eradication of exotic species such as *Melanoides cf tuberculata* in Milagro, Guayaquil, Naranjal and, especially, Simón Bolívar (Amarillo river) where good diversity of macroinvertebrates were founf and also *Pomacea canaliculata was* registered in the counties Samborondón, Yaguachi, Pedro Carbo, Playas and Naranjito. This programme would help avoid loss of native macrobenthic diversity and economic losses in the agricultural sector relate with rice production.

Conitnuing evaluation is important mainly in lowlands basin, sites such as Estero San Camilo Durán), Guayas River and Daule and Babahoyo subwatershed by evidence of the negative influence of industrial and urban activities. The the lack of enforcement of effluent management of industrial origin seems not to be developedvery effectively, so an analysis of strengths, weaknesses, opportunities and threats capabilities enabling better environmental departments at the level of autonomous governments needs to improve their role in the conservation of natural resources.

The aspect of continuous monitoring of compliance is essential on water quality and verification meeting environmental management plans for the productive sector in the basin of Guayas. It is suggested that in the absence of the current legal standard for the control of pollutants, nutrients in the sediment continue using the EPA criteria for assessing environmental damage. The Environment Ministry, aquatic ecosystem researchers and the ecuadorian academic community must work in the development of standards for evaluating the quality of sediments in estuarine and river waters in Ecuador, including standardization of internationally recognized methodologies (number of replicates for each study site) so since there might be underestimating of some important biological groups due to the lack of a national legal laws. This will allow collected data to contribute to build a platform of technological information useful in making decisions on sustainable use of natural resources in the basin of the Guayas River and build a predictive statistical model such as: RIVPACS (River Invertebrate Prediction and Classification System) to estimate the ecological health of new running water sites to avoid the environmental damage and preventing the loss of habitat and biodiversity of rivers in the Guayas province.

Based on the precautionary principle, it is suggested that environmental impact studies must include analysis of sediments related to chemical and biological studies and these should have as minimum three replicates for each study site to have more representative samples according to the nature of each site, and in avoiding underestimation of benthic macroinvertebrate community structure and prevent loss of real data to evaluate the health aquatic environment.

Future surveys should include work in relation to distribution of alien species and research which environmental factors impact on the reproductive development of these, and which native and commercial species are bioaccumulating harmful substances such as pesticides (which are generating sources of diffuse pollution), and how can the development of a socioeconomic and environmental comprehensive project in Guayas province (despite being of great socioeconomic importance) can help integrating physical, chemical and biological parameters that would enable sustainable use of bio-aquatic resources in the province of Guayas.

Estero Salado Estuary

In relation with community of benthic macroinvertebrates, there is evidence that these have suffered natural stress caused by the characteristics from the estuaries. However, the imposed stress by anthropogenic sources as changes of salinity is negatively affecting these organisms. There is a replacement of estuarine species by opportunistic species; there are changes in composition, abundance and diversity of these organisms in the inner branches where there are domestic and industrial effluents.

The composition of the macrobenthic fauna is influenced by physical and chemical characteristic of aquatic environment; Salinity, and water supply for domestic and industrial use determine the limits of tolerance of such biota. Thus the branches with greater tidal influence (Reserva de Producción Faunística Manglares El Salado) presente a taxacenosis characteristic of the estuarine area where the diversity of taxa allowed the presence of bryozoans, tunicates, sponges, bivalves, gastropods, crustaceans, polychaetes, nematodes. While, in the branches entering the urban area, the composition is totally different, with freshwater problems of organic pollution where the most representative macroinvertebrates are Diptera insects, at the levels of eggs, pupae and larvae, and oligochaetes, gastropods in oligotrophic environments.

In the RPFMS, common species were *Cosmiconcha* sp., *Costoanachis nigricans*, *Cylinchna* sp., *Balanus* sp., *Micronereis sp.* While in the urban area were: larvae of Dolichopodidae and Ceratopogonidae, the species *Psychoda* sp., *Pericoma* sp., *Culicoides* sp., *Alluaudomyia* sp., *Stilobezzia* sp., *Melanoides cf tuberculata, Heleobia* sp., and *Capitella cf capitata.* These are confirmed as bioindicators of aquatic polluted environments in tropical zone.

The Protected Area 'Reserva de Producción Faunística Manglares El Salado' showed the importance and utility for conservation of scarce remanents of mangrove and fauna associated with estuarine ecosystem and for that reason there should be implementation of monitoring of the waters and sediments for conservation purposes, showing the results of biocenosis with more diversity of taxa as poripheros, bryozoa, crustaceans, polychaeta bivalve, gastropods, nematodes, and showing the incidence of tide in renovation of water and inputs of phytoplankton and zooplankton support food webs and local fisheries. Conversely, the inner branches in the zone I section A and B of Estero Salado were where there were a distinct composition and diversity of aquactic insects showing the importance of salinity in the supervival of characteristic estuarine species.

The branches of zone I are differentiated by the type of pollutants and their prevalence, and section A characterized by the predominance of cadmium (Annex 27), especially observed in Bosques El Salado (residence zone) while section B is found the predominance of total petroleum hydrocarbon (Annex 28), lead (Annex 29), mercury (Annex 30), phosphates (Annex 31) and alkalinity (Annex 32) in the sediments from Miraflores (Industrial zone) and Aventura Plaza (Residence zone). It is the organic xenobiotics which presented the highest concentrations and, although present in protected areas, were more prevalent in urban areas.

Sites located in urban areas had higher concentrations of TPH in comparison with protected area (RPFMS). The highest concentrations were registered in Miraflores in 2007 probably at the moment of industrial discharges to channel attached to sports fields of the Ecuadorian Federation. However, the sediments in section A registered high concentrations in the residence zone in Parque Ecológico de Urdesa and Bosque Protector Salado Norte.

A lower concentration of dissolved oxygen was found in the urban area in Miraflores (0.06mg/L) and Alban Borja (0.05 mg/L) in 2007 so too Miraflores (0.19 mg/L) and Urdesa (0.35 mg/L) in the 2009 (Annex 33). However, sites as Kennedy-Universidad de Guayaquil reached greater concentrations with a concentration of 1.46 mg/L, due probably to the influence of the tidal and currents.

Despite the degradation of waters and sediments of the estuary's internal branches A and B (as the last remaining mangrove present in this area) (Urban Area), these still contribute as habitat and shelter for invertebrates, fish, birds, reptiles and mammals.

The tidal action produces a still important role in the water column that allows the increase of dissolved oxygen in these anoxic branches incorporates nutrients and the wide horizons of life, producing an increasing resilience of the estuarine organisms and generates possibility of survival and growth. The flow of the tide brings water from outer estuary providing phytoplankton, ichthyoplankton and zooplankton to sustain the energy flow to top ecological web links. Therefore it is suggested to avoid environmental unsustainable solutions such as constructions of bike paths in the estuarine channels, closing estuarine branches and directing the efforts to control, monitoring and enforcement of legal sanctions based on TULSMA and the Precautionary principle to prevent the discharge of wastewater without any treatment into the Estero Salado.

The techniques of bioaugmentation and biostimulation showed no significant difference in the degradation of hydrocarbons considering that the main factor that led to this was the lack of control variables in the natural medium as a continuous steady input of hydrocarbons and heavy metals as evidenced in Aventura Plaza sediments. This, and any other environmental restoration applied to contaminated sludge, may not have good results in reducing of TPH or be effectively evaluated under similar conditions, Hence, it is recommended to try the Estero Salado native bacterial consortium under controlled conditions in the laboratory such as testing with different concentrations of TPH, pH, temperature, salinity, and assessing minimal doses of nitrates and phosphates necessary to improve biostimulation in a microcosm simulating dry and rainy seasons. Later, with the knowledge gained, apply the technique in the field under strict control of effluents both in areas with higher and lower incidence of tide. This would assist robust evaluation of the effectiveness of bioremediation techniques in the natural conditions.

Sediments from Aventura Plaza showed very poor levels of macrobenthic diversity, the composition was given mainly by the presence of species of Diptera and other taxa characteristic of organic pollution and oligotrophic environments such as: Ceratopogonidae, Dolichopodidae*,* *Pericoma* sp, *Probezzia* sp., *Ablabesmyia* sp., *Chironomus* sp., oligochaeta and *Melanoides cf tuberculata* (exotic specie). There was an increase in the diversity of invertebrates throughout the period of bioremediation especially in station 3 but this diversity was given by the presence of insects generated by contribution of domestic waste waters in the study area. Similar composition and abundance of organisms were found along the bioremediation time. So the contribution of effluents from domestic and industrial wastes contribute in an important way to the community structure of macroinvertebrates, probably much more at low tide where there is less incidence of dilution in estuarine waters, so this would be a factor on the macrobenthic community more than the technique bioremediation.

The artificial substrate based on sponges indicate that this allows the fixation of bivalves and strong adhesion between the bissus and the surface in sponges was observed, this could provide a suitable substrate and source of food and nutrients are retained for longer in this substrate for the growth of species typical of the estuary. So it is necessary to develop a second phase to evaluate which native species settle and as the aggregator works in areas with higher incidence of tide and different stressors, since by the filtering action of mussels could in the near future to help the natural cleansing water in areas where they form larger aggregates.

This study will contribute to the Ministry with evidence necessary for the development of a remedial environmental management to prevent further environmental deterioration with organic xenobiotics and inorganics on the aquatic biota and to increase their concentrations could mean the total loss of aquatic and native biodiversity of the Estero Salado and could also affect human health with vapours of heavy metals and hydrocarbons emitted when the temperature increases and at low tide in residential areas bordering the estuary of Urdesa, Miraflores, Bosques El Salado and Kennedy.

This also will provide as input to the study on the economic estimate of the loss and environmental damage in this historic landmark estuary of great importance for the city of Guayaquil.

It is necessary to implement an environmental project in the long term based on the economic and political sustainability continued from studies in Guayaquil ecological project with a clear outline of specific actions and studies to ecological restoration of estuarine branches entering the city of Guayaquil where a management group is formed and where the participation of universities, academics, institutions that have direct competence in the management of this resource are led by the environmental authority (Ministry of Environment). This will help to develop viable solutions for the recovery of this important estuary.

The inclusion of Zone I and II in the National Protected Area System (SNAP) should be the beginning of this etrategy of recovery because it will give the legal base, policy for the survival of this long-term project.

It is essential that among the studies to be carried out in the: **Legal Area regarding** maximum values of pollutaning agents in sediments for freshwater, estuarine and marine waters, and: **Environment Area**) measurements of bioavailability of hydrocarbon and heavy metals concentrations in sediments, diffusion of contaminants from sediments (potential for spreading to food, bioaccumulation and biomagnification), biochemical, sub-lethal and ecotoxicology effects on biota mainly more sentinel as bivalves (Mytilidae), crustacean (shrimp, amphipods) advancing not only in ecological indicators but also genotoxic, physiological, morphological changes to comprehensively assess the environmental damage.

APPENDICES

Appendix A - Tables and Figures relevant to Chapter 3 to 6
(Pages 179-222)

Appendix B - Copy of publication Cárdenas-Calle, M. &Mair J.M, 2014
- Cover technical reports committed to Decentralized Autonomous Government of Guayas Provinces and Ministry of Environment of Ecuador
(Pages 223-236)

Annex 1. Multiannual mean values of climatological variables in Guayaquil during period 2007 to 2012.

Year	Heliofania (mm)	Temperature (oC)	Humidity (%)	Rainfall (mm)	Cloudiness (Octaves)	Evaporation (mm)	Mean Wind velocity (Km/h)
2007	1207	26.1	73	840	6	1631.6	0
2008	1043.1	26.1	74	1506.5	7	1481	10
2009	1404.1	26.4	72	1301.8	6	1703.5	27
2010	1030.9	26.4	75.5	1027.1	7	1029	26
2011	1265.4	26.4	73	705.5	7	1616.3	3
2012	1283.8	26.5	77	1650	6	1296.7	4
Multiannual Mean	1206	26	74	1172	7	1460	12

FAMILIY	GENUS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
STRATIOMYDAE	*Stratiomydae*									4						
CULICIDAE	*Anopheles*			3	2											
	Culicidae			3												
	Culex			13												
PSYCHODIDAE	Psychodidae									1						
	Elasmothemis	2														
	Libellulidae		1													
	Ischnura										1					
MEGAPODAGRIONID	*Megapodagrionidae*	11														
PROTONEURIDAE	*Neoneura*	1														
GOMPHIDAE	*Phyllocycla*										3					
HYDROPHILIDAE	Hydrophilidae	1				2	3	1		3		2				
	Hydroptila						3									
	Hydroptilidae	2	1								8	1				
HYDROPTILIDAE	*Oxyethira*										1					
	Smicridea	2														
HYDROPSYCHIDAE	*Oecetis*	2														
STAPHYLINIDAE	*Staphylinidae*															1
DYTISCIDAE	Dytiscidae	1										1				
NOTERIDAE	*Hydrocanthus*			2												
PSEPHENIDAE	*Psephenus*	1	1			1										
	Psephenidae															
ELMIDAE	*Heterelmis*	2				1						1				
	Cylloepus	15			1											
HYDROPHILIDAE	*Enochrus*											3				
	Hydrophilidae				3											
CURCULIONIDAE	*Curculionidae*										1	1				1
CORYDALIDAE	*Corydalus*								1							
ND	*Ostracodo*			8					2	1	161		3			2
ISOTOMIDAE	Isotomidae			4						1	135		2			
OCYPODIDAE	*Uca panamensis*											1				1
PALAEMONIDAE	*Macrobrachium panamense*													5	1	
	Macrobrachium carcinus															1
HYDROBIIDAE	*Heleobia*															
	Amnicola	317										1		4	1	6
	Hydrobiidae	26												10		
THIARIDAE	*Melanoides cf tuberculata*													2		
	Thiaridae	28		2							61					
PHYSIDAE	*Physa*	8														
LYMNAEIDAE	*Lymnaea*									1	4					
	Pseudosuccinea	1														
PLANORBIDAE	Planorbidae											3				
SPHAERIIDAE	*Musculium*	2														
	Pisidium	0		2						1	65	4		16	1	6
PISCICOLIDAE	*Pscicolidae*	3														
ND	*Planaria*	2				2										
ND	*Oligochaeta*	11														

FAMILIY	**GENUS**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
MESOVELIIDAE	*Mesovelia*										**8**					**3**
	Mesoveliidae										6					
	Microvelia			1				1			4	1				
	Rhagovelia	9														
CORIXIDAE	*Tenagobia*		50													
NOTONECTIDAE	*Martarega*															1
OCHTERIDAE	*Ochterus*			1												
BELOSTOMATIDAE	*Belostoma*	2										1	1			
	Belostomatidae	2														
HIDROMETRIDAE	*Veliometra*					1										
HEBRIDAE	*Lipogomphus*															3
GERRIDAE	*Trepobates*										1					
LEPTOHYPHIDAE	*Leptohyphes*	1														
	Tricorythodes	16														
	Traverhyphes	12														
	Yaurina	85														
	Leptohyphidae	20									1					
BAETIDAE	*Americabaetis*															1
	Baetidae	1									1		1			
	Callibaetis		4		22											
CHIRONOMIDAE	*Polypedilum*	9							1		1					7
	Chironomus				10	63			1							1
	Tanytarsus									31						
	Chironomidae			7	4	9	1	1		2	5					
	Dicrotendipes			2												
CERATOPOGONIDAE	*Allauydomyia*				1		1			12	1					1
	Stilobezzia							1			2					

FAMILIY	**GENUS**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
MESOVELIIDAE	*Mesovelia*			**18**														
VELIIDAE	*Platyvelia*																	
	Microvelia			**2**								**5**			**1**		**2**	**1**
	Rhagovelia																**73**	
CORIXIDAE	*Tenagobia*			**8**					**8**									
OCHTERIDAE	*Ochterus*																	**1**
BELOSTOMATIDAE	*Belostoma*					**1**												
HEBRIDAE	*Lipogomphus*																	**1**
GERRIDAE	*Trepobates*					**1**											**1**	
	Leptohyphodes			**8**														
	Allenhyphes				**2**													**1**
	Tricorythodes			**13**	**5**				**1**								**7**	**8**
	Traverhyphes				**1**													
	Leptohyphidae			**23**	**1**												**5**	**5**
	Camelobaetidius								**17**									
	Baetidae													**1**			**1**	
	Callibaetis			**21**			**2**							**15**		**14**		
POLYMITARCYIDAE	*Camprurus*									**1**								
CHIRONOMIDAE	*Polypedilum*				**1**													
	Endotribelos																	**1**
	Pseudochironomus								**6**									
	Paratanytarsus								**12**									
	Chironomidae			**7**		**1**	**1**		**16**						**2**			**3**
	Dicrotendipes			**5**														
CERATOPOGONIDAE	*Allauydomyia*			**2**			**1**											
	Stilobezzia							**8**										
STRATIOMYDAE	*Stratiomydae*																	**1**
TIPULIDAE	*Tipula*											**1**						
CULICIDAE	*Anopheles*															**1**		
PSYCHODIDAE	Psychodidae			**2**														
LIBELLULIDAE	*Orthemis*								**1**									
	Leptobasis					**1**												

FAMILIY	**GENUS**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
CALOPTERYGIDAE	*Hetaerina*																**6**	**7**
GOMPHIDAE	*Phyllocycla*													**1**				
PYRALIDAE	*Pyralidae*												**1**					**3**
HYDROPHILIDAE	Hydrophilidae					**1**									**1**			
	Hydroptilidae			**2**										**3**				**2**
HYDROPTILIDAE	*Oxyethira*																**3**	**2**
	Smicridea								**1**								**1**	
HYDROPSYCHIDAE	*Oecetis*			**4**													**2**	**8**
DYTISCIDAE	Dytiscidae			**5**														
PSEPHENIDAE	*Psephenus*			**1**														
	Stegoelmis				**1**													
	Elmidae			**6**		**1**												
	Heterelmis																**1**	
	Cylloepus				**3**													**6**
CURCULIONIDAE	*Curculionidae*			**2**														
ND	*Ostracodo*								**56**									
ISOTOMIDAE	Isotomidae								**70**									
OCYPODIDAE	*Uca panamensis*		**2**				**1**											
PALAEMONIDAE	*Macrobrachium panamense*									**2**			**1**					
	Macrobrachium gallus			**3**						**6**			**14**	**12**			**4**	
	Macrobrachium carcinus	**1**	**1**							**1**			**20**					
	Zoeas de caridea			**4**						**4**			**3**					
HAUSTORIDAE	*Haustoriidae*					**1**	**1**			**1**	**3**	**3**						
HYALELLIDAE	*Hyalella*						**1**		**16**									

FAMILIY	GENUS	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
BOPYRIDAE	*Probopyrus*												1					
AMPULLARIIDAE	*Pomacea canaliculata*								18			5	1					
	Pomacea sp.														2			
HYDROBIIDAE	*Heleobia*														4			
	Amnicola					22	1					1			2			
	Hydrobiidae				1										25			
THIARIDAE	*Melanoides cf tuberculata*																	
	Thiaridae			18					5						2415		5	23
PHYSIDAE	*Physa*																4	1
LYMNAEIDAE	*Lymnaea*			1											2			
	Pseudosuccinea			1														
	Hebetancylus						1											
	Gundlachia																	1
CORBICULIDAE	*Corbicula cf fluminea*						1				1							
SPHAERIIDAE	*Musculium*								20	3							6	1
	Pisidium			20	1	22	3		23	2	1	6	1				9	25
	Sphaeriidae									1					18		2	
GLOSSIPHONIDAE	*Helobdella*												1					
ND	Planaria														18			
ND	Oligochaeta														10			

FAMILIY	**GENUS**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**
MESOVELIIDAE	*Mesovelia*			**1**							
	Mesoveloidea					**1**					**1**
	Microvelia			**1**		**2**					
	Rhagovelia	**33**		**4**		**95**	**3**	**1**	**2**	**2**	**20**
CORIXIDAE	*Tenagobia*		**5**	**1**		**107**	**5**				**155**
NAUCORIDAE	*Cryphocricos*	**2**									
	Limnocoris	**3**	**3**		**1**	**23**	**3**		**1**	**1**	**4**
NOTONECTIDAE	*Martarega*				**1**						
GERRIDAE	*Trepobates*		**11**		**2**			**1**		**1**	**1**
LEPTOHYPHIDAE	*Leptohyphes*	**10**				**5**		**1**		**7**	**2**
	Allenhyphes										**2**
	Tricorythodes	**85**				**8**	**3**		**37**	**13**	**24**
	Leptohyphidae	**4**					**4**	**2**		**15**	**2**
LEPTOPHLEBIIDAE	*Thraulodes*							**1**	**3**		
BAETIDAE	*Americabaetis*							**2**			
	Baetodes					**2**					
	Baetidae							**1**			
	Callibaetis	**2**		**1**					**5**	**1**	**19**
POLYMITARCYIDAE	*Camprurus*										
CHIRONOMIDAE	*Polypedilum*	**1**						**1**	**3**		
	Chironomidae	**61**		**7**			**1**				**2**
CERATOPOGONIDAE	*Allauuydomyia*	**5**				**70**	**4**				
STRATIOMYDAE	*Stratiomydae*					**6**	**2**				

FAMILIY	**GENUS**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**
CULICIDAE	*Culex*	**1**									
SIMULIIDAE	*Simuliidae*	**1**									
LIBELLULIDAE	Libellulidae					**1**					
COENAGRIONIDAE	*Coenagrionidae*										**1**
CALOPTERYGIDAE	*Hetaerina*	**3**							**2**		
PROTONEURIDAE	*Neoneura*	**1**									
GOMPHIDAE	*Phyllocycla*								**1**		
	Progomphus									**1**	
HYDROPHILIDAE	Hydrophilidae	**2**				**19**	**2**				**3**
	Tropisternus					**8**					
HYDROPTILIDAE	*Oxyethira*					**4**					
	Smicridea	**1**								**2**	**11**
HYDROPSYCHIDAE	*Oecetis*	**20**						**1**		**21**	
LEPTOCERIDAE	*Leptoceridae*	**2**									
	Anastomoneura	**2**							**1**		
STAPHYLINIDAE	*Staphylinidae*	**1**									
	Psephenidae	**7**		**1**		**1**	**3**				
ELMIDAE	*Macrelmis*			**1**							
ELMIDAE	*Neoelmis*	**5**									
	Stegoelmis										**1**
	Hexanchyorus	**1**								**1**	
	Heterelmis	**2**				**4**					
	Cylloepus	**10**		**1**		**24**	**4**	**3**	**1**	**5**	**1**
	Disersus						**1**				
CHRYSOMELIDAE	Chrysomelidae										**1**
CURCULIONIDAE	Curculionidae					**11**					
LEPTOCERIDAE	Leptoceridae										**1**
BUPRESTIDAE	Buprestidae	**1**									
CICADELLIDAE	Cicadellidae					**1**					
ND	Ostracodo										**1**
HYDROBIIDAE	*Pomacea* sp.									**6**	
	Heleobia									**1**	
	Amnicola									**20**	
	Hydrobiidae	**2**								**1**	
THIARIDAE	*Melanoides cf tuberculata*									**1**	
	Thiaridae			**2**				**42**		**188**	
LYMNAEIDAE	*Lymnaea*	**4**									
	Pseudosuccinea	**8**									
ANCYLIDAE	*Antillorbis*					**1**					
	Hebetancylus									**1**	
	Uncancylus	**1**									
	Laevapex									**3**	
	Ancylidae									**5**	
SPHAERIIDAE	*Musculium*							**25**			
	Pisidium	**15**		**2**		**1**		**42**		**0**	
ND	Hirudinea	**1**									

Annex 3. Analysis Summary of Descriptive Statistic of Richness (R), Abundance (N), Diversity (H´) and Eveness (J´) registered in the Subwatershed of Guayas River during November of 2012 and March of 2013.

Depende Variable: S
Factor : Subwatershed

Name /Subwatershed	Subwatershed	Count	Average	Median	Standard desviation	Standard error	Minimun	Maximum
Out the watershed	0	6	15.83	16	4.7	1.92	10	21
Daule	1	22	8.4	7	5.65	1.21	1	23
Yaguachi	3	6	12.6	10	11	4.49	3	32
Macul	5	1	30	30	0	30	30	30
Jujan	6	4	12.75	14.5	3.94	3.94	2	20
Drenajes menores	7	4	5.75	5.5	3	3	3	9
	Total	43	10.72	9	1.14	1.14	1	32

Depende Variable: N
Factor : Subwatershed

Name /Subwatershed	Subwatershed	Count	Average	Median	Standard desviation	Standard error	Minimun	Maximum
Out the watershed	0	6	192.6	187.5	143.6	58.62	35	394
Daule	1	22	64.5	26	110.4	23.55	3	470
Yaguachi	3	6	140.3	32.5	190.6	77.8	4	457
Macul	5	1	595	595	0	595	595	595
Jujan	6	4	687	116.5	1209	604.8	15	2500
Drenajes menores	7	4	19.75	21	11.12	5.56	5	32
	Total	43	159.04	37	393.9	60.08	3	2500

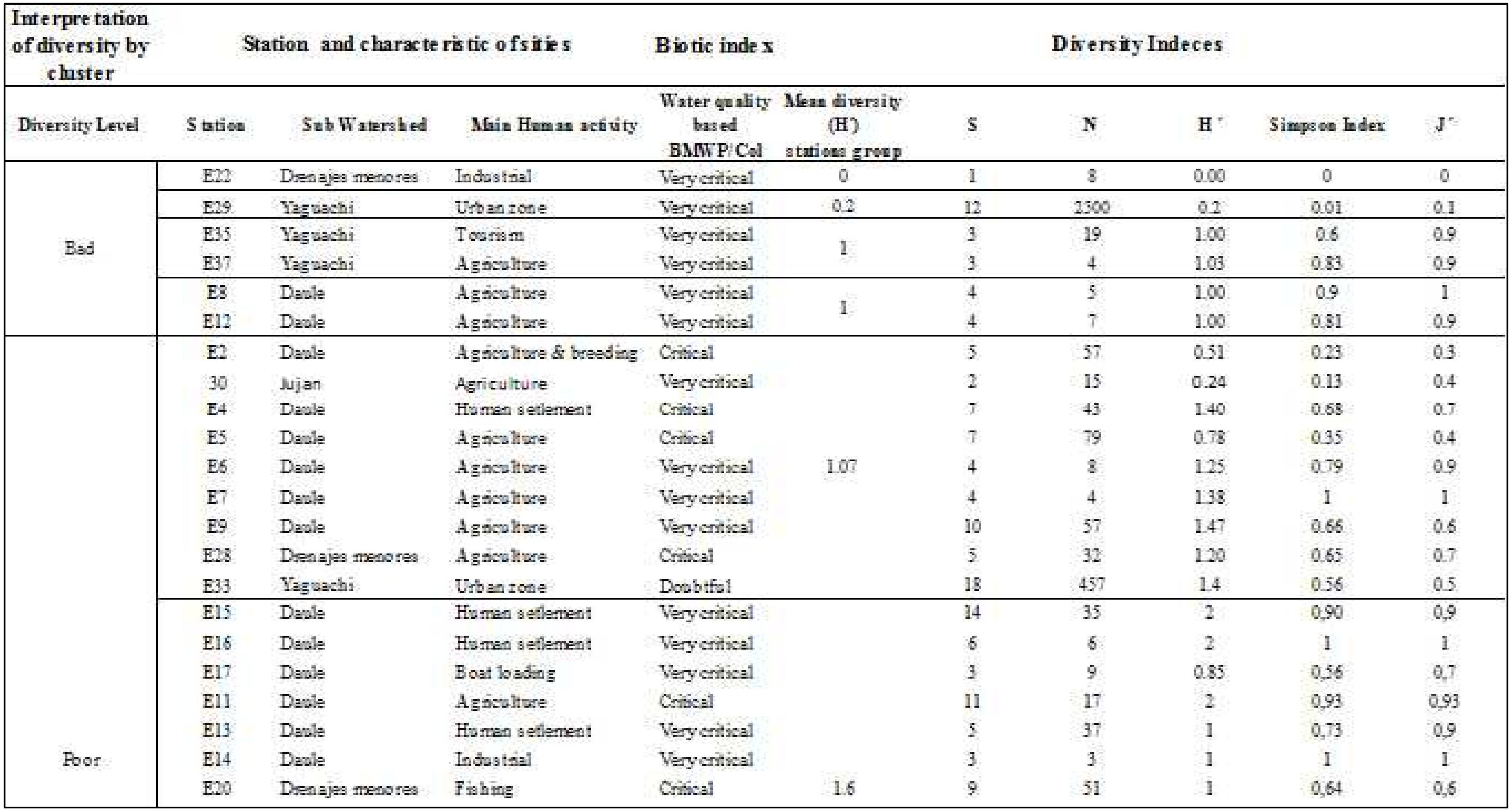
Depende Variable: H´
Factor : Subwatershed

Name /Subwatershed	Subwatershed	Count	Average	Median	Standard desviation	Standard error	Minimun	Maximum
Out the watershed	0	6	1.73	1.53	0.42	0.17	1.32	2.39
Daule	1	22	1.51	1.14	0.66	1.14	0	2.73
Yaguachi	3	6	1.56	1.42	0.58	0.24	1	1.44
Macul	5	1	1.8	1.8	0	1.8	1.08	0
Jujan	6	4	1.16	1.04	1.1	0.55	2.36	2.16
Drenajes menores	7	4	1.44	1.4	0.43	0.22	0.97	0.97
	Total	43	1.52	1.47	0.63	0.09	0	2.73

Depende Variable: J´
Factor : Subwatershed

Name /Subwatershed	Subwatershed	Count	Average	Median	Standard desviation	Standard error	Minimun	Maximum
Out the watershed	0	6	0.65	0.6	0.19	0.08	0.5	1
Daule	1	22	0.77	0.9	0.26	0.06	0	1
Yaguachi	3	6	0.77	0.8	0.16	0.06	0.5	0.9
Macul	5	1	0.5	0.5	0	0	0.5	0.5
Jujan	6	4	0.48	0.5	0.3	0.15	0.1	0.8
Drenajes menores	7	4	0.85	0.9	0.05	0.05	0.7	0.9
	Total	43	0.73	0.8	0.24	0.04	0	1

Annex 4. Resume of indices and main characteristic of studied sites in Guayas river watershed during 2012-2013. Diversity Indexes: Biotic Index (BMWP/Col), Species Richness (S); Total Number of Individuals (N); Shannon Wiener (H´); Simpson Index (1-Lamda´) and Equitability (J´).



Interpretation of diversity by cluster	Station and characteristic of sities			Biotic index		Diversity Indeces				
Diversity Level	Station	Sub Watershed	Main Human activity	Water quality based BMWP/Col	Mean diversity (H') stations group	S	N	H´	Simpson Index	J´
Bad	E22	Drenajes menores	Industrial	Very critical	0	1	8	0.00	0	0
	E29	Yaguachi	Urban zone	Very critical	0.2	12	2300	0.2	0.01	0.1
	E35	Yaguachi	Tourism	Very critical	1	3	19	1.00	0.6	0.9
	E37	Yaguachi	Agriculture	Very critical		3	4	1.03	0.83	0.9
	E8	Daule	Agriculture	Very critical	1	4	5	1.00	0.9	1
	E12	Daule	Agriculture	Very critical		4	7	1.00	0.81	0.9
Poor	E2	Daule	Agriculture & breeding	Critical	1.07	5	57	0.51	0.23	0.3
	30	Jujan	Agriculture	Very critical		2	15	0.24	0.13	0.4
	E4	Daule	Human setlement	Critical		7	43	1.40	0.68	0.7
	E5	Daule	Agriculture	Critical		7	79	0.78	0.35	0.4
	E6	Daule	Agriculture	Very critical		4	8	1.25	0.79	0.9
	E7	Daule	Agriculture	Very critical		4	4	1.38	1	1
	E9	Daule	Agriculture	Very critical		10	57	1.47	0.66	0.6
	E28	Drenajes menores	Agriculture	Critical		5	32	1.20	0.65	0.7
	E33	Yaguachi	Urban zone	Doubtful		18	457	1.4	0.56	0.5
	E15	Daule	Human setlement	Very critical	1.6	14	35	2	0,90	0,9
	E16	Daule	Human setlement	Very critical		6	6	2	1	1
	E17	Daule	Boat loading	Very critical		3	9	0.85	0,56	0,7
	E11	Daule	Agriculture	Critical		11	17	2	0,93	0,93
	E13	Daule	Human setlement	Very critical		5	37	1	0,73	0,9
	E14	Daule	Industrial	Very critical		3	3	1	1	1
	E20	Drenajes menores	Fishing	Critical		9	51	1	0,64	0,6

	E21	Drenajes menores	Fishing	Critical		10	13	2	0,95	1
	E24	Drenajes menores	Agriculture	Very critical		9	21	1.97	0,88	0,9
	E25	Drenajes menores	Agriculture	Very critical		3	5	1.00	0,7	0,9
	E26	Drenajes menores	Urban zone	Very critical		6	21	1.60	0,82	0,9
	E27	Yaguachi	Agriculture	Very critical		9	43	1.43	0,69	0,7
	E43	Out Watershed	Agriculture	Acceptable		19	252	1.50	0.6	0.5
	E19	Drenajes menores	Agriculture	Doubtful	1.65	9	16	2	0.88	0.9
	E41	Out Watershed	Agriculture	Acceptable		10	56	1.32	0.56	0.6
	E1	Macul	Agriculture	Acceptable	1.88	30	595	2	0.68	0.5
	E42	Out Watershed	Agriculture	Acceptable		20	296	1.51	0.58	0.5
	E18	Drenajes menores	Agriculture	Very critical		23	176	3	0.92	0.9
	E40	Out Watershed	Agriculture	Very critical		13	123	1.55	0.73	0.6
	E31	Jujan	Agriculture	Very critical		17	132	1.83	0.68	0.6
Moderate	E3	Daule	Agriculture	Very critical	2.06	12	48	2	0.87	0.9
	E10	Daule	Agriculture & Breeding	Doubtful		20	470	2	0.76	0.58
	E23	Out Watershed	Fishing	Critical		15	270	2.23	0.86	0.8
	E36	Yaguachi	Agriculture	Critical		11	22	2.10	0.87	0.9
	E32	Jujan	Agriculture	Acceptable	2.32	20	101	2.36	0.87	0.8
	E34	Yaguachi	Tourims	Acceptable		32	297	2.44	0.85	0.7
	E38	Out Watershed	Tourims	Acceptable		21	394	2.10	0.83	0.7
	E39	Out Watershed	Tourims	Doubtful		12	35	2.39	0.93	1

Annex 5. Resumen of Water quality of 43 sites of Guayas provinces using the Biotic Index BMWP/COL.

Station (No)	County	River	Water quality
1	El Empalme	Rio Macul	**Acceptable**
2	Balzar	Rio Pucon	**Critical**
3		Rio Puca	**Very critica**
4		Rio Daule frente al recinto San Felipe	**Critical**
5		Río Daule Salida de Balzar	**Critical**
6	Colimes	Rio Colimes antes de unirse al río Daule	**Very critica**
7	Palestina	Rio Daule antes de llegar al pueblo de palestina	**Very critica**
8	Santa Lucía	Rio Daule antes de llegar al pueblo viniendo de Palestina	**Very critica**
9	Pedro Carbo	Rio Procel frente al Recinto la Providencia	**Very critica**
10		Rio Pedro Carbo	**Doubtful**
11	Lomas de Sargentillo	Rio Bachillero	**Critical**
12	Isidro Ayora	Rio Paco	**Very critica**
13	Daule	Río Daule Antes de la union del río Banife	**Very critica**
14		Rio Daule - Pascuales	**Very critica**
15	Nobol	Rio Daule frente al Santuario Narcisa de Jesus	**Very critica**
16		Río Daule Malecon del Santuario de la Narcisa de Jesús	**Very critica**
17		Zona de desembarque de canoas en el Malecon de Nobol	**Very critica**
18	Guayaquil	Rio Chongon	**Doubtful**
19		Rio Cangaguilla	**Critical**
20		Rio Guayas a la altura de la isla Santay Estero La Bocana	**Critical**
21		Rio Guayas -Isla Santay Hda La Puntilla	**Critical**
22	Durán	Estero San Camilo Km 2 salida norte de Durán a Guayaquil .	**Very critica**
23	Playas	Rio del Mate entre Guayaquil y Playas	**Critical**
24	Samborondón	Ramales del rio Vinces que se une con el rio Babahoyo	**Very critica**
25		Rio Babahoyo antes de la union con el río Yaguachi (Hacienda Monterrey)	**Very critica**
26		Rio Babahoyo- Ciudad Celeste	**Very critica**

27	Yaguachi	Rio Yaguachi antes de desembocar al río Babahoyo frente a Samborondon	**Very critica**
28	Salitre	Pueblo Nuevo aguas arriba del pueblo	**Crítica**
29	Milagro	Río Milagro bajo el Puente de Milagro	**Very critica**
30	Jujan:Alfredo Baquerizo Moreno	Rio Jujan	**Very critica**
31		Rio Chilintomo	**Doubtful**
32	Simón Bolívar	Rio Los Amarillos	**Aceptable**
33		Rio Chimbo	**Doubtful**
34	Bucay: Gral Antonio Elizalde	Rio San Antonio - Matilde Esther	**Acceptable**
35		Poza Agua Clara que luego sus aguas se vierten al rio Chimbo	**Very critica**
36	Naranjito	Rio Venecia	**Crítica**
37	Marcelino Maridueña	Unión del río Chimbo y Chanchan	**Very critica**
38	El Triunfo	Rio Dos bocas	**Acceptable**
39		Rio San Isidro	**Doubtful**
40	Naranjal	Rio Ruidoso	**Very critica**
41		Rio Cañar	**Acceptable**
42		Rio Blanco	**Acceptable**
43	Balao/Tengel	Balao Rio Gala	**Acceptable**

Annex 6. Physicochemical parameters of Temperature (^oC), pH, Oxygen (mg/L), Biochemical Oxygen Demand (mg/L) and Totals Suspended Solids (mg/L) for the water samples collected at Inner branches of Estero Salado between June and July of 2007.

Site	Date	Time	Soil Depth (m)	Temperature (oC)	pH	Dissolved Oxygen (mg/l)	BOD 5 (mg/l)	Totals Suspended Solids (mg/l)
Puerto Hondo camaronera	07-jun-07	12:20	2	25.1	7.34	3.25	0.17	163
Muelle Puerto Hondo	07-jun-07	12:45	1	25.1	7.36	3.15	0.19	163
Terminal Portuario Internacional	06-jun-07	12:15	3	25.1	6.23	3.17	0.18	147
Muelle Puerto Azul	06-jun-07	13:45	3.50	26.2	7.94	2.33	0.18	169
Puerto Azul	06-jun-07	14:30	2	29.1	8	2.91	0.18	151
Estación Tres Bocas	06-jun-07	16:00	1	26.2	7.95	3.54	0.18	293
Estero del Muerto	06-jun-07	17:05	0,8	26.2	7.97	4.97	0.17	189
Miraflores	12-jul-07	14:00	0.5	28.3	7.5	0.06	22	22
Alban Borja	12-jul-07	15:50	0.5	26.8	7.3	0.05	64	116

Annex 7. Statistical parameter (mean, 95% confidence level for the mean, minimum and maximum value) of contaminants concentrations in sediments collected at the inner branches of Estero Salado.

Parameter	Year	Minimum	Maximum	Mean	Median	SD	Limit of Tolerance
TPH (mg/Kg-1)	2009	69	1124	315.96	202.76	321.55	(1125/69)
	2011	237	2825	1249.45	822.77	1086.59	
	2011-2012	227	994	560.33	556	262.46	(994/227)
Hg (mg/Kg-1)	2009	0.1	0.89	0.20	0.1	0.23	(0.89/0.1)
	2011	0.14	0.3	0.27	0.3	0.05	
	2011-2012	0.1	0.34	0.19	0.16	0.08	(0.34/0.1)
Cd (mg/Kg-1)	2009	0.7	1.84	1.04	0.78	0.43	(1.84/0.7)
	2011	2.57	4.88	3.72	3.79	0.68	
	2011-2012	0.1	0.72	0.27	0.26	0.15	(0.72/0.1)
Pb (mg/Kg-1)	2009	19	37.89	20.57	19	5.45	(37.89/19)
	2011	29.9	10.26	40.18	39.58	10.26	(59.33/29.9)
	2011-2012	12.02	53.9	23.17	19.62	12.52	(53.9/12.02)

Annex 8. Statistical parameter (mean, 95% confidence level for the mean, minimum and maximum value) of nutrients concentrations in sediments collected at the inner branches of Estero Salado.

Parameter	Year	Minimum	Maximum	Mean	Median	SD	Limit of Tolerance (Upper- Lower) with 95% confidence
Nitrate (mg/Kg-1)	2009	0.10	0.80	0.23	0.10	0.22	(0.8/0.1)
	2011	0.50	6.32	1.84	0.89	2.12	(6.32/0.5)
	2011-2012	0.50	30.00	7.47	6.10	7.88	(30/0.5)
Nitrite (mg/Kg-1)	2009	0.07	0.49	0.08		0.16	
	2011	0.029	2.22	0.58	0.09	0.88	(2.22/0.03)
	2011-2012	0.01	0.17	0.06	0.03	0.05	(0.17/0.01)
Phosphate (mg/Kg-1)	2009	0.06	1.18	0.62	0.6	0.36	(1.18/0.06)
	2011	2.00	15.90	7.65	5.75	4.90	(15.9/2.0)
	2011-2012	8.30	151.23	50.25	24.98	52.08	(151.23/8.3)

Annex 9. Results of PCA for the environmental variables responsible of the differences between sites in the inner branches of Estero Salado during 2009 and 2011.

Lambda	Eigenvalue	Proportion	Cumulative
PC1	8.2	0.82	0.82
PC2	1.8	1.18	1
COEFFICIENTS			
Variables	PC1	PC2	
TPH	0.33	0.21	
Hg	0.35	0.08	
Cd	0.33	-0.26	
Pb	0.33	-0.24	
NO3	0.33	0.14	
NO2	0.34	0.15	
PO4	0.29	-0.42	
H2S	-0.33	-0.24	
OM	0.04	0.74	
pH	0.35	-0.07	

Annex 10. Table of spatial variation of macroinvertebrates taxa registered in sediments at the two areas in Estero Salado Estuary: Protected area (I) and urban area (II) at November 2009.

ETB19: Estación Tres Bocas boya 19; ETBS: Estación Tres bocas Muelle suinsa;ETBPA: Estación Tres Bocas Puerto Azul;TPIc:Terminal Portuario Internacional centro;TPIm: Terminal Portuario Internacional antes del Muelle;TPIh: Terminal Portuario Internacional Puerto Hondo: CUAc: Cuarenta centro; CUAe: Cuarentena este;CUAo: Cuarentena oeste; URG: Urdesa;KUG: Kennedy-Universidad de Guayaquil and MIR: Miraflores.

Area	Protected Area (I)									Urban Area (II)		
Taxa	**ETBb19**	**ETBS**	**ETBPA**	**TPIC**	**TPI m**	**TPIh**	**CUAc**	**CUAe**	**CUAo**	**URD**	**KUG**	**MIR**
Nematode sp1.	0	0	0	1	0	0	1	0	0	0	9	0
Capitella cf capitata	0	0	0	0	0	0	0	0	0	380	17	199
Polychaeta sp1.	10	14	2	0	0	0	6	0	0	43	0	0
Eunice sp.	0	0	7	0	0	0	0	0	0	0	0	0
Nephtyidae	0	0	0	0	0	0	0	0	2	0	0	0
Nepthys squamosa	0	0	0	8	0	0	0	2	0	0	0	0
Pericoma sp.	0	0	0	0	0	0	0	0	0	15	112	123
Probezzia sp.	0	0	0	0	0	0	0	0	0	0	25	0
Pupa Psychodidae	0	0	0	0	0	0	0	0	0	3	40	6
Phoridae	0	0	0	0	0	0	0	0	0	0	0	1
Dolichopodidae	0	0	0	0	0	0	0	0	0	0	1	0
Ephydridae	0	0	0	0	0	0	0	0	0	0	1	0
Tipulidae	0	0	0	0	0	0	0	0	0	2	3	0
Hydracarina	0	0	0	0	0	0	0	0	0	1	3	0
Uca sp.	0	0	5	0	0	0	0	0	0	0	0	0
Podura aquatica	0	0	0	0	0	0	0	0	0	0	164	0
Crepidula sp.	0	0	0	0	0	0	0	0	0	0	1	0
Tellina cf *suffusa*	0	0	1	0	0	0	0	0	0	0	0	0
Tellina sp.	0	0	18	0	1	0	0	0	0	0	0	0
Tellina simulans	0	0	0	1	0	0	0	0	0	0	0	0
Cyrenoida inflata	0	0	0	0	0	0	0	0	0	0	18	0
Ameae sp.	0	0	0	0	0	2	0	0	0	0	0	0
Detracia graminea	0	0	0	0	0	0	0	0	0	0	16	0
Littorina aberrans	0	0	0	0	0	0	0	0	0	0	37	0
Melanoides cf tuberculata	0	0	0	0	0	0	0	0	0	0	3	0
Cyclostremiscus cf beauii	0	0	0	4	0	0	0	0	0	0	0	0
Costoanachis nigricans	0	0	48	0	0	0	0	0	0	0	0	0
Cylichna sp.	0	7	14	13	0	0	0	0	0	0	0	0
Cerithidea mazatlanica	1	0	2	0	0	14	0	0	0	0	0	0
Cerithidea valida	0	0	12	0	0	0	0	0	0	0	0	0
Cosmioconcha sp.	1	0	70	0	0	0	0	0	0	0	0	0
Anachis sp.	0	0	6	0	1	0	0	0	0	0	0	0
Melampus cf *mousleyi*	0	1	0	0	0	0	0	0	0	0	0	0
Gastropod sp1.	0	1	0	0	0	0	0	0	0	0	0	0
Natica sp.	0	0	0	0	0	0	1	0	0	0	0	0
Drepanotrema	0	0	0	0	0	0	0	0	0	0	0	1
Ophiuroidea	0	0	13	0	0	0	0	0	0	0	0	0
N	12	23	198	27	2	16	8	2	2	444	450	330

Annex 11.Table of spatial variation of macroinvertebrates taxa registered in sediments at the urban area in Estero Salado Estuary: Residence and Industrial zone at January of 2011.

PEU: Parque Ecológico Urdesa, BPSN: Bosque Salado Norte, BES: Bosque El Salado, PMIR: Puente Miraflores-Urdesa, KUG: kennedy-Universidad de Guayaquil.

Taxa	Urban Area				
	Residence			Industrial	
	PEU	BPSN	BES	PMIR	KUG
Pericoma sp.	0	**0**	**6**	**6**	**16**
Hydrophorus sp.	0	**0**	**39**	**25**	**15**
Probezzia sp.	0	**0**	**0**	**0**	**1**
Ceratopogonidae	0	**0**	**0**	**0**	**8**
Culicoides sp.	0	**0**	**48**	**201**	**14**
Pupa Diptera	2	**0**	**5**	**58**	**0**
Larva Diptera	0	**0**	**2**	**1**	**0**
Podura aquatica	0	**2**	**0**	**0**	**416**
Talitridae	0	**0**	**0**	**5**	**0**
Nemertine sp1	0	**0**	**6**	**0**	**3**
Nematode sp1	0	**0**	**8**	**0**	**1**
Capitella cf capitata	0	**0**	**0**	**365**	**13**
Micronereis sp.	0	**0**	**1**	**284**	30
Bivalve sp1	0	**0**	**23**	**0**	**184**
Mytilposis sp.	0	**0**	**1**	**0**	**91**
Hidrobiidae	1	**2**	18	**0**	**24**
Planorbidae	0	**0**	**0**	**0**	**13**
Melampus cariolanus	0	**0**	**0**	**0**	**4**
Marinula concinna	0	**0**	**10**	**0**	**19**
Detracia graminea	0	**0**	**0**	**0**	**2**
Melanoides cf tuberculata	3	**2**	**1**	**0**	**104**
Gastropod sp1	0	**0**	**0**	**0**	**12**
ND	0	**0**	**0**	**0**	**4**

Annex 12. Test and their significance levels (P) for ANOVA for the Number of species, number of individuals and diversity indexes of the benthic macroinvertebrates at the two area areas in Estero Salado Estuary: Protected Area (I) and Urban Area (II) at November 2009. Significant results are highlighted in bold.

Parameter	ANOVA Test	Test Statistic	P
Number of species (S)	Kruskal Wallis	4.20	**<0.04**
Number of individuals (N)	Kruskal Wallis	6.32	**<0.011**
Shannon-Wiener Index (H´)	Fisher	0.73	0.414
Simpson Index	Fisher	0.06	0.810
Eveness (J)	Fisher	0.09	0.775

Annex 13. Statistical parameters (minimum, maximum, mean, median, standard deviation and 95% confidence level for the mean of the Number of species, number of individuals and diversity indexes of the benthic macroinvertebrates at the two area areas in Estero Salado Estuary: Protected area (I) and urban area (II) at November 2009.

Parameter	Minimum	Maximum	Mean	Median	SD	95%Confidence Interval for Mean
Number os species	1	15	4.92	3.5	4.36	(15 -1)
Number of individuals	2	450	126.17	19.5	180.52	(450 -2)
Shannon-Wiener Index	0	1.9	0.81	0.72	0.62	(1.9-0)
Simpson Index	0	1	0.54	0.6	0.3	(1-0)
Eveness	0	1	0.47	0.32	0.48	(1-0)

Annex 14. Statistical parameters (minimum, maximum, mean, median, standard deviation and 95% confidence level for the mean of the Number of species, number of individuals and diversity indexes of the benthic macroinvertebrates macroinvertebrates in sediments at the urban area in Estero Salado Estuary: Residence and Industrial zone at January of 2011.

Parameter	Minimum	Maximum	Mean	Median	SD	95%Confidence
Number of species	3	19	9.2	6.47	8	(19 -3)
Number of individuals	6	970	418.4	165	468	(970-6)
Shannon-Wiener Index	1.01	2	1.47	1.39	0.43	(2-1.010)
Simpson Index	0.71	0.83	0.77	0.76	0.05	(1-0.64)
Eveness	0.64	1	0.81	0.78	0.14	(0.83-0.71)

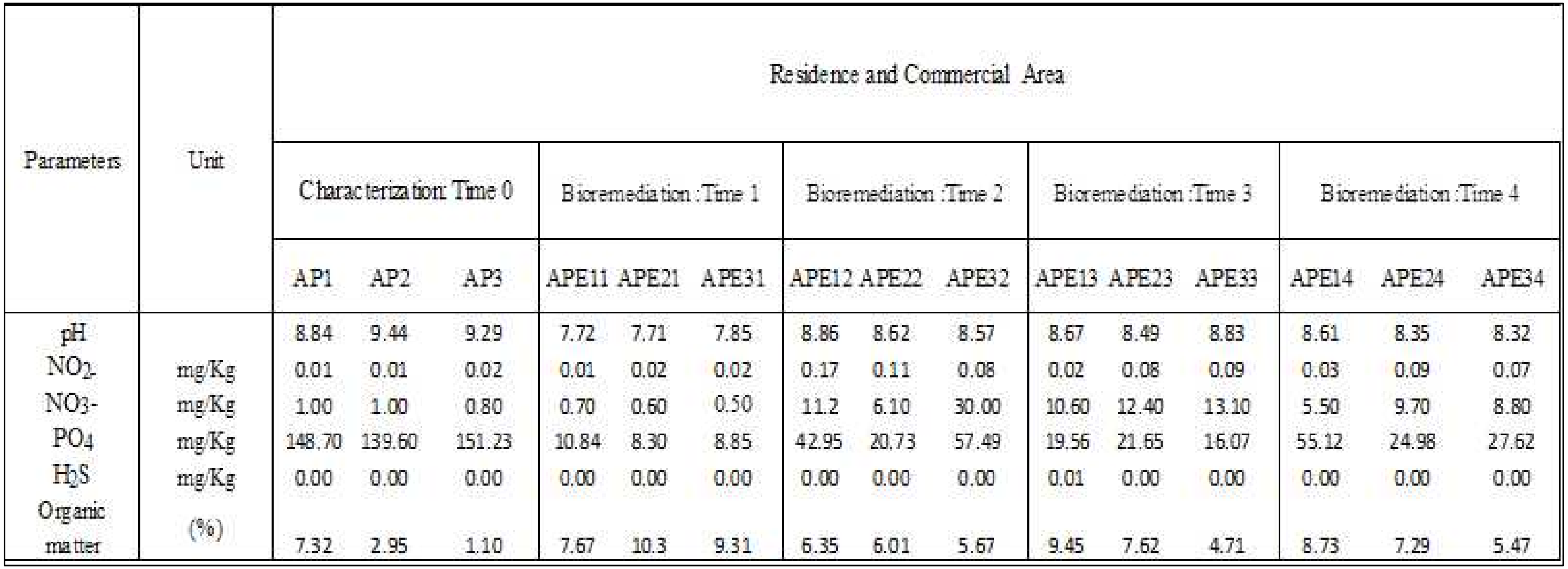
Annex 15. Test and their significance levels (P) for ANOVA for the Number of species, number of individuals and diversity indexes of the benthic macroinvertebrates in sediments at the urban area in Estero Salado Estuary: Residence and Industrial zone at January of 2011. Significant results are highlighted in bold.

Parameter	**ANOVA Test**	**Test Statistic**	**P**
Number of species (S)	Kruskal Wallis	3.16	**0.750**
Number of individuals (N)	Kruskal Wallis	7.11	**<0.007**
Shannon-Wiener Index (H´)	Kruskal Wallis	0.75	0.386
Simpson Index	Fisher	3.93	0.082
Eveness (J)	Kruskal Wallis	6.75	**<0.009**

Annex 16. Total Hydrocarbon Petroleum and Heavy Metal concentration (mg/Kg^{-1} dry weight) in superficial mangrove sediments in Urban Area (Residential and Commercial zone: Aventura Plaza) in the inner branches of Estero Salado from December 2011 to March of 2012.

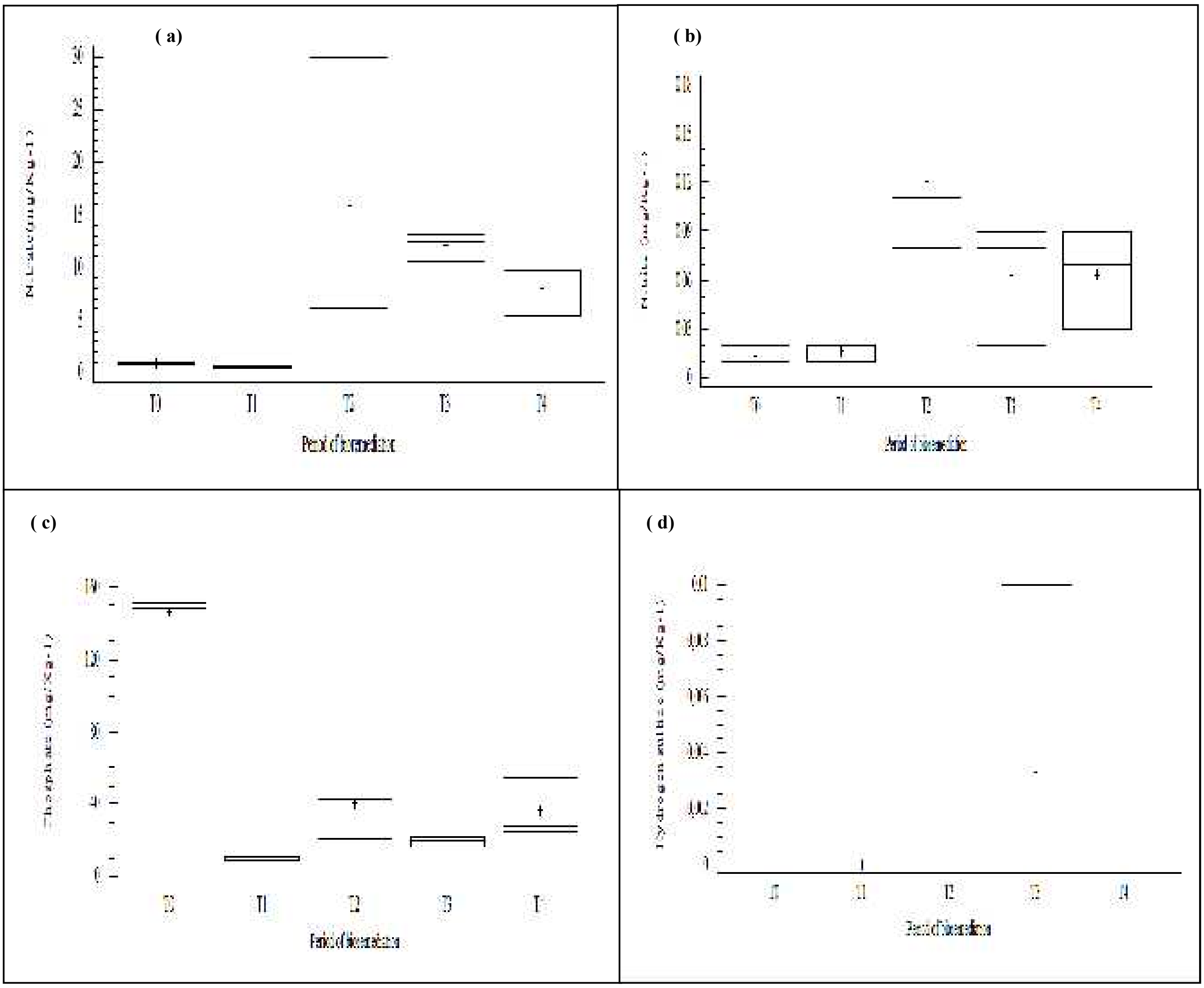
Group substance		Residential and Commercial Zone																	
		Characterization: Time 0			Bioremediation :Time 1			Bioremediation :Time 2			Bioremediation :Time 3			Bioremediation :Time 4			EQS-Ecuador (mg/kg-1)		<ERL - EPA (ug/kg-1)
		AP1	AP2	AP3	APE11	APE21	APE31	APE12	APE22	APE32	APE13	APE23	APE33	APE14	APE24	APE34	Residence zone	Industrial and Commercial zone	
Hydrocarbon (mg/Kg-1)	TPH	*439.07	708.14	*254.04	583	454	356	529	979	679	710	686	253	994	274	227	230	620	NA
Heavy Metals (mg/kg-1)	Hg	0.27	0.18	0.14	0.27	0.15	0.14	0.17	0.13	0.34	0.13	0.33	0.17	0.10	0.17	0.13	1	10	150
	Cd	0.33	0.26	0.16	0.33	0.35	0.22	0.20	0.23	0.30	0.11	0.72	0.10	0.30	0.27	0.10	4	10	1200
	Pb	20.50	17.16	13.42	25.0	53.9	19.65	15.25	47.43	19.3	12.24	33.87	15.54	22.62	19.62	12.02	140	150	47000

Annex 17. pH, HS, Nutrients (mg/Kg dry weight) organic matter in superficial sediments and superficial water in Aventura Plaza in the inner branches of Estero Salado from December 2011 to March of 2012.

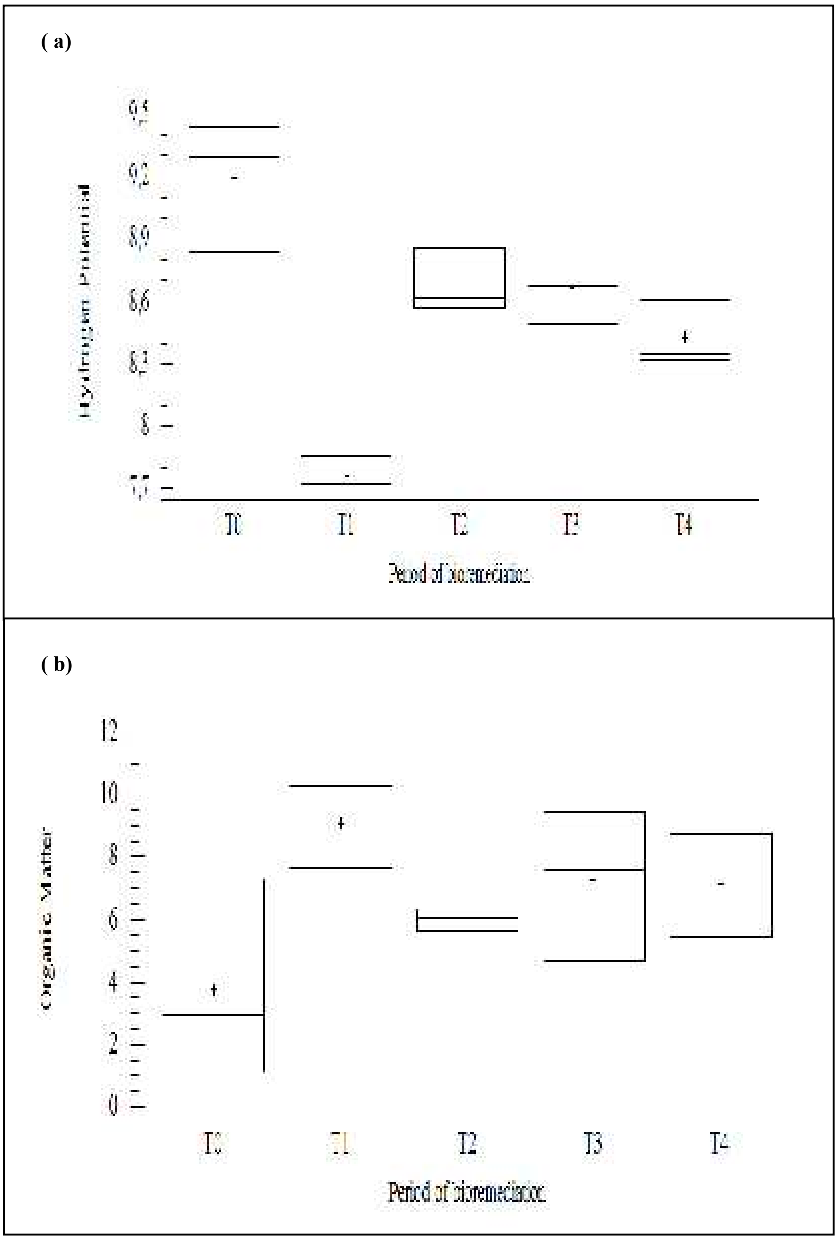


Parameters	Unit	Residence and Commercial Area														
		Characterization: Time 0			Bioremediation :Time 1			Bioremediation :Time 2			Bioremediation :Time 3			Bioremediation :Time 4		
		AP1	AP2	AP3	APE11	APE21	APE31	APE12	APE22	APE32	APE13	APE23	APE33	APE14	APE24	APE34
pH		8.84	9.44	9.29	7.72	7.71	7.85	8.86	8.62	8.57	8.67	8.49	8.83	8.61	8.35	8.32
NO_2-	mg/Kg	0.01	0.01	0.02	0.01	0.02	0.02	0.17	0.11	0.08	0.02	0.08	0.09	0.03	0.09	0.07
NO_3-	mg/Kg	1.00	1.00	0.80	0.70	0.60	0.50	11.2	6.10	30.00	10.60	12.40	13.10	5.50	9.70	8.80
PO_4	mg/Kg	148.70	139.60	151.23	10.84	8.30	8.85	42.95	20.73	57.49	19.56	21.65	16.07	55.12	24.98	27.62
H_2S	mg/Kg	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Organic matter	(%)	7.32	2.95	1.10	7.67	10.3	9.31	6.35	6.01	5.67	9.45	7.62	4.71	8.73	7.29	5.47

Annex 18. Medians for (a) Nitrate; (b) Nitrite; (c) Phosphate and (d) Hydrogen Sulfide in sediments at characterization time (To) and bioremediation time (T1-T4), from December 2011 to March 2012, at Aventura Plaza – Estero Salado inner estuary.



Annex 19. Medians for pH and organic matter in sediments at characterization time (To) and bioremediation time (T1-T4), from December 2011 to March 2012, at Aventura Plaza – Estero Salado inner estuary.



Annex 20. Table of spatial variation of macroinvertebrates taxa registered in superficial mangrove sediments in Aventura Plaza in the inner branches of Estero Salado from December 2011 to March of 2012.

	Residential and Commercial Area														
	Characterization : Time 0			Bioremediation: Time 1			Bioremediation: Time 2			Bioremediation: Time 3			Bioremediation: Time 4		
Taxa	APE10	APE20	APE30	APE11	APE21	APE31	APE12	APE22	APE32	APE13	APE23	APE33	APE14	APE24	APE34
Melanoides cf tuberculata	0	0	0	5	27	787	5	1	12	32	15	1	35	26	3
Heleobia sp.	0	0	0	0	0	0	6	0	0	61	2	0	25	0	0
Gastropod sp.1	0	0	0	64	5	5	0	0	0	2	0	0	1	3	7
Marinula acuta	0	0	0	1	11	1	1	0	1	0	0	0	6	0	0
Marinula sp.	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1
Cyrenoida inflata	0	0	0	29	8	1	0	0	0	7	7	0	16	1	2
Mytella guyanensis	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0
Oligochaeta	15	16	390	286	409	412	447	36	596	805	374	31	1174	80	734
Nereidae	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Nereis sp.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Palitridae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Tylidae	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
Podura aquatica	0	0	0	4	2	7	0	0	0	0	0	0	0	0	0
Ablabesmyia sp.	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Chironomus sp.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Pericoma sp.	0	0	0	3	7	3	4	32	42	18	13	0	3	3	7
Probezzia sp.	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0
Ceratopogonidae	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Dolichopodidae	0	0	0	17	1	3	0	0	0	1	0	1	1	0	2
Staphylinidae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Pupa	0	0	0	7	2	2	0	5	4	4	1	2	0	0	1
Larva	0	0	0	1	0	7	0	0	0	0	0	0	0	0	0
Eggs of insects	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Hydracarina	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
Nematode	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Nemertine	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0
Undetermited	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
N	15	16	390	435	473	1240	463	74	658	931	412	35	1263	113	758

Annex 21. Statistical parameters (minimum, maximum, mean, median, standard deviation and 95% confidence level for the mean of the Number of species, number of individuals and diversity indexes of the benthic macroinvertebrates macroinvertebrates in superficial mangrove sediments in Aventura Plaza in the inner branches of Estero Salado from December 2011 to March of 2012.

Parameter	Minimum	Maximum	Mean	Median	SD	95%Confidence Interval for Mean
Number of species	1	14	5.93	5	3.95	(14 -1)
Number of individuals	15	1262	482.47	427	416.72	(1262-15)
Shannon-Wiener Index	0	1.19	0.43	0.37	0.35	(1.19-0)
Simpson Index	0	0.52	0.21	0.17	0.19	(0.69-0)
Eveness	0	0.69	0.24	0.25	0.19	(0.52-0)

Annex 22. Test and their significance levels (P) for ANOVA for the Number of species, number of individuals and diversity indexes of the benthic macroinvertebrates registered in superficial mangrove sediments in Aventura Plaza in the inner branches of Estero Salado from December 2011 to March of 2012. Significant results are highlighted in bold.

Parameter	ANOVA Test	Test Statistic	P
Number of species (S)	Fisher	11.90	**<0.008**
Number of individuals (N)	Kruskal Wallis	5.9	0.206
Shannon-Wiener Index (H´)	Fisher	4.59	**<0.023**
Simpson Index	Kruskal Wallis	9.08	0.082
Eveness (J)	Fisher	1.88	0.189

Annex 23. Taxa detected by SIMPER at distinguishing between the benthic macroinvertebrate assemblage in sediments in Aventura Plaza in the inner branches of Estero Salado from December 2011 to March of 2012. The period of bioremediation in which the abundance were greater and high ratios of dissimilarity to standard deviation (Diss/SD) have been highlighted in bold for each taxa and the % contribution of each one to the dissimilarity between the composition in each period are given .

Distinguishing Taxa	Bioremediation Period			
	Time 0	**Time 1**		
	Av.Abund	**Av.Abund**	**Diss/SD**	**Contrib%**
Oligochaeta	9.21	**19.14**	**1.36**	26.85
Melanoides tuberculata	0	**11.83**	**1.12**	23.97
Gastropod sp.1	0	**4.16**	**1.38**	9.89
Cyrenoida inflata	0	**3.07**	**1.55**	7.61
Dolichopodidae	0	**2.29**	**1.52**	5.3
Pericoma sp.	0	**2.04**	**2.32**	4.98
Marinula acuta	0	**1.77**	1.2	4.59
Podura aquatica	0	**2.02**	**6.79**	4.57
Nemertea	0	**0.94**	0.66	2.25
	Time 0	**Time 2**		
	Av.Abund	**Av.Abund**	**Diss/SD**	**Contrib%**
Oligochaeta	9.21	**17.19**	**1.45**	52.88
Pericoma sp.	0	**4.71**	**1.42**	27.11
Melanoides tuberculata	0	**2.23**	**3.76**	10.53
	Time 0	**Time 3**		
	Av.Abund	**Av.Abund**	**Diss/SD**	**Contrib%**
Oligochaeta	9.21	**17.76**	**1.56**	51.9
Melanoides tuberculata	0	**3.51**	**3.8**	15.26
Heleobia sp.	0	**3.07**	0.94	9.59
Pericoma sp.	0	**2.62**	**1.26**	9.44
Cyrenoida inflata	0	**1.76**	**1.21**	6.48
	Time 0	**Time 4**		
	Av.Abund	**Av.Abund**	**Diss/SD**	**Contrib%**
Oligochaeta	9.21	**23.43**	**2.02**	52.73
Melanoides tuberculata	0	**4.25**	**1.44**	16.27
Pericoma sp.	0	**2.04**	**2.4**	7.55
Gastropod sp.1	0	**1.79**	**1.8**	6.98
Cyrenoida inflata	0	**2.14**	**2.77**	6.55

Distinguishing Taxa	**Bioremediation Period**			
	Time 1	**Time 2**		
	Av.Abund	**Av.Abund**	**Diss/SD**	**Contrib%**
Melanoides tuberculata	**11.83**	2.23	0.87	22.38
Oligochaeta	**19.14**	17.19	**1.09**	19.23
Gastropod sp.1	**4.16**	0	**1.37**	10.89
Cyrenoida inflata	**3.07**	0	**1.52**	8.31
Pericoma sp.	2.04	**4.71**	**1.51**	7.3
Dolichopodidae	**2.29**	0	**1.5**	5.87
Podura aquatica	**2.02**	0	**5.22**	5.07
Marinula acuta	**1.77**	0.67	0.8	3.39
Nemertea	**0.94**	0	0.66	2.49
Probezzia sp.	**0.94**	0	**1.28**	2.25
Mytella guyanensis	**0.82**	0	0.66	2.15
Heleobia sp.	0	**0.82**	0.66	2.02
	Time 1	**Time 3**		
	Av.Abund	**Av.Abund**	**Diss/SD**	**Contrib%**
Oligochaeta	**19.14**	**17.76**	**1.12**	22.69
Melanoides tuberculata	**11.83**	3.51	0.86	21.83
Gastropod sp.1	**4.16**	0.47	**1.13**	9.98
Heleobia sp.	0	**3.07**	0.89	6.17
Cyrenoida inflata	**3.07**	1.76	0.97	5.82
Podura aquatica	**2.02**	0	**3.64**	5.07
Pericoma sp.	2.04	**2.62**	**2.06**	5.05
Marinula acuta	**1.77**	0	**1.16**	4.96
Dolichopodidae	**2.29**	0.67	0.99	4.07
Nemertea	**0.94**	0	0.64	2.48
Probezzia sp.	**0.94**	0	**1.22**	2.25
	Time 1	**Time 4**		
	Av.Abund	**Av.Abund**	**Diss/SD**	**Contrib%**
Oligochaeta	19.14	**23.43**	**3.28**	29.75
Melanoides tuberculata	**11.83**	4.25	0.85	21.93
Gastropod sp.1	**4.16**	1.79	0.87	6.96
Cyrenoida inflata	**3.07**	2.14	**1.14**	5.57
Podura aquatica	**2.02**	0	**4.32**	5.4
Marinula acuta	**1.77**	0.82	**1.17**	4.75
Dolichopodidae	**2.29**	0.8	1	4.52
Heleobia sp.	0	**1.67**	0.66	3.66
Nemertea	**0.94**	0	0.65	2.63
Probezzia sp.	**0.94**	0	**1.26**	2.41
Mytella guyanensis	**0.82**	0	0.65	2.28
Tylidae	**0.58**	0	0.66	1.33

Distinguishing Taxa	**Bioremediation Period**			
	Time 2	**Time 3**		
	Av.Abund	**Av.Abun**	**Diss/SD**	**Contrib%**
Oligochaeta	17.19	**17.76**	**1.12**	45.47
Pericoma sp.	**4.71**	2.62	0.87	17.31
Heleobia sp.	0.82	**3.07**	**1.09**	10.66
Melanoides tuberculata	2.23	**3.51**	**1.45**	8.46
Cyrenoida inflata	0	**1.76**	**1.23**	6.39
Dolichopodidae	0	**0.67**	0.95	3.57
	Time 2	**Time 4**		
	Av.Abund	**Av.Abund**	**Diss/SD**	**Contrib%**
Oligochaeta	17.19	**23.43**	**1.44**	47.04
Pericoma sp.	**4.71**	2.04	**1.24**	11.43
Melanoides tuberculata	2.23	**4.25**	**1.17**	10.41
Gastropod sp.1	0	**1.79**	**1.91**	7.34
Cyrenoida inflata	0	**2.14**	**2.22**	7.33
Heleobia sp.	0.82	**1.67**	0.96	6.25
Marinula acuta	0.67	**0.82**	**1.4**	3.46
	Time 3	**Time 4**		
	Av.Abund	**Av.Abund**	**Diss/SD**	**Contrib%**
Oligochaeta	17.76	**23.43**	**1.29**	50.15
Heleobia sp.	**3.07**	1.67	**1.1**	10.4
Melanoides tuberculata	3.51	**4.25**	0.88	10.18
Pericoma sp.	**2.62**	2.04	**1.84**	8
Cyrenoida inflata	**1.76**	2.14	**1.72**	6.59
Gastropod sp.1	0.47	**1.79**	**1.18**	6.56

Annex 24. Results of PCA for the main environmental variables that describe the community structure of benthic macroinvertebrates observed in sediments in Aventura Plaza in the inner branches of Estero Salado from December 2011 to March of 2012. Significant results are highlighted in bold.

Lambda	Eigenvalue	Proportion	Cumulative
PC1	5.83	0.36	0.36
PC2	2.54	0.16	0.52
PC3	2.25	0.14	0.66
PC4	1.58	0.1	0.76
PC5	1.41	0.09	0.85
PC6	0.85	0.05	0.90
COEFFICIENTS			
Variables	PC1	PC2	PC3
TPH	-0.06	0.17	0.12
Hg	0.02	0.32	0.29
Cd	-0.07	0.26	**0.49**
Pb	-0.2	0.22	**0.36**
Oil &Greases	0.31	-0.17	0.33
OM	-0.33	-0.08	0.1
pHs	**0.38**	0.05	-0.11
NO3	0.01	**0.39**	-0.32
NO2	0.01	**0.46**	**-0.33**
PO4	**0.36**	-0.15	0.2
HS	-0.06	-0.19	-0.28
O2	-0.3	**-0.25**	0.2
S	**-0.36**	-0.22	-0.04
N	-0.19	**-0.3**	-0.19
H´	-0.36	0.07	0.03
J´	-0.28	0.29	-0.05

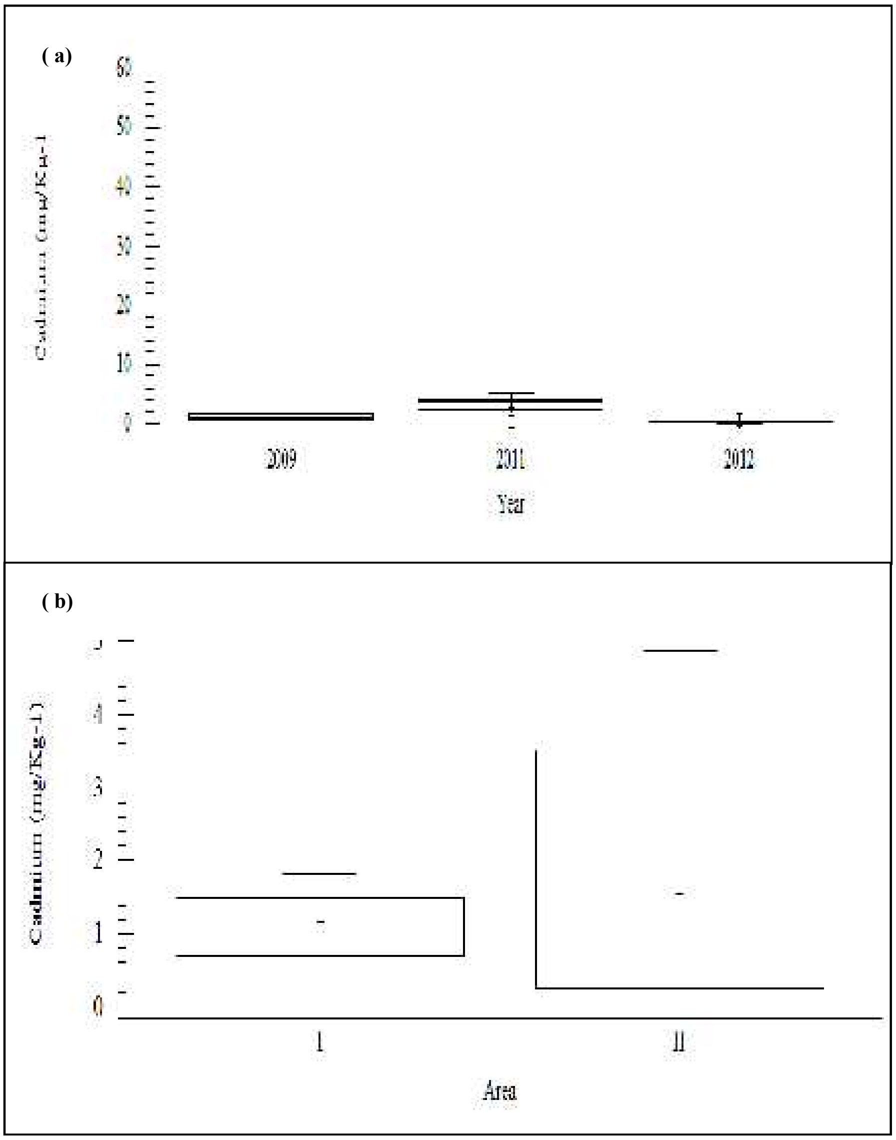
Annex 25. Environmental parameters recorded at the University of Guayaquil and Aventura Plaza stations during the September to December 2012.

Universidad de Guayaquil station				
Date	**pH water**	**PH sediment**	**Temperature (oC)**	**Salinidad (psu)**
15/09/2012	7.6	7.1	25.9	15
15/10/2012	7.5	7.0	26.4	15
15/11/2012	7.0	7.0	27.1	22
15/12/2012	6.9	6.5	26.5	20
Aventura Plaza station				
Date	**pH water**	**PH sediment**	**Temperature (oC)**	**Salinidad (psu)**
14/09/2012	7.1	7.0	26.1	14
15/10/2012	7.3	7.0	26.0	14
15/11/2012	7.7	7.0	26.2	14
15/12/2012	7.1	6.9	26.9	16

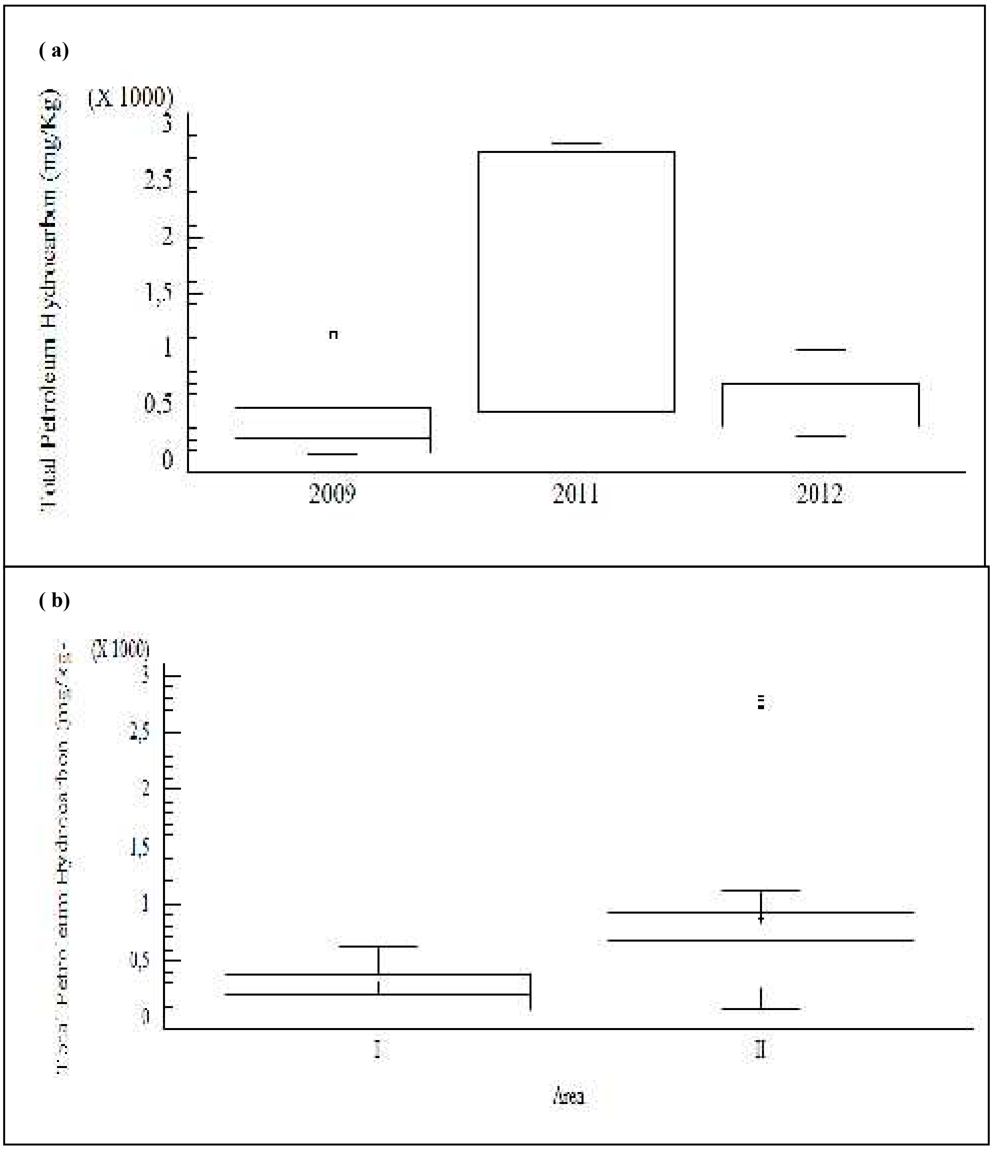
Annex 26. Abundace and distribution of benthic macroinvertebrates during the recolonization in artificial substrate.

Time	Month 0						Month 1						Month 2						Month 3					
Stations	Aventura Plaza			Universidad de Guayaquil			Aventura Plaza			Universidad de Guayaquil			Aventura Plaza			Universidad de Guayaquil			Aventura Plaza			Universidad de Guayaquil		
Taxa	**SAPAC**	**SAPBC**	**SAPCC**	**SUGAC**	**SUGBC**	**SUGCC**	**OAPAL**	**OAPBE**	**OAPBO**	**OUGBL**	**OUGCE**	**OUGCO**	**NAPCL**	**NAPBE**	**NAPCO**	**NUGCL**	**NUGBE**	**NUGBO**	**DAPCL**	**DAPCE**	**DAPCO**	**DUGCL**	**DUGAE**	**DUGCO**
Arthropoda																								
Pericoma	32	6	24				121	163	25	5	16	7	1553	739	337	4	69	17	35	182	17	229	7	144
Psychoda																2			1	4	1		1	2
Pupa											2			1					3	2	1	4	2	1
Podura aquatica			2				192	714	90	8	83	1	471	1366	87	22	141	1	2	113	58	67	132	76
Hydrachnidae							4	332	11		1		125	338	243	37	85	3	66	191	174	136	292	58
Dermaptera							15	18	89				3	177	158	3	13		14	34	318	5	21	21
Stilobezzia							3	11			3		79	319	8	8	3		6	46	1	21	1	36
Dolichopodidae	2	4	1				2	2	1	1			37	5	11					4		24		38
Hemiptero																1							1	
Naucoridae											1													
Amphipoda														2		2	3		1		3			
Penaeus																1								
Zoea de Brachyura																3	3		1					
Ostracoda													1							2			3	
Balanus																			1					
Annelida																								
Oligochaeta sp 1				147	67	131																		
Oligochaeta sp 2	1	2					51	44	153	446	17	437	541	446	1235	922	321	1161	725	1946	812	143	614	1522
Capitella				4				32	1	4	4	36	3	214	29	71	16	11	7	264	21	3	14	18
Nereis										1														
Mollusca																								
Amnicola																3								
Melampus					2								2						2					
Detracia											2			1		1				2				
Melanoides					2		3	1					2		1	1			4			9		
Heleobia		7		5		19	829	193	168	15	19	57	446	44	375	45	489	36	444	253	134	27	145	15
Bivalvo ND										1					2	12								
Mytilopsis								21		2	166			274		12	337	3	21	185	6		375	
Cyrenoida inflata													1							2		4		3
Polymesoda				1	1																			
Nematoda									1					14	2		419	81		27	616	2	182	223

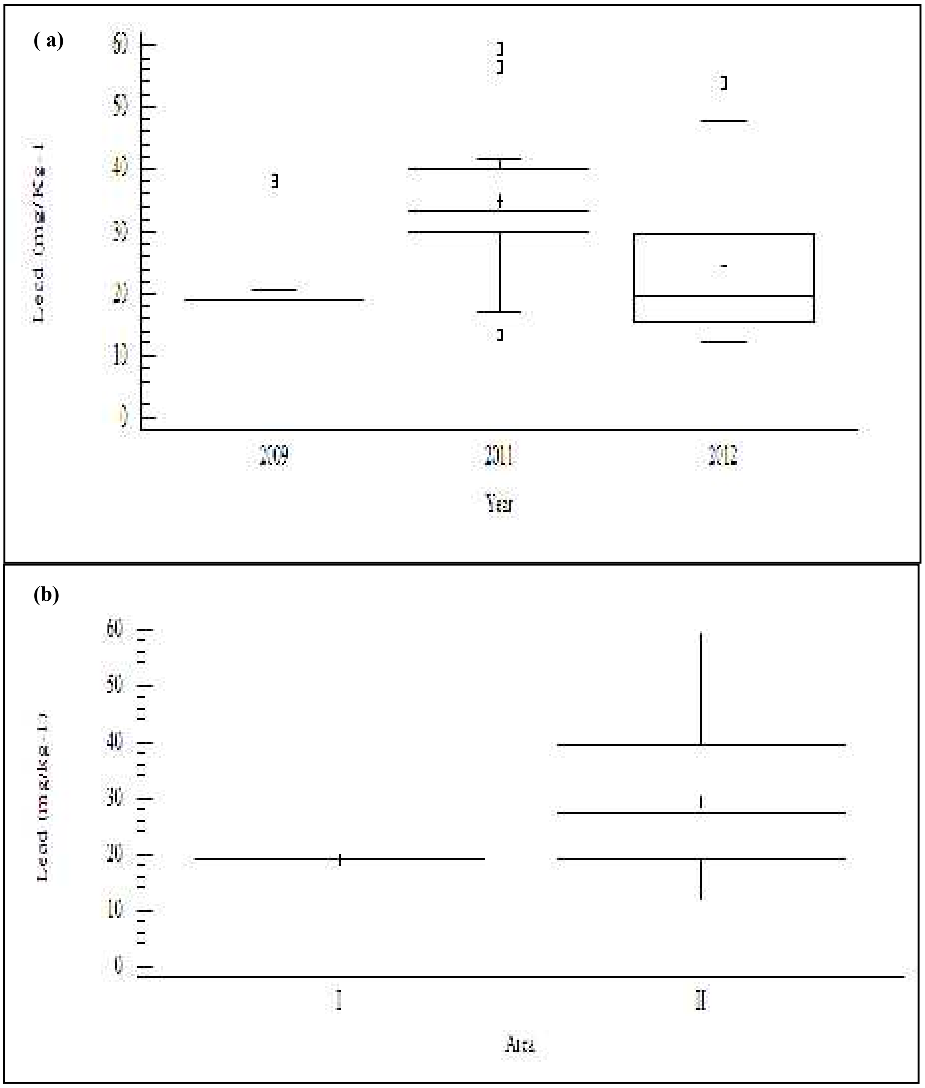
Annex 27. Medians for cadmium (a) comparison between years and (b) protected area (I) and urban area (II) in sediments of inner branches of Estero Salado from 2009 to 2012.



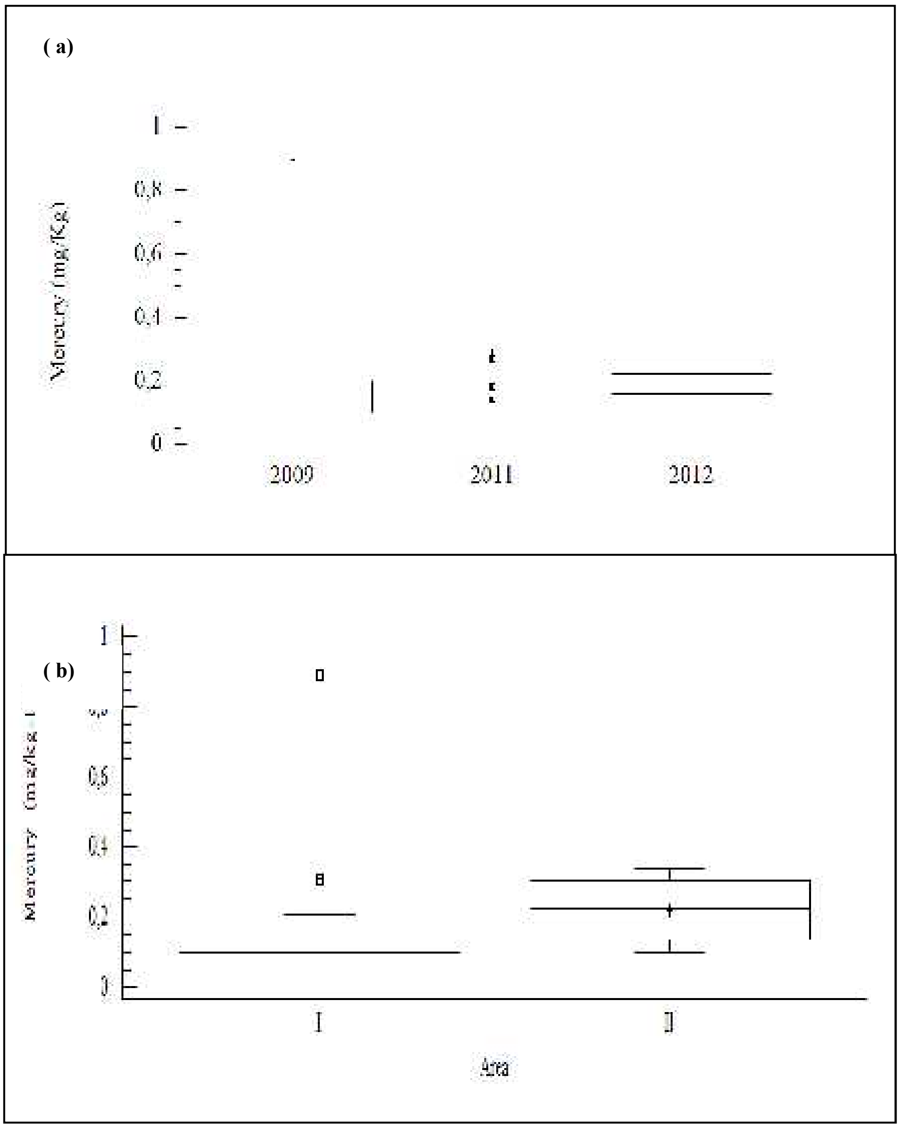
Annex 28. Medians for Total Petroleum Hydrocarbon (a) comparison between years and (b) protected area (I) and urban area (II) in sediments of inner branches of Estero Salado from 2009 to 2012.



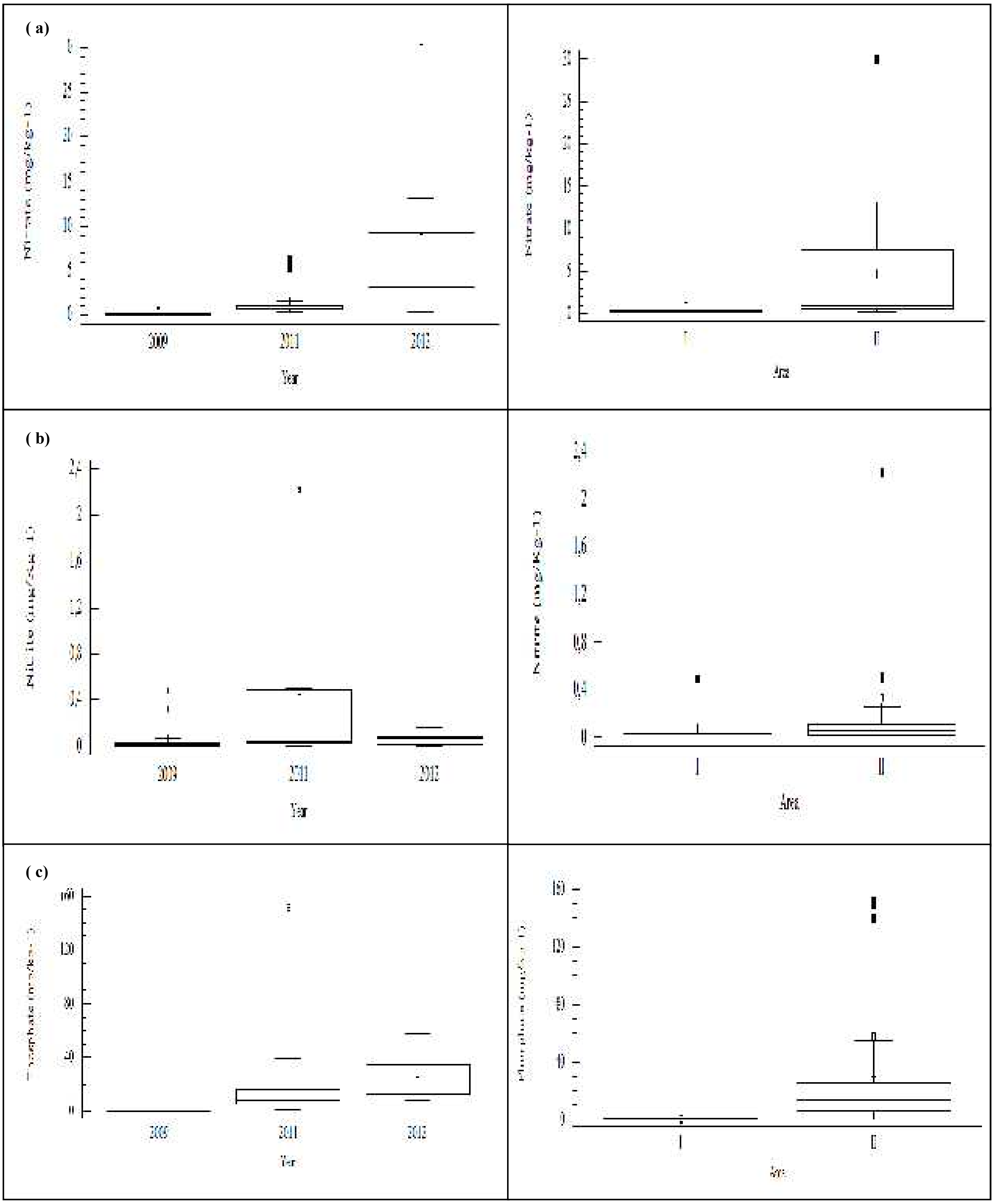
Annex 29. Medians for lead (a) comparison between years and (b) protected area (I) and urban area (II) in sediments of inner branches of Estero Salado from 2009 to 2012.



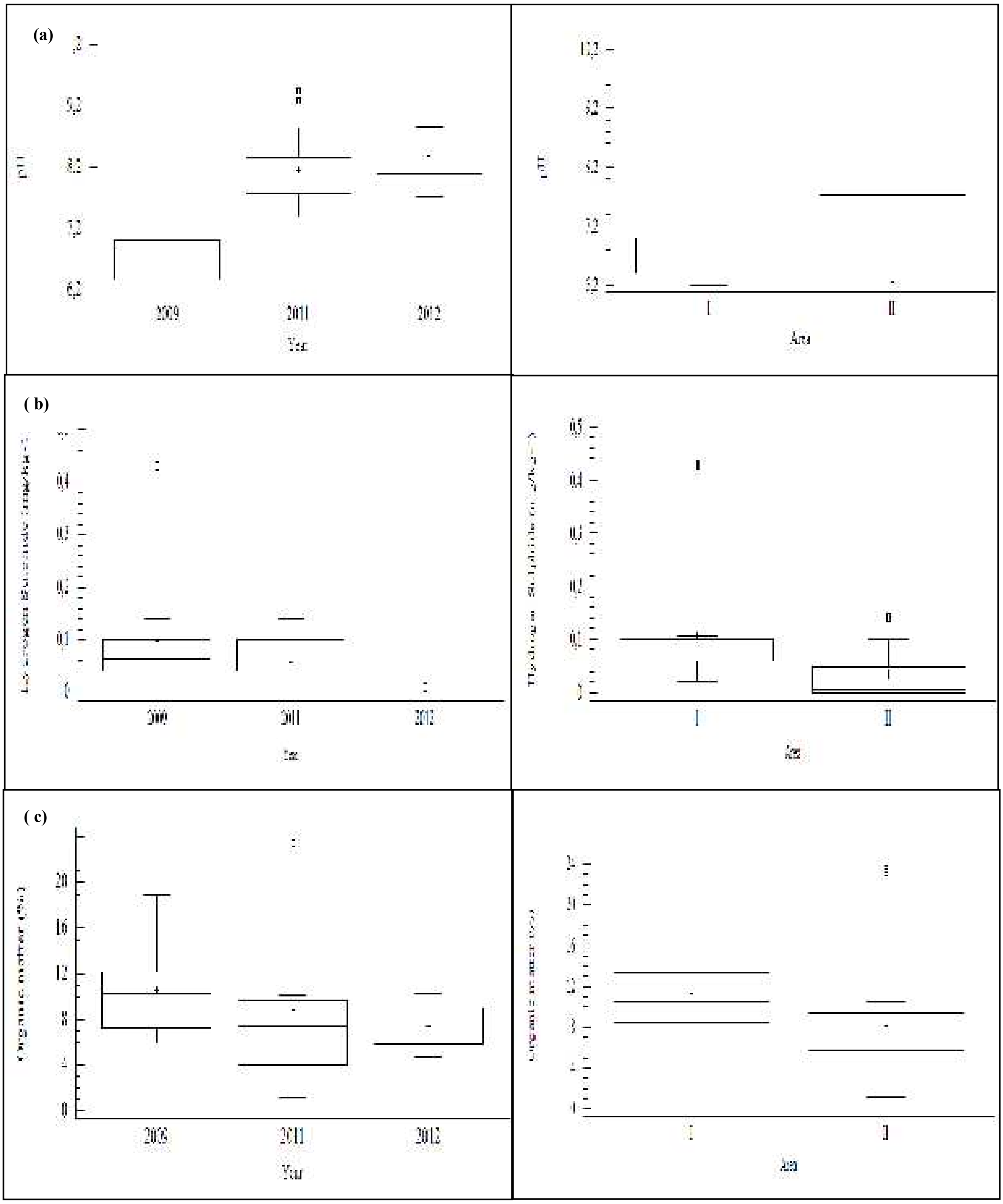
Annex 30. Medians for total mercury (a) comparison between years and (b) protected area (I) and urban area (II) in sediments of inner branches of Estero Salado from 2009 to 2012.



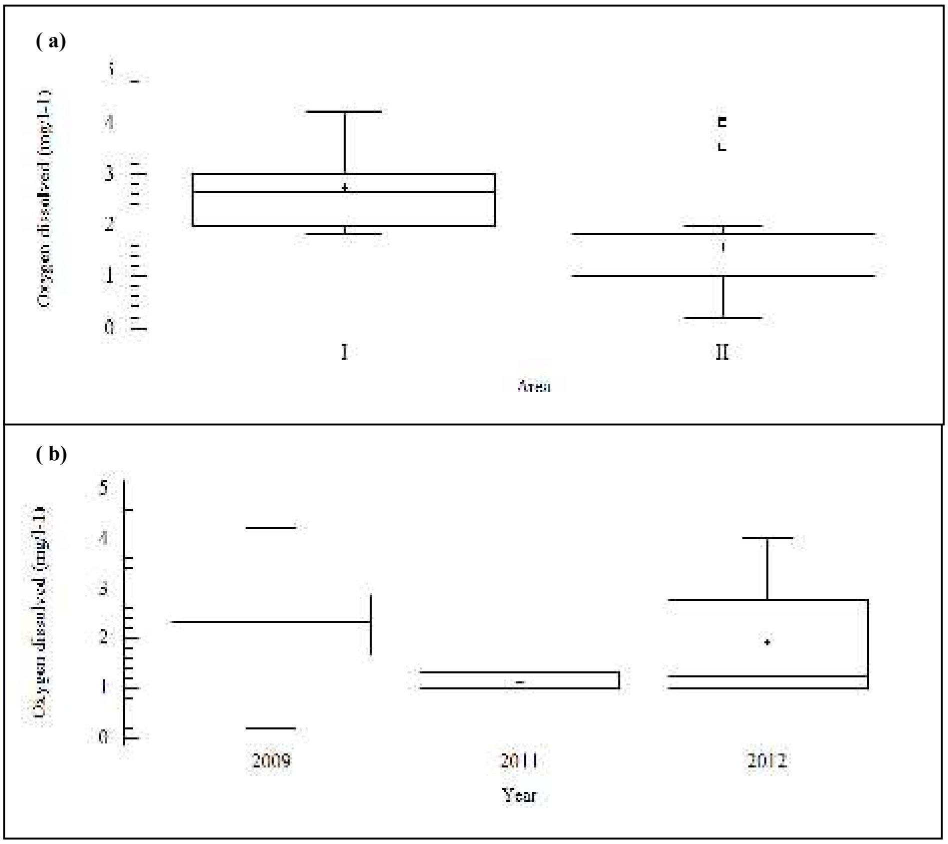
Annex 31. Medians for Nitrate (a), Nitrite (b) and Phosphate (c) (1) comparison between years and (2) protected area (I) and urban area (II) in sediments of inner branches of Estero Salado from 2009 to 2012.



Annex 32. Medians for pH (a) hydrogen sulphide (b) and organic matter (c) comparison between years and protected area (I) and urban area (II) in sediments of inner branches of Estero Salado from 2009 to 2012.



Annex 33. Medians for oxygen dissolved (a) years (b) area: protected area (I) and urban area (II) in superficial water of branches of Estero Salado from 2009 to 2012.



Annex 34. Paper of Chapter 7 publicated in Intropica-Santa Marta Colombia Magazine.

Rev. Intropica	ISSN 1794-161X	9	118 - 128	Santa Marta, Colombia, Enero - Diciembre de 2014

CARACTERIZACIÓN DE MACROINVERTEBRADOS BENTÓNICOS DE DOS RAMALES ESTUARINOS AFECTADOS POR LA ACTIVIDAD INDUSTRIAL, ESTERO SALADO-ECUADOR

BENTHONIC MACROINVERTEBRATES CHARACTERIZATION ON TWO ESTUARINE BRANCHES AFFECTED BY INDUSTRIAL POLLUTION, ESTERO SALADO-ECUADOR

Maritza Cárdenas-Calle y James Mair

RESUMEN

El propósito de este estudio fue evaluar la composición, abundancia y diversidad de los macroinvertebrados en dos zonas afectadas por aguas residuales industriales y domésticas en dos ramales estuarinos en la ciudad de Guayaquil: Aventura Plaza y la Universidad de Guayaquil. Se realizaron tres muestreos por sitio, utilizando una draga Van Veen de 0,1 m durante la marea baja en septiembre de 2012. No se encontraron diferencias significativas entre las varianzas de la riqueza y abundancia de especies, registrándose la mayor abundancia en el ramal estuarino cercano a la Universidad de Guayaquil, donde el taxón más abundante fueron los oligoquetos y donde se presentó la más alta temperatura y mayor salinidad. Los sitios estudiados fueron muy pobres y poco diversos debido posiblemente a las emisiones de aguas residuales de uso doméstico e industrial que alteran parámetros claves para la supervivencia de especies estuarinas como la salinidad, la temperatura y el pH.

PALABRAS CLAVES: contaminación difusa, Estero Salado, macroinvertebrados

ABSTRACT

The purpose of this study was to assess composition, abundance and diversity of macroinvertebrates on two areas affected by industrial and domestic wastewater into two estuarine branches in the city of Guayaquil: Aventura Plaza and the Universidad de Guayaquil. Three samplings per site were performed using a Van Veen grab from 0.1 m at low tide in September 2012. No statistical differences were detected between in the variances of richness and abundance species, the most abundant was the nearest branch to estuarine Universidad de Guayaquil, where the most abundant taxa were Oligochaeta, presented the higher temperature and higher salinity. While the study sites were very poor and few diverse, could be related to emissions from municipal sewage and industrial uses that changed key parameters for the survival of estuarine species such as salinity, temperature and pH.

KEYWORDS: diffuse pollution, diversity, Estero Salado, macroinvertebrates

INTRODUCCIÓN

Los manglares constituyen uno de los ecosistemas acuáticos de mayor productividad biológica, esto es unas 20.000 Kcal.m^{-2}.año^{-1} (Montaño y Sanfeliu, 2008). Se encuentran en las zonas tropicales y subtropicales y albergan una gran diversidad de especies de fauna y flora (Márquez y Jiménez, 2002), que se han adaptado a vivir en ambientes salinos (Boullion, 2011), tales como mangles, moluscos, crustáceos, equinodermos, tunicados y otros invertebrados que están bien adaptados a cambios de mareas, salinidad, temperatura, suelos anaeróbicos y que sustentan la alimentación, crecimiento, reproducción y supervivencia de especies acuáticas y niveles tróficos superiores, incluyendo al hombre.

En el Ecuador los manglares forman parte de una variedad de hábitats que se extienden a lo largo de aproximadamente 2900 km de línea de costa (Boothroyd

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et al., 1994), siendo uno de los estuarios de mayor importancia el Estero Salado por formar parte del Golfo de Guayaquil, situado en la provincia del Guayas y representado por seis especies de mangle, de los cuales tres son conocidos comúnmente como mangles rojos *Rhizophora mangle*, *R. harrisonii*, *R. racemosa*, *Conocarpus erectus* (mangle jelí), *Laguncularia racemosa* (mangle blanco) (Valverde y Pérez, 2012) y *Avicennia germinans* (mangle negro) (Pérez, 2012), así como otras formaciones vegetales hidrohalófitas (ej. *Salicornia fruticosa*, *Cryptocarpus pyriformes*, *Batis maritima*). Las aguas del Golfo son biológicamente fértiles y soportan importantes pesquerías artesanales del Golfo de Guayaquil como las de peces como el barrilete (*Katsuwonus pelamis*), aleta amarilla (*Thunnus albacares*) (Stevenson, 1981); moluscos como *Ostrea columbiensis* (Mora y Reinoso, 1981), *Anadara tuberculosa*, *A. similis*, *Mytella guyanensis*, *M. strigata*; y crustáceos como el cangrejo *Ucides occidentalis*.

El Golfo de Guayaquil, es considerado el estuario más grande de la costa sudamericana del Pacífico. Posee una extensión de 13701 km^2 (11711 km^2 de superficie de agua y 1990 km^2 de islas e islotes) (CAAM, 1996; Stevenson, 1981). El Golfo está dividido naturalmente en estuario exterior e interior. El Estero Salado y el sistema del río Guayas forman parte del estuario interior, el Estero Salado se inicia en la ciudad de Guayaquil y se extiende hacia el Sur-Oeste, hasta el Canal del Morro (Cruz, 1992) y tiene una extensión de 74 km (Stevenson, 1981).

Entre los principales cambios que ha sufrido el Estero Salado debido a su cercanía a Guayaquil se encuentran alteraciones en sus características físicas, químicas y biológicas tales como: la reducción de sus ramales, falta de renovación de sus aguas (Roldán, 1978), incremento de aguas residuales de uso doméstico sin tratamiento previo (EMAG, 1978); aumento de concentraciones de nitrógeno, fósforo, bacterias fecales y virus (Twilley, 1989). Y una amplia gama de químicos persistentes que se descargan al estero (Pérez, 2000) como: hidrocarburos, aceites, grasas (Cárdenas, 2010; Rodríguez, 2005; Thiákos, 2000), blanqueadores, metales pesados como Al, As, Cr, Ni, Zn y Pb (Fernández-Cadena et al., 2014), detergentes, cloraminas (CAAM, 1996), afectando negativamente la calidad del agua del Estero así como la composición, abundancia, diversidad y distribución de la biota acuática planctónica y bentónica (Cárdenas, 2010; Hidroestudios, 2003).

Entre los principales estudios realizados sobre macroinvertebrados en el Estero Salado, se encuentran descripciones de moluscos realizadas por Olsson (1961) y Keen (1971); listados de especies de la fauna sublitoral bentónica del Estero Salado interior incluyendo moluscos, anélidos, crustáceos, foraminíferos (Cruz, et al., 1980); estado de las poblaciones de ostiones en tres zonas del estuario interior del Golfo de Guayaquil (Mora y Reinoso, 1981); bivalvos del Golfo de Guayaquil (Cruz, 1983); estudios descriptivos de especies de bivalvos en el Estero Salado y el Estero Cascajal (Cruz, 1986); estado del recurso malacológico de la zona intralitoral del Golfo de Guayaquil (Cruz, 1992); malacofauna existente alrededor de la ciudad de Guayaquil durante 2003 (Cruz, 2003).

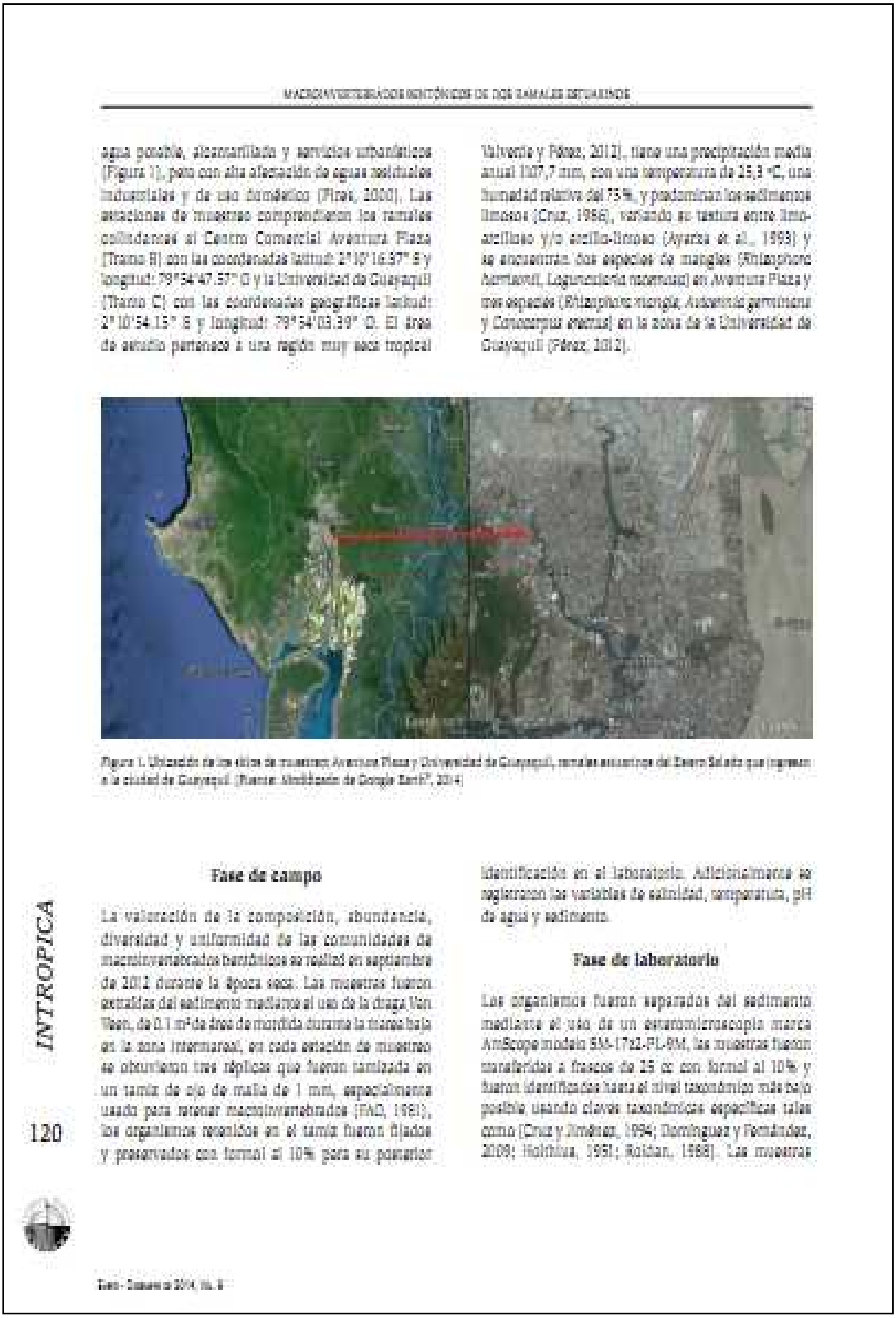
Otros estudios han incluido el análisis del efecto de la contaminación hidrocarburífera en la estructura comunitaria de macroinvertebrados bentónicos en el Estero Salado (Cárdenas, 2010); así como el estudio de los niveles de coliformes totales y *Escherichia coli* en bivalvos de interés comercial (Sigüencia, 2010); el levantamiento de la batimetría y caracterización del sedimento del Estero Salado y su relación con la diversidad macrobentónica (INOCAR, 2012), entre otros estudios.

Entre los factores que inciden en la distribución de las comunidades macrobentónicas se encuentran la salinidad, tipo de sustrato, corrientes, disponibilidad de nutrientes (Cruz et al., 1980). En este estudio se describe los parámetros de temperatura, salinidad, pH del agua y sedimentos; así como la estructura (composición, abundancia, diversidad y uniformidad) de macroinvertebrados en dos zonas afectadas por aguas residuales industriales y domésticas cercanas a la ciudad de Guayaquil: Avenida Plaza y la Universidad de Guayaquil.

MATERIALES Y MÉTODOS

Descripción del área de estudio

Los sitios de estudio fueron dos ramales estuarinos que ingresan a la ciudad de Guayaquil denominados por las autoridades de control ambiental como tramo B y C, estos forman parte de la zona I que incluyen áreas de asentamientos humanos próximos de servicios de



agua potable, alcantarillado y servicios urbanísticos (Figura 1), pero con alta afectación de aguas residuales industriales y de uso doméstico (Pérez, 2000). Las estaciones de muestreo comprendieron los ramales colindantes al Centro Comercial *Aventura Plaza* (Tramo B) con las coordenadas latitud: 2°10'16.37" S y longitud: 79°54'47.57" O y la Universidad de Guayaquil (Tramo C) con las coordenadas geográficas latitud: 2°10'54.13" S y longitud: 79°54'03.39" O. El área de estudio pertenece a una región muy seca tropical Valverde y Pérez, 2012), tiene una precipitación media anual 1107,7 mm, con una temperatura de 25,3 °C, una humedad relativa del 73 %, y predominan los sedimentos limosos (Cruz, 1986), variando su textura entre limo-arcilloso y/o arcillo-limoso (Ayarza et al., 1993) y se encuentran dos especies de mangles (*Rhizophora harrisonii, Laguncularia racemosa*) en *Aventura Plaza* y tres especies (*Rhizophora mangle, Avicennia germinans* y *Conocarpus erectus*) en la zona de la Universidad de Guayaquil (Pérez, 2012).

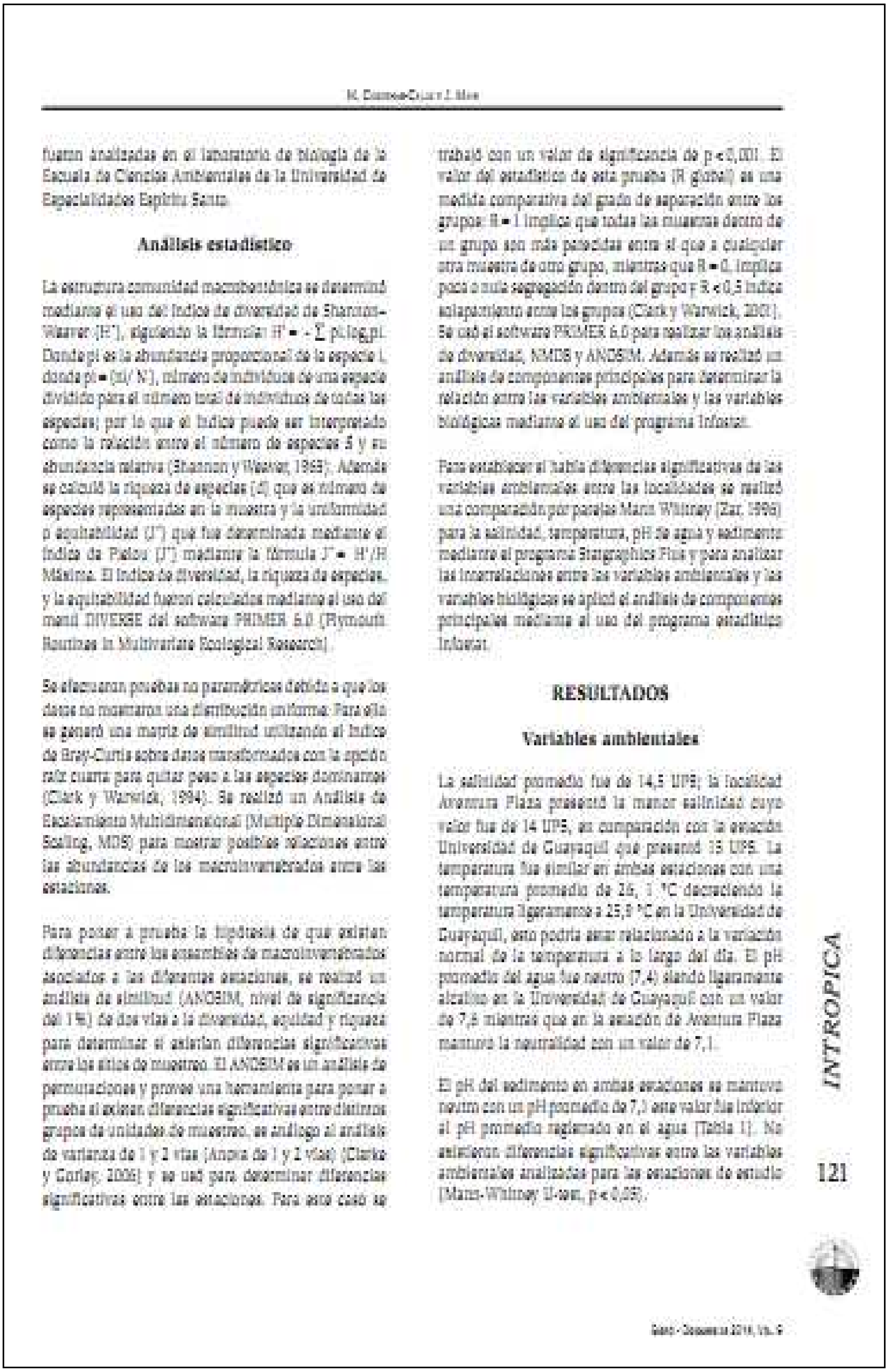
Figura 1. Ubicación de los sitios de muestreo: Aventura Plaza y Universidad de Guayaquil, ramales estuarinos del Estero Salado que ingresan a la ciudad de Guayaquil. (Fuente: Modificación de Google Earth®, 2014)

Fase de campo

La valoración de la composición, abundancia, diversidad y uniformidad de las comunidades de macroinvertebrados bentónicos se realizó en septiembre de 2012 durante la época seca. Las muestras fueron extraídas del sedimento mediante el uso de la draga Van Veen, de 0.1 m² de área de mordida durante la marea baja en la zona intermareal, en cada estación de muestreo se obtuvieron tres réplicas que fueron tamizada en un tamiz de ojo de malla de 1 mm, especialmente usado para retener macroinvertebrados (FAO, 1981), los organismos retenidos en el tamiz fueron fijados y preservados con formol al 10% para su posterior identificación en el laboratorio. Adicionalmente se registraron las variables de salinidad, temperatura, pH de agua y sedimento.

Fase de laboratorio

Los organismos fueron separados del sedimento mediante el uso de un estereomicroscopio marca AmScope modelo SM-1TSZ-FL-9M, las muestras fueron transferidas a frascos de 25 cc con formol al 10% y fueron identificados hasta el nivel taxonómico más bajo posible usando claves taxonómicas específicas tales como (Cruz y Jiménez, 1994; Domínguez y Fernández, 2009; Holthius, 1951; Roldán, 1988). Las muestras



M. Cornejo-Cajas y J. Mair

fueron analizadas en el laboratorio de biología de la Escuela de Ciencias Ambientales de la Universidad de Especialidades Espíritu Santo.

Análisis estadístico

La estructura comunidad macrobentónica se determinó mediante el uso del índice de diversidad de Shannon-Weaver (H'), siguiendo la fórmula: H' = - ∑ pi.log.pi. Donde pi es la abundancia proporcional de la especie i, donde pi = (ni/ N), número de individuos de una especie dividido para el número total de individuos de todas las especies; por lo que el índice puede ser interpretado como la relación entre el número de especies S y su abundancia relativa (Shannon y Weaver, 1963). Además se calculó la riqueza de especies (d) que es número de especies representadas en la muestra y la uniformidad o equitabilidad (J') que fue determinada mediante el índice de Pielou (J') mediante la fórmula J' = H'/H Máxima. El índice de diversidad, la riqueza de especies, y la equitabilidad fueron calculados mediante el uso del menú DIVERSE del software PRIMER 6.0 (Plymouth Routines in Multivariate Ecological Research).

Se efectuaron pruebas no paramétricas debido a que los datos no mostraron una distribución uniforme. Para ello se generó una matriz de similitud utilizando el índice de Bray-Curtis sobre datos transformados con la opción raíz cuarta para quitar peso a las especies dominantes (Clark y Warwick, 1994). Se realizó un Análisis de Escalamiento Multidimensional (Multiple Dimensional Scaling, MDS) para mostrar posibles relaciones entre las abundancias de los macroinvertebrados entre las estaciones.

Para poner a prueba la hipótesis de que existen diferencias entre los ensambles de macroinvertebrados asociados a las diferentes estaciones, se realizó un análisis de similitud (ANOSIM, nivel de significancia del 1%) de dos vías a la diversidad, equidad y riqueza para determinar si existían diferencias significativas entre los sitios de muestreo. El ANOSIM es un análisis de permutaciones y provee una herramienta para poner a prueba si existen diferencias significativas entre distintos grupos de unidades de muestreo, es análogo al análisis de varianza de 1 y 2 vías (Anova de 1 y 2 vías) (Clarke y Gorley, 2006) y se usó para determinar diferencias significativas entre las estaciones. Para este caso se trabajó con un valor de significancia de p < 0,001. El valor del estadístico de esta prueba (R global) es una medida comparativa del grado de separación entre los grupos: R = 1 implica que todas las muestras dentro de un grupo son más parecidas entre sí que a cualquier otra muestra de otro grupo, mientras que R = 0, implica poca o nula segregación dentro del grupo y R < 0,5 indica solapamiento entre los grupos (Clark y Warwick, 2001). Se usó el software PRIMER 6.0 para realizar los análisis de diversidad, NMDS y ANOSIM. Además se realizó un análisis de componentes principales para determinar la relación entre las variables ambientales y las variables biológicas mediante el uso del programa Infostat.

Para establecer si había diferencias significativas de las variables ambientales entre las localidades se realizó una comparación por parejas Mann Whitney (Zar, 1996) para la salinidad, temperatura, pH de agua y sedimento mediante el programa Statgraphics Plus y para analizar las interrelaciones entre las variables ambientales y las variables biológicas se aplicó el análisis de componentes principales mediante el uso del programa estadístico Infostat.

RESULTADOS

Variables ambientales

La salinidad promedio fue de 14,5 UPS; la localidad *Aventura Plaza* presentó la menor salinidad cuyo valor fue de 14 UPS, en comparación con la estación Universidad de Guayaquil que presentó 15 UPS. La temperatura fue similar en ambas estaciones con una temperatura promedio de 26, 1 °C decreciendo la temperatura ligeramente a 25,9 °C en la Universidad de Guayaquil, esto podría estar relacionado a la variación normal de la temperatura a lo largo del día. El pH promedio del agua fue neutro (7,4) siendo ligeramente alcalino en la Universidad de Guayaquil con un valor de 7,6 mientras que en la estación de Aventura Plaza mantuvo la neutralidad con un valor de 7,1.

El pH del sedimento en ambas estaciones se mantuvo neutro con un pH promedio de 7,3 este valor fue inferior al pH promedio registrado en el agua (Tabla 1). No existieron diferencias significativas entre las variables ambientales analizadas para las estaciones de estudio (Mann-Whitney U-test, p < 0,05).

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Tabla 1. Variables ambientales y análisis de comparación de medianas usando la prueba de Mann-Whitney U-test.

Variable	Estación		Valor p	U- test
	Aventura Plaza	Univ. De Guayaquil		
Salinidad	14	15	< 0,05	0,1
Temperatura	28,1	25,9	< 0,05	0,66
pH agua	7,1	7,8	< 0,05	0,66
pH sedimento	7	7,1	< 0,05	0,66

Variables biológicas

La comunidad macrobentónica estuvo representada por 461 individuos, representados por 6 especies, 6 géneros, 8 familias y 5 clases del zoobentos, distribuidas en el sedimento y representadas por 3 phyla: Arthropoda, Annelida y Mollusca. Los anélidos fueron el grupo más abundante, con el 76 %, seguido de los artrópodos con el 16 % y los moluscos con el 8 % (Figura 2).

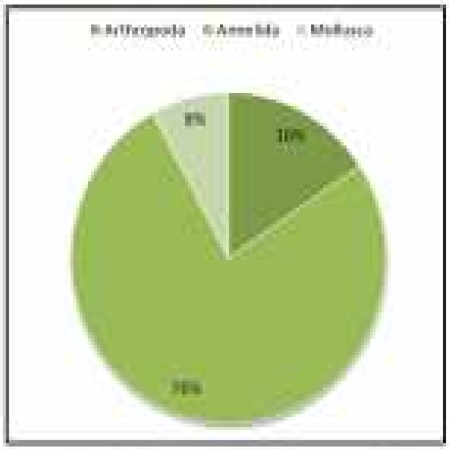


Figura 2. Composición porcentual de la fauna macrobentónica de dos ramales del Estero Salado influenciados por actividades humanas en Guayaquil, Ecuador.

La estación con mayor abundancia de organismos fue la Universidad de Guayaquil con 379 individuos el grupo taxonómico más abundante fue el de los oligoquetos representados por familia Enchitraeidae (Tabla 2). Mientras que en la estación *Aventura Plaza* se registró la menor abundancia de macroinvertebrados con 82 individuos representados por la clase Insecta, especialmente por los dípteros de la familia Psychodidae con el género *Pericoma* con 62 individuos, con una menor abundancia se registró a las familias Dolichopodidae (7 individuos) y Poduridae (2 individuos) con la especie *Podura aquatica* y una pupa no identificada.

Los moluscos estuvieron representados por las clases gastropoda y bivalvia, siendo los gasterópodos los más abundantes, pertenecientes a la familia Hydrobiidae representado por el género *Heleobia*, la familia Thiaridae con la especie *Melanoides tuberculata* y la familia Ellobiidae con la especie *Melampus* cf. *carolianus*. Los gasterópodos se encontraron en mayor abundancia en la estación Universidad de Guayaquil (28 individuos) en comparación con la estación *Aventura Plaza* que sólo presentó 7 individuos del género *Heleobia*. Los bivalvos estuvieron representados por la familia Corbiculidae por el género *Polymesoda inflata* y un espécimen no identificado.

El análisis de la abundancia y riqueza de especies permitió distinguir un patrón espacial que establece que no hay similitud entre las comunidades macrobentónicas presentes en las localidades de estudio. El valor de estrés obtenido fue de 0 (Figura 3), ratificando que se trata de dos comunidades distintas, en la cual la estación *Aventura Plaza* se caracterizó por la presencia de insectos dípteros del género *Pericoma* mientras que los oligoquetos estuvieron presentes en la estación Universidad de Guayaquil.

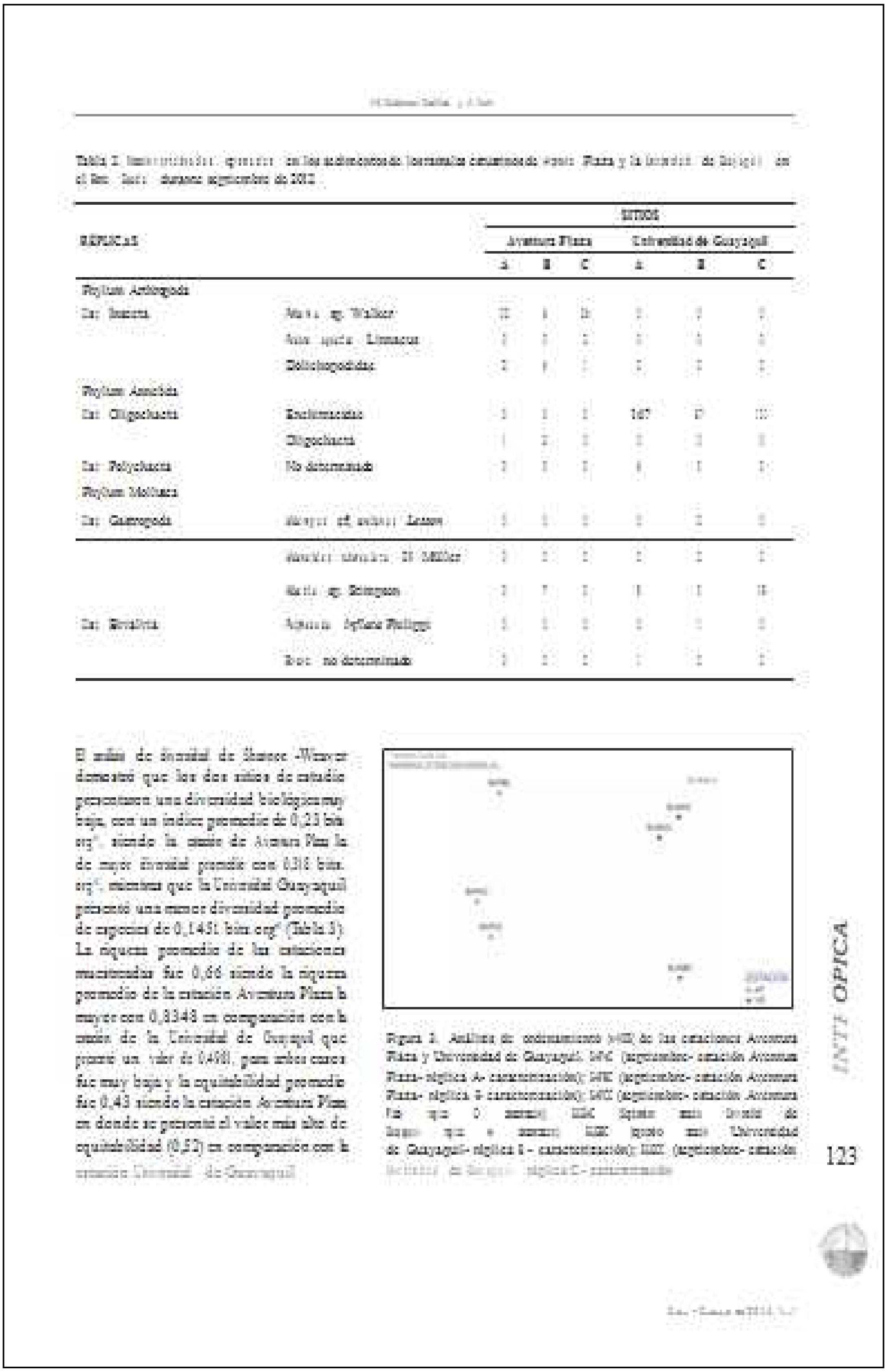


Tabla 2. [illegible] en los sedimentos de los manglares [illegible] Plaza y la [illegible] de Guayaquil en el [illegible] durante septiembre de 2012.

RÉPLICAS		SITIOS					
		Aventura Plaza			Universidad de Guayaquil		
		A	B	C	A	B	C
Phylum Arthropoda							
Cl. Insecta	[illegible] sp. Walker	22	1	11	0	0	0
	[illegible] [illegible] Linnaeus	0	0	0	0	0	0
	Dolichopodidae	0	9	0	0	0	0
Phylum Annelida							
Cl. Oligochaeta	[illegible]	0	0	0	167	17	[illegible]
	Oligochaeta	1	2	0	0	0	0
Cl. Polychaeta	No determinado	0	0	0	6	1	0
Phylum Mollusca							
Cl. Gastropoda	[illegible] cf. [illegible] Lesson	0	0	0	0	0	0
	[illegible] [illegible] Müller	0	0	0	0	0	0
	[illegible] sp. Simpson	0	7	0	1	1	16
Cl. Bivalvia	[illegible] [illegible] Philippi	0	0	0	0	0	0
	Bivalvo no determinado	0	0	0	0	0	0

El índice de diversidad de Shannon-Weaver demostró que los dos sitios de estudio presentaron una diversidad biológica muy baja, con un índice promedio de 0,23 bits org^{-1}, siendo la estación de Aventura Plaza la de mayor diversidad promedio con 0,38 bits org^{-1}, mientras que la Universidad Guayaquil presentó una menor diversidad promedio de especies de 0,1451 bits org^{-1} (Tabla 3). La riqueza promedio de las estaciones muestreadas fue 0,66 siendo la riqueza promedio de la estación Aventura Plaza la mayor con 0,8348 en comparación con la estación de la Universidad de Guayaquil que presentó un valor de 0,498, para ambos casos fue muy baja y la equitabilidad promedio fue 0,43 siendo la estación Aventura Plaza en donde se presentó el valor más alto de equitabilidad (0,52) en comparación con la estación Universidad de Guayaquil.

Figura 3. Análisis de ordenamiento (nMDS) de las estaciones Aventura Plaza y Universidad de Guayaquil. [illegible] (septiembre- estación Aventura Plaza- réplica A- caracterización); [illegible] (septiembre- estación Aventura Plaza- réplica B- caracterización); [illegible] (septiembre- estación Aventura [illegible] réplica C- [illegible]); [illegible] (septiembre- estación Universidad de Guayaquil- réplica A- [illegible]); [illegible] (septiembre- estación Universidad de Guayaquil- réplica B - caracterización); [illegible] (septiembre- estación Universidad de Guayaquil- réplica C - caracterización).

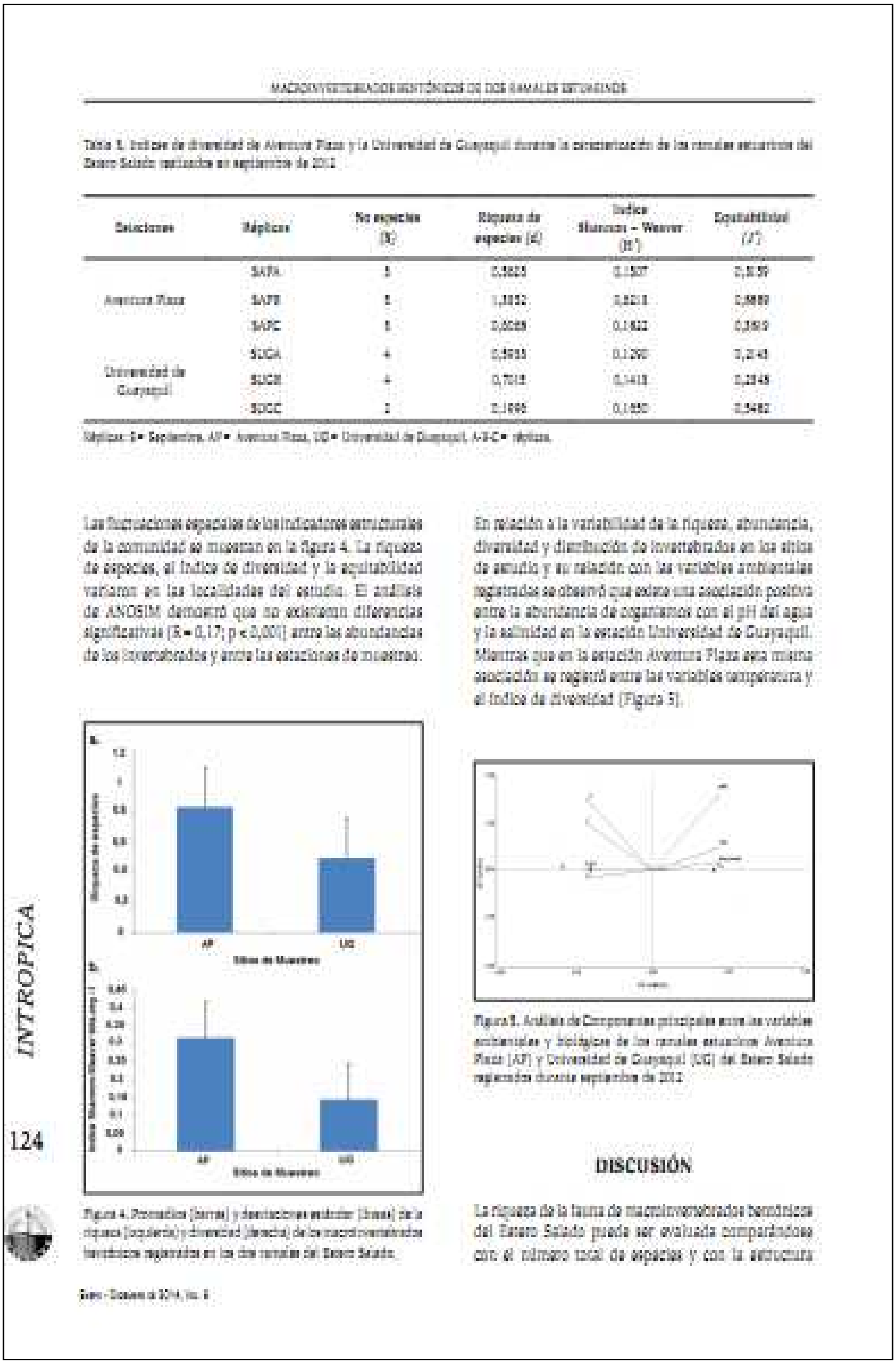


Tabla 8. Índices de diversidad de Aventura Plaza y la Universidad de Guayaquil durante la caracterización de los canales estuarinos del Estero Salado realizados en septiembre de 2012

Estaciones	Réplicas	No especies (S)	Riqueza de especies (d)	Índice Shannon – Weaver (H')	Equitabilidad (J')
Aventura Plaza	SAPA	5	0,5623	0,1507	0,8159
	SAPB	5	1,2852	0,8213	0,8889
	SAPC	5	0,6088	0,1822	0,3919
Universidad de Guayaquil	SUGA	4	0,5933	0,1290	0,2143
	SUGB	4	0,7015	0,1413	0,2348
	SUGC	2	0,1698	0,1850	0,5482

Réplicas: S= Septiembre, AP= Aventura Plaza, UG= Universidad de Guayaquil, A-B-C= réplicas.

Las fluctuaciones espaciales de los indicadores estructurales de la comunidad se muestran en la figura 4. La riqueza de especies, el índice de diversidad y la equitabilidad variaron en las localidades del estudio. El análisis de ANOSIM demostró que no existieron diferencias significativas (R = 0,17; p < 0,001) entre las abundancias de los invertebrados y entre las estaciones de muestreo.

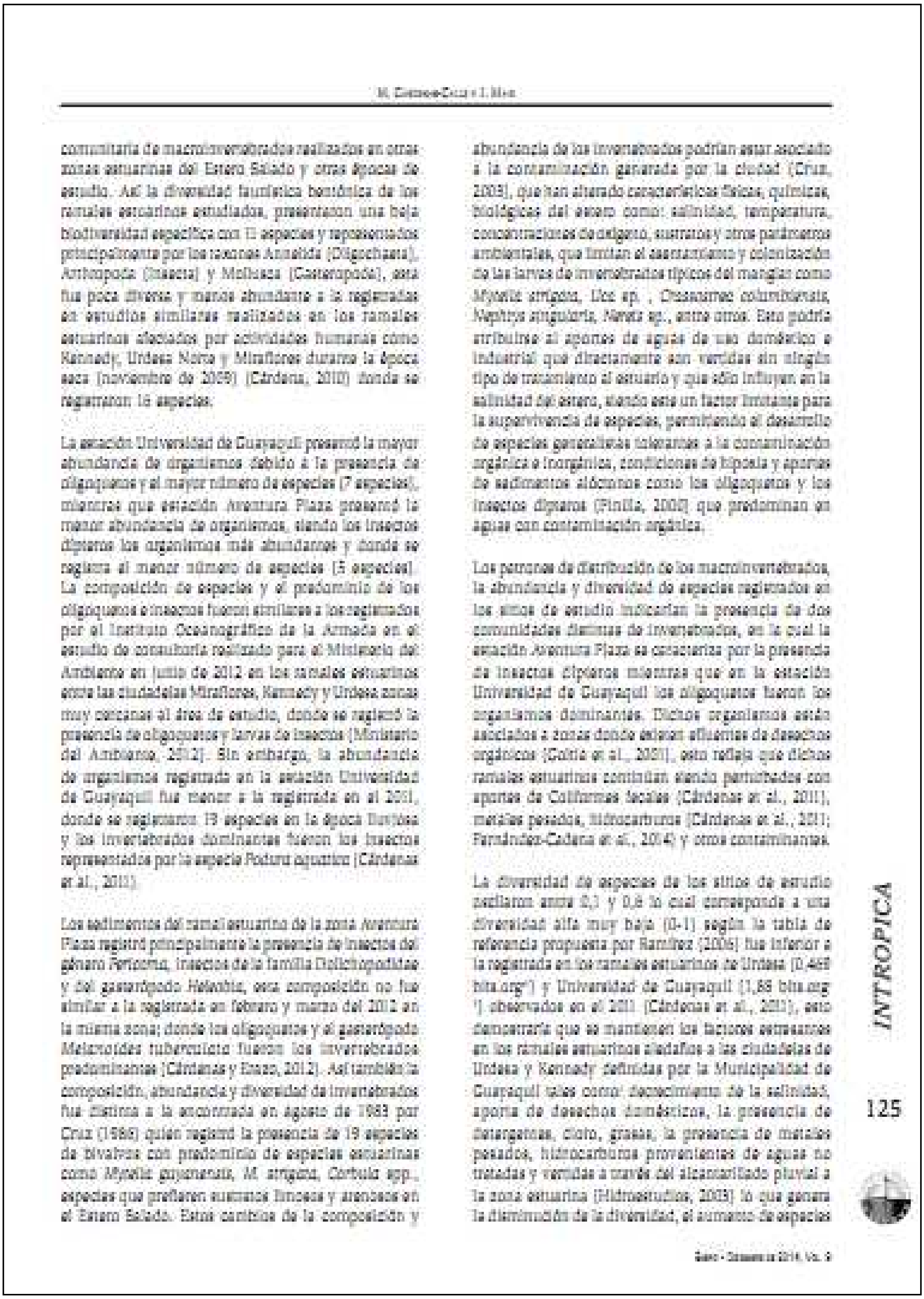
Figura 4. Promedios (barras) y desviaciones estándar (líneas) de la riqueza (izquierda) y diversidad (derecha) de los macroinvertebrados bentónicos registrados en los dos canales del Estero Salado.

En relación a la variabilidad de la riqueza, abundancia, diversidad y distribución de invertebrados en los sitios de estudio y su relación con las variables ambientales registradas se observó que existe una asociación positiva entre la abundancia de organismos con el pH del agua y la salinidad en la estación Universidad de Guayaquil. Mientras que en la estación Aventura Plaza esa misma asociación se registró entre las variables temperatura y el índice de diversidad (Figura 5).

Figura 5. Análisis de Componentes principales entre las variables ambientales y biológicas de los canales estuarinos Aventura Plaza (AP) y Universidad de Guayaquil (UG) del Estero Salado registrados durante septiembre de 2012.

DISCUSIÓN

La riqueza de la fauna de macroinvertebrados bentónicos del Estero Salado puede ser evaluada comparándolos con el número total de especies y con la estructura



comunitaria de macroinvertebrados realizados en otras zonas estuarinas del Estero Salado y otras épocas de estudio. Así la diversidad faunística bentónica de los ramales estuarinos estudiados, presentaron una baja biodiversidad específica con 11 especies y representados principalmente por los taxones Annelida (Oligochaeta), Arthropoda (Insecta) y Mollusca (Gasteropoda), esta fue poca diversa y menos abundante a la registradas en estudios similares realizados en los ramales estuarinos afectados por actividades humanas como Kennedy, Urdesa Norte y Miraflores durante la época seca (noviembre de 2009) (Cárdenas, 2010) donde se registraron 16 especies.

La estación Universidad de Guayaquil presentó la mayor abundancia de organismos debido a la presencia de oligoquetos y el mayor número de especies (7 especies), mientras que estación Aventura Plaza presentó la menor abundancia de organismos, siendo los insectos dípteros los organismos más abundantes y donde se registra el menor número de especies (5 especies). La composición de especies y el predominio de los oligoquetos e insectos fueron similares a los registrados por el Instituto Oceanográfico de la Armada en el estudio de consultoría realizado para el Ministerio del Ambiente en junio de 2012 en los ramales estuarinos entre las ciudadelas Miraflores, Kennedy y Urdesa zonas muy cercanas al área de estudio, donde se registró la presencia de oligoquetos y larvas de insectos (Ministerio del Ambiente, 2012). Sin embargo, la abundancia de organismos registrada en la estación Universidad de Guayaquil fue menor a la registrada en el 2011, donde se registraron 19 especies en la época lluviosa y los invertebrados dominantes fueron los insectos representados por la especie *Podura aquatica* (Cárdenas et al., 2011).

Los sedimentos del ramal estuarino de la zona *Aventura* Plaza registró principalmente la presencia de insectos del género *Pericoma*, insectos de la familia Dolichopodidae y del gasterópodo *Heleobia*, esta composición no fue similar a la registrada en febrero y marzo del 2012 en la misma zona; donde los oligoquetos y el gasterópodo *Melanoides tuberculata* fueron los invertebrados predominantes (Cárdenas y Erazo, 2012). Así también la composición, abundancia y diversidad de invertebrados fue distinta a la encontrada en agosto de 1983 por Cruz (1986) quien registró la presencia de 19 especies de bivalvos con predominio de especies estuarinas como *Mytella guyanensis*, *M. strigata*, *Corbula* spp., especies que prefieren sustratos limosos y arenosos en el Estero Salado. Estos cambios de la composición y abundancia de los invertebrados podrían estar asociado a la contaminación generada por la ciudad (Cruz, 2003), que han alterado características físicas, químicas, biológicas del estero como: salinidad, temperatura, concentraciones de oxígeno, sustratos y otros parámetros ambientales, que limitan el asentamiento y colonización de las larvas de invertebrados típicos del manglar como *Mytella strigata*, *Uca* sp. , *Chasmagnathus columbiensis*, *Nephtys singularis*, *Nereis* sp., entre otros. Esto podría atribuirse al aportes de aguas de uso doméstico e industrial que directamente son vertidas sin ningún tipo de tratamiento al estuario y que sólo influyen en la salinidad del estero, siendo este un factor limitante para la supervivencia de especies, permitiendo el desarrollo de especies generalistas tolerantes a la contaminación orgánica e inorgánica, condiciones de hipoxia y aportes de sedimentos alóctonos como los oligoquetos y los insectos dípteros (Pinilla, 2000) que predominan en aguas con contaminación orgánica.

Los patrones de distribución de los macroinvertebrados, la abundancia y diversidad de especies registrados en los sitios de estudio indicarían la presencia de dos comunidades distintas de invertebrados, en la cual la estación *Aventura* Plaza se caracteriza por la presencia de insectos dípteros mientras que en la estación Universidad de Guayaquil los oligoquetos fueron los organismos dominantes. Dichos organismos están asociados a zonas donde existen afluentes de desechos orgánicos (Goitia et al., 2001), esto refleja que dichos ramales estuarinos continúan siendo perturbados con aportes de Coliformes fecales (Cárdenas et al., 2011), metales pesados, hidrocarburos (Cárdenas et al., 2011; Fernández-Cadena et al., 2014) y otros contaminantes.

La diversidad de especies de los sitios de estudio oscilaron entre 0,1 y 0,8 lo cual corresponde a una diversidad alfa muy baja (0-1) según la tabla de referencia propuesta por Ramírez (2006) fue inferior a la registrada en los ramales estuarinos de Urdesa (0,469 bits.org^{-1}) y Universidad de Guayaquil (1,66 bits.org^{-1}) observados en el 2011 (Cárdenas et al., 2011), esto demostraría que se mantienen los factores estresantes en los ramales estuarinos aledaños a las ciudadelas de Urdesa y Kennedy definidas por la Municipalidad de Guayaquil tales como: decrecimiento de la salinidad, aporte de desechos domésticos, la presencia de detergentes, cloro, grasas, la presencia de metales pesados, hidrocarburos provenientes de aguas no tratadas y vertidas a través del alcantarillado pluvial a la zona estuarina (Hidroestudios, 2003) lo que genera la disminución de la diversidad, el aumento de especies

oportunistas y el cambio de las comunidades biológicas propias de las zonas estuarinas.

En relación a la variabilidad de la abundancia de invertebrados en los sitios de estudio se observó que la mayor abundancia de invertebrados está asociada a variables como el pH del agua y la salinidad como se observó en el caso del ramal de la Universidad de Guayaquil donde se registró especies estuarinas como *Polymesoda inflata* (Russell-Hunter, 2013), *Melampus* cf *carolianus* que habita en la arena húmeda entre la vegetación del manglar (Paredes et al., 2005). Sin embargo, también se registró especies de aguas continentales como oligoquetos y gasterópodos como *Heleobia* sp. y *Melanoides tuberculata* considerada esta última especie como invasora originaria del África y de Asia (Domínguez y Fernández, 2009) que se ha distribuido también en América del Sur a países como Venezuela, Colombia, Argentina, Uruguay, Brasil (Peso et al., 2010).

Al realizar comparaciones de las comunidades entre las localidades de estudio se observa que la estación Aventura Plaza presenta una comunidad distinta y atípica a una zona estuarina donde predominan los insectos acuáticos y oligoquetos, donde disminuyen la abundancia y la diversidad de las especies. Por lo que se hace necesario la implementación de un programa de monitoreo de la calidad de los efluentes de origen doméstico e industrial en las zonas de estudio y zonas aledañas, dirigido a evaluar parámetros físicos - químicos como: salinidad, temperatura, oxígeno disuelto, pH, detergentes, materia orgánica metales pesados, aceites - grasas, sulfuros e hidrocarburos y parámetros biológicos como coliformes fecales e invertebrados para determinar el impacto de las actividades antropogénicas sobre la salud del ecosistema estuarino. De esta forma se logrará conocer la calidad de las aguas del Estero Salado, conocer las fuentes de los agentes estresores y sus implicaciones en el deterioro del ecosistema acuático. Así también generará información técnica que permitirá a las autoridades ambientales ejecutar medidas legales pertinentes hacia aquellos que incumplen la normativa ambiental ecuatoriana en relación a la calidad ambiental.

AGRADECIMIENTOS

Los autores agradecen al Centro de Investigaciones de la Universidad de Especialidades Espíritu Santo por los fondos otorgados para la realización de este estudio, se agradece al apoyo de campo y laboratorio del estudiante de la Escuela de Ciencias Ambientales de la Universidad de Especialidades Espíritu Santo Sr. Julián Pérez por su tiempo en la separación de las muestras biológicas y al Master René Rodríguez Director de la Escuela de Ciencias Ambientales de la Universidad Espíritu Santo por la revisión de este manuscrito y al Dr. Luis Troccoli Investigador del Instituto Nacional de Pesca Programa Prometeo por sus recomendaciones en el análisis estadístico.

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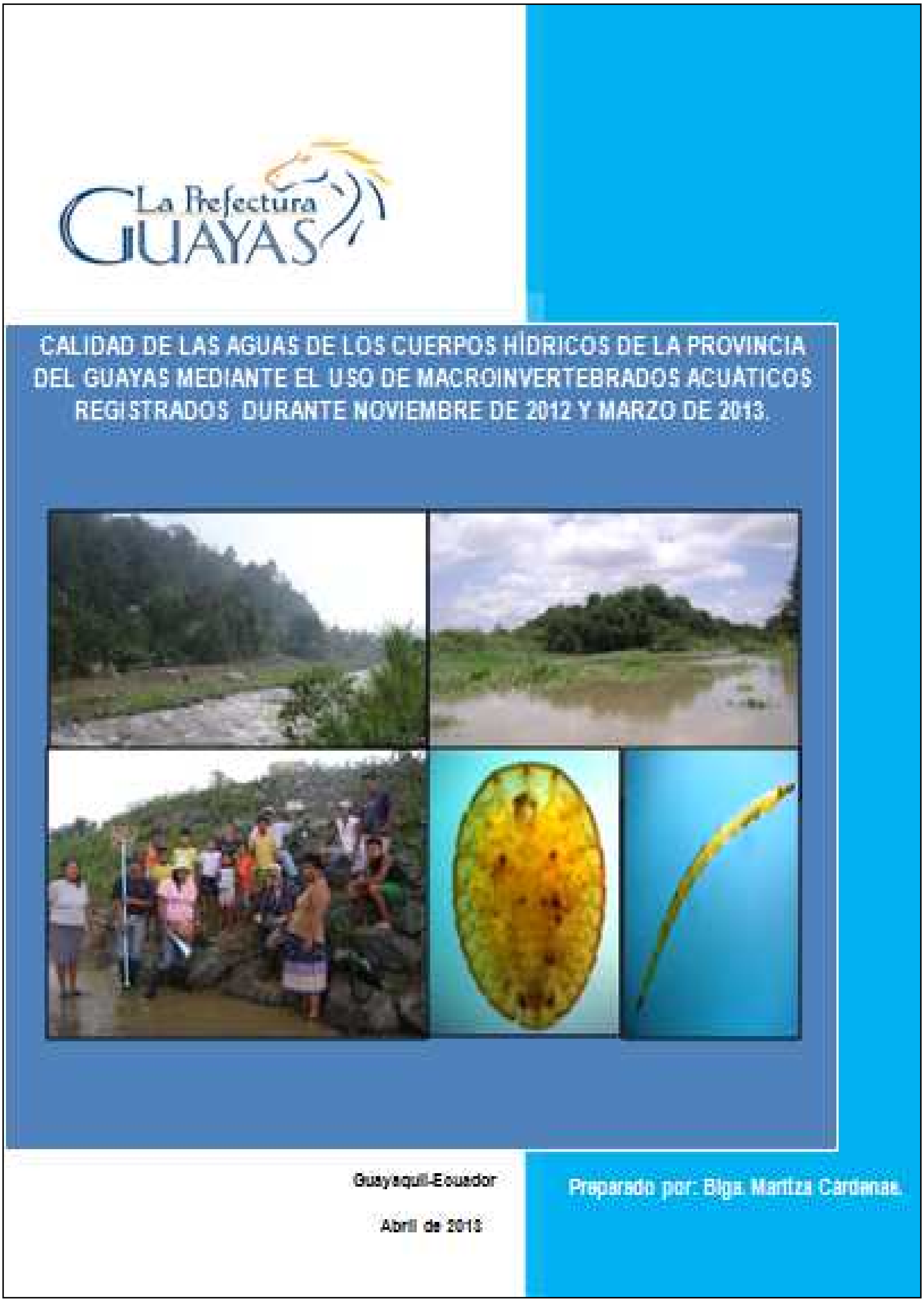
Fecha de recepción: 01/08/2014
Fecha de aceptación: 26/11/2014

Para citar este artículo: Cárdenas-Calle, M. y Mair, J. 2014. Caracterización de macroinvertebrados bentónicos de dos ramales estuarinos afectados por la actividad industrial, Estero Salado-Ecuador. Revista Intropica 9: 118 - 128

Annex 35. Technical report on water quality of water bodies by using macroinvertebrates recorded in 2012 and 2013 and biomonitoring protocol prepared for the Prefecture of Guayas



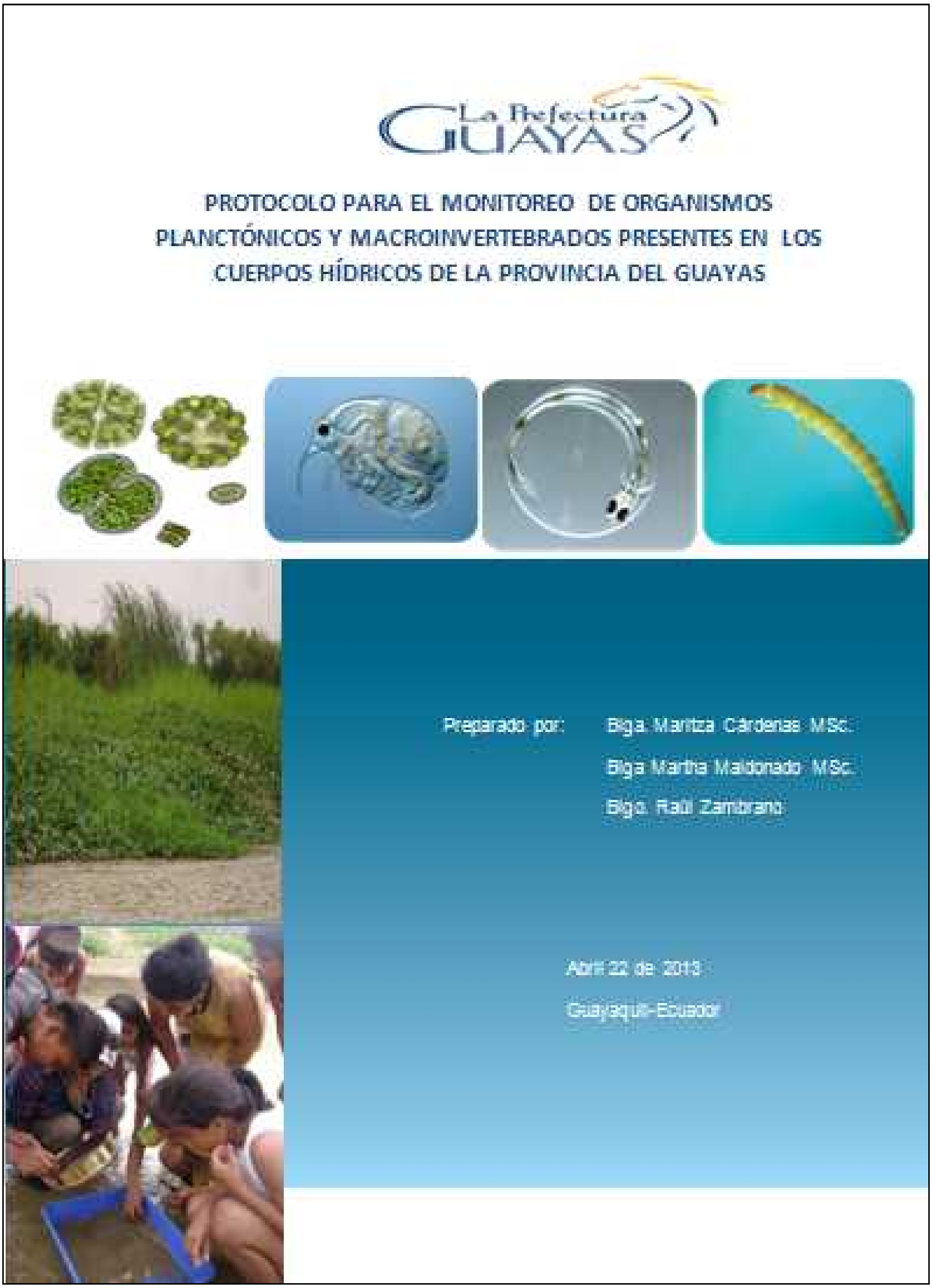
La Prefectura
GUAYAS

CALIDAD DE LAS AGUAS DE LOS CUERPOS HÍDRICOS DE LA PROVINCIA DEL GUAYAS MEDIANTE EL USO DE MACROINVERTEBRADOS ACUÁTICOS REGISTRADOS DURANTE NOVIEMBRE DE 2012 Y MARZO DE 2013.

Guayaquil-Ecuador

Abril de 2013

Preparado por: Blga. Maritza Cardenas.



La Prefectura GUAYAS

PROTOCOLO PARA EL MONITOREO DE ORGANISMOS PLANCTÓNICOS Y MACROINVERTEBRADOS PRESENTES EN LOS CUERPOS HÍDRICOS DE LA PROVINCIA DEL GUAYAS

Preparado por: Blga. Maritza Cárdenas MSc.
Blga. Martha Maldonado MSc.
Blgo. Raúl Zambrano

Abril 22 de 2013
Guayaquil-Ecuador

Annex 36. Technical report on the bioremediacion of polluted sediments by using native bacteria consortia from Estero Salado in 2012 prepared for the Ministry of Environment of Ecuador.

ESCUELA SUPERIOR POLITECNICA DE CHIMBORAZO

CENTRO DE SERVICIOS TÉCNICOS Y TRANSFERENCIA TECNOLÓGICA AMBIENTAL

[MINISTERIO DEL AMBIENTE DEL ECUADOR

UNIDAD GESTORA DE PROYECTOS DE REMEDIACIÓN AMBIENTAL Y SOCIAL

ESCUELA SUPERIOR POLITÉCNICA DE CHIMBORAZO

CENTRO DE SERVICIOS TÉCNICOS Y TRANSFERENCIA TECNOLÓGICA AMBIENTAL

ESTUDIO A NIVEL PILOTO SOBRE TRATAMIENTOS DE BIORREMEDIACIÓN DE LODOS DESARROLLANDO Y UTILIZANDO CONSORCIOS BACTERIANOS NATIVOS DEL ESTERO SALADO

SEGUNDO PRODUCTO

Informe técnico mensual sobre las actividades realizadas

USO DE TECNOLOGIAS BIOLOGICAS DE REMEDIACION

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