

Temporal variations in the level of chlordecone in seawater and marine organisms in Martinique Island (Lesser Antilles)

Charlotte R. Dromard (1,5), Jean-Pierre Allenou (2), Nathalie Tapie (3), H  l  ne Budzinski (3), Nicolas Cimmaterra (2), Pauline De Rock (2), Salim Arkam (1), S  bastien Cordonnier (1), Jean-Louis Gonzalez (4), Yolande Bouchon-Navaro (1,5), Claude Bouchon (1,5), Emmanuel Thouard (2)

- (1) Unit   Biologie des organismes et   cosyst  mes aquatiques (BOREA), Universit   des Antilles, MNHN, Sorbonne Universit  , Universit   de Caen Normandie, CNRS, IRD, BP 592, 97157 Pointe-  -Pitre, Guadeloupe –_charlotte.dromard@univ-antilles.fr (+590 690 841 573)
- (2) IFREMER, Unit   Biodiversit   et Environnement de la Martinique, 79 route de Pointe Fort, 97231 Le Robert
- (3) UMR CNRS 5805 EPOC – OASU,   quipe LPTC, Universit   de Bordeaux, 351 Cours de la lib  ration, 33405 Talence Cedex
- (4) IFREMER, D  partement Oc  anographie et Dynamique des Ecosyst  mes, Laboratoire environnement ressources de Provence-Azur-Corse, Z.P. de Br  gaillon CS20330, 83507 La Seyne/mer cedex
- (5) Laboratoire d'excellence « CORAIL », Universit   des Antilles, Guadeloupe, France

ABSTRACT

The present study, conducted in the Galion Bay in Martinique, aims to highlight the temporal and seasonal variations of chlordecone contamination (an organochlorine pollutant) in the ambient environment (seawater) but also in the marine organisms in three main coastal marine habitats (mangroves, seagrass beds and coral reefs). To this end, two methodologies were used to measure and compare the chemical contamination of seawater during 13 months (spot samplings and POCIS technique). In parallel, concentrations of chlordecone and isotopic ratios (C and N) were carried out on marine organisms, collected during two contrasting climatic periods (dry and rainy), to evidence seasonal variations. The results showed that the contamination of seawater displayed significant variations over time and depended on environmental factors such as water flows, which imply dilution and dispersion phenomena. Concerning the marine organisms, the level of contamination varied considerably between the two seasons in seagrass beds with higher levels of contamination during the rainy season. Reef organisms were more moderately affected by this pollution, while mangrove organisms

showed a high level of chlordecone whatever the season. Finally, isotope analyses highlighted that bioamplification along marine food-webs occurs at each season and each station.

Keywords: organochlorine pollution, mangrove, seagrass, coral reef, passive samplers, stable isotopes

INTRODUCTION

In the French West Indies, aquatic environments have been exposed to a chronic contamination by chlordecone, an organochlorine pollutant used as insecticide in banana plantations from 1972 to 1993. This very persistent pollutant was spread during 20 years at the bottom of banana trees, leading to a transfer of the molecule from field to underground waters, rivers and coastal areas, due to runoff, leaching and erosion phenomena (Lesueur-Jannoyer *et al.*, 2016). Chlordecone is a pervasive issue in the French West Indies, which has caused major environmental pollutions but also risks to human health (Multigner *et al.*, 2016). Measurements carried out on the soils of Guadeloupe (Rochette *et al.*, 2020) and Martinique have made it possible to map the land surfaces according to their contamination risk (Desprats *et al.*, 2004). The soils located in the south of Basse-Terre in Guadeloupe present high risks of chlordecone contamination, totaling around 6,200 ha of polluted soils. In Martinique, soils at high risk of contamination are mainly located in the north of the island and 12,000 ha are considered to be polluted by chlordecone (Cabidoche and Lesueur Jannoyer, 2011). The presence of this molecule in the natural environment is worrying, particularly because of its toxicity to living organisms. Numerous pathologies, such as reproductive disorders or neurotoxicity syndromes, have been described in birds and mammals (Epstein, 1978; Huff and Gerstner, 1978). Chlordecone is also carcinogenic in rats and mice (Epstein, 1978). More recently, human studies have shown positive associations between chlordecone exposure and the risk of prostate cancer (Multigner *et al.*, 2010), prematurity (Kadhel *et al.*, 2014) or cognitive development delays in children (Dallaire *et al.*, 2012; Boucher *et al.*, 2013). The chemico-physical properties of the molecule make it extremely stable and therefore highly persistent in the natural environment, as well as having a high sorption capacity on soil organic matter (Kenaga, 1980; Cabidoche *et al.*, 2009). Once applied, chlordecone is stored in soils for a period varying from several decades to several centuries depending on the nature of the soil and the model used for estimations (Cabidoche *et al.*, 2006; 2009; Comte *et al.*, 2021). Thus, although the use of chlordecone was banned in 1993, the molecule is still present in agricultural soils of Guadeloupe and Martinique and the population is still exposed to this pollutant on a daily basis. The tropical climate of the two islands, characterized by abundant rainfall during the wet season (July to November), leads to significant runoff and infiltration episodes that allow chlordecone molecules to disperse easily in the environment and reach the marine environment *via* rivers and marine resurgences (Cattan *et al.*, 2008; Cabidoche, 2011). Most recently, Mottes *et al.* (2020) and Sabatier *et al.* (2021) showed that soil erosion is an important pathway for chlordecone transfer from crops to the coastal areas. Thus, the first studies carried out on the aquatic environment revealed

significant contamination of freshwater organisms (Kermarrec, 1980; Coat, 2009) but also of coastal marine organisms (Coat *et al.*, 2006; Bouchon and Lemoine, 2007; Bertrand *et al.*, 2009; Bodiguel *et al.*, 2011; Bertrand *et al.*, 2012; 2013; Dromard *et al.*, 2016). Between 2008 and 2013, numerous reports were published (Bertrand *et al.*, 2009; 2010a; 2010b; 2012; 2013), making it possible to target the marine species with a high risk of contamination as well as the maritime areas most affected by this pollution, in order to draw up fishing restriction and ban zones. In parallel with studies focused on mapping the areas and species impacted, some works have focused on the transfer mechanisms of the molecule from lands to coastal areas and the processes that lead to an accumulation of chlordecone in marine organisms, also called “bioaccumulation” (Bodiguel *et al.*, 2011; Dromard *et al.*, 2015). The results of these studies, during which marine organisms belonging to different trophic levels and coming from different marine ecosystems, revealed a decreasing gradient of contamination from the coast (mangrove) to the open sea (coral reefs) (Dromard *et al.*, 2017). This information indicates that quantities of chlordecone coming from the river is diluted upon arrival in coastal areas and decrease with the distance from the estuary. This fact involves a contamination of marine organisms through a “bathing” or “bioconcentration” phenomenon (*i.e.*, uptake from surrounding water through contact with the teguments in polluted waters), which is predominant in mangrove food-webs and decreases in more remote ecosystems such as seagrass beds and coral reefs (Dromard *et al.*, 2015; 2018). The second process of bioaccumulation is a “bioamplification” or “biomagnification” phenomenon (*i.e.* a trophic contamination during the consumption of contaminated prey), which can be observed in most of the food-webs contaminated with organochlorine or metallic pollutants (Gray 2002). This trophic contamination can lead to an impact on remote ecological systems such as pelagic food-web as demonstrated by the study from Mendez-Fernandez *et al.* (2018), who showed a contamination of marine mammals by chlordecone, even if concentrations found very relatively low. Thus, bioaccumulation of chlordecone in marine organisms is the result of two processes that may combine: bioamplification and “bathing” contamination (Dromard *et al.*, 2018). While methods for measuring chlordecone in organic matrices (animal or vegetal) have long been mastered, the analyze of seawater has long remained a challenge. Since a decade, several methodologies are developed and tested *in situ* to lead to an accurate measurement of chlordecone in water from rivers and coastal areas (Gonzalez *et al.* 2014, 2019). Analytical projects permitted to develop and improve methods for the analysis of chlordecone in water and to arrive at a protocol for analysis by direct injection in “LC-MS/MS” with a limit of quantification of less than 1 ng.l⁻¹. In parallel, these development research allowed the use of “POCIS” technique (Polar Organic Chemical Integrative Samplers) to measure chlordecone in an integrative approach (Gonzalez *et al.* 2019). In order to improve the understanding of chlordecone contamination of the marine environment, the present study was proposed 1) to investigate the level of contamination of the ambient environment over time, *i.e.* to follow contamination of waters in the river and coastal areas during 13 months, and 2) to study bioaccumulation processes in living organisms during two contrasting season (the rainy and the dry season).

MATERIALS AND METHODS

Study site

The study was conducted in the Galion Bay, a semi-enclosed bay located on the East coast of Martinique Island (Lesser Antilles). Four stations were chosen to evaluate the level of contamination of the ambient environment: a river station (ST-R), and three marine stations (ST1, ST2, ST3) with an increasing distance from the source of pollution that is the estuary (Fig. 1). The river station is a station monitored in the framework of the observatory “OPALE” (Observatoire des Pollutions Agricoles aux Antilles – Observatory for Agricultural Pollutions in Antilles), which is a structure following multiple physico-chemical variables along the watershed of the Galion River, as a workshop-site (Mottes *et al.* 2020). Three other stations were chosen to study the contamination of marine organisms, in order to represent the three coastal marine habitats found in the bay: a mangrove, a seagrass bed and a coral reef station (Fig. 1). Mangrove station is a canal colonized by *Rhizophorae mangle* trees. Seagrass beds are located in a shallow site (1 meter deep) and are characterized by a meadow of *Thalassia testudinum* seagrass. The coral reef station is a small fringing reef, presenting a drop off (5 m deep maximum) exposed to the dominant current and wind.

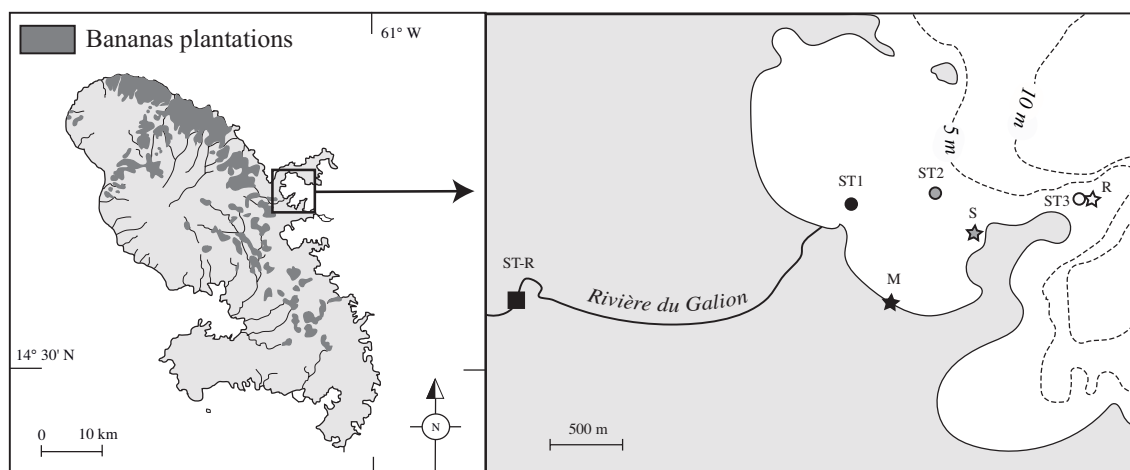


Fig. 1 Location of the three stations chosen to evaluate seawater contamination (circle, ST1, ST2, ST3) and marine organisms (stars) in mangrove (M, black), seagrass beds (S, grey) and coral reef (R, white). The black square indicates the river station (ST-R)

Sampling campaigns and measurements for seawater samples

The level of contamination of seawater and water from the river was followed during one year, from March 2018 to March 2019 on four stations (ST-R, ST1, ST2 and ST3). Two sampling techniques were applied for water analysis in order to have access to the spot concentrations of chlordecone (“spot sampling”) and the time-weighted average (TWA) concentrations of chlordecone (“passive sampling”).

For the spot sampling method, water (1 l) was sampling every three weeks in order to achieve instantaneous contamination at the sampling time. Chlordecone was analyzed by LC-MS/MS (adapted from Dromard *et al.*, 2018 and Hubas *et al.*, 2022) by direct

injection of 40 μL of water sample after adding of the internal standard of CLD 13C10 (Gonzalez *et al.*, 2019). The analytical method was validated in terms of extraction recoveries (78% to 108%, samples of fortified mineral water from 10 to 1000 ng.l^{-1}) and in terms of limits of quantifications (LOQ: 1.5 ng.l^{-1}). For each series of analysis, blank experiments (complete procedure but without matrix) were performed. Control calibrating standards (10 à 1000 ng.l^{-1}) were also injected every 15 samples and analytical blanks were performed.

Passive samplers used in this study (for the passive sampling method) were POCIS (Polar Organic Chemical Integrative Sampler) (Alvarez *et al.*, 2004). They allowed to access to the integrated concentration of chlordecone during a period of exposition of three weeks (Gonzalez *et al.*, 2014, 2019). POCIS were home made by sandwiching 200 mg of OASIS HLB sorbent between two membranes that were maintained by two stainless steel rings. Prior to use, sorbent was cleaned with methanol, dried under vacuum and spiked with Performance Reference Compounds (PRC) Caffeine C13, Deisopropyl-atrazine D5 (DIA D5), Salbutamol D3 (Belles *et al.*, 2014). The theory and modelling of POCIS are not described in details in this paper. To sum up, during the phase of linear uptake (3 to 5 weeks), the quantity of chlordecone accumulated in the sampler is proportional to the concentration of chlordecone in water following the equation:

$$M_s(t) = C_w R_s t$$

where M_s : quantity of accumulated Chlordecone (ng), C_w : concentration of chlordecone in water (ng.l^{-1}), R_s : Sampling Rate (l.j^{-1}), t (time) and the sampling rate is corrected by the PRC approach (Mazzella, 2010 and Belles *et al.*, 2014).

More methodological information can be found in Alvarez *et al.* (2004), Mazzella (2010) and Belles *et al.* (2014). After exposure, POCIS were sent frozen to the laboratory EPOC for analyses according to the protocols from Tapie *et al.* (2011), Belles *et al.* (2014) and Dufour (2017). To sum up, the POCIS sorbent was transferred into an empty glass SPE tube with polyethylene frits and was dried using the Visprep SPE vacuum manifold (Supelco) for 30 min. Then, chemicals were eluted successively with 10 mL MeOH, 10 mL MeOH/DCM (50/50; v/v) mixture and 10 mL DCM in a receiving vial which contained internal standards (CLD 13C10). The extract was concentrated to 200 μL of ACN under nitrogen flow for analysis on (LC-MS/MS) (Gonzalez *et al.*, 2019). The analytical method was also validated in terms of extraction recoveries (75% to 97%, OASIS HLB sorbent fortified with 5 to 500 ng of chlordecone) and in terms of limits of quantifications (0.8 ng.g^{-1} of sorbent, and 10 pg.l^{-1} extrapolated in water). For each series of analysis, blank experiments (complete procedure but without matrix) were performed. No significant contamination was observed. Control calibrating standards (5 à 500 ng.g^{-1}) were also injected every 15 samples and analytical blanks were performed.

Weekly measurements (semi-integrative samplings) were also done in the river station (ST-R) by the Observatory OPALE. Concentrations of chlordecone were determined by chromatography coupled to mass spectrometry in tandem (HPLC-MS/MS) by the laboratory LDA26 (Valences, France) (Mottes *et al.* 2020). These weekly concentrations were used to calculate the daily flow of chlordecone. The daily quantity of chlordecone transferred from the land to the marine environments, or "daily flow", was then calculated by multiplying the daily river flow (public data from Hydro.eaufrance

website) by the concentration of chlordecone measured every week at the river station by OPALE, following the equation:

$$\text{Daily flow (g.d}^{-1}\text{)} = \text{daily river flow (m}^3\text{.d}^{-1}\text{)} \times \text{concentration in chlordecone (g.m}^{-3}\text{)}$$

Linear regressions were also carried on between the concentration in chlordecone (in ng.l⁻¹) measured at the four stations (ST-R, ST1, ST2 and ST3) by the punctual sampling method, and the river flow (in m³.s⁻¹) at same date, in order to test the influence of the river flow on the transfer of chlordecone in surrounding aquatic habitats.

Sampling campaigns and measurements for marine food-webs

Two sampling campaigns were organized to collect marine organisms: in October 2018 during the wet season and in March 2019 during the dry season. During both campaign, animal and vegetal organisms were collected at each station according to their trophic level (primary producers, primary consumers, secondary consumers, decomposers). Principal terrestrial sources of organic matter were also collected in the bay that is sediment and Particulate Organic Matter (POM). In total, 329 samples were collected (176 during the dry season and 153 during the wet season), representing 62 species. Between 3 and 8 individuals per species or category of sample (sediment, POM) were used for analyses. Fish were collected using nets in seagrass beds and mangroves, fish pots and spear fishing in reef areas. Benthic invertebrate, vegetal matter and sediments were sampled by hand in snorkeling. Seawater (20 l per station) was collected in jerricans and filtered on Whatman GF/C filters to collect POM. For each living sample, a piece of tissue (muscle for animals, leaves or *thalli* for vegetal) was conditioned and conserved in a freezer until analyses (-20°C). Concentrations of chlordecone were quantified with liquid chromatography coupled to mass spectrometry in tandem (UPLC-MS/MS), by the laboratory LABOCEA (Quimper, France). Chlordecone concentrations were expressed in µg.kg⁻¹ (wet weight). The LOD and the LOQ were respectively 0.5 and 1 µg.kg⁻¹ with this method for living animal or vegetal and measurement precision was 0.1 µg.kg⁻¹ when data were superior to the LOQ. The LOQ for sediments was 10 µg.kg⁻¹ and 20 µg.kg⁻¹ for POM.

Stable isotope analyses

A subsample of each animal and vegetal organisms, POM filters and sediments collected was dried in an oven (60°C during 48h) for stable isotopes analyses. All samples were ground with an agate pestle and a mortar, using a grid of 1 mm mesh, to obtain a homogeneous powder. Powder samples were then packed into tin capsules. Nitrogen and carbon isotope ratios were determined by a continuous flow mass spectrometer (Thermo Fisher™, delta V Advantage) coupled with an elementary analyzer (Thermo Fisher™, Flash EA 1112) to measure carbon and nitrogen concentrations ([C]% and [N]%). Analyses were performed by the laboratory LIENS (La Rochelle, France). Isotopic ratios were expressed in standard delta notation (δ values in ‰) according to the following formula:

$$\delta = [(R_{\text{sample}} / R_{\text{standard}} - 1)] \times 1000$$

where R is the ratio of a heavy isotope to a light isotope ($^{15}\text{N}:^{14}\text{N}$ or $^{13}\text{C}:^{12}\text{C}$), R_{sample} is measured for sample and R_{standard} is an international standard (Vienna Pee Dee belemnite limestone carbonate for carbon and atmospheric air for nitrogen) (Fry 2006).

Statistical analyses

To compare seasonal variations of the concentrations of chlordecone in marine organisms, a subset of the database, clustering only species or genus that have been collected both during the wet and the dry season, was selected. As data were not normally distributed (tested with Shapiro-Wilks test), comparisons of chlordecone concentrations between seasons were tested using with Mann-Whitney tests. Species from similar genera, and exhibiting similar ecology, were pooled together to obtain a higher number of samples for comparisons.

Then, considering the whole database, relationships between concentrations in chlordecone and nitrogen isotopic signature (a *proxy* of the trophic level, Fry 2006) were tested using simple linear regressions, in order to assess bioaccumulation phenomenon along the three studied food-webs (Borgå *et al.* 2012). Coefficients “ a ” (slope) were used to calculate “Trophic Magnification factors (TMF)”, following the equation: $\text{TMF} = 10^a$ (adapted from Sun *et al.*, 2015). TMF superior to 1 indicates a biomagnification phenomenon along a food web, that is an increase of chlordecone concentration by trophic pathway (consumption of contaminated preys). Coefficient “ b ” was used to evaluate the ambient level of contamination, following the conversion equation: 10^b . All analyses were performed using R Studio (V.1.2.5033), with packages “*pgirmess*” and “*ggplot2*”.

RESULTS

Variations in the level of contamination measured in freshwater and seawater

Concentrations in chlordecone measured after spot sampling (direct analysis with UPLC-MS/MS) varied from 257 to 882 ng.l^{-1} in the river station (mean concentration \pm SE = $557.0 \pm 171.4 \text{ ng.l}^{-1}$) and the highest concentrations were observed in July and November 2018. In the marine stations, concentrations in chlordecone varied from 1.3 to 91.5 ng.l^{-1} with a decreasing gradient of concentrations from ST1 to ST3 (mean \pm SE = $46.0 \pm 19.5 \text{ ng.l}^{-1}$ in ST1, $33.0 \pm 14.3 \text{ ng.l}^{-1}$ in ST2 and $7.6 \pm 5.6 \text{ ng.l}^{-1}$ in ST3), that is from the coast to the open sea (Fig. 2).

The passive sampling method presented integrated concentrations of chlordecone during a period of three weeks. Concentrations varied between 83.5 and 1449 ng.l^{-1} in station ST-R (mean \pm SE = 679.0 ± 415.0), between 6.5 and 187 ng.l^{-1} in station ST1 (mean \pm SE = $29.0 \pm 16.6 \text{ ng.l}^{-1}$), between 3.5 and 160 ng.l^{-1} in station ST2 (mean \pm SE = $14.0 \pm 9.3 \text{ ng.l}^{-1}$) and between 0.95 and 11.5 ng.l^{-1} in station ST3 (mean \pm SE = $2.8 \pm 1.7 \text{ ng.l}^{-1}$)(Fig. 3). A decreasing gradient in the level of contamination in the ambient environment has also been detected with this method, from the coast to the open sea.

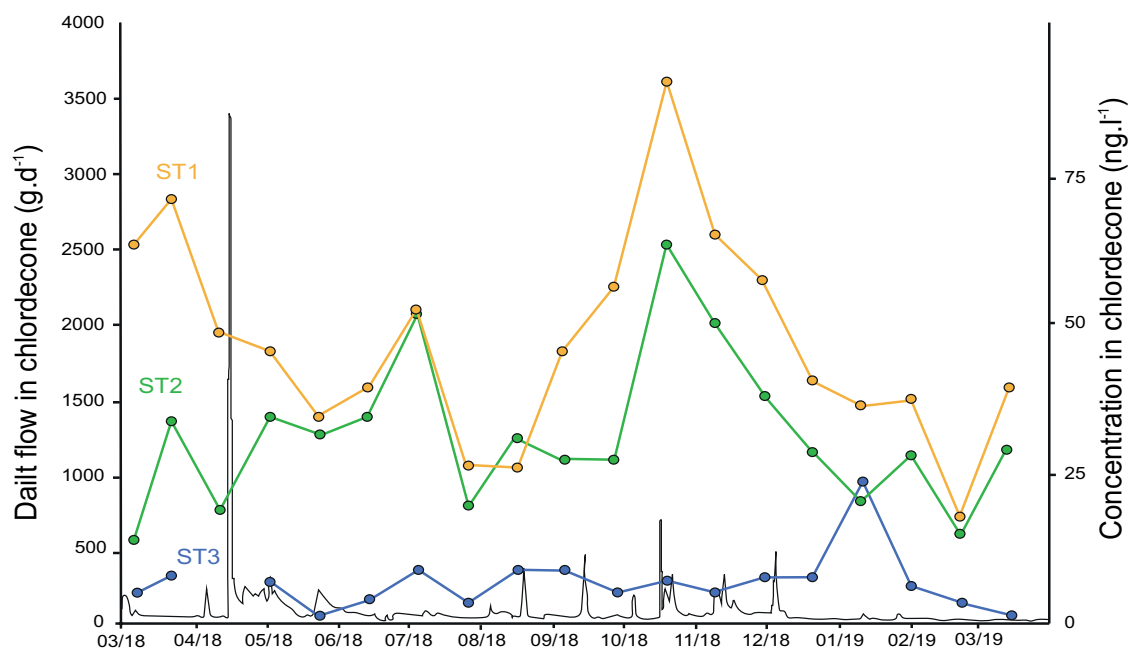


Fig. 2 Concentrations of chlordecone (in ng.l^{-1}) measured in seawater using spot samplings method in the three marine stations (ST1 In orange, ST2 in green and ST3 in blue) and daily flow of chlordecone (in g.d^{-1}) estimated in the river (in black)

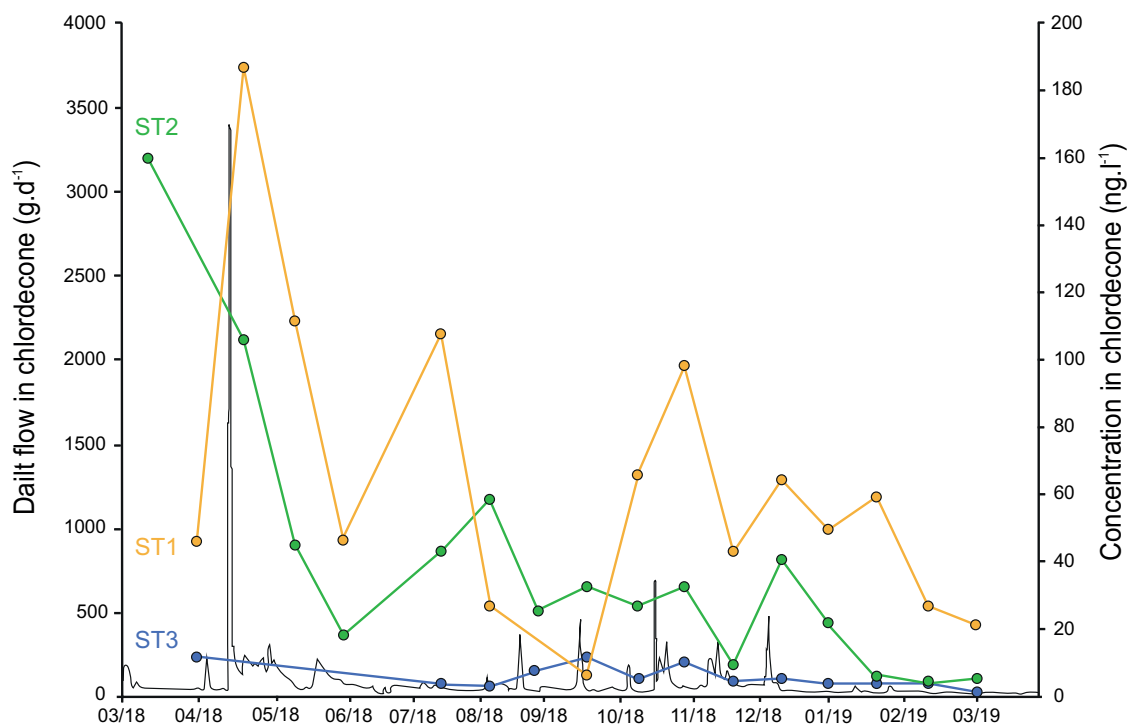


Fig. 3 Concentrations of chlordecone (in ng.l^{-1}) measured in seawater using POCIS method in the three marine stations (ST1 In orange, ST2 in green and ST3 in blue) and daily flow of chlordecone (in g.d^{-1}) estimated in the river (in black)

Transfer of chlordecone between land and marine environments

The quantity of chlordecone transferred from the land to the marine environments was estimated during the period of the study. The estimated daily flow of chlordecone presented high variations, with a minimum value equal to 5 g and a maximum equal to 3400 g (Fig. 2 and 3). The total quantity of chlordecone transferred from the Galion catchment basin to the sea was estimated to 27 kg of chlordecone between January 2018 and March 2019.

A positive relationship was shown between the value of the river flow and the concentration of chlordecone measured in the marine stations ST1 and ST2 (linear regressions: $R^2=0.57$ and $R^2=0.64$, $p<0.01$ for both analyzes). The highest flow values are responsible for higher concentrations of chlordecone in the mangrove and seagrass stations (ST1 and ST2). On the other hand, at the reef station (ST3), that is the most distant station from the estuary, the linear regression shows no correlation between the two parameters ($R^2=0.09$, $p=0.7$). In the same way, no correlation was also found in the river station ($R^2=0.36$, $p=0.13$).

Seasonal variations in the level of contamination of marine organisms

The quantities of chlordecone released from the Galion catchment basin, over the two-month period preceding the sampling campaigns, were estimated at 3.4 kg during the wet season and at 0.9 kg during the dry season. In the mangrove station, nine categories of marine organisms, representing 69 samples, were collected and analyzed, in addition to POM and sediments. Mean concentration of chlordecone all organisms pooled was $1310 \mu\text{g.kg}^{-1}$ in the wet season and $1518 \mu\text{g.kg}^{-1}$ in the dry season. Samples of POM were more contaminated during the dry season, as well as oysters *Crassostrea rhizophorae* (primary consumer), crabs from the genus *Callinectes* (decomposer), and fish from the genus *Sphoeroides* (secondary consumer). On the contrary, the fish species *Bairdiella ronchus* and *Mugil curema* (tertiary and secondary consumer respectively, as well as the two species of shrimps collected, did not exhibit significant differences in chlordecone concentrations between seasons (Table 1).

Table 1. Median concentrations of chlordecone (min-max in $\mu\text{g.kg}^{-1}$) measured in marine organisms at the mangrove station, during the wet season and the dry season. P-value indicate the significance of the differences between season tested with Mann-Whitney tests; NS: non-significant differences. n is the total number of samples per species, genus or category.

Samples	n	Wet season	Dry season	p-value
POM	6	75 (74-78)	186 (181-199)	<0.05
Sediment	8	90 (62-111)	86 (46-88)	<0.05
<i>Crassostrea rhizophorae</i>	6	955 (882-969)	712 (675-755)	<0.05
<i>Mugil curema</i>	5	411.5 (407-416)	999 (523-1019)	NS
<i>Callinectes</i> spp.	10	2603 (1619-3922)	3688.5 (2957-4392)	<0.05
Shrimp Sp 1	5	752 (718-924)	1230 (886-1332)	NS
Shrimp Sp 2	9	1098.5 (967-1230)	904 (566-1083)	NS
<i>Atherinella brasiliensis</i>	2	1712	1800	-
<i>Gerres cinereus</i>	3	1262.5 (664-1861)	1160	NS

<i>Sphoeroides</i> spp.	7	1746 (1679-2005)	2413 (2258-2620)	<0.05
<i>Bairdiella ronchus</i>	8	661 (526-1571)	1710 (1416-1800)	NS

In seagrass bed environment, 50 samples representing seven genera or species were studied for comparisons between seasons. Mean concentration of chlordecone during the wet season was 255.5 $\mu\text{g.kg}^{-1}$ and 132.9 $\mu\text{g.kg}^{-1}$ during the dry season. Chlordecone was not detected in POM and sediments samples from the seagrass habitat. Primary producers (*Padina* spp. and *Thalassia testudinum*), two primary consumers: the sponge *Amphimedon compressa* and the sea cucumber *Holothuria mexicana* as well as the detritivorous lobster *Panulirus* spp. exhibited higher concentrations in chlordecone during the wet season (Table 2). No difference of chlordecone level was observed between seasons for two genera of fishes, *Acanthurus* spp. (primary consumer) and *Holocentrus* spp. (secondary consumer).

Table 2. Median concentrations of chlordecone (min-max in $\mu\text{g.kg}^{-1}$) measured in marine organisms at the seagrass station, during the wet season and the dry season. P-values indicate the significance of the differences between season tested with with Mann-Whitney tests; NS: non-significant differences. n is the total number of samples per species, genus or category.

Samples	n	Wet season	Dry season	p-value
<i>Padina</i> spp.	6	27 (23-34)	8 (6.4-8)	<0.05
<i>Thalassia testudinum</i>	6	37 (36-47)	9.1 (8-10)	<0.05
<i>Acanthurus</i> spp.	11	122 (82-813)	82 (39-124)	NS
<i>Amphimedon compressa</i>	6	82 (70-367)	13 (11-14)	<0.05
<i>Holothuria Mexicana</i>	7	24 (16-29)	7.1 (6.4-8.8)	<0.05
<i>Panulirus</i> spp.	6	571 (396-571)	284 (277-346)	<0.05
<i>Holocentrus</i> spp.	8	473.5 (398-1454)	457.5 (415-582)	NS

At the coral reef station, eight species or genus of marine organisms (60 samples) were studied for seasonal variations. Mean concentrations of chlordecone during wet and dry seasons were 234.8 and 193.3 $\mu\text{g.kg}^{-1}$ respectively. Chlordecone was not detected in POM and sediments samples from the reef habitat. There was no difference of chlordecone concentrations between dry and wet season for all the studied organisms (Table 3).

Table 3. Median concentrations of chlordecone (min-max in $\mu\text{g.kg}^{-1}$) measured in marine organisms at the coral reef station, during the wet season and the dry season. P-value indicate the significance of the differences between season tested with with Mann-Whitney tests; NS: non-significant differences. n is the total number of samples per species, genus or category.

Samples	n	Wet season	Dry season	p-value
<i>Acanthurus</i> spp.	15	89 (46-170)	75 (50-177)	NS
<i>Sparisoma viride</i>	8	107.5 (71-144)	60 (57-141)	NS
<i>Tripneustes ventricosus</i>	7	309.5 (38-424)	22 (7-48)	NS
<i>Panulirus</i> spp.	9	346 (283-402)	536 (257-605)	NS
<i>Holocentrus</i> spp.	9	380.5 (275-363)	400 (206-400)	NS
<i>Scorpaena plumieri</i>	2	242	311	NS
<i>Aulostomus maculatus</i>	4	207	377 (337-413)	NS
<i>Caranx crysos</i>	6	478.5 (129-504)	6.5 (0-13)	NS

Processes of bioaccumulation

All samples collected during the study were used for linear regressions, that is 329 samples (176 during the dry season and 153 during the wet season). Linear regressions presenting log-transformed concentrations in chlordecone ($\text{Log}_{10}[\text{chlordecone}]$) function of nitrogen isotopic signatures ($\delta^{15}\text{N}$) were drawn for each habitat: mangrove (Fig. 4a), seagrass bed (Fig. 4b) and coral reefs (Fig. 4c).

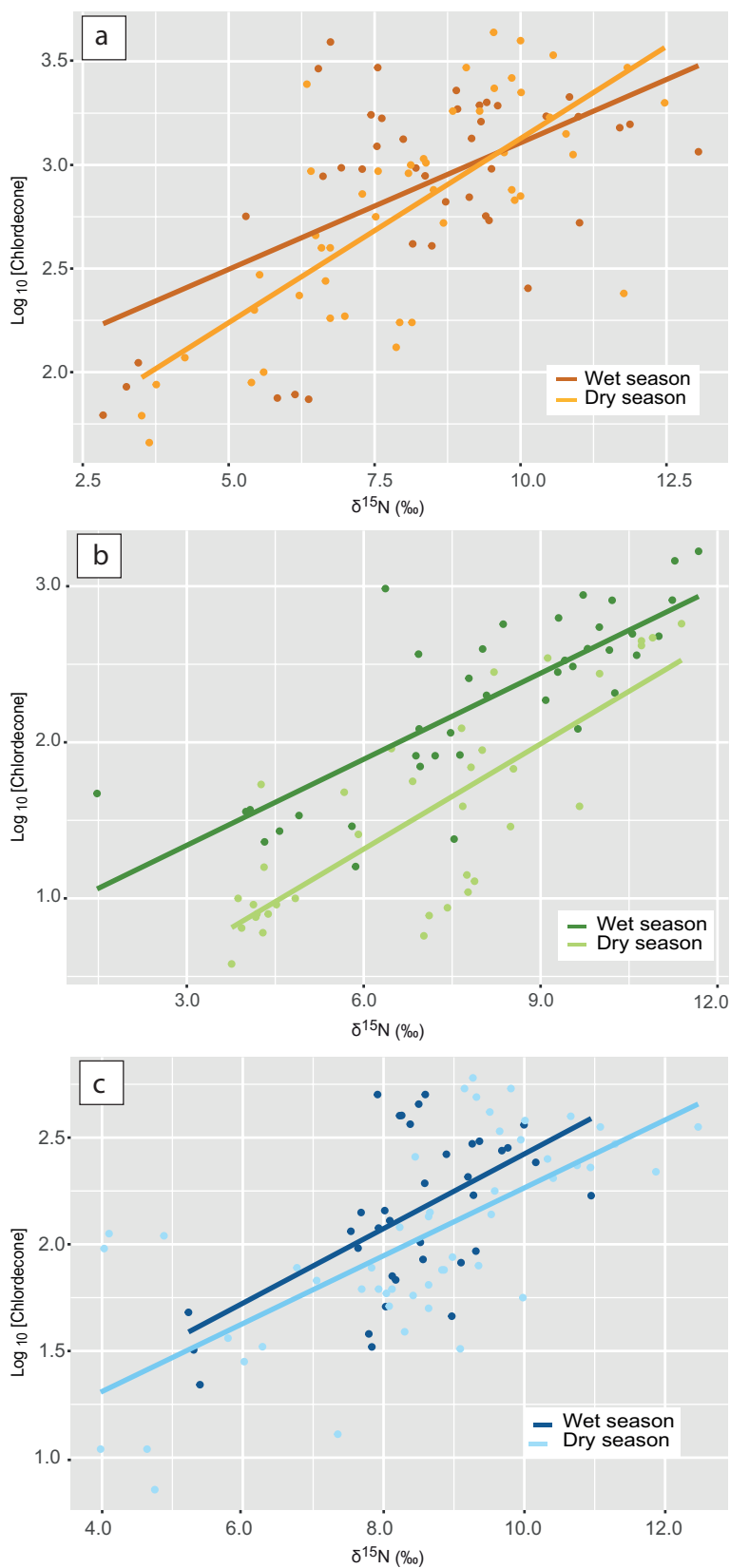


Fig. 4 Linear regressions between log-transformed concentrations values of chlordecone and nitrogen isotopic signatures (‰) in different marine habitats: mangrove (a), seagrass beds (b) and coral reef (c), during the wet and the dry season

For each habitat and each season, results of the linear regressions showed a positive correlation between the nitrogen signature, that is a trophic level proxy, and the level of contamination by chlordecone. The values of the "b" coefficients (ordinates at the origin) reflect the ambient level of contamination (Table 4). Thus, the b coefficients were high in the mangrove station (> 1), intermediate in the seagrass beds (0.78) and low in the reef (0.67). These coefficients correspond to an estimated concentration of chlordecone in the ambient environment equal to 77.62 $\mu\text{g.kg}^{-1}$ in mangrove, 6.03 $\mu\text{g.kg}^{-1}$ in seagrass beds and 4.68 $\mu\text{g.kg}^{-1}$ during the wet season (Table 4). At the mangrove and seagrass stations, the b coefficients were higher during the wet season rather than during the dry season. At the coral reef station, the b coefficients were equal in both seasons.

The "a" coefficients, corresponding to the slopes of the regression lines, gave an indication of the degree of bioaccumulation along the food chain and made it possible to calculate the "Trophic Magnification Factor" (TMF)(Table 4). All the TMF indices calculated, regardless of the station or season, were greater than 1. These indices ranged from 1.32 to 1.66. There was also a difference in TMF between the two seasons. Indeed, in mangroves and in seagrass beds, TMF indices were higher during the dry season. At the reef station, the two TMF indices calculated in the dry and wet seasons were relatively similar (1.48 and 1.45).

Table 4. Results of linear regressions between log-transformed concentrations values of chlordecone and nitrogen isotopic signatures. "a" is the slope, "b" is the intercept, "R²" is the coefficient of correlation, TMF: Trophic Magnification Factor, p-values indicate the significance of the test.

	Mangrove		Seagrass bed		Coral reef	
	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season
Slope "a"	0.12	0.18	0.18	0.22	0.17	0.16
Intercept "b"	1.89	1.35	0.78	-0.03	0.67	0.67
10 ^b	77.62	22.38	6.03	0.93	4.68	4.68
R ² adjusted	0.30	0.54	0.61	0.61	0.30	0.46
p-value	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001
TMF (10 ^a)	1.32	1.51	1.51	1.66	1.48	1.45

DISCUSSION

The present study aimed to measure the level of chlordecone in water from different aquatic environments (river and marine habitats) and marine organisms in the coastal areas and compare these values over time or between seasons.

The first part of the present study focused on the measurements of chlordecone concentrations in waters during 13 months, at different stations (river and three marine stations). To do so, two methods of sampling were used. The first method (spot samplings analyzed with UPLC-MS/MS) was very efficient. With a limit of quantification

lowered to 1.5 ng.l^{-1} , this method allowed the quantification of chlordecone on all the samples collected during the present study. The data obtained provided reference values for other methods under development. The second method, using "POCIS", proved to be very effective, allowing quantification of all the samples even at low concentrations. This method offers two advantages: it allows the integration of contamination over time and thus highlights fleeting episodes of contamination, and it offers a lower limit of quantification than the other methods (10 pg.l^{-1}). The results highlighted differences in the behavior of the tool between the river station and the marine stations, in particular a stronger desorption of the tracers, like PRC (Performance Reference Compound), at the marine stations (Dufour, 2017). This point deserves to be studied at greater depth, for example by *in situ* calibration in marine water at the study site. Finally, three-week immersion period may be too long, with the risk of a loss of accuracy. For example, the highest concentrations measured in the river in November 2018 by the punctual measurements were not reflected in the results of POCIS.

The total estimated flows of chlordecone reaching the marine environment from the Galion catchment basin, over the period from January 2018 to March 2019, was estimated at 27 kg of chlordecone. This result is consistent with those obtained by Mottes *et al.* (2020) for the period 2016-2017. In this late study, annual flows of chlordecone were estimated at 33 to 55 kg for the Rivière du Galion and at 19 to 25 kg per year for the Rivière La Digue, which also contributes to chlordecone inputs in the Galion Bay (Mottes *et al.*, 2020). It should be noted that inputs from the La Digue River, which is also a major contributor to chlordecone inputs in Galion Bay, was not taken into account in our study. Flows of chlordecone are very variable over time. They depend very strongly on the river flow, which varies quickly, even on a daily basis. Thus, the Galion Bay receives continuous chlordecone inputs from the Galion catchment basin, but with very variable intensities.

In the marine environment, these irregular inputs of chlordecone from the river generated fluctuating concentrations in coastal areas, particularly at ST1 and ST2. At station ST3 ("offshore" station), the levels of contamination were considerably lower than ST1 and ST2. This result could be explained by physical phenomena of dilution and dispersion offshore, which are themselves strongly conditioned by the wind (direction and intensity) and the oceanic swell. A more detailed knowledge of local hydrodynamic conditions would be necessary to better understand the mechanisms of marine contamination and, in particular, the area of influence of outputs from the Galion River on the marine environment outside the bay. Globally, a decreasing gradient of seawater contamination was observed from the coast (ST1) to the open sea (ST3). This decreasing gradient of contamination was also reflected in marine organisms, as attested by the linear regression (coefficients *b*) and as it has been evidenced before in Guadeloupe (Dromard *et al.*, 2017). Indeed, the global level of contamination was high in the mangrove system, compared to the two other habitats.

The second objective of this study was to compare the level of contamination in marine organisms at two contrasted seasons (dry and wet seasons). Thus, a seasonal

comparison was conducted on targeted species. At the mangrove and seagrass stations, the level of contamination (coefficients b) was higher during the wet season, probably indicating a higher influence of the river and higher chlordecone inputs, in relation to the higher river flow and quantities of chlordecone reaching the sea. Indeed, the estimated quantity of chlordecone reaching the sea few months before samplings marine organisms during the wet season (August to October 2018) was 3432 g whereas this quantity was equal to 904 g during the dry season (January to March 2019). These results were attested by the concentrations of chlordecone measured in seawater with the spot sampling method, and during which concentrations measured during the rainy season (October 18) were two time higher than those measured during the dry season (March 19). In mangrove and seagrass beds, targeted marine organisms displayed different behavior facing the contamination. In mangrove, some species showed highest level of contamination during the dry season like the fish *Sphoeroides* spp. or crabs *Callinectes* spp., probably due to their diet (invertebrates' feeders) or their link with the sediment or detritus that could accumulate contaminants. Others species, such as filters feeders *Crassostrea rhizophorae* exhibited highest concentrations in chlordecone during the wet season, probably because the number of organic particles in the water column, on which chlordecone binds, is greater during the rainy season (more erosion in the river). In seagrass station, majority of the marine organisms tested were more contaminated during the wet season compared to the dry season, probably because chlordecone inputs are higher during the wet season and because they may decontaminate themselves during the dry season. At the reef station, the b coefficients were equal in both seasons, suggesting a stable ambient level of contamination throughout the year, due to the distance of this station from to the source of pollution, that is the river estuary. Likewise, targeted species exhibited similar level of contamination between wet and dry season. These results have been confirmed by the measurements in seawater that showed similar concentrations throughout the year.

Trophic Magnification factors, calculated with the slopes of the linear regression (coefficient a) were all greater than 1, attesting to the existence of biomagnification phenomenon at each site, at each season (Broman *et al.*, 1992; Sun *et al.*, 2015). These indices TMF ranged from 1.32 to 1.66. There was also a difference in TMF between the two seasons. Indeed, in mangrove and seagrass beds, the indices were higher during the dry season. These results may suggest the relative dominance of the trophic contamination phenomenon during the dry season and the relative dominance of the "bathing" contamination phenomenon during the wet season. At the reef station, the two TMF indices calculated in the dry and wet seasons are relatively similar (1.48 and 1.45), demonstrating the low influence of the river flow on the contamination of marine organisms at this station, and the dilution of the contamination with the distance from the river estuary.

At a larger temporal scale, previous studies have evidenced high variability in the chlordecone input from crops to surface or grounds waters (Cattan *et al.*, 2019; Sabatier *et al.*, 2021). These results lead to the prediction of a large irregularity in the release of chlordecone in the coastal environment. However, the retention time of chlordecone in

marine ecosystems, the last outlets of the pollution, is not predictable in the light of our current knowledge.

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Statements & Declarations

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Authors contributions

All authors contributed to the study conception and design. Field work, material preparation and data collection were performed by Charlotte Dromard, Jean-Pierre Allenou, De Rock Pauline, Salim Arkam, Sébastien Cordonnier, Nicolas Cimmaterra, Yolande Bouchon-Navaro, Claude Bouchon and Emmanuel Thouard. Samples analyses, statistical analyses and results illustrations were conducted by Charlotte Dromard, Nathalie Tapie, Hélène Budzinski, De Rock Pauline, Jean-Louis Gonzalez. The first draft of the manuscript was written by Charlotte Dromard and Pauline De Rock, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethical approval

We attest that we have no conflicts of interests regarding this study.

This study doesn't involve human participants. Animals were collected to measure concentration of chlordecone (fish and invertebrates). These animals were sacrificed according to a strict ethical protocol, including rapid death of the organisms by decerebration (for vertebrates) immediately after collection.

Consent to participate and publish

All authors consented to participate to this study, to write this article and to publish it in the revue Environmental Science and Pollution Research. All authors agreed the content of this manuscript and consent to submit it.

Availability of data and materials

Data are available on requests from the authors.

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