Anthropogenic Contaminations in the mangrove of Guadeloupe (Lesser Antilles): Use of a Biomarker of Genotoxicity for Monitoring

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ABSTRACT

Mangroves are hotspots of primary production which act as a buffer between marine and terrestrial environment. They shelter a unique ecosystem characterized by an exceptional biodiversity. In Guadeloupe, mangroves spread over 3000 hectares. Presently the mangrove ecosystem of Guadeloupe is under serious threat because many anthropogenic activities are concentrated on the coastline and contribute to the contamination of the whole coastal ecosystems by polluting substances.

Biomarkers have become valuable ecotoxicological tools to assess the toxicity and bioavailability of pollutants. In this study, two approaches are used to evaluate the importance of damage caused to mangroves ecosystem. The first one is the quantification of the pollutants (heavy metals, polyaromatics hydrocarbons and pesticides) notably in sentinel species (*Crassostrea rhizophorae*) and the second one is the measure of the impact on natural populations by using a biomarker of genotoxicity. Here we present an adaptation of this biomarker to assess DNA damage in different species as mangrove oysters (*Crassostrea rhizophorae*) and fishes (*Ocyurus chrysurus*). The quantification of DNA single strand breaks (SSB) by the comet assay can be used as a rapid and sensitive indicator of genotoxicant exposure. The xenobiotic concentrations should be correlated to comet assay results in order to locate risk zone. At present, complementary monitoring studies targeting other biomarkers of oxidative stress (catalase, GST, TBARS) are carried out to assess more precisely the impact of contamination of guadeloupean mangroves by human activities.

KEY WORDS: Mangrove, pollution, genotoxicity

Contaminaciones Antropogénicas en los Manglares de Guadalupe (Las Antillas Menores): Biomonitoreo por la Utilización de un Maracador Biológico de Genotoxicidad

Los mangles son lugares de producción primaria que actúan como tampon entre el ambiente marino y terrestre. Abrigan un ecosistema único caracterizado por un excepcional biodiversidad. En Guadalupe, el manglar se extiende sobre cerca de 3000 hectáreas. Actualmente los manglares de Guadalupe están bajo seria amenaza porque muchas de las actividades antropogénicas están concentradas en la línea de la costa y contribuyen a la contaminación de los ecosistemas costeros por sustancias contaminantes. Los marcadores biológicos son instrumentos preciosos para la evaluación de la toxicidad y la biodisponibilidad de los agentes contaminantes.

En este estudio, dos enfoques fueron abordados para evaluar la importancia de los daños causados a los manglares. El primero fue la cuantificación de los agentes contaminantes (metales pesados, pesticidas) en sedimentos y en la especie centinela (*Crassostrea rhizophorae*). El segundo fue la evaluación del impacto a nivel de las poblaciones naturales utilizando un marcador biológico de genotoxicidad. Este marcador biológico fue adaptado para evaluar el daño de ADN en varias especies como son : ostras de mangle (*Crassostrea rhizophorae*), pesces (*Ocyurus chrysurus*). La cuantificación de las roturas simples de las ramas del ADN por la prueba cometa puede ser utilizada como un indicador rápido y sensible a la exposición a sustancias con genotoxicidad. Las concentraciones de las sustancias xenobióticas pueden ser correlacionadas con los resultados de las pruebas cometas con el fin de localizar las zonas de riesgo.

Actualmente, se conducen estudios complementarios que se dirigen a otros contaminantes (HAPs). Estos emplean tambien marcadores biológicos de estrés oxydativo (catalasa, Glutathion-S-Transférasa, TBARS) con el fin de evaluar más precisamente la importancia de la contaminación de los manglares Guadalupeños por las actividades antropológénicas.

PALABRAS CLAVES: Manglar, polución, genotoxicidad

Contaminations D'origine Anthropique des Mangroves de Guadeloupe (Petites Antilles): Biosurveillane par L'utilisation d'un Biomarquer de Génotoxicité

Les mangroves sont de hauts lieux de productivité primaire qui jouent un rôle de tampon entre les milieux marin et terrestre. Elles abritent un écosystème unique caractérisé par une exceptionnelle biodiversité. En Guadeloupe, la mangrove s'étend sur environ 3000 hectares. Actuellement, les mangroves de Guadeloupe sont sérieusement menacées, car de nombreuses activités anthropiques se concentrent sur les zones côtières, contribuant ainsi à la pollution de l'ensemble des écosystèmes côtiers. Les biomarqueurs sont de précieux outils permettant d'évaluer la toxicité et la biodisponibilité des contaminants. Dans cette étude, deux approches ont été utilisées afin de déterminer l'importance des dommages causés aux mangroves. La première est la quantification des polluants (métaux lourds, pesticides) au sein du sédiment et d'une espèce sentinelle (*Crassostrea rhizophorae*). La deuxième est l'évaluation de l'impact au niveau des populations naturelles en utilisant un biomarqueur de génotoxicité. Ce biomarqueur a été adapté à différentes espèces : l'huître de palétuvier (*Crassostrea rhizophorae*), des poissons (*Diapterus rhombeus* ; *Ocyurus chrysurus*) et des plantes (*Rhizophorae mangle*), pour évaluer les dommages causés à l'ADN. La quantification des cassures simples brins de l'ADN réalisée grâce au test comète, peut être utilisée comme un indicateur rapide et sensible de l'exposition des organismes à des substances génotoxiques. Les concentrations des contaminants peuvent être corrélées avec les résultats des tests comètes afin de localiser les zones à risque. Actuellement, des études complémentaires ciblant d'autres contaminants (HAPs) et employant des biomarqueurs de stress oxydatif (catalase, Glutathion-S-Transférase, TBARS) sont conduites afin d'évaluer plus précisément

l'importance de la contamination des mangroves guadeloupéennes par les activités anthropiques.

MOTS CLÉS: mangrove, pollution, *Crassostrea rhizophorae*

INTRODUCTION

In Guadeloupe, mangroves are well developed along the shore. This exceptional ecosystem spreads over 3000 hectares of Guadeloupe's coastline. Mangroves are a hotspot of primary production (Imbert and Rollet 1989) which act as a buffer between terrestrial and marine ecosystems. They are characterized by an exceptional biodiversity. In Guadeloupe, industrial, agricultural, and urban developments coupled with the small size of the island contribute to concentrate the anthropogenic activities on the coastlines where most of the discharges of the island occur.

The main goal of this study is to establish a measure of anthropogenic pressure gradient, through an assessment of heavy metals, poly aromatic hydrocarbons (PAHs) and pesticides contaminations. It attempts to measure effects induced by pollution on organisms. To establish this gradient, bioaccumulation of contaminants was mainly assessed by studying a sentinel species, the mangrove oyster (*Crassostrea rhizophorae*). The effects induced by pollution were measured by using a biomarker of genotoxicity.

MATERIALS AND METHODS

Study Area

In this study, seven stations located in the shoreline mangrove were selected according to the location of the main streams and of the others most important sites of human activities (Figure 1). The Fajou Islet was defined as a control station because of its relative distance from the coastline.

Collection and Analytical Methods

On each station, oysters were hand sampling in order to the measure heavy metals (cadmium, mercury, lead) and polycyclic aromatic hydrocarbons (PAHs) concentrations with the help of respectively atomic absorption spectrometry and gas chromatography. For pesticides, organisms were collected with fish net and fish trap before being analyzed with the help of liquid chromatography.

Genotoxicity Analysis: The Comet Assay

The comet assay is a technique developed to detect DNA strand breaks which permits to identify genotoxicity of xenobiotic substances. Cells are included in agarose gel and then lysed in an alkaline solution prior to be submitted to electrophoresis. After neutralization, DNA is stained with ethidium bromide and then observed with a microscope. DNA damage is assessed according to a visual classification method. Comets are classified into four categories based on the length of migration and/or the perceived relative proportion of the DNA in the tail (**Figure 2**). A numerical value is assigned to each category: A (for an undamaged nucleus) to D (for a severely damaged nucleus). The numerical value permits to calculate a mean score for each station studied. For each individual, a hundred cells were observed. The mean score can fluctuate between 0 and 300. In this study, the comet assay was applied on gill cells of mangrove oysters (*Crassostrea rhizophorae*) (n = 10) and liver cells of yellowtail snapper (*Ocyurus chrysurus*) (n = 5).

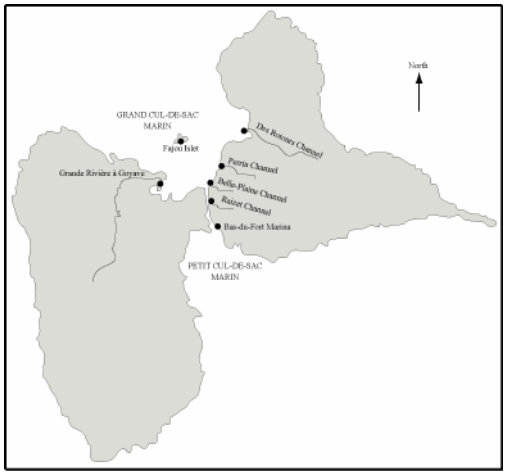

Figure 1. The study zone. Seven stations: Des Rotours Channel (DRC); Perrin Channel (PC); Belle Plaine Channel (BPC); Grande Rivière à Goyave (GRG) ; Raizet Channel (RC); Bas-du-Fort Marina (BFM).

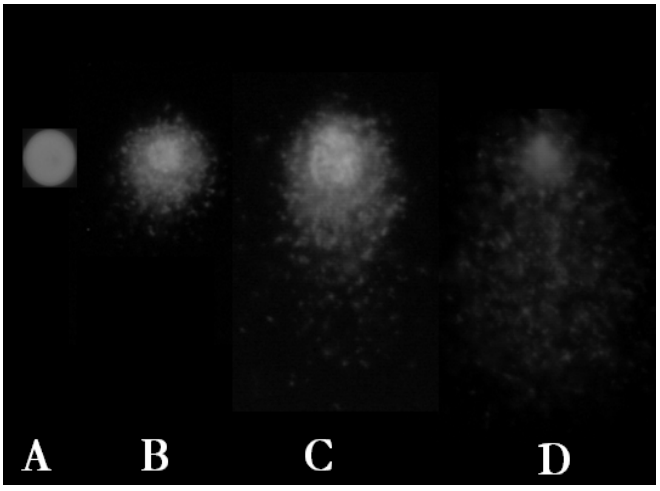

Figure 2. The different cell damage stages (A-D) used for scoring the comet assay (visual approach).

RESULTS

Metals, Pesticides and Polycyclic Aromatic Hydrocarbons Concentrations

Table 1 presents the mean heavy metals and PAHs (particularly Benzo(α)Pyrene:BαP) concentrations. Grande Rivière à Goyave shows the highest concentration of cadmium (0.55 mg/kg). Moreover, at this station, high concentrations of chlordecone, a very persistent pesticide, were recorded in crabs (*Callinectes exasperates*) (0.03 mg/kg) and shrimps (*Penaeus schmitti*) (0.079 mg/kg) (Bouchon and Lemoine 2007). Perrin Channel shows the highest concentration of mercury (0.25 mg/kg). High concentrations of dithiocarbamate were detected as well into fishes (*Archosargus rhomboidalis*) (0.40 mg/kg) and moray (*Gymnothorax funebris*) (0.39 mg/kg) (Bouchon and Lemoine 2007). The highest concentrations of lead were recorded at Raizet Channel (0.80 mg/kg) and Bas-du-Fort Marina (0.81 mg/kg). At this last one, very high concentrations of PAHs and Benzo(α)Pyrene were found (respectively 961.16 and 65.4 ng/g). It is important to underline that these values exceed the limit values determined by European legislation.

Table 1. Mean heavy metal and PAH concentrations at selected stations.

	Heavy metals (mg/kg)			Hydrocarbons (ng/g)	
Stations	Cadmium	Mercury	Lead	Σ PAHs	BαP
Des Rotours Channel (DRC)	0,14	0,1	0,15	66,86	2,03
Fajou Islet (FI)	0,27	0,06	0,2	117,2	3,1
Grande Rivière à Goyave (GRG)	0,55	0,09	0,16	142,56	12,9
Raizet Channel (RC)	0,19	0,11	0,8	163,03	2,6
Bas-du-Fort Marina (BFM)	0,17	0,06	0,81	961,16	65,4
Perrin Channel (PC)	0,21	0,25	0,14	-	-
Belle Plaine Channel (BPC)	0,17	0,06	0,11	-	-

Genotoxicity Analysis

Six stations were selected to carry out the comet assay on mangrove oysters (Figure 3). During the rainy season 2005, the highest level of DNA damage (193.75) is obtained at Bas-du-Fort Marina (BFM). During the following dry season 2006, the whole mean scores increased except for Grande Rivière à Goyave (GRG). The highest ones were recorded at BFM (253.80) and Raizet Channel (RC) (237.30). Conversely, during the dry season 2007, about one year later, the whole mean scores decreased. During this season, the level of DNA damage is the most important (196.90) at Raizet Channel (RC).

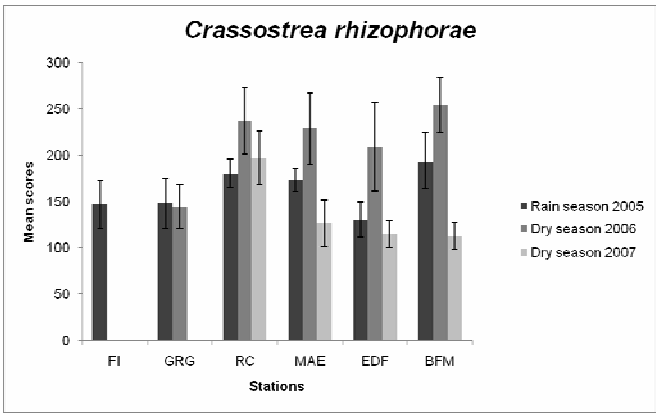

Figure 3. Levels of DNA damage recorded in mangrove oysters (*Crassostrea rhizophorae*) gill cells at six stations at different seasons. Mean scores (n=10 oysters per station, 100 cells per oyster) calculated using points assigned to four different comet classes (0, 1, 2 and 3).

Figure 4 presents the results of comet assay applied on yellowtail snapper (*Ocyurus chrysurus*) (Quistin 2007). In this study two stations were selected. One supposed to be free from pollution and the other one located near an industrial zone. The comet assay did not show significant differences between the stations.

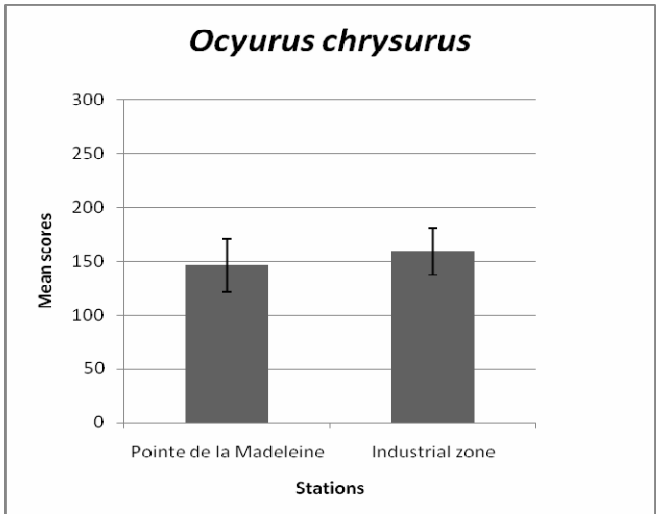

Figure 4. Levels of DNA damage recorded in yellowtail snapper (*Ocyurus chrysurus*) gill cells at six stations at different seasons. Mean scores (n = 10 oysters per station, 100 cells per oyster) calculated using points assigned to four different comet classes (0, 1, 2 and 3).

DISCUSSION

Table 2 presents a comparison of results between Guadeloupe and other Caribbean and South American countries. Concerning non essential metals cadmium, mercury and lead the concentrations are lower in Guadeloupe. Cadmium bioaccumulated in oysters coming from Grande Rivière à Goyave probably originate from anthropic sources, namely a rum distillery and illegal dumps. Moreover, heavy metal dispersion is probably enhanced at this station by the large amount of suspended materials and organic matter present in the stream. Lead is a metal commonly used in batteries. High concentrations of this metal recorded at Raizet Channel (RC) could partially originate from wrecked cars found in Grand-Camp's dump. Indeed, RC collects rain runoff from Pointe-à-Pitre, one of the main Guadeloupean towns and receives Grand-Camp's dump (31 hectares) lixiviation products. In the last few years, the frequentation of the Bas-du-Fort Marina (BFM) has considerably increased, generating higher heavy metal and hydrocarbons pollution levels (wastes from boat careening, car park and gas station drained into the stream).

Cadmium, like mercury and lead, are highly toxic non-essential metal (Depledge and Rainbow, 1990; Pruski and Dixon, 2002). Bihari and Fafandel (2004) show that Benzo(α)Pyrene is a genotoxic substance. The relative proportions of the different levels of DNA damage varies according to the station and the season. Between 2005 and 2006, RC and BFM were stations where high concentrations of genotoxic substances (heavy metal, PAHs) were recorded. The comet assay is very sensitive and shows that a high level of genotoxicity occurs where the anthropogenic activities are important.

The biomarker of genotoxicity used in these studies is a good diagnostic tool which permits complementary studies to be carried out: the use of biomarkers of oxidative stress (Catalase, Glutathion-S-Transferase, Thiobarbituric Acid Reaction Species) and accurate researches of pollutants. This study asks the question of the choice of the biological models. Even if the yellowtail snapper (*Ocyurus chrysurus*) gives responses concerning the level of DNA damages, it is a non-sedentary organism, conversely to the mangrove oyster which is a good sentinel specie for this kind of study.

Table 2. A comparison of heavy metal concentrations (mg/kg) from Guadeloupe and other Caribbean and South American countries.

Stations	Cd	Hg	Pb	References
Guadeloupe	0.14-0.55	0.05-0.11	0.16-0.81	*this study*
Dominican Republic	0.35-2.57	0.49-7.02	0.08-1.46	*Sbriz et al., 1998*
Brazil	0.8-1.9	-	1.8-7.8	*Silva et al., 2001*

CONCLUSION

Human activities release pollutants into the biotope. Among these pollutants, genotoxins are bioaccumulated and are responsible of disturbances at the individual scale, notably genotoxicity. Accumulation of unrepaired DNA lesion could explain the embryotoxicity of certain chemical pollutants. As embryotoxicity exerts a direct impact on recruitment rate, genotoxicity could be closely related to modifications of the structure of marine communities and then in long-term could produce a possible impact upon ecosystems.

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